

The Role of Synovial Inflammation in Osteoarthritis

by

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**Submitted in fulfilment of the requirements for the degree of Doctor of Philosophy
(Medical Research)**

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Statement of Originality

This thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in the thesis, and to the best of my knowledge and belief no material previously published or written by another person except where due acknowledgement is made in the text of the thesis, nor does the thesis contain any material that infringes copyright.

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Statement of Authority of Access and Regarding Published Work

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Statement of Co-Authorship

This thesis includes papers for which Xia Wang (XW) was not the sole author. XW was the lead in the research of each manuscript; however, she was assisted by the co-authors whose contributions are detailed below.

Chapters 4

Wang X, Jin X, Han W, Cao Y, Halliday A, Blizzard L, Pan F, Antony B, Cicuttini F, Jones G, Ding C. Cross-sectional and longitudinal associations between knee joint effusion-synovitis and knee pain in older adults. *Journal of Rheumatology* 2016 Jan;43(1):121-30.

The contribution of each author:

XW was responsible for data collection, data acquisition and management, carried out data analysis and interpretation, prepared the initial manuscript draft, and completed manuscript revisions.

CY, HW and AH participated in acquisition of data, and critically revised the manuscript for important intellectual content.

XJ, LB, FP, BA and FC participated in data analysis and interpretation, and critically revised the manuscript for important intellectual content.

CD and GJ designed and carried out the study conception, participated in data analysis and interpretation, assisted with the initial manuscript draft, and critically revised the manuscript.

All authors made final approval of the version of the article being published.

Chapter 5

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XW was responsible for data collection, data acquisition and management, carried out data analysis and interpretation, prepared the initial manuscript draft, and completed manuscript revisions.

HW and AH participated in acquisition of data, and critically revised the manuscript for important intellectual content.

XJ, LB and FC participated in data analysis and interpretation, and critically revised the manuscript for important intellectual content.

CD and GJ designed and carried out the study conception, participated in data analysis and interpretation, assisted with the initial manuscript draft, and critically revised the manuscript.

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Chapter 6

Wang X, Blizzard L, Jin X, Chen Z, Zhu Z, Halliday A, Cicuttini F, Jones G, Ding C. Quantitative knee effusion-synovitis assessment in older adults: associations with knee structural abnormalities. *Arthritis & Rheumatology* 2016 Apr;68(4):837-44.

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Chapter 7

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CD designed and carried out the study conception, participated in data analysis and interpretation, assisted with the initial manuscript draft, and critically revised the manuscript.

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Chapter 8:

Wang X, Cicuttini F, Jin X, Wluka E A, Han W, Zhu Z, Blizzard L, Antony B, Winzenberg T, Jones G, Ding C. Effect of vitamin D on effusion-synovitis in knee osteoarthritis: a secondary analysis of a randomised controlled trial. (Abstract accepted for American College of Rheumatology Annual Meeting 2015, San Francisco, U.S.A.)

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LB, FC, AW, TW and GJ participated in data analysis and interpretation, and critically revised the manuscript for important intellectual content.

CD designed and carried out the study conception, participated in data analysis and interpretation, assisted with the initial manuscript draft, and critically revised the manuscript.

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Statement of Ethical Conduct

The research associated with this thesis abides by the international and Australian codes on human and animal experimentation, the guidelines by the Australian Government's Office of the Gene Technology Regulator and the rulings of the Safety, Ethics and Institutional Biosafety Committees of the University.

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Abstract

Osteoarthritis (OA) is a multifactorial joint disease and one of the leading causes of pain and disability in older adults. It has long been hypothesised that synovium plays an important role in the initiation and progression of OA. This thesis aims to investigate the associations between synovial inflammation in the knee and important disease outcomes of OA, and the therapeutic effects of vitamin D supplementation on synovial inflammation.

This thesis utilises data from two clinical studies. The first one is a cohort study with a total number of 977 participants randomly selected from the local community (51% female; aged 62 ± 7 years) at baseline. 404 participants were followed up approximately 2.7 ± 0.4 years later. Synovial inflammation (assessed as effusion-synovitis), cartilage volume, cartilage defects, tibial bone area, and bone marrow lesions (BMLs) were assessed using magnetic resonance imaging (MRI). X-ray was used to assess radiographic changes including joint space narrowing (JSN) and osteophytes. Questionnaires were used to assess knee pain, knee function, knee stiffness, physical activity, and other disease status.

Another study is a multi-centre, parallel, randomised, placebo-controlled trial which was conducted in symptomatic knee OA patients aged 63 ± 7 years (208 females). 413 patients were allocated to either a 50,000 IU monthly vitamin D₃ capsule (n=209) or placebo (n=204) for 24 months. Knee effusion-synovitis, cartilage defects, cartilage volume and BMLs were assessed using MRI. Knee symptoms were assessed at baseline and months 3, 6, 12, 24 using Western Ontario and McMaster University Index of OA (WOMAC) questionnaire. Serum 25-hydroxylvitamin D (25-(OH)D) was assayed by Liaison method utilising a direct competitive chemiluminescent immunoassay.

In Chapter 4 the cross-sectional and longitudinal associations between site-specific effusion-synovitis and knee pain were examined in older adults. We found that effusion-synovitis in the suprapatellar pouch was consistently associated with weight-bearing and non-weight-bearing pain while those in central and posterior sites were only associated with non-weight-bearing (inflammatory) knee pain. These associations were independent of age, sex, body mass index (BMI), cartilage defects, BMLs and ROA.

In Chapter 5, we examined if effusion-synovitis was associated with knee structural changes cross-sectionally and longitudinally in older adults. Suprapatellar pouch effusion-synovitis at baseline predicted the development/progression of cartilage defects and BMLs as well as cartilage volume loss. Effusion-synovitis was independently associated with cartilage defects after adjustments for other structural abnormalities. The associations between

effusion-synovitis and BMLs and cartilage volume were largely dependent on cartilage defects, suggesting potential causal pathways.

In Chapter 6, natural history and clinical significance of quantitatively measured effusion-synovitis were described in older adults. The mean (\pm SD) size of effusion-synovitis in participants was 1.64 cm² (\pm 1.34 cm²) at baseline with 29% improving and 22% worsening in size over 2.7 years. Males had larger effusion-synovitis size than females. Baseline effusion-synovitis also positively associated with changes in cartilage defects and BMLs, while it is negatively associated with change in cartilage volume. No associations were found between baseline structural alterations and change in effusion-synovitis size.

In Chapter 7, we further examined clinical relevance of effusion-synovitis using effusion-synovitis volume measurement in patients with knee OA. Baseline BML, cartilage defect, JSN and osteophyte scores were significantly associated with the change in effusion-synovitis volume. However, neither baseline effusion-synovitis volume nor score was significantly associated with changes in BMLs, cartilage defects or cartilage volume. There were no significant associations between knee effusion-synovitis volume and measures at patellofemoral joint.

In Chapter 8, the effects of vitamin D supplementation on effusion-synovitis were determined in patients with knee OA. Baseline effusion-synovitis volume was 8.0 ml with a prevalence of 52%. After a 24-month intervention, the vitamin D group had a less increase in total effusion-synovitis volume than the control group (between group difference: -1.94 ml, $p=0.02$). The between-group differences were particularly significant in suprapatellar pouch region, and in patients who had effusion-synovitis at baseline.

In conclusion, the series of relevant studies indicate that synovial inflammation plays a pivotal role in the pathogenesis of knee OA. Synovial inflammation contributed to knee pain and predicted the development or progression of disease outcomes such as cartilage defects and BMLs in older adults, most of them being in early stages of osteoarthritic changes; in contrast, cartilage defects and bone abnormalities accelerated progression of effusion-synovitis only in those with established OA. Vitamin D as an attractive therapeutic intervention has shown a beneficial effect on halting the worsening of effusion-synovitis in knee OA patients. Our work suggests that effusion-synovitis can be used as a disease diagnostic feature and an outcome measure in OA trials. Targeting on the effusion-synovitis can be implicated for future development of disease-modifying OA drugs (DMOADs). Future work is required to confirm the relationship between effusion-synovitis and joint structural changes in patients with early and/or late stages of OA in longer-term cohort studies. Clinical

trials are also needed to examine whether treatments such as vitamin D supplementation can reduce progression of effusion-synovitis (as the primary outcome) in knee OA patients with inflammatory phenotype.

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Publications Arising from the Thesis

Chapter 4: Wang X, Jin X, Han W, Cao Y, Halliday A, Blizzard L, Pan F, Antony B, Cicuttini F, Jones G, Ding C. Cross-sectional and longitudinal associations between knee joint effusion-synovitis and knee pain in older adults. *Journal of Rheumatology*. 2016 Jan;43(1):121-30.

Chapter 5: Wang X, Blizzard L, Halliday A, Han W, Jin X, Cicuttini F, Jones G, Ding C. Association between MRI-detected knee joint regional effusion-synovitis and structural changes in older adults: a cohort study. *Annals of the Rheumatic Diseases*. 2016 Mar;75(3):519-25.

Chapter 6: Wang X, Blizzard L, Jin X, Chen Z, Zhu Z, Halliday A, Cicuttini F, Jones G, Ding C. Quantitative knee effusion-synovitis assessment in older adults: associations with knee structural abnormalities. *Arthritis & Rheumatology* 2016 Apr;68(4):837-44.

Chapter 7: Wang X, Cicuttini F, Jin X, Wluka E A, Han W, Zhu Z, Antony B, Blizzard L, Winzenberg T, Jones G, Ding C. Associations between knee effusion-synovitis and joint structural changes in patients with knee osteoarthritis: a cohort study (Manuscript under revision).

Chapter 8: Wang X, Cicuttini F, Jin X, Wluka E A, Han W, Zhu Z, Blizzard L, Antony B, Winzenberg T, Jones G, Ding C. Knee effusion-synovitis volume measurement and effects of vitamin D supplementation in patients with knee osteoarthritis. *Osteoarthritis & Cartilage* 2017 Mar 6 (in press).

Other Publications

Wang X, Hunter D, Xu J, Ding C. Metabolic triggered inflammation in osteoarthritis.

Osteoarthritis & Cartilage. 2015;23(1):22-30.

Zhu, Z; Laslett, L; Jin, X; Han, W; Antony, B; **Wang, X**; Lu, M; Cicuttini, F; Jones, G; Ding, C. Association between MRI-detected osteophytes and changes in knee structures and pain in older adults: a cohort study. Osteoarthritis & Cartilage 2017 Jan 21.

Jin X, Jones G, Cicuttini F, Wluka A, Zhu Z, Han W, Antony B, **Wang X**, Winzenberg T, Blizzard L, Ding C. Effect of vitamin d supplementation on tibial cartilage volume and knee pain among patients with symptomatic knee osteoarthritis: A randomized clinical trial.

JAMA. 2016;315(10):1005-13.

Zhu, Z; Otahal, P; Wang, B; Jin, X; Laslett, L; Wluka, A; Antony, B; Han, W; **Wang, X**; Winzenberg, T; Cicuttini, F; Jones, G; Ding, C. Cross-sectional and longitudinal associations between serum inflammatory cytokines and knee bone marrow lesions in patients with knee osteoarthritis. Osteoarthritis & Cartilage 2017 Apr;25(4):499-505.

Jin X, Ding C, **Wang X**, Antony B, Laslett L, Blizzard L, Cicuttini F, Jones G. Longitudinal associations between adiposity, lean mass and change in knee pain: Tasmanian Older Adult Cohort Study. Seminars in Arthritis and Rheumatism. 2016 Apr;45(5):564-9.

Pan F, Han W, **Wang X**, Liu Z, Jin X, Antony B, Cicuttini F, Jones G, Ding C. A longitudinal study of the association between infrapatellar fat pad maximal area and changes in knee symptoms and structure in older adults. Annals of the Rheumatic Diseases. 2015 Oct;74(10):1818-24.

Wang J, Han W, **Wang X**, Pan F, Liu Z, Halliday A, Jin X, Antony B, Cicuttini F, Jones G, Ding C. Mass effect and signal intensity alteration in the suprapatellar fat pad: associations with knee symptoms and structure. Osteoarthritis & Cartilage. 2014;22(10):1619-26.

Han W, Aitken D, Zhu Z, Halliday A, **Wang X**, Antony B, Cicuttini F, Jones G, Ding C. Signal intensity alteration in the infrapatellar fat pad at baseline for the prediction of knee

symptoms and structure in older adults: a cohort study. *Annals of the Rheumatic Diseases*. 2015 Nov 26. [Epub ahead of print].

Cao Y, Jones G, Han W, Antony B, **Wang X**, Cicuttini F, Ding C. Popliteal cysts and subgastrocnemius bursitis are associated with knee symptoms and structural abnormalities in older adults: a cross-sectional study. *Arthritis Research & Therapy*. 2014;16(2): R59.

Han W, Cai S, Liu Z, Jin X, **Wang X**, Antony B, Cao Y, Aitken D, Cicuttini F, Jones G, Ding C. Infrapatellar fat pad in the knee: is local fat good or bad for knee osteoarthritis? *Arthritis Research & Therapy*. 2014;16(4): R145.

Wang J, Antony B, Zhu Z, Han W, Pan F, **Wang X**, Jin X, Liu Z, Cicuttini F, Jones G, Ding C. Association of patellar bone marrow lesions with knee pain, patellar cartilage defect and patellar cartilage volume loss in older adults: a cohort study. *Osteoarthritis & Cartilage*. 2015;23(8):1330-6.

Deveza LA, Ding C, Jin X, **Wang X**, Zhu Z, Hunter DJ. (2016) Chapter 20. Laboratory tests. *Oxford Textbook of Osteoarthritis and Crystal Arthropathy* (3rd edition). London, Oxford University Press. (Book chapter)

Scientific Presentations Arising from the Thesis

International

- 2016** European Congress of Rheumatology (EULAR): Annual Scientific Meeting 2016, London, UK. **“Effect of vitamin D on effusion-synovitis in knee osteoarthritis: a randomized controlled trial”**
(Poster presentation)
- 2015** American College of Rheumatology (ACR): Annual Scientific Meeting 2015. San Francisco, U.S. **“Effect of vitamin D on effusion-synovitis in knee osteoarthritis: a randomized controlled trial.”**
(Poster presentation)
- European Congress of Rheumatology (EULAR): Annual Scientific Meeting 2015, Rome, Italy. **“The natural history and clinical significance of knee effusion-synovitis change: a 2.7-year older adults cohort study.”**
(Poster presentation)
- 2014** Annual European Congress of Rheumatology EULAR 2014, Paris, France.
“Cross-sectional and longitudinal associations between knee joint effusion and knee pain in older adults”
“Cross-sectional and longitudinal associations between knee joint effusion and osteoarthritic structural changes in older adults”
(2 Oral presentations)

National

- 2016** Australian Rheumatology Association (ARA): 57th Annual Scientific Meeting with the Rheumatology Health Professionals Association 2016, Darwin Convention Centre, Northern Territory. **“Effect of vitamin D supplementation on effusion-synovitis in knee osteoarthritis: a randomised controlled trial”**
(Oral presentation)

- 2015** Australian Rheumatology Association (ARA): 55th Annual Scientific Meeting 2015, Adelaide, Australia. **“The natural history and clinical significance of knee effusion-synovitis change: a 2.7-year older adults cohort study”**
(Poster presentation)
- 14th National Emerging Research in Aging (ERA) Conference 2015, Melbourne, Australia. **“Effect of vitamin D supplementation on effusion-synovitis in knee osteoarthritis: a randomised controlled trial”**
Rapid Fire Presentation Competition: “Synovial inflammation in osteoarthritis”
(2 oral presentations)
- 5th Australia-China Biomedical Research Conference, 2nd Hobart Satellite Meeting.
“Cross sectional and longitudinal associations between knee joint effusion and osteoarthritic changes in older adults.”
(Poster presentation)
- 2013** Australia Chinese Association for Biomedical Sciences (ACABS): Five minutes science competition, Melbourne, Australia. **“Infrapatellar fat pad fat: Can local fat actually be good for osteoarthritis?”**
(Oral presentation)

Local

- 2015** University of Tasmania: 2015 Graduate Research Conference, Sandy Bay, Australia.
“Associations between MRI-detected knee joint effusion and osteoarthritic changes in older adults.”
(Poster presentation)
- Tasmanian Health Higher Degree Research Student (THRSC) conference. Hobart, Australia. **“Effect of vitamin D supplementation on effusion-synovitis in knee osteoarthritis: a randomised controlled trial”**
(Oral presentation)

2014 Tasmanian Health Higher Degree Research Student (THRSC) conference. Hobart, Australia. **“Association between MRI-detected knee joint effusion-synovitis and structural changes in older adults: a cohort study”**

(Oral presentation)

Menzies Student Showcase series of events: “Menzies Digest” 5-minute presentations, Hobart, Australia. **“MRI-detected knee synovial inflammation in Osteoarthritis”**

(Oral presentation)

Awards Resulting from the Thesis

- 2016** Winner of the “2015 Ten of The Best Research Award” of Menzies Institute for Medical Research. A travel grant provided to Ph.D. candidate to participate in conferences.
- “2015 Chinese Government Award for Outstanding Students (Non Government-Sponsored) Abroad” (USD 6,000). A prestigious award from the Chinese government for outstanding academic achievement made by students studying overseas.
- 2015** Second place of “Best Poster Award” at 2nd Hobart Satellite Meeting of 5th Australia-China Biomedical Research Conference.
- 2015** Winner of the “2014 Ten of The Best Research Award” of Menzies Institute for Medical Research.
- 2014** Graduate Research Travel and Conference Fund grant from University of Tasmania. A travel grant provided to Ph.D candidate to participate in international conferences.
- 2013-2016** International postgraduate research scholarship, University of Tasmania. A prestigious award from the Australian Government.
- 2012-2015** Living allowance scholarship, University of Tasmania.

List of Abbreviations

25-(OH)D	25-hydroxyvitamin D
2D	two-dimensional
3D	three-dimensional
ACL	anterior cruciate ligament
ACR	American College of Rheumatology
ADAMTS	a disintegrin and metalloproteinase with thrombospondin motifs
AIHW	Australian Institute of Health and Welfare
BLOKS	Boston Leeds Osteoarthritis Knee Score
BMD	bone mineral density
BMI	body mass index
BML	bone marrow lesion
BMP	bone morphogenetic protein
CE	contrast enhance
CGP-39	cartilage glycoprotein-39 (also known as chitinase-3-like protein 1 and YKL-40)
CI	confidence interval
COMP	cartilage oligomeric matrix protein
COX-2	cyclooxygenase-2
CRP	C-reactive protein
CS	chondroitin sulfate
CTX-II	type II collagen C-terminal telopeptide
CV	coefficient of variation
DMOAD	disease-modifying osteoarthritis drug
DXA	dual-energy x-ray absorptiometry
ESR	erythrocyte sedimentation rate

EGF	endothelial growth factor
EULAR	European League Against Rheumatism
FS	fat suppressed/saturated
FSE	fast spin echo
GAG	glycosaminoglycan
GAIT	Glucosamine/chondroitin Arthritis Intervention Trial
GEE	generalised estimating equations
Glc-Gal-PYD	glucosyl-galactosyl-pyridinoline
GRE	gradient-recalled echo
HA	hyaluronic acid
HDL	high-density lipoprotein
ICAM-1	intercellular adhesion molecule 1
IL	interleukin
IL-1Ra	interleukin 1 receptor antagonist
ICC	intraclass correlation coefficient
JSN	joint space narrowing
JSW	joint space width
KOSS	Knee Osteoarthritis Scoring Systems
KJ	kilojoule
K-L	Kellgren and Lawrence
LF	lateral femoral
LSC	least significant criterion
LT	lateral tibial
MF	medial femoral
MMP	matrix metalloproteinase
MOAKS	MRI Osteoarthritis Knee Score

MOST	Multicentre Osteoarthritis Study
MRI	magnetic resonance imaging
MT	medial tibial
NAG	N-acetylglucosaminidase
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NGF	nerve growth factor
NHMRC	National Health and Medical Research Council
NO	nitric oxide
NSAIDS	non-steroidal anti-inflammatory drugs
OA	osteoarthritis
OARSI	Osteoarthritis Society International Radiographic Atlas
OR	odds ratio
p.a.	per annum
PCL	posterior cruciate ligament
PD	proton density
PGE₂	prostaglandin E 2
PR	prevalence ratio
RA	rheumatoid arthritis
RCT	randomised controlled trial
ROA	radiographic osteoarthritis
ROI	region of interest
RR	relative risk
SAPL	surface-active phospholipids
SE	standard error
SD	standard deviation
STIR	short tau inversion recovery

TASOAC	Tasmanian Older Adult Cohort study
TKR	total knee joint replacement
TNF	tumour necrosis factor
US	United States
VAS	visual analogue score
VCAM-1	vascular cell adhesion molecule 1
VEGF	vascular endothelial growth factor
VIDEO	Vitamin D Effect on Osteoarthritis Study
WOMAC	Western Ontario and McMaster Universities Osteoarthritis Index
WORMS	Whole-Organ Magnetic Resonance Imaging Score

Table of Contents

Statement of Originality	II
Statement of Authority of Access and Regarding Published Work.....	III
Statement of Co-Authorship	IV
Statement of Ethical Conduct.....	VIII
Abstract.....	IX
Acknowledgements	XII
Publications Arising from the Thesis	XIV
Other Publications	XV
Scientific Presentations Arising from the Thesis	XVII
Awards Resulting from the Thesis	XX
List of Abbreviations	XXI
Table of Contents	XXV
List of Tables	XXX
List of Figures.....	XXXII
Synopsis.....	1
Chapter 1 - Introduction	6
1.1 Overview of osteoarthritis.....	7
1.1.1 Disease prevalence and burden.....	9
1.1.2 Clinical symptoms.....	9
1.1.3 Risk factors.....	10
1.1.4 Treatment and management.....	10
1.2 Knee OA diagnostic criteria.....	11
1.2.1 Radiography.....	11
1.2.2 Clinical criteria.....	12
1.2.3 Magnetic resonance imaging.....	14
1.3 Synovium in OA	14
1.3.1 Physiology and function.....	15
1.3.2 Synovial anatomy of the knee joint	16
1.3.3 Biological markers.....	20
1.3.4 Clinical significance	23
1.3.5 MR Imaging assessment.....	25
1.3.6 Synovial inflammation as a treatment target.....	32

1.4	Summary	34
Chapter 2 - Research questions		36
Chapter 3 - Methodology.....		38
3.1	Prelude.....	39
3.2	TASOAC study population and design.....	39
3.2.1	<i>Anthropometrics.....</i>	<i>41</i>
3.2.2	<i>Knee pain assessment</i>	<i>41</i>
3.2.3	<i>Magnetic Resonance Imaging.....</i>	<i>41</i>
3.2.4	<i>X-ray</i>	<i>49</i>
3.2.5	<i>Summary of outcome factors, study factors, and covariates</i>	<i>50</i>
3.2.6	<i>Ethical considerations</i>	<i>50</i>
3.2.7	<i>Statistical analysis</i>	<i>50</i>
3.3	VIDEO Study population and design.....	51
3.3.1	<i>Intervention.....</i>	<i>52</i>
3.3.2	<i>Randomisation</i>	<i>52</i>
3.3.3	<i>Blinding.....</i>	<i>53</i>
3.3.4	<i>Trial monitoring and safety assessments</i>	<i>53</i>
3.3.5	<i>Anthropometrics.....</i>	<i>53</i>
3.3.6	<i>Knee symptoms.....</i>	<i>53</i>
3.3.7	<i>Magnetic Resonance Imaging.....</i>	<i>53</i>
3.3.8	<i>X-ray</i>	<i>54</i>
3.3.9	<i>Serum 25-(OH) D.....</i>	<i>55</i>
3.3.10	<i>Summary of outcome factors, study factors, and covariates.....</i>	<i>55</i>
3.3.11	<i>Sample size and role of the candidate in the VIDEO study</i>	<i>55</i>
3.3.12	<i>Ethical considerations.....</i>	<i>56</i>
3.3.13	<i>Trial registration.....</i>	<i>56</i>
3.3.14	<i>Protocol.....</i>	<i>56</i>
3.3.15	<i>Statistical analysis.....</i>	<i>56</i>
Chapter 4 – Regional knee effusion-synovitis and knee pain in older adults: a cohort study		57
4.1	Introduction	58
4.2	Materials and Methods	59
4.2.1	<i>Participants and study design.....</i>	<i>59</i>
4.2.2	<i>Knee pain</i>	<i>59</i>

4.2.3	<i>Knee MRIs</i>	59
4.2.4	<i>Knee X-rays</i>	59
4.2.5	<i>Anthropometrics</i>	59
4.2.6	<i>Statistical analysis</i>	59
4.3	Results	61
4.3.1	<i>Participants and study design</i>	61
4.3.2	<i>Cross-sectional associations</i>	63
4.3.3	<i>Longitudinal associations</i>	68
4.4	Discussion	72
Chapter 5 - Regional knee effusion-synovitis and knee structural abnormalities in older adults: a cohort study		75
5.1	Introduction	76
5.2	Materials and Methods	77
5.2.1	<i>Participants and study design</i>	77
5.2.2	<i>Knee MRI sequences</i>	77
5.2.3	<i>Knee MRI measurements</i>	77
5.2.4	<i>Knee X ray</i>	78
5.2.5	<i>Anthropometrics</i>	78
5.2.6	<i>Statistical analysis</i>	78
5.3	Results	79
5.3.1	<i>Characteristics of participants</i>	79
5.3.2	<i>Effusion-synovitis and cartilage defects</i>	81
5.3.3	<i>Effusion-synovitis and cartilage volume</i>	85
5.3.4	<i>Effusion-synovitis and BMLs</i>	87
5.3.5	<i>Radiographic changes</i>	89
5.3.6	<i>Other analyses</i>	89
5.4	Discussion	90
Chapter 6 – Quantitative knee effusion-synovitis and knee structural abnormalities in older adults: a cohort study		94
6.1	Introduction	95
6.2	Materials and Methods	96
6.2.1	<i>Characteristics of participants</i>	96
6.2.2	<i>Knee MRI sequences</i>	96
6.2.3	<i>Knee MRI measurements</i>	96

6.2.4	<i>Knee pain</i>	97
6.2.5	<i>Knee X-ray</i>	97
6.2.6	<i>Anthropometrics</i>	97
6.2.7	<i>Statistical analysis</i>	97
6.3	Results	98
6.3.1	<i>Characteristics of participants</i>	98
6.3.2	<i>Natural history of effusion-synovitis</i>	100
6.3.3	<i>Associations between effusion-synovitis and knee structures</i>	102
6.4	Discussion	107
Chapter 7 – Associations between knee effusion-synovitis and joint structural changes in patients with knee osteoarthritis: a cohort study 110		
7.1	Introduction	111
7.2	Materials and Methods	112
7.2.1	<i>Participants and study design</i>	112
7.2.2	<i>Knee symptoms</i>	112
7.2.3	<i>Knee MRI sequences</i>	112
7.2.4	<i>Knee MRI measurements</i>	112
7.2.5	<i>Knee X-ray</i>	113
7.2.6	<i>Anthropometrics</i>	113
7.2.7	<i>Serum 25-(OH) D</i>	113
7.2.8	<i>Statistical analysis</i>	113
7.3	Results	114
7.3.1	<i>Characteristics of participants</i>	114
7.3.2	<i>Quantitative and semi-quantitative measures of effusion-synovitis</i>	116
7.3.3	<i>Association between effusion-synovitis and knee symptoms</i>	116
7.3.4	<i>Association between effusion-synovitis and knee structures</i>	119
7.4	Discussion	123
Chapter 8 – Effects of vitamin D supplementation on effusion-synovitis in patients with knee osteoarthritis and low vitamin D levels: a randomised controlled trial..... 126		
8.1	Introduction	127
8.2	Materials and Methods	129
8.2.1	<i>Trial design</i>	129
8.2.2	<i>Participants</i>	129
8.2.3	<i>Interventions</i>	129

8.2.4	<i>Outcomes.....</i>	129
8.2.5	<i>Randomisation</i>	130
8.2.6	<i>Blinding.....</i>	130
8.2.7	<i>Trial monitoring and safety assessment.....</i>	130
8.2.8	<i>Sample size calculation.....</i>	130
8.2.9	<i>Statistical analysis</i>	130
8.3	Results	131
8.3.1	<i>Characteristics of participants</i>	131
8.3.2	<i>Outcome</i>	134
8.4	Discussion	139
Chapter 9 - Summary and future directions		141
9.1	Summary	142
9.2	Future directions.....	144
9.2.1	<i>Synovial biomarker for diagnosis and treatment.....</i>	144
9.2.2	<i>Inflammatory phenotype of OA.....</i>	146
9.2.3	<i>Challenges in treating synovial inflammation</i>	146
Bibliography		148
Appendices.....		165

List of Tables

Table 1.1. Criteria for diagnosis of knee osteoarthritis (43).....	13
Table 1.2. Biomarker related to synovium in osteoarthritis.....	22
Table 1.3. Summary of semi-quantitative MRI scoring system for synovial abnormalities in knee OA.	30
Table 3.1. Summary of outcome factors, study factors, and covariates used in this thesis.....	50
Table 3.2. Summary of outcome factors, study factors, and covariates used in this thesis.....	55
Table 4.1. Characteristics of participants.....	62
Table 4.2. Cross-sectional associations between knee joint effusion-synovitis and knee pain at baseline.	64
Table 4.3. Cross-sectional associations between knee joint effusion-synovitis and knee pain (highest quartile) at baseline.	65
Table 4.4. Longitudinal associations between knee joint effusion-synovitis and knee pain. ..	69
Table 4.5. Longitudinal associations between knee joint effusion-synovitis and knee pain. ..	70
Table 4.6. Longitudinal associations between knee joint effusion-synovitis and knee pain. ..	71
Table 5.1. Characteristics of sample at baseline.	80
Table 5.2. Associations between knee effusion-synovitis and cartilage defects.	82
Table 5.3. Associations between knee effusion-synovitis and cartilage volume.....	86
Table 5.4. Associations between knee effusion-synovitis and cartilage volume.....	88
Table 6.1. Characteristics of participants at baseline.....	99
Table 6.2. Natural history of knee effusion-synovitis in total sample and gender subgroups.	101
Table 6.3. Associations between baseline knee structural factors and baseline effusion-synovitis.	103
Table 6.4. Associations between baseline knee effusion-synovitis and change in structural factors.....	104
Table 6.5. Associations between baseline knee effusion-synovitis and change in structural factors.....	105
Table 6.6. Associations between knee structural factors and effusion-synovitis.	106
Table 7.1. Participant characteristics at baseline.	115
Table 7.2. The Associations between change in effusion-synovitis and baseline structures.	120
Table 7.3. The associations between baseline effusion-synovitis and change in structures..	121
Table 7.4. The associations between effusion-synovitis and other structures.	122

Table 8.1. Participant characteristics at baseline.	133
Table 8.2. Two-year changes in total knee effusion-synovitis between vitamin D and placebo groups.....	135
Table 8.3. Two-year changes in regional knee effusion-synovitis between vitamin D and placebo groups.	137
Table 8.4. Changes in knee effusion-synovitis exceeding MCID or LSC over two years. ...	138

List of Figures

Figure 1.1. Articular structures that are affected in OA (8).....	8
Figure 1.2. Kellgren Lawrence (K-L) grading scale on radiographic knee OA (40).....	12
Figure 1.3. The schema of the synovial membrane (57).....	16
Figure 1.4. The synovial anatomy of a knee joint (59).....	17
Figure 1.5. The T2/PD weighted MR images show multiple synovial recesses in different subregions of a knee joint (53).....	19
Figure 1.6. Joint effusion.	26
Figure 1.7. Synovitis in Hoffa's fat pad.....	27
Figure 1.8. Joint effusion-synovitis.	28
Figure 3.1. Flow chart describing recruitment, participation rates, and withdrawal reasons for TASOAC participants.....	41
Figure 3.2. Typical T2-weighted fat-saturation fast spin echo sagittal images for effusion-synovitis in different subregions.....	43
Figure 3.3. Examples of changes in effusion-synovitis area (cm ²).....	45
Figure 3.4. MRI acquired from the knee, with superimposed colour data showing the area of high signal.....	47
Figure 3.5. Flow chart describing recruitment, participation rates, and withdrawal reasons for VIDEO participants.	52
Figure 4.1. Associations between knee effusion-synovitis in different subregions and pain. .66	
Figure 4.2. Prevalence ratio for associations between knee effusion-synovitis in different subregions and weight-bearing/non-weight-bearing pain at baseline.....	67
Figure 5.1. Longitudinal associations between joint effusion-synovitis in suprapatellar pouch and structural measures over 2.6 years.	83
Figure 7.1. Scatter plot of longitudinal associations between change in effusion-synovitis volume and change in WOMAC score over 24 months in adjusted analyses.	117

Synopsis

OA is the most common form of arthritis and a leading cause of musculoskeletal pain and disability. Although its signature pathologic feature is articular cartilage loss, many other joint structures, including subchondral bone, ligaments, menisci, periarticular muscles, peripheral nerves, and synovium were also involved. It was hypothesised that synovial inflammation plays an important role in disease pathogenesis of early OA. There is emerging evidence to support this; however, it remains controversial whether synovial inflammation precedes cartilage damage. This thesis examines the role of effusion-synovitis in preclinical and clinical OA using data from a prospective population-based study in community-dwelling older adults and a clinical trial in patients with knee symptomatic OA. Specifically, it compares quantitative and semi-quantitative effusion-synovitis measures in different joint subregions and explores how these measures relate to disease severity and progression. This synopsis presents an overview of the content of each chapter. It further investigates the effects of vitamin D supplementation on effusion-synovitis in knee symptomatic OA patients using data from a RCT.

Chapter 1 provides an overview of knee OA with a focus on the synovial membrane. A working definition is provided and the economic impact, burden of disease, symptoms, risk factors, treatment and management options are discussed. A description of the radiographic and clinical criteria for the diagnosis of knee OA is presented. Notably, this chapter presents an overview of the physiological and pathological roles of joint fluid and how it relates to clinical outcomes in OA. It then provides an outline of different MRI assessment systems in measuring synovial abnormalities. Lastly, this chapter summed current treatments that targeted on synovial inflammation in OA research.

Chapter 2 lists the research questions to be addressed in the thesis.

Chapter 3 describes two studies, the Tasmanian Older Adult Cohort (TASOAC) and the Vitamin D Effect on Osteoarthritis study (VIDEO), which are used in this thesis, respectively. It describes the study population and its design, as well as the protocols for measurement of factors, which are common to multiple chapters in this thesis. Additional factors, which are unique to each chapter, are described in more detail in the methodology section of the subsequent chapters.

Chapter 4 describes the cross-sectional and longitudinal associations between knee regional effusion-synovitis and knee pain in older adults. Data from a population-based random sample (N = 880, mean age 62 years; 50% females) was used. Baseline knee joint effusion-synovitis was graded (0-3) using T2-weighted MRI in suprapatellar pouch, central portion, posterior femoral recess, and subpopliteal recess. Effusion-synovitis of the whole joint was defined as a score of ≥ 2 in any subregion. Other knee structural (including cartilage, bone marrow and menisci) lesions were assessed by MRI at baseline. Knee pain was assessed by WOMAC questionnaire at baseline and 2.6 years later. Multivariable analyses were performed after adjustment for age, gender, BMI and other structural lesions. The prevalence of effusion-synovitis was 67%. Suprapatellar pouch effusion-synovitis was significantly and independently associated with increased total and non-weight-bearing knee pain in both cross-sectional and longitudinal (for an increase in total knee pain of ≥ 5 : RR, 1.26 per grade, 95% CI: 1.04, 1.52) analyses, and increased weight-bearing knee pain in the longitudinal analysis only. Effusion-synovitis in posterior femoral recess and central portion were independently associated with increases in non-weight-bearing pain (RR: 1.63 per grade, 95% CI: 1.32, 2.01; RR: 1.29 per grade, 95% CI: 1.01, 1.65, respectively) in longitudinal analyses only. In conclusion, knee joint effusion-synovitis has independent associations with knee pain in older adults. Suprapatellar pouch effusion-synovitis is associated with non-weight-bearing and weight-bearing knee pain, while posterior femoral recess and central portion effusion-synovitis are only associated with non-weight-bearing pain.

Chapter 5 describes the cross-sectional and longitudinal associations between knee regional effusion-synovitis and structural changes in older adults. A total of 977 subjects were randomly selected from the local community (mean 62 years, 50% female) at baseline and 404 were followed up 2.6 years later. T2-weighted MRI was used to assess knee effusion-synovitis in four subregions: suprapatellar pouch, central portion, posterior femoral recess and subpopliteal recess. Knee cartilage defects, cartilage volume and bone marrow lesions (BMLs) were measured using MRI at baseline and follow-up. Cross-sectionally, effusion-synovitis in most subregions was significantly associated with higher risks of cartilage defects, BMLs and reduced cartilage volume. Longitudinally, suprapatellar pouch effusion-synovitis at baseline predicted an increase in cartilage defects ($p < 0.01$), loss of cartilage volume ($p = 0.04$) and an increase in BMLs ($p = 0.02$) in multivariable analyses. The significant

associations of effusion-synovitis with cartilage volume and BMLs disappeared after adjustment for cartilage defects. Effusion-synovitis in the whole knee joint ($p<0.01$) and subpopliteal recess ($p<0.05$) was consistently associated with longitudinal changes in cartilage defects but not in cartilage volume and BMLs. To sum up, there are independent associations between knee joint effusion-synovitis and knee cartilage defects in both cross-sectional and longitudinal analyses, suggesting a potential causal relationship. The associations of effusion-synovitis with BMLs and cartilage volume were largely dependent on cartilage defects, suggesting potential causal pathways.

Chapter 6 describes the natural history of quantitatively measured knee effusion-synovitis and the longitudinal associations between effusion-synovitis and knee structural factors including cartilage defects, cartilage volume, subchondral BMLs and meniscal pathology in older adults. A total of 406 subjects were randomly selected (mean 62 years, 50% females) at baseline and followed up 2.7 years later. T2 or T1-weighted fat saturation MRI was used to assess the knee effusion-synovitis maximal area, cartilage defects, cartilage volume, BMLs and meniscal pathology at baseline and follow-up. Multivariable generalized linear regressions were performed to analyse the associations between the maximal area of effusion-synovitis and other joint structural factors after adjustment for age, gender, BMI, tibial bone area and/or radiographic OA (ROA). Over 2.7 years of follow-up, the size of effusion-synovitis increased in 29%, remained stable in 50% and decreased in 22% of the participants. Baseline effusion-synovitis maximal area was significantly associated with changes in knee cartilage defects (β : 0.18, 95% CI: 0.07, 0.29), BMLs (β : 0.17, 95% CI: 0.05, 0.30), and cartilage volume (β : -0.40, 95% CI: -0.71, -0.09) but not with change in meniscal pathology. In contrast, baseline structural measures were not associated with change or increase in effusion-synovitis maximal area. Knee effusion-synovitis was not static in older adults. It was predictive of, but not predicted by, other structural abnormalities suggesting a potential role in knee early osteoarthritic changes.

Chapter 7 investigates the associations between effusion-synovitis and joint structural abnormalities in patients with knee OA over 24 months. A total of 413 symptomatic OA patients (age 63.2 ± 7.0 years, 208 females) were recruited. Knee pain, function and stiffness were assessed by WOMAC. Knee effusion-synovitis volume and score (0-3), cartilage defects, cartilage volume and BMLs were assessed using T1/T2-weighted MRI. Baseline JSN and osteophytes were assessed. Multivariable linear regression or multilevel generalised mixed-

effects linear or ordinal logistic regression models were used to analyse the longitudinal associations. Total effusion-synovitis volume increased significantly from baseline (8.0 ± 8.5 ml) to follow-up (9.0 ± 10.5 ml). Change in effusion-synovitis volume was positively correlated with changes in knee pain, function and stiffness scores (all $p < 0.05$). In the generalised mixed-effects models, knee effusion-synovitis was positively associated with total BMLs (volume: $\beta=1.16$; score: $OR=1.74$, both $p < 0.001$) and total cartilage defects (volume: $\beta=2.25$; score: $OR=2.26$, both $p < 0.001$) scores, and negatively associated with total cartilage volume. Baseline total BML, cartilage defect, JSN and osteophyte scores were positively associated with change in effusion-synovitis volume (all $p < 0.05$). Baseline cartilage defects and JSN were also associated with change in effusion-synovitis score (all $p < 0.05$). However, neither baseline effusion-synovitis volume nor score was significantly associated with change in above structures. Knee joint effusion-synovitis was associated with symptoms, and was predicted by cartilage and subchondral bone abnormalities. These suggest that synovial inflammation is clinically relevant and can be the result of joint structural abnormalities in patients with established knee OA.

Chapter 8 examines the effect of vitamin D supplementation on synovial inflammation in patients with knee OA and low vitamin D levels over 24 months. Symptomatic knee OA patients with a low 25-(OH)D level (12.5 to 60 nmol/l) were recruited for a multi-centre, randomised, placebo-controlled and double-blind trial. 413 patients (age 63.2 ± 7.0 years, 208 females) were allocated to either 50,000IU monthly vitamin D₃ orally ($n=209$) or placebo ($n=204$) for 24 months. Changes in knee effusion-synovitis volume and score (0-3) were assessed using MRI. The least significant change criterion (LSC) was used to define as an increase or a decrease in effusion-synovitis volume. At baseline, mean total effusion-synovitis volume was 8.0 ml with a prevalence of 48% (score ≥ 2). Over 24 months, total effusion-synovitis volume remained stable in the vitamin D group (mean change: 0.26 ml) but increased significantly in the placebo group (mean change: 2.20 ml). There was a significant difference between groups (-1.94 ml, 95% CI: -3.54, -0.33). This effect was evident in those with baseline effusion-synovitis (difference: -2.04 ml, 95%CI: -3.83, -0.25) and in the suprapatellar pouch (difference: -2.49 ml, 95%CI: -4.74, -0.25). The proportions with increases in total (relative risk: 0.66, 95%CI: 0.49, 0.90) and suprapatellar (relative risk: 0.64, 95%CI: 0.44, 0.93) effusion-synovitis volume were lower while the proportion with the decrease in suprapatellar pouch effusion-synovitis was higher in the vitamin D group than in

the placebo group. This secondary analysis suggested that vitamin D supplementation can retard the progression of effusion-synovitis in patients with knee OA and low 25-(OH)D levels. Effusion-synovitis volume is clinically related to osteoarthritic changes and it has a modifiable nature suggesting it can be utilised as a sensitive outcome measure in OA trials.

Chapter 9 summarises the findings of the thesis and also provides a number of potential directions for future research based on these findings.

Chapter 1 - Introduction

1.1 Overview of osteoarthritis

Osteoarthritis (OA) is the most common musculoskeletal disorder, affecting approximately 1.8 million Australians (8% of the population) (1). It is one of the leading causes of impaired mobility among older adults, characterised by pain and declining physical function (2), and eventually, in some cases, will lead to joint replacement surgery. A number of risk factors, including age, female sex, obesity, injury, quadriceps weakness and malalignment, contribute to both the onset and progression of OA (3).

Primary OA is an idiopathic disease afflicting a few or many joints, which is known as, generalised OA. Secondary OA is attributable to a cause, such as injury, congenital or developmental disorders of joints, but might have similar disease processes as primary OA (4). Our recent narrative review suggests that metabolic dysfunction or low-grade inflammation contributes to form a potential OA phenotype (Appendix 1), sharing similar aetiology as obesity and diabetes (5).

As a multifactorial disease, it is increasingly recognised that OA affects all structures within a joint, with multiple causal pathways being involved in its aetiology (6). Figure 1.1 shows the articular cartilage loss, bone remodelling as well as abnormalities related to peri-articular structures including muscles, meniscus, ligaments, synovium and subchondral bone marrow. Cartilage and subchondral bone are susceptible to abnormal external mechanical stress and internal biochemical and morphological changes, thus lose the function of absorbing biomechanical forces. Similarly, muscles, ligaments and menisci can fail due to injury or weakness, causing a breakdown in biomechanical function and amplification of physical stresses. The synovium in OA can exhibit inflammatory responses, which further damage surrounding tissues through the release of pro-inflammatory mediators into the synovial fluid (7). This interdependence among tissues underpins the multifactorial nature of the disease, with the loss of normal function in one tissue directly influencing another. Thus, OA rarely has a single cause and often presents a variety of pathological features and symptoms.

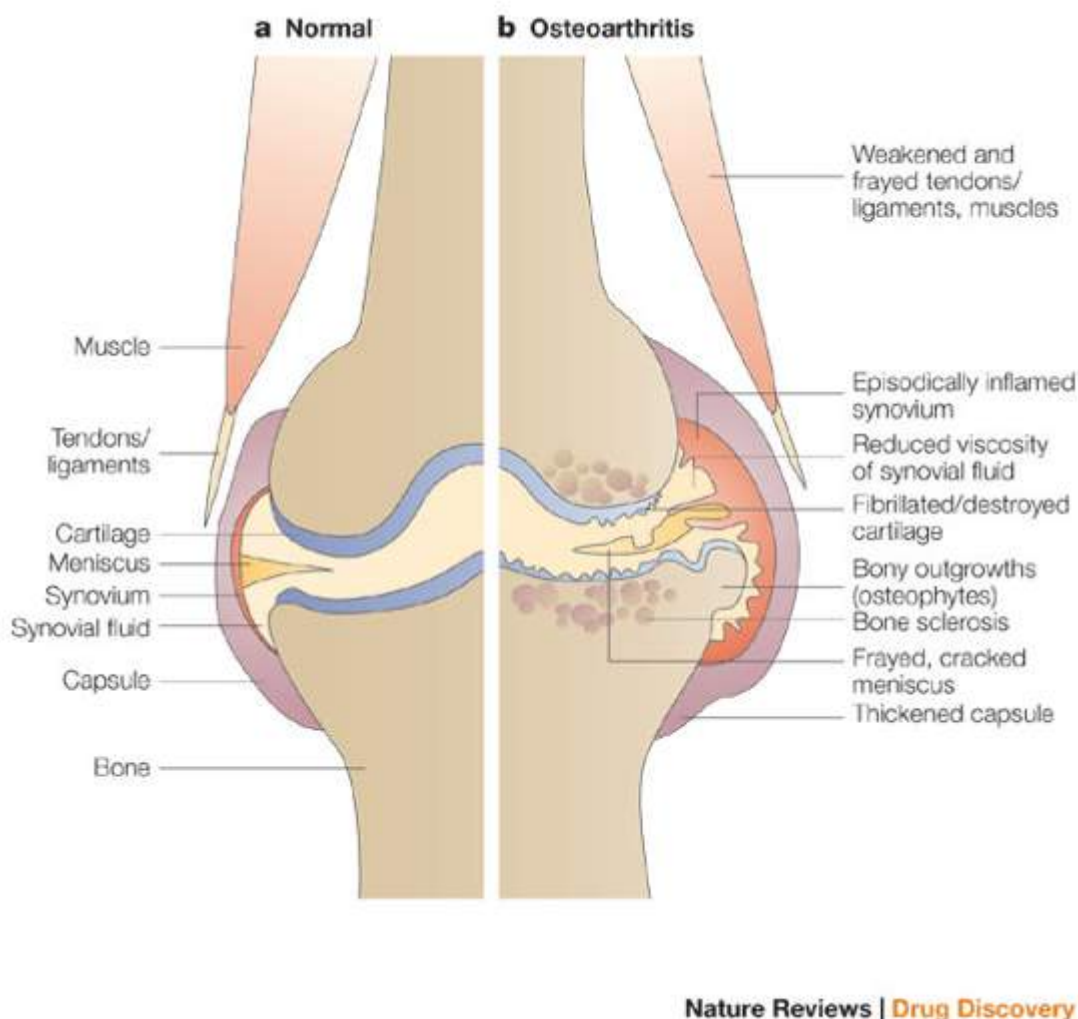


Figure 1.1. Articular structures that are affected in OA (8).

The development and progression of OA can be classified into four stages (9). The changes to the healthy joint in the first stage often involve abnormal growth of the subchondral bone and disruption to cartilage homeostasis, reflected by increased bone and cartilage biomarkers in the blood. This is followed by a preclinical stage involving structural changes in the cartilage and surrounding tissues which are now detectable by magnetic resonance imaging (MRI). The third stage is represented by definite cartilage loss and bone remodelling including outgrowths known as osteophytes, both of which are visible on radiographs. The cartilage fragments and breakdown products build up in the synovial fluid, interfering with the mechanical operation of the joint and stimulating a pro-inflammatory response from the synovium and adjacent tissues. The fourth or end-stage is total joint

dysfunction, which is characterised by catastrophic damage to the articulating surfaces and a loss of joint function, which can only rely on joint replacement. Systemic metabolic and inflammatory factors predominate in the early stages of OA, whereas mechanical factors seem to be more important in the later stages (9).

1.1.1 Disease prevalence and burden

Approximately 10-12% of the adult population have symptomatic OA. The risk of disability attributable to knee OA alone is greater than that attributable to any other medical condition in people aged over 65 years (10). Estimates published in 2012 suggest that 250 million people worldwide are affected by knee OA (1). On the basis of international radiological survey data, the Australian Institute of Health and Welfare (AIHW) estimated that there are more than 40,000 new cases of radiological OA each year, adding to the large pool of prevalent OA, which will continue to grow as our population ages (11). Prevalence of symptomatic OA increases to 10% of men and 20% of women aged 45–65 years. Radiological prevalence surveys suggest much higher rates than this, with changes of OA being present on X-ray in more than 50% of people aged over 65 years (10).

Based on AIHW disease expenditure data, \$1.6 billion was attributed to OA in 2008-09 (the most recent year for which data are available). This expenditure consisted of: \$1,256 million on admitted patient costs (76.7%); \$282 million on out-of-hospital-costs (17.2%); \$99 million on prescription pharmaceuticals (6.0%) (1). Furthermore, the expenditure on OA may not be fully captured due to lack of comprehensive information, such as on allied health costs and over-the-counter medications. In 2012-13, 40,255 total knee and 25,169 hip replacement procedures were performed (1). The symptoms and disability of patients will lead to loss of wellbeing and health which further contributes to the costs of OA. It is critical that both the current economic climate and rapidly increasing burden from OA call for urgent attention to this matter.

1.1.2 Clinical symptoms

The most common symptom in OA is joint pain. Patients also experience stiffness, swelling, tenderness, inflammation, crepitus, instability, loss of motion, and muscle weakness. These symptoms lead to a limitation of mobility, physical and psychological

disability and impaired quality of life. Patients often experience major difficulties in daily activities including walking, running and stair-climbing (12).

1.1.3 Risk factors

OA used to be regarded as a degenerative joint disease resulting from “wear and tear” and the inevitable effects of aging (13). Women are more likely to have knee and hand OA than men and also have more severe OA (14). Other systemic risk factors include race/ethnicity, genetics, congenital or developmental condition (15).

One of the most well-agreed risks in OA is obesity, especially for weight-bearing joints. Outside the mechanical loading, being overweight can also lead to OA through increasing the metabolic and inflammatory effects (16). Past joint injury is a consistent risk factor that can result in damage to cartilage as well as other tissues (17). Trans-articular fracture, meniscal tear requiring meniscectomy or anterior cruciate ligament (ACL) injury can result in increased risks of knee OA and musculoskeletal symptoms (18). Additional risk factors include occupational activity, and mechanical factors such as physical activity, adduction moment, malalignment and muscle weakness (19, 20).

1.1.4 Treatment and management

Most guidelines have recommended various options including non-pharmacological, pharmacological and surgical therapies for OA treatment. Since there were no effective disease-modifying osteoarthritis drugs (DMOADs) approved for OA structural improvement, current therapies are mostly palliative and are concerned with controlling pain and improving function. Acetaminophen, oral and topical non-steroidal anti-inflammatory drugs (NSAIDs), tramadol and opioids have variably been recommended for symptom relief and control joint inflammation (21). According to a population-based study, the drugs most frequently used for OA therapy are chondroitin (21.2%), glucosamine (15.8%) and oral NSAIDs (14.4%), and the incidence of the use of opioids, cyclooxygenase-2 (COX-2) inhibitors and chondroitin increased over 5-year period, whereas all others decreased (22). Injections of hyaluronic acid (HA) into the knee joint have been approved by the Food and Drug Administration (FDA) for the treatment of OA, but data on efficacy are inconsistent. Recent meta-analyses reported statistically significant but limited efficacy (23). Glucosamine and chondroitin sulphate are widely used for the treatment of OA, although their mechanisms of action are unclear. Most

RCTs have reported moderate effects in reducing OA symptoms with either compound but larger than with placebo with little toxicity (24).

According to most current guidelines, the mainstay of treatment and what represents the call for everyone with OA should consist of non-pharmacologic approaches first, typically including weight loss, lifestyle modification, physiotherapy, and exercise (25-27). There is strong evidence to show that exercise has beneficial effects on pain and function, and a combination of strengthening, aerobic and functional exercise is recommended (28). Exercise helps to lose weight, strengthen muscles, increase the range of motion, improve proprioception, balance, cardiovascular fitness, and relief emotional stress (29, 30). Importantly, exercise has similar effects to analgesic and anti-inflammatory medications, but has fewer contraindications and adverse events than drugs and surgery (29, 31). However, exercise might not dramatically modify disease progression, additional physiotherapies are recommended in assisting with OA interventions include taping, bracing, wedged insoles, and manual therapy (32-34).

If conservative therapy fails, surgery therapies should be considered, which included arthroscopy, cartilage repair, osteotomy, and knee arthroplasty. Determining which of these procedures is most suitable depends on location, stage of OA and comorbidities etc. Arthroscopic lavage is often carried out, but does not change disease progression. If OA is limited to one compartment, uni-compartmental knee arthroplasty or unloading osteotomy can be considered (35). Ultimately, when other treatment has failed and OA advanced greatly, knee arthroplasty is performed. Due to its irreversible nature, though, only applied in the most severe cases (36).

1.2 Knee OA diagnostic criteria

The knee joint is the weight-bearing site that is often affected by pain, which is typically attributed to OA and, a leading cause of chronic disability in older persons (37-39). This thesis will focus on knee OA.

1.2.1 Radiography

Conventional X-ray remains the main test for the diagnosis of knee OA. Most of the developed radiological scoring systems include the assessment of osteophytes, joint space narrowing (JSN) and width, subchondral sclerosis, and bony attrition. The most commonly

used method is the Kellgren and Lawrence (K-L) grading system (40). The cut-off point of two or more comprises the radiological definition of OA in clinical studies as shown in Figure 1.2.

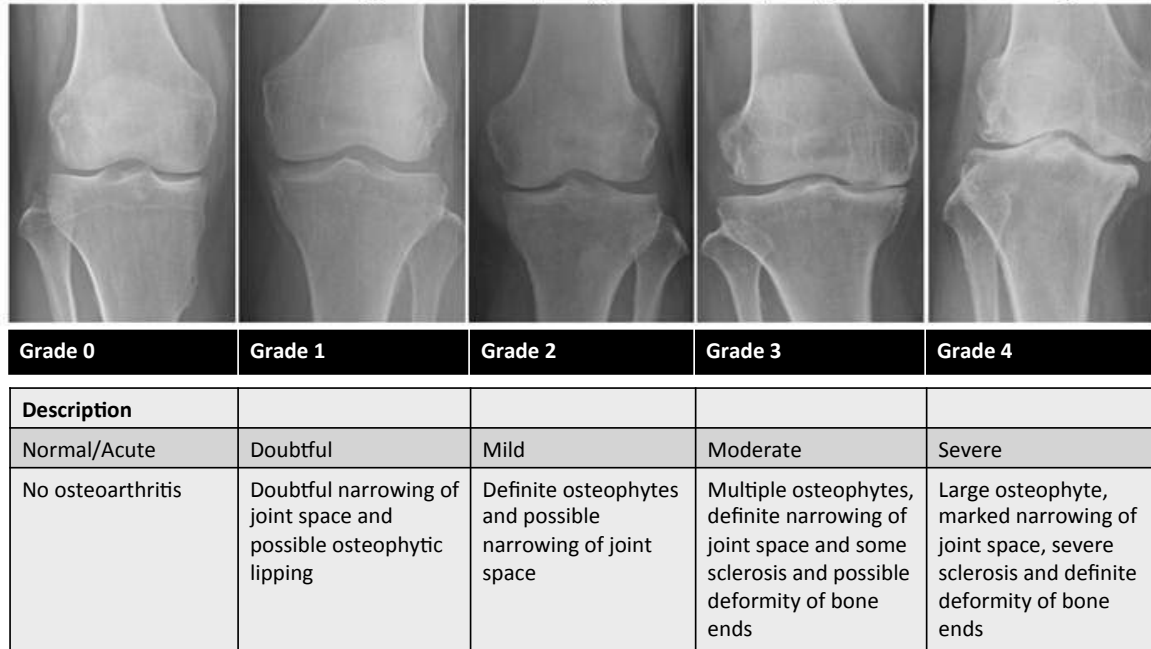


Figure 1.2. Kellgren Lawrence (K-L) grading scale on radiographic knee OA (40).

More recently developed scales, such as the Osteoarthritis Society International (OARSI) radiographic atlas (41), define each discernible radiographic characteristic of OA including osteophyte and JSN, in the hand, hip and knee. This system has the advantage of scoring each radiographic feature separately, whereas a composite measure such as the K-L grade may hinder the understanding of each separate radiographic component of OA.

1.2.2 Clinical criteria

Diagnostic criteria for OA have been developed by the American College of Rheumatology (4). These criteria include a combination of the patient's age, signs and symptoms on physical exam, radiographic and/or laboratory evidence. When the radiograph is used along with physical exam, the sensitivity and specificity of this method are 91% and 86%, respectively (Table 1.1). More recently, The European League Against Rheumatism (EULAR) OA Task Force suggests that a confident clinical diagnosis of knee OA may be made according to three symptoms (persistent knee pain, morning stiffness and reduced function) and three signs (crepitus, restricted movement and bony enlargement) (42).

Table 1.1. Criteria for diagnosis of knee osteoarthritis (43).

Clinical criteria					
	<ul style="list-style-type: none"> - Age older than 50 years - Activity-related joint pain - Bony enlargement - Bony tenderness - Crepitus - No palpable warmth - Stiffness for < 30 minutes 				
Laboratory criteria					
	<ul style="list-style-type: none"> - Erythrocyte sedimentation rate < 40 mm/hour - Rheumatoid factor < 1:40 - Synovial fluid analysis: clear, viscous, white blood cell count < 2,000/μL (2.00×10⁹ per L) 				
Radiographic criteria					
	- Presence of osteophytes				
		Sensitivity (%)	Specificity (%)	LR+	LR–
	- Pain plus ≥ 3 clinical criteria	95	69	3.1	0.07
	- Pain plus ≥ 5 clinical or laboratory criteria	92	75	3.7	0.11
	- Pain plus ≥ 5 clinical or laboratory criteria, plus osteophyte	91	86	6.5	0.1

LR+ = positive likelihood ratio; LR– = negative likelihood ratio.

1.2.3 Magnetic resonance imaging

The diagnosis of OA by radiographs used to be a “gold standard” to define and monitor disease progression in many epidemiological and clinical studies. However, the continued use of plain radiography has many limitations. Many studies have shown that radiographic features such as JSN and osteophytes do not correlate well with clinical symptoms (44, 45). It has been reported that less than 50% of people with evidence of OA on X-rays have symptoms related to these findings (46). Radiographs are also insensitive to early disease alterations and require longer study duration to detect significant changes. By the time that radiographic changes are detected, 10% of knee cartilage has already been lost (47). JSN is influenced by many joint structures including cartilage and meniscus, which cannot be separated by radiographs.

Magnetic resonance imaging (MRI) has proven to be an important tool in OA research and has revolutionised the understanding of OA pathology. MRI can visualise all tissues in the joint involved in OA including cartilage, menisci, bone marrow, and soft tissues. While costly, MRI is free of ionising radiation and has good tissue contrast and anatomical resolution allowing for the non-invasive examination of joint structures. MRI is a useful tool to study early stages or even pre-OA. There is increasing evidence to demonstrate that structural changes can be measured both reliably and with good responsiveness on MRI (48). Numerous studies have examined the use of MRI in effusion-synovitis, meniscus pathology, cartilage morphology alteration, bone marrow lesions (BMLs), osteophytes, cartilage composition, and other markers along with their correlations with clinically defined OA (49). Despite the growing pool of information, there is little uniformity in the diagnostic application of MRI and a lack of its confirmed diagnostic utility, as noted in the “Evidence Based Recommendations for the Diagnosis of Knee OA” published by EULAR in 2009 (42). The OARSI Food and Drug Administration (FDA) OA initiative has developed a MRI definition of knee OA which has been used in several recent studies (50), it suggests that further testing should focus on comparisons other than the radiograph, that may capture later stage disease and thus nullify the potential for detecting early disease that MRI may afford.

1.3 Synovium in OA

An important emerging theme in OA is broadening the focus of the disease from cartilage only to the whole joint. The cartilage, bone and soft tissues are each involved in

pathological processes that lead to progressive joint degradation. The synovial abnormalities have been observed at multiple stages of OA, suggesting it plays an important role in OA pathogenesis supported by increasing evidence (51, 52). This thesis investigates the role that synovium membrane plays in OA using data from a prospective population-based study of older adults and a RCT of knee OA patients. It focuses on MRI-detected synovial measures, included synovial fluids (effusion) and synovitis in different locations of a knee joint, and investigated how it is related to disease severity and progression. A detailed introduction specific to each research question will be presented at the start of Chapters 4 to 8. This section presents an overview of how synovial membrane involved in healthy and osteoarthritic joint and describes the clinical impact of synovial inflammation on OA-related symptoms and structural changes.

1.3.1 Physiology and function

The articular capsule surrounding synovial joint consists of a thick outer layer, the fibrous capsule and a more delicate inner layer, the synovial membrane. The highly vascular synovial membrane lines the non-articular portions of the synovial joint as well as the intra-articular ligaments and tendons (53). The normal synovium provides a deformable packing that allows movement of adjacent non-deformable tissues (54). Composed of thin connective tissue, it includes the continuous surface layer of cells (intima) and the underlying tissue (subintima). The intima consists of macrophages and fibroblasts while the subintima includes blood, lymphatic vessels and nerve fibres. The microscopic anatomy of normal synovial tissue often falls into three main types based on the content of the subintima layer: fibrous, areolar and adipose. Areolar synovium may also have specialized viscoelastic properties for coping with stretching, rolling and folding it undergoes during joint movement (54). (Figure 1.3). Furthermore, the cellular elements of the synovium were responsible for the secretion of the viscid synovial fluid, which lubricates and nourishes the joint. Synovial fluid is an ultrafiltrate or dialysate of plasma, which contains hyaluronan (hyaluronic acid), lubricin (also known as proteoglycan-4), surface-active phospholipids (SAPL), proteinases and collagenases (55). The synovial fluids also have a role in the removal of metabolic wastes (e.g. carbon dioxide and products of matrix turnover) and intra-articular particulate matter (i.e. cartilaginous debris), which could be diffused in the fluid or deposited within the membrane (54, 56).

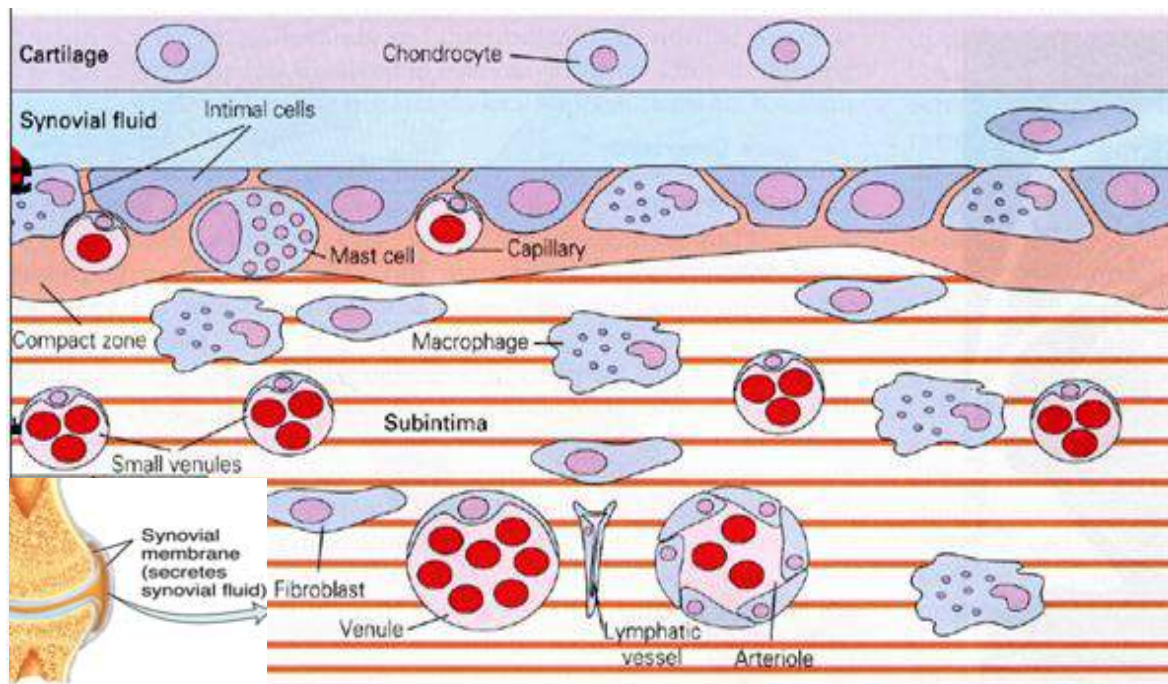


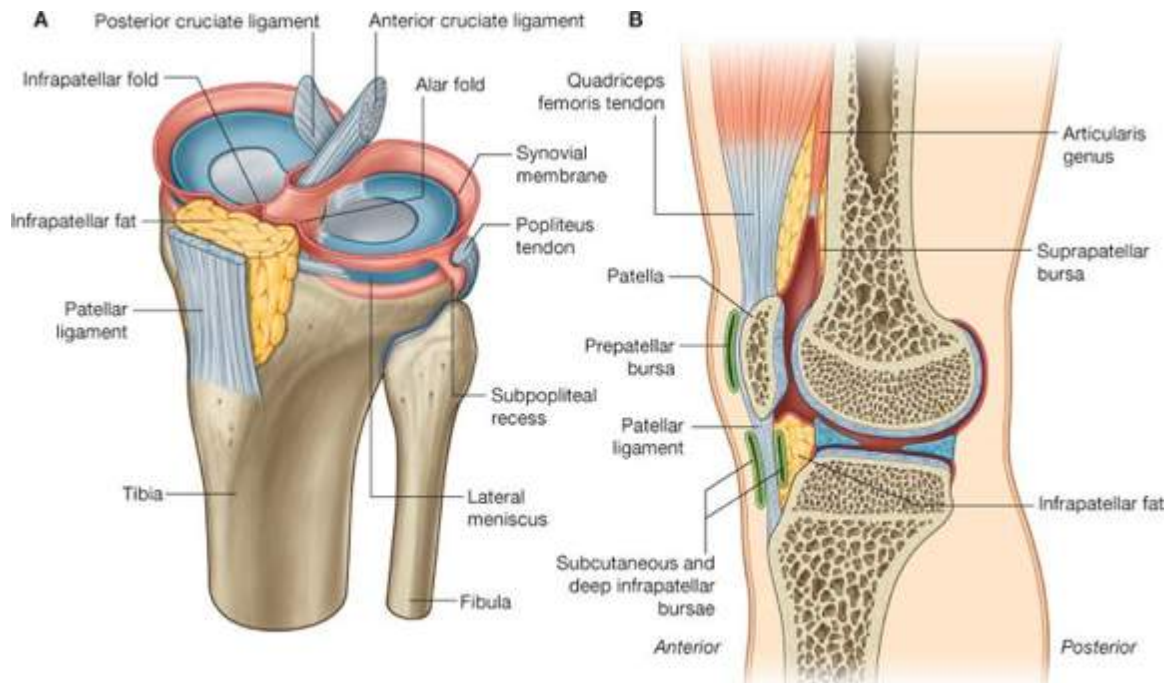
Figure 1.3. The schema of the synovial membrane (57).

Joint effusion may result from mechanical irritation by worn cartilage and bone, with the normal composition of the synovial fluid and excessive production of hyaluronan by intimal fibroblasts stimulated by frictional forces, such as in OA. On the other hand, joint effusion in an inflammatory synovitis is likely to be an accumulation of exudate similar to a pleural effusion, for example, an overspill from the inflammatory edema in synovial tissue created by increased vascular permeability (54). However, recent evidence suggests that low-grade inflammatory and immune reaction also contribute to the pathogenesis of OA, implicating that these two mechanisms of effusion development may not be as distinct as originally thought (58).

1.3.2 Synovial anatomy of the knee joint

The synovial membrane of the knee joint attaches to the margins of the articular surfaces and to the superior and inferior outer margins of the menisci. It lines the joint capsule except posteriorly where cruciate ligaments were found (Figure 1.4A). The two cruciate ligaments, which attach in the inter-condylar region of the tibia below and the inter-condylar fossa of the femur above, are outside the articular cavity, but enclosed within the fibrous membrane of the knee joint. Posteriorly, the synovial membrane reflects off the fibrous membrane of the joint capsule on either side of the posterior cruciate ligament (PCL)

and loops forward around both ligaments thereby excluding them from the articular cavity. Anteriorly, the synovial membrane is separated from the patellar ligament by an infrapatellar fat pad (53) (Figure 1.4B).



Drake: Gray's Anatomy for Students, 2nd Edition.
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Figure 1.4. The synovial anatomy of a knee joint (59).

(A) Superolateral view of the synovial membrane (showed in red). (B) Paramedial sagittal section through the knee.

Understanding of the synovial anatomy allow not only to illustrate a variety of structural changes involving them, but also to make the investigations possible regarding the location-specific pathology of a joint. Figure 1.5 demonstrates the synovial lining of the knee joint which forms several interconnected compartments. First, in the central portion of the joint, the synovial fluid can be observed between the patella and femoral shaft (yellow arrow), extending medially and laterally deep to the patellar retinacula (Figure 1.5A). The fluid can also accumulate (yellow arrow) in the deep part of the central recess, around the ACL and PCL (Figure 1.5B-C). Second, the synovial fluid can be detected in the suprapatellar pouch which extending superiorly from the upper surface of the patella (yellow arrow). This large pouch is formed between the quadriceps tendon anteriorly and femur posteriorly (Figure 1.5D-E). Third, the synovial membrane extends posteriorly from lateral and medial sides of the femur (yellow arrow) and forms recesses above the posterior portions of both condyles (Figure 1.5F-G). In the midline, the posterior capsular recess may become

an extension from the medial tibiofemoral compartment that behind the PCL (Figure 1.5B). Sub-popliteal recess as a small pouch can be seen between the lateral meniscus posteriorly and the popliteus tendon. Occasionally, the lining is continuous with the synovial membrane of the proximal tibiofibular joint. There is also a recess that frequently observed during arthrography superior to the posterior third of the medial meniscus (53). Anatomic variation of synovitis has been reported and it was most commonly observed posterior to the PCL and in the supra-patellar (60), but whether synovitis was widespread or more localised in OA was still unknown. Hence, one of our research aims was to investigate joint effusion and synovitis at different anatomic sites.

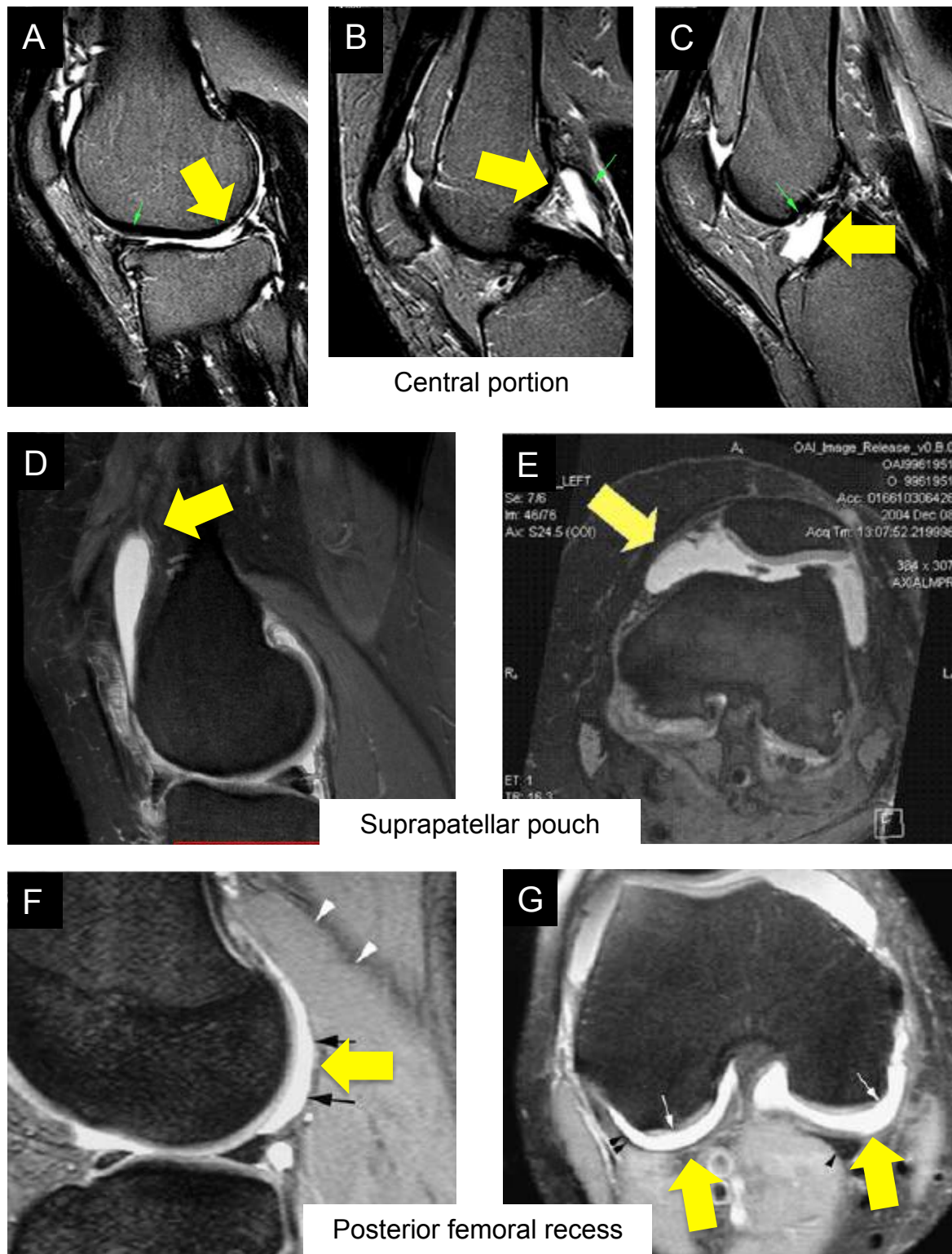


Figure 1.5. The T2/PD weighted MR images show multiple synovial recesses in different subregions of a knee joint (53).

(A) The central portion of the joint (arrow), between the patella and femoral shaft, extending medially and laterally deep to the patellar retinacula. The deep part of the central recess (arrow), around the ACL (B) and PCL (C). (D-E) The suprapatellar pouch, between the quadriceps tendon anteriorly and femur posteriorly (arrow), extending superiorly from the

upper surface of the patella. (F-G) The posterior femoral portions, above the lateral and medial sides of femur condyles (arrow).

1.3.3 Biological markers

In OA, inflammation occurs mainly in a restricted area of a periarticular joint. However, some biomarkers of inflammation can be detected in the circulation, especially in cases with generalised OA. For example, cartilage debris might trigger a systemic inflammatory response, leading to the release of pro-inflammatory mediators by the synovium. Also, the inflamed OA synovium itself could release synovial molecules (Table 1.2).

A high concentration of C-reactive protein measured using a high sensitivity assay (hs-CRP) was suggested to be predictive of rapid disease progression in early knee OA (61, 62). It was positively associated with higher degree of inflammatory cell infiltration of the synovial tissue and also with higher levels of interleukin (IL)-6 in synovial fluid (63). In OA, the concentration of IL-6 in synovial fluid was positively correlated with the total leukocyte count (64). However, the effects of hs-CRP in predicting OA progression disappeared when other factors, such as age, BMI and serum IL-6, were taken into account (65). According to our recent meta-analysis, it remains inconclusive whether hs-CRP level could be a surrogate biomarker in OA, as it was correlated with clinical symptoms but not structural alterations (66).

HA is an important component of articular cartilage, and the principle constitute of synovial fluid. HA is widely distributed throughout many body tissues, indicating its presence in the serum can be caused by conditions other than arthritis. When synovial alterations such as inflammation and hyperplasia occur, the permeability of the membrane is altered, which likely contributes to the decreased concentrations of HA and lubricin in synovial fluid. Hence, serum HA concentrations have been used as a marker of synovitis (67). An increase in serum levels of HA has been reported in patients with OA, but no correlation was found between serum levels of HA and CRP (68). A previous study has shown no correlation between serum levels of HA and the erythrocyte sedimentation rate (ESR) in patients with either hip or knee OA (69). The lack of associations supports the conceptualisation of HA as a marker of localised joint inflammation, as opposed to systemic inflammation.

Serum levels of cartilage oligomeric matrix protein (COMP), a member of the thrombospondin family of glycoproteins, have been shown to be increased in individuals with

knee OA and synovitis, compared to those without synovitis (70). Cartilage glycoprotein 39 (CGP-39, also known as chitinase 3 like protein 1 and as YKL-40) is a 40 kDa glycoprotein secreted by chondrocytes and synoviocytes. Increased levels of CGP39 have been found in the synovial fluid and serum of patients with severe knee OA (71). Masuhara et al. has found the concentrations of matrix metalloproteases (MMPs) were elevated in the synovial cells, synovial fluid, plasma and serum in patients with rapidly destructive hip OA, a typical form of inflammatory OA (73). Finally, the secretion of glucosyl-galactosyl-pyridinoline (Glc-Gal-PYD), a glycosylated analog of pyridinoline, which specifically reflects the degradation of synovial tissues, was also found increased in the urine sample of knee OA patients (74).

Table 1.2. Biomarker related to synovium in osteoarthritis.

Sources	Observations	Examples of related factors
Serum/synovial fluid	Increased levels of hs-CRP	
	Production and/or release of pro-inflammatory cytokines	TNF, IL-1 β , IL-6, IL-8, IL-15, IL-17, IL-21
	Production of adipokines	visfatin, leptin, adiponectin
	Decreased release of anti-inflammatory cytokines	IL-4, IL-10, IL-13, IL-1Ra
	Increased activity of MMPs and ADAMTS	MMP-1, MMP-3, MMP-9, MMP-13
	Increased production of PGE ₂ and nitric oxide	
	Release of growth factors	EGF and VEGF
	Release of pro-inflammatory and pain neurotransmitters	Substance P, neuropeptide Y, NGF
Synovial tissue/cells	Non-collagenous proteins	CGP-39, HA
	Involvement of macrophages in osteophyte formation via BMPs	
	Increased levels of MMPs in synoviocytes	MMP-3, MMP-9
	Increased expression of adhesion molecules in the synovium	ICAM-1, VCAM-1
Other	Fragments of type II collagen and aggrecans	
	Other potential surrogate biomarkers of synovial inflammation	Glc-Gal-PYD

Abbreviations: ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; BMP, bone morphogenetic protein; CGP-39, cartilage glycoprotein-39 (also known as chitinase-3-like protein 1 and YKL-40); CRP, C-reactive protein; EGF, endothelial growth factor; ICAM-1, intercellular adhesion molecule 1; IL, interleukin; IL-1Ra, interleukin 1 receptor antagonist; MMP, matrix metalloproteinase; NGF, nerve growth factor; PGE₂, prostaglandin E₂; TNF, tumor necrosis factor; VCAM-1, vascular cell adhesion molecule 1; VEGF, vascular endothelial growth factor.

1.3.4 Clinical significance

Research efforts have provided substantial evidence that synovial inflammation is associated with greater symptoms such as pain and degree of joint dysfunction, and may promote more rapid cartilage degradation and non-cartilaginous abnormalities.

1.3.4.1 Association with symptoms

Despite different approaches employed for detection and characterisation of synovitis (e.g. imaging or histologic assessment), evidence underpins the associations between synovial inflammation and knee symptoms in patients with OA. Torres et al. investigated the relationship between knee pain and specific joint pathology detected by MRI in OA patients. They noted that synovitis or effusion, meniscal tears, bone attrition and BMLs, were highly correlated with knee pain measured on a visual analog scale (VAS) (75). Others specifically examined the relationship between pain and MRI-detected synovitis and noted that changes in pain scores over time varied with changes in synovitis, strengthening the notion of a relationship (76, 77). Furthermore, there was a 9-fold greater risk of having painful knee OA conferred by higher grades of synovitis using contrast-enhanced (CE) MRI (78). We reported an independent association between effusion-synovitis using non-CE MRI and knee symptoms measured by the WOMAC score (which measures knee pain walking, climbing, sitting, standing and at night) in older adults with high risk of OA (Appendix 3). Among those associations non-weight-bearing pain was best correlated with synovial inflammation (79). The relationship between symptoms and synovitis was more likely to be localised to the infra-patellar and supra-patellar areas (80). There is conflicting evidence regarding association of pain with the severity of synovitis as determined on non-CE MRI (76, 81-85). Possible reasons for such discrepancies may include the use of different scoring methods for synovitis and pain, the use of different pulse sequences in different studies, and the limited specificity of signal alterations in Hoffa's fat pad for detection of synovitis (86).

Synovial inflammation has also been suggested to contribute in knee joint dysfunction using performance-based measures of walking and stair-climbing times (87). One study of patients receiving total knee replacement (TKR) with end-stage knee OA found that synovitis was closely correlated with the current functional impairment and disability (88). Despite some disagreements in the literature, the majority of available studies provide compelling

evidence that synovial inflammation is a rational target for therapeutic intervention to control joint symptoms in OA. Future work should help to define specific disease phenotypes or patient populations for whom targeting synovitis may have the greatest benefits.

1.3.4.2 Association with structures

The causal pathway of low-grade inflammation in non-osteochondral tissues and osteochondral abnormalities has become an emerging topic in recent years. Ayral et al. demonstrated that the progression of cartilage pathology was more advanced at one-year follow-up in patients with synovial inflammation. Approximately 50% of the patients had reactive (proliferation of opaque villi) or inflammatory (hyper-vascularisation and/or proliferation of hypertrophic and hyperemic villi) synovial abnormalities, which were documented by the visual appearance of the synovial membrane during baseline arthroscopy (89). There was also evidence to suggest that synovitis and/or effusion were predictors of progression to TKR in prospective studies (90, 91).

However, the causal relationship between synovial inflammation and early osteoarthritic abnormalities was still inconclusive. A study of symptomatic OA patients failed to corroborate the associations between longitudinal changes in synovitis and cartilage (76). However, another study of early OA patients without radiographic changes has demonstrated that effusion and synovitis were associated with subsequent development of cartilage loss at 30 months (92). A nested case-control study including patients with and without ROA has found that the occurrence of ROA was associated with the presence of effusion and/or synovitis at baseline and follow-up, supporting its role in predicting the development and incident of ROA (93). Our group has reported that in an older population with early stage of OA, effusion-synovitis not only independently predicted progression of cartilage defects over three years, but also preceded the worsening of subchondral BMLs (94). Miller et al. have demonstrated that knee effusion was associated with medial meniscal extrusion (95). However, a cross-sectional study of individuals who had or were at risk for knee OA reported that knee effusion was not associated with meniscal extrusion after adjusting for covariates (96). Another cross-sectional study reported that knees without OA but with meniscal pathology exhibited joint effusion to a significantly higher degree than those without meniscal damage. The association persisted for knees without cartilage damage. The prevalence of effusion is further increased when present in two compartments (97). It is suggested that the concomitant occurrence of synovial activation and early structural damage

may help to understand the pathophysiology of a degenerative joint disease at an early stage, but this needs longitudinal evidence to confirm.

In addition to being a potential risk factor for knee OA, synovial inflammation is also thought to be triggered by release of detritus from cartilage and bone deteriorations throughout the course of OA (98, 99). Once cartilage degradation has begun, the synovial cells phagocytose the breakdown products released into the synovial fluid, resulting in the synovial membrane becoming hypertrophic and hyperplastic. These enzymes then activate synovial cells to cause the release of pro-inflammatory cytokines, collagenases and other hydrolytic enzymes. Consequently, a vicious positive feedback loop involving cartilage breakdown and synovial inflammation occurs (100).

Although the majority of published studies support a relationship between synovitis and joint structural damage, reasons for some disparate results are likely related to differences in patient populations, sample size, study time period as well as the methods and anatomical areas of assessing synovial abnormalities which will be introduced in the following section.

1.3.5 MR Imaging assessment

MRI-assessment of synovitis in knee OA is well-documented in the literature (53, 101-105). The advantage of MRI over ultrasound and X-ray is that it can visualise synovial changes located deep within the joints without being obscured by bony structures.

1.3.5.1 MRI methods

MRI is uniquely able to directly depict all anatomic structures of the joint which has been increasingly recognised in OA research. MRI signs of synovial inflammation included increased synovial thickness/volume, increased signal intensity after intravenous gadolinium injection (enhancement), and increased water content (synovial effusion), alone or in combination (106). Non-CE MRI is still the most commonly used modality to assess synovitis in large epidemiological OA studies, although it has some limitations compared with CE MRI (78, 107-110). One distinct disadvantage of non-CE MRI is that it cannot differentiate inflamed synovium from joint effusion as shown in Figure 1.6. Only inflammatory synovium will manifest enhancement, while effusion will remain hypo-intense on T1-weighted sequences after contrast administration (111). Synovitis can be assessed indirectly using a surrogate marker in non-CE MRI, e.g. hyper-intense signal changes within

Hoffa's fat pad on fat-suppressed (FS) proton density (PD) or T2-weighted fast spin echo (FSE) sequences which was shown in Figure 1.7 (112) (76, 113). Alternatively, synovitis can be evaluated in combination with effusion on these sequences, but inflamed synovium was not distinguishable from joint fluid filling the joint cavity surrounded by synovium because they both showed equivalent high signals (114, 115). Because of this, the phrase “effusion-synovitis” has been proposed recently (Figure 1.8) (115).

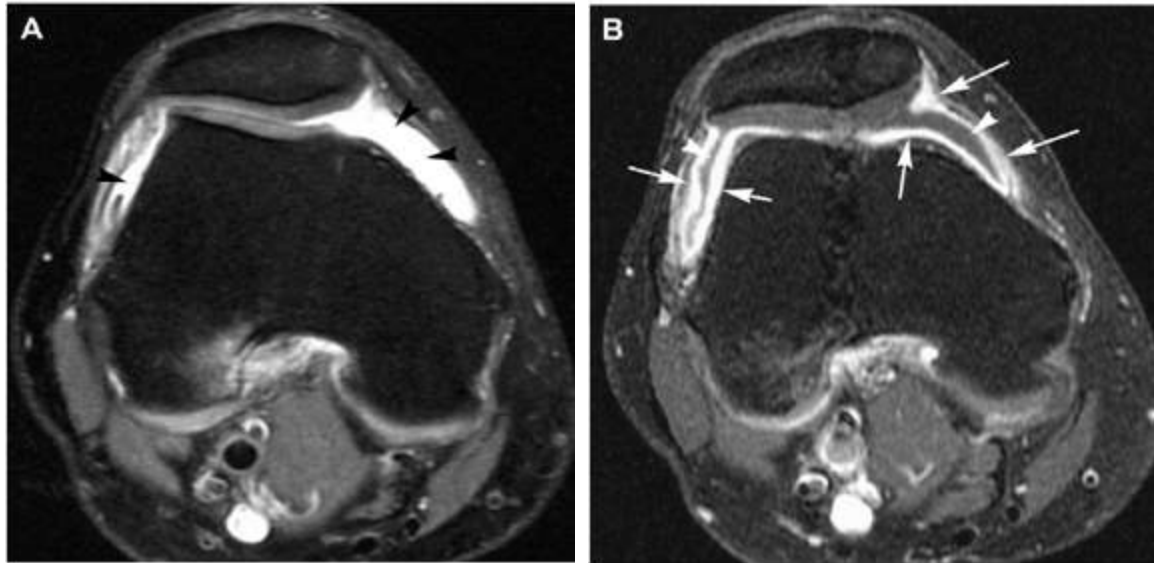


Figure 1.6. Joint effusion.

(A) Axial PD FS image. Marked bright signal intensity within the joint and convexity of the joint capsule suggestive of a large joint effusion are depicted (arrowheads). (B) Axial T1 FS CE image of the same knee at the same slice position. The CE image shows marked synovial thickening depicted as hyper-intense tissue lining along the joint capsule. Only a small amount of effusion is observed (arrowheads) (111).

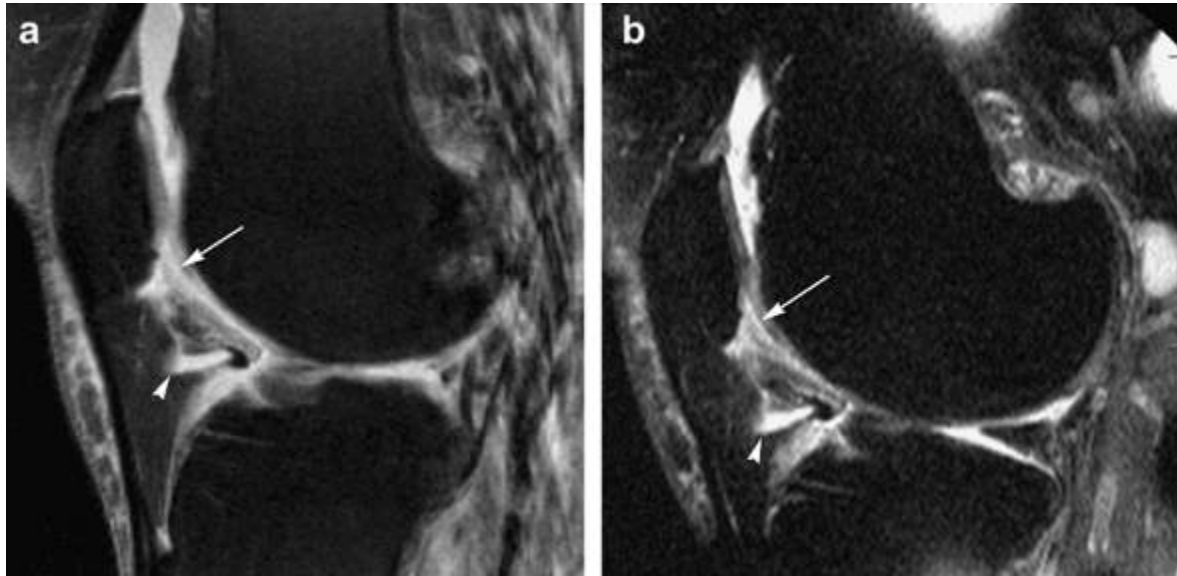


Figure 1.7. Synovitis in Hoffa's fat pad.

Equivalent delineation of hyper-intense signal changes in the infrapatellar (a and b, arrow) and intercondylar (a and b, arrowheads) regions. (a) Sagittal PD FS image at 1.0 T MRI. (b) Same slice on the 1.5 T system (112).

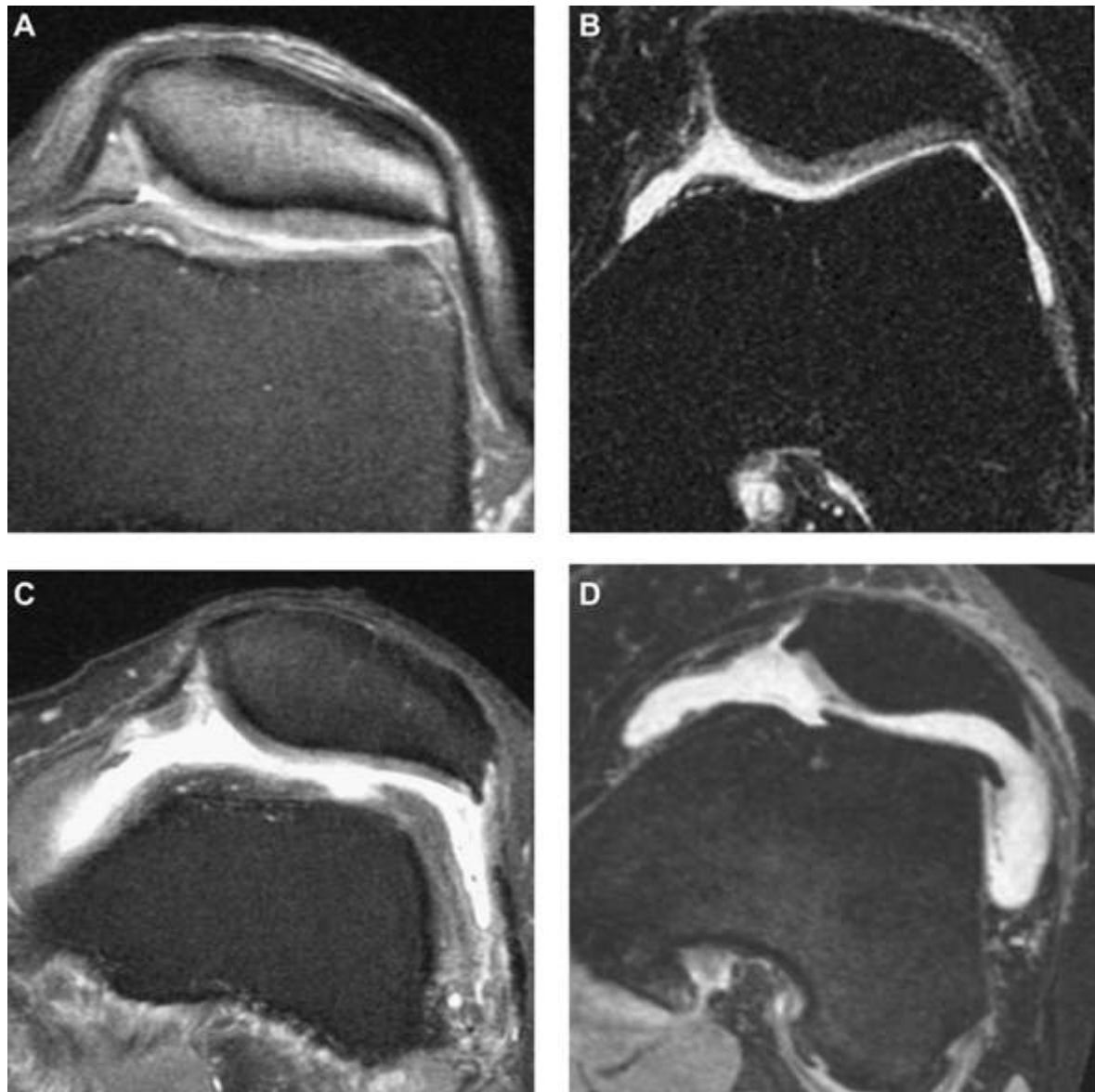


Figure 1.8. Joint effusion-synovitis.

Hyper-intensity within the articular cavity represents a composite of effusion and synovial thickening that cannot be distinguished from each other in the absence of contrast. Grade 0 = none (A); grade 1 = small (B); grade 2 = medium (C) and grade 3 = large (D) (115).

There are few studies using dynamic CE MRI, in which early enhancement (i.e. initial distribution of gadolinium) accurately reflects the inflammatory activity of the joint (106, 116, 117). With static CE MRI, the extent of synovial enhancement may be misinterpreted if the assessment is performed on images acquired at a late phase. Because the signal intensity of joint effusion also enhances with time, as gadolinium passes into the joint space by diffusion of fluid from synovial capillaries (118). One study showed that dynamic CE-MRI with derived pharmacokinetic parameters can provide useful information in differentiating

synovitis in hand OA from rheumatoid arthritis (RA) (119). Another MRI-based study using a dynamic CE sequence found that quantitative synovial volume was strongly correlated with BMLs volume in knee OA patients (120). Thus, the clinical value of using dynamic CE T1-weighted MRI to measure synovitis is required to assess in future OA studies.

1.3.5.2 Assessment systems

Several methods are available for semi-quantitative or quantitative assessment of effusion and/or synovitis in OA using non-CE and CE MRI (107, 108, 115, 121, 122). Semi-quantitative scoring systems of knee OA that specify the synovial abnormalities are summarised in Table 1.3.

Table 1.3. Summary of semi-quantitative MRI scoring system for synovial abnormalities in knee OA.

Author (year)	Acronym	Evaluation	Location	Scores	CE-MRI
Kaneke (1993)	-	Effusion	Central portion (para-ACL and para-PCL)	0-1	No
			Suprapatellar pouch	0-1	No
			Posterior femoral recess	0-1	No
			Subpopliteal recess	0-1	No
Hill (2001)	-	Effusion	Joint cavity	0-3	No
Peterfy (2004)	WORMS	Effusion/synovial thickening	Joint cavity	0-3	No
Loeullie (2005)	MRI synovitis score	Synovial thickening	Medial and lateral suprapatellar recess	0-6	Yes
			Trochlear groove	0-3	Yes
			Medial and lateral femoral gutters	0-6	Yes
Kornaat (2005)	KOSS	Effusion	Joint recesses	0-2	Yes
		Synovial thickening	Synovial joint cavity	0-1	Yes
Rhodes (2005)	-	Synovitis	Para-patellar recesses	0-3	Yes
Hunter (2008)	BLOKS	Effusion	Joint cavity	0-3	Yes
		Synovitis	Joint cavity	0-3	No
Pelletier (2008)	-	Synovial thickening	Medial and lateral recesses	0-6	No
			Medial and lateral suprapatellar bursa	0-6	No
Meredith (2009)	-	Effusion	Joint cavity	0-3	No
		Synovitis	Joint cavity	0-3	No
Baker (2010)	-	Synovial thickening	Medial and lateral para-patellar recesses	0-3	Yes
			Suprapatellar pouch	0-3	Yes
			Infrapatellar fat pad	0-3	Yes
			Medial and lateral posterior femoral condyles	0-2	Yes
Baker (2010)	WORMS	Effusion-synovitis	Joint cavity	0-3	No
Hunter (2011)	MOAKS	Effusion-synovitis	Joint cavity	0-3	No
		Hoffa-synovitis	Hoffa's fat pad	0-3	No
Guermazi (2011)	-	Synovial thickening	Medial and lateral para-patellar recesses	0-2	Yes
			Suprapatellar	0-2	Yes
			Infrapatellar	0-2	Yes
			Intercondylar	0-2	Yes
			Medial and lateral peri-meniscal	0-2	Yes
			Adjacent to ACL/PCL	0-2	Yes

Abbreviations: ACL: anterior cruciate ligament; PCL: posterior cruciate ligament; WORMS: Whole Organ Magnetic Resonance Imaging Score; KOSS: Knee Osteoarthritis Scoring Systems; BLOKS: Boston Leeds Osteoarthritis Knee Score; MOAKS: MRI Osteoarthritis Knee Score.

A histological correlation study comparing three scoring systems for evaluating synovitis and joint effusion on MRI reported that only the scoring from CE images correlated with microscopically proven synovitis (105). Studies have shown that effusion and synovitis often coexist in OA-affected joints but they seem to be two distinct entities (111, 113). Two studies reported that signal alterations in Hoffa's fat pad seen on non-CE sequences were a sensitive (71-97%) but not a specific (10-55%) sign of peripatellar synovitis, compared with CE sequences (80, 86). A CE MRI-based study showed definite synovitis might be present independent of effusion (111). Also, in a non-ROA cohort, baseline joint effusion, but not synovitis, predicted the development of tibiofemoral cartilage loss (92).

Rhodes et al. demonstrated that semi-quantitative scoring of OA synovitis using CE T1-weighted images was closely related to the quantitative synovial volume (107). This scoring method was subsequently modified and was used in another study which demonstrated that synovitis in the peri-patellar region had a strong correlation with knee pain severity (78). However, these evaluations were limited to synovitis of the peri-patellar region. Guermazi et al. have assessed synovitis at 11 sites of the entire knee joint in OA patients using CE MRI (108). It has shown that moderate to severe synovitis had a significant association with the maximum WOMAC pain score. More recently, the same group has reported that synovitis was strongly associated with tibiofemoral ROA and widespread MRI-detected cartilage degradation (110). Another study by Roemer et al. has demonstrated an association between meniscal damage of the posterior horns and localised posterior synovitis (123). Moreover, synovitis severity evaluated using this scoring method was validated by arthroscopic and synovial biopsy findings in knee OA patients (124).

There is a lack of longitudinal studies using MRI to evaluate changes in synovial inflammation before and after treatment. So far, there was only one RCT using CE MRI to monitor the efficacy of a bradykinin receptor-2 antagonist in symptomatic knee OA (125). Apart from a significant improvement in the VAS pain score after therapy, no significant change was observed in the severity of synovitis as detected on CE-MRI. Moreover, histopathologic studies are needed to establish a better cut-off between physiologic and pathologic enhancement of synovial tissue (126, 127). To date, only three such studies have been published (88, 105, 109). There is still a lack of longitudinal studies using MRI to assess synovitis in knee OA and its relationship with progression of structural damage, such as cartilage loss and JSN. Current evidence regarding this was still inconsistent. Whether synovitis in knee OA predicts the progression of structural deteriorations is still inconclusive.

Since the synovial inflammation has a potential to regress, it would be a potential target in clinical practice and medical research. The following section will provide an overview of different treatments targeting on synovial inflammation.

1.3.6 Synovial inflammation as a treatment target

1.3.6.1 Developed strategies

Classical non-targeted strategies, such as the use of NSAIDs, intra-articular injection or systemic administration of steroids, can modulate pain and function during both acute flares and chronic complaints in OA (128, 129). However, the complications caused by drug side-effects and repeated injections became problematic during long-term use. Some recommendations agree with the use of glycosaminoglycan (GAG) compounds like chondroitin sulfate (CS), glucosamine sulfate, and hyaluronic acid (HA). They are characterised by a delayed, but significant effect on pain and function in knee OA. Glucosamine and CS have been described as having disease-modifying effects in knee OA.

CS, a natural glycosaminoglycan, has demonstrated to control the 3 aspects of synovial inflammation: cell infiltration and action, biochemical mediators release and angiogenesis. In synovial fluid of patients requiring joint aspiration, treatment orally for 10 days with CS (800 mg/day) significantly increased the hyaluronate concentration and the intrinsic viscosity, while decreased collagenolytic activity, phospholipase A2 and N-acetylglucosaminidase (NAG) (130). In several arthritic animal models, CS has been found to significantly decrease the inflammatory and histo-pathological lesions of synovial membrane (131, 132). There is little information about the effect of CS in OA patients with moderate pain and manifestations of synovitis (joint swelling and effusion). The multicentre, double-blind, placebo- and celecoxib-controlled Glucosamine/chondroitin Arthritis Intervention Trial (GAIT) assessed the effect of CS and glucosamine alone or in combination on joint swelling and/or effusion in 1583 patients with mild to severe knee OA (133). The patients received 1200 mg of CS, or 1500 mg of glucosamine or both CS and glucosamine, or 200 mg of celecoxib or placebo, daily for 24 weeks. The results showed that CS diminished the percentage of patients with joint swelling and/or effusion from 28.3% at baseline to 12.4% at the end of 24 weeks of treatment. It is of interest that the beneficial effect of CS was statistically significant in the patients with mild pain (WOMAC pain scores 125-300). It has been suggested the direct anti-inflammatory effect on the synovium in OA, perhaps by

reducing the nuclear translocation of the transcription factor NF- κ B in synoviocytes and macrophages thus decreasing inflammatory activation in these cells (130).

Glucosamine, as a constituent of the natural glycosaminoglycan, can be provided to OA patients. It was first thought to be effective during cartilage repair by enhancing cartilage extracellular matrix components, such as aggrecan and collagen type II (134). Glucosamine has showed the potency of reducing synovial inflammation, including cell infiltration and hyperplasia (135). Interestingly, it inhibited IL-6 production, whereas up-regulated IL-10 in synovial inflammation of mouse collagen-induced arthritis (136). The anabolic properties of glucosamine were demonstrated in human OA synovial explants where it stimulated HA synthase activity and GAG production in human synovial cells (137, 138). The latest *in vitro* study showed glucosamine inhibited PGE₂, nitric oxide (NO), and MMPs in synoviocytes, chondrocytes and macrophages (139) (140). Although there is a body of evidence supporting the anti-inflammatory effects of glucosamine in animal models and *in vitro* studies, clinical data supporting the effect of glucosamine on synovitis in OA patients are still lacking.

It has been suggested that intra-articular injection of HA restores synovial fluid viscoelasticity, thus improves clinical symptoms and joint functionality (141). The anti-inflammatory properties of HA consisted many cellular aspects of synovitis (141). In addition, HA was proven to reduce NO in synovial fluid of OA patients when concomitantly improving WOMAC pain and physical function scores (142). Moreover, the adhesion molecules induced by IL-1 β , TNF- α and IFN, such as ICAM-1 and VCAM-1, were decreased in the synovial fluid of patients treated with HA (143). The number of lining cells, macrophages, lymphocytes, mast cells, and fibrin was also significantly reduced when examining synovial biopsies from OA patients 6 months after the last injection of HA (144). On the contrary, Schumacher et al. showed no differences in the severity of synovitis and the amount of synovial fluid between patients treated with and without HA, even if the treatment was proven to be beneficial on walking pain (145). This may be due to the short time between the examination and the last injection. Indeed, HA is known to have a long onset of action.

Natural supplements have been advocated as novel treatments for OA recently. *In vivo* evidence reported that functional VDR signalling inhibited synovial inflammation, cartilage damage and bone erosion in arthritis by modulating monocyte function (146). Primary cultures of rheumatic synovial cells showed that VDR expression was found in the macrophages and fibroblasts. However, 25-(OH)D did not appear directly to affect the MMP or prostanoid production by unstimulated synovial fibroblasts or chondrocytes *in vitro*, rather

than through modulating the cytokine-induced MMP and PGE₂ production (147). A RCT has shown that vitamin D supplementation significantly reduced serum levels of IL-6 and modulated adipokines in obese adolescents with low circulating 25-hydroxyvitamin D (25-(OH)D) levels (148). Several epidemiological studies showed that lower serum levels of 25-(OH)D were associated with higher prevalence of ROA (149, 150) and accelerated disease deteriorations (151-153). However, evidence remains controversial regarding the efficacy of vitamin D supplementation from limited OA trials. One study suggested it had beneficial effects on symptoms (154), while another showed no effects on symptoms and cartilage loss (155). Nevertheless, none of these studies has investigated the effects of vitamin D on synovial inflammation yet, leaving a window of opportunity in the future trial design.

1.3.6.2 Developing strategies

The synovial membrane is a promising target for novel strategies to prevent structural alterations and treat clinical symptoms (156). Designed to interfere with specific targets, the novel therapies include anti-fibrotic agents, biologic agents such as anti-TNF monoclonal antibodies, anti-NGF antibodies, anti-proteases (anti-MMPs and ADAMTS) and bradykinin-blocking agents (157). Studies are underway to assess the effects of systemic TNF blocking agents in OA, although local IL-1 blockade has not been markedly beneficial (158). Data concerning the role of IL-6 in OA pathogenesis suggests that IL-6 targeted therapy could be an interesting approach for treating OA (159, 160). Specific aspects (e.g., control of inflammation and angiogenesis) could be identified when targeting synovitis in OA.

1.4 Summary

OA is a leading cause of disability among older adults, affecting one in eight adults (161). By 2050, the prevalence of OA is projected to increase to 11.2% of males and 14.5% of females, affecting nearly 3.8 million Australians (162). This highly prevalent disease and the attendant disability have a formidable effect on individuals and on society. Until recently, it has been recognised that cartilage damage is no longer the only initial pathologic feature in OA, while many non-cartilaginous structures are also involved. Synovial membrane, which contains metabolically highly active cells (synoviocytes), is physiologically important as it nourishes chondrocytes and removes metabolites of matrix degradation via the synovial fluid. Biomechanically, it lubricates the joint during movement and buffers the loading forces. As

synovial abnormalities are thought to occur at early stages of the disease, it could be an important risk factor in OA disease initiation and progression with a great potentially treatment value.

The following chapters investigate the role synovial inflammation plays in knee OA using data from a cohort study of community-dwelling older adults and a RCT of knee OA patients with low vitamin D levels. Comprehensive analyses have been performed examining the associations between effusion-synovitis and measures of cartilage defect, cartilage volume loss, BML change, meniscal pathology, knee pain and function. Novel investigation about the therapeutic effects of vitamin D on synovial inflammation has been examined in knee OA patients. The following sections will provide an overview of the different synovial inflammation measures used in this thesis.

The research questions, which directed this work, will be described in the next chapter.

Chapter 2 - Research questions

In a population-based sample of community-dwelling adults who were aged 50–80 years examined at baseline and approximately 3 years later:

1. What are the cross-sectional and longitudinal associations between MRI-detected regional effusion-synovitis and knee pain?
2. What are the cross-sectional and longitudinal associations between MRI-detected regional effusion-synovitis and structural measures including cartilage defect, cartilage volume loss and BMLs?
3. What is the natural history of MRI-detected effusion-synovitis and what structural factors are associated with the longitudinal change of effusion-synovitis?

In a multicentre, randomised, placebo-controlled and double-blind clinical trial which was performed over 2 years in symptomatic knee OA patients with low vitamin D levels:

1. What are the associations between disease outcomes and MRI-detected effusion-synovitis using both quantitative and semi-quantitative measures?
2. Does vitamin D supplementation have effects on MRI-detected effusion-synovitis volume?

Chapter 3 - Methodology

3.1 Prelude

Chapter 4-6 in this thesis arose from analyses conducted in older adults, using data from the Tasmanian Older Adult Cohort (TASOAC) study. Chapter 7-8 arose from the analyses conducted in OA patients, using data from a randomised, placebo-controlled, and double-blind clinical trial (Vitamin D Effect on Osteoarthritis, VIDEO study). Both studies are similar in some aspects of methodology, particularly in the outcome factors, study factors and covariates. This chapter describes each study population and design, as well as the protocols for measurement of factors which are common to multiple chapters in this thesis. Additional factors which are unique to each chapter are described in more details within the methodology section of each of the subsequent chapters.

It should be noted that the following chapters are presented in the form in which they were submitted to, or accepted by, peer-reviewed journals for publication. Thus, throughout these chapters there are some differences in the description of methods, analyses, results, and interpretations, due chiefly to requests from journal reviewers.

3.2 TASOAC study population and design

The work in this thesis was conducted as part of the TASOAC study, an ongoing prospective, population-based study aimed at identifying the environmental, genetic, and biochemical factors associated with the development and progression of osteoarthritis (OA) at multiple sites (hand, knee, hip, and spine). The cohort consisted of both males and females aged between 50 and 80 years (mean \pm standard deviation (SD) age = 62 ± 7 years), selected from the roll of electors in southern Tasmania (population 229,000) using stratified simple random sampling without replacement. Electoral rolls represent the most complete population information available in Australia because voting in federal and state elections is compulsory. The sample was stratified by sex to provide equal numbers of men and women, and equal distribution was drawn from urban and rural areas in southern Tasmania. As TASOAC was designed to examine community-dwelling older adults, institutionalised older adults were excluded. Participants were also excluded if they reported contraindication for MRI, as these tests were required to examine OA progression.

Figure 3.1 provides an overview of participant recruitment and withdrawal during the study period. 2,135 initially eligible participants were identified from which 1,904 were able to be contacted. Of all initially eligible participants, 1,100 enrolled in the study, and 1,099

attended a baseline clinic between March 2002 and September 2004. The overall response rate for participation in Phase 1 is 57%, which is similar to response rates from studies with equivalent response burdens conducted around the same time period, such as the North West Adelaide Health Study at 58% (163), and the Australian Diabetes, Obesity and Lifestyle Study at 52% (164). Follow-up data was collected for 875 eligible participants (80%) at a subsequent clinic approximately 2.6 years later (range: 1.4-4.8 years). The MRI machine was decommissioned halfway through the follow-up period; therefore, MRI scans were only available for approximately half of the follow-up participants. As a result, the sample size used in Chapters 4–6 of this thesis varies depending on the available data for each of the research questions.

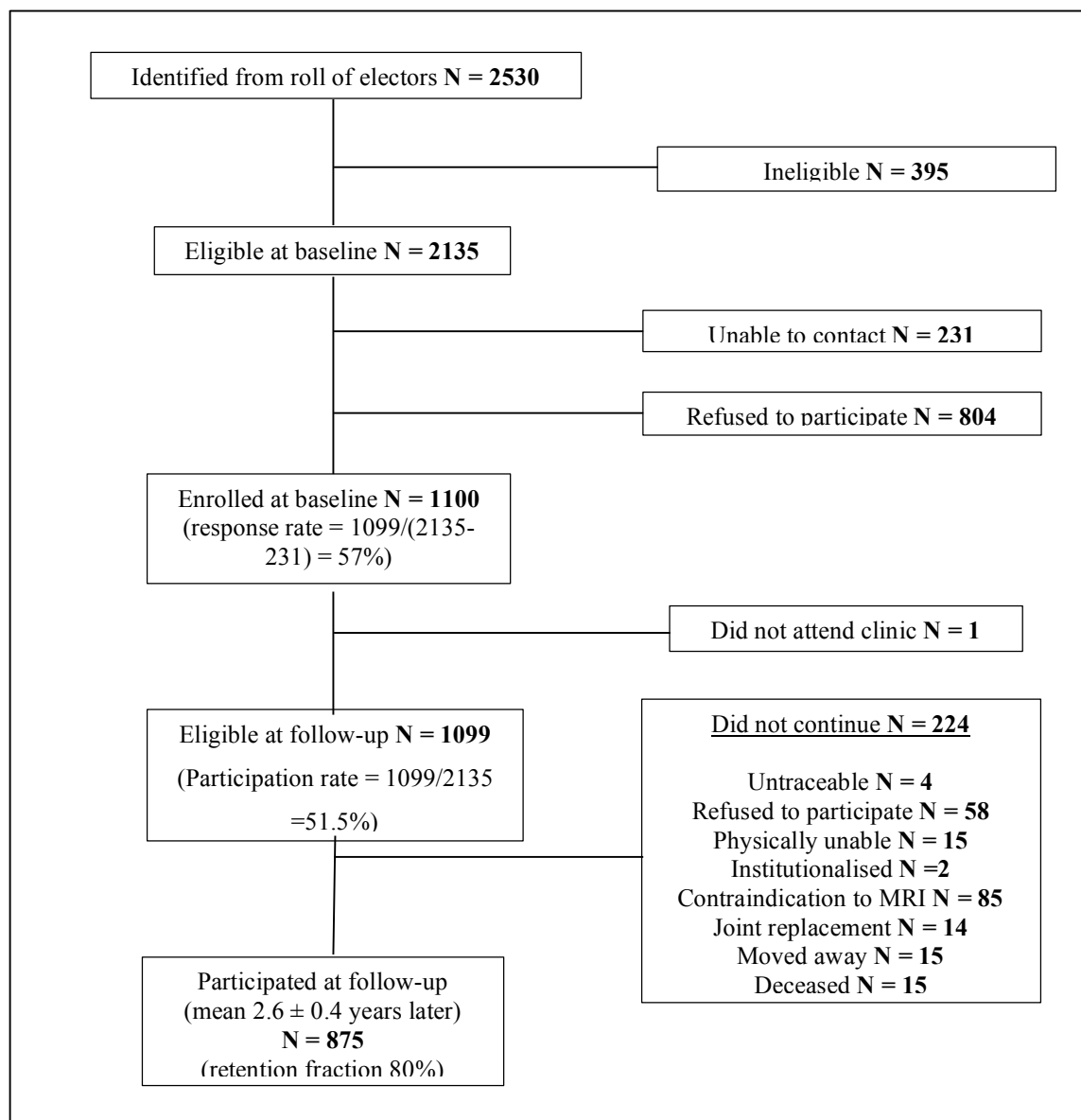


Figure 3.1. Flow chart describing recruitment, participation rates, and withdrawal reasons for TASOAC participants.

3.2.1 Anthropometrics

Each subject's body weight was measured to the nearest 0.1 kg (with shoes, socks, and bulky clothing removed) using a single pair of electronic scales (Seca Delta Model 707, Bradford, MA, USA). Height was measured to the nearest 0.1 cm (with shoes and socks removed) using a stadiometer. BMI was calculated as kg/m^2 .

3.2.2 Knee pain assessment

Self-reported knee pain (walking on flat surface, going up/down stairs, at night in the bed, sitting/lying and stand upright) was assessed by knee-specific Western Ontario McMaster osteoarthritis index (WOMAC) (165). It was demonstrated in Appendix 2 with a 10-point pain scale from 0 (no pain) to 9 (most severe pain). Total pain score (0-45) was created by summing all the subscale scores.

The five WOMAC pain subscales were clinically categorised into weight-bearing pain (including pain on flat surface, on stairs and standing) and non-weight-bearing pain (including pain at night and sitting) as suggested by a previous study (166).

The presence of knee pain was defined as a pain score of 1 or greater. Change in knee pain score was calculated as (follow-up value - baseline value), with a change in score of ≥ 1 indicating increased knee pain (47). We have calculated the smallest statistically significant difference for the change in total WOMAC knee pain score (167) to be 0.8 for our population, so we defined an increase in pain as a change in score ≥ 1 .

3.2.3 Magnetic Resonance Imaging

MRI of the right knee was acquired with a 1.5T whole-body magnetic resonance unit (Picker, Cleveland, OH, USA) using a commercial transmit/receive extremity coil. Image sequences included the following: (1) a T1-weighted fat saturation 3-dimensional (3D) gradient-recalled acquisition in the steady state, flip angle 30° , repetition time 31 ms, echo time 6.71 ms, field of view 16 cm, 60 partitions, 512×512 -pixel matrix, acquisition time 5 min 58 s, one acquisition; sagittal images were obtained at a slice thickness of 1.5 mm

without an inter-slice gap; and (2) a T2-weighted fat saturation two-dimensional (2D) fast spin echo, flip angle 90°, repetition time 3067 ms, echo time 112 ms, field of view 16 cm, 15 partitions, 228 × 256–pixel matrix; sagittal images were obtained at a slice thickness of 4 mm with an inter-slice gap of 0.5–1.0 mm.

3.2.3.1 Effusion-synovitis

Knee effusion-synovitis was assessed as the amount of intra-articular fluid-equivalent signal on T2-weighted MRI. We distinguish knee effusion-synovitis in 4 different subregions according to the anatomy of the joint synovial cavity (53). They are suprapatellar pouch: a large pouch is formed between the posterior suprapatellar fat pad (quadriceps femoris tendon) and the anterior surface of the femur; central portion: lies between the central femoral and tibial condyles, around the ligaments and menisci; posterior femoral recess: lies behind the posterior portion of each femoral condyle and the deep surface of the lateral and medial heads of the gastrocnemius; subpopliteal recess: lies posteriorly between the lateral meniscus and the popliteal tendon.

i. Ordinal assessment for effusion-synovitis

Effusion-synovitis in each subregion was scored individually according to WORMS, grading collectively from 0 to 3 in terms of the estimated maximal distention of the synovial cavity: 0 refers to normal; 1 to < 33% of maximum potential distention; 2 to 33% – 66% of maximum potential distention; 3 to > 66% of maximum potential distention (114). Figure 3.2 demonstrates examples of the ordinal assessment. There is an obvious distention of the synovial cavity when effusion-synovitis of grade 2 is present, so pathological effusion-synovitis was defined as any score of ≥ 2 (92). Total effusion-synovitis of the whole joint was defined as a score of ≥ 2 in any subregion. Two independent observers who scored all images were blinded to the patients' information. The intraclass reliability assessed as weighted κ in 50 randomly selected images were 0.63 – 0.75 in different subregions, and the interclass reliability were 0.65 – 0.79 (94) (expressed as weighted kappa 0.6=threshold for good validity (168)).

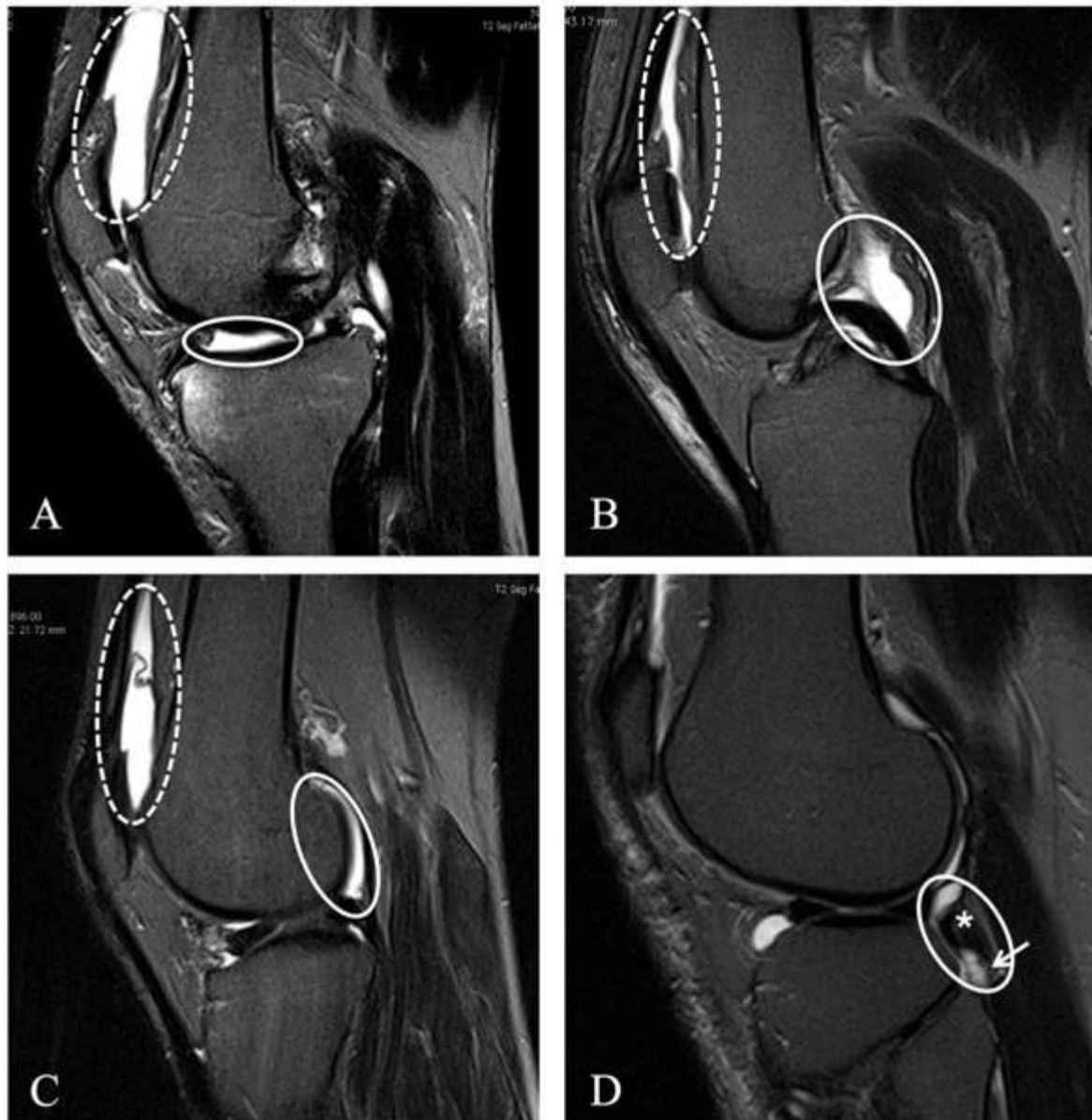


Figure 3.2. Typical T2-weighted fat-saturation fast spin echo sagittal images for effusion-synovitis in different subregions.

(A) Grade 3 effusion-synovitis in medial suprapatellar pouch (dash circle) and grade 2 central portion effusion-synovitis (solid circle); (B) grade 1 midline suprapatellar pouch effusion-synovitis (dash circle) and grade 2 central effusion-synovitis around posterior cruciate ligament (solid circle); (C) grade 2 effusions in lateral posterior femoral recess (solid circle) and suprapatellar pouch (dash circle); (D) grade 2 subpopliteal effusion-synovitis (solid circle) around popliteal tendon (asterisk), extending to popliteal bursa (arrow).

ii. Areal assessment for effusion-synovitis

The size of effusion-synovitis (cm²) in each of subregions (referred as regions of interest, ROIs) was directly generated in the entire series of images using OsiriX software. A total area of all the ROIs in the same slice was summed as effusion-synovitis area of this slice. A maximal area in one slice was selected to represent effusion-synovitis area of the knee. A change in the effusion-synovitis area was calculated by subtracting baseline maximal area from follow-up maximal area (Figure 3.3). A musculoskeletal researcher (XW, 3 years experience) and an orthopaedic clinician (WH, 7 years experience) measured the maximal area of effusion-synovitis under the guidance of an experienced radiologist (AH, >20 years experience) and were blinded to the patients' information. 100 randomly selected images were assessed with at least two weeks interval between the readings, and the intra-observer (expressed as the intraclass correlation coefficient, ICC) and inter-observer reproducibility was 0.81 and 0.60, respectively (169).

The validity of effusion-synovitis maximal area measurement was tested by comparing this maximal area with total proxy volume generated using the OsiriX software in 30 randomly selected participants (2-D sagittal T2-weighted images with inter-slice gaps did not allow calculating the real volume). There was a high correlation ($r=0.83$, $p<0.001$) between these two measures. We also compared the maximal area with semi-quantitative scores of effusion-synovitis, and found that the correlation was high ($r=0.71$, $p<0.001$). Changes in effusion-synovitis size were defined as increase, stable and decrease based on the least significant change criterion (LSC) (170), which takes into account measurement error and the correlation between the baseline and follow-up measurements. The formula was as follows:

$$LSC = 1.96 \times \sigma \sqrt{2(1 - \rho)}$$

(σ = the standard error of the mean; ρ = the serial correlation).

LSC was calculated to be 0.49 cm² (where $\sigma = 0.3419$ and $\rho = 0.7328$) in this study. Therefore, an increase in effusion-synovitis size was defined as a change in effusion-synovitis area of ≥ 0.49 cm². A decrease in effusion-synovitis size was defined as a change in effusion-synovitis area of ≤ -0.49 cm².

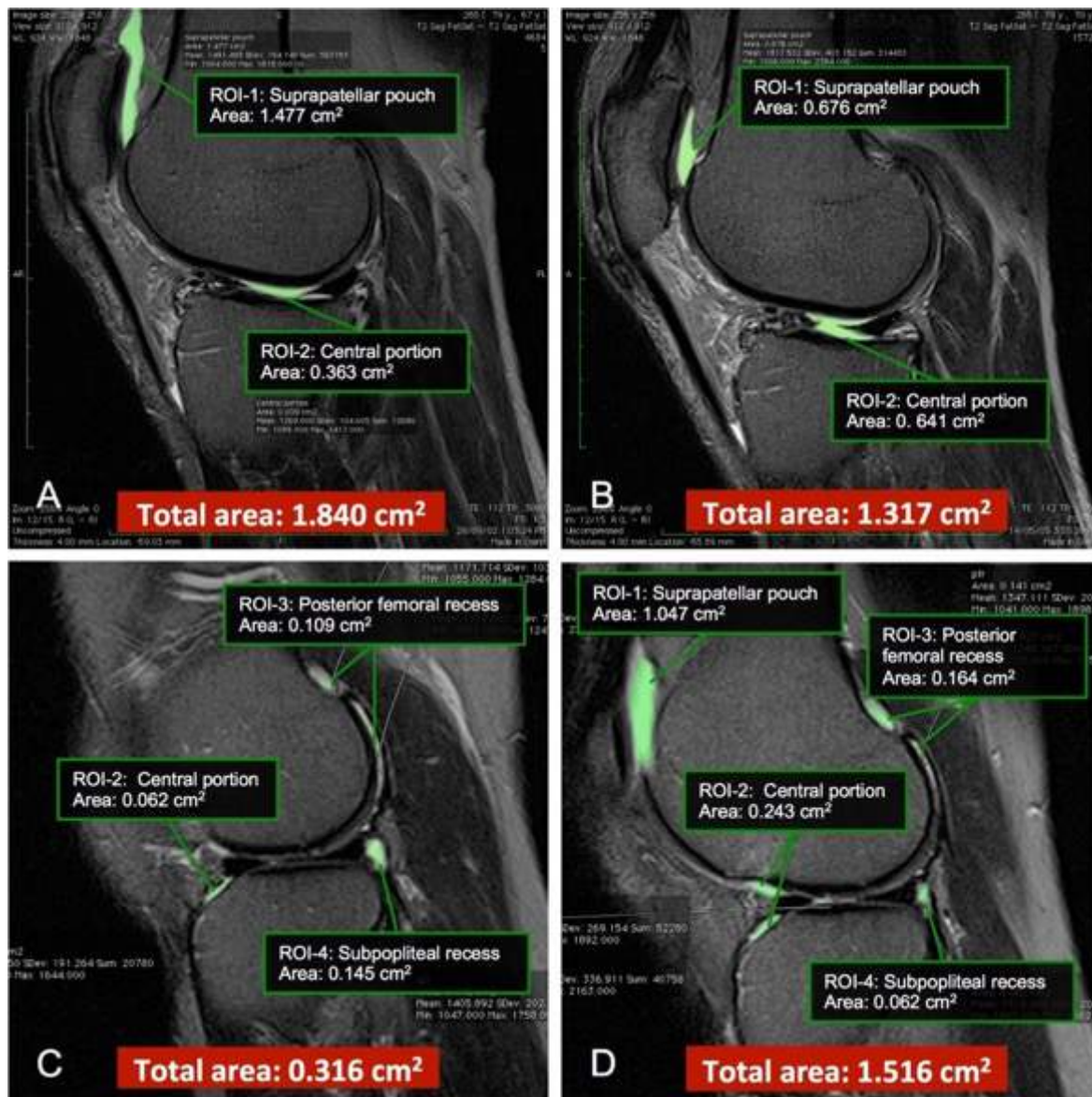


Figure 3.3. Examples of changes in effusion-synovitis area (cm²).

Effusion-synovitis size decreased from baseline to follow-up (A-B) or increased from baseline to follow-up (C-D). The ROIs included suprapatellar pouch (ROI-1), central portion (ROI-2), posterior femoral recess (ROI-3) and subpopliteal recess (ROI-4).

iii. Voluminal assessment for effusion-synovitis

Each subregion of effusion-synovitis was also measured the maximum area of the lesion as demonstrated in Figure 3.4. The volumes of individual joint subregions were isolated from the total volume by selecting each region of interest (ROI) according to the intra-articular fluid-equivalent signal on a section-by-section basis. The final 3-D volume rendering was generated using commercial in-house OsiriX Lite imaging software cursors (32-bit version 5.9, Pixmeo SARL, Geneva, Switzerland) (171) (Figure 3.4). The readers

were blinded to the treatment allocation and patients' information. To analyse the reliability of measurement, two independent readers assessed 40 randomly selected images with at least a 4-week interval between readings. The intra-class correlation coefficients (ICCs) were 0.96-0.97 and inter-rater correlation coefficients were 0.93-0.99 in different subregions.

Change in effusion-synovitis volume was calculated as follows: Absolute change (ml) = (follow-up volume) – (baseline volume); Relative change per annum (%p.a.) = [(absolute change)/(baseline volume)]/(time interval between 2 scans) x 100.

The semi-quantitative increase or decrease was defined by LSC as previously described (e.g., an increase in effusion-synovitis volume was defined as a change in effusion-synovitis volume of ≥ 1.81 ml; and a decrease in effusion-synovitis volume was defined as a change ≤ -1.81 ml). The minimal clinically important difference (MCID) was estimated for effusion-synovitis volume (both the absolute and the relative annual change). A reduction of mean WOMAC function score ≥ 7 was used as an anchor to determine the cut-off of effusion-synovitis volume in patients who actually experienced clinically significant improvement (167).

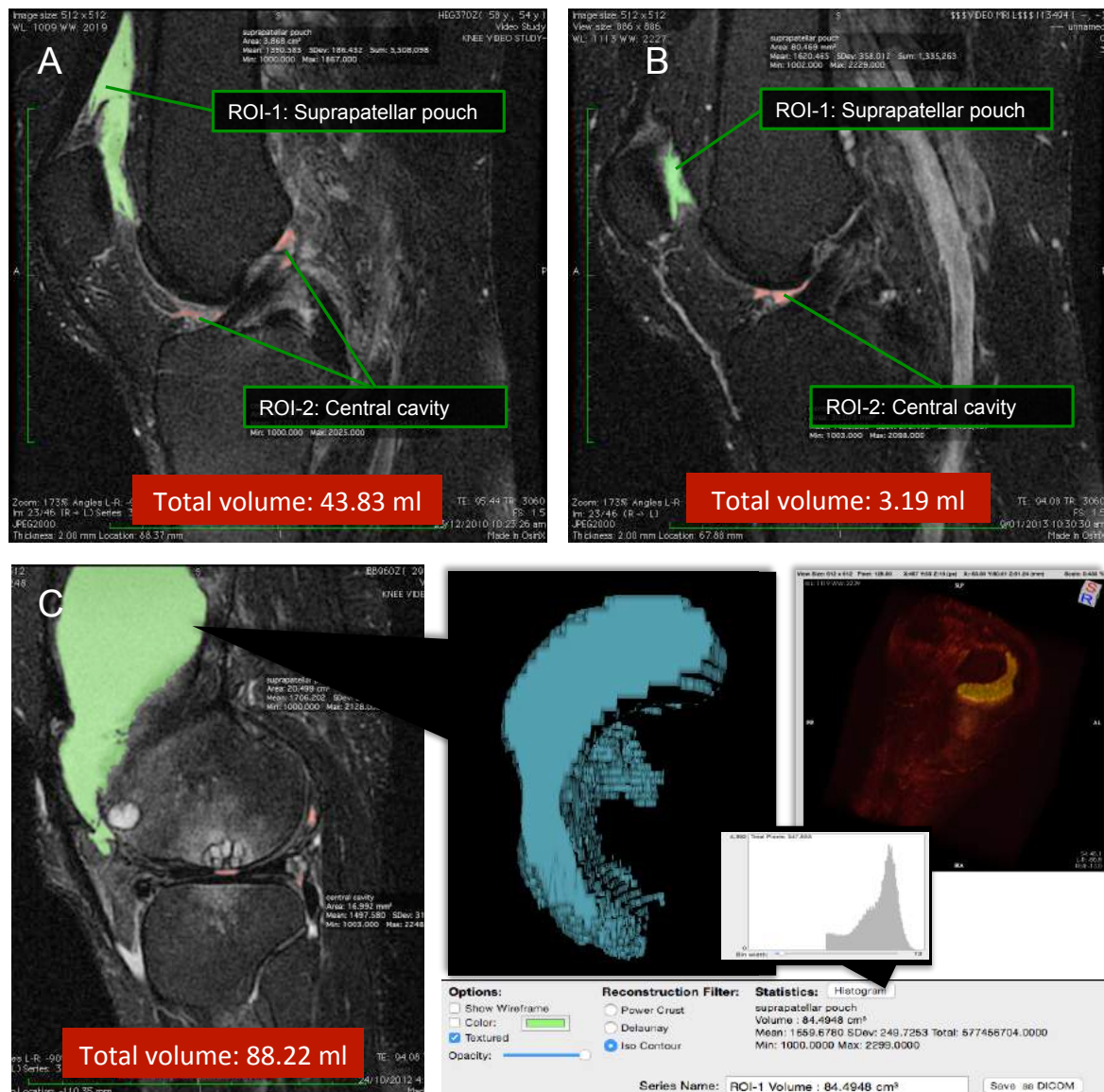


Figure 3.4. MRI acquired from the knee, with superimposed colour data showing the area of high signal.

The images were obtained before (A) and 24 months after (B) intervention. Data were analysed in two regions of interests (ROIs), which were located in the suprapatellar pouch (ROI-1, pixels shown in green) and the central joint cavity (ROI-2, pixels shown in red), respectively. The total volume was generated from the area of each ROI in the entire series of images using OsiriX software (C).

3.2.3.2 BMLs

Subchondral BMLs were assessed on T2-weighted MR images using OsiriX software and were defined as areas of increased signal adjacent to the subcortical bone at the medial

tibial, medial femoral, lateral tibial, and lateral femoral sites. BMLs were scored using both an ordinal scoring system and an areal scoring system. Each BML was scored on the basis of lesion size (e.g. a lesion was scored as grade 1 if it was only present on one slice, grade 2 if present on two consecutive slices, or grade 3 if present on 3 or more consecutive slices). The BML with the highest score was used if more than one lesion was present at the same site. Intra-observer repeatability was assessed in 50 subjects with at least a one-week interval between the two readings. The intraclass correlation coefficients (ICCs) were 0.94, 1.00, 0.89, and 0.96 at the medial tibial, medial femoral, lateral tibial, and lateral femoral sites, respectively (172).

3.2.3.3 Cartilage defects

Cartilage defects were assessed on T1 and T2-weighted MR images (score range, 0–4) at the medial tibial, medial femoral, lateral tibial, and lateral femoral sites, as previously described (173) as follows: grade 0 = normal cartilage; grade 1 = focal blistering and intra-cartilaginous low or high-signal intensity area with an intact surface and base; grade 2 = irregularities on the surface or base and loss of thickness <50%; grade 3 = deep ulceration with loss of thickness >50%; and grade 4 = full-thickness chondral wear with exposure of subchondral bone. A cartilage defect also had to be present on at least two consecutive slices. The cartilage was considered to be normal if the band of intermediate signal intensity had a uniform thickness. The highest score was used if more than one defect was present on the same site. One observer scored the cartilage defects.

Intra-observer repeatability was assessed in 50 subjects with an interval of at least one week between the two measurements. ICCs were 0.93, 0.92, 0.95, and 0.80 at the medial tibia, medial femur, lateral tibia, and lateral femur, respectively.

3.2.3.4 Cartilage volume

Knee tibial cartilage volume was assessed on T1-weighted MR images by means of image processing on an independent workstation using OsiriX software as previously described (47). The volumes of individual cartilage plates (medial tibia and lateral tibia) were isolated from the total volume by manually drawing disarticulation contours around the cartilage boundaries on a section by section basis. These data were then resampled by means of bilinear and cubic interpolation (area of 312×312 mm and 1.5 mm thickness, continuous

sections) for the final 3D rendering. The CV in our hands for this method of measurement was 2.1% for the medial tibia and 2.2% for the lateral tibia (47).

3.2.3.5 Meniscal pathology

Meniscal lesions were assessed by a trained observer on MR images as previously described (47, 174). The proportion of the menisci affected by a tear, partial or full extrusion was scored laterally and medially at the anterior horn, body, and posterior horn. These scores were summed to create a total meniscal pathology score (47).

3.2.3.6 Tibial bone area

Knee tibial plateau bone area was assessed on T1-weighted MR images and defined as the cross-sectional surface area of the tibial plateau, as previously described (29, 73, 117). Medial and lateral bone area was measured manually by reformatting the whole sagittal image to the axial plane. The area was then measured on 3 slices closest to the tibial cartilage and the mean of all 3 areas was used as an estimate of the tibial plateau bone area. The slice thickness on the axial images was 0.625 mm. In a previous study, the coefficient of variation (CV) in our hands for this method of measurement was 2.2–2.6% (47).

3.2.4 X-ray

A standing anteroposterior semi-flexed view of the right knee with 15° of fixed knee flexion was performed. Radiographs were assessed using the atlas developed by Altman et al (175). Each of the followings was assessed on a scale of 0–3: medial JSN, lateral JSN, medial femoral osteophytes, medial tibial osteophytes, lateral femoral osteophytes, and lateral tibial osteophytes. Each score was determined by consensus of two readers who simultaneously assessed the radiograph with immediate reference to the atlas. Intra-observer repeatability was assessed in 40 subjects with an interval of at least one week between the two measurements. ICCs ranged from 0.65 – 0.85. The presence of ROA was defined as any score ≥ 1 for JSN or osteophytes (172).

3.2.5 *Summary of outcome factors, study factors, and covariates*

Table 3.1 summarises the variables used in each chapter of this thesis.

Table 3.1. Summary of outcome factors, study factors, and covariates used in this thesis.

Chapter	Outcome factors	Study factors	Covariates
4	Pain	Effusion-synovitis	Age, sex, BMI, ROA, cartilage defects, BMLs, meniscal lesions
5	Cartilage defects, cartilage volume, BMLs	Effusion-synovitis	Age, sex, BMI, cartilage defects, cartilage volume, bone area, BMLs, ROA
6	Effusion-synovitis	Cartilage defects, cartilage volume, BMLs, meniscal lesions, osteophytes, JSN	Age, sex, BMI, bone area, ROA

*Measurement protocol described in “Materials and Methods” section of the relevant chapter.

3.2.6 *Ethical considerations*

All procedures in TASOAC were approved by the Southern Tasmanian Health and Medical Human Research Ethics Committee (Ethics Approval Number: H6488). Written informed consent was obtained from all participants prior to enrolment in the study.

3.2.7 *Statistical analysis*

T-tests and chi-squared tests were used to compare differences in means and proportions as appropriate. Standard diagnostic checks of model fit and residuals were routinely performed, and data points with large residuals and/or high influence were investigated for data errors. A p value less than 0.05 (two-tailed) is considered statistically significant. A more detailed description of statistical analyses performed is presented in their relevant chapters. All statistical analyses were performed on Intercooled Stata versions 12 or 13 for Mac (StataCorp., College Station, USA).

3.3 VIDEO Study population and design

The work in this thesis was conducted as part of the VIDEO study, a randomised, double-blind, placebo-controlled clinical trial primarily aimed at determining whether vitamin D supplementation reduces the loss of knee cartilage volume (~2% per year less loss than placebo group) and progression of knee pain in symptomatic knee OA patients (176). Participants were recruited in Tasmania and Victoria, Australia, using a combined strategy, including working with general practitioners, specialist rheumatologists and orthopaedic surgeons, and advertising through media and community groups. Eligible participants were randomly allocated to either treatment or matching placebo group in a 1:1 ratio. A telephone pre-screen assessed knee pain, anticipated knee and hip surgery, participation in other studies and comorbidities. Eligible participants were subsequently screened in a clinic visit including knee radiographs and a blood test for serum 25-(OH)D level.

Eligible participants were aged between 50 to 79 years with symptomatic knee OA for at least 6 months and pain of at least 20 mm on a 100 mm on a visual analogue scale (VAS) and were recruited from August 2010 to December 2011. All individuals were assessed according to the American College of Rheumatology (ACR) criteria for symptomatic knee OA (4). Participants also had an ACR function class rating of I, II and III (177) and relatively good health, with a score of 0 to 2 on a 5-point Likert scale (from 0 indicating very good health to 4 indicating very poor health) according to the global investigator assessment of disease status. Participants were included if their serum 25-(OH)D levels > 12.5 nmol/l or < 60 nmol/l. Exclusion criteria included grade 3 knee ROA according to Altman's atlas (175), contraindication to MRI, rheumatoid or psoriatic arthritis, lupus, cancer, severe cardiac or renal impairment, hypersensitivity to vitamin D, conditions affecting oral drug absorption, anticipated knee or hip surgery within the next two years, history of significant trauma of knees (e.g. arthroscopy or injury to ligaments or menisci within one year preceding the study) and history of taking vitamin D or an investigational drug within the last 30 days.

Figure 3.5 provides an overview of participant recruitment and withdrawal during the study period. A total of 599 participants were screened for eligibility and 413 subjects were randomly assigned to either the vitamin D or the placebo group. Over 24 months, 28 (13.4%) in the vitamin D group and 45 (22.1%) in the placebo group withdrew from the study. A total of 340 patients (82.3%) completed the follow-up. The number of patients who discontinued treatment allocation was larger in the placebo group than the vitamin D group (21 versus 8). The major reason for discontinuation was non-adherence to the protocol when low 25-(OH)D

levels were disclosed to the participants by their general practitioners. Intention-to-treat analyses were performed in all 413 randomised participants. The mean age of participants was 63.2 ± 7.0 years, with 208 (50%) females and a mean BMI of 29.6 ± 5.0 kg/m².

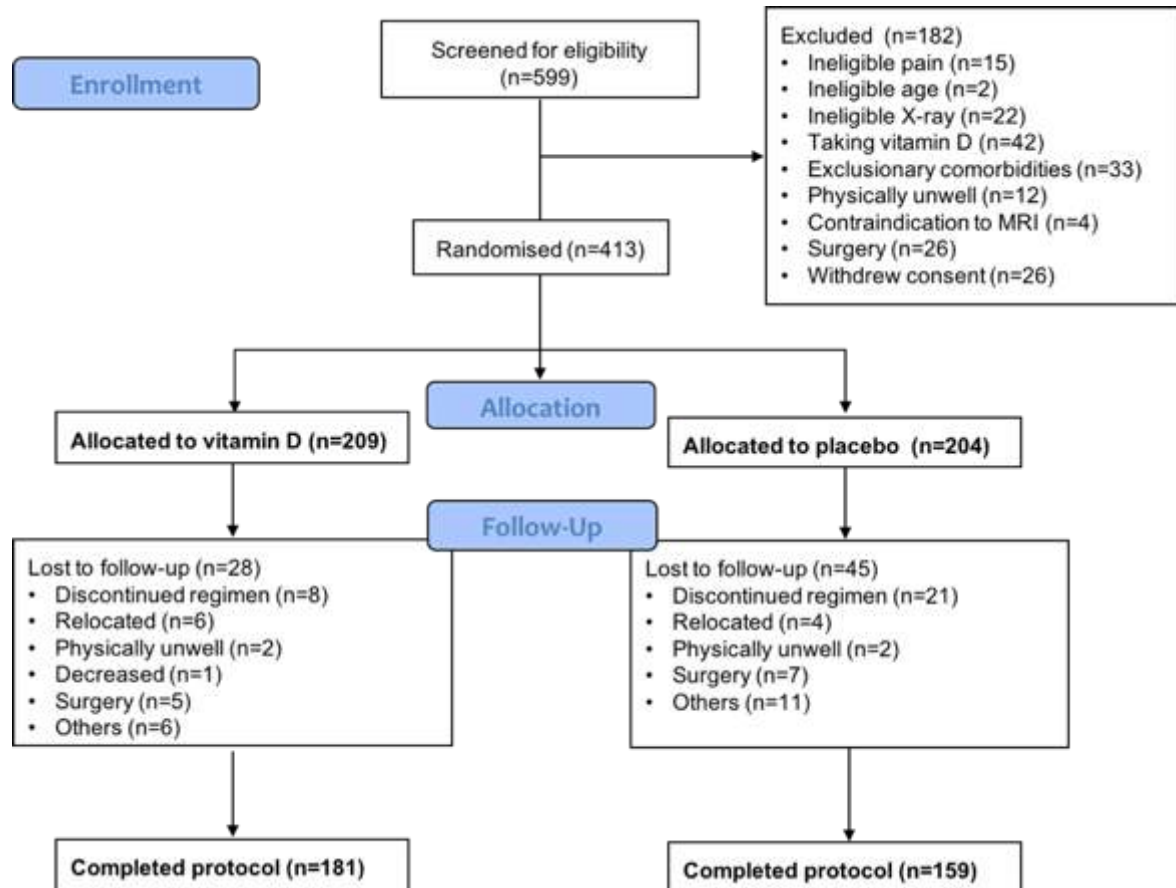


Figure 3.5. Flow chart describing recruitment, participation rates, and withdrawal reasons for VIDEO participants.

3.3.1 Intervention

Participants in the intervention group were given a monthly capsule of 50,000 IU (1.25 mg) vitamin D₃ (cholecalciferol) for 24 months (178). The vitamin D₃ compound was purchased from Nationwide Compounding Pharmacy, Melbourne, Australia. Participants in the control group received an identical inert placebo provided by the same company.

3.3.2 Randomisation

Participants were allocated to either vitamin D or placebo arm at a ratio of 1:1 based on computer-generated random numbers. Allocation concealment was ensured by a central

automated allocation procedure with security in place to ensure allocation data cannot be accessed or influenced by any person from the investigative team.

3.3.3 *Blinding*

Participants, research coordinators and investigators were all blinded to treatment assignment. The blinding procedure was maintained until all the data were collected, cleaned, confirmed for accuracy and statistical analyses were performed.

3.3.4 *Trial monitoring and safety assessments*

The project manager visited and monitored each site to examine trial procedures, ensure data quality and compliance with the trial protocol. Spontaneously reported adverse events were recorded throughout the study. Intensity and relationship with the study medication were ascribed.

3.3.5 *Anthropometrics*

Height and weight were measured as described in section 3.2.1, and BMI was calculated.

3.3.6 *Knee symptoms*

Knee symptoms were assessed from baseline to month 24, using the WOMAC score as described in section 3.2.2. The WOMAC system quantifies the degree of pain (5 questions), functional impairment (17 questions) and stiffness (2 questions) in patients with OA. Each question was assessed in a 100 mm visual analogue format to create a total score (179).

3.3.7 *Magnetic Resonance Imaging*

T1 and T2 weighted MRI scans were taken using the same protocol as described in section 3.2.3. Proton density-weighted coronal fat-suppressed, FSE, flip angle 90°, repetition time 2640 msec, echo time 37 msec, FOV 16 cm, 30 slices, 256 × 256-pixel matrix, acquisition time 5 min 26 sec, 1 acquisition, slice thickness of 3 mm.

3.3.7.1 Effusion-synovitis

Knee effusion-synovitis was assessed on T2-weighted and proton density-weighted MR images, as previously described in section 3.2.3.1 .

As the MRI sequence used to determine effusion-synovitis at the site in Victoria was obtained in the coronal plane, the subregional effusion-synovitis was unable to be differentiated in Victorian participants. Hence, subregional analyses were only performed in participants from Tasmania.

3.3.7.2 BMLs

BMLs were assessed on T2-weighted MR images as previously described in section 3.2.3.2 .

3.3.7.3 Cartilage defects

Cartilage defects were assessed on T1 and T2-weighted MR images as previously described in section 3.2.3.3 .

3.3.7.4 Cartilage volume

Tibial cartilage volume was assessed on T1-weighted MR images as previously described in section 3.2.3.4 .

3.3.7.5 Tibial bone area

Tibial plateau bone area was assessed on T1-weighted MR images as previously described in section 3.2.3.6 .

3.3.8 X-ray

A Knee x-rays were obtained and scored for JSN and osteophytes using the same protocol as described in section 3.2.4.

3.3.9 Serum 25-(OH) D

Serum 25-(OH)D was assayed by Liaison method utilising a direct competitive chemiluminescent immunoassay (DiaSorin Inc., Stillwater, Minnesota, USA). The intra-assay and inter-assay CVs were 3.2% and 6.0%.

3.3.10 Summary of outcome factors, study factors, and covariates

Table 3.2 summarises the variables used in each chapter of this thesis.

Table 3.2. Summary of outcome factors, study factors, and covariates used in this thesis

Chapter	Outcome factors	Study factors	Covariates
7	Effusion-synovitis	Pain*, function*, stiffness*, cartilage defects, cartilage volume, BMLs Osteophytes JSN	Age, sex, BMI, ROA, study site, 25-(OH)D*
8	Effusion-synovitis	Vitamin D or placebo effect*	--

*Measurement protocol described in “Materials and Methods” section of the relevant chapter.

3.3.11 Sample size and role of the candidate in the VIDEO study

As the TASOAC study was in progress before the commencement of the Ph.D. candidature formal sample size calculations were not performed during the design of this thesis. The statistical power calculation is based on formula provided by Cohen (180). Using primary outcome cartilage volume loss in the VIDEO study, the sample size was calculated as 200 per group (176). From our previous study in older people with knee pain, 29% of participants experienced an increase in effusion-synovitis size (181). With 400 recruited patients (200 in each arm), we have 80% power ($\alpha=0.05$) to detect a 13% difference in the increase in effusion-synovitis size.

Whilst the candidate was involved in VIDEO data acquisition during candidature, data acquisition was also completed prior to and during the candidature by a number of other VIDEO staff and volunteers, including Changhai Ding, Graeme Jones, Flavia Cicuttini, Anita E Wluka, Tania Winzenberg, Jodi Barling, Kay Nguo, Benny Antony, Yuelong Cao, Judy Hankin and Alice Noone. The candidate gratefully acknowledges the efforts of Xingzhong Jin in cleaning the vitamin D data, cartilage volume, cartilage defect, BMLs and bone area data; Weiyu Han and Zhaohua Zhu in measuring of radiographic data.

3.3.12 Ethical considerations

Ethics approval was received from the Tasmania Health and Human Medical Research Ethics Committee (reference number H1040) and Monash University Human Research Ethics Committee (reference number CF10/1182-2010000616). Informed written consent was obtained from all participants.

3.3.13 Trial registration

ClinicalTrials.gov identifier: NCT01176344;

Australian New Zealand Clinical Trials Registry: ACTRN12610000495022.

3.3.14 Protocol

Cao Y, Jones G, Cicuttini F, et al. Vitamin D supplementation in the management of knee osteoarthritis: study protocol for a randomized controlled trial. *Trials* 2012;13:1.

3.3.15 Statistical analysis

All statistical analyses were performed similarly as described in section 3.2.7.

**Chapter 4 – Regional knee effusion-synovitis and knee pain in older adults:
a cohort study**

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**Chapter 5 - Regional knee effusion-synovitis and knee structural
abnormalities in older adults: a cohort study**

5.1 Introduction

OA is characterized by joint pain and structural changes including cartilage loss and subchondral bone abnormalities. Synovial activation, namely proliferation and inflammation, has emerged as a critical component of OA and a potential predictor of disease progression (89). It has been linked to the signs of disease flare-up, such as joint swelling and inflammatory pain (100), though not all consistent (77, 83).

Synovial inflammation can occur at both the early and late stages of OA (51). *In vitro* and animal studies have demonstrated that inflamed synovium produces catabolic and pro-inflammatory mediators such as cytokines, nitric oxide, prostaglandin E2 and neuropeptides, which lead to excess production of the proteolytic enzymes responsible for cartilage breakdown, disturbing the homeostasis of cartilage matrix degradation and repair (206, 207). Cartilage breakdown products in turn exacerbate synovial inflammation, creating a vicious circle (208). These suggest that synovial inflammation can be a cause or a result of knee structural changes; however, epidemiological and clinical studies have shown inconsistent associations between synovial inflammation and knee focal cartilage loss (76, 92, 209). It is not known whether synovial inflammation is associated with diffuse cartilage loss assessed by cartilage volume and subchondral bone abnormalities such as BMLs, and whether synovial inflammation is causally related to these structural changes.

Clinically, synovial pathology is usually manifested as thickening of synovium (synovitis) and/or excess synovial fluid (effusion) (185), which are usually assessed as a whole according to the extent of capsular distention using non-contrast-enhanced magnetic resonance imaging (MRI) (111). To date, it is still unknown whether effusion/synovitis is distributed in a homogeneous fashion within the joint cavity. It is also unclear whether effusion/synovitis in different anatomical subregions have different impacts on intra-articular pathology. MRI is able to detect minor effusion/synovitis in the whole joint, which allows us to determine whether effusion/synovitis in different locations is associated with MRI-detected osteoarthritic alterations. The aim of this study, therefore, was to describe the cross-sectional and longitudinal associations between knee regional effusion-synovitis and structural changes in older adults.

5.2 Materials and Methods

5.2.1 *Participants and study design*

This study used subjects from the TASOAC study as described in section 3.2.

5.2.2 *Knee MRI sequences*

T1 and T2 weighted fat saturated MRIs sequences as described in section 3.2.3.

5.2.3 *Knee MRI measurements*

5.2.3.1 *Effusion-synovitis*

Baseline effusion-synovitis was scored in four subregions as described in section 3.2.3.1 and displayed in Figure 3.2.

5.2.3.2 *Cartilage volume*

Baseline and follow-up cartilage volume was measured on MRI as described in section 3.2.3.4 .

5.2.3.3 *Cartilage defects*

Baseline and follow-up cartilage defects were assessed from 0-4 on MRI as described in section 3.2.3.3 .

5.2.3.4 *BMLs*

Baseline and follow-up BMLs were assessed from 0-3 on MRI as described in section 3.2.3.2 .

5.2.3.5 *Tibial bone area*

Baseline and follow-up tibial bone area was measured on MRI as described in section 3.2.3.6 .

5.2.4 *Knee X ray*

Baseline knee radiographs were taken and scored for ROA as described in section 3.2.4.

5.2.5 *Anthropometrics*

Height, weight and BMI was measured as previous described in section 3.2.1.

5.2.6 *Statistical analysis*

Comparisons of baseline characteristics by effusion-synovitis status were carried out using unpaired student t-tests or chi-square tests (as appropriate). The statistically significant differences between subjects who completed the study and who were lost to follow-up were evaluated using t-test or chi-square tests (as appropriate).

We examined the associations between effusion-synovitis and baseline/change in cartilage volume using linear regression. Logistic regression analysis and generalized linear models were used to estimate prevalence ratios (PR) or relative risks (RR) (195) between effusion-synovitis and JSN, osteophytes, cartilage defects and BMLs. The associations were adjusted for age, gender, BMI, and ROA, and further adjusted for tibial bone area (for cartilage volume), cartilage defects and/or BMLs (where appropriate). Standard diagnostic checks of model fit and residuals were routinely performed, and data points with large residuals and/or high influence were investigated for data errors. Interactions between gender or ROA, and effusion-synovitis were investigated by testing the statistical significance of the coefficient of a product term (effusion \times gender or ROA) after adjustment for confounders.

5.3 Results

5.3.1 *Characteristics of participants*

At baseline, 977 subjects (50% females) were included in this study. The average age was 62.3 years, and the mean BMI was 27.7 kg/m². Prevalence of knee joint effusion-synovitis (≥ 2) was 67% in the whole joint cavity (42.9% in suprapatellar pouch, 48.8% in central portion, 10.3% in posterior femoral recess and 14.4% in subpopliteal recess). As shown in Table 5.1, participants with or without effusion-synovitis (score 0-1 vs. 2-3) were similar in terms of gender, BMI, ROA, total cartilage volume and BMLs; however, participants with effusion-synovitis were older, had larger tibial bone size, and had higher proportions with osteophytes and cartilage defects.

Over 2.7 years (range from 1.3 to 4.8 years), 115 subjects did not continue at follow-up because of death (n=28), joint replacement (n=15), being physically unable (n=28), moving away (n=20) and other reasons such as refusing to continue or giving no reason. In subjects who completed the follow-up study, the first 404 subjects had the second MRI scan, but the others did not because the local MRI machine was decommissioned and became unavailable for research purposes. There were no significant differences in demographic factors, including age, gender, BMI and diseases status, between participants who remained in the study and who dropped out. There were no significant interactions between demographic factors and effusion-synovitis on joint structural changes.

Table 5.1. Characteristics of sample at baseline.

Characteristics	Knee effusion-synovitis		p value
	Absent (Grade 0-1) N=323	Present (Grade 2-3) N=654	
Age	61.14 (7.03)	62.90 (7.47)	<0.01
Female (%)	53	49	0.28
Body mass index	27.55 (4.32)	27.84 (4.84)	0.37
Tibial bone area (ml)	3.26 (0.48)	3.37 (0.50)	<0.01
Total cartilage volume (ml)	8.29 (1.98)	8.28 (1.95)	0.94
Knee radiographic osteoarthritis (%)	58	60	0.51
Any joint space narrowing (%)	57	60	0.41
Any osteophytes (%)	8	13	0.01
Any cartilage defect (%)	47	61	<0.01
Any bone marrow lesion (%)	26	32	0.27

Two-tailed t-tests are used for differences between means; chi-square tests are used for proportions. Results are shown as mean (SD) or percentage. Data in bold denote statistically significant results.

5.3.2 *Effusion-synovitis and cartilage defects*

Cross-sectionally, knee effusion-synovitis in all of the subregions, including suprapatellar pouch, central portion, posterior femoral recess and subpopliteal recess, was significantly associated with cartilage defects in multivariable analyses (Table 5.2). The significant associations apart from that in central portion remained significant after further adjustment for BMLs (Table 5.2).

Longitudinally, effusion-synovitis in whole knee joint and 2 joint subregions (except for central portion and posterior femoral recess) was significantly associated with worsening cartilage defects and remained significant after further adjustment for baseline BMLs and cartilage defects (Table 5.2). There was a dose-response association between suprapatellar pouch effusion-synovitis and an increase in cartilage defects (Figure 5.1A).

Table 5.2. Associations between knee effusion-synovitis and cartilage defects.

	Multivariable*	Multivariable**
<i>Baseline cartilage defects</i>	PR (95%CI)	PR (95%CI)
Whole knee joint	1.21 (1.11, 1.31)	1.15 (1.06, 1.25)
Suprapatellar pouch	1.18 (1.10, 1.27)	1.13 (1.05, 1.22)
Central portion	1.11 (1.02, 1.20)	1.06 (0.97, 1.15)
Posterior femoral recess	1.18 (1.00, 1.39)	1.18 (1.01, 1.39)
Subpopliteal recess	1.17 (1.07, 1.28)	1.14 (1.04, 1.24)
<i>Any increase in cartilage defects</i>	RR (95%CI)	RR (95%CI)
Whole knee joint	1.13 (1.03, 1.25)	1.11 (1.01, 1.23)
Suprapatellar pouch	1.23 (1.12, 1.34)	1.19 (1.09, 1.31)
Central portion	1.01 (0.91, 1.12)	1.01 (0.91, 1.12)
Posterior femoral recess	1.05 (0.85, 1.29)	1.03 (0.84, 1.27)
Subpopliteal recess	1.23 (1.14, 1.34)	1.20 (1.10, 1.30)

Dependent variable: baseline and increase in cartilage defects (yes vs. no). Independent variable: knee effusion-synovitis (per grade).

*Adjusted for age, gender, BMI and radiographic osteoarthritis. **Further adjusted for baseline bone marrow lesions and cartilage defects (for longitudinal analyses only).

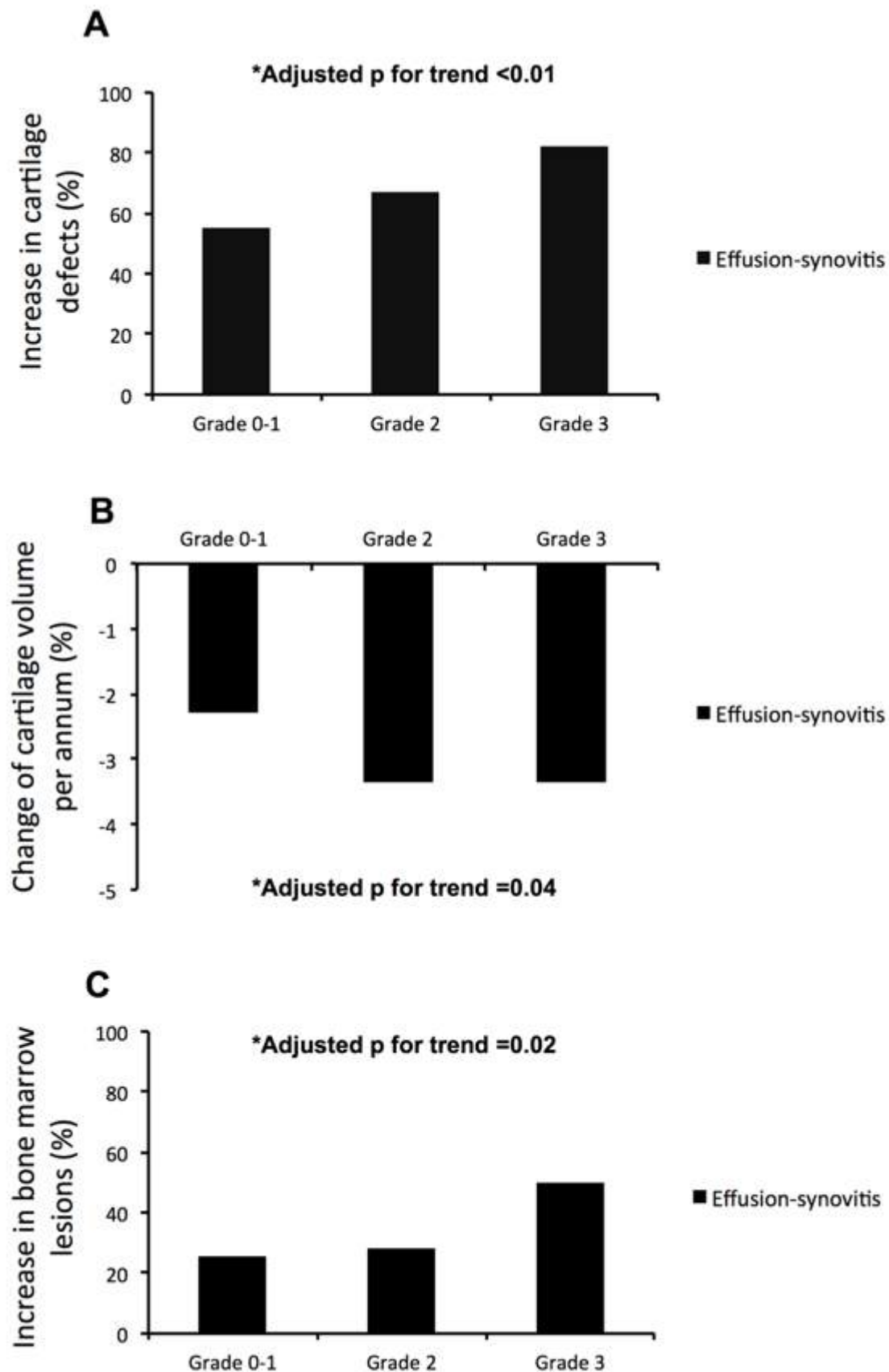


Figure 5.1. Longitudinal associations between joint effusion-synovitis in suprapatellar pouch and structural measures over 2.6 years.

(A) Increases in cartilage defects (%) (B) Changes in cartilage volume per annum (%) (C) Increases in bone marrow lesions (%). Number of participants in each grade: N= 51 (grade 0-1), N=74 (grade 2), N = 27 (grade 3) * p values were those after adjustment for baseline age, gender, BMI and radiographic osteoarthritis.

5.3.3 *Effusion-synovitis and cartilage volume*

Cross-sectionally, effusion-synovitis in whole joint, suprapatellar pouch and posterior femoral recess was negatively and significantly associated with total tibial cartilage volume after adjustment for age, gender, BMI, ROA, bone area and BMLs (Table 5.3). These associations became non-significant after further adjustment for cartilage defects (Table 5.3). Effusion-synovitis in central portion and subpopliteal recess was not significantly associated with total cartilage volume (Table 5.3).

Longitudinally, effusion-synovitis in suprapatellar pouch was significantly associated with change in cartilage volume after adjustment for age, gender, BMI and ROA (Table 5.3). Grade 2 and 3 suprapatellar pouch effusion-synovitis was associated with greater loss of cartilage volume than grade 1 effusion-synovitis (Figure 5.1B). The magnitude of association decreased by 11% and became of borderline significance ($p=0.07$) after further adjustment for baseline cartilage volume, tibial bone area and BMLs (Table 5.3) (decreased by 7% after sole adjustment for BMLs). The magnitude of association decreased by a further 17% after adjustment for cartilage defects (Table 5.3). Effusion-synovitis in other subregions was not significantly associated with change in knee cartilage volume (Table 5.3).

Table 5.3. Associations between knee effusion-synovitis and cartilage volume.

	Multivariable*	Multivariable**	Multivariable#
<i>Baseline cartilage volume</i>	β (95%CI)	β (95%CI)	β (95%CI)
Whole knee joint	-0.11 (-0.24, 0.01)	-0.16 (-0.28, -0.04)	-0.07 (-0.18, 0.04)
Suprapatellar pouch	-0.15 (-0.28, -0.02)	-0.20 (-0.32, -0.08)	-0.08 (-0.19, 0.04)
Central portion	-0.05 (-0.18, 0.09)	-0.05 (-0.18, 0.08)	-0.02 (-0.13, 0.10)
Posterior femoral recess	-0.32 (-0.62, -0.03)	-0.37 (-0.64, -0.10)	-0.21 (-0.46, 0.05)
Subpopliteal recess	-0.11 (-0.30, 0.07)	-0.10 (-0.27, 0.07)	0.01 (-0.14, 0.17)
<i>Changes in cartilage volume (% p.a.)</i>			
Whole knee joint	-0.16 (-0.67, 0.34)	-0.12 (-0.62, 0.38)	-0.03 (-0.53, 0.48)
Suprapatellar pouch	-0.54 (-1.06, -0.02)	-0.48 (-1.00, 0.05)	-0.40 (-0.92, 0.13)
Central portion	0.14 (-0.39, 0.67)	0.17 (-0.35, 0.69)	0.27 (-0.25, 0.80)
Posterior femoral recess	-0.11 (-1.25, 1.03)	-0.16 (-1.29, 0.96)	-0.10 (-1.22, 1.02)
Subpopliteal recess	-0.58 (-1.28, 0.12)	-0.44 (-1.14, 0.26)	-0.37 (-1.07, 0.32)

Dependent variable: baseline and change in cartilage volume (ml). Independent variable: knee effusion-synovitis (per grade).

*Adjusted for baseline age, gender, BMI and radiographic osteoarthritis. **Further adjusted for baseline tibial bone area, bone marrow lesions and cartilage volume (for longitudinal analyses only). #Further adjusted for baseline cartilage defects. p.a.: per annum.

5.3.4 Effusion-synovitis and BMLs

As shown in Table 5.4, in multivariable analyses, effusion-synovitis in most of the subregions (except posterior femoral recess) was significantly associated with BMLs at baseline, but became non-significant after further adjustment for cartilage defects.

After 2.6 years follow-up, only suprapatellar pouch effusion-synovitis was significantly associated with increases in BMLs after adjustment for age, gender, BMI and ROA and baseline BMLs (Table 5.4). Grade-3 effusion-synovitis was significantly associated with greater increase in BMLs than grade 1 and 2 effusion-synovitis (Figure 5.1C). The significant association disappeared after further adjustment for cartilage defects (Table 5.4).

Table 5.4. Associations between knee effusion-synovitis and cartilage volume.

	Multivariable*	Multivariable**	Multivariable#
<i>Baseline bone marrow lesions</i>	PR (95%CI)		PR (95%CI)
Whole knee joint	1.28 (1.13, 1.44)	-	1.11 (0.99, 1.25)
Suprapatellar pouch	1.26 (1.13, 1.41)	-	1.07 (0.96, 1.19)
Central portion	1.19 (1.06, 1.34)	-	1.10 (0.98, 1.24)
Posterior femoral recess	1.04 (0.79, 1.37)	-	0.92 (0.71, 1.20)
Subpopliteal recess	1.24 (1.07, 1.43)	-	1.09 (0.96, 1.24)
<i>Any increase in bone marrow lesions</i>	RR (95%CI)	RR (95%CI)	RR (95%CI)
Whole knee joint	1.15 (0.94, 1.42)	1.14 (0.92, 1.40)	1.04 (0.85, 1.27)
Suprapatellar pouch	1.27 (1.04, 1.55)	1.23 (1.01, 1.51)	1.10 (0.90, 1.36)
Central portion	1.13 (0.92, 1.39)	1.13 (0.91, 1.39)	1.06 (0.87, 1.30)
Posterior femoral recess	1.10 (0.69, 1.74)	1.08 (0.69, 1.71)	1.01 (0.65, 1.58)
Subpopliteal recess	1.15 (0.88, 1.50)	1.12 (0.86, 1.47)	1.09 (0.84, 1.42)

Dependent variable: baseline and increases in bone marrow lesions (yes or no). Independent variable: knee effusion-synovitis (per grade).

*Adjusted for age, gender, BMI, radiographic osteoarthritis and **baseline bone marrow lesions (for longitudinal analyses only). # Further adjusted for baseline cartilage defects.

5.3.5 Radiographic changes

Radiographic changes including JSN and osteophytes were assessed cross-sectionally. Effusion-synovitis in whole joint (PR: 1.36, 95% CI: 1.12, 1.66), suprapatellar pouch (PR: 1.44, 95% CI: 1.20, 1.71), posterior femoral recess (PR: 1.51, 95% CI: 1.04, 2.18) and subpopliteal recess (PR: 1.37, 95% CI: 1.11, 1.69) was significantly associated with greater moderate-severe JSN in multivariable analyses. Effusion-synovitis in whole joint (PR: 1.58, 95% CI: 1.07, 2.35), suprapatellar pouch (PR: 1.97, 95% CI: 1.38, 2.81), and subpopliteal recess (PR: 1.53, 95% CI: 1.00, 2.34) was also significantly associated with osteophytes in multivariable analyses. All these significant associations disappeared after adjustment for cartilage defects, but remained largely unchanged after adjustment for BMLs.

5.3.6 Other analyses

Neither in gender nor ROA subgroup analyses, no significant differences were found in terms of the associations between effusion-synovitis and changes in cartilage volume, increases in cartilage defects and BMLs. Furthermore, the associations remained largely unchanged if the dichotomised effusion-synovitis scores were used as the independent variable.

5.4 Discussion

To the best of our knowledge, this is the first study to report the associations between subregional effusion-synovitis and multiple knee structural changes in older adults. Knee joint effusion-synovitis was associated cross-sectionally with increased knee cartilage defects, BMLs, and reduced cartilage volume; and longitudinally, with increases in knee cartilage defects and BMLs, and a loss of cartilage volume. The longitudinal associations were observed mainly for the suprapatellar pouch, and, to a lesser extent, the subpopliteal recess, but not in posterior femoral recess and central portion. The significant associations with cartilage defects were independent of BMLs, but the associations with cartilage volume, BMLs and radiographic changes were largely mediated by cartilage defects. A dose-response association was observed between suprapatellar pouch effusion-synovitis and cartilage defects. Our results suggest that knee joint effusion-synovitis, particularly in suprapatellar pouch, is causally related to knee cartilage defects and this then leads to BMLs and cartilage loss over time in older adults.

Previous assessment for effusion-synovitis was usually limited to the suprapatellar pouch, but an anatomical atlas describes some relatively isolated synovial regions that lie within the knee joint cavity (53, 193). In patients with traumatic injuries, 99% effusions were found in the central portion, 76% knees in the suprapatellar pouch with only 9% in the posterior femoral recess and 2% in subpopliteal recess (60). Using contrast-enhanced MRI, Roemer et al. reported that the most common sites for definite synovitis (≥ 2) were posterior to the posterior cruciate ligament (PCL, 71.2%) and the suprapatellar region (59.5%) (111). These were consistent with our findings that the prevalence of knee joint effusion-synovitis (≥ 2) was higher in the suprapatellar pouch and central portion, and lower in the posterior femoral recess and subpopliteal recess. These regional separations allowed us to examine the anatomical distribution patterns of synovial inflammation within the joint cavity, in order to investigate whether synovial inflammation in different anatomical subregions has different effects on intra-articular pathology.

So far, there are a few studies that have evaluated the roles of knee effusion and/or synovitis in knee osteoarthritic changes, and the findings were inconsistent. Hill et al. utilized fat-suppressed spin-echo proton density and T2-weighted MRI to assess synovitis and reported that change in synovitis was associated with change in knee pain, but not with focal cartilage loss in patients with symptomatic OA (76). Loeuille et al. reported that synovial thickening grade detected by contrast-enhanced T1-weighted MRI was associated with

cartilage lesion (assessed by arthroscopy) only in the medial tibiofemoral compartment, not in the lateral tibiofemoral and patellofemoral compartments (109). In a 30-month cohort study, Roemer et al. assessed synovitis or effusion using fat-suppressed fast spin-echo intermediate-weighted MRI at baseline and found that synovitis or effusion might increase the risk of fast focal cartilage loss in 347 individuals who had or were at higher risk for knee OA, but the result was only of statistically borderline significance ($p=0.07$) (209). This group further examined the associations between effusion-synovitis and focal cartilage loss in 514 subjects with neither cartilage damage nor tibiofemoral ROA. Over 30 months follow-up, they found that effusion-synovitis was associated with an increased risk for focal cartilage loss in tibiofemoral compartment but not in the patellofemoral compartment (92). Most recently, a cross-sectional association of whole knee (across 11 sites) synovitis assessed by contrast-enhanced MRI with widespread cartilage damage was found in the same study (210). These studies did not assess diffuse cartilage volume loss using quantitative MRI measurement, mostly assessed synovitis/effusion around suprapatellar area, and did not examine other structural changes such as BMLs.

Using fat suppressed T2-weighted fast spin echo MRI, we found that joint effusion-synovitis in suprapatellar pouch was consistently associated with focal (as assessed by cartilage defects) and diffuse (as assessed by reduced cartilage volume) cartilage loss in older adults in both cross-sectional and longitudinal analyses, indicating that suprapatellar pouch effusion-synovitis could induce focal cartilage loss observed largely at an earlier stage as well as diffuse cartilage loss seen mainly at a later stage of OA. The associations with cartilage volume loss decreased in magnitude by 17% after further adjustment for cartilage defects and by 7% after adjustment for BMLs suggesting that effects of effusion on cartilage volume loss are largely through cartilage defects.

Additionally, our study explored the effects of effusion-synovitis in other subregions. We found that effusion-synovitis in subpopliteal recess was cross-sectionally and longitudinally associated with increased cartilage defects, but its association with cartilage volume loss was not significant. These indicated that subpopliteal recess effusion-synovitis might only cause focal cartilage loss. Furthermore, although effusion-synovitis in the central portion and posterior femoral recess was cross-sectionally associated with increased cartilage defects, no longitudinal associations with cartilage defects and cartilage volume were found, suggesting that effusion-synovitis in these 2 subregions might not play a significant role in inducing cartilage loss.

The pivotal role that subchondral BMLs play in knee pain and disease progression in knee OA has been reported (183, 211), but the causes of the marrow signal changes are still uncertain. Hypothetically, synovial fluid can be forced under pressure into subchondral bone marrow through defects in cartilage or bone, increasing marrow fluid and eventual trabecular bone excavation, and thus cause BMLs, which is similar to the mechanism of cyst formation (184). Yet very little clinical evidence has emerged about the relationship between effusion-synovitis and subchondral BMLs. There is only one cross-sectional study reporting that quantitative synovial membrane volume measured in T1-weighted contrast-enhanced MRI was significantly associated with the volume of subchondral BMLs (120), but no cohort studies have reported this association so far. Our study confirmed the significant cross-sectional associations between effusion-synovitis in most joint regions and BMLs, and was the first to report that longitudinal association between suprapatellar pouch effusion-synovitis and increases in BMLs over time. The significant cross-sectional and longitudinal associations disappeared after further adjustment for cartilage defects, indicating that joint effusion may lead to BMLs via cartilage defects. This would be consistent with our previous study where defects predicted BML worsening (212).

This study reported the most consistent associations between suprapatellar pouch effusion-synovitis and cartilage defects, cartilage volume loss and BML, suggesting this may be the most important site or the most easily detected (197). The volume necessary was large (but common in our unselected sample) as the associations were confined to grade 2 or above effusions. Effusion-synovitis in subpopliteal recess may also cause focal cartilage loss. Although effusion-synovitis in major subregions was associated with JSN and osteophytes, causal relationships are unclear due to the cross-sectional nature of these findings.

The strengths of the present study lie in the comprehensive MRI and radiographic measurements and the effort taken to untangle site specific and structural associations. There are several potential limitations in our study. First, we had knee MRI scans in 977 subjects at baseline, and only 404 subjects at follow-up owing to decommissioning of the MRI scanner; however, there were no differences in demographic factors between those who remained in the study and who dropped out. Second, we did not have radiographic assessments at follow-up, because x-ray is insensitive to detect the changes over this short period, so we were not able to examine the associations between effusion-synovitis and changes in radiographic changes in this study. Lastly, measurement error may influence results. However, all measures were highly reproducible suggesting this is unlikely.

There are dose-dependent and independent associations between knee joint effusion-synovitis and knee cartilage defects in both cross-sectional and longitudinal analyses, suggesting a potential causal relationship. The associations of effusion-synovitis with BMLs and cartilage volume were largely dependent on cartilage defects suggesting potential causal pathways.

Chapter 6 – Quantitative knee effusion-synovitis and knee structural abnormalities in older adults: a cohort study

6.1 Introduction

OA contributes greatly to overall disability in our aging society. Accumulating evidence shows that OA is not a degenerative disease but involves dynamic biological and inflammatory processes (213). Synovial inflammation, which is directly responsible for clinical symptoms, is a feature of early and advanced knee OA (51, 214). Low-grade synovial inflammation may contribute to the progression of chondropathy and the deterioration of other joint structures by producing a number of pro-inflammatory mediators (206, 207). Additionally, the histological changes in synovial tissue are heterogeneous at different locations of the affected joint (109). It is unclear whether synovial morphological changes are the origin or consequence of joint structural abnormalities in OA (215). Biologically, it is suggested that synovial inflammation was induced by debris from cartilage breakdown (216). However, increasing evidence suggests that synovial abnormalities can precede joint pathological changes, especially cartilage loss (92, 209, 217). We recently reported that effusion-synovitis (a MRI feature reflecting synovial inflammation) was independently associated with knee cartilage defects cross-sectionally and longitudinally, suggesting a possible causal relationship (94). The natural history of MRI-detected effusion-synovitis in older people has not been reported so far. It is unknown if knee structural abnormalities including cartilage defects, reduced cartilage volume, bone marrow lesions (BMLs) and meniscal lesions can predict effusion-synovitis.

Joint effusion can be visually distinguished from synovitis using contrast-enhanced (CE) MRI after intravenous gadolinium injection, but this assessment is not commonly used for research because of potential side effects and high cost. Recently, the term “effusion-synovitis” has been proposed for effusion and synovitis because these two features cannot be differentiated by non-CE MRI (115). We assessed regional effusion-synovitis using a semi-quantitative scoring system in four compartments of the knee: suprapatellar pouch, central portion, posterior inter-condylar recess and subpopliteal recess, and found that regional effusion-synovitis scores were well correlated with osteoarthritic features (79, 94). However, a quantitative measurement of effusion-synovitis has not been established.

The aims of this study were, therefore, to describe the natural history of effusion-synovitis using a quantitative measure in a population-based sample, and to investigate the longitudinal associations between effusion-synovitis and knee structural factors including cartilage defects, cartilage volume, subchondral BMLs and meniscal pathology over 2.7 years in older adults.

6.2 Materials and Methods

6.2.1 *Characteristics of participants*

This study used subjects from the TASOAC study as described in section 3.2.

6.2.2 *Knee MRI sequences*

T1 and T2 weighted fat saturated MRIs sequences as described in section 3.2.3.

6.2.3 *Knee MRI measurements*

6.2.3.1 *Effusion-synovitis*

Baseline and follow-up semi-quantitative effusion-synovitis was scored in four subregions as described in section 3.2.3.1 and displayed in Figure 3.2. Quantitative effusion-synovitis area was measured in the whole joint at baseline and follow-up as described in section 3.2.3.1 and displayed in Figure 3.3.

6.2.3.2 *Cartilage volume*

Baseline and follow-up total cartilage volume was measured on MRI as described in section 3.2.3.4 .

6.2.3.3 *Cartilage defects*

Baseline and follow-up cartilage defects were assessed from 0-4 on MRI as described in section 3.2.3.3 .

6.2.3.4 *BMLs*

Baseline and follow-up BMLs were assessed on MRI as described in section 3.2.3.2 .

6.2.3.5 *Meniscal pathology*

Baseline and follow-up meniscal pathology was assessed on MRI as described in section 3.2.3.5 .

6.2.3.6 Tibial bone area

Baseline and follow-up tibial bone area was measured on MRI as described in section 3.2.3.6 .

6.2.4 Knee pain

Knee pain was assessed using the WOMAC scoring system as described in section 3.2.2.

6.2.5 Knee X-ray

Knee radiographs were taken and scored for ROA as described in section 3.2.4.

6.2.6 Anthropometrics

Height, weight and BMI was measured as previous described in section 3.2.1.

6.2.7 Statistical analysis

Comparisons of baseline characteristics among groups with increased, stable and decreased effusion-synovitis were carried out using one-way ANOVA. The statistically significant differences between subjects who completed the study and who were lost to follow-up were evaluated using t-test or chi-square tests (as appropriate). Univariable and multivariable linear regressions were used to assess the associations between effusion-synovitis and structural abnormalities at baseline, between baseline effusion-synovitis and changes in structural abnormalities, or between baseline structural abnormalities and change in effusion-synovitis, before and after adjustment for age, gender, BMI, tibial bone area and/or ROA.

Standard diagnostic checks of model fit and residuals were routinely performed, and data points with large residuals and/or high influence were investigated for data errors. Interactions between ROA and exposure factors on changes in outcomes were investigated by testing the statistical significance of the coefficient of a product term (an exposure \times ROA) after adjustment for confounders.

6.3 Results

6.3.1 *Characteristics of participants*

A total of 1,100 subjects (51% female) aged between 50 and 80 years (mean 63 years) participated in the TASOAC study. More than half participants did not have MRI at follow-up because local MRI machine was decommissioned and became unavailable for research purposes. There were 63 participants (age: 64 ± 7 ; female sex: 56%; BMI: $27.8 \pm 5.6 \text{ kg/m}^2$) who were lost to follow-up before the MRI machine was decommissioned. There were no significant differences in baseline characteristics between these subjects and those who completed follow-up MRIs (all $p > 0.05$). The current study consisted of a sample of 406 subjects (age: 63 ± 7 years; female sex: 50%; BMI: $27.7 \pm 4.5 \text{ kg/m}^2$; and ROA: 57%) who had MRI measures at baseline and 2.7 years later. Prevalence of JSN and osteophytes in these subjects was 56% (grade 1: 41%, grade 2: 12% and grade 3: 3%) and 8% (grade 1: 5%, grade 2: 2% and grade 3: 1%), respectively. There were no significant differences in baseline demographics, cartilage defects, BMLs and cartilage volume between the subjects who were included in the current study and the rest of the cohort (data not shown).

Baseline characteristics of the study sample are shown in Table 6.1. There were no significant differences in female sex, BMI, knee pain, cartilage defects, BMLs and meniscal lesions and ROA among those with increased, stable and decreased effusion-synovitis. Those with stable effusion-synovitis were younger, and those with decreased effusion-synovitis had higher baseline maximal area of effusion-synovitis.

Table 6.1. Characteristics of participants at baseline.

Characteristics	Knee effusion-synovitis			P value
	Decrease (n=88)	Stable (n=201)	Increase (n=117)	
Age (year)	63.6 (7.1)	61.7 (6.7)	63.5 (7.9)	0.03
Female (%)	45	54	46	0.28
BMI (kg/m ²)	27.5 (4.6)	27.8 (4.0)	27.4 (5.0)	0.65
WOMAC knee pain score (0-45)	3.3 (5.4)	3.4 (6.7)	3.7 (6.8)	0.87
WOMAC knee pain present (%)	55%	46%	50%	0.51
Cartilage volume (ml)	8.5 (1.8)	8.4 (1.9)	8.5 (2.0)	0.78
Cartilage defect (%)	56	45	47	0.22
Bone marrow lesion (%)	38	36	33	0.78
Meniscal lesion (%)	93	84	86	0.12
Effusion-synovitis (cm ²)	2.9 (1.7)	1.2 (0.9)	1.4 (1.0)	<0.01
Knee ROA (%)	56	54	63	0.27

Values expressed as mean (standard deviation) unless percentages.

X² test was used for the comparisons.

BMI, body mass index; WOMAC, western Ontario and McMaster universities; ROA, radiographic osteoarthritis.

Bold p value indicates statistically significant difference at $\alpha=0.05$.

6.3.2 *Natural history of effusion-synovitis*

At baseline, the mean (\pm SD) size of effusion-synovitis was 1.64 cm² (\pm 1.34 cm²), ranging from 0.04 cm² to 8.91 cm². Male subjects had larger baseline and follow-up effusion-synovitis size compared to females (Table 6.2). Over 2.7 years follow-up, the mean size of effusion-synovitis changed to 1.75 cm² (\pm 1.29 cm²), with 29% (N=88) increasing in size, 50% (N=201) remaining stable and 22% (N=117) decreasing in size in total sample (Table 6.2). In males, 31% increased in size, 46% remained stable and 23% decreased in size of effusion-synovitis. In comparison, 27% increased in size, 53% remained stable and 20% decreased in size of effusion-synovitis in females (Table 6.2).

Table 6.2. Natural history of knee effusion-synovitis in total sample and gender subgroups.

Effusion-synovitis area	Total sample	Male	Female	
	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)	P value
Baseline (cm ²)	1.64 (1.51, 1.76)	1.78 (1.59, 1.96)	1.49 (1.31, 1.67)	<0.01
Follow-up (cm ²)	1.75 (1.62, 1.87)	1.92 (1.73, 2.11)	1.57 (1.41, 1.74)	<0.01
	Percentage (95% CI)	Percentage (95% CI)	Percentage (95% CI)	
Decrease (%)	21.67 (17.92, 25.97)	23.53 (18.18, 29.87)	19.80 (14.85, 25.91)	0.67
Stable (%)	49.51 (44.64, 54.38)	45.59 (38.84, 52.50)	53.47 (46.53, 60.27)	0.26
Increase (%)	28.82 (24.60, 33.44)	30.88 (24.89, 37.59)	26.73 (21.06, 33.29)	0.62

T-test and X² test was used for the comparisons.

Bold p value indicates statistically significant difference between males and females at $\alpha=0.05$.

6.3.3 *Associations between effusion-synovitis and knee structures*

Cross-sectionally, effusion-synovitis maximal area was significantly and positively associated with cartilage defects and BMLs, and negatively with cartilage volume, after adjustment for age, gender, BMI, tibial bone area and/or ROA (Table 6.3). No significant associations were found between effusion-synovitis and meniscal lesions at baseline.

Baseline effusion-synovitis maximal area was significantly and positively associated with changes in knee cartilage defects and BMLs over 2.7 years, and negatively with change in knee cartilage volume in multivariable analyses (Table 6.4). No significant association was found between baseline effusion-synovitis area and change in meniscal lesions.

In contrast, baseline structural measures were not significantly associated with change in effusion-synovitis maximal area (Table 6.5) or an increase in effusion-synovitis area defined using LSC (Table 6.6).

There were no significant interactions between effusion-synovitis maximal area and ROA on changes in other joint structures, or between other joint structures and/or ROA on change in effusion-synovitis maximal area (data not shown), so subjects with and without ROA were combined for analyses.

Table 6.3. Associations between baseline knee structural factors and baseline effusion-synovitis.

	Baseline effusion-synovitis area	
	Univariable β (95% CI)	Multivariable* β (95% CI)
<i>Baseline structural factors</i>		
Cartilage defect	0.21 (0.16, 0.26)	0.15 (0.10, 0.21)
Cartilage volume	0.02 (-0.05, 0.08)	-0.18 (-0.28, -0.07)
Bone marrow lesion	0.14 (0.04, 0.25)	0.10 (0.00, 0.19)
Meniscal lesion	0.11 (0.01, 0.22)	0.06 (-0.04, 0.16)

Independent variable: baseline structural factors (score or cartilage volume)

Dependent variable: baseline effusion-synovitis area (continuous variable)

*Adjusted for age, gender, body mass index, tibial bone area and radiographic osteoarthritis.

Bold p value indicates statistically significant difference at $\alpha=0.05$.

Table 6.4. Associations between baseline knee effusion-synovitis and change in structural factors.

	Baseline effusion-synovitis area	
	Univariable β (95% CI)	Multivariable* β (95% CI)
<i>Change in structural factors</i>		
Cartilage defect	0.18 (0.09, 0.27)	0.18 (0.07, 0.29)
Cartilage volume	-0.47 (-0.74, -0.21)	-0.40 (-0.71, -0.09)
Bone marrow lesion	0.23 (0.12, 0.35)	0.17 (0.05, 0.30)
Meniscal lesion	-0.05 (-0.14, 0.05)	-0.02 (-0.13, 0.09)

Independent variable: baseline effusion-synovitis area (continuous variable)

Dependent variable: change in structural factors (score or cartilage volume)

*Adjusted for age, gender, body mass index, tibial bone area and radiographic osteoarthritis.

Bold p value indicates statistically significant difference at $\alpha=0.05$.

Table 6.5. Associations between baseline knee effusion-synovitis and change in structural factors.

	Change in effusion-synovitis area	
	Univariable β (95% CI)	Multivariable* β (95% CI)
<i>Baseline structural factors</i>		
Cartilage defect	-0.04 (-0.09, 0.00)	-0.02 (-0.08, 0.03)
Cartilage volume	0.00 (-0.06, 0.06)	-0.01 (-0.11, 0.09)
Bone marrow lesion	-0.04 (-0.13, 0.05)	-0.04 (-0.13, 0.05)
Meniscal lesion	-0.04 (-0.13, 0.05)	-0.03 (-0.13, 0.06)

Independent variable: baseline structural factors (score or cartilage volume)

Dependent variable: change of effusion-synovitis area (continuous variable)

*Adjusted for age, gender, body mass index, tibial bone area and radiographic osteoarthritis.

Bold p value indicates statistically significant difference at $\alpha=0.05$.

Table 6.6. Associations between knee structural factors and effusion-synovitis.

	Effusion-synovitis	
	Model A	Model B
	Multivariable* OR (95% CI)	Multivariable* OR (95% CI)
<i>Structural factors</i>		
Cartilage defect	0.92 (0.58, 1.47)	1.32 (1.07, 1.64)
Bone marrow lesion	0.87 (0.54, 1.39)	1.27 (1.06, 1.53)
Meniscal lesion	0.85 (0.42, 1.71)	0.99 (0.78, 1.27)

Model A:

Independent variable: baseline scores of structural factors

Dependent variable: increase in effusion-synovitis (increase vs. no increase)

Model B:

Independent variable: baseline effusion-synovitis area

Dependent variable: change in structural factors (increase vs. no increase)

*Adjusted for age, gender, BMI, tibial bone area and ROA.

BMI, body mass index; ROA, radiographic osteoarthritis.

Bold p value indicates statistically significant difference at $\alpha=0.05$.

6.4 Discussion

This longitudinal study investigated the natural history and clinical significance of effusion-synovitis in a randomly selected older cohort. We assessed effusion-synovitis by measuring the maximal area, and found that the proportions of worsening (29%) or improving (22%) effusion-synovitis were similar, with 49% remaining stable. Baseline effusion-synovitis was associated with changes in cartilage measures and subchondral BMLs, but baseline cartilage measures and BMLs were not associated with change in effusion-synovitis. This suggests a potential causal pathway from effusion-synovitis to cartilage and bone abnormalities, but not vice versa, in older adults.

This study was the first study to report the natural history of effusion-synovitis in a community-based cohort. Previous studies mainly investigated the prevalence of synovial inflammation in asymptomatic or OA cohorts, which were not generalizable to the older population. Besides, the prevalence of synovial inflammation has ranged from 10% to 80% at different sites according to different definitions (92, 108, 111, 113, 218, 219). The conflicting data may be due to variations in assessment techniques, grading systems, populations and study designs. To our knowledge, the information for incidence or progression of synovial inflammation in OA was very limited. Hill et al. reported that about 15% to 20% of the knee synovitis score improved and 20% worsened at 30-month follow-up in symptomatic OA patients (76). In our study, the increase (incidence and progression) in effusion-synovitis was observed in nearly one third of the participants, and the decrease was seen in 22% of the participants.

Unlike the previous scoring system, we assessed effusion-synovitis by measuring its maximal area. 2-D sagittal T2-weighted images with an inter-slice gap of 0.5 to 1.0 mm did not allow measuring the real volume of joint effusion-synovitis. Joint effusion-synovitis can be dynamic, and this is why maximal effusion-synovitis areas at baseline and follow-up were observed on slightly different slices (Figure 1C and 1D); however, maximal effusion-synovitis area measurement is simple and reproducible, and has construct and predictive validities. Furthermore, the maximal area was highly correlated with the proxy volume and the semi-quantitative scores of effusion-synovitis. This suggests that this maximal effusion-synovitis area measurement has concurrent validity. The external validity was further tested in another study (Vitamin D Effect on Osteoarthritis Study), in which MRI images had no inter-slice gaps. It showed a very high correlation ($r=0.97$) between 2D- maximal area and 3D- volume of effusion-synovitis. The proxy volume of effusion-synovitis in our sample

(mean \pm SD: 4.7 \pm 2.8 ml) underestimated the real volume measured in other studies [17.7 \pm 7.0 ml (220) and 25.5 \pm 22.3 ml (190)]. Therefore, we did not use the proxy volume as the outcome in this study. Furthermore, we calculated the variations of effusion-synovitis from baseline to follow-up using the LSC (170). This may minimize the measurement errors and offer a more reasonable tool to describe the natural history of effusion-synovitis.

Our findings confirmed that synovial inflammation could be a precursor of OA, which was consistent with our recent findings that effusion-synovitis score assessed using semi-quantitative measurements was associated with increased incidence/progression of cartilage degradation and BMLs (94). Synovial inflammation has been shown to be predictive of OA outcomes such as radiographic changes and total knee replacement (91, 93), and was independently linked to knee symptoms (83, 85). Although we found that cartilage defects and BMLs were associated with effusion-synovitis cross-sectionally, we did not find significant associations between baseline cartilage, bone and meniscal measures and change in effusion-synovitis. Hypothetically, structural degradation products such as cartilage fragments could irritate synovial membrane and trigger the pro-inflammatory processes within the joint capsule (216), but this may happen in a subset of OA, e.g., those with post-traumatic or injury-related phenotypes, or in late-stage OA. Our current findings indicate that in a community-based older population, the inflammatory phenotype of OA was prevalent, and inflammation may initiate early osteoarthritic changes. Furthermore, considering its potential ability to regress or resolve, effusion-synovitis can be a promising therapeutic target for slowing the disease progression of knee OA.

The strength of this study is that we studied a community-based population with a large sample size using a quantitative measurement to assess change in effusion-synovitis size over time. It also has several potential limitations. First, loss of follow-up MR images was caused by unavailability of local MRI machine for research purposes. Assuredly there were no significant differences in demographic characteristics between included participants and those who lost to follow-up. Second, we used non-CE MRI, which was unable to differentiate synovial fluid and synovial thickening, so measurement of effusion-synovitis may not actually reflect synovitis status. Third, we did not assess synovitis at other sites (e.g. Hoffa's fat pad) that may be associated with osteoarthritic changes; however, effusion-synovitis is a potentially better marker of synovial activation than Hoffa's synovitis (92). Lastly, we did not evaluate the re-scan reproducibility for the MR images, which would potentially influence the quantified area measurement that goes beyond the field of view.

In conclusion, knee joint effusion-synovitis was not static in older adults. It was predictive of, but not predicted by, other structural abnormalities suggesting a potential role in knee early osteoarthritic changes.

Chapter 7 – Associations between knee effusion-synovitis and joint structural changes in patients with knee osteoarthritis: a cohort study

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Chapter 8 – Effects of vitamin D supplementation on effusion-synovitis in patients with knee osteoarthritis and low vitamin D levels: a randomised controlled trial

8.1 Introduction

Osteoarthritis (OA) was generally thought of as a ‘non-inflammatory’ type of arthritis; however, localised low-grade inflammation is now known to be an important factor in OA pathogenesis (103, 208, 222). The development of chronic inflammation in OA following joint injury or metabolic dysfunction may contribute to the formation of a cycle of local tissue lesions, inflammation and repair (224). Notably, synovial activation (effusion and/or synovitis) has been considered as a precursor of OA outcomes such as radiographic changes and total knee replacement (91, 93). It is independently associated with clinical symptoms, such as knee pain and physical function (83, 85). Studies have demonstrated the link between synovial inflammation and structural changes of knee OA (51, 92, 97, 110), suggesting that reducing synovial inflammation may be a potential avenue for slowing disease progression in knee OA. This is extremely important, as there are no proven treatment options to modify disease progression in OA so far.

Importantly, comparing to articular and bony alterations, synovial inflammation has a greater potential to regress or resolve (225) which creates a treatment opportunity. Previous pharmaceutical managements such as non-steroidal anti-inflammatory drugs (NSAIDs) and intra-articular steroid injection have been recommended for OA patients particularly those with joint effusion (234); however, use of these can result in side-effects and drug intolerance, especially during long-term use (235, 236). It is therefore important to identify safer and more cost-effective interventions targeting synovial inflammation in OA (237).

In observational studies vitamin D deficiency has been associated with cartilage loss and pain (150, 153). In animal models, vitamin D supplementation has a protective effect in OA by reducing the expression of pro-inflammatory cytokines (238). Furthermore, an exercise-interventional study has found that vitamin D sufficiency increases anti-inflammatory cytokines response to muscular injury (239). So far, RCT evidence on the efficacy of vitamin D supplementation for knee OA is limited and inconsistent. While one study suggested it had beneficial effects on symptoms (154), another showed no effects on symptoms and cartilage loss (155). In our recent Vitamin D Effect on Osteoarthritis (VIDEO) study in patients with knee OA and low serum vitamin D levels vitamin D supplementation over 24 months had no significant effect on knee pain or cartilage morphology but might have modest effects on knee function loss and bone marrow lesions (240). However, none of these studies has investigated the effects of vitamin D on synovial inflammation. We hypothesised that vitamin D may reduce synovial inflammation in patients with knee OA.

The aim of this post-hoc analysis was, therefore, to examine the effect of vitamin D supplementation over 24 months on effusion-synovitis (a MRI marker of synovial inflammation) in the VIDEO study.

8.2 Materials and Methods

8.2.1 Trial design

This study is a post-hoc analysis of the original VIDEO study which has been described in section 3.3.

8.2.2 Participants

This chapter used participants from the VIDEO study as described in section 3.3.

8.2.3 Interventions

Participants in the intervention group were given a monthly capsule of 50,000 IU (1.25 mg) vitamin D₃ (cholecalciferol) for 24 months (178) as described in section 3.3.1.

8.2.4 Outcomes

The co-primary efficacy endpoint measures of the originally specified trial were MRI assessment of knee cartilage volume changes from baseline to month 24, as well as the WOMAC) score (165). Volume and score of knee effusion-synovitis were used as outcomes in this post-hoc analysis.

8.2.4.1 Knee MRI sequences

The MRI sequences used in this study have been described in section 3.3.7.

8.2.4.2 Knee MRI measurements of effusion-synovitis

Quantitative measurement

Quantitative effusion-synovitis volume was measured at baseline and follow-up as described in section 3.2.3.1 and displayed in Figure 3.4.

Semi-quantitative measurement

Effusion-synovitis score was assessed at baseline and follow-up as described in section 3.2.3.1 and displayed in Figure 3.2.

8.2.5 Randomisation

Participants were allocated to either vitamin D or placebo arm at a ratio of 1:1 based on computer-generated random numbers as described in section 3.3.2.

8.2.6 Blinding

As described in section 3.3.3, participants, research coordinators and investigators were all blinded to treatment assignment.

8.2.7 Trial monitoring and safety assessment

The project manager visited and monitored each site to examine trial procedures, which was previously described in section 3.3.4.

8.2.8 Sample size calculation

Sample size calculation has been described in section 3.3.11.

8.2.9 Statistical analysis

Baseline characteristics were compared between two groups with the use of Student's t-tests or Chi-square tests. Independent t-tests were used to compare changes in effusion-synovitis volume from baseline to follow-up between groups. In secondary analyses, the minimal clinically important difference (MCID) was estimated for effusion-synovitis volume (both the absolute and the relative annual change). A reduction of mean WOMAC function score ≥ 7 was used as an anchor to determine the cut-off of effusion-synovitis volume in patients who actually experienced clinically significant improvement (167). A least significant change (LSC) criterion was used to define an increase, stable or a decrease in effusion-synovitis volume. This takes into account measurement error and the correlation between the baseline and follow-up measurements (170). The formula was as follows:

$$LSC = 1.96 \times \sigma \sqrt{2(1 - \rho)}$$

(σ = the standard error of the mean; ρ = the serial correlation). For example, LSC of total effusion-synovitis volume was calculated to be 1.81 ml (where $\sigma = 1.17$ and $\rho = 0.69$) in this study. Therefore, participants were categorised as having an increase in effusion-synovitis

volume if change in effusion-synovitis volume was $\geq +1.81$ ml, having a decrease if change in effusion-synovitis was ≤ -1.81 ml, and having a stable effusion-synovitis if change in effusion-synovitis volume was between -1.81 and $+1.81$ ml. Logistic regression was applied to examine the effects of intervention when dichotomised outcomes of effusion-synovitis (improvement versus no improvement; increase versus no increase, or decrease versus no decrease) were used as described in section 3.2.3.1 .

For intention-to-treat analysis, multiple imputations were used to address missing data due to loss to follow-up and non-response. Imputations were performed separately for each treatment group and each outcome, using baseline values, age, gender, BMI and serum 25-(OH)D level. All the data analysis was performed on Stata V13.0 (Stata Corp., College Station, Texas, USA). A two-sided p value of 0.05 was considered statistically significant.

8.3 Results

8.3.1 *Characteristics of participants*

The flowchart of participants through the study is described in Figure 3.5. Of 599 participants were screened for eligibility, 413 subjects were randomly assigned to either vitamin D or placebo group. Over 24 months, 28 (13.4%) in the vitamin D group and 45 (22.1%) in the placebo group withdrew from the study. Three hundred and forty patients (82.3%) completed the follow-up. The number of patients who discontinued treatment allocation was larger in the placebo group than the vitamin D group (21 versus 8). The major reason for discontinuation was non-adherence to the protocol when low 25-(OH)D levels were disclosed to the participants by their general practitioners. Intention-to-treat analyses were performed in all 413 randomized participants. The mean age of participants was 63.2 ± 7.0 years, with 208 (50%) females and a mean BMI of 29.6 ± 5.0 kg/m². Using a semi-quantitative grading assessment, baseline prevalence of effusion-synovitis (score ≥ 2) was 47.7%, which were similar in two groups (48.5% in vitamin D versus 46.8% in placebo). Participants' characteristics at baseline were comparable between two groups (Table 8.1). Other characteristics of the participant at baseline were comparable between two groups (Table 8.1) (240).

Table 8.1. Participant characteristics at baseline.

<i>Total Sample</i>	Vitamin D (N = 209)	Placebo (N = 204)	P value
Age (y)	63.55 (6.88)	62.85 (7.22)	0.32
Women (%)	51	50	0.92
Body mass index (kg/m ²)	29.57 (5.39)	29.64 (4.62)	0.88
Radiographic osteoarthritis (%)	96	96	0.84
Plasma 25-hydroxyvitamin D (nmol/l)	43.74 (11.80)	43.81 (12.66)	0.95
Effusion-synovitis volume (ml)	7.93 (7.81)	7.98 (9.20)	0.95
Effusion-synovitis prevalence (%)	49	47	0.60

Results are shown as mean (SD) or percentage unless stated otherwise.

Student t-test or χ^2 -test was used for the comparison.

8.3.2 Outcome

8.3.2.1 Intention-to-treat analysis

The serum 25-(OH) D levels increased by an average 40.6 ± 19.5 nmol/l in the vitamin D group but only 6.7 ± 17.9 nmol/l in the placebo group throughout the study period. This has been described in detail elsewhere (240). In the total study sample, total effusion-synovitis volume increased from baseline (8.0 ± 8.5 ml) to follow-up (9.0 ± 10.5 ml). The mean effusion-synovitis volume increased from 8.0 ± 9.2 ml to 10.0 ± 12.3 ml in the placebo group ($p=0.08$), but remained stable in the vitamin D group (8.0 ± 7.8 ml to 8.0 ± 8.4 ml).

There were statistically significant differences in absolute and relative effusion-synovitis volume change between groups (-1.94 ml over 24 months or -45% p.a.) (Table 8.2). The statistically significant differences were only evident in patients who had baseline effusion-synovitis (score ≥ 2), not in those without baseline effusion-synovitis (Table 8.2).

Table 8.2. Two-year changes in total knee effusion-synovitis between vitamin D and placebo groups.

Effusion-synovitis measures	Vitamin D	Placebo	Between Group Difference*	
	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)	P value
Whole sample	N = 209	N = 204		
Volume, absolute change (ml)	0.26 (-0.82, 1.34)	2.20 (1.01, 3.38)	-1.94 (-3.54, -0.33)	0.02
Volume, relative change (p.a.) [#]	16% (-8%, 39%)	60% (31%, 89%)	-45% (-82%, -7%)	0.02
Grade, absolute change (0-3)	0.04 (-0.06, 0.14)	0.14 (0.03, 0.25)	-0.10 (-0.25, 0.05)	0.19
Those with baseline effusion-synovitis	N = 106	N = 108		
Volume, absolute change (ml)	0.13 (-1.09, 1.35)	2.17 (0.88, 3.46)	-2.04 (-3.83, -0.25)	0.03
Volume, relative change (p.a.) [#]	9% (-1%, 18%)	28% (17%, 38%)	-19% (-33%, -5%)	0.01
Grade, absolute change (0-3)	-0.05 (-0.15, 0.06)	0.07 (-0.04, 0.18)	-0.12 (-0.27, 0.04)	0.14
Those without baseline effusion-synovitis	N = 103	N = 96		
Volume, absolute change (ml)	0.80 (-0.22, 1.82)	1.97 (0.64, 3.30)	-1.17 (-0.50, 2.85)	0.17
Volume, relative change (p.a.) [#]	60% (-144%, 264%)	351% (69%, 632%)	-290% (-57%, 638%)	0.10
Grade, absolute change (0-3)	0.57 (0.30, 0.83)	0.74 (0.40, 1.09)	-0.18 (-0.26, 0.62)	0.43

All analyses compared baseline vs. 2-year outcomes.

*The results in this table were generated on an imputed dataset.

[#]Relative change = (absolute change/baseline value)/time interval.

Bold p value indicates statistically significant difference at $\alpha=0.05$.

CI, confidence interval; p.a., per annum.

8.3.2.2 *Regional effusion-synovitis analysis*

In subregional analyses, the absolute changes in volume and score of effusion-synovitis in suprapatellar pouch were less in the vitamin D than the placebo group, and the between group differences were statistically significant (Table 8.3). In contrast, there were no significant differences between two groups in changes in effusion-synovitis volume and score in other joint cavity (Table 8.3).

Clinical improvements in effusion-synovitis volume (absolute and percentage changes) were further defined using MCID. The proportions with improvements in percentage changes of total and suprapatellar effusion-synovitis were significantly higher in the vitamin D compared to placebo group (Table 8.3 and Table 8.4). In contrast, there were no statistically significant differences between groups in the improvements in effusion-synovitis in other regions of the joint (Table 8.3 and Table 8.4). When effusion-synovitis volume was categorised as an increase or a decrease by LSC, the proportions with an increase of effusion-synovitis volume in total, suprapatellar or other regions were higher in the placebo group, while the proportion with a decrease of suprapatellar effusion-synovitis volume was higher in the vitamin D group (Table 8.4).

Per protocol analysis comparing those reached a 25-(OH)D level over 60 nmol/l at month 3 to those who did not (253 versus 146) showed similar results of change in effusion-synovitis volume (data not shown).

Table 8.3. Two-year changes in regional knee effusion-synovitis between vitamin D and placebo groups.

Effusion-synovitis measures	Vitamin D (N = 129)	Placebo (N = 132)	Between Group Difference*	
	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)	P value
<i>Suprapatellar pouch</i>				
Volume, absolute change (ml)	0.04 (-1.46, 1.53)	2.53 (0.84, 4.22)	-2.49 (-4.74, -0.25)	0.03
Volume, relative change (p.a.) [#]	19% (-111%, 149%)	148% (-6%, 302%)	-129% (-330%, 72%)	0.21
Grade, absolute change (0-3)	-0.02 (-0.14, 0.11)	0.18 (0.05, 0.31)	-0.20 (-0.38, -0.02)	0.03
<i>Other cavity</i>				
Volume, absolute change (ml)	0.12 (-0.13, 0.38)	0.40 (0.13, 0.67)	-0.28 (-0.65, 0.09)	0.14
Volume, relative change (p.a.) [#]	10% (-15%, 34%)	39% (11%, 68%)	-30% (-67%, 8%)	0.12
Grade, absolute change (0-3)	0.04 (-0.07, 0.16)	0.15 (0.03, 0.28)	-0.11 (-0.28, 0.06)	0.21

All analyses compared baseline vs. 2-year outcomes.

*The results in this table were generated on an imputed dataset.

[#]Relative change = (absolute change/baseline value)/time interval.

Bold p value indicates statistically significant difference at $\alpha=0.05$.

CI, confidence interval; p.a., per annum.

Table 8.4. Changes in knee effusion-synovitis exceeding MCID or LSC over two years.

Effusion-synovitis measures	Vitamin D	Placebo	RR of achieving difference in vitamin D compared to placebo group*	
	Percentage (N)	Percentage (N)	RR (95% CI)	P value
<i>Whole joint</i>				
Improvement in absolute change by MCID	53% (96)	44% (76)	1.22 (0.98, 1.52)	0.07
Improvement in relative change by MCID	70% (126)	60% (104)	1.16 (1.00, 1.36)	0.05
Increase by LSC	26% (47)	39% (68)	0.66 (0.49, 0.90)	0.01
Decrease by LSC	21% (38)	18% (31)	1.16 (0.76, 1.77)	0.48
<i>Suprapatellar pouch</i>				
Improvement in absolute change by MCID	47% (53)	40% (45)	1.17 (0.86, 1.58)	0.31
Improvement in relative change by MCID	67% (76)	53% (59)	1.27 (1.02, 1.57)	0.03
Increase by LSC	26% (29)	39% (44)	0.64 (0.44, 0.93)	0.02
Decrease by LSC	46% (25)	30% (19)	1.56 (1.09, 2.22)	0.02
<i>Other cavity</i>				
Improvement in absolute change by MCID	41% (46)	32% (36)	1.27 (0.89, 1.80)	0.19
Improvement in relative change by MCID	59% (67)	49% (54)	1.22 (0.95, 1.56)	0.11
Increase by LSC	28% (32)	39% (44)	0.70 (0.49, 0.99)	0.04
Decrease by LSC	44% (25)	34% (23)	0.41 (0.33, 0.50)	0.29

All analyses compared baseline vs. 2-year outcomes

*The results in this table were generated on an imputed dataset.

Relative change = (absolute change/baseline value)/time interval.

MCID, minimal clinically important difference was defined as the mean of the change among patients with functional improvement.

LSC, least significant change criterion was used to correct measurement errors between multiple measures. CI, confidence interval.

Bold p value indicates statistically significant difference at $\alpha=0.05$.

8.4 Discussion

This post-hoc analysis of the VIDEO study suggests that vitamin D supplementation retards the progression of effusion-synovitis over 24 months in vitamin D deficient knee OA patients. This is the first study using quantitative effusion-synovitis as an outcome measure in a clinical trial of OA. Most importantly, effusion-synovitis is able to regress or resolve suggesting its potential as a new approach for OA treatment.

The initial report from the trial suggested that vitamin D supplementation over two years did not have a major effect on cartilage morphology and knee pain in OA patients with low serum vitamin D levels (240). Results from this secondary analysis suggest a beneficial effect of vitamin D supplementation on effusion-synovitis particularly in the suprapatellar region in knee OA patients with vitamin D deficiency. Not surprisingly, the effect was only evident in those with baseline effusion-synovitis. The effect size (1.9 ml) was small but statistically significant. The results were largely consistent when an improvement defined by MCID or an increase or decrease defined by LSC was used as an outcome, suggesting that effect of vitamin D supplementation on effusion-synovitis was not due to a measurement error and is large enough to be of clinical importance. The possible biological mechanism is that vitamin D could alter the inflammatory status by modulating pro-inflammatory mediators (239) through VDRs signalling pathways in the inflammatory OA phenotype.

In our previous studies, we reported that effusion-synovitis, particularly in suprapatellar pouch, predicted worsening of knee pain, which was independent of other joint structural changes (169). In addition, it was significantly associated with cartilage defects and bone marrow lesions (94) over time. Effusion-synovitis in other regions was inconsistently associated with the progression of structural abnormalities, indicating effusion-synovitis in other regions was less clinical relevant possibly due to the limited joint space (94). Therefore, therapies targeting suprapatellar effusion-synovitis are most likely to have effect on disease progression and symptoms in knee OA. Effusion-synovitis volume in suprapatellar pouch responded well to vitamin D treatment in this trial. Its capacity to resolve over time indicates that it is sensitive to change. Our findings suggest that suprapatellar pouch effusion-synovitis can be used as an outcome measure in future clinical trials.

There were several limitations to our study. First, as this is a post-hoc analysis it requires confirmation in further studies (241). Nonetheless, the results of the current study are biologically plausible. Further, the sample size in the original trial had sufficient power to

address the research question in the current study. Indeed, we were able to detect quite small changes in effusion-synovitis even if the sample size decreased by 50% after exclusion of those without baseline effusion-synovitis. Second, MRI coronal planes were used at one clinical site so regional effusion-synovitis was unable to be measured in all participants. This may reduce the power to detect significant effects of vitamin D supplementation on regional effusion-synovitis.

In conclusion, vitamin D supplementation may retard progression of effusion-synovitis in patients with knee OA and low 25-(OH)D levels. Effusion-synovitis volume is clinically relevant to OA so vitamin D treatment has potential to improve clinical outcomes of knee OA. Effusion-synovitis volume can be utilised as an outcome measure in future OA trials.

Chapter 9 - Summary and future directions

9.1 Summary

OA is one of the most common joint disorder and the leading causing of pain and disability in adults. The social and economic burden associated with OA is increasing in the ageing population. Despite its large impact, there are no proven treatments, which stop or delay the progression of the disease. In Australia, OA affects over 1.9 million people costing the economy around \$1.6 billion annually (1). OA manifests as alterations of the whole joint structure, including degradation of cartilage, subchondral bone, menisci and synovium. The diagnosis of OA is currently based on radiographic criteria (e.g., JSN, JSW) and clinical symptoms (e.g., pain and loss of function). However, the limitations of radiography (e.g., technical issues, precision and sensitivity) have led to research into alternative parameters for monitoring OA that could serve as biomarkers in drug development (242). It is now recognised that synovial changes occurring earlier in the disease process may precede structural damage which could be a potential disease indicator (89, 93). This thesis has described a series of prospective associations between synovial inflammation and disease progression and severity, and examined the effects of vitamin D supplementation on synovial inflammation in knee OA. It has presented novel and important findings which are summarised below.

Chapter 4 describes the cross-sectional and longitudinal correlates between subregional effusion-synovitis and knee pain. Knee effusion-synovitis was measured on MRI at multiple sites, including suprapatellar pouch, central portion, posterior femoral recess and subpopliteal recess. Of these subregions, suprapatellar pouch effusion-synovitis, and posterior femoral recess effusion-synovitis, to a lesser extent, were found to be consistently associated with the prevalence and change in knee pain. These associations were found to be independent of cartilage defects and BMLs. In contrast, central portion and subpopliteal recess effusion-synovitis were not consistently associated with knee pain. Moreover, the associations of knee effusion-synovitis with non-weight-bearing was stronger than those with weight-bearing knee pain.

Chapter 5 describes the cross-sectional and longitudinal correlations between subregional effusion-synovitis and knee structures. Knee structural features measured on X-ray and MRI including osteophytes, JSN, cartilage defects, cartilage volume and BMLs were associated with effusion-synovitis. The longitudinal associations were observed mainly in the

suprapatellar pouch, to a lesser extent, the subpopliteal recess, but not in posterior femoral recess and central portion. The significant associations with cartilage defects were independent of BMLs, but the associations with cartilage volume, BMLs and radiographic changes were largely mediated by cartilage defects. Our results suggest that knee effusion-synovitis, particularly in suprapatellar pouch, may be causally related to cartilage defects, which would lead to BMLs and cartilage loss over time.

Chapter 6 investigated the natural history and clinical significance of effusion-synovitis in community-dwelling older cohorts. The size of effusion-synovitis was quantitatively measured according to its maximal area on MRI. It was found that the proportions of worsening (29%) or improving (22%) effusion-synovitis were similar, with 49% remaining stable. Baseline effusion-synovitis was associated with changes in cartilage measures and subchondral BMLs, but baseline cartilage measures and BMLs were not associated with a change in effusion-synovitis. This suggests a potential causal pathway from effusion-synovitis to cartilage and bone abnormalities, but not vice versa. Our findings confirmed that effusion-synovitis could be a precursor of OA, which was consistent with our previous findings that effusion-synovitis score assessed using semi-quantitative measurements was associated with increased incidence/progression of cartilage degradation and BMLs. Overall, these findings highlighted that effusion-synovitis is an attractive target for therapeutic intervention.

Chapter 7 describes the dynamic changes of effusion-synovitis over 24 months and the relationship between effusion-synovitis and OA symptoms and structures in established knee OA patients. Quantitative volume measures and semi-quantitative scores of effusion-synovitis were used. We found that baseline knee osteoarthritic outcomes, including osteophytes, JSN, cartilage defects, cartilage volume and BMLs were all significantly associated with changes in effusion-synovitis, but baseline effusion-synovitis was not associated with changes in osteoarthritic outcomes over 24 months. It is suggested that synovial inflammation is clinically relevant and can be the result of joint structural abnormalities in established knee OA.

Chapter 8 examined the effects of vitamin D supplementation on effusion-synovitis in knee OA patients with low vitamin D levels. This post-hoc analysis of the VIDEO study showed that vitamin D supplementation reduced the progression of effusion-synovitis over 24 months in vitamin D deficient knee OA patients. It is the first multi-centre RCT to examine

the effect of vitamin D on joint effusion-synovitis detected by MRI in patients with symptomatic OA. Given the fact that currently there are no disease-modifying drugs for OA, our study has provided direct evidence showing that monthly oral vitamin D supplementation is cost-effective and safe with a high compliance in controlling effusion-synovitis particularly in those with an inflammatory OA phenotype, which requires duplication by future studies.

In conclusion, this series of related analyses of a prospective population-based study of community-dwelling older adults provide considerable insight into the role synovial inflammation plays in OA, suggesting that synovial inflammation is an attractive target for therapeutic intervention. Most importantly, a post-hoc analysis from a RCT in knee OA patients showed the beneficial effects of vitamin D supplementation in treating effusion-synovitis. Recommendations for the future direction are provided in the following section.

9.2 Future directions

This thesis has presented several novel findings from a large prospective study of older community-dwelling adults and a RCT of vitamin D supplementation. It is suggested that effusion-synovitis plays a key role in the prediction of osteoarthritic outcomes and could serve as a therapeutic target. Knowledge of its role and relationships advance toward diagnostic criteria and clinical solutions related to knee OA.

9.2.1 Synovial biomarker for diagnosis and treatment

Imaging markers, from magnetic resonance and ultrasound, may be useful biomarkers in the evaluation of OA and in drug development in the field (102, 103, 202). From Chapter 4 to Chapter 6, we established both qualitative and quantitative assessments for synovial inflammation termed as effusion-synovitis in multiple knee joint locations on MRI. It provides evidence for the inflammatory pathways in early OA. It suggested that effusion-synovitis independently predicted worsening of knee pain after 2.6 years, regardless of other structural abnormalities. It also demonstrated a novel cascade that effusion-synovitis would cause cartilage defect development and/or progression, further leading to BMLs and diffuse cartilage loss over time. A promising trend is the use of MRI to monitor disease progression and onset by assessing those effusion-synovitis as well as other early structural changes (188, 243, 244). However, the widespread use of MRI is limited by cost, availability and the

absence of a validated international atlas. In the future, the correlations between imaging data and clinical outcomes needs to be summarised by systematic literature reviews. A comparison of synovitis detected by MRI and other imaging techniques (e.g. ultrasonography) in terms of their relative sensitivity, specificity and prognostic value would also be required.

Chapter 6 and Chapter 7 examined the natural history and clinical significance of effusion-synovitis, which found that effusion-synovitis was not static with similar proportions of both worsening and improving. Effusion-synovitis is expected to be an important disease endpoint in the future trial design. Chapter 8 showed that vitamin D was protective against progression of knee effusion-synovitis. This finding is novel but is from a post-hoc analysis from a RCT. Future intervention trials are required with knee effusion-synovitis as the primary endpoint. Notably, the effusion-synovitis is variable and requires repeated assessments at multiple time points, which we were unable to do in our current study. Future studies are needed to determine which time interval is best to describe the variation of effusion-synovitis.

Another attractive alternative is the soluble biochemical markers in blood, urine or synovial fluid samples, which could reflect dynamic and quantitative changes in synovial metabolism or inflammation, and, are therefore related to disease progression. In the setting of OA, a biochemical marker could be either an effector molecule (i.e., an operator of joint damage), the result of joint damage, or both, as in the case of cartilage extracellular matrix fragments, such as HA, that serve as both biomarkers and stimuli of the innate immune chronic wound healing response in the osteoarthritic joint (245). In our current study, we didn't include systematic or localised soluble biomarkers that related to synovium. The relative impact of soluble biomarkers (e.g. cytokine, HA) produced by synovial tissue in OA pathogenesis needs to further study. Although there are disadvantages to sampling synovial fluid (e.g., discomfort to the patient; dislike of the procedure by practitioners; short half-life of some biomarkers) (246), in a research setting it can provide the most proximal quantitative data through biomarker analyses of the disease process and thereby can be invaluable for providing biological insights in the disease.

9.2.2 *Inflammatory phenotype of OA*

OA can result from an extremely diverse range of pathologies, resulting in a heterogeneous mix of pathological processes and tissue subtypes amongst diagnosed patients. This creates a challenge for clinical trials that a treatment applied to a particular study group may only be effective in a small subset of that group. Therefore, identifying subtypes or phenotypes of OA is likely to assist in targeting treatment to patients who are the most likely to benefit (personalised medicine) (247). For example, when patients with low-grade inflammation were labelled as having an “inflammatory OA phenotype” (5), then synovitis may be a direct treatment biomarker that responds to anti-inflammatory interventions. Phenotypes postulated by other authors are post-traumatic (acute or repetitive), metabolic, ageing, genetic, symptomatic (248), and progressive/prognosis; each with different aetiological features, causal pathways, affected sites and effective treatments. One current major challenge is lack of universal criteria for phenotypes and characterisation for different stages of OA. To facilitate interpretation and comparability of biomarker studies across trials, study participants need to be described in detail with respect to symptoms, structure, function, and other known risk factors and medications.

9.2.3 *Challenges in treating synovial inflammation*

Although effusion-synovitis is proving to be an attractive target in OA, more clinical trials are needed to test if some relevant interventions are cost-effective for effusion-synovitis in knee OA. Many of the studies on the role of synovitis in OA cited in this thesis were recent, but several issues remain to be elucidated by future research. First, there is no standard in defining synovial inflammation as a study outcome. Numerous variations existed according to different assessment techniques, grading systems, populations and study designs and possibly the histological heterogeneity of synovial enhancement (e.g., effusion, hyperplasia, fibrosis, and inflammation) (249). In our study, it is possible that effusion-synovitis resolution may occur with changes in some histological profiles but not others. The challenge is to identify which histological change will be responsive to therapy. More research is also needed to define the roles of the various cell types present in the synovial tissue. In addition, the role of synovial neovascularisation in the pathogenesis of OA was not fully understood. Finally, the influence of mechanical stress on synovial components remains

to be fully elucidated. While synovial inflammation is not a strictly specific feature of such a multifactorial joint disease, it is unlikely to constitute the primary endpoint for all OA phenotypes. However, it could be a valuable endpoint for inflammatory OA phenotype in future drug development.

In conclusion, the analyses of data from a prospective cohort study and a RCT indicate that synovial inflammation plays a significant role in knee OA pathogenesis. This thesis supports a role for synovial changes in the early stages of the disease and its value of being a treatment target. Features of the synovial inflammation contribute to knee pain and predict important disease outcomes such as cartilage defect and BMLs. It can help identify subgroups and be used as an endpoint in future clinical trials. There remains a clear need for more research in the field.

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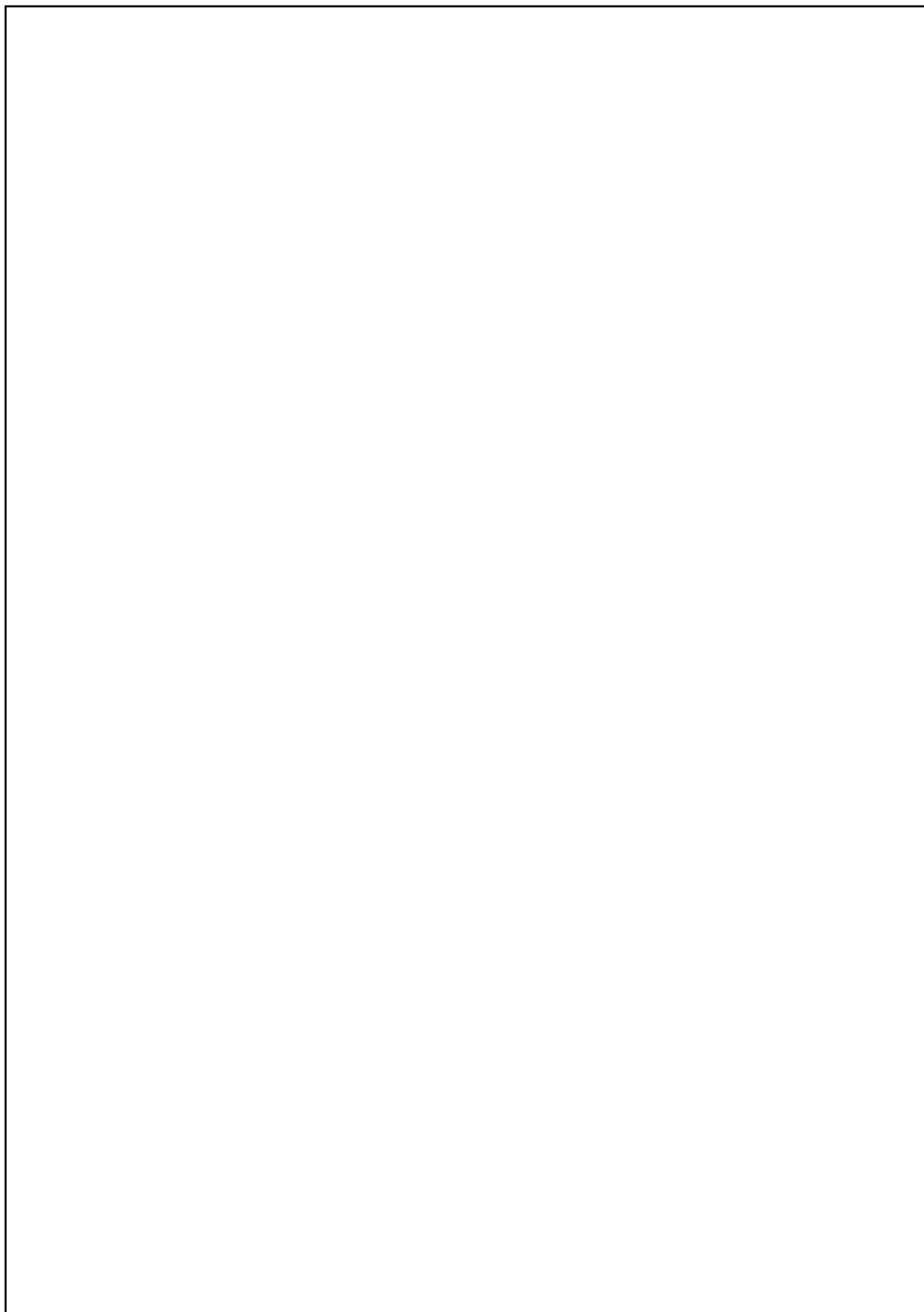
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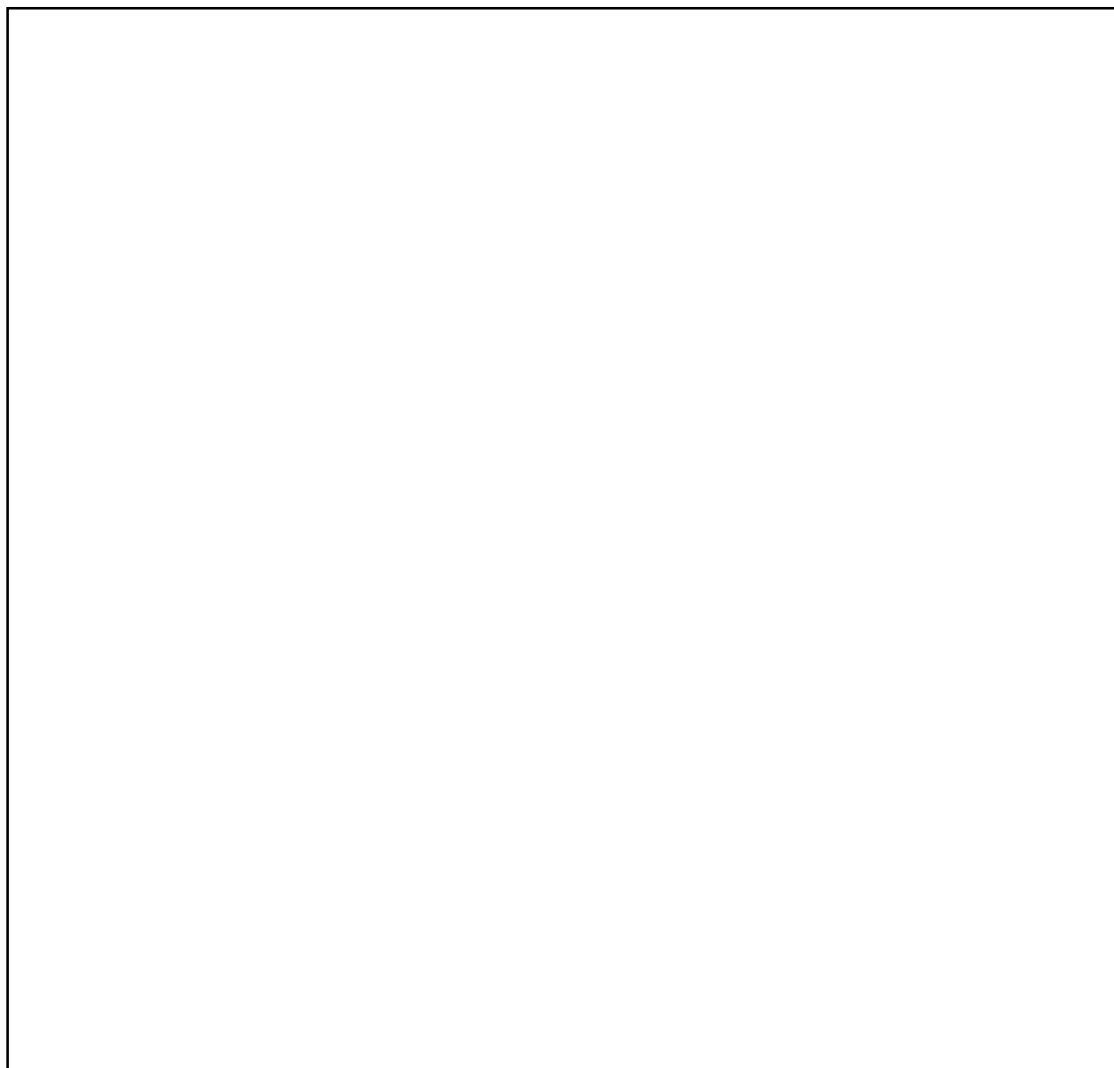
Appendices

Appendix 1 has been removed
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Wang, X., Hunter, D., Xu, J., Ding, C.,
2015, Metabolic triggered inflammation
in osteoarthritis, Osteoarthritis &
cartilage, 23(1), 22-30

Appendix 2 Example of the Western Ontario and McMaster Universities Osteoarthritis (WOMAC) index





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It is an article published as: Wang, X., Jin, X., Han, W., Cao, Y., Halliday, A., Blizzard, L., Pan, F., Antony, B., Cicuttini, F., Jones, G., Ding, C., 2016. Cross-sectional and longitudinal associations between knee joint effusion-synovitis and knee pain in older adults. *Journal of rheumatology*, 43(1), 121-30

Appendix 4 has been removed for
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Wang, X., Blizzard, L., Halliday, A., et al.,
2016, Association between MRI-detected
knee joint regional effusion-synovitis and
structural changes in older adults: a cohort
study, *Annals of the rheumatic diseases*,
75(3), 519-525

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Wang, X. , Blizzard, L. , Jin, X. , Chen, Z. ,
Zhu, Z. , Han, W. , Halliday, A. , Cicuttini,
F. , Jones, G., Ding, C., 2016. Quantitative
assessment of knee effusion-synovitis in
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structural abnormalities, Arthritis &
rheumatology, 68(4), 837-844

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Wang, X., Cicuttini, F., Jin, X., Wluka, E. A., Han, W., Zhu, Z., Blizzard, L., Antony, B., Winzenberg, T., Jones, G., Ding, C. 2017. Knee effusion-synovitis volume measurement and effects of vitamin D supplementation in patients with knee osteoarthritis. *Osteoarthritis & cartilage*, 25(8), 1304-1312