AN ULTRASONOGRAPHIC STUDY OF KNEE JOINTS: FEATURES OF INFLAMMATION AND THEIR RELATIONSHIP TO RADIOGRAPHIC OSTEOARTHRITIS AND PAIN

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Abstract

Background. Knee osteoarthritis (OA) can result in considerable pain and disability for some people. Inflammation within the joint may be partly responsible for the pain associated with OA and a link between inflammation and disease progression has been suggested. Ultrasound (US) imaging has been successfully employed in the evaluation of knee joint effusion, synovial hypertrophy and power doppler signal (PDS) which are said to represent joint inflammation. The associations between US features of inflammation, knee pain and radiographic OA have yet to be firmly established.

Objectives. The objectives of this thesis were to compare the frequency of US features of inflammation in 4 groups from a community sample, [1] those with normal knees (controls) [2] knee pain - without radiographic OA (KP) [3] radiographic OA (without pain) (ROA) and [4] symptomatic OA (SOA). Associations between US features, knee pain, radiographic change and clinical signs of inflammation could then be explored. Secondary objectives were to determine if US features change in tandem with fluctuations in knee pain (1) over time and (2) with improved pain following a therapeutic intervention in people with SOA.

Methods. In a cross-sectional multiple group comparison study, 243 participants were divided into 4 groups based on the presence of absence of knee pain and ROA. All underwent an US examination for

effusion, synovial hypertrophy, peri-articular cysts and PDS. The presence or absence of features, absolute measures (millimetres) and grade of PDS (0-3) was recorded for both knees. Radiographs and clinical evaluation of knee pain, biomechanical stiffness and function were also undertaken.

Follow-up examination of control and SOA groups was undertaken at 3 months. Participants with SOA were then invited to take part in a randomised placebo-controlled study of intra-articular (IA) cortico-steroid and a saline placebo.

Results. The frequency of US features in the control group (effusions (29%) synovial hypertrophy (8%), popliteal cysts (12%) and PD signal (2%)) was not significantly different from those in the KP group. US features were more common in ROA and higher again in SOA (effusion 81% and 92% respectively, synovial hypertrophy 41% and 82%, popliteal cysts 22% and 39%). PDS was not significantly different between ROA (6.3%) and SOA (16%).

Synovial hypertrophy was the only US feature independently associated with knee pain after adjusting for ROA (aOR 6.6; 95% CI 2.85, 15.11). All grey-scale features were strongly associated with ROA and remained so after adjusting for pain (effusion aOR 13.39, 95%CI 6.14, 29.02; synovial hypertrophy aOR 14.39, 95%CI 6.28, 32.94; popliteal cysts aOR 2.82, 95%CI 0.76, 10.43). PDS was not association with either knee pain or radiographic OA.

Change in pain severity was not found to correlate with and change in US measures among the participants followed up at 3 months or following improved pain among participants in the intervention study.

Conclusion. These findings show that US features suggestive of inflammation are higher in participants with SOA but was only significant for synovial hypertrophy. Synovial hypertrophy was confirmed as an independent risk factor for knee pain but was not found to be responsive to temporal changes in pain or improved pain following an IA cortico-steroid or placebo injection. Further studies to understand the contribution of US features of inflammation to pain in knee OA are warranted.

Declaration

This is to certify that work submitted in this thesis is the result of original research. It has been conducted substantially by myself with assistance as outlined below. It has not already been accepted for any degree and no publications for this thesis have been undertaken. All authors and works to which reference has been made are fully acknowledged.

Study design, ethical application, data collection, analysis, writing and general administration were conducted primarily by myself with support from Professor Michael Doherty, Dr Weiya Zhang and Helen Richardson. Sally Doherty provided support as the x-ray reader on the project and as the sonographer for the intervention study. Dr Khalid Latief provided support as the second sonographer in evaluating the inter-observer reliability for the study. Supervision of this thesis was undertaken by Professor Michael Doherty and Dr Weiya Zhang.

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List of Abbreviations

ACR	American College of Rheumatology
aOR	Adjusted odds ratio
BMI	Body Mass Index
BML	Bone marrow lesions
CC	Chondrocalcinosis
CD	Colour doppler
CI	Confidence Interval
COMP	Cartilage oligomeric peptide
CPCD	Calcium pyrophosphate crystal deposition
CRP	C-reactive protein
ES	Effect size
EULAR	European league against rheumatism
GDF	Growth differentiation factor
GP	General practioner
GUG	Get Up and Go
HA	Hyaluronic acid
IA	Intra-articular
ICC	Intraclass correlation co-efficients
ICOAP	Intermittent and Constant Osteoarthritis Pain
JSN	Joint space narrowing
JSW	Joint space width
K&L	Kellgren & Lawrence
KP	Knee pain
LDA	Logically derived drawing atlas
LED	Light emitting diode
MRI	Magnetic resonance Imaging
NICE	National institute for health and clinical excellence
NSAID	Non-steroidal anti-inflammatory drugs
OA	Osteoarthritis
OARSI	Osteoarthritis Research Society International
OMERACT	Outcome Measures in Rheumatoid Arthritis Clinical Trials
OR	Odds ratio
PATB	Pes anserine tendino-bursitis
PD	Power doppler
PDS	Power doppler signal
PFJ	Patello-femoral joint
RA	Rheumatoid arthritis
RCT	Randomised controlled trial
ROA	Radiographic OA
SD	Standard deviation

SOA	Symptomatic osteoarthritis
SWD	Short-wave diathermy
TFJ	Tibio-femoral joint
TKR	Total knee replacement
US	Ultrasound
VAS	Visual analogue scale
WOMAC	Western Ontario and McMaster Universities OA Index

1 Introduction

This chapter lays out the rationale for the studies undertaken for this PhD. The chapter commences with a description of osteoarthritis (OA) and describes the current burden of the disease in the United Kingdom and worldwide. Risk factors for the development and progression of the disease are outlined, and the pathology of the disease is described and current theories regarding the patho-aetiology, particularly that of joint inflammation are discussed. The use of ultrasound (US) as a measure for evaluating joint inflammation is discussed in terms of the metrics of a good outcome measure, the prevalence of ultrasound features in patient populations and the relationships between ultrasound features, clinical symptoms and structural pathology. An overview of current guidelines on the management of OA is presented. Finally the aims and objectives of this current work are presented.

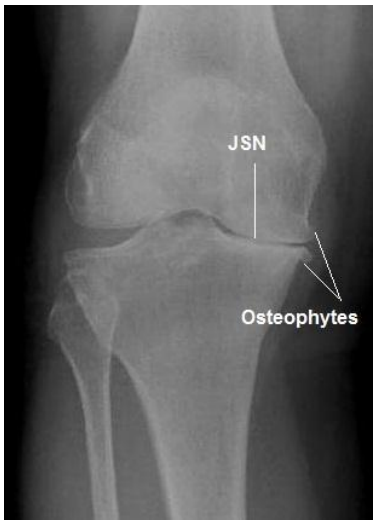
1.1 Osteoarthritis (OA)

Arthritis is a large and growing public health burden throughout the world and OA is the major source of that burden (Brooks 2006). Lifetime risk for the development of symptomatic OA suggests that nearly 1 in 2 people are at risk of painful knee OA (Murphy, Schwartz et al. 2008) and 1 in 4 for painful hip OA (Murphy, Helmick et al. 2006). In the United States it is estimated that some 27 million adults have clinical OA in one or more joints (Lawrence, Felson et al. 2008) and in

the UK 10% of all musculoskeletal consultations with GPs reportedly are for OA (Parsons and Symmons 2010).

To many, OA is recognised as an age-related painful joint condition affecting the hips, knees or hands that is accompanied by structural changes on x-rays resulting in loss of function and ultimately ending in joint replacement (in the case of hip and knee joints) (Figure 1-1). However, although the prevalence and incidence of OA certainly increases with age, it is not an inevitable consequence of aging and while pain and disability can be devastating for some, structural changes can occur without symptoms in others.

Figure 1-1 Tibio-femoral x-ray showing osteophytes and joint space narrowing (JSN) of the medial tibio-femoral compartment



Despite its associations with loss of function and disability, OA has been conserved throughout our evolutionary history and is also present in other animals with synovial joints (Hutton 1987; Rogers and Dieppe

1994). Clinically OA is now recognised as a syndrome of joint pain, rather than a single disease entity, that presents with a variety of signs and symptoms triggered by an initial mechanical or biological insult (Table 1-1).

Table 1-1 Common symptoms and signs of OA

Symptoms	Signs
Pain	Crepitus
Stiffness	Restricted movement
Functional Impairment	Tenderness
Anxiety, depression	Bony swelling
	Deformity
	Muscle wasting/weakness
	Soft tissue swelling
	Increased joint temperature
	Joint instability

OA can affect any synovial joint but typically affects the large weight-bearing joints in the lower limb and the interphalangeal joints of the hand. Prevalence rates for OA vary in their estimates but all rise with age, are higher in women than men and are higher for radiographic OA compared to symptomatic OA. Radiographic hand OA has been reported in as many as 67% of women over 55 years but symptomatic disease is present in around just 9% (Arden and Nevitt 2006). Knee OA is less frequent, with around 42% of women and 31% of men showing radiographic changes and pain affecting around half of these (Peat, McCarney et al. 2001; Arden and Nevitt 2006; Dillon, Rasch et

al. 2006). Hip OA is less common again with rates of 3-5% in the elderly (Arden and Nevitt 2006).

Incidence rates for radiographic and symptomatic OA also increase with age and are higher for women. Most published data has been on the knee joint where the incidence of new radiographic OA is around 2%, symptomatic OA around 1% and progression in around 2.5-3% per year (Felson, Zhang et al. 1995; Cooper, Snow et al. 2000). Incidence rates are higher in those with knee pain at baseline and are higher in the patello-femoral joint (9.6%) than the tibio-femoral joint (7.2%) (Duncan, Peat et al. 2011).

1.2 Knee OA

Defining knee OA has been dogged by the common discordance between symptoms and radiographic evidence of the condition. Clinical criteria for the classification of knee OA have been published by the American College of Rheumatology (Altman, Asch et al. 1986). Pain is the major inclusion factor and is required on most days of the previous month. Other symptoms include crepitus on movement, morning stiffness of less than 30 minutes, age over 50, bony tenderness, bony enlargement and no palpable warmth of which 3 items are required. However, these criteria have been criticised for reflecting more advanced disease and poor agreement with radiographic OA, therefore under-estimating the true prevalence of the condition. This has been

borne out by several epidemiological studies. The Chingford study, for example, reported the prevalence of clinical symptomatic OA to be 2.3% in a cohort of women aged between 45 and 65, but radiographically defined OA was present in 17% (Hart, Spector et al. 1991). A later cross-sectional study of adults with knee pain found that only 41% of people with symptomatic radiographic OA fulfilled the ACR clinical criteria for knee OA (Peat, Thomas et al. 2006).

Radiographs remain the cornerstone of defining knee OA in most population studies and are usually considered alongside the absence or presence of symptoms leading to the use of the separate terms radiographic OA (ROA) and symptomatic OA (SOA) respectively. However controversies also exist in the use of radiographs particularly with respect to the scoring methods employed. The most widely used is the Kellgren and Lawrence (K&L) grading scheme which awards a global score from 0 to 4 based on the presence of several features as listed in Table 1-2 (Ball, Jeffrey et al. 1963).

However, it has been criticised for several reasons. Firstly, it gives emphasis to the presence of osteophytes over joint space narrowing and secondly, assumes a fixed sequence of development and hierarchy of change. Importantly the original atlas also omits the patello-femoral joint, a compartment with a reported prevalence of isolated SOA of 8% in women and 2% in men (McAlindon, Snow et al. 1992) and the site in which it is suggested that knee OA is most likely to start (Duncan, Peat et al. 2011). Furthermore, differences between grades tend to be gross

reducing the ability to detect early radiographic features and responsiveness over time.

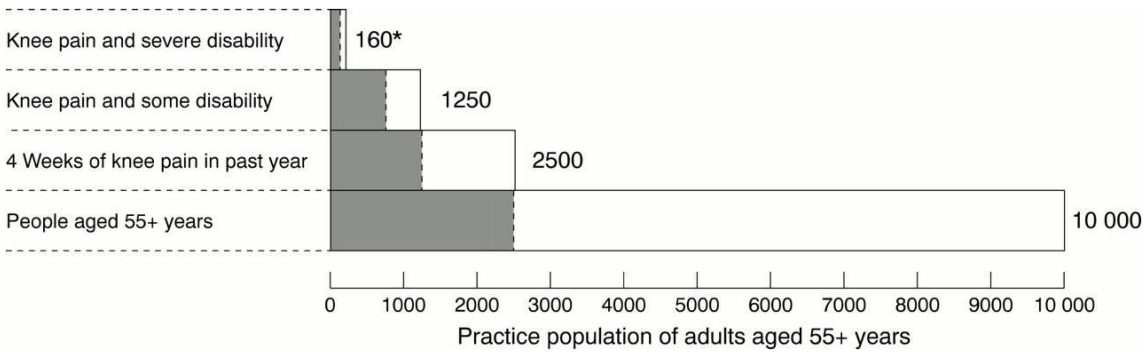
Table 1-2 Kellgren & Lawrence grading system for OA

Grade	Description
Grade 0	Normal
Grade 1	Doubtful narrowing of joint space, possible osteophyte
Grade 2	Definite osteophyte, possible narrowing
Grade 3	Moderate multiple osteophytes, definite narrowing, some sclerosis, possible deformity of bone ends
Grade 4	Large osteophytes, marked narrowing, severe sclerosis, definite deformity of bone ends

Efforts to improve on this have been made with the development of photographic atlases (Altman, Hochberg et al. 1995) but these have also had issues in terms of using an ordinal and not interval grading of features and a lack of skyline views for the patello-femoral joint. More recently an atlas of logically devised line drawings has been developed (LDA) (Nagaosa, Mateus et al. 2000; Wilkinson, Carr et al. 2005). Advantages over other grading systems include illustrations for normal shape and joint space width for men and women, maximum osteophyte representation and mathematically calculated intervals for joint space width and size of osteophyte. Despite this, the K&L system with various adjustments remains the most widely used and reported. However, lack of a universally agreed consistent method for scoring and defining radiographic knee OA remains an issue.

Although most epidemiological studies focus on the prevalence of ROA it has been suggested that prevalence of knee pain is more important for healthcare planning and delivery (Peat, McCarney et al. 2001). In the UK, 1 in 3 people over the age of 40 years old develops significant knee pain within 12 years and the annual incidence is about 3% (Ingham, Zhang et al. 2011). The estimated prevalence of knee pain in the over 40's is between 20% and 28% with around half of these reporting a resulting disability and a similar proportion showing ROA (McAlindon, Cooper et al. 1992; Tennant, Fear et al. 1995; O'Reilly, Muir et al. 1996). The prevalence staircase shown in Figure 1-2 summarises the UK estimates of knee pain, ROA and disability for older adults (Peat, McCarney et al. 2001). Prevalence of disability is 50% higher in those with bilateral compared to unilateral symptoms (White, Zhang et al. 2010).

Figure 1-2 Prevalence staircase (Peat G et al 2001; permission granted).



Shading represents the proportion in each category with radiographic evidence of knee osteoarthritis. *The proportion with radiographic evidence in this category is not known, though seems likely to be high.

1.3 Risk factors for knee OA and knee pain

Large scale population studies have identified risk factors for both the development and progression of knee OA. They can be broadly categorised into systemic factors which increase an individual's susceptibility to OA, and mechanical factors that interfere with the joint integrity and function (Figure 1-3) (Arden and Nevitt 2006). In reality, however, these factors are not always discrete from each other and frequently interact to modify overall risk. Risk factors for the development of structural knee OA may differ from those for progression of structural change and these are outlined in Table 1-3 (Doherty M. 2001). The complexity of the disease is further compounded by the fact that knee pain and the resultant disability also have a number of different risk factors, including poor self-efficacy, depression and anxiety (McAlindon, Cooper et al. 1992; McAlindon, Cooper et al. 1993; Odding, Valkenburg et al. 1998; Sharma, Cahue et al. 2003).

Figure 1-3 Risk factors for Knee OA

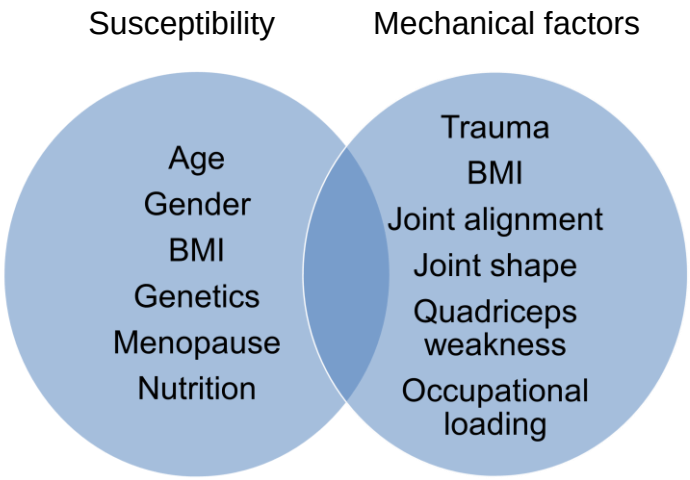


Table 1-3 Risk factors for development and progression of knee OA

Development	Progression
Heredity (unidentified genetic factors)	Obesity
Ageing	Low bone density
Female sex	Low intake of vitamins C and D
Heberden's node/hand OA	Indomethacin (possibly other non-steroidal anti-inflammatory drugs)
Obesity	
High bone density	
Trauma/meniscectomy/ligament rupture	Instability
Varus/valgus laxity	Varus/valgus malalignment
Occupation/sports • Professional soccer, mining • Repetitive knee bending (especially carrying loads)	Chondrocalcinosis/calcium pyrophosphate crystal deposition
Quadriceps weakness	Knee effusion/warmth

1.3.1 Systemic risk factors

Increasing age, female gender and obesity are well recognised as risk factors for the onset of knee OA. Age and gender were first confirmed as risk factors by the Framingham cohort study (Felson, Naimark et al. 1987) and later the associations with obesity were recognised (Anderson and Felson 1988; Felson, Anderson et al. 1988). More recently a systematic review and meta-analysis has confirmed these as important risk factors for the onset of OA (Blagojevic, Jinks et al. 2010). Pooled odds ratio (OR) for knee OA conferred an increased risk for women of 1.81 (95% CI, 1.32 -2.55). Pooled OR for age was not possible due to the varied categorisation used to report age but the methodological quality of the studies and overall agreement in the

direction of risk led the authors to conclude that older age was an important risk factor (Blagojevic, Jinks et al. 2010).

Increased BMI imparts its risk for knee OA primarily as a mechanical factor. Pooled OR for BMI >30 compared to a normal BMI were 2.63 (95% CI, 2.28-3.05 (Blagojevic, Jinks et al. 2010). The result was supported by a more recent meta-analysis, where pooled OR was 3.91 (95%CI 3.32 – 4.56) with more studies included (Muthuri, Hui et al. 2011). Furthermore it was estimated that about 50% of knee OA in the US and 42% in the UK may be avoided should obesity be prevented in the population. Obesity is also associated with hand OA which would suggest that its mechanism of action is not entirely biomechanical. Obesity is known to contribute to an inflammatory systemic environment which has a catabolic effect on cartilage metabolism. The presence of circulating cytokines and adipokines are elevated in obese individuals and are associated with cartilage degeneration (Lee and Kean 2012). Odds ratios for the onset of knee OA for those with hand OA is 1.49 (95% CI, 1.05- 2.10) (Blagojevic, Jinks et al. 2010).

Other reported systemic factors include genetics, hormonal status in women and nutrition. Familial clustering of knee and hand OA has long been recognised and siblings of people with symptomatic knee OA are almost 3 times more likely to have the condition compared to age and sex matched individuals from the community (Neame, Muir et al. 2005). Multiple genes are likely to contribute to the overall susceptibility and environmental factors will also interact and modulate their effects

(Spector and MacGregor 2004). Polymorphisms of certain common genes are now being identified, and most of those identified significant associations by genome wide studies, such as growth differentiation factor 5 (GDF5), may influence early development and growth of the musculoskeletal system suggesting that variation in growth and shape of bones and joints biomechanically may help explain some of the recognised heritability of OA (Sandell 2012).

The rise of incident OA in women after menopausal age is suggestive of a role for reduced sex hormones as a risk factor (Arden and Nevitt 2006) and this is strengthened by studies which report that hormone replacement therapy confers a negative effect on the risk of incident ROA (Spector, Nandra et al. 1997) and increased cartilage volumes on MRI (Wluka, Davis et al. 2001).

The role of nutrition in the development of OA is also an area of growing interest. While poor nutrition from an unhealthy diet is often associated with obesity which imparts an increased risk of OA, the potentially beneficial effects of specific nutritional components are less clear. Vitamins C and E are thought to protect against oxidative stress which has been proposed to prevent the normal division of chondrocytes during cartilage turnover (McAlindon and Biggee 2005). This in turn may contribute to the incidence and progression of OA.

1.3.2 Mechanical risk factors

As previously stated obesity is perhaps the most convincing risk factor for knee OA. Its primary mechanism is via the overloading of the joint during weight-bearing activities and this is evident from the dose-response increase in risk for the development of knee OA. Pooled odds ratios for risk increase from 2.18 (95 CI, 1.86-2.55) for BMI > 25 (overweight) to 2.63 (95% CI, 2.28-3.05) for BMI > 30 (obese) compared to a normal BMI (Blagojevic, Jinks et al. 2010).

Joint injury is also widely accepted as a risk factor for knee OA. The disruption of normal joint biomechanics, joint stability, proprioceptive sensibility and muscle strength can all lead to abnormal loading on the joint and recurrent micro trauma which may contribute to the risk of onset of OA. One study reported the risk of OA as nearly five times higher for those with a history of previous knee injury (OR 4.8 (95% CI 1.0-24)) (Cooper, Snow et al. 2000). The results were confirmed by a recent meta-analysis of 24 observational studies (7 cohort studies, 5 cross-sectional studies and 12 case controlled studies). Pooled OR was 4.20 (95%CI 3.11-5.66), with 3.74 (95%CI 2.16-6.74) for cohort studies, 3.34 (5%CI 1.95-5.75) for cross sectional studies and 5.34 (95%CI 3.16-9.02) for case control studies respectively (Muthuri, McWilliams et al. 2011).

Mechanical mal-alignment of the knee in the frontal plane results in either a varus or valgus deformity. Whilst it has not been demonstrated that mal-alignment is an independent risk factor for onset of OA

(Hunter, Niu et al. 2007) it has been demonstrated as a risk factor in the overweight and obese (Brouwer, Tol et al. 2007). It has also been reported as a risk for disease progression in which a four-fold increase was observed in individuals with varus mal-alignment and an almost fivefold increase for those with valgus mal-alignment (Sharma 2001). However, a later study reported a smaller risk for varus alignment (OR 2.9 (95% CI 1.07-8.88) and no increased risk for valgus alignment (Brouwer, Tol et al. 2007). More recently an association has been found between self-reported varus or valgus knee malalignment during young adulthood was and subsequent development of knee OA in later life (McWilliams, Doherty et al. 2010). A higher risk was found for varus knee alignment (aOR= 5.16, 95%CI 2.87, 9.41) which was associated with medial tibiofemoral OA than valgus alignment (aOR 3.16, 95%CI 1.04, 9.64) which was associated with lateral tibiofemoral and lateral patellofemoral OA.

Studies of muscle strength agree that quadriceps weakness is associated with knee OA and knee pain but it is unclear to what extent this is an independent risk factor. A decrease in overall strength occurs with normal aging from as early as the third decade but is accelerated by as much as 30% per decade after the age of 60 (Murray, Gardner et al. 1980). A further decrease in quadriceps strength by as much as 30% has been reported in SOA (Messier, Glasser et al. 2002). Whether strong quadriceps muscles can negate the risk of OA is not clear. One study reported that although strength was not protective against the

development of ROA, it was protective for SOA in women (Segal, Torner et al. 2009).

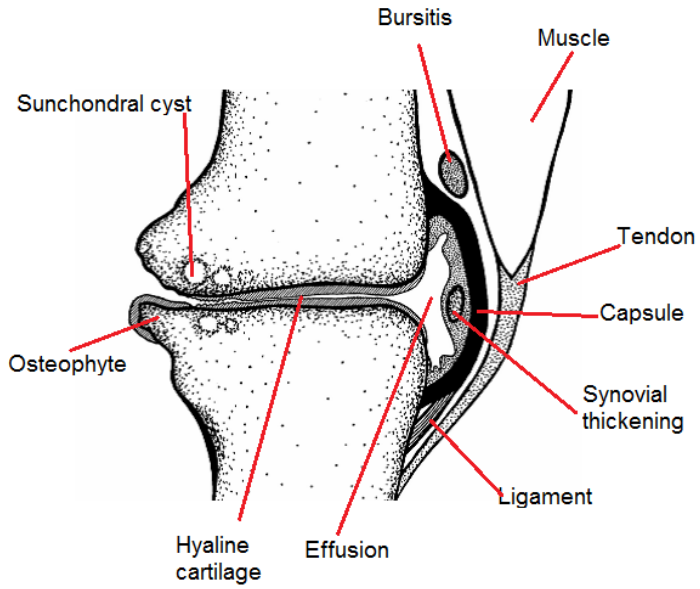
Occupational loading and leisure activity are also known to be risk factors for both incidence and progression. Regular kneeling (OR 3.4, 95% CI 1.3-9.1) or squatting (OR 6.9, 95% CI 1.8-26.4) for more than 30 minutes per day, or climbing more than ten flights of stairs per day (OR 2.7, 95% CI 1.2-6.1) confer significant risks even after adjusting for BMI and hand OA (Cooper, McAlindon et al. 1994). Mixed results have been reported for leisure or sports participation. Regular participation in sports has been reported as a risk for onset but not for progression (Cooper, Snow et al. 2000; Chapple, Nicholson et al. 2011) whereas regular physical activity (as opposed to sport) was not found to influence the risk in either direction (Felson, Niu et al. 2007). A recent meta-analysis which examined a variety of occupational factors from 51 studies reported the risk of knee OA increased by 60% for all occupational risks (McWilliams, Leeb et al. 2011). Any risks associated with occupation and leisure activity are likely to be confounded by other risk factors, particularly previous injury, muscle strength, BMI and mal-alignment (McWilliams, Doherty et al. 2010).

1.4 Pathology of OA

While OA is typically characterised by the degradation and loss of articular cartilage, it is now widely appreciated that the processes leading to the pathological end point are active, dynamic and involve all

the tissues that comprise the organ of the joint (Figure 1-4). What was once described as a ‘wear and tear’ process is now more appropriately referred to as “tear, flare and repair”, embodying the aetiological role of the initial biomechanical insult to the joint, the role of inflammation in pain and progression, and the mechanical adaptations of the joint tissues to compensate for the initial insult (Birrell, Howells et al. 2011).

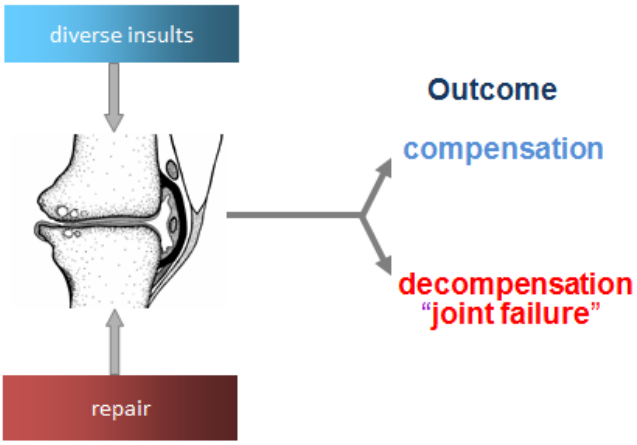
Figure 1-4 OA joint showing involvement of all joint tissues



The natural history of the condition is slow allowing what is now considered an inherent repair process to occur. The remodelling and production of new bone and the mechanical adaptations of the joint tissue often compensate for the initial insult that triggered the need for the joint to repair (“compensated OA”). For some however, the mediating effect of one’s individual risk factors coupled with the overwhelming insult(s) or continued microtrauma in the joint means the joint cannot compensate effectively and then more commonly may

associate with symptoms and loss of function (“decompensated OA” or “joint failure” Figure 1-5).

Figure 1-5 Osteoarthritis as an inherent repair process

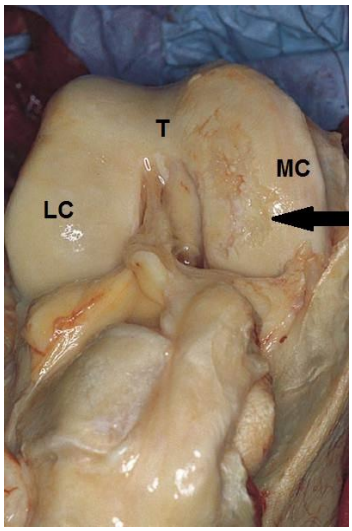


1.4.1 Articular cartilage

Articular cartilage is subject to turnover throughout our lifespan. Maintenance of this is controlled by chondrocytes which are both mechano-sensitive and osmo-sensitive (Abramson and Attur 2009). Under normal conditions they produce aggregan, type II collagen and other components that comprise the cartilage matrix and release anabolic factors responsible for the growth or stimulation of cartilage repair. Under abnormal mechanical stresses, they become stimulated to produce a range of catabolic and inflammatory mediators which lead to its degradation (Martel-Pelletier, Boileau et al. 2008). This is further enhanced by pathological changes in the subchondral bone and synovium.

The degradation of articular cartilage predominantly affects focal areas of cartilage subjected to abnormal mechanical force, with other areas remaining intact (Figure 1-6). Progressive changes can then be observed in adjacent areas until in advanced OA more of the joint surface may be involved (Pritzker, Gay et al. 2006). Histologically, oedema and fibrillation of the superficial layers of the cartilage are the earliest changes to be seen. Focal lesions within the superficial layer and flaking of small fragments of cartilage into the synovial fluid then appear as the superficial matrix is exposed to shearing forces across the joint. Vertical fissures begin to extend into the mid layers of the cartilage which can branch and extend into deeper zones as it progresses. Cell death and proliferation can be observed adjacent to the fissures. Matrix loss is progressive until the non-mineralised articular cartilage is eroded exposing mineralised cartilage or bone.

Figure 1-6 Medial compartment knee OA showing articular cartilage loss.

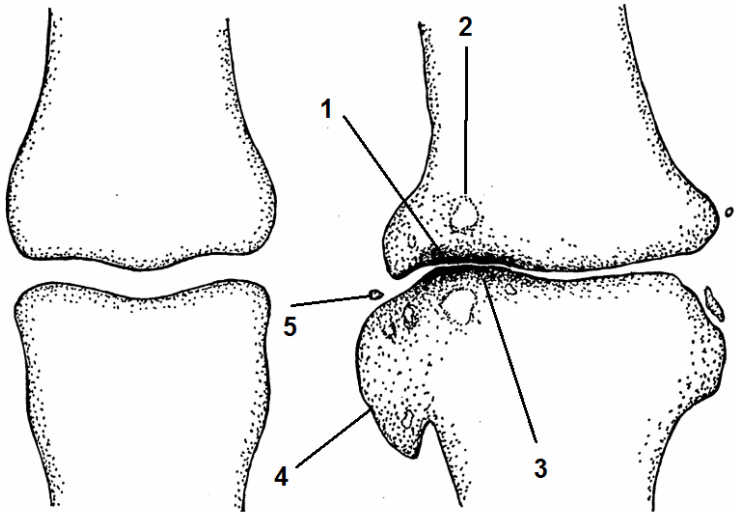


Photograph shows medial compartment (MC) OA with loss of articular cartilage exposing the underlying bone (thick arrow). Note the normal cartilage at the lateral compartment (LC) and trochlea (T).

1.4.2 Bony changes

Osteophyte formation and subchondral sclerosis are the key pathological features of the bone in OA (**Error! Not a valid bookmark self-reference.**). The development of osteophytes, bony outgrowths capped by fibro-cartilage, may be due a number of processes. Osteophytes can be induced by mechanical instability in animal studies and it is proposed that they are simply features of remodelling in an attempt to stabilise the joint (Aspden 2008). Other theories include the abnormal repair of stress fractures in the subchondral bone near the joint margins (Abramson and Attur 2009) or as a mechanical response to increased loading of the bone at the joint margins transmitted via biochemical signalling (Aspden 2008).

Figure 1-7 Bony features of OA



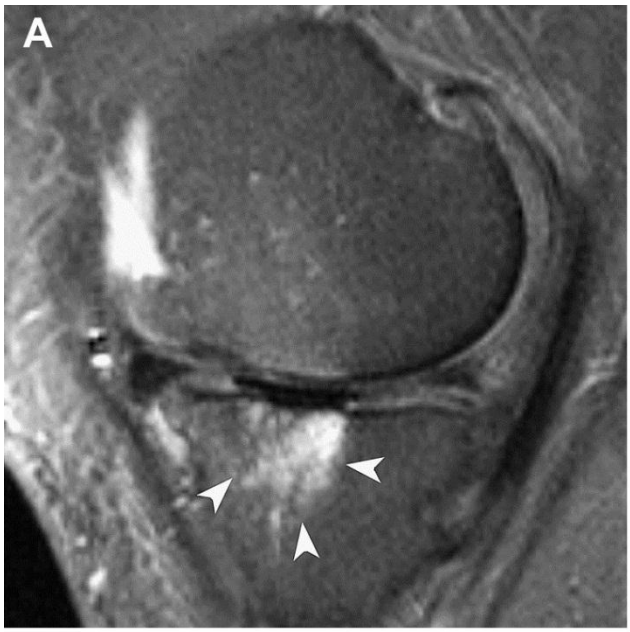
Normal joint

osteoarthritis joint showing (1) focal joint space narrowing (2)subchondral cysts (3) sclerosis (4) osteophyte (5) osteochondral bodies

Sclerosis describes the radiographic appearance of dense bone tissue at the joint margin which may extend into the deeper subchondral bone. Sequentially it is detectable after loss of joint space and eburnation of the exposed subchondral bone and is thought to represent bone remodelling in response to increased stress and micro-fractures.

Bone marrow lesions (BMLs) represent further pathology of bone in OA. These lesions, which are observable on magnetic resonance imaging (MRI) represent areas of abnormal bone with excessive fibrosis, focal osteonecrosis and remodelling and are consistent with bony trauma such as micro-fractures and chronic abnormal loading (Figure 1-8). The presence of BMLs is associated with painful knee OA having been found in over 75% of persons with symptomatic OA compared to 30% of those with asymptomatic disease, and their frequency increases with radiographic severity (Felson, Chaisson et al. 2001; Felson, McLaughlin et al. 2003). Joint mal-alignment which results in increased abnormal loading of the joint is also associated with BMLs in the corresponding joint compartment and with progressive joint space narrowing (Felson, McLaughlin et al. 2003). The sequence of bony pathology in OA is not clear and while many theories suggest they occur in response to changes in the articular cartilage they may occur concurrently or even precede it (Aspden 2008).

Figure 1-8 MRI showing subchondral bone marrow lesion in OA knee (white arrowheads). (Roemer, Frobell et al. 2009) permission granted.



1.4.3 Synovial and peri-articular pathology

In addition to the pathological changes occurring within the cartilage and bone, changes in other tissues may also contribute to the disease process and associated symptoms and disability. The synovium and its outer layer the capsule undergo hyperplasia and thickening, excess synovial fluid produced by the activated synovium leads to capsular swelling. This can cause pain, stiffness and lead to arthrogenic inhibition of the quadriceps muscle which in turn can progress to muscle weakness and atrophy (Stokes and Young 1984; Hurley and Newham 1993).

Enthesitis has also gained some attention as a possible pathoetiological mechanism for OA. Enthesis are the insertion sites of

tendons, ligaments, fascia or articular capsule into bone, and their function is largely to dissipate biomechanical forces between the joint and bone. Pathological changes at the enthesis and adjacent tissues are recognised as a common feature within the spondylarthritides, but modest changes have also been demonstrated in hand and knee OA. Histological changes including synovial villus formation and inflammatory cell infiltration were commonly observed in elderly cadaveric specimens (Benjamin and McGonagle 2007). Whilst in an MRI study, Tan et al (2008) reported enthesopathy almost universally in the joints of early and established hand OA, the most common of which were changes in the joint collateral ligaments. Subtle ligamentous changes were also common in asymptomatic adjacent joints and in the joints of older control subjects, which the authors and others suggest may play an initiating or modulating role in the pathogenesis of OA or represent a subset of OA patients with systemic enthesopathy (Tan, Grainger et al. 2005; McGonagle, Tan et al. 2008).

In SOA knees, MRI scans have found central BMLs adjacent to the insertion of the anterior cruciate ligament (ACL) which are highly prevalent (54%) and strongly related to ligament pathology, though many knees with central BMLs showed no evidence of ACL tear (58%) (Hernández-Molina, Guermazi et al. 2008). A subsequent study by Gibson et al (2012) was unable to demonstrate an association between enthesopathy at the hand and central BMLs at the knee, questioning the theory that OA maybe a systemic enthospathic disease and

supporting the belief that enthesopathy in OA is biomechanically induced (Gibson, Guermazi et al. 2012; Haugen 2012).

A recent review also suggested that the infra-patellar fat pad should be considered as an active joint tissue in knee OA which is capable of influencing the degradation of cartilage by stimulating the production of pro-inflammatory mediators (Clockaerts, Bastiaansen-Jenniskens et al. 2010).

1.5 Inflammation in OA

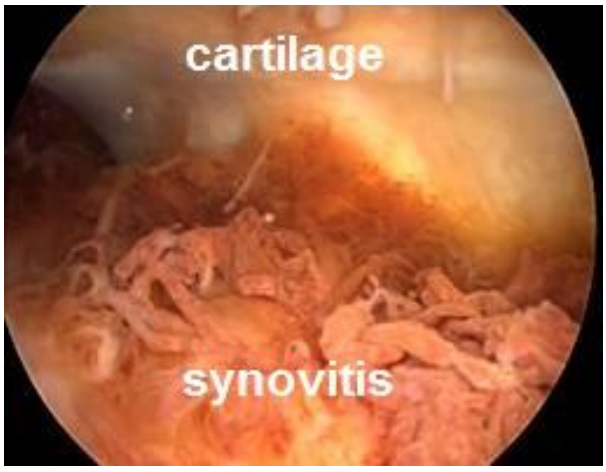
1.5.1 Synovitis

Over the past decade or so, there has been an increasing interest in the pathological changes occurring in the synovium and its possible role in the onset and progression of the disease. Though not as florid as the synovitis observed in rheumatoid arthritis, the synovium and outer layer the capsule undergo hyperplasia and thickening and excess synovial fluid produced by the activated synovium leads to capsular swelling Figure 1-9. Synovitis is often localised to areas adjacent to damaged cartilage, bone or entheses and is generally regarded as a secondary response to cartilage and biomechanical insults and represents the flare component in the ‘tear, flare and repair’ analogy (Porcheret, Healey et al. 2011).

However, the detection of elevated cytokines early in the disease process has led to the suggestion that there may be an underlying primary inflammatory process (Aspden, 2008). It has also been demonstrated that the synovium itself produces some of the chemokines that degrade cartilage (Samuels *et al.*, 2008).

Arthroscopic evidence of synovitis has been reported in around half of patients with symptomatic knee OA and may be predictive of structural progression. A prospective study of 422 patients with primary symptomatic OA found that those with synovitis on arthroscopy at baseline showed greater deterioration in cartilage, compared to those with none or mild synovitis after 12 months (Ayral, Pickering et al. 2005).

Figure 1-9 Arthroscopic image of synovitis in the knee joint



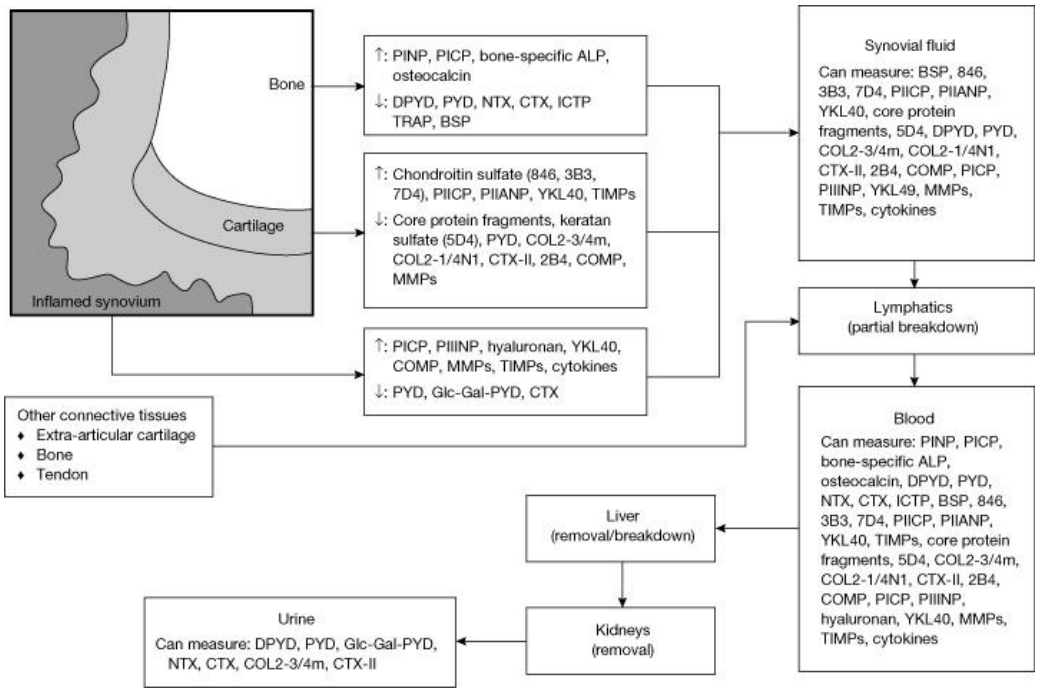
Histological evaluation has demonstrated features of mild synovitis in OA including hyperplasia of the synovial lining, increased vascular

density and higher density of proliferating cells compared to normal controls. The outer layer, the fibrous capsule, shows hyperplasia and overall thickening (Pessler, Chen et al. 2008). Synovial biopsies from patients with early OA (defined as knee pain < 1 year, normal radiographs and early but defined chondropathy on arthroscopy) showed greater evidence of synovitis than patients with late stage OA (undergoing knee joint arthroplasty) (Benito, Veale et al. 2005) suggesting that inflammation may be more important in early disease and contribute to disease progression in some patients.

1.5.2 Biomarkers

Biochemical biomarkers are molecules of connective tissue matrices released during the process of tissue turnover (Garnero, Piperno et al. 2001). They hold promise of being useful clinical tools to aid in the diagnosis of disease and disease prognosis, monitoring of disease progression and response to treatment, as well as helping our understanding of the underlying disease mechanisms, particularly inflammation. A range of biochemical markers of bone, synovium and cartilage have been measured and reported in OA (Figure 1-10) though no definitive markers have yet been identified which could be successfully used in research or clinical practice (Doherty, Jones et al. 2004).

Figure 1-10 Potential biomarkers in the osteoarthritis process.(Doherty, Jones et al. 2004)



Hyaluronic acid (HA) is most commonly associated with synovial tissue. It is a glycosaminoglycan produced by fibroblasts and other cells within connective tissue. It has a structural role in the connective tissue matrix and is involved in intercellular communication and increased levels are considered to reflect increased synovial activity (Attur, Samuels et al. 2010). Increased serum HA levels can be found in patients with a wide range of conditions including liver disease, cancer, hypothyroidism and a variety of arthropathies (Turan, Bal et al. 2007).

An early study of serum HA levels in OA found that they were twice that found in healthy age-matched controls and showed a correlation with gross function (Steinbrocker's classification) and an articular index representing the amount of cartilage involved (Goldberg, Huff et al.

1991). Further indications that serum HA was linked with structural damage was demonstrated by a correlation with disease duration ($p = 0.036$) (Sharif, George et al. 1995; Turan, Bal et al. 2007), minimum joint space width ($p = 0.049$) and medial joint space width (Sharma, Hurwitz et al. 1998). No direct association has been found with radiographic grade (Turan, Bal et al. 2007), though patients whose disease had progressed over a five year period, were shown to have significantly higher levels of HA at baseline compared with those whose disease had not progressed ($p=.019$) (Sharif, George et al. 1995). However the confounding effects of co-morbidities were not fully explored.

Though not a specific tissue biomarker, serum C-reactive protein (CRP) levels rise in response to inflammation. CRP is the most sensitive available routine test for the acute phase response and is often used to help differentiate between “inflammatory” conditions such as rheumatoid arthritis (RA) and “non-inflammatory” conditions such as OA. However more sensitive testing techniques have showed low elevated levels in conditions with low-grade inflammation (Pearle, Scanzello et al. 2007). Several studies have reported modest but significantly higher CRP concentrations in patients with knee OA compared to those without (Sharif, George et al. 1995; Spector, Hart et al. 1997; Sharif, Shepstone et al. 2000; Sowers, Jannausch et al. 2002). It was also reported that raised CRP could be predictive of disease progression over 4 (Spector, Hart et al. 1997) and 8 years

(Sharif, Shepstone et al. 2000). However, low levels of CRP failed to show a correlation with histological grading of synovial infiltration for patients undergoing hip or knee arthroplasty (Pearle, Scanzello et al. 2007) and the most recent and convincing evidence on this has shown that the association between knee OA and CRP is completely driven by BMI (Kerkhof, Bierma-Zeinstra et al. 2010).

1.5.3 Clinical signs and symptoms

Clinical signs and symptoms indicative of inflammation can be observed in OA. Pain, tenderness, mild swelling and self-reported stiffness are common and warmth and synovial thickening can be apparent in some patients, some of the time.

1.5.3.1 Effusion

Whilst small joint effusions in OA are not uncommon, substantial effusions are infrequent and their presence would usually raise concerns of associated synovitis, for example gout or OA with calcium pyrophosphate crystal deposition.

Under normal conditions the volume of synovial fluid in the joint is balanced by articular flux, that is, it is controlled by the concentration of solutes within the synovial fluid and its rate of clearance. Clearance from the joint occurs via a combination of the microvascular network and by the synovial lymphatic system. In the normal turnover of articular

cartilage, smaller molecules from the cartilage matrix pass into draining blood vessels as well as lymph but larger molecules such as aggrecan are cleared more slowly by the lymphatic system (Levick and McDonald 1995; Simkin and Bassett 1995).

Acute injury or insult to the cartilage results in an increase in the catabolic and inflammatory mediators accelerating the normal turnover of the cartilage matrix which is cleared in the main by the lymphatic system. The subsequent effusion is an attempt to restore equilibrium to the synovial fluid solutes and resolution of the insult usually sees a return to normal in the balance and volume of synovial fluid. Where resolution fails to occur such as in inflammatory joint disease or with repeated micro-trauma, this can lead to an elevated level of flux and chronic effusion. Levels of lymphatic clearance in knees with rheumatoid arthritis (RA) has been reported as almost twice that in knee joints with OA, reflecting the high turnover (Wallis, Simkin et al. 1987).

1.5.3.2 Stiffness

Joint stiffness of limited duration is a common complaint in people with knee OA and one of 6 criteria used for clinical diagnosis of knee OA (Altman 1991). Patient reported stiffness is associated with decreased function (Odding, Valkenburg et al. 1998; Hall, Mockett et al. 2006) yet its evaluation is often limited to patient reporting and scoring on the WOMAC Index (Bellamy, Buchanan et al. 1988). The underlying basis for joint stiffness is not clear though its prolonged duration in RA

compared to OA would suggest that the underlying inflammation is a major contributing factor.

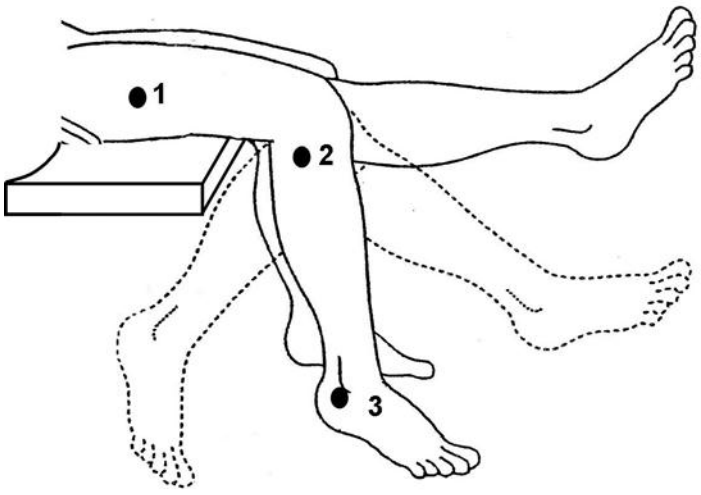
The use of the term stiffness is open to a variety of interpretations but for use in a clinical situation Thompson et al suggests that stiffness is the “resistance to passive motion at a joint throughout the normal range of motion in a functional plane” as opposed to a restricted range of movement (Thompson, Wright et al. 1978). Other terminology, such as flexibility, elasticity, plasticity and viscosity have specific characteristics and definitions that are related to stiffness but do not always translate easily to the clinical interpretation of stiffness.

A method of quantifying joint stiffness by calculating stiffness and damping co-efficient during a pendulum test (a relaxed swinging of the knee joint in sitting) has been described by Oatis et al (1993). This method utilises a mechanical spring-damper system to model the behaviour of a joint. The stiffness of the spring represents behaviour of the joint as it deforms or bends and the peri-articular structures represent the damper. The effect of a damper is to reduce the amplitude of oscillations. Mathematically it is modelled as a force synchronous with the velocity of the object but opposite in direction to it.

During the pendulum test the angular displacement of the knee is measured using motion analysis equipment while the relaxed lower-leg is released from a position of joint extension and allowed to oscillate freely until it comes to a resting state (Figure 1-11). Stiffness and damping co-efficients can then be calculated using displacement data

and anthropometric measures of the participant. Stiffness is the force or moment needed to produce a rotational deformation and is expressed in newton-meter/radian. A higher stiffness co-efficient indicate greater forces are required to produce a movement and are synonymous increased stiffness of a joint. The damping co-efficient reflects the time-dependent nature of the moment expressed in newton-meter-second/radian. Larger damping co-efficients indicate larger moments required to produce more rapid movement (Oatis, Wolff et al. article in press 2013).

Figure 1-11 Knee joint displacement during the pendulum test. (Adapted from (Valle, Casabona et al. 2006).



The solid line represents the leg at the starting position (knee extended) and at the final position (knee flexed). 3-D motion analysis systems are most commonly used to capture displacements data from the positions of small reference markers attached to the anatomical reference points. The numbers represent the positions of the markers: 1, greater trochanter; 2, head of fibula; 3, lateral malleolus.

These co-efficients represent the passive resistance resulting from the articular cartilage, viscosity of the synovial fluid, thickening of the joint capsule and the surrounding ligaments and musculature. While they do not enable the source of the resistance to be determined, a previous animal study reported that the joint capsule contributes almost half of the overall resistance, the muscles around 40% and tendons 10% (Johns and Wright 1962). The contribution of viscous and frictional stiffness from within the joint is thought to be much smaller, around 1% and 10% respectively (Wright and Johns 1960).

A number of small pilot studies have utilised the pendulum test to explore biomechanical stiffness in patients with knee joint disease. In one study (Oatis, Wolff et al. 2006) participants with knee OA were shown to have significantly higher damping co-efficients when compared to age and sex-matched controls ($p=.035$) but the difference in mean stiffness co-efficients was not significant (Oatis, Wolff et al. 2006). A type II error was highly probable and a power calculation established that 50 participants with knee OA would be required to show a significant difference between controls and cases. Furthermore, OA was self-reported by participants and so correlation with radiographic severity could not be examined, and confounding variables such as muscle strength were not measured. However, damping co-efficients were found to be associated with patient reported stiffness using the WOMAC ($r= 0.85$, $p=.003$) and it was suggested that this measure may reflect the patients' perception of joint stiffness.

In contrast, a study by Burks et al (2206) reported a small but significant correlation between the WOMAC stiffness subscale and the stiffness co-efficient (r=0.36, p=0.05). However, the analysis for this study was limited and no data was presented for the measures of stiffness or damping to infer any differences between participants with and without knee OA (Burks and Keegan 2006).

A similar method was used in a study evaluating stiffness in knees affected by RA compared to healthy controls (Valle, Casabona et al. 2006). This study found that the amplitude of limb oscillations was reduced in RA knees and that this coincided with a significant increase in knee joint stiffness ($p<.0001$) which correlated to disease severity ($R^2=0.68$). Damping co-efficients were lower in the RA group but not significantly so.

The use of an objective measure of knee joint stiffness has been limited to small studies which have been unable to draw any firm conclusions regarding the relationship of stiffness and damping co-efficients measures with either structural joint change or symptoms. The application of this novel technique could help draw inferences as to the role of inflammation in joint stiffness in knee OA.

1.5.4 Imaging

Radiographs are the usual imaging technique of choice in the study of OA but do not allow for the evaluation of soft tissue pathology or inflammation. Other imaging techniques have been utilised in this

pursuit, though by far the most robust technique is magnetic resonance imaging (MRI).

Infra-red thermography has demonstrated differences in temperature between OA and non-OA hand joints. A decline in joint temperature with increasing K&L score was noted and led the authors to suggest that early OA may represent an inflammatory phase of the disease (Varju, Pieper et al. 2004). A decrease in knee temperature associated with decreasing knee pain was also demonstrated by thermography following intra-articular steroid injection for knee OA (Dieppe, Sathapatayavongs et al. 1980).

Abnormal bone scintigraphic uptake patterns around the subchondral bone (early phase indicating increased bone perfusion and late phase indicating increased bone turnover) have been shown to correspond to increased signal on MRI scans in both chronic knee pain (Boegård, Rudling et al. 1999) and knee OA (McAlindon, Watt et al. 1991). It has been suggested that this may reflect abnormal perfusion in areas of active subchondral bone as well as in areas of synovitis (McCrae, Shouls et al. 1992). Increased uptake on bone scans was also found to be correlated with serum levels of cartilage oligomeric peptide (COMP) a marker of cartilage turnover) ($r=0.56$, $p=0.002$) (Petersson, Boegård et al. 1998).

MRI is the gold standard for the imaging of synovitis, particularly when it is gadolinium contrast enhanced which allows the thickened synovium to be distinguished from fluid within the joint. Good correlations have

been demonstrated between synovitis on MRI and findings on arthroscopy and subsequent histological examination in patients with OA knees (Fernandez-Madrid, Karvonen et al. 1995; Loeuille, Chary-Valckenaere et al. 2005).

Synovitis on MRI has been reported in 50% or more of knees with SOA (Baker, Grainger et al. 2010). Findings agree that synovial hypertrophy is more common in those with SOA than ROA ($p=0.002$) (Hill, Gale et al. 2001) and that increased synovitis correlates with radiographic severity (Torres, Dunlop et al. 2006; Pelletier, Raynauld et al. 2008). However synovitis has also been observed in knees with mild or no radiographic changes. The association with knee pain has been demonstrated using pain severity scales and pain scores derived from the WOMAC index where extensive synovitis conferred a 9 fold increased odds for knee pain ($p<0.001$) (Fernandez-Madrid, Karvonen et al. 1995; Hill, Gale et al. 2001; Torres, Dunlop et al. 2006; Baker, Grainger et al. 2010).

Longitudinal changes in synovitis and knee pain have shown a modest but direct correlation between increased synovitis and increased pain severity ($r=0.21$, $p<0.0003$) (Hill, Hunter et al. 2007). However a more recent study reported that although increased synovitis increased the risk of more frequent knee pain the reverse was not observed i.e. improved synovitis did not convey a decrease in the risk of frequent pain (Zhang, Nevitt et al. 2011).

Effusions on MRI are common and have been observed in over two thirds of people with normal knees (Hill, Gale et al. 2001). Moderate and large effusions are less common but are more common in those with SOA (54.6%) compared to ROA (15.6%). Hill (2007) reported that changes in effusion over time were not associated with changes in pain and Zhang (2011) reported that there was no association between change in effusion and change in frequency of knee pain in the either direction.

Other MRI features associated with knee pain include bone marrow lesions (BMLs) which are present in 35% of those with SOA compared to just 2% of those without. (Felson, Chaisson et al. 2001). Unique to this feature, in comparison to effusions and synovitis, is that a change in BML scores over time is associated with a concomitant change in knee pain presence and severity, in either direction (Zhang, Nevitt et al. 2011).

Other peri-articular pathologies such as popliteal cysts and pes anserine bursitis are also more commonly observed in patients with SOA compared to ROA though the overall prevalence is low (less than 15%) and they have not been found to be associated with pain severity. (Hill, Gale et al. 2003).

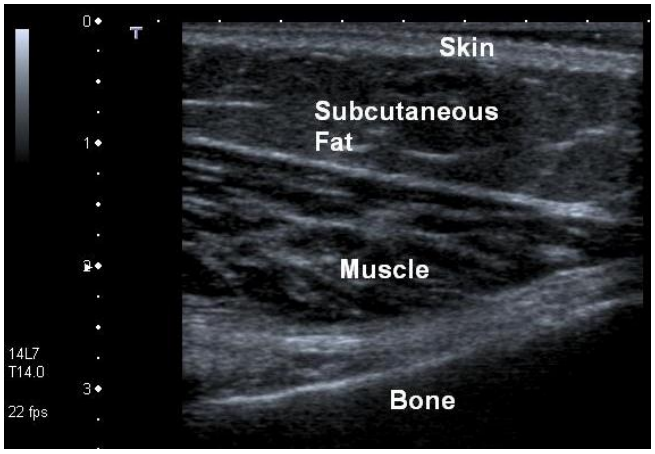
1.6 Ultrasound assessment of inflammation in knee OA

Over the past decade ultrasound (US) imaging has gained popularity as an imaging tool for the musculoskeletal system, particularly in the

assessment of joint disease. In OA the advantage of US over traditional radiographs is that it allows the evaluation of joint effusion and synovitis as well as peri-articular lesions such as bursitis.

Grey-scale or B-mode US is the most common mode used in musculoskeletal imaging and produces a two-dimensional scan where reflected waves are represented by white images. The acoustic impedance of bone is such that US waves are all reflected and appear as white, whereas articular cartilage and liquids like synovial fluid do not reflect any sound waves at all and will appear as black. Reflection from muscle and fat vary depending on their density and water content and appear as different shades of grey (Schmidt and Backhaus 2008), Figure 1-12.

Figure 1-12 Grey-scale US image showing appearance of different tissues

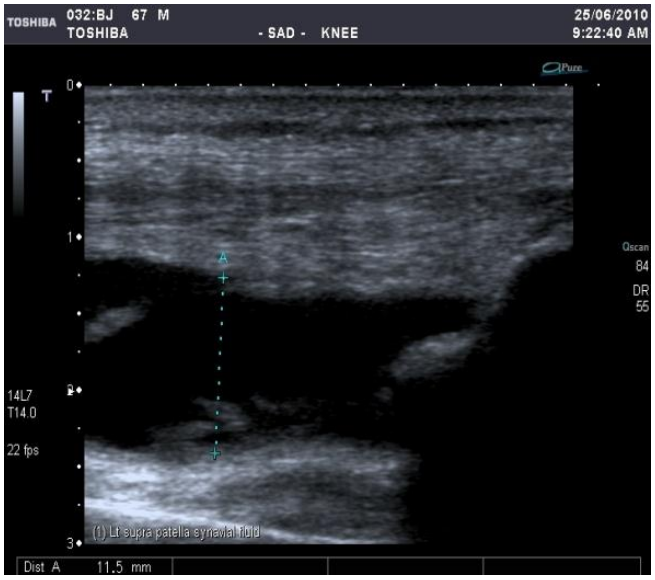


1.6.1 Grey scale features: Effusion

Effusions in the knee are observed in the supra-patellar recesses and appear as abnormal anechoic areas that are displaceable and

compressible but do not exhibit Doppler signal (Wakefield, Balint et al. 2005) (Figure 1-13). They are usually recorded as present or absent or measured on a continuous scale at their maximal depth in millimetres.

Figure 1-13 Grey-scale US image of an effusion in the supra-patellar pouch (taken from study participant).



The use of US to detect knee effusions is more sensitive than clinical examination (Hauzeur, Mathy et al. 1999; Kane, Balint et al. 2003) and can detect small volumes of fluid in as many as 77% of normal healthy knees (Schmidt, Schmidt et al. 2004). The validity of US effusion has been established by comparison with MRI in a number of studies across a range of knee pathologies (Ostergaard, Courtpayen et al. 1995; Scheel, Schmidt et al. 2005). In OA knees, MRI has been found to be more sensitive than US in detecting effusion (effusion was detected in 85% of 58 symptomatic OA knees on MRI compared to 70% on US) but a good correlation between the two was found ($r=0.63$, $p<0.001$) (Tarhan and Unlu 2003).

Cadaver studies have shown that MRI can detect volumes as small as 4ml (Schweitzer, Falk et al. 1992) within the knee joint whereas the smallest volume detected by US was 7.4 ml (Delaunoy, Feipel et al. 2003). However this is unlikely to have any clinical significance and agreement between MRI and US in the detection of knee effusion is excellent (Scheel, Schmidt et al. 2005).

Reliability for the US detection of effusion has been evaluated for both intra and inter-observer agreement across a range of knee conditions. A study of 23 European experts in musculoskeletal sonography demonstrated an overall agreement of 91% for presence of knee effusion and synovitis (Naredo, Moller et al. 2006). A more recent study of inter-observer reliability specifically in knee OA, was undertaken by two sonographers who evaluated 18 participants (34 knees) within a six week period (Abraham, Goff et al. 2011). High inter-observer reliability was found for both the presence of effusion (kappa= 0.65, 0.77 for right and left knees respectively) and for direct measures of effusion size (ICC = 0.70, 0.85 for right and left knees respectively). Wu et al reported similar reliability for the intra-observer reliability of repeat scans of 12 OA knee participants acquired on the same day kappa = 0.78 (Wu, Shao et al. 2012).

The prevalence of US detected effusion in knee OA varies in the literature though this can be attributed in part to variations in defining criteria and scanning protocols. In the most extensive US study of knee OA to date, a large cross-sectional European study by EULAR,

clinically assessed effusion was present in 44% of 600 patients with SOA (D'Agostino, Conaghan et al. 2005). US detected effusion (defined as present if maximal depth ≥ 4mm) was present in 43.5% of participants (mean of 7.5mm, SD 5). Multivariate analysis showed that the probability of US detected effusion was increased for more severe OA, K&L grade ≥3 (OR=1.91, 95%CI 1.32-2.77), sudden aggravation in knee pain in the previous two weeks (OR=2.7, 95%CI 1.76-4.16) and a moderate knee effusion on clinical examination (OR=1.77) which the authors propose is suggestive of an "inflammatory flare". Clinical features including morning stiffness and pain severity were not found to be good predictors of effusion.

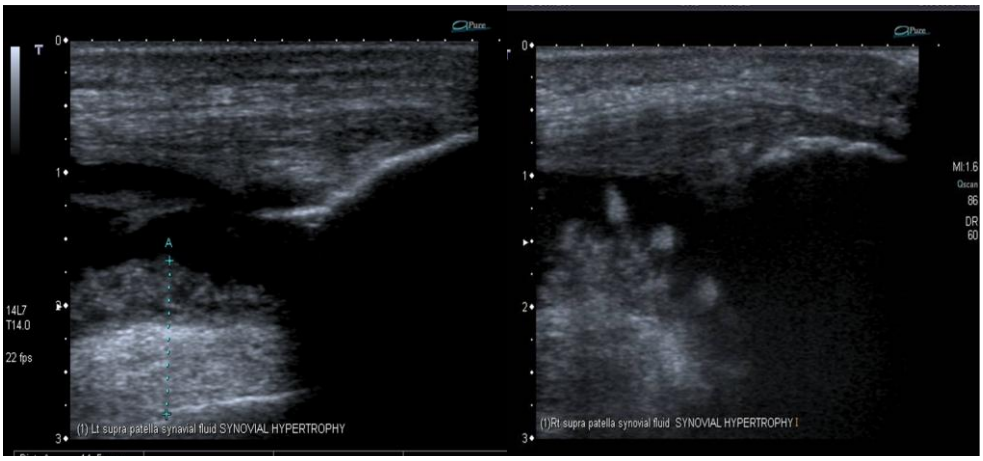
Similar observations on the frequency of effusions in participants with SOA were reported by Naredo et al (2005) (47% of 50 participants), Iaggnocco (2010) (43% of 82 participants) and Wu et al (2012) (33% of 112 knees) who used the same cut-off value of 4mm for defining the presence of effusion. Higher frequencies were reported by some studies which used smaller (>2mm) or no cut-off values, specifically 72% (Mermerci, Garip et al. 2011), 79% (de Miguel Mendieta, Cobo Ibáñez et al. 2006) and 86% (Kristoffersen, Torp-Pedersen et al. 2006). Effusions were less frequently seen in patients with ROA without knee pain (35%) (de Miguel Mendieta, Cobo Ibáñez et al. 2006) and those with normal knees (0-16%)(Tarhan and Unlu 2003; Naredo, Cabero et al. 2005) though numbers for these groups were small.

1.6.2 Grey scale: Synovitis

In the knee joint, synovitis tends to occur in the supra-patellar pouch, but can also be visualised in the medial and lateral recesses of the pouch and the medial and lateral aspects of the knee joint. Synovium cannot be seen on US unless it is hypertrophic when it appears as “abnormal hypo-echoic (relative to sub-dermal fat, but sometimes may be iso-echoic or hyper-echoic) intra-articular tissue that is non-displaceable and poorly compressible and which may exhibit Doppler signal” (Wakefield, Balint et al. 2005). Its appearance can vary from flattened, thickened synovium to frond like protrusions into the synovial fluid (Figure 1-14). Pathology of the synovium can be assessed using grey-scale US and graded according to thickness or measured directly in millimetres.

Figure 1-14 US images of synovial hypertrophy

A **B**



(A) showing thickened flattened synovium (B) showing frond like protrusions into the synovial fluid

Most US studies of synovitis are concerned with establishing the validity of US findings by comparison with macroscopic and microscopic evaluation of the joint and findings on MRI. This has been convincingly demonstrated in a number of studies.

In a study of 60 patients with a variety of knee pathologies the sensitivity and positive predictive value for US was 98% compared to macroscopic evaluation on arthroscopy (Karim, Wakefield et al. 2004). The specificity and negative predictive value were both 88%, with an overall accuracy of 97%. This was considerably higher than the comparison for clinical examination and arthroscopy which resulted in a sensitivity of 85% and specificity of 25% and an overall accuracy of 77%. Intra- and inter-observer reproducibility for the presence or absence of US detected synovitis read from stored scans was 0.85 and 0.71 ($p<0.05$) respectively.

A comparison of US detected synovial thickening with MRI findings demonstrated a moderate but highly significant correlation between US and MRI measurements of thickened synovium in symptomatic OA knees ($r= 0.66$, $p<0.0001$) (Tarhan and Unlu 2003).

There have also been studies which have sought correlations between sonographic severity and biomarkers of synovium, cartilage and bone (Jung, Do et al. 2006; Kumm, Tamm et al. 2009). While HA is commonly associated with synovitis, COMP is more associated with cartilage breakdown but has been correlated with clinical signs of

synovitis (joint tenderness, non-bony swelling and warmth) in knee OA (Vilím, Vytášek et al. 2001).

Jung et al (2006), set out to examine whether abnormal features detected by US correlated with serum levels of HA and COMP. Fifty one patients with primary knee OA were examined using US by a single sonographer and fasting blood samples which were analysed for serum COMP and HA. Pain was also measured on a VAS and Lesquene's functional index. A range US features were examined though the scanning protocol and measurements were peculiar to the study and therefore the results cannot easily be compared or generalised. Synovial hypertrophy was measured in the supra-patellar recess where thickness >3.1mm was considered abnormal. Frequency data for synovial hypertrophy were not reported but serum HA was significantly higher in those with synovial proliferation than those without ($p=0.03$) and in those with larger effusion ($p=0.02$). Serum HA was positively correlated with capsular distension ($r=0.468$, $p=0.001$) and the length of medial and lateral osteophytes ($r=0.484$, $p< 0.001$; $r= 0.315$, $p<0.05$). Serum COMP showed a positive but not statistically significant correlation with US parameters. The results reflect what has been reported in other studies, specifically that there is a relationship between serum biomarkers and radiographic severity of knee OA and that biomarkers are elevated in people with US detected inflammation.

Kumm et al (2009) investigated the association between US findings and several bone and cartilage (COMP) biomarkers in 106 participants

with early knee OA. Ultrasound assessment followed EULAR guidelines and features were recorded as present or absent. Effusion and synovial hypertrophy were observed in 25% and 31% respectively. Several significant associations between US findings and biomarkers (including effusion and COMP) were reported but the strength of these associations was not included and therefore the validity of the findings remains uncertain. Furthermore, most associations were between US measures of bone and cartilage and markers of bone and cartilage turnover as opposed to inflammation.

Prevalence of synovial hypertrophy in knee OA is infrequently reported in the literature. Studies by Naredo et al (2005) and de Miguel (2006) surprisingly did not address synovitis in their studies of US features and pain. Synovitis was reported in the EULAR study where it was defined as hypo-echoic synovial hypertrophy (≥4mm thickness) and diffuse or nodular appearance (D'Agostino, Conaghan et al. 2005). Hypertrophy was observed in 16.7% of participants though a further 37% showed nodular or diffuse hypertrophy <4mm. The mean depth of hypertrophy in those with hypertrophy was 5.8mm (SD 3) compared to 1.3mm (SD 1.5) in those without. A strong association was found between synovial hypertrophy and joint effusion ($p<.001$) that remained strong even when continuous measures of depth (mm) were used ($r=0.51$, $p<0.001$) (D'Agostino, Conaghan et al. 2005).

Multivariate analysis showed that subjects with more severe OA (K&L score ≥ grade 3) and a moderate clinical effusion had an increased likelihood of synovitis on US (OR 2.2, 95%CI 1.33-3.64).

Other studies that have reported frequency data for synovitis in OA show wide variation. Tarhan et al (2003) reported hypertrophy in 34% of symptomatic knees using US, whereas Mermerci et al (2011), reported a much higher occurrence of 72% though this was a measure which incorporated effusion and hypertrophy and was considered an "inflamed supra-patellar pouch". Iagnocco (2010) used the same definition and cut-off values reported by D'Agostino and found synovitis to be present in 22% of participants with SOA.

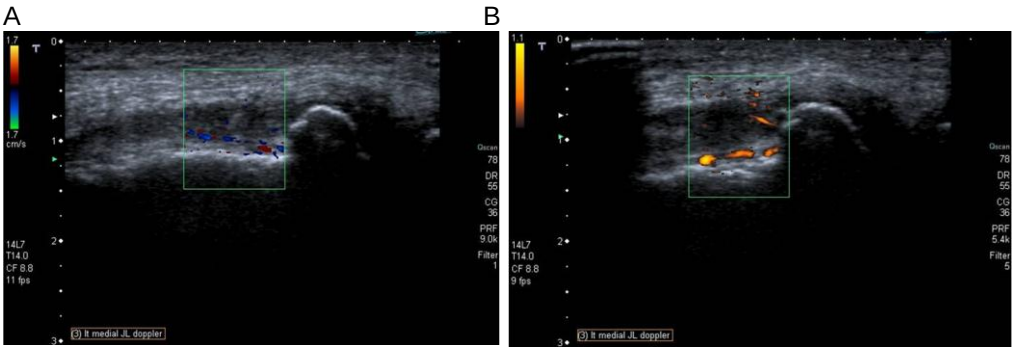
More recently, Wu et al (2012) reported on a study of 56 patients with bilateral knee OA of the same grade in each knee. Synovial hypertrophy was examined using US in the median longitudinal plane, and medial and lateral recesses of the suprapatellar pouch and was found in 93% of symptomatic knees and 63% of asymptomatic knees. Intra and inter-observer reliability was established as substantial (kappa was 0.80 and 0.79 respectively). A linear regression model with generalised estimating equation (GEE) was used to examine the association between synovitis and pain VAS scores. Synovitis in the medial compartment had a positive linear relationship with VAS pain scores on motion; pain VAS at rest and pain WOMAC scores after adjusting for age, sex, BMI and K&L grade. Of other US features only effusion was associated with pain on motion (Wu, Shao et al. 2012).

1.6.3 Power Doppler assessment of synovitis

The application of Doppler mode to greyscale US has added another dimension to the assessment of inflammation in OA. The Doppler principle refers to the fact that sound waves alter in frequency when they are reflected by moving objects. This principle is largely employed in the study of blood flow where sound waves are reflected off moving erythrocytes. Within rheumatology this has been applied in the detection and evaluation of hyperaemia (increased blood flow) in the synovium, which is considered to represent inflammation (Schmidt and Backhaus 2008).

Colour Doppler (CD) combines information on the velocity of the blood flow with grey-scale imaging to produce a colour signal where the direction of blood flow is indicated by red signals for flow directed towards and blue signals for flow directed away the transducer. Power Doppler (PD) measures the shift in energy caused by the Doppler effect, rather than velocity or direction of blood flow, and is therefore very sensitive to slower flow rates within small vessels (Torp-Pedersen and Terslev 2008), (Figure 1-15). For this reason, PD is more commonly utilised in musculoskeletal imaging.

Figure 1-15 US image showing colour (A) and power (B) Doppler activity at the medial knee joint line



Grading of Doppler signal is usually recorded dichotomously as present or absent or is scored on a four-point semi-quantitative scale (0-3) representing normal, mild moderate or marked Doppler signal. More recently this subjective scale has been improved by addition of descriptive bands for each grade (Table 1-4) (Iagnocco, Meenagh et al. 2010). Establishing reliability of Doppler in evaluating synovial perfusion is difficult due the subjectivity of the grading used (absolute values are generally not assigned) and is further complicated by the type of US equipment used, its settings and the experience of the operator.

Table 1-4 Semi-quantitative grading system for synovitis

Grade	Definition
Grade 0	Normal – no Doppler signal within the synovium (grey scale area)
Grade 1	Mild – up to 3 single spots or up to 2 confluent spots or one confluent and 2 single spots
Grade 2	Moderate – more than Grade 2 but <50% of the grey scale area
Grade 3	Marked – Doppler signal in >50% of the grey scale area

Most studies utilising US Doppler are concerned with inflammatory conditions and usually report on participants with a range of pathologies some of which include OA as a comparison. One such early study of Doppler was carried out in a small group of patients undergoing total knee replacement (TKR) for either OA (10) or RA (10) (Schmidt, Volker et al. 2000) where US assessments from two sonographers were compared to surgical and histological evaluation. The presence of

effusion and synovial hypertrophy were evaluated using conventional grey-scale US and measured in mm. Colour and or power doppler signal were graded on a scale of 0-3, where 0= normal and 1-3 represented subjectively increasing perfusion.

There was good agreement between sonographers and surgeons for grey-scale US features. Twelve out of thirteen surgically confirmed effusions were also detected by US. Synovial hypertrophy was observed in 4 OA patients by sonographers. Rates of agreement between the two sonographers was 80% and between sonographers and surgeons were between 80-85% and. No correlations were found between greyscale measures of effusion, synovial thickening, and the surgical or histological findings of pannus, therefore highlighting that grey scale US cannot differentiate pannus from non-destructive synovial hypertrophy.

Synovial perfusion detected by both CD and PD provided a higher correlation between pathological findings and the detection of pannus by histology. Mild or moderate perfusion was more common in patients without pannus, more marked or intense perfusion was more common in those with pannus. Pannus was present histologically in 8/10 patients with RA and 1/10 with OA. There was no correlation between the number of vessels seen on histology and the extent of synovial hypertrophy or intensity of perfusion in colour Doppler.

These findings were confirmed in a further study of OA and RA knees (n=23) undergoing TKR (Walther, Harms et al. 2001). US effusion,

synovial hypertrophy and PD signal were graded and samples of the synovium were graded for vascularity. Digital imaging analysis was also applied to both the histological samples and the US PD images. RA patients showed a significantly higher degree of synovial hypertrophy ($p<0.01$) than patients with knee OA but no significant difference in effusion. There was a very good correlation between semi-quantitative evaluation of PD signal and the pathologist's grading of vascularity ($r=0.89$ $p<0.01$) and between visual and digital analysis of PD signal ($r=0.89$ $p<0.01$). The findings also demonstrated that while moderate correlations existed between thickness of the synovial membrane, vascularity on histology and PD signal, having a thickened synovium or joint effusion does not mean that inflammation is present.

In contrast, a study of joints with known inflammatory disease found no significant correlation between US and histological evaluations (Koski, Saarakkala et al. 2006). Grey scale and PD findings were reported and graded on a semi-quantitative scale of 0-3 and compared to histological grading of synovial biopsy samples in a range of joints. Grey scale effusion, synovial hypertrophy and PD signal were found in 80%, 89% and 83% of patients with histological inflammation but no significant correlation was found between US and histological grading, though the authors state that a negative doppler signal does not preclude the possibility of synovitis. The authors also questioned the use of semi-quantitative grading for Doppler signal as this bears no correlation to

histopathological findings and instead suggested a dichotomous scale which demonstrated better sensitivity.

A larger study of Doppler activity focused on patients with knee OA (n=71) and 10 healthy volunteers (Kristoffersen, Torp-Pedersen et al. 2006). Patients studied had been referred to secondary care and fulfilled ACR criteria for knee OA and had radiographic osteophytes. Effusion, synovial hypertrophy and colour Doppler signal within the synovium was recorded as present or absent. Synovial hypertrophy was demonstrated in all patients and effusion in 86% (though no criteria or cut-off values were stated). Colour Doppler activity was recorded in 73% of patients, of whom more than half had a diastolic blood flow profile which the authors stated was indicative of inflammatory blood flow. By contrast, a trace effusion was observed in 1 control knee and a small isolated spot of colour Doppler signal in 2 control knees but no synovial thickening was found.

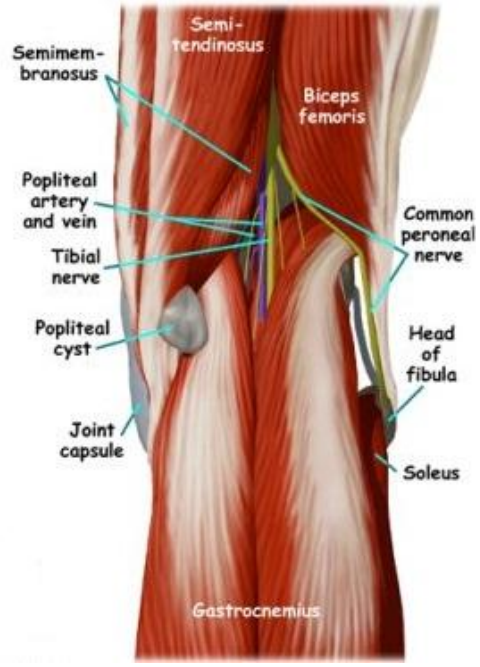
The authors reported no associations between US features and radiographic appearance, knee pain severity (VAS), function (WOMAC/Lequesne score) or CRP values, though they called for larger studies with longitudinal follow-ups to address this. There were a number of methodological issues in this study particularly regarding the number of US examiners, reliability of US findings, definitions and cut-off points for effusion and synovial hypertrophy, and lack of detail regarding radiographic assessment and healthy volunteers. The results should therefore be considered with caution.

Song et al reported a prevalence of PD signal in 63% of 41 participants with symptomatic knee OA and reported even greater sensitivity being found using contrast-enhanced US which detected synovitis in 95% of patients (Song, Burmester et al. 2008). Other studies to have reported Doppler activity in knee OA have shown show a very low prevalence. Iagnocco et al (2010) reported PD in only 3% of SOA participants while Mermerci et al (2011) reported no PD activity in any of the 143 SOA patients they assessed. It is not clear from the methodologies of these studies why there should be such a wide variation in the prevalence of PD signal.

1.6.4 Bursitis

Popliteal or “Baker’s” cysts are frequently reported in US studies of knee joints. A popliteal cyst is a composite of 2 bursae: the sub gastrocnemius bursa between the medial gastrocnemius tendon and medial femoral condyle and a second bursa between the medial gastrocnemius and semimembranosus tendon (Figure 1-16). The sub gastrocnemius bursa is the point of communication with the posterior joint capsule and the posterior extension represents the second bursa. The validity of US in detecting popliteal cysts is well established and the reported sensitivity is very high when compared to MRI (84-100%) (Ward, Jacobson et al. 2001; Tarhan and Unlu 2003). Inter-observer agreement among sonographers in the detection of popliteal cysts is also high (k=0.82, Naredo et al 2006; and k=1, (Scheel, Schmidt et al. 2005).

Figure 1-16 Anatomy of popliteal fossa and location of popliteal cyst



The reported prevalence of popliteal cysts in those with symptomatic knee OA ranges from 6.6% (Iagnocco, Meenagh et al. 2010) to 42.6% (Mermerci, Garip et al. 2011) and is low (2%) in age matched controls (Chatzopoulos, Moralidis et al. 2008). As with other US features, variations in definition, scanning protocols and the use of a minimal cut-off measure may explain the differences in prevalence.

De Miguel (2006) and Naredo (2005) both used the same criteria for defining Baker's but reported different rates of occurrence. De Miguel reported a prevalence of 37% patients with SOA and a rate of 15% in those with ROA whereas Naredo (2005) reported a lower occurrence of 22.2% for SOA and 0% in control participants with normal knees. The presence of a Baker's cyst increased the risk of presence of pain by an OR of 5.5 (95% CI 1-31.05) but did not associate with pain severity.

Frequency of popliteal cysts was considerably higher in those studies which did not define minimum cut-off points for the cyst size. Chatzopoulos et al (2008) reported popliteal cysts in 37% of patients with chronic knee OA. The mean size of cyst was 4.0cm (SD 1.1) cm (range 1.4–6.2) taken from its maximum diameter. The prevalence of cysts was not influenced by age, gender, symptom duration or limited range of movement. Knee effusions within the supra-patellar recess were almost invariably present and even when classified as large were not significantly different in prevalence in those with and without Baker's cysts. Early phase bone scans were able to discriminate between OA and non-OA knees and abnormally elevated synovial perfusion was observed more frequently in those with Baker's cysts suggesting that the presence of Baker's cysts is associated with synovial inflammation and its grade, and may play a role in its patho-aetiology.

Mermerci et al (2011) reported Baker's cysts to be present in 42.6% of 94 patients with SOA and 6.1% with ROA. Pain severity scores were significantly higher in those with Baker's cysts than those without.

Frequency of bursitis at other sites around the knee joint in SOA have been reported for infra-patellar superficial and deep bursae (8.6%) and the pes anserine bursa (6.2%) (de Miguel Mendieta, Cobo Ibáñez et al. 2006).

1.6.5 Responsiveness of Ultrasound

Surprisingly the responsiveness of US in knee OA has only been looked at in a small number of studies. Responsiveness can be considered in terms of internal and external dimensions. Internal responsiveness is the ability to demonstrate temporal changes or changes in response to an intervention whereas external responsiveness is the extent to which those changes correlate with the changes of other established measurements (Keen, Mease et al. 2011).

The effect of an intra-articular corticosteroid (IACS) injection on popliteal cyst size and synovial hypertrophy was measured in 30 patients with a diagnosis of knee OA and US confirmed popliteal cyst (Acebes, Sánchez-Pernaute et al. 2006). The cross-sectional area of the cyst, cyst wall thickness (hypertrophy), knee pain, range of movement (ROM) and circumferential swelling were measured at baseline and after 4 weeks. Internal responsiveness of US measures was demonstrated by the significant improvements in thickness of synovial hypertrophy ($p<0.01$) and popliteal cyst size ($p<0.05$) following the intervention. The decrease in cyst area was correlated with increased ROM ($r=0.38$, $p<0.05$) thus showing a degree of external responsiveness. Significant improvements in knee pain and swelling ($p<0.01$) were also reported but these did not correlate with US measures. The initial size of the cyst was not found to predict clinical or sonographic improvement.

Yoon et al (2005) investigated the effects of local corticosteroid injection in pes anserine tendino-bursitis (PATB) syndrome in patients with knee OA. Twenty six patients were clinically diagnosed with PATB based on history of knee pain in the previous two weeks, pain on activity and tenderness on palpation of the anserine bursa. US assessment included thickness of anserine bursa (mean of 3 measures), presence of tendonitis, and presence of bursitis (≧2mm). Pain VAS scores, global patient and physician assessments of improvement and WOMAC scores were also measured. Only 2 (8%) patients were found to have evidence of a bursitis on US examination. Other US findings included supra-patellar effusion (85%), osteophytes (62%), popliteal cysts (15%) and infra-patellar bursitis (4%).

Local steroid injection was administered to 17 patients though it is not clear how or why these participants were chosen. Significant improvements in knee pain, WOMAC pain and function scores were reported following injection (but the paper does not state a time point for re-assessment). Global patient response showed that no patients were worse following injection, 2 had complete symptom relief, 6 were good, 1 was fair and 8 remained unchanged. No significant changes were found in US measures.

Another study investigated the effects of shortwave diathermy (SWD) in 36 patients with knee OA on synovial hypertrophy in the supra-patellar pouch (Jan, Chai et al. 2006). SWD is purported to reduce inflammation and pain in OA via changes in the microcirculation and

resultant changes in tissue temperature so US would seem an ideal outcome measure to demonstrate these effects. US was used to measures synovial sac thickness (this incorporated both the synovium and synovial fluid) at the midline, the medial and lateral para-patellar recess and the sum of the thicknesses calculated and knee pain was assessed using a 10 cm VAS.

Participants in the study self-selected one of 3 treatment arms - SWD alone, SWD with NSAIDs as needed and a control group (no intervention). The intervention groups received their treatments over 30 sessions with follow-up US assessments at the end of the 10^{th}, 20^{th} and 30^{th} sessions and the control was re-assessed every 2-3 weeks. There were no significant difference between groups at baseline in terms of demographic or US measures though pain VAS was lower in the control group. Change in synovial sac thickness was reported as a percentage change in thickness from baseline. Changes in pain were reported as difference in pain from baseline.

The results showed that for both treatment groups there was a decrease in synovial sac thickness of up to 72% which was not observed in the control group, thus demonstrating some internal responsiveness of the measure. US measures of thickness continued to decrease with increasing treatment sessions ($p<0.0001$). Knee pain also showed a significant decrease in both treatment groups compared to the control group ($p<0.005$) but with no significant difference between

the groups. Correlation between synovial thickness and knee pain was weak (spearman r=0.17, p=0.05).

There are some obvious caveats to this study. The self-selection of treatment arms by participants may have influenced expectancy and thus may bias towards greater reported improvements. A standardised protocol was used for scanning and patient positioning but US measurement was peculiar to the study. It is not clear why the authors used a composite measure of synovial sac thickness rather than separate measures of effusion and synovial thickening. Furthermore reliability of US was not evaluated in this study and it is not explicitly stated whether the US assessments were carried out by one or more observers. In terms of the statistical analysis the data were not treated consistently with some data expressed as a percentage change (US measures) and others as actual difference (pain VAS).

A trial by Pendleton et al set out to evaluate whether US detected synovitis could be a useful clinical predictor of response to intra-articular cortico-steroid (IACS) injection (Pendleton, Millar et al. 2008). A baseline US assessment was carried out in 86 patients with confirmed symptomatic knee OA. Grey-scale features and PD signal were examined by a single sonographer according to published guidelines at the time (Backhaus, Burmester et al. 2001). The presence or absence of clinical effusion, US detected effusion, synovial hypertrophy, pes-anserine bursitis and popliteal cyst were recorded. Pain and function were assessed using the WOMAC questionnaire.

Follow-up evaluation took place at 1 and 6 weeks but only included pain and function in the re-assessment. There was no placebo arm to the trial, the authors' justification being that there is sufficient evidence of a positive effect of IACS in knee OA.

At baseline effusion were detected in 79% of patients using US (compared to 46% clinically), synovial hypertrophy was detected in 62%, popliteal cysts in 36% and PD signal was observed in 6% of patients. Pes anserine bursitis was found in 12% and patellar tendinopathy in less than 5%. Higher WOMAC scores at baseline were associated with significant improvements in pain and function at week 1 and 6 ($p<0.01$) but the presence of US effusion or synovial hypertrophy did not predict response to IACS injection. It is unfortunate that this study did not repeat the US assessment at the follow-up time points since this may have demonstrated responsiveness of US measures.

The ability of US features to predict response to IACS injection was also considered in a randomised placebo study of 79 military veterans with symptomatic knee OA (Chao, Wu et al. 2010). Baseline evaluation included the WOMAC questionnaire and grey-scale US evaluation of the supra-patellar pouch. Inflammation was defined as the presence of synovial hypertrophy with or without effusion. Pathological effusion was defined as $\geq$5mm. Assessments were carried out at baseline, 4 and 12 weeks. A small sub-sample (n=13) also provided blood samples for biomarker analysis at baseline (which included inflammatory cytokines

and chemokines, serum metalloprotease 1 and 3, and C-reactive protein levels.

Participants were randomised to the treatment (IACS injection) or placebo group (saline injection). WOMAC pain scores were comparable at baseline though total WOMAC scores were slightly higher (denoting worse function) in the treatment group. Significant improvements were observed in the treatment group between baseline and 4 weeks for WOMAC pain scores (p=0.001) and VAS pain scores (p=0.03) compared to placebo but this was not maintained at 12 weeks.

In the treatment group 47% of patients had evidence of synovitis on US at baseline; these were called “inflammatory” patients. There was no difference in the presence or absence of synovitis at 4 and 12 weeks for these participants. At 4 weeks there was no significant difference in improvement of WOMAC scores between those with and without synovitis but there was a significant improvement in pain subscale among “non-inflammatory” patients at 12 weeks. The presence of effusion at baseline did not have an effect on response. Biomarker analysis found no differences between “inflammatory” and “non-inflammatory” patients.

The authors concluded that the results suggest that “non-inflammatory” patients, that is those without synovial hypertrophy may benefit more from IACS than those with. However the generalisability of this study is questionable as the population comprised mainly male military veterans (97%) and the prevalence of secondary OA due to trauma was not

reported. Furthermore, the definition of “inflammatory” was limited by the scope of the assessment which was restricted to grey-scale US of the supra-patellar pouch in the midline. Further exploration of the joint as well as the use of Doppler US may have resulted in a different number of patients being categorised as “inflammatory”.

1.7 Management of OA

Universally guidelines on the management of OA promote an individualised patient-centred approach utilising both non-pharmacological and pharmacological modalities (American College of Rheumatology Subcommittee on Osteoarthritis (2000; Jordan, Arden et al. 2003; Zhang, Moskowitz et al. 2008; Hochberg, Altman et al. 2012). In 2008, the National Institute of Health and Clinical Excellence (NICE) recommendations published recommendations which included core treatments applicable to all persons with OA, specifically education and advice, exercise and weight loss in those who are overweight or obese (shown in inner circle, Figure 1-17) (NICE 2008). Relatively safe pharmacological treatments which include paracetamol and topical NSAIDs (middle circle) should be considered before the use of other adjunctive treatments (shown in the outer circle, Figure 1-17). These include pharmaceutical options, self-management techniques, surgery and other non-pharmaceutical treatments. Despite this, wide variation in management practice within Europe is reported (Denoeud, Mazieres et al. 2005; Mazieres, Scmidely et al. 2005). The efficacy of these

therapeutic intervention have most recently been reviewed by the OARSI Treatment Guideline Committee (Zhang, Nuki et al. 2010) and a summary of effect sizes for pain relief in shown in Table 1-5.

Figure 1-17 NICE summary of recommended treatments in the management of OA (NICE 2008)

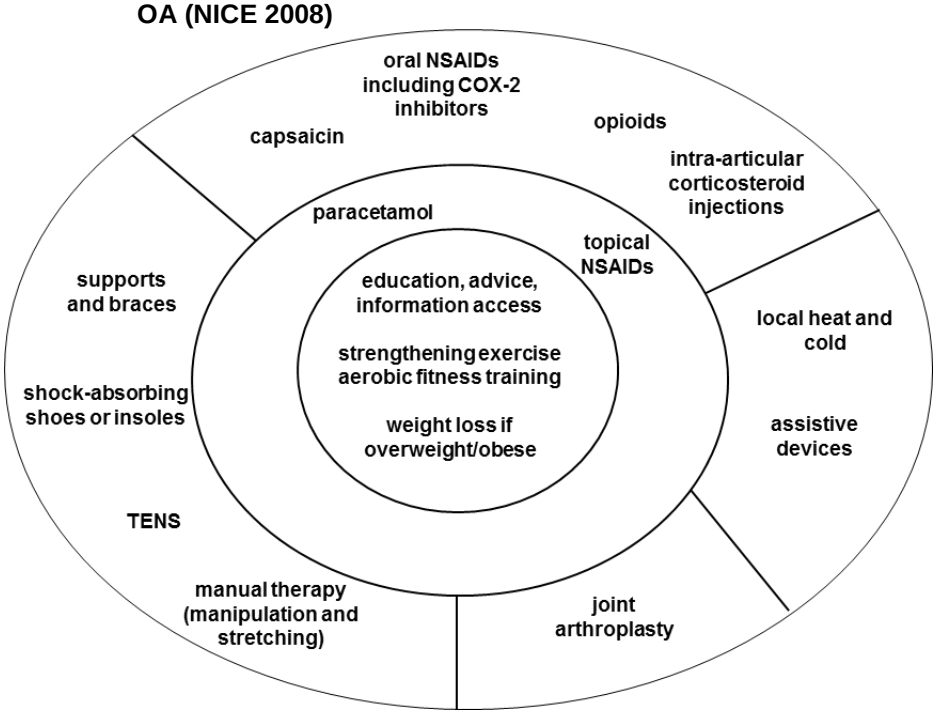


Table 1-5 Effect sizes with 95% CI for relief of pain for pharmacological and non-pharmacological treatments for OA. (Zhang, Nuki et al. 2010)

Modality	Joint	Effect size (95% CI)
Education	Hip & Knee	0.06 (0.03, 0.10)
Aerobic Exercise	Knee	0.52 (0.34, 0.70)
Strengthening	Knee	0.32 (0.23, 0.42)
Weight loss	Knee	0.20 (0.00, 0.39)
Acetaminophen (paracetamol)	Hip & Knee	0.14 (0.05, 0.23)
Oral Non-steroidal anti-inflammatory drugs	Hip & Knee	0.29 (0.22, 0.35)
Topical non-steroidal anti-inflammatory drugs	Knee	0.44 (0.27, 0.62)
Intra-articular corticosteroid injection	Knee	0.58 (0.34, 0.75)
Surgical Lavage/debridement	Knee	0.21 (-0.12, 0.54)

Intra-articular corticosteroid injections are recommended as an adjunct for the relief of moderate to severe pain. The effects size for the treatment of pain is relatively large and is strongest for the first week following the injection (0.72, 95% CO 0.42, 1.01), diminishing over subsequent weeks with a pooled ES of 0.58 (0.34, 0.75). Guidelines by the American College of Rheumatology (2000; Hochberg, Altman et al. 2012) suggest that intra-articular injection of corticosteroid may be of particular benefit to patients with signs of local inflammation but whether inflammatory features can predict treatment response has yet to be substantiated.

Further to this, the effect size of placebo should not be under-rated since it is often higher than the additional benefit conferred by the specific effect of an individual treatment. A systematic review of 198 randomised controlled trials reported an overall effect size of 0.51 (95% CI 0.46, 0.55) for placebo compared to 0.03 (-0.13, 0.18) for untreated controls (Zhang, Robertson et al. 2008). The magnitude of the placebo response varies between treatments but is higher with increased effect size of the active treatment, increased baseline symptom severity and invasive route of delivery.

To date, no disease-modifying OA drugs have been found to reverse or halt the progression of OA, though several drugs have demonstrated some effects. They can be divided in to those targeting cartilage, inflammatory pathways and subchondral bone. Identification of sub-groups of patients who may benefit from such drugs and improved

outcome measures are important areas for development in the future (Davies, Graham et al. 2013).

1.8 Study Rationale

Ultrasound evaluation of symptomatic OA knees has shown that grey-scale features of joint effusion and synovial hypertrophy are not uncommon (43-79%), and that Doppler activity may be present in a subset of patients. Most studies have been carried out in hospital-referred patients who are likely to represent patients with more severe structural changes and pain. The prevalence of these features has not yet been fully explored in community participants.

The relationship between US features, ROA and knee pain suggest that US features are more common as radiographic severity increases and in the presence of knee pain but the extent to which they reflect joint inflammation is as yet unconvincing. Furthermore their responsiveness to change has not yet been determined and therefore their potential role for monitoring disease progression or outcome following intervention is unclear.

1.9 Thesis Aims

The aims of the thesis are to determine the frequency of US detected inflammatory features in the knees of community-derived adults and the

relationship of these features to knee pain, structural damage on x-ray, and symptoms and signs that support inflammation (pain, clinical effusion, self-reported stiffness and biomechanically assessed stiffness).

1.9.1 Primary Objectives

To determine in a community sample:

1. The frequency of US features of joint inflammation (effusion, synovial hypertrophy and PD signal) in normal knees, painful knees, radiographic OA knees and symptomatic OA knees.

2. To determine if US features of inflammation are associated with structural change on x-ray, knee pain or clinical symptoms and signs of inflammation (night pain, clinical effusion, self-reported stiffness and biomechanically assessed stiffness) and function.

1.9.2 Secondary Objectives

1. To determine if US features of inflammation change over time (over 3 months) in tandem with fluctuations in knee pain in SOA knees and healthy control knees.

2. To determine if US features of inflammation change following an accepted intervention for knee OA pain (IACS injection) or a placebo injection in SOA knees.

1.9.3 Hypotheses

Hypothesis 1. US features of inflammation will be more common in knees with radiographic changes, irrespective of pain status, and will be more pronounced in people with knee pain.

Hypothesis 2. US features of inflammation will be independently associated with an increased risk for knee pain

Hypothesis 3. Changes in US features of inflammation in SOA knees will correlate with changes in reported knee pain over time and also following an intervention for knee pain.

2 Methods

This chapter starts by describing the training undertaken in musculoskeletal ultrasonography prior to the onset of the study. Following a statement of ethical approval for the study, recruitment for the study is described. Study designs are then described for:

- establishing the intra and inter-observer reliability of US measures and biomechanical assessment of joint stiffness and damping co-efficients
- an evaluation of diurnal variation of US measures
- a cross-sectional multiple group comparison study and follow-up analysis
- an intervention study looking at pain and US response following intra-articular injection of a cortico-steroid and placebo using a randomised cross-over design

Detailed protocols for the clinical, US and radiographic assessments undertaken are presented. The methods used for checking data accuracy and the treatment of missing data are described. Finally, study sample size and power are stated and the statistical analysis is described.

2.1 Ultrasonographic Training

Prior to the start of the study, US training was undertaken over a four month period under the supervision of Dr Philip Courtney, Consultant Rheumatologist and Dr Khalid Latief, Consultant Radiologist at Nottingham University Hospital NHS Trust. Training included:

- One to one teaching sessions with demonstrations on a variety of patients and joints whilst attending a rheumatology clinic.
- Self - directed learning using recommended texts (Bradley and O'Donnell 2002; Martino, Silvestri et al. 20006), DVDs (Arthritis Research UK DVD – Musculoskeletal Ultrasound: a beginner's guide to normal peripheral joint anatomy) and on-line educational resource available on the website of the EULAR Working Group for Musculoskeletal Ultrasound in Rheumatology http://www.sameint.it/eular/ultrasound)
- One to one teachings on the principles of musculoskeletal ultrasound and practical skills for scanning at the knee joint including patient positioning, transducer alignment, multiplanar scanning, optimising images, detection of effusion, synovial hypertrophy, bursitis, osteophytes and cartilage damage, use of power and colour Doppler, and recognising and minimising artefacts and saving images and cine-clips.

Over 40 hours training was accrued including scanning of normal knees, knee joints with osteoarthritis and knees joints with inflammatory pathology referred to the rheumatology clinic at Nottingham University

Hospital NHS Trust. Competency was not formally assessed at the end of the training period although both trainers were satisfied with the quality and interpretation of the US images produced by the author at end of the training period. At present there is no standardised model of training in musculoskeletal US but similar approaches that undertaken here have been shown to achieve competency (Filippucci, Unlu et al. 2003; Atchia, Birrell et al. 2007). Intra and inter-observer reliability was formally assessed for key US measures.

2.2 Ethics

Ethical approval for the study was granted by the Derbyshire Research Ethics Committee in December 2009 (Ref 09/H0401/83). Research & Development Approval was granted by the Nottingham University Hospitals NHS Trust in January 2010 (CSP Ref: 28550). All participants gave their informed written informed consent. Supporting documentation, participant information sheets and consent forms are included in Appendix 1.

2.3 Recruitment

Participants were recruited from databases held by the Division of Academic Rheumatology, of community dwelling men and women over the age of 50. All persons had previously participated in community studies of knee pain or knee OA (as either cases or controls) and had

consented to being approached for future research (Limer, Tosh et al. 2009; Doherty, Hawkey et al. 2011; Ingham, Zhang et al. 2011).

Participants were purposefully recruited with the aim of attaining fifty participants in each of the four comparison groups (control knees, knee pain, radiographic OA, symptomatic OA) for the main cross-sectional study. A phased approach was used, recruiting as many participants as possible from a single study source before recruiting from the next. Potential participants were invited to take part in this study. A reminder letter was sent to non-responders after 6 weeks.

Exclusion criteria for the study included:

- Diagnosis of inflammatory arthritis
- Clinical Hip OA
- Steroid injection to either knee within the previous 3 months
- Knee arthroplasty
- Significant neurological disease (e.g. Parkinson's disease, multiple sclerosis, stroke)
- Chronic widespread pain / diagnosis of Fibromyalgia
- Steinbrocker Grade IV

Participants were allocated to one of four comparison groups based on their current knee pain and radiographic data (which may have changed since their original involvement in the previous studies). While participants were not matched on an individual basis, there was an attempt to match (where available) gender and age within a 5 year age band across the four groups. Group numbers were monitored

throughout the study and recruitment continued until the comparison group was full or no further participants could be recruited for that group.

The primary source of study participants was a cohort study of incident knee pain in the community (Ingham, Zhang et al. 2011). This study followed-up participants from two earlier studies, a survey of knee pain in the community and an exercise intervention study for knee pain, both of which were recruited direct from the community using a questionnaire (O'Reilly, Muir et al. 1996; Thomas, Muir et al. 2002). During 2007- 2008, 5,479 participants were followed-up of which 3,109 responded and 424 were x-rayed. After removing those who had not given additional consent to be approached (322) and those who had undergone TKR (76), 328 potential participants with knee pain data and radiographs were identified.

The second source of recruitment was a randomised controlled trial of non-prescription analgesics for people with chronic knee pain (Doherty, Hawkey et al. 2011). Original study participants (n=892) were recruited direct from the community between 2007-2008, were over 40 years of age and had moderate knee pain for most of the previous 3 months but were not under direct medical supervision for their pain. Radiographic OA was present in 63% (n=559) of participants. Exclusions criteria included concomitant rheumatic disease, joint misalignment, recent joint disease-modifying drugs and gastrointestinal, renal or hepatic conditions.

Thirdly, participants were recruited from a population based case-control study (Genetics of OA and Lifestyle (GOAL) study). Between 2002 -2006 Caucasian men and women aged between 45 and 86, were recruited as cases (hip or knee OA) (n=2049) or controls (n=1123) for genetic association and gene-environmental interaction studies of knee and hip OA (Limer, Tosh et al. 2009; Valdes, McWilliams et al. 2010; Valdes, De Wilde et al. 2011). All cases and controls were excluded if they had rheumatoid arthritis, ankylosing spondylitis, Paget's disease of the bone adjacent to the hip or knee joints, trauma directly to the index joint, Perthe's disease, childhood hip dysplasia, polio or congenital lower limb deformities.

2.4 Study designs

2.4.1 Intra and Inter-observer reliability of US measures

Intra-observer reliability in the acquisition of the inflammatory US measures was tested for the study assessor (MH) by scanning the knees of 14 participants (28 knees) on two separate days within a seven day period.

Inter-observer reliability was tested against a consultant radiologist with clinical expertise in musculoskeletal ultrasound (Dr Khalid Latief (KL), Consultant Musculoskeletal Radiologist at Nottingham University Hospitals NHS Trust). The knees of 5 participants (10 knees) were scanned independently by MH and KL on the same day.

Participants were scanned at the same time of day for the key US features listed in Table 2-1. Both investigators were blinded to previous measures and each other's measurements. Repeat WOMAC and ICOAP questionnaires and a pain VAS were also completed at each visit.

Intra and inter-reliability of structural features (the presence of osteophytes, and measurement of FAC both in transverse and longitudinal planes) were assessed in a subset of 10 knees.

Table 2-1 US Measures included in Reliability study

Inflammatory US Features	**Dichotomous**	**Continuous data**	**Ordinal data**
Effusion	absent/present	Max diameter (mm)	
Synovial hypertrophy	absent/present	Max thickness (mm)	
Popliteal cyst	absent/present	Max depth (mm)	
PD Signal	absent/present	-	Grade 0-3
Structural US features			
Osteophytes	absent/present	Depth (mm)	
FAC Transverse view		Thickness (mm)	
FAV longitudinal view		Thickness (mm)	

PD signal was recorded for each of 3 sites (suprapatellar pouch, medial joint line and lateral joint line).
Osteophytes were recorded in any compartment.

2.4.2 Reliability of biomechanical assessed stiffness measures

Intra-observer reliability of the pendulum test used to calculate biomechanical stiffness and damping co-efficient was carried out on ten participants (20 knees with and without knee OA). Both knees were

tested on two separate days within one week. Testing was carried out under the same conditions, at the same time of day by the same assessor (MH).

2.4.3 Diurnal variation of US measures

Ten knees from 5 participants with bilateral SOA underwent US examination of both knees at two time points, morning (between 9 and 10am) and afternoon (3 and 4 pm) during the same day. The same participants were re-examined on two further days, morning and afternoon, one week apart. Knees were examined by one sonographer (MH) for the features listed in Table 2-1. WOMAC and ICOAP questionnaires and a pain VAS were completed in the morning session of each assessment day.

2.4.4 Cross-sectional comparative study

The main study had a cross-sectional multiple group comparison design and was implemented to achieve the primary objectives (Figure 2-1). Participants underwent clinical, ultrasound and radiographic assessment of both knees. The study population comprised of four comparison groups based on the presence or absence radiographic OA and the presence or absence of knee pain (Table 2-2).

Radiographic OA was determined from knee x-rays taken in a standardised posterior-anterior, semi-flexed weight bearing and skyline views of both knees. Radiographic scoring was undertaken blind to the

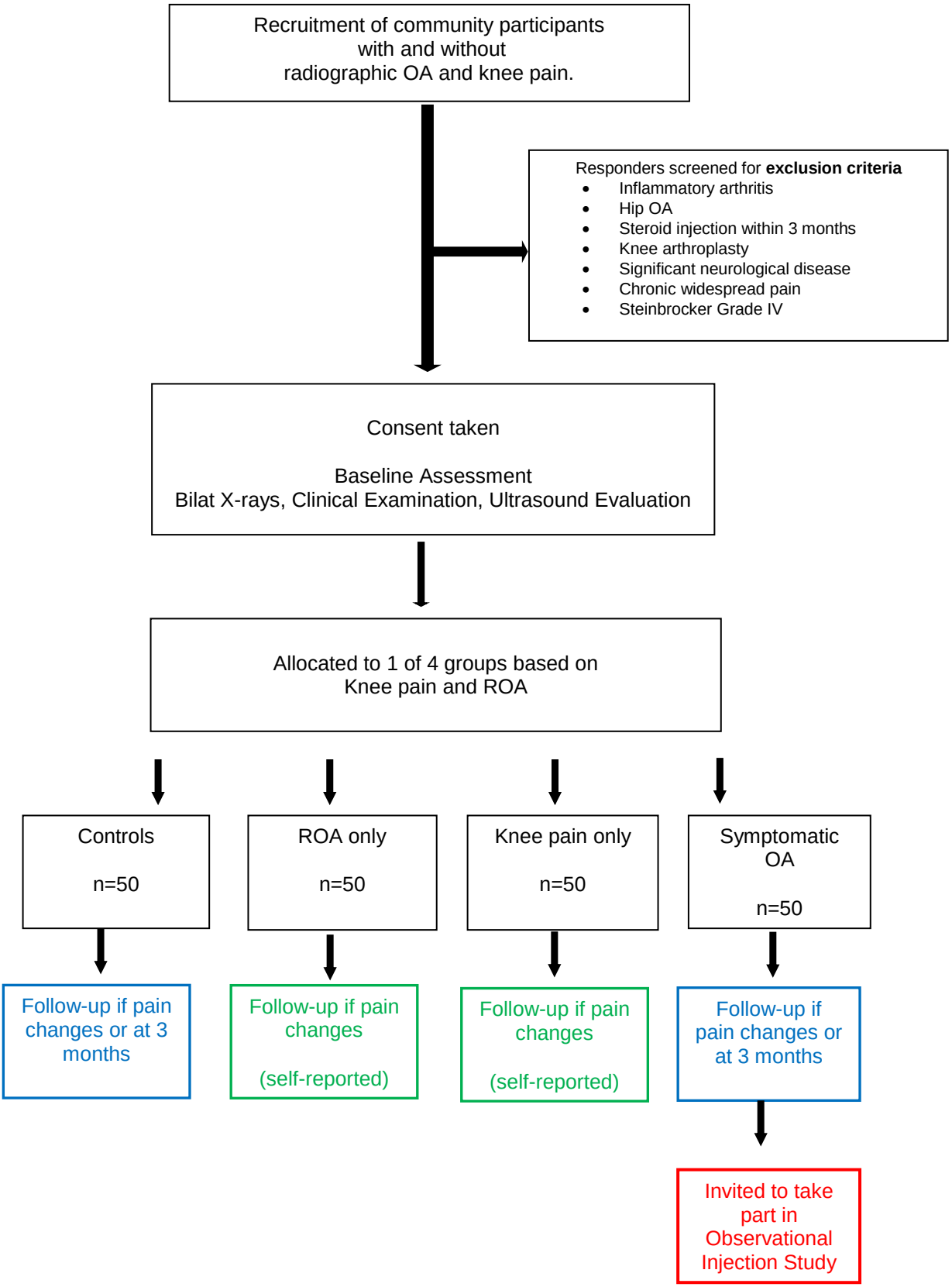
clinical and pain status of the participants. For the purpose of allocating participants to groups for analysis ROA was classified according to the overall Kellgren & Lawrence grade. Grades0/1 were classified as none/minimal radiographic change and grade 2/3/4 were classified as positive.

Knee pain was classified according to responses on the WOMAC Index. Reporting of at least moderate pain during the previous week in one or more of the pain items of the index was considered pain positive. Those reporting no pain or mild pain in this section were classified as pain negative.

Table 2-2 Study groups for cross-sectional comparative study.

	ROA absent	**ROA present**
Knee Pain absent	*Controls*	ROA *(Radiographic OA without knee pain)*
Knee Pain present	KP *(Knee pain only)*	SOA *(Symptomatic OA)*

Figure 2-1 Flow chart of cross-sectional multiple group comparison study**, follow-up evaluation and entry into intervention study**



2.4.5 Follow-up evaluation

A follow up evaluation (at 3 months) of participants in the control and SOA groups examined temporal changes in pain and US findings. In addition, participants who self-report a significant change in pain (in either direction) during the 3 months following initial assessment were re-assessed. A significant change in symptom pain was defined as an increase or decrease in the worst item score reported in the WOMAC Pain subscale.

2.4.6 Intervention study

After completing the multiple group comparison study and follow-up evaluation, participants with symptomatic OA were invited to take part in an intervention study (Figure 2-2). Participants were randomly allocated to a two treatment sequence (balanced randomisation using 2 blocks): intra-articular saline followed by corticosteroid injection or intra-articular corticosteroid followed by saline injection. A cross-over design was used to ensure each participant received both treatments. The purpose of this intervention was not examine the efficacy of the treatments but to change the pain status and to observe the relationship between the changes of pain status and US features of inflammation. There is little systematic evidence to guide the choice and dose of corticosteroid but Methylprednisolone is commonly used within clinical practice in England and the manufacturers recommended dose

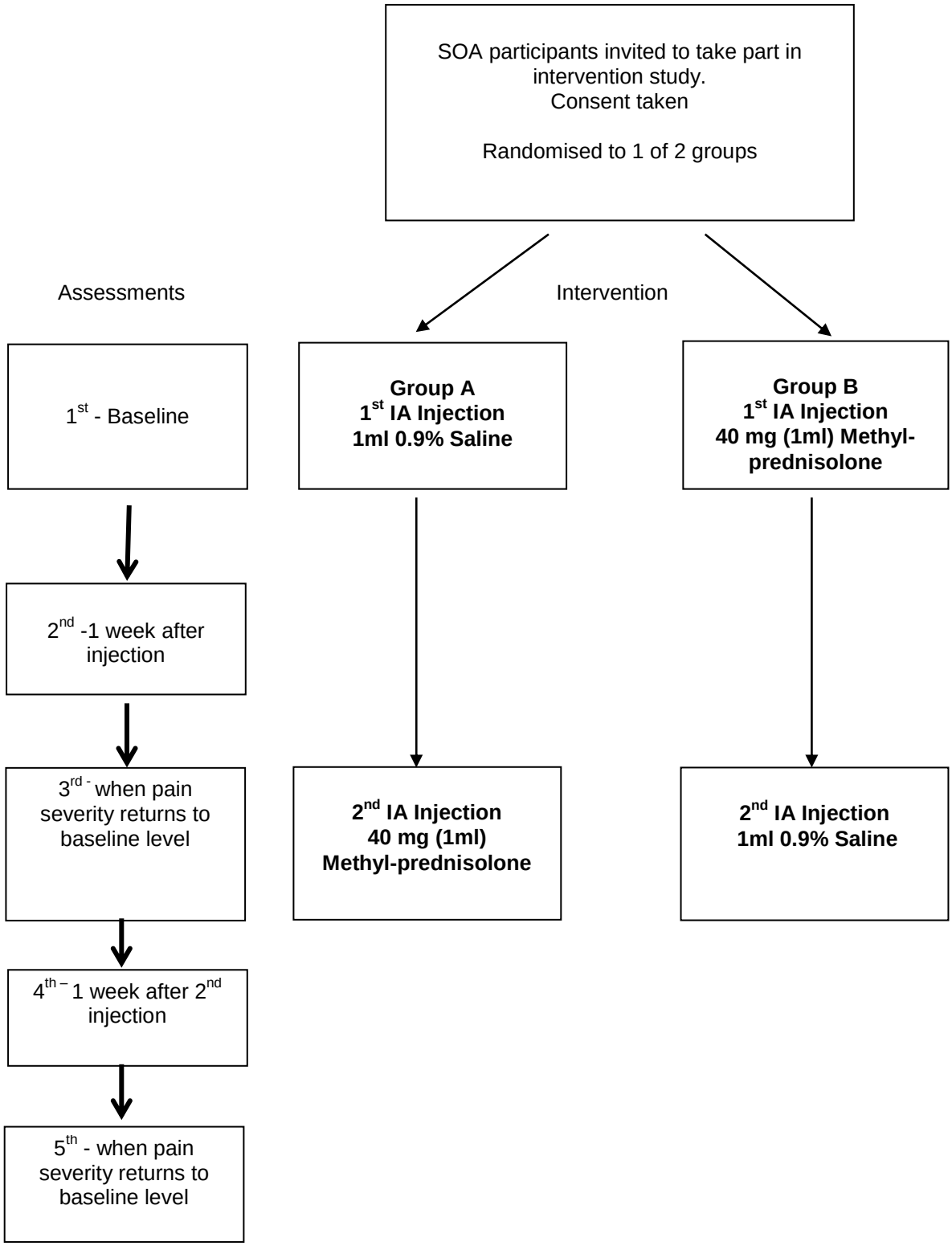
of 40mg has been shown to be effective for pain relief (Jones and Doherty 1996; Pyne, Ioannou et al. 2004). A recent review concluded that there is no evidence to support the use of corticosteroid doses beyond those recommended by the manufactures (Douglas 2012).

All participants consenting to the intervention study had a baseline assessment of both knees which included assessment of clinical effusion, WOMAC and ICOAP questionnaires, visual analogue scales (VAS) for knee pain and a US examination of both knees (for effusion, synovial hypertrophy, PD signal and popliteal bursitis).

Participants were then randomised to receive the first intra-articular injection of either 40mg (1ml) methylprednisolone or placebo 1ml (0.9%) saline, to their most painful knee. Where both knees were equally painful, one knee was chosen randomly (computer generated). Injections were delivered by an experienced rheumatology consultant (MD) and both participants and assessors were blinded to the order of the injections.

A second assessment was performed one week following the 1st injection and a third when the partcipants reported their pain to have returned to their pre-injection level which was monitored by a weekly phone call. At the third assessment, the 2nd injection was also administered. A fourth assessment was performed one week following the 2nd injection and a final fifth assessment when the participant reported their pain severity had returned to their pre-injection level again.

Figure 2-2 Flow chart of intervention study



2.5 Clinical assessments

Participants attended the Clinical Sciences Building at Nottingham City Hospital for their assessments (Table 2-3). All assessments for the cross-sectional group comparison study and follow-up evaluations were carried out by the author (MH) who was blind to participants' radiographic status but aware of their pain status at the baseline assessment. All assessments for the injection study were carried out a research nurse (SD) with experience in sonography who was aware of the participant's pain and radiographic status (all had symptomatic knee OA).

All participants were asked where appropriate to discontinue taking non-steroidal anti-inflammatory drugs (NSAIDs) 48 hours before attending. This would ensure an adequate washout period of five times the half-life period for most short-acting NSAIDs (including in ibuprofen, and diclofenac) (Brater 1988). Paracetamol could be taken for rescue pain-relief up to the evening before their appointment.

Table 2-3 Summary of Clinical Assessments

Anthropometric measures	Body mass index (BMI) (kg/m^2)
Pain	WOMAC Knee Index (Pain subscale) ICOAP Questionnaire (Knee version) Current knee pain - VAS (mm) Current medication Current pattern of analgesic use
Knee joint examination	Knee pain map pain & tenderness Joint deformity Effusion Warmth Range of movement
Function	WOMAC Knee Index (Function subscale) Muscle strength –Quadriceps and Hamstring muscles Get Up and Go (GUG) test 3m (seconds) Get Up and Go (GUG) test 50ft (seconds)
Stiffness	WOMAC Knee Index (Stiffness subscale) Duration of morning stiffness (sec) Biomechanical assessed stiffness co-efficient (Nm/rad) and damping co-efficient(Nm/rad/sec)
Ultrasound Evaluation	Joint effusion (present/absent) (mm) Grey scale synovitis (present/absent) (mm) Power Doppler signal (present/absent) (grade 0-3) Bursitis (present/absent) (mm) Osteophytes (present/absent) (mm) Femoral Articular Cartilage (mm)
Radiographic Evaluation	Standardised bilateral weight bearing tibio-femoral and skyline patello-femoral x-rays

2.5.1 Body Mass Index (BMI)

BMI (kg/m^2) was calculated from height measured using a standard stadiometer to the nearest 0.5cm and weight using a set of standard aviary scales (to the nearest 0.1kg).

BMI was classified as normal (BMI <25 kg/m2), overweight (BMI 25 - 30kg/m2) and obese (BMI >30 kg/m2).

2.5.2 Knee pain

A variety of pain measures were used in the clinical assessment. The presence of unilateral or bilateral knee pain and the most symptomatic knee (Index knee) if any, was recorded. Two questionnaires relating to knee pain and disability experienced over the past week were administered (Appendix 2).

2.5.2.1 Western Ontario MacMaster (WOMAC) OA Index

The WOMAC index for the knee joint (Bellamy, Buchanan et al. 1988) is a 24 item questionnaire relating to pain, stiffness and function. Items are rated on a five point Likert scale and subscales calculated for pain (0-20), stiffness (0-8) and function (0-68). Knee pain was classified according to responses on the WOMAC Index as previously described by Baker et al (2010). Reporting of at least moderate pain during the previous week in one or more of the five pain items of the index was considered pain positive. Those reporting no pain or mild pain in this section were classified as pain negative.

2.5.2.2 Intermittent and Constant Osteoarthritis Pain (ICOAP) Knee Questionnaire

The ICOAP questionnaire (Hawker, Davis et al. 2008) is an 11-item tool designed to assess knee pain taking both constant and intermittent pain into account. Items are scored on a 5 point Likert scale with 5 items considering constant pain (subscore 0-20) and 6 items considering intermittent pain (subscore 0-24). Intermittent KP was scored dichotomously using the ICOAP intermittent subscale. Those who reported at least moderate intensity intermittent knee pain were considered positive. Those reporting none or mild intermittent pain intensity were considered negative. Constant KP was scored dichotomously using the ICOAP constant subscale. Those who reported at least moderate intensity constant pain were considered positive. Those reporting none or mild constant pain intensity were considered negative.

2.5.2.3 Night pain

The presence of night pain was scored dichotomously as present or absent using question 3 from the WOMAC index. Those reporting at least moderate pain at night were considered night pain positive. Those reporting no pain or mild pain at night were considered night pain negative.

2.5.2.4 Pain severity

A current knee pain score was taken from a 100mm visual analogue scale (VAS) where 0 represented NO PAIN and 100mm represented EXTREME PAIN.

Change in knee pain for follow-up evaluations was defined as an increase or decrease in pain VAS of 15mm or an increase or decrease of the worst item score reported in the WOMAC Pain subscale.

2.5.3 Drugs history and current medication

Current medication related to knee pain was documented. Participants were asked to describe their pattern of analgesic use from the following options (Blamey, Jolly et al. 2009):

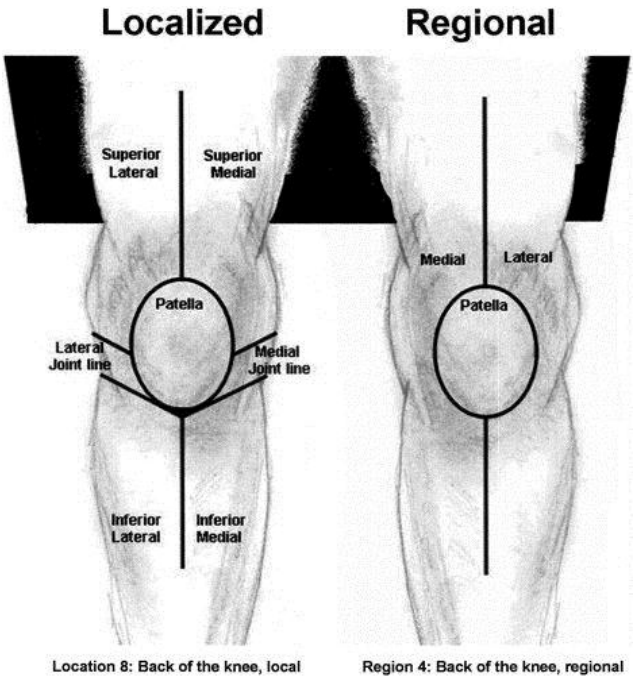
- Always, every day
- When pain gets too bad
- Depends on how bad pain is
- Every day when having a bad patch, otherwise only as needed
- Usually before bed
- Usually before exercise

2.5.4 Knee pain location

Knee pain was recorded on a knee pain map and the pattern of the knee pain characterised as localised, regional or diffuse (Thompson, Boudreau et al. 2009) (Figure 2-3). The use of this interviewer-administered assessment of knee pain location has been shown to be a reliable method for the identification of localised and regional pain

patterns in patients with knee pain (κ = 0.7-0.9 and 0.7-0.8 respectively).

Figure 2-3 Knee pain map



Localised pain was defined by the use of 1 or 2 fingers to point to a specific area of pain, whereas regional pain was defined by the use of all of the fingers or the whole hand to cover larger region. Participants who were not able to identify areas of localized or regional pain and/or who said that the pain was “all over” the knee were classified as having diffuse pain.

Participants were allowed to identify multiple areas of localized and/or regional pain but were classified as diffuse if: >3 areas of localized pain or >2 areas of regional pain or 1 area of localised and 1 non-overlapping regional area of pain.

2.5.5 Joint tenderness

Site(s) of maximal tenderness on palpation were recorded on a separate knee pain map.

2.5.6 Joint deformity

Valgus or varus alignment was documented according to validated line drawings (Ingham, Moody et al. 2010).

2.5.7 Effusion

Joint effusion was assessed and graded using the “Stroke test” (Sturgill, Snyder-Mackler et al. 2009). The stroke test was performed with the participant in sitting on a plinth with their knees extended. An upward stoke was applied from the medio-femoral joint line towards the suprapatellar pouch 2-3 times followed by a single downwards stroke on the lateral distal thigh just superior to the suprapatellar pouch. An observed wave of fluid at the medial side of the knee was indicative of an effusion. The effusion was graded according to a scale where 0=no/trace wave produced, 1= large bulge produced, 2= spontaneous wave without down stroke, 3= unable to move effusion from medial aspect of the knee. The inter-rater reliability of this method (κ = 0.61 95%CI 0.54 -0.81) has been shown to be higher than other methods of assessing clinical effusion (fluctuation test κ = 0.37 ; patellar tap test κ = 0.21 (Fritz, Delitto et al. 1998)). For analysis, clinical effusion was dichotomised as present (≥ grade 1) or absent (grade 0/trace).

2.5.8 Joint Warmth

The knee joint was palpated anteriorly and along the medial and lateral joint lines with the dorsal aspect of the examiner's hand for increased temperature relative to the surrounding tissue and contra-lateral knee joint.

2.5.9 Range of Movement (ROM)

Knee ROM was measured using a standard clinical goniometer in degrees using a standardised protocol (Clarkson 2005).

The axis of the goniometer was placed over the lateral condyle of the femur, and the arms of the goniometer in line with the lateral malleolus of the ankle and the greater trochanter of the femur.

Maximal active knee joint flexion was measured with the participant in supine in reclined long sitting. Maximal knee joint extension was measured with the participant's foot elevated on a small bolster to allow for hyperextension if present. Overall ROM was calculated as the angle (in degrees) between full available extension and flexion.

For analysis overall ROM was divided by tertiles using SPSS to create third groups. Tertile 1 represented greatest ROM whilst tertile 3 represented those with the most restricted ROM.

2.5.10 Joint instability

Medial and lateral collateral and cruciate ligaments were tested for instability (Doherty and Doherty 1992).

2.5.11 Muscle strength

Maximal isometric strength of the quadriceps and hamstring muscles was tested using a manual muscle tester (MMT) (Nicholas Manual Muscle Tester; Lafayette Instruments). The MMT displayed the maximal force generated in kilograms (Figure 2-4).

Figure 2-4 Manual muscle manometer

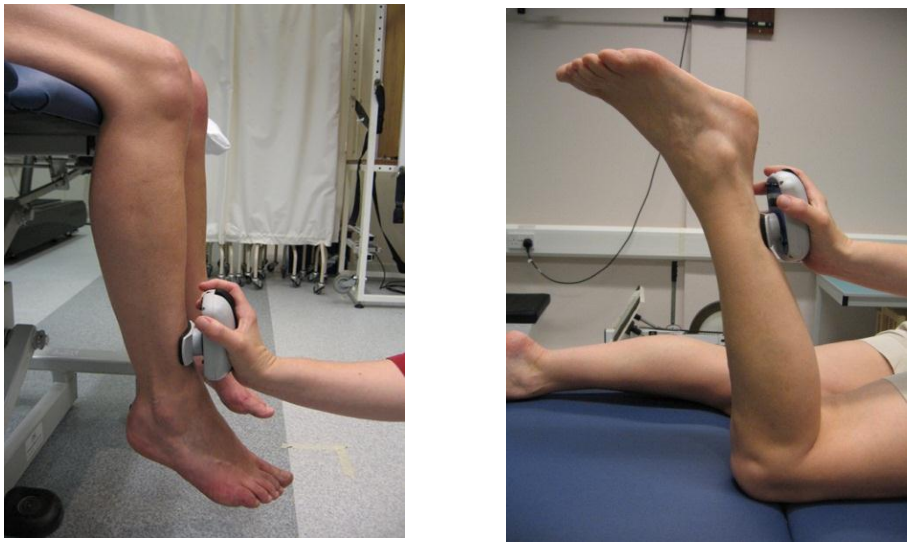
Quadriceps testing was performed with the participant in upright sitting on the edge of an examination plinth with the knee joints in 90° flexion (Figure 2-5 A). The MMT was placed on the distal tibia above the ankle joint. Participants were asked to push their leg against the pad of the manometer as strongly as possible for a period of 5 seconds. The examiner was positioned in a lunge position using their body weight to

prevent any movement of the lower leg and knee joint, maintaining a static contraction.

Hamstring strength was measured with participants in prone lying with the knee joint flexed to 90° (Figure 2-5 B). The MMT was placed at the distal calf above the insertion of the Achilles tendon. Participants were asked to push their calf against the pad as strongly as possible for 5 seconds. The examiner stood facing caudally to resist the movement.

Figure 2-5 Strength testing using the MMT: (A) Quadriceps (B) Hamstrings

A **B**



Three attempts were recorded on each limb for quadriceps and hamstring muscles and the average force calculated. For analysis average strength scores were divided by tertiles to create third groups for comparison. Tertile 1 represented those with strongest muscles whilst tertile represented those with the weakest muscles.

2.5.12 Function - WOMAC Function subscale

A summated score (0-68) for function was calculated for WOMAC function from the 17 questions in the index.

2.5.13 Function: Get Up and Go (GUG) tests

Two simple tests of lower limb performance were assessed. The Get Up and Go (50ft) test which measured the time in seconds it took to rise from a chair and walk 50ft as fast as possible along a level and unobstructed walk way (Hurley, Scott et al. 1997). A second Get Up and Go (3m) test measured the time it took to rise from a chair, walk 3m, turn around, walk back and sit down (Mathias, Nayak et al. 1986). Participants were allowed to use a walking aid where required.

Scores for the 3m test were dichotomised into those who could complete the test in 10 seconds or under (normal mobility) and those who took longer than 10 seconds to complete the test (impaired mobility) (Podsiadlo and Richardson 1991).

2.5.14 Morning stiffness

Maximal duration of early morning stiffness in or around the knee was recorded in minutes. A dichotomous variable was created for morning stiffness lasting longer than 30 minutes.

2.5.15 WOMAC – Stiffness subscale

A summated score (0-8) was calculated for WOMAC stiffness from the 2 questions in the index.

A dichotomous score was also calculated. Those reporting at least moderate stiffness during the previous week in one or more of the stiffness items of the index was considered positive. Those reporting none or mild stiffness in this section were classified as negative.

2.5.16 Biomechanical measures of joint stiffness

Two objective measures of knee joint stiffness (stiffness and damping co-efficients) were obtained by tracking the angular displacement of the knee during a “passive pendulum test” using the Coda motion analysis system (Figure 2-6).

Figure 2-6 Coda Motion analysis system camera

Infra-red LED (light emitting diode) markers were attached to both limbs at the greater trochanter, lateral knee joint line on the femoral condyle, and lateral malleolus of each limb using double sided adhesive tape (Figure 2-7). Participants sat on the edge of plinth with the test knee positioned in a loosely packed position (around 75° knee flexion) and their contra-lateral limb supported on a stool. The test limb was

extended passively by the investigator just short of full extension before releasing the lower leg and allowing it to oscillate freely until it came to a stop (Figure 2-8).

Figure 2-7 LED markers used for capturing notion data

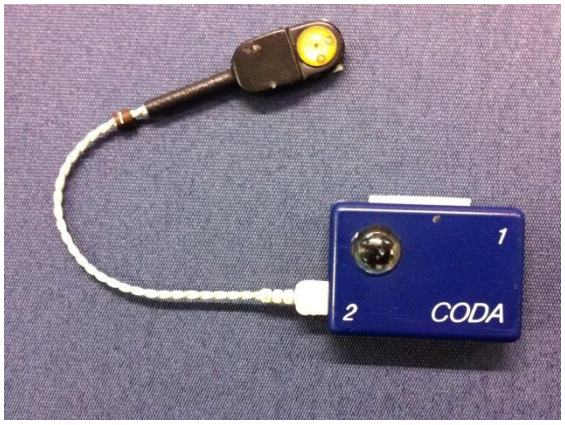
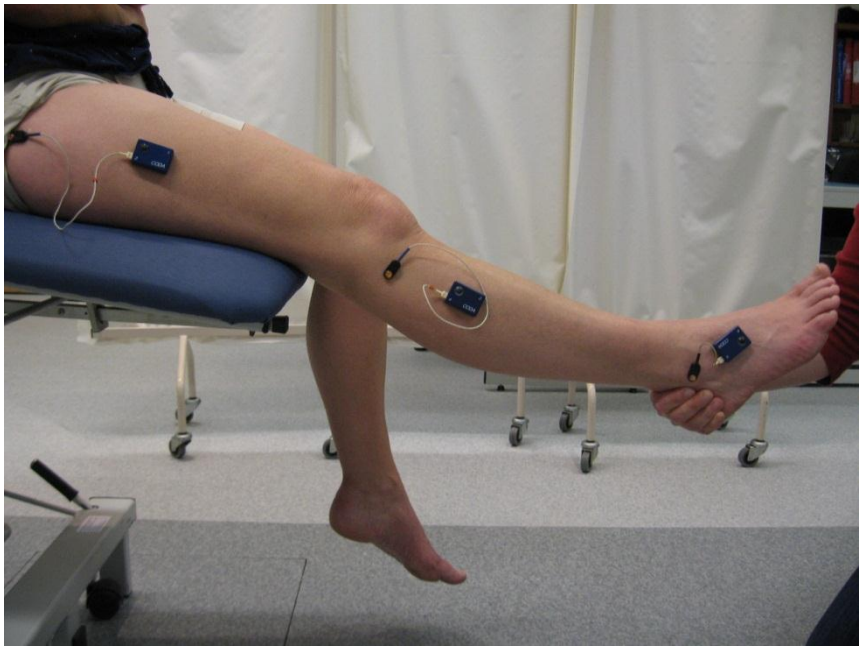


Figure 2-8 Participant with LED markers in situ at onset of pendulum test



Motion data was collected at a frequency of 60Hz for a period of 10 seconds during the test. Average stiffness and damping co-efficient were calculated using the following previously published equations:

$$\kappa = I \times \omega^2$$

where κ = the stiffness co-efficient expressed as Newton meters per radian (Nm/rad), I is the moment of inertia of the leg-foot segment with respect to the knee joint and ω is the natural frequency of the oscillation. The moment of inertia is calculated through the measurements of the total body weight and lower leg length.

$$c = 2 \times \zeta \times \omega \times I$$

where c is the damping co-efficients expressed as Newton meters per radian per second (Nm/rad/sec), and ζ is the viscous damping factor calculated using the angular displacement of the knee over time (Oatis 1993).

Three valid tests for each participant were required to calculate average stiffness and damping co-efficients. A valid test was defined by the pattern of the displacement graph of the knee joint during the pendulum test which displays a smooth sinusoidal decaying oscillation over time (Figure 2-9). Aberrations in the pattern of the graph can be due to poor visualisation of the markers by the Coda cameras, or by muscle activity around the knee joint (Figure 2-10). Where this was identified the test was repeated. Where 3 valid tests could not be completed the data was not included in the analysis.

The average scores from 3 trials of the passive pendulum test were used to calculate stiffness and damping co-efficients. These scores were divided by tertiles to create three 3 groups. Tertile 1 represented those with the least stiff knees and tertile 3 represented those with the stiffest.

Figure 2-9 Oscillation data of valid trial showing sinusoidal decaying of amplitude

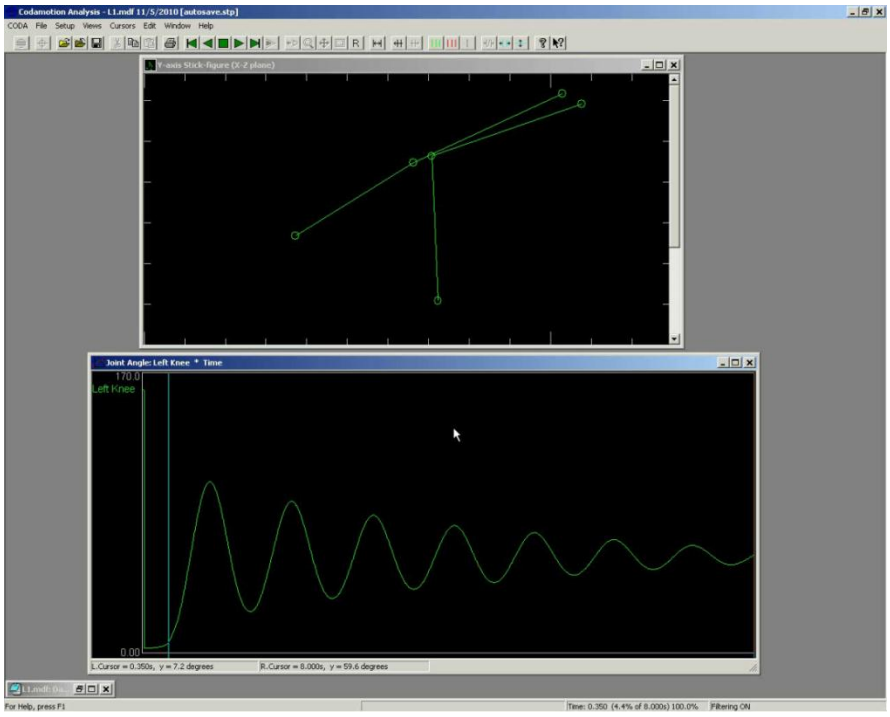
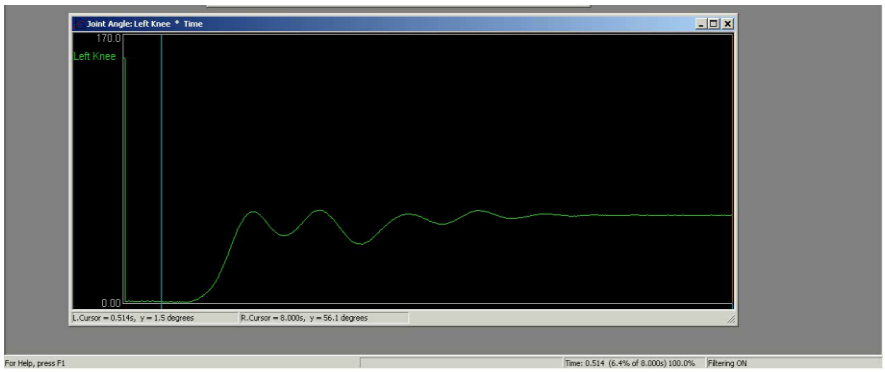


Figure 2-10 Oscillation data from an un-relaxed limb and therefore unsuccessful trial



2.6 Radiographic assessment

Radiographs of both knees were obtained for each participant. Standardised, semi-flexed weight-bearing tibio-femoral x-rays (using an adapted Rosen template) and skyline patella-femoral x-rays were taken with knees flexed (with a jig) in lying. Radiographs were taken at the Radiology Department at the Nottingham City Hospital.

Radiographs were scored by a blinded single trained reader (SAD) with established reliability. X-rays were scored using the Nottingham logically derived Line Drawing Atlas (Wilkinson, Carr et al. 2005) (scoring sheet is located in Appendix 3). Individual features were scored for in all three compartments; osteophytes (0-5) and joint space narrowing (0-5), and summated scores were calculated for osteophytes (0-40) and joint space narrowing (0- 20), and for the tibio-femoral (0-30) and patello-femoral (0-30) compartments. A global x-ray score for the whole knee joint was also calculated (0-60). The total number of osteophytes was counted (0-8). The presence or absence of chondrocalcinosis in the fibrocartilage and or hyaline cartilage, subluxation (lateral/medial) and attrition was scored as present or absent.

An overall Kellgren & Lawrence grade (0-4) was also given for the tibio-femoral and patello-femoral compartments of each knee (Ball, Jeffrey et al. 1963).

Table 2-4 Definitions of the severity according to Kellgren & Lawrence.

Grade 0	**Normal**
Grade 1	Doubtful narrowing of joint space, possible osteophyte
Grade 2	Definite osteophyte, possible narrowing
Grade 3	Moderate multiple osteophytes, definite narrowing, some sclerosis, possible deformity of bone ends
Grade 4	Large osteophytes, marked narrowing, severe sclerosis, definite deformity of bone ends

2.7 Ultrasound assessment

Ultrasound examinations of both knees was performed using the Toshiba Aplio SSA-770A machine by a single assessor (MH) using a multi-frequency (7-12 MHz) linear array transducer with standard image windows. A standardised research protocol, reflecting current EULAR definitions and measurements (D'Agostino, Conaghan et al. 2005) was followed. Definitions for pathological features published by the OMERACT group (Wakefield, Balint et al. 2005) were used throughout and though these were published with inflammatory arthritis in mind they also reflect the pathology seen to a lesser severity in osteoarthritis.

Power doppler settings were standardised with a pulse repetition frequency (PRF) of 1000-1300 Hz with a wall filter of 5. For optimum sensitivity, PD gain was set manually with the transducer focused on the area interest. Gain was increased manually until the colour box was uniformly filled with colour, then reduced until the background signal was removed.

Motion artefacts during PD assessment were minimised by ensuring participants were comfortable during the examination and that the positioning of the examiner was also comfortable. Random noise on the power Doppler setting was minimised by setting the gain levels just below the level where noise was generated.

Participant positioning was standardised so that a supine position was adopted for ventral and lateral scans and a prone position for dorsal scans. The knee joint was maintained in 30° flexion for ventral scans and lateral scans (using a bolster behind the knee) and was standardised to 90° flexion for imaging of the intercondylar sulcus. A combination of both longitudinal and transverse transducer planes were used throughout the examination. Generous amounts of scanning gel were used to ensure a minimal pressure between the transducer and participant's skin which can affect measures of joint effusion as well PD signal. Focus points for both grey-scale and doppler examination were adjusted throughout for the features examined.

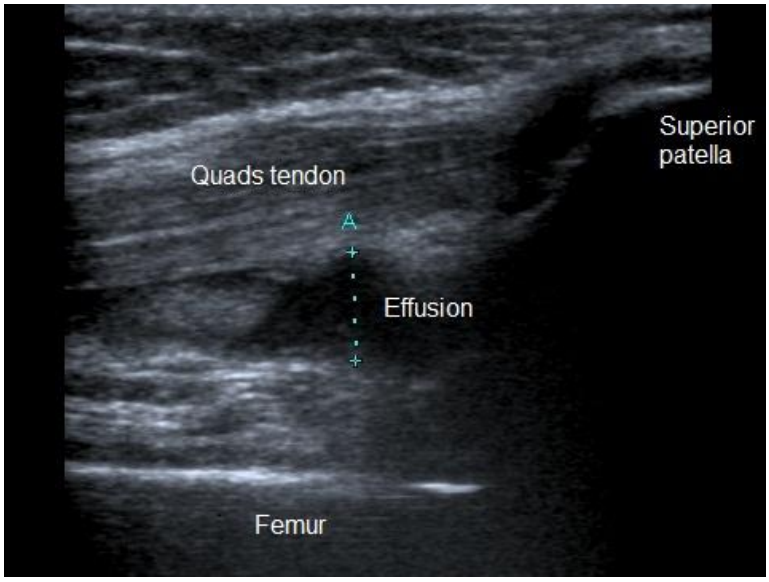
The following features were examined:

2.7.1 Effusion

Effusion is defined as an abnormal hypoechoic or anechoic (relative to sub dermal fat, but sometimes maybe isoechoic or hyperechoic) intra-articular material that is displaceable and compressible but does not exhibit Doppler signal (Wakefield, Balint et al. 2005) (Figure 2-11).

Maximal depth of effusion (mm) was measured using a longitudinal scanning plane and recorded as absent if < 4mm and present if ≥ 4mm (D'Agostino, Conaghan et al. 2005).

Figure 2-11 US scan image of suprapatellar pouch showing joint effusion



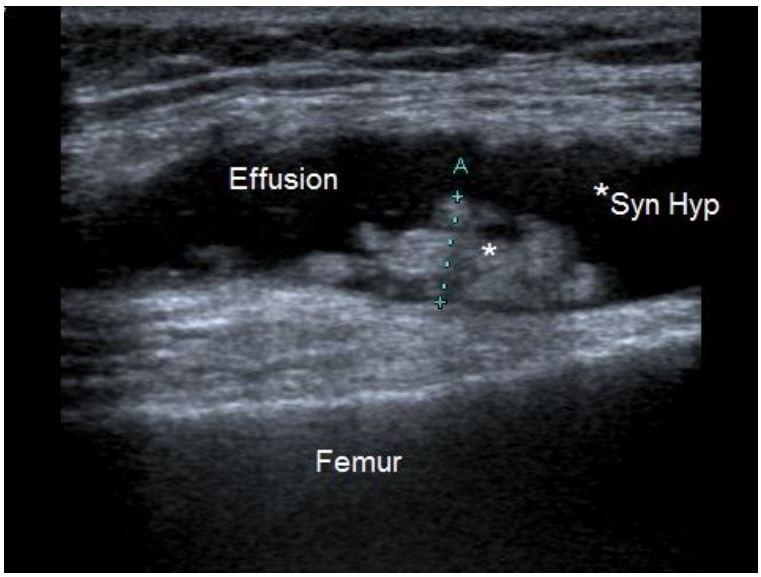
2.7.2 Synovial hypertrophy

Synovial hypertrophy is defined as abnormal hypoechoic (relative to sub dermal fat, but sometimes maybe isoechoic or hyperechoic) intra-articular tissue that is non-displaceable and poorly compressible and which may exhibit Doppler signal (Wakefield, Balint et al. 2005) (Figure 2-12).

Supra-patellar recesses, medial and lateral joint lines were scanned for synovial thickening with the knee joint in 30° flexion. Maximal synovial thickness (mm) was measured on the longitudinal scan and

dichotomised as absent if <4mm or present if ≥ 4mm (D'Agostino, Conaghan et al. 2005).

Figure 2-12 US scan image of synovial hypertrophy



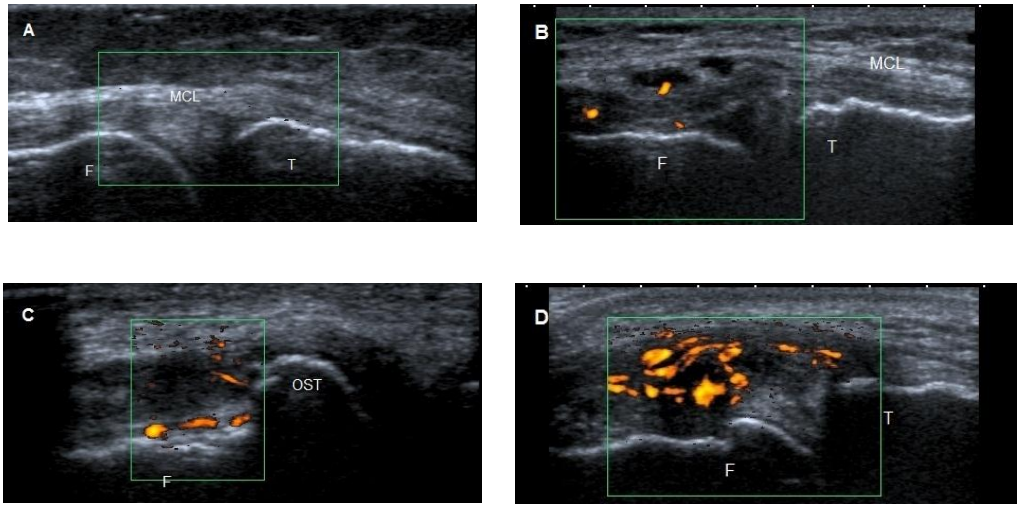
2.7.3 Power Doppler Signal

PD was used to detected abnormal blood flow within the hypertrophic synovium. Standard transverse and longitudinal scans of the medial and lateral recesses of the supra-patellar pouch and medial and lateral joint lines were used focusing on areas of synovial hypertrophy. Signal within the synovium was recorded as absent or present and graded 0-3 (Iagnocco, Meenagh et al. 2010) (Table 2-5) (Figure 2-13).

Table 2-5 Semi-quantitative grading for PD signal

Grade	Definition
Grade 0	Normal – no Doppler signal within the synovium (grey scale area)
Grade 1	Mild – up to 3 single spots or up to 2 confluent spots or one confluent and 2 single spots
Grade 2	Moderate – more than Grade 2 but <50% of the grey scale area
Grade 3	Marked – Doppler signal in >50% of the grey scale area

Figure 2-13 US scan images of medial knee joint line showing grades of PDS within the synovium. A: Normal, B: Grade 1, C: Grade 2, D: Grade 3

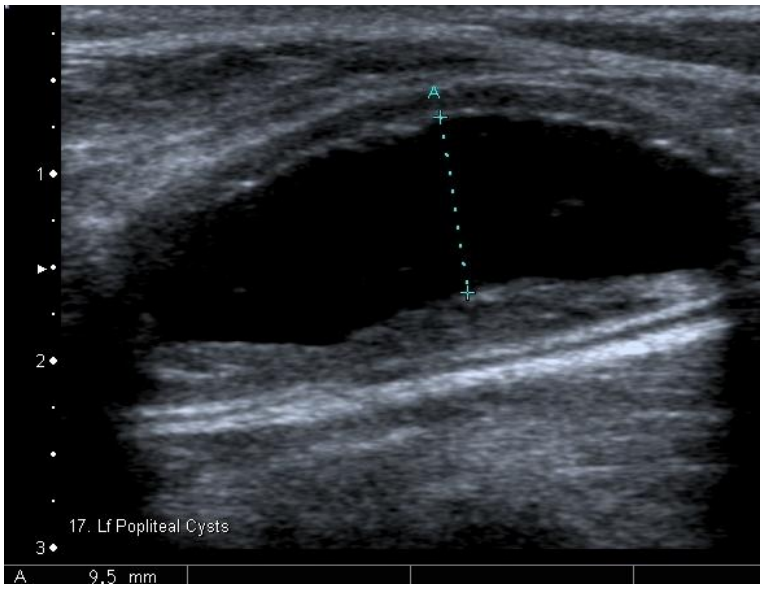


2.7.4 Bursitis

Bursae can have a hypoechoic, anechoic or mixed echolucency appearance when present. Three sites were examined for the presence of bursitis.

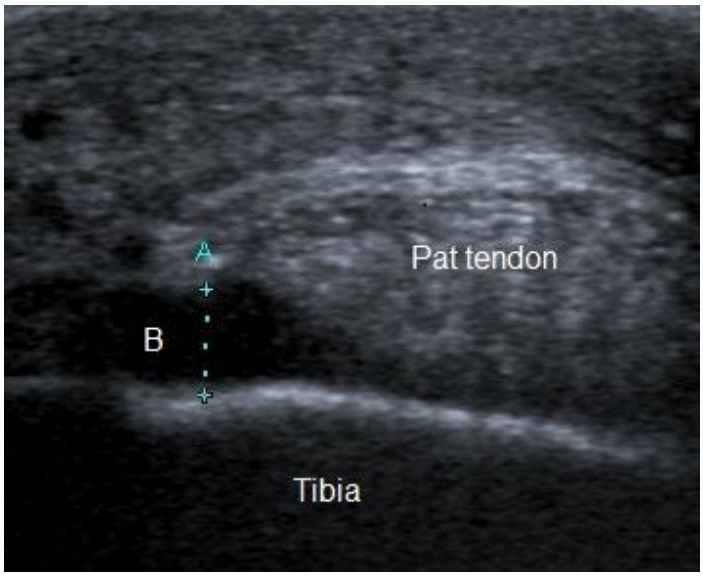
The popliteal fossa was examined with the participant in prone lying and the knee joint in extension. When present the popliteal bursa lies between the semimembranosus and medial gastrocnemius tendons (Chatzopoulos, Moralidis et al. 2008) . Maximum depth was measured (mm) in the longitudinal planes (Schmidt, Schmidt et al. 2004) and recorded as absent if <4mm and present if ≥ 4mm (de Miguel Mendieta, Cobo Ibáñez et al. 2006) (Figure 2-14).

Figure 2-14 US scan image of popliteal cyst



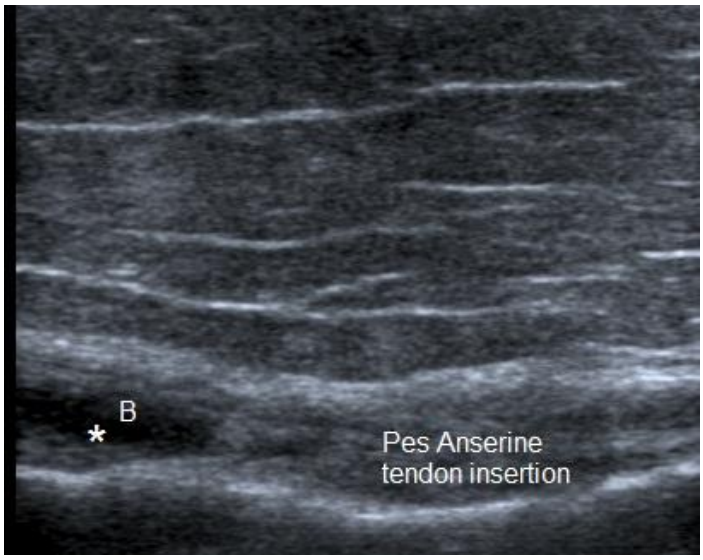
The insertion of the patella tendon was examined with the knee joint in 30° flexion. The patellar bursa appears hypoechoic with a slit like hypoechoic appearance centrally. Patellar bursitis was recorded as present or absent if≥ 4mm, and maximal depth in mm was recorded (Figure 2-15).

Figure 2-15 US scan image of patellar tendon bursae (B) taken in transverse plane



The insertion of the pes-anserine tendon, inferior and anterior on the tibia to the insertion of the medial collateral ligament was also examined in 30° flexion. Bursitis was recorded as present or absent if ≥ 2mm, and maximal depth in mm was measured where present (de Miguel Mendieta, Cobo Ibáñez et al. 2006).

Figure 2-16 US scan image of Pes anserine bursae (B)



2.7.5 Additional US features

The following additional features were noted during the US assessment

2.7.5.1 Osteophytes

Osteophytes are defined as cortical protrusions located at the edges of the joint surfaces seen in 2 planes (Keen, Wakefield et al. 2008). The presence was recorded as present or absent and the location noted. A measure representing the size of the osteophyte was taken from the apex of the osteophyte to a line connecting the medial or lateral edges of the femur and tibia (Jung, Do et al. 2006) (Figure 2-17).

2.7.5.2 Femoral articular cartilage

Normal cartilage has a homogenous hypoechoic appearance with a smooth contour parallel to the bone (O'Connor and Grainger 2002). The sharpness of the synovial space-cartilage interface and the clarity of the cartilaginous layer is reduced in OA joints, the articular cartilage is narrowed and an increased intensity of the bone-cartilage interface can be observed (Grassi, Lamanna et al. 1999). Thickness of the femoral articular cartilage was measured in two planes 1) in the transverse plane at 10mm to the medial and lateral midpoint of the intra-condylar groove (Ostergaard, Courtpayen et al. 1995) (Figure 2-18) and 2) in the longitudinal plane taken at the mid-area of the medial and lateral femoral condyles at the centre-point of the concavity (Yoon, Kim et al. 2008).

2.7.5.3 Chondrocalcinosis

Calcification within the hyaline cartilage can be detected using US where it appears as fine linear echogenic foci within the normally hypoechoic articular cartilage (Sofka, Adler et al. 2002). The appearance of chondrocalcinosis was noted during the examination of the femoral articular cartilage and recorded as present or absent (Figure 2-19).

Figure 2-17 US image of osteophytes on medial joint line

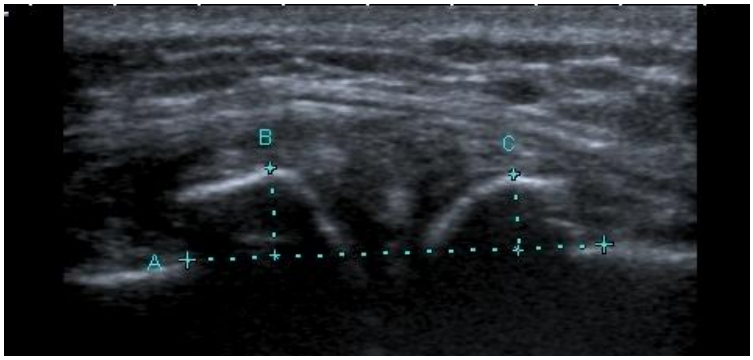


Figure 2-18 US image of femoral articular cartilage (FAC) (transverse image)

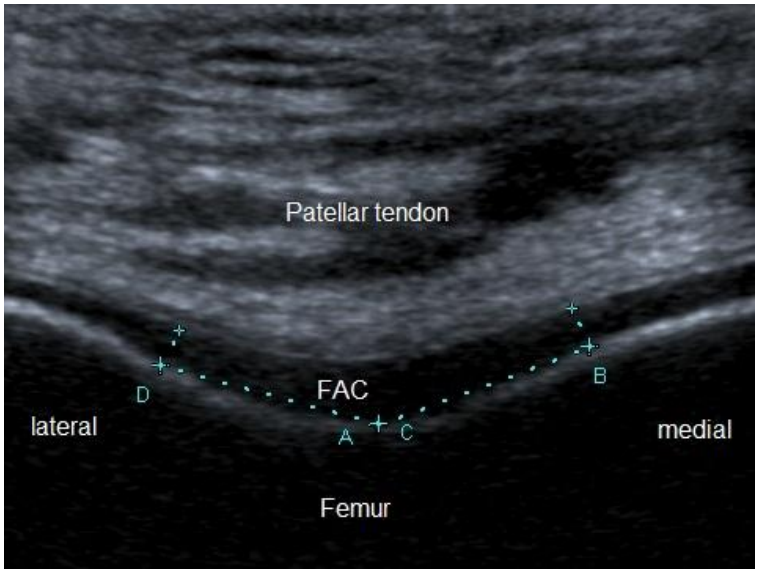
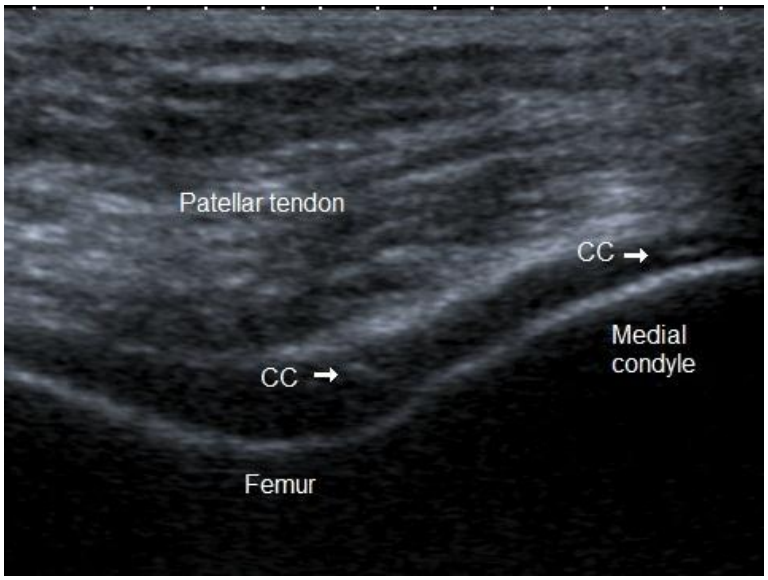


Figure 2-19 US image of chondrocalcinosis (CC→) within FAC



2.8 Statistical analysis

2.8.1 Sample size

There was limited previous work on which to base a sample size for the cross-sectional comparison study. Three published studies which have investigated US changes in symptomatic OA of which only one had an independent control sample of non-symptomatic OA (D'Agostino, Conaghan et al. 2005; Naredo, Cabero et al. 2005; de Miguel Mendieta, Cobo Ibáñez et al. 2006). Furthermore, all three studies reported on different US features and had different definitions and defined cut-off values. The single common feature reported was joint effusion in the supra-patellar pouch. The reported occurrence ranged from 43% (D'Agostino, Conaghan et al. 2005) to 79% (de Miguel Mendieta, Cobo Ibáñez et al. 2006) in subjects with symptomatic OA.

The power analysis was based on a 2 x 2 fixed effects analysis of variance. Fifty participants were required for each cell (200 in total) for 4 groups to achieve a balanced design and detect the minimum difference between groups with 90 % power and less than 5% type I errors.

Main effects: Knee pain included 2 levels, with 100 cases per level. The effect size (f) was 0.35, which yielded power of 1.00. Radiographic OA included 2 levels, with 100 cases per level. The effect size (f) was 0.25, which yielded power of 0.94.

Interactions: It was not the purpose of the study to look at the interaction between pain and x-ray changes but to look at the spectrum and difference of prevalence between groups.

For the follow-up evaluation, the power analysis was based on the null hypothesis that there was no correlation between changes in US synovitis and knee pain. The criterion for significance was set at 0.05 and the test was two tailed. The proposed sample size for the SOA group to be followed up was 50, which yielded a power of 97.9%. The calculation was based on an assumed correlation of 0.5 within the population, the observed value being test against the theoretical value of 0.00.

No formal power calculations were carried out for the injection study as this was a small observational study. The data from this study may be used in a power calculation to determine the sample size needed for a larger study.

2.8.2 Analysis: Intra-observer and Inter-observer reliability

Intra-observer reliability was examined whereby measures from day 1 (MH) were compared to the measures from day 2 (MH).

Inter-observer reliability was examined whereby measures from day 2 (MH) were compared to measures from day 2 (KL).

Cohen's kappa test was used to examine agreement of dichotomous data and a weighted kappa for ordinal data. The strength of agreement was classified according to criteria in Table 2-6 (Landis and Koch 1977).

Table 2-6 Strength of agreement for the values of kappa

Value of kappa	Strength of agreement
0 - 0.2	Slight
0.2 - 0.4	Fair
0.41 - 0.6	Moderate
0.61 - 0.8	Substantial
0.81 - 1.0	Almost perfect

For continuous variables, intra-class correlation co-efficients (ICC) and 95% confidence intervals were reported. Criteria for describing the strength of ICC reliability indices have been proposed where less than 0.5 indicates poor reliability, between 0.5 - 0.75 moderate reliability, between 0.75 - 0.90 good reliability and > to 0.90 excellent (Portney and Watkins 2003).

Bland & Altman plots were also used to explore the intra and inter-observer agreement in continuous US measurements. The plots show the difference between the two measures as a function of the average of the two measurements with 95% limits of agreement (Bland and Altman 2010).

2.8.3 Analysis: Diurnal variation of US measure

A repeated-measures ANOVA and post-hoc tests with Bonferonni corrections or non-parametric equivalent (Friedman's ANOVA and Wilcoxon signed-rank test with Bonferonni adjustment) were performed to determine whether within participant measures differed significantly over the 3 weeks or between morning and afternoon measures.

2.8.4 Analysis: Cross-sectional comparison study

Data for this study are described for index (most symptomatic or a randomly chosen knee) and contralateral (non-index knee) knees are presented. As data relating to pain severity was collected only for the index knee and not each individual knee, analyses were carried out using data from the index knee joint using standard analysis techniques. These analyses are therefore both knee- and subject-specific.

More sophisticated statistical methods can be use which can take into account the correlation between data from two joints coming from the same person such as random effects modelling, marginal modelling and generalised estimating equations (GEE) (Sutton, Muir et al. 1997) but as that data was not collected these methods are not suitable for this study.

2.8.4.1 Primary analysis – difference between groups

Primary analysis was the analysis based on the study design and compared the differences between groups. Chi-square test was used for nominal or frequency data. Where the expected frequencies were less than 5, Fisher's exact test were reported. Post-hoc comparisons were made using the z-test with adjusted p values (Bonferroni method).

Continuous variables that were normally distributed were compared using the one-way ANOVA and post-hoc Bonferroni tests performed. Welch's F statistic and post-hoc Games-Howell test were reported where variance between groups were unequal.

Non-normally distributed data were analysed using the Kruskal-Wallis test and selected post-hoc Mann Whitney tests with a Bonferroni correction performed.

2.8.4.2 Secondary analysis

2.8.4.2.1 Pain and US features.

Associations between knee pain and the presence of US features were investigated using logistic regression. All odds ratios (OR) were adjusted for age, sex and BMI and ROA (K&L ≥ Grade 2). Similar analysis adjusting for these features have been previously used in the analysis of cross-sectional studies examining associations between MRI features and knee pain (Hernández-Molina, Guermazi et al. 2008;

Hernández-Molina, Neogi et al. 2008).Crude OR and adjusted OR for each adjustment are presented.

The same adjusted models were used to evaluate the associations between US features and night pain, intermittent and constant knee pain.

The relationships between pain severity and continuous US measures were examined using correlation co-efficients. Pearson correlation co-efficient is reported for parametric data and Spearman's correlation co-efficient for non-parametric or ordinal data.

2.8.4.2.2 ROA and US features

Associations between radiographic OA (as defined by K&L ≥ grade 2) and the presence of US features were also examined using logistical regression. All OR were adjusted for age, sex, BMI and knee pain. Chondrocalcinosis on radiographs was also examined as an individual feature, as clinically it is associated with inflammation.

Relationships between radiographic severity (as determined by radiographic scoring from the Nottingham LDA) and continuous US measures were examined using non-parametric correlation co-efficients.

2.8.4.2.3 Clinical signs and symptoms and US features

Associations between clinical signs and symptoms which support inflammation (clinical effusion, self-reported morning stiffness and

biomechanically assessed stiffness and damping co-efficients) and US features were also explored. OR were adjusted for age, sex BMI, the presence of knee pain and ROA.

2.8.4.2.4 Knee joint stiffness

Joint stiffness was explored as a clinical symptom that is commonly associated with joint inflammation. Univariate analysis identified variables associated with biomechanically measured stiffness and damping co-efficients and multiple linear regressions was used to explore their contribution in the overall variance of stiffness.

2.8.5 Analysis: Follow-up evaluation

Change in knee pain was defined as an increase or decrease in maximal pain rating on the WOMAC pain subscale. Change in reported symptoms was examined in control and SOA groups separately.

Associations between change in knee pain at follow-up and change in presence or absence US features were examined using Chi-square analysis for both groups.

Further exploration of possible relationships between change in pain VAS scores and change in continuous measures of US effusion, synovial hypertrophy and popliteal cyst were examined using a correlation matrix.

Correlations between change in pain VAS, US features and biomechanical assessed stiffness and damping co-efficients were also explored.

2.8.6 Analysis: Intervention study

The primary outcome measure was change in knee pain VAS from the time of the injection to 1 week after the injection.

Whilst it was not the intention of this study to examine the efficacy of the steroid against the placebo injection, standardised statistical methods for analysis of a cross-over trial were carried out. An order effect was excluded by comparing the response to the steroid and placebo injection in each intervention period using unpaired t –tests or Mann-Whitney U test.

Response following the steroid and placebo injection and the differences between them were then examined using paired t-tests or the non-parametric equivalent and Chi-square test for dichotomous data.

Responders to the steroid and placebo injections were defined as those whose pain VAS scores decreased by 15mm or greater, one week after injection. Associations between response to injection and presence of baseline US features were examined using simple logistical regression. Correlations between change in pain VAS scores and change in continuous US measures were explored using Spearman's correlation rho. Individual responses in VAS pain scores and US variables

following both steroid and placebo injections were then examined for trends.

2.9 Data management

2.9.1 Data accuracy

Completeness and accuracy of the data was checked in a sample of 30 participants. Hard copy records were compared against the study database by two persons independently (15 data records each). Each data input was examined for errors and omission and these were recorded in a separate document. The errors for each section of the assessment were totalled and a percentage error calculated. Errors in each section are shown in Table 2-7 below. An error below 2% was considered acceptable. There was no difference in the overall number of errors detected by each person.

Table 2-7 Percentage errors detected

Assessment section	% Errors detected
Clinical history/physical examination	<0.5
Knee pain map	<0.5
Knee tenderness map	1.42
WOMAC questionnaire	<0.5
ICOAP questionnaire	0
Ultrasound assessment	<1.0
Biomechanical assessment of joint stiffness	1.6
X-ray scores	0

2.9.2 Missing data

Missing data was entered as a discrete value (99) in the database and was included in the analysis. Missing data in the US assessment was usually due to difficulty in positioning the participant, for example some participants were unable to prone lie for examination of the popliteal fossa.

Some participants failed to complete all questions in the WOMAC Index. Missing values were dealt with as recommended by the authors. Where one pain, one stiffness or up to 3 physical function items were missing, the average value for that subscale was substituted. This method is similar to that employed by other indices such as the SF36 and AIMS2 (Bellamy 1995).

3 Results

The results for this thesis are reported in sections as follows:

Section 3.1 Recruitment

Section 3.2 Reliability of outcome measures:

- intra and inter-observer reliability of ultrasound assessment
- inter-observer reliability of ultrasound assessment
- intra-observer reliability of biomechanical assessed stiffness and damping co-efficient

Section 3.3 Diurnal variation in US measures

Section 3.4 Cross-section multiple group comparison study

- primary analysis: difference between groups
- secondary analysis: associations between knee pain, structural change and US features

Section 3.5 Follow-up evaluation

- correlations and associations between changing knee pain and change in US features

Section 3.6 Intervention with corticosteroid or placebo injection

- response following interventions
- correlations and associations between response following intervention and change in US features

3.1 Recruitment

Of 1090 potential participants approached to take part in the study, 241 were enrolled in the study. An overview of the recruitment is presented in Figure 3-1 and a detailed breakdown of recruitment in Table 3-1. Two additional participants were recruited from other sources. One was a spouse of a study participant with knee pain and without ROA, and the other was a patient with knee pain who was referred to the OA out-patient clinic at Nottingham University Hospital with knee pain but without radiographic changes.

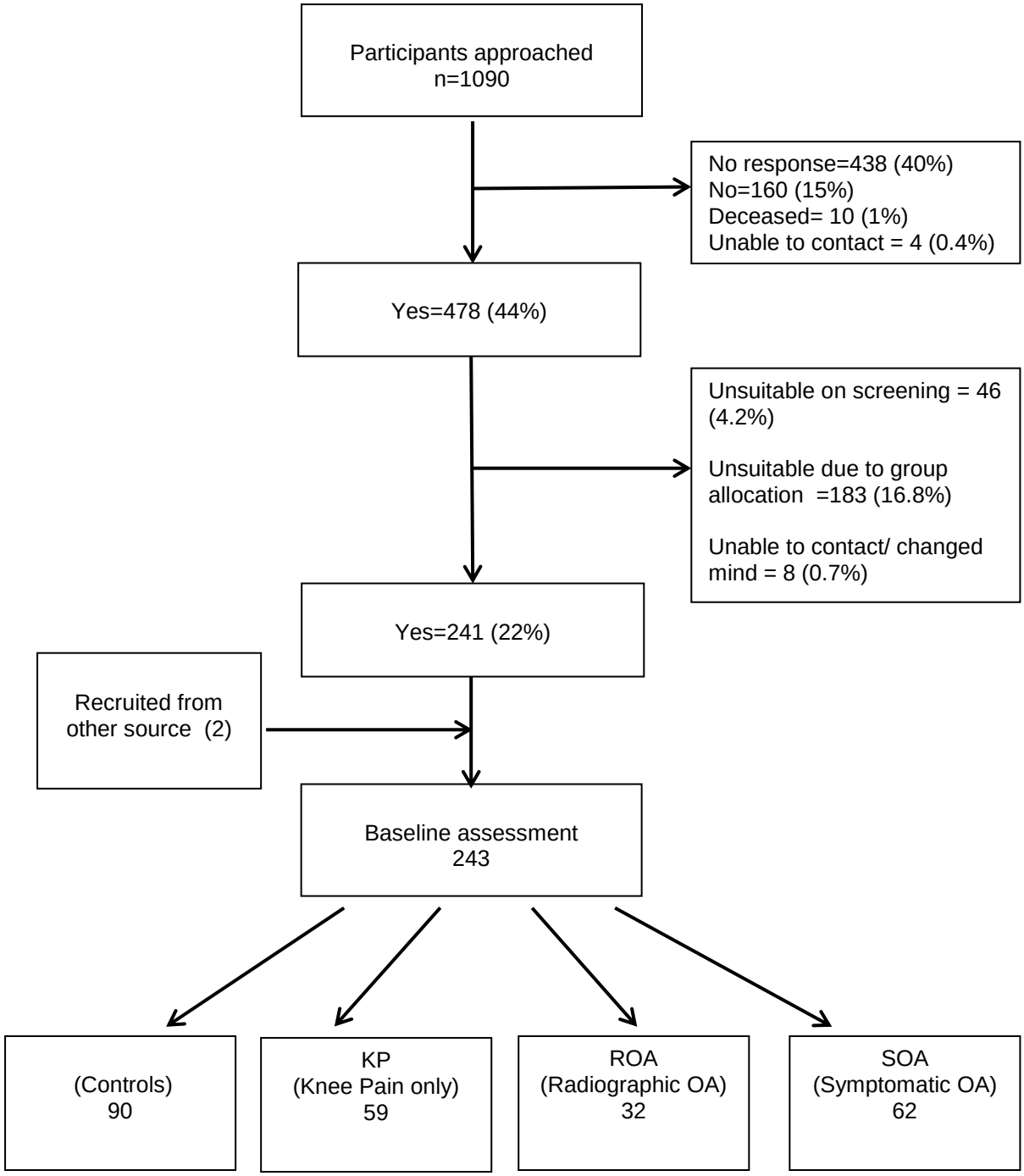
The characteristics of participants invited for the current study, those who did not reply, those who declined those who were unsuitable and those who were enrolled are shown in Table 3-2 along with the baseline characteristics of their original studies. At the point of invitation, potential participants from the community knee pain trial were younger (mean age, 58 SD(7) years) than other participants, and the potential participants from the incident KP cohort had a higher proportion of females (m: f = 29:71%)

Response rates varied across the 3 sources of recruitment with the highest proportion of positive responders coming from the incident knee pain study (67.5%) followed by the community KP trial (57.8%) and then the GOAL database (33.4%). The ratio of males to females recruited was similar across the three sources (1:2.2), and mean age reflected the differences observed at the point of invitation.

Conversely, non-responders were highest from the GOAL database, followed by the community knee pain trial and incident knee pain cohort (52%, 26% and 12% respectively), age and gender were representative of those who were approached. The proportion of those who replied but declined to take part was similar across the three recruitment sources (14-17%) and also closely reflected the age and gender of those initially invited to participate.

The proportion of participants who were unsuitable due to exclusion criteria were more common in the incident KP cohort compared to the community KP trial and GOAL database (12.6%, 6.3% and 1.6% respectively). Mean age reflected those invited to participate, though the proportion of females was higher from the incident knee pain cohort and GOAL database. More men were excluded from the community knee pain trial than women.

Figure 3-1 Overview of Recruitment



(KP= knee pain only; ROA= radiographic OA; SOA= symptomatic OA)

Table 3-1 Breakdown of recruitment

	Total invites sent out n=1090		
	Incident KP cohort N = 265 n (%)	**Community KP trial N = 95 n (%)**	**GOAL database N = 730 n (%)**
No response	32 (12.1%)	25 (26.3%)	381 (52.1%)
Reply 'No'	46 (17.4%)	14 (14.7%)	100 (13.7%)
Deceased	4 (1.5%)	1 (1%)	5 (0.7%)
Unable to contact	4 (1.5%)	0	0
Reply 'yes'	179 (67.5%)	55 (57.8%)	244 (33.4%)
Unable to contact	8 (3%)	0	0
Excluded	28 (10.6%) - *TKR (14)* - *Hip OA (8)* - *Neurological (2)* - *Fibromyalgia (3)* - *Cancer (1)*	6 (6.3%) - *Hip OA (3)* - *Neurological (1)* - *Fibromyalgia (1)* - *Inflammatory Arthritis (1)*	12 (1.6%) - *Hip OA (1)* - *TKR (1)* - *Fibromyalgia (3)* - *Inflammatory Arthritis (1)* - *Care Home (1)* - *Acute knee injury(3)* - *Chronic other pain (2)*
Unsuitable for Group	0	18 (18.9%)	165 (22.6%)
Recruited and included	**143 (54%)**	**31 (33%)**	**67 (9.2%)**
Control	50	6	35
KP	16	25	16
ROA	20	0	12
SOA	57	0	5

(TKR = Total knee replacement; KP= knee pain; ROA= radiographic OA; SOA= symptomatic OA)

Table 3-2 Characteristics of individuals selected for recruitment

Recruitment source	Incident KP cohort	Community KP trial	GOAL database
Baseline data collected	1996-2001	2007-2008	2002-2006
Population (n)	3109	892	3171
Age at baseline			
Mean (SD) years	57 (9)	60.6 range (40-84)	66.5 (7.9)
Gender male: female %	44.5: 55.5%	49: 51%	52: 48%
Invited for current study (n)	265	95	730
*Mean age (SD) years	72.5 (8.1)	58 (6.9)	70 (8.0)
male:female %	29:71%	42 % 58%	47: 53%
No reply			
*Mean age (SD) years	72.4 (9.1)	59.9 (7.0)	70 (9.0)
male:female %	28: 72%	44:56%	48:52%
Reply No			
*Mean age (SD) years	73.9 (7.9)	57.7 (3.6)	72 (8.0)
male:female %	24:73%	36:64	45:56%
Excluded			
*Mean age (SD) years	73.4 (7.2)	59.6 (8.0)	69 (9.0)
male:female %	18.5 :81.5%	55:45%	25:75
Reply Yes			
*Mean age (SD) years	72 (8.0)	56.1 (7.0)	71 (8.0)
male:female %	31:69%	32:68%	31:68%

*** age at onset of recruitment for current study 01/03/2010**
(KP= knee pain)

3.2 Reliability of outcome measures

3.2.1 Intra and inter-observer reliability of US assessment

3.2.1.1 Participants

Fourteen participants (28 knees) mean age 67.5 years (range 56-71), 12 female and 2 males had their knees scanned twice by the same examiner (MH) within a 7 day period. 13 knees were identified as normal, 3 knees had knee pain only, 2 had ROA only and 10 had SOA.

Five participants (ten knees), mean age 71.4 years (range 66-77), 4 women and 1 man took part had their knees scanned independently by two examiners (MH and KL) on the same day. 5 knees were identified as normal, 3 had knee pain and 2 had SOA.

3.2.1.2 Results

Kappa co-efficients for intra- and inter-observer agreement for the dichotomous presence or presence of US features and weighted kappa values for PD signal are shown in Table 3-3.

Table 3-3 Intra- and Inter-observer agreement for the presence of US features

	Kappa	
Inflammatory US Features	**Intra-observer**	**Inter-observer**
Effusion	0.76 p<0.001	0.78 p=0.001
Synovial hypertrophy	0.79 p<0.01	0.78 p<0.01
Popliteal cyst	0.70 p<0.001	0.62 p=0.04
PD Signal (unweighted)	1.0 p<0.001	0.76 p<0.001
Grade PD Signal (weighted)	1.0 p<0.001	0.78 p<0.001
Structural US Features		
Osteophytes	0.80 p<0.001	0.55 <0.01

The level of agreement for effusion and synovial hypertrophy was substantial, and for popliteal cysts was moderate for inter-observer reliability and substantial for intra-observer reliability. Intra-observer agreement was better for PD signal than inter-observer agreement. There was moderate agreement between different observers for presence of osteophytes, and good agreement within the same observer.

Intra-class correlation co-efficients for the continuous measures of effusion, synovial hypertrophy and bursitis are shown in Table 3-4. The ICC values demonstrate that the reliability for both intra and inter-observer measures were good to excellent for all US features of inflammation. ICCs for the measurement of structural features was good for osteophytes. Measurement of femoral articular cartilage depth had higher ICCs when measured in the transverse plane compared to the longitudinal plane, and was higher for intra compared to inter-observers.

Table 3-4 Intra-class correlation co-efficients (ICC) and 95% confidence interval (CI) for reliability of continuous US features

	ICC (95% CI)	
Inflammatory US Features	**Intra-observer**	**Inter-observer**
Effusion (mm)	0.91 (0.81-0.96)	0.88 (0.60-0.97)
Synovial hypertrophy (mm)	0.95 (0.9-0.98)	0.84 (0.50-0.96)
Popliteal cyst (mm)	0.82 (0.66-0.91)	0.80 (0.60-0.90)
Structural US Features		
Osteophytes (mm)	0.86 (0.76-0.92)	0.79 (0..5-0.89)
Femoral articular cartilage (mm)		
Transverse view	0.71 (0.41-0.87)	0.52 (0.12-0.78)
Longitudinal view	0.66 (0.33-0.84)	0.14 (-0.41-0.44)

Bland & Altman plots were also used to explore the intra and inter-observer agreement for inflammatory US features (Figure 3-2, Figure 3-3**).**

The plots show the difference between the two measures as a function of the average of the two measurements. The solid line indicates the mean difference of the paired measures; the distance from zero provides an estimate of the bias from the two methods (Bland and Altman 2010). Values close to zero indicate lesser bias. The dashed lines indicate the 95% limits of agreement. A summary of the mean differences and 95% limits of agreement for intra and inter-observer measures are presented in Table 3-5.

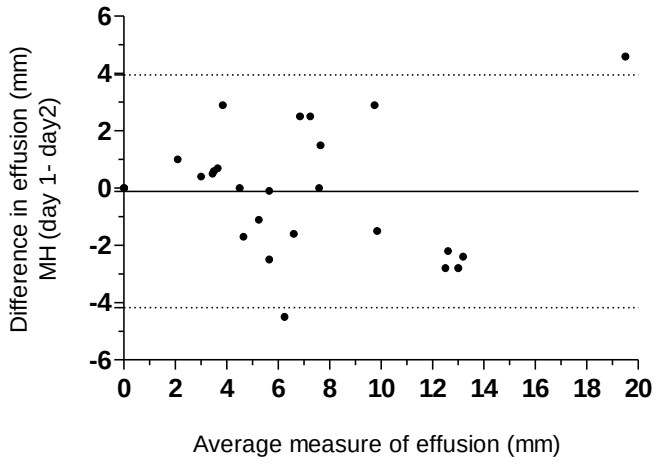
Table 3-5 Summary of mean differences and 95% limits of agreement for intra and inter-observer measures of continuous US measures

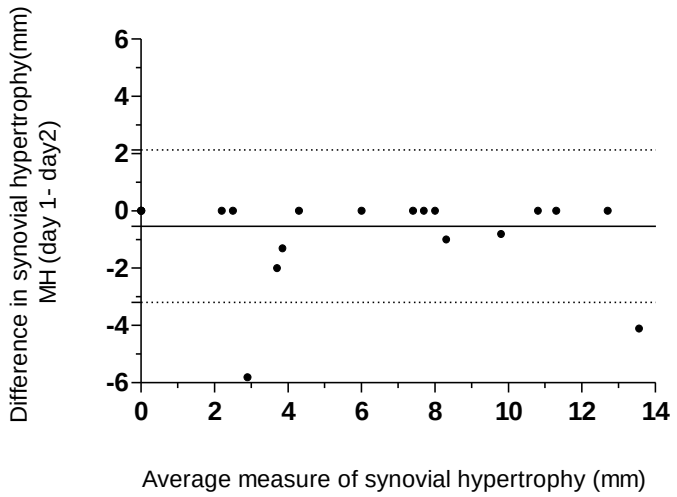
	Intra-observer agreement (MH, day1- day2)		**Inter-observer agreement (MH-KL)**	
US measures (mm)	mean difference (SD)	95% limits of agreement	mean difference (SD)	95% limits of agreement
Effusion	-0.1 (2)	(-4.0, 4.0)	1.4 (2.1)	(-2.7, 5.5)
Synovial hypertrophy	0.5 (1.4)	(-3.2, 2.1)	1.3 (2.6)	(-3.8, 6.3)
Bursitis	0.2 (1.5)	(-2.8, 3.2)	0.5 (1.0)	(-1.4, 2.3)

For intra-observer agreement the mean discrepancy between observations on day 1 and day 2 was less than 1mm for all measures. For inter-observer agreement between MH and KL, the mean difference was less than 1.4mm for all measures. There was a visible trend on all three plots where measures taken by MH were higher than that of KL

indicating a degree of systematic bias. However the magnitude of the difference is unlikely to be clinically significant.

Figure 3-2 Bland_Altman plots for intra-observer agreement of US measure of effusion, synovial hypertrophy and popliteal cysts.





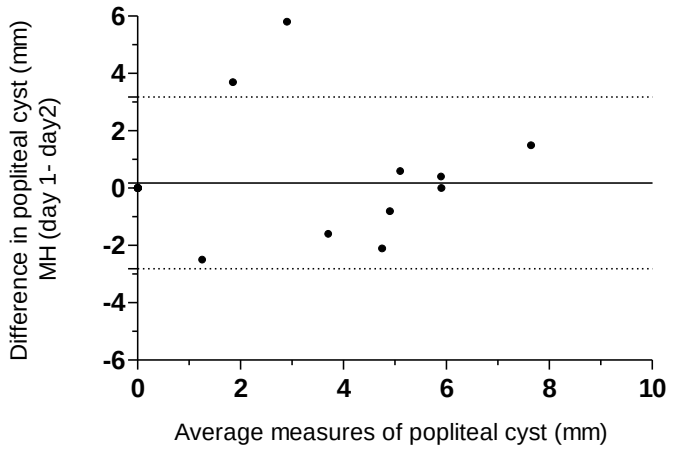
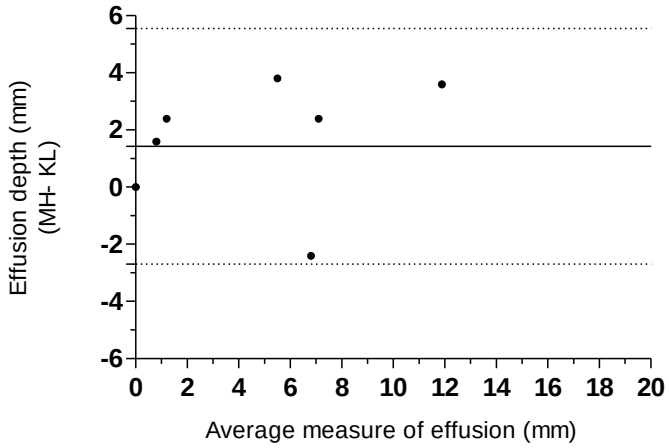
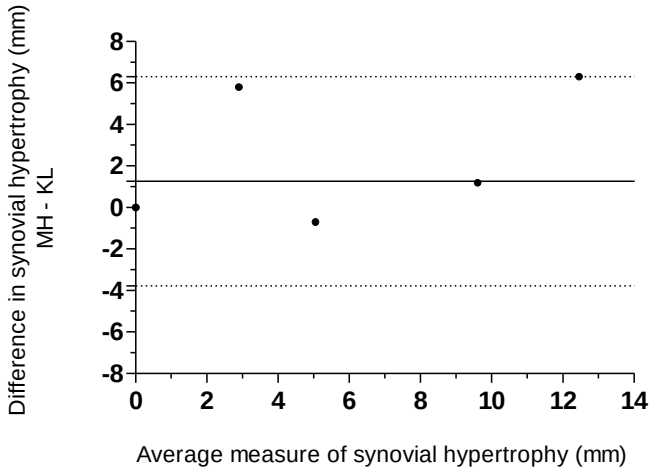
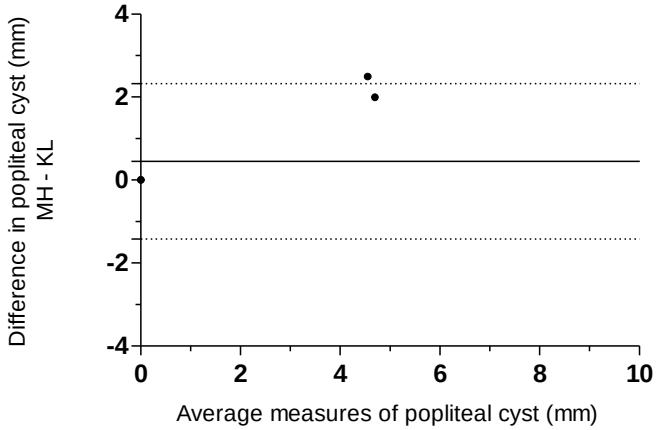


Figure 3-3 Bland-Altman plots for inter-observer agreement of US measures of effusion, synovial hypertrophy and bursitis.







3.2.2 Reliability of biomechanically assessed stiffness

3.2.2.1 Participants

10 subjects participated in this test-retest study, 4 women and 6 men, aged 61-77 (mean age 69.3). Twenty knees were tested of which 14 had radiographic changes and 13 were painful. Four participants were unable to complete valid test trials on both days and were excluded from the analysis.

3.2.2.2 Results

Intra-class correlation co-efficients and 95% confidence intervals for the stiffness and damping between days are presented in Table 3-6. Reliability for damping co-efficinets was very good and excellent for stiffness co-efficient.

Table 3-6 Intra-class correlation co-efficients (ICC) and 95% confidence intervals (CI) for intra-observer reliability for stiffness and damping co-efficients

	ICC	(95% CI)
Damping	0.85	(0.52 - 0.96)
Stiffness	0.96	(0.85 - 0.99)

For the damping co-efficient, the mean difference between measures on day 1 and day 2 was 0.02 Nm /rad/sec (SD 0.06) with 95% limits of agreement from -0.09 to 0.14 (Figure 3-4). For the stiffness co-efficient, the mean difference was 0.35 Nm/rad (SD 1.05) with 95% limits of agreement from -1.70 to 2.40 (Figure 3-5).

Figure 3-4 Bland & Altman plot of damping co-efficient (Nm/rad/sec) taken on 2 days by the same observer

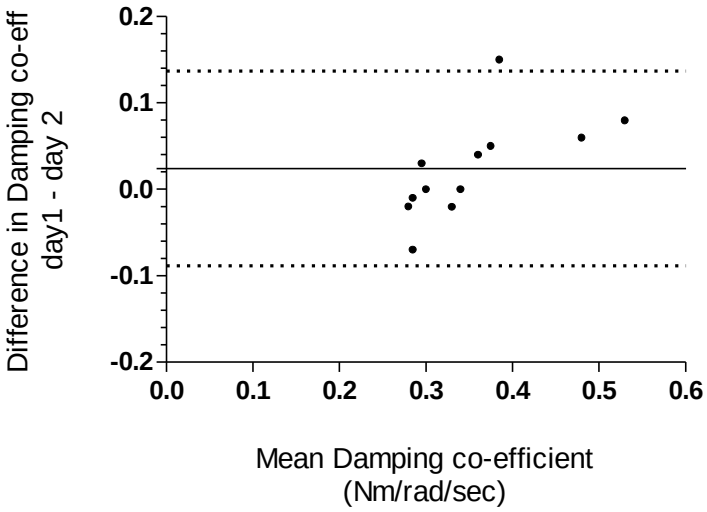
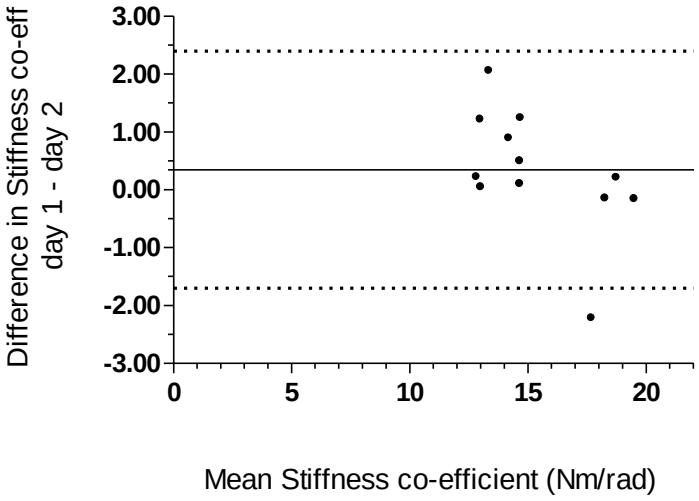


Figure 3-5 Bland & Altman plot of stiffness co-efficient (Nm/rad) taken on 2 days by the same observer



3.3 Diurnal variation of US measures

3.3.1 Participants

Five participants, mean age 70.4 (range 61-76), 4 women and 1 man took part in this study, all with bilateral symptomatic knee OA. US assessments took place in the morning and were repeated in the afternoon, on 3 separate occasions, one week apart. Effusions and synovial hypertrophy were observed in all knees, popliteal cysts were found in 3 knees. PD signal was not detected in any of the knees assessed and so this was not subjected to statistical analysis.

3.3.2 Results

Descriptive data for pain and US variables are presented in Table 3-7. WOMAC, ICOAP and VAS pain scores were found to be normally distributed (Shapiro Wilks test $p>0.05$). Repeated-measures ANOVA showed no significant differences in participant reported WOMAC scores, ICOAP scores or VAS scores between weeks 1, 2 and 3.

US measures of synovial hypertrophy were normally distributed ($p>0.05$) but effusion and popliteal cysts were not and were analysed using Friedman's ANOVA.

Friedman's ANOVA found no statistical difference between measures for effusion $X^2(5)=1.31$, $p=0.93$ or for popliteal cysts $X^2(5)=6.06$, $p=0.33$. Repeated measures ANOVA for synovial hypertrophy showed no significant difference between measures $F=1.13$, $p=0.36$.

Table 3-7 Descriptive data for pain and US variables in diurnal variation study

	Week 1		Week 2		Week 3	
Pain variables	**Mean (SD)**		**Mean (SD)**		**Mean (SD)**	
Pain VAS (mm)	41.00 (27.82)		56.00 (19.11)		51.40 (21.31)	
WOMAC Pain scores	7.60 (2.70)		8.00 (4.30)		8.80 (5.07)	
ICOAP Intermittent score	11.80 (4.66)		11.90 (5.07)		12.40 (6.66)	
Constant score	7.80 (3.70)		9.60 (4.16)		9.20 (3.56)	
US measures	**am**	**pm**	**am**	**pm**	**am**	**pm**
Synovial hypertrophy (mm)						
Mean(SD)	6.25 (3.29)	7.09 (2.68)	7.29 (3.61)	7.04 (2.99)	5.89 (3.22)	6.77 (3.28)
Effusion (mm)						
Mean(SD)	8.13 (6.19)	8.08 (5.19)	8.47 (4.80)	8.31 (4.76)	7.90 (4.29)	7.95 (6.05)
Median (range)	5.15 (4.00,21.80)	6.60 (3.10,17.00)	6.45 (3.10,17.20)	6.80 (4.40,16.30)	7.70 (3.80,16.00)	5.75 (2.70,18.20)
Popliteal cyst (mm)						
Mean(SD)	2.35 (3.41)	2.18 (3.03)	2.27 (3.15)	2.73 (3.92)	2.68 (3.79)	2.32 (3.21)
Median (range)	0.00 (0-8.40)	0.00 (0-6.60)	0.00 (0-6.90)	0.00 (0-9.20)	0.00 (0-8.90)	0.00 (0-10.32)

3.4 Results: Cross-sectional comparison study

This section describes the results of the cross-sectional multiple group comparison study which set out to achieve the primary objectives of this body of work. Participant recruitment, baseline demographics, clinical signs and symptoms, radiographic scores and US findings are presented. Primary analysis of differences between controls (those without knee pain or radiographic changes) and participants with painful knees, ROA and SOA are reported. Secondary analysis exploring the associations between knee-pain, US features and structural damage are reported.

3.4.1 Participant demographics

A total of 243 participants were included in the analysis of the study, 157 (64.6%) women and 86 (35.4%) men. Participant characteristics are presented for each study group according to the index knee (most symptomatic or randomly chosen knee) (Table 3-80).

There was no significant difference in the number of men and women within each group ($p=0.29$). One way ANOVA showed there was a significant difference in age across the groups ($p<0.001$). Bonferroni post-hoc comparisons revealed that the KP group was significantly younger compared to all other groups $p<0.001$ (Figure 3-6). The control group had a significantly lower BMI $p<0.05$, but the other groups were not significantly different to each other (Figure 3-7).

Table 3-8 Participant demographics – comparison between groups

Group		Controls		KP		ROA		SOA		p
	N (%)	90	(37%)	59	(24%)	32	(13%)	62	(26%)	
Gender										
Men	*n (%)*	27	(30%)	26	(44.1%)	13	(40.6%)	20	(32.3%)	
Women	*n (%)*	63	(70%)	33	(55.9%)	19	(59.3%)	42	(67.7%)	0.29
Age (years)										
	mean (SD)	71	(7.9)	63.8	(8.8)	73.1	(7.9)	73.9	(7.78)	<0.001
	Median (range)	70	(51-90)	64	(50-81)	71	(61.9)	74	(56-91)	
BMI (kg/m^2)										
	mean (SD)	26.5	(4.4)	28.5	(4.0)	29.60	(5.3)	29.21	(4.1)	<0.001
	median (range)	25.9	(19.1-39.6)	28.5	(21.7 – 40)	28.37	(22.5 - 41.4)	28.97	(20 - 40.9)	

KP= knee pain only; ROA= radiographic OA; SOA= symptomatic OA
BMI=Body mass index

Figure 3-6 Mean Age with 95% confidence intervals (CI) for each comparison group.

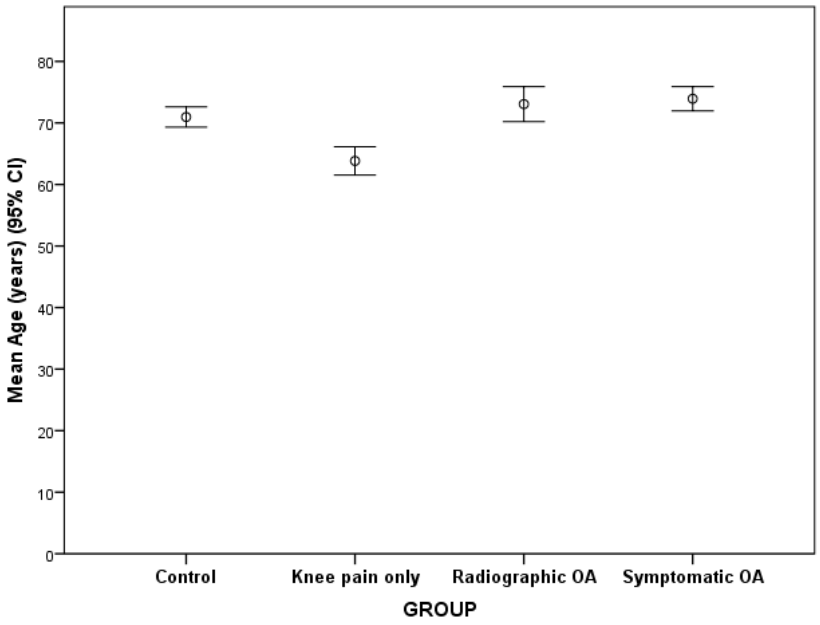
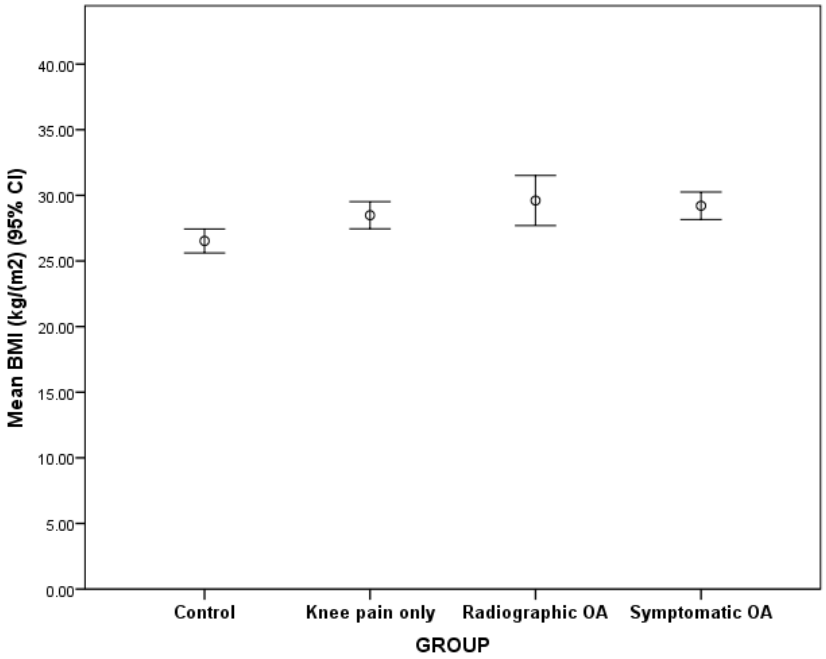


Figure 3-7 Mean body mass index (BMI) with 95% confidence intervals (CI) for each comparison group.



3.5 Primary Analysis

3.5.1 Radiographic evaluation – comparison between groups

3.5.1.1 Index knee

Table 3-9 summarises the radiographic scores for the index knee using the logically derived Line Drawing Atlas. Mean summated scores are presented for individual features of osteophytes and joint space narrowing, and for tibio-femoral and patello-femoral compartments. A global score for all features in all compartments is also presented. The median (range) is presented for the number of osteophytes observed and frequency data for the presence of radiographic chondrocalcinosis is given. Descriptive data for Kellgren & Lawrence grading is also presented.

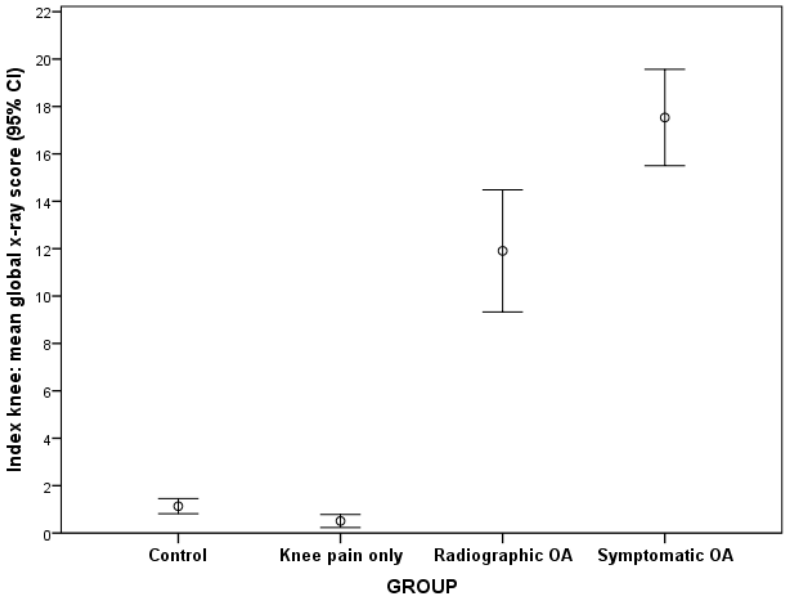
One way-ANOVA showed significant differences between groups for global radiographic scores ($p<0.001$). Post-hoc tests found no significant difference between control and KP groups for all summated scores. Participants with SOA had significantly worse x-rays compared to those with ROA (mean difference = 5.6 for global scores, $p=0.005$) (Figure 3-8).

Table 3-9 Summated radiographic scores for index knee – comparison between groups

Summated radiographic scores		Controls 90		KP 59		ROA 32		SOA 62		P
Global Score (0-60)										
	Mean (SD)	1.1	(1.5)	0.5	(1.1)	11.9	(7.14)	17.5	(8.0)	<0.001
	Median (range)	0.5	(0-7)	0	(0-5)	11	(3-30)	16	(4-38)	
Osteophytes (0-40)										
	Mean (SD)	0.6	(.1.0)	0.2	(.8)	7.9	(5.9)	12.4	(7.0)	=0.001
	Median (range)	0	(0-6)	0	(0-5)	7	(1-25)	11	(2-30)	
Joint Space Narrowing (0-20)										
	Mean (SD)	0.6	(1.0)	0.3	(0.6)	4.0	(2.1)	5.2	(2.1)	<0.001
	Median (range)	0	(0-5)	0	(0-3)	4	(0-9)	5	(1-11)	
Tibio-femoralJoint (0-30)										
	Mean (SD)	0.8	(1.2)	0.3	(0.7)	4.6	(3.9)	8.6	(5.5)	<0.001
	Median (range)	0	(0-6)	0	(0-3)	4	(0-18)	8	(0-22)	
Patellofemoral joint (0-30)										
	Mean (SD	0.4	(0.8)	0.2	(0.8)	7.3	(5.7)	9.0	(4.9)	<0.001
	Median (range)	0	(0-4)	0	(0-5)	6.5	(0-19)	9	(0-19)	
Number of Osteophytes										
	Median (range)	0	(0-5)	0	(0-3)	4	(1-8)	6	(1-8)	=0.001
Chondrocalcinosis	***N (%)***	5	(5.6%)	3	(5.8%)	1	(3.1%)	8	(12.9%)	=0.27
Kellgren & Lawrence Grade	***N (%)*** **G0**	71	(79.0%)	54	(91.5%)	0		0		
	G1	19	(21.0%)	5	(8.5%)	0		0		
	G2	0		0		9	(28.1%)	5	(8.1%)	
	G3	0		0		15	(46.9%)	23	(37.1%)	
	G4	0		0		8	(25.1%)	34	(54.8%)	

(KP= knee pain only; ROA= radiographic OA; SOA= symptomatic OA)

Figure 3-8 Index knee: mean global x-ray scores for each comparison group



Comparison of individual features found the number of osteophytes was significantly higher in the SOA group (p=0.001), as was the summated score for osteophytes (mean difference = 4.5, p<0.001) (Figure 3-9). There was no significant difference for joint space narrowing (p=0.09) (Figure 3-10).

For comparison by joint compartment, tibio-femoral joint scores were also significantly worse in the SOA group (mean difference = 4.0, p<0.001) (Figure 3-11) but not patello-femoral joint score (p=0.5) (Figure 3-12). No difference was found in the frequency of chondrocalcinosis between any groups (p=0.27).

Figure 3-9 Index knee: mean summated scores for osteophytes for each comparison group

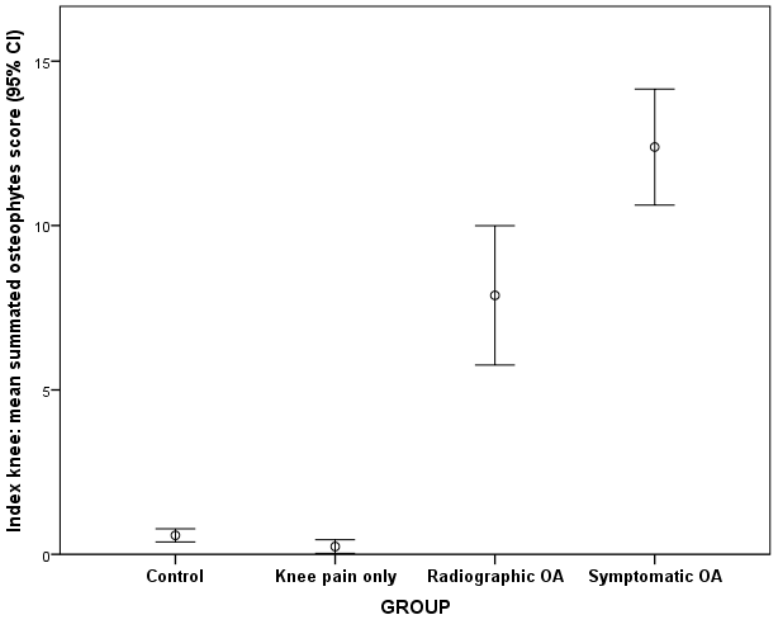


Figure 3-10 Index knee: mean summated score for joint space narrowing (JSN) for each comparison group

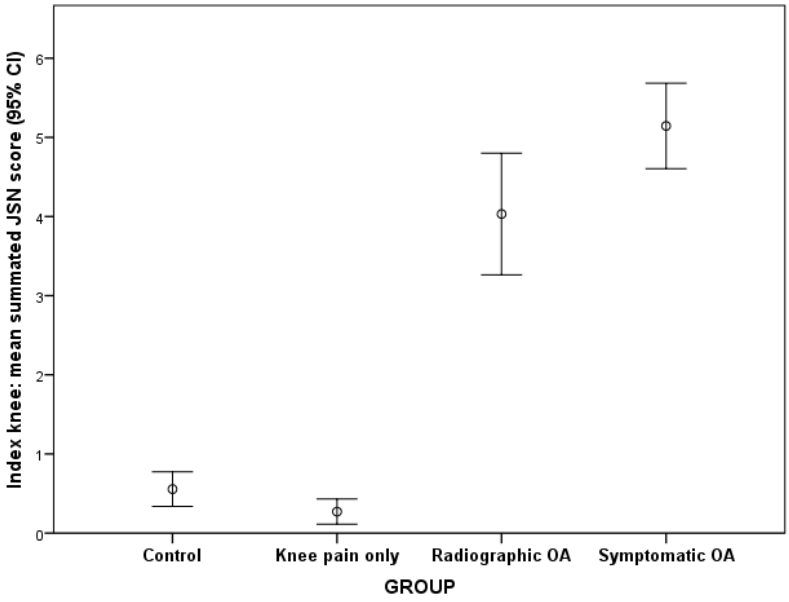


Figure 3-11 Index knee: mean summated scores for tibio-femoral joint (TFJ) for each comparison group

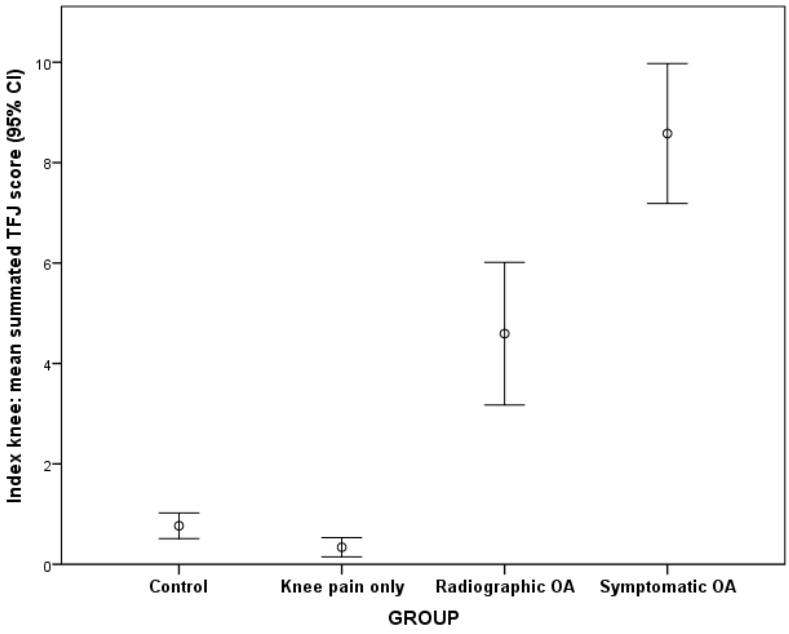
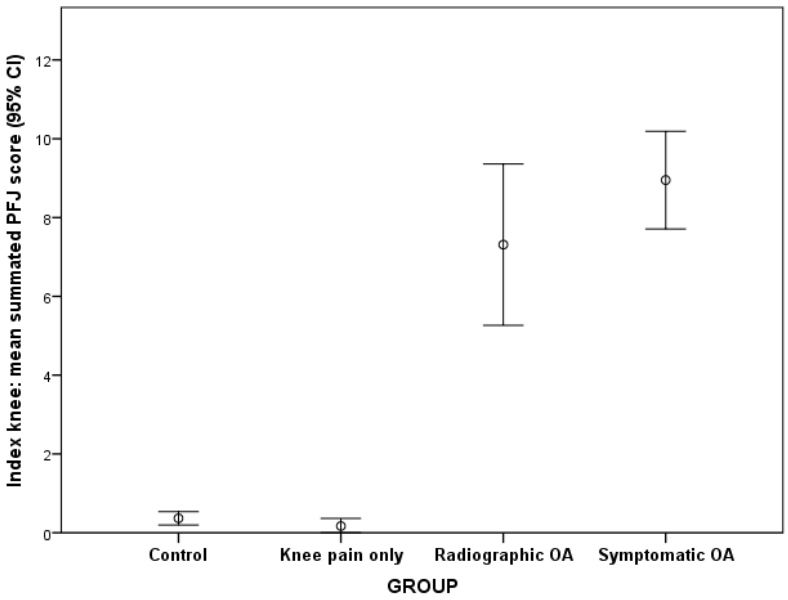


Figure 3-12 Index knee: mean summated scores for patello-femoral joint (PFJ) for each comparison group



3.5.1.2 Contra-lateral knee

Table 3-10 summarises the radiographic scores of the contra-lateral (non-index) knees.

A Kruskal-Wallis test showed significant difference in the summated knee scores between groups ($p<0.001$) (Figure 3-13). Post-hoc Mann-whitney U tests revealed the SOA group had higher global scores and summated scores for osteophytes (Figure 3-14), joint space narrowing (Figure 3-15) and the tibio-femoral joint (Figure 3-16) in the contra-lateral knee compared to those in the ROA group ($p<0.05$). There was no significant difference for patello-femoral joint scores between SOA and ROA groups (Figure 3-17).

Kellgren & Lawrence scores for the contra-lateral (non-index) knee showed no significant differences between control and knee pain groups. The SOA group had a higher proportion of participants with G3 and G4 KL scores than all other groups ($p<0.05$) but was not significantly different to ROA group for grade G2. There was no significant difference between all groups for G0 and G1.

No significant differences were observed in the proportion of participants with radiographic chondrocalcinosis in the contra-lateral knee ($p=0.52$).

Table 3-10 Summated radiographic scores for contra-lateral knee – comparison between groups

Summated radiographic scores		Controls 90		KP 59		ROA 32		SOA 62		p
Global Score (0-60)										
	Mean (SD)	1.07	(2.0)	0.5	(1.1)	8.67	(6.9)	12.2	(8.3)	
	Median (range)	0	(0-12)	0	(0-5)	8	(0-23)	12.5	(0-36)	<0.001
Osteophytes (0-40)										
	Mean (SD)	0.6	(1.3)	0.3	(0.6)	5.8	(5.4)	8.3	(6.6)	
	Median (range)	0	(0-9)	0	(0-2)	5.5	(0-19)	7	(0-28)	<0.001
Joint Space Narrowing (0-20)										
	Mean (SD)	0.5	(1.0)	0.2	(0.7)	2.9	(2.5)	4.0	(2.6)	
	Median (range)	0	(0-5)	0	(0-4)	3	(0-11)	4	(0-10)	<0.001
Tibio-femoralJoint (0-30)										
	Mean (SD)	0.6	(1.2)	0.2	(0.6)	3.1	(3.5)	5.3	(4.9)	
	Median (range)	0	(0.7)	0	(0-3)	2	(0.13)	4	(0.19)	<0.001
Patellofemoral joint (0-30)										
	Mean (SD	0.5	(1.26)	0.3	(0.8)	5.6	(4.9)	7.0	(5.3)	
	Median (range)	0	(0-6)	0	(0-4)	6	(0-20)	6	(0-18)	<0.001
Number of Osteophytes										
	Median (range)	0	(0-5)	0	(0-2)	3.5	(0-8)	5	(0-8)	
Chondrocalcinosis	***N (%)***	5	(5.6%)	3	(5.1%)	0	(0%)	9	(14.5%)	
Kellgren & Lawrence Grade	***N (%)*** **G0**	69	(76.7%)	53	(89.8%)	9	(28.1%)	7	(11.3%)	
	G1	13	(14.4%)	5	(8.5%)	2	(6.3%)	5	(8.1%)	
	G2	4	(4.4%)	1	(1.7%)	3	(9.4%)	13	(21.0%)	
	G3	3	(3.3%)	0	(0%)	16	(50%)	13	(21.0%)	
	G4	1	(1.1%)	0	(0%)	2	(6.3%)	24	(38.7%)	

(KP= knee pain only; ROA= radiographic OA; SOA= symptomatic OA)

Figure 3-13 Contra-lateral knee: mean global x-ray scores for each comparison group

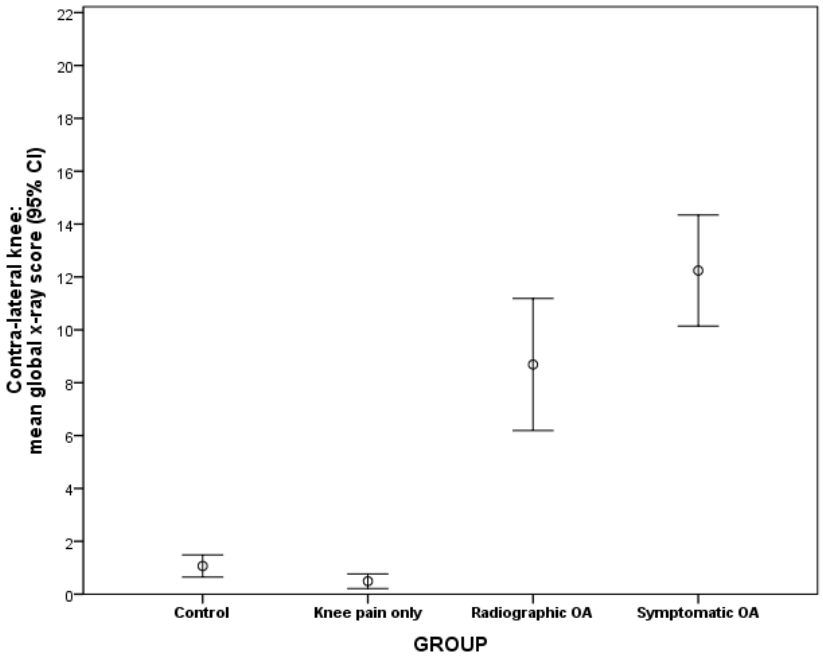


Figure 3-14 Contra-lateral knee: mean summated scores for osteophytes for each comparison group

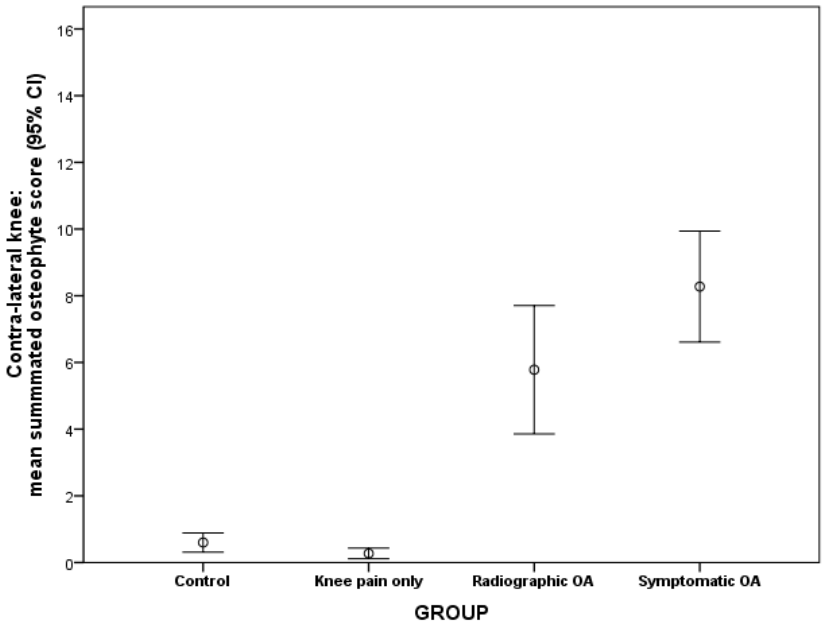


Figure 3-15 Contra-lateral knee: mean summated score for joint space narrowing (JSN) for each comparison group

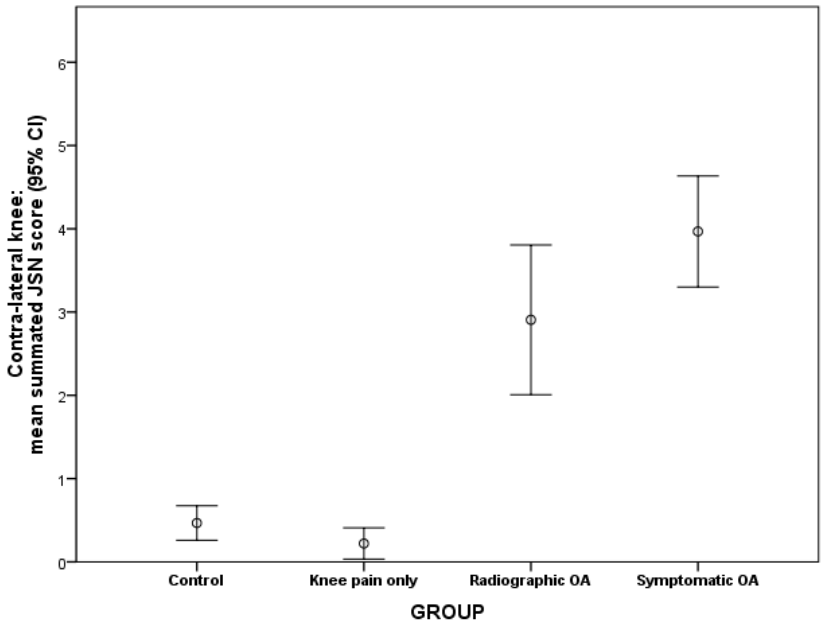


Figure 3-16 Contra-lateral knee: mean summated scores for tibio-femoral joint (TFJ) for each comparison group

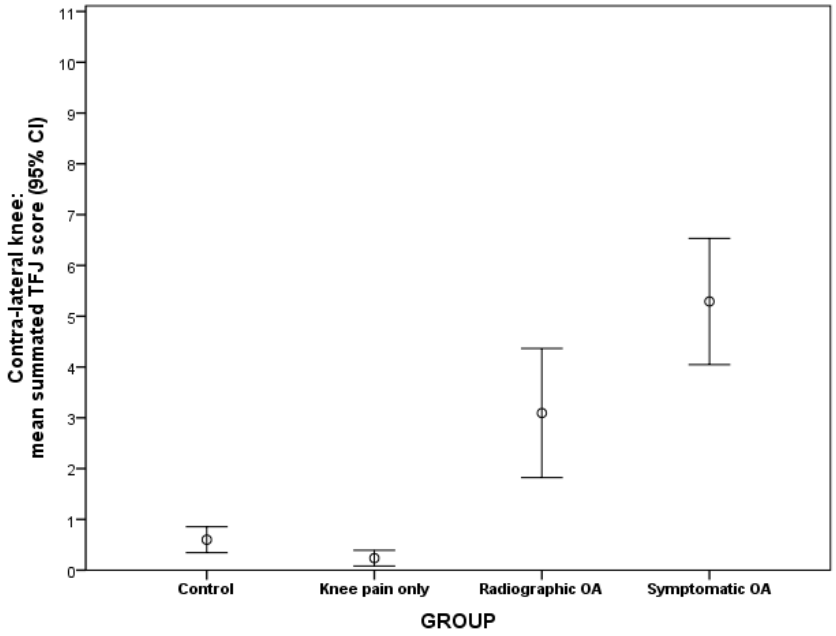
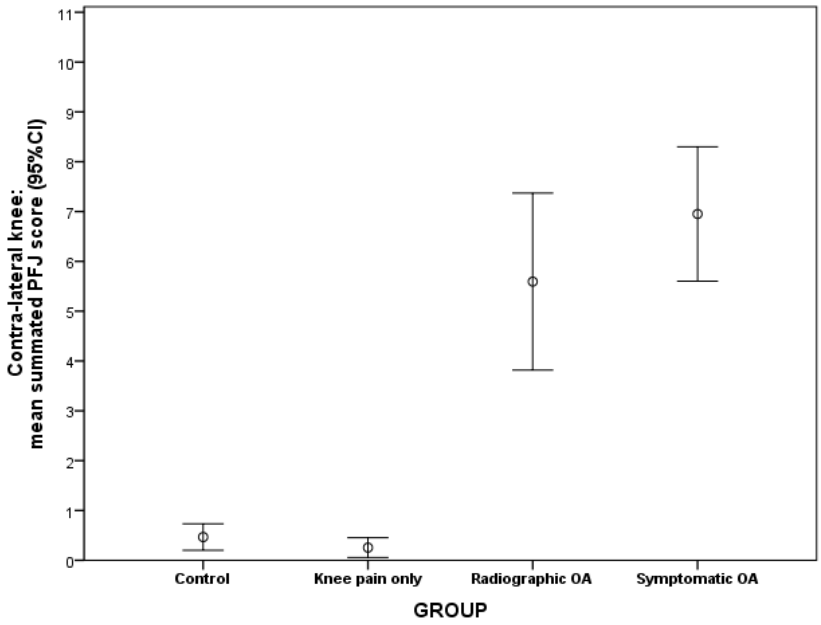


Figure 3-17 Contra-lateral knee: mean summated scores for patella-femoral joint (PFJ) for each comparison group



3.5.2 Pain evaluation – comparison between groups

Half of all participants (n=122) reported having at least moderate pain in the previous week, of which 79 (65%) had pain bilaterally. Participants with pain were asked to consider which knee was most symptomatic – left, right or neither, 49% reported their right knee, 36.5% reported their left knee and 14.5% reported neither knee as more symptomatic. Contra-lateral knee pain was present in 36 (61%) of participants with KP and 43 (69%) of those with SOA. Pain severity in the contra-lateral knee was recorded.

Measures of pain included a 100mm VAS, the pain section of the WOMAC index and the ICOAP questionnaire which has subscales for intermittent and constant knee pain. Descriptive data are presented in Table 3-11. All pain measures demonstrated significant differences between those with and without knee pain ($p<0.001$). No significant differences were observed between those with KP and SOA for measures of knee pain, (Figure 3-18 - Figure 3-21) patterns of knee pain, and type of current pain medication used ($p<0.05$). Pain VAS measures for the contra-lateral knee were not recorded.

Table 3-11 Descriptive Pain data for Index knee – comparison between groups

Group n	Controls 90		KP 59		ROA 32		SOA 62		p
VAS (mm)									
Mean (SD)	6.6	(11.0)	48.9	(22.0)	7.2	(14.4)	48.2	(24.6)	<0.001
WOMAC (0-20)									
Mean (SD)	1.0	(1.5)	8.0	(3.3)	0.9	(1.3)	8.0	(3.2)	
Median (range)	0	(0-8)	8	(2-15)	0	(0-5)	8	(2-15)	<0.001
ICOAP Subscales									
Constant (0-20)									
Mean (SD)	0.5	(1.2)	5.9	(4.8)	0.2	(0.6)	6.9	(5.2)	
Median (range)	0	(0-5)	6	(0-17)	0	(0-2)	6	(0-18)	<0.001
Intermittent (0-24)									
Mean (SD)	1.6	(2.5)	10.2	(4.1)	2.0	(3.1)	10.6	(5.5)	
Median (range)	0	(0-11)	10	(0-21)	0	(0-14)	10.5	(0-24)	<0.001
Knee pain pattern			**n**	**%**			**n**	**%**	
Localised			19	(32.2%)			16	(25.8%)	<0.05
Regional			31	(52.5%)			27	(43.5%)	<0.05
Diffuse			9	(15.3%)			19	(30.6%)	<0.05
Current pain medication	**n**	**(%)**	**n**	**(%)**	**n**	**(%)**	**n**	**(%)**	
None	84	(93.3%)	19	(32.2%)	26	(81.3%)	17	(27.4%)	<0.05
Paracetamol only	2	(2.2%)	12	(20.3%)	1	(3.1%)	14	(22.6%)	<0.05
Oral NSAID only	1	(1.1%)	8	(13.6%)	1	(3.1%)	6	(9.7%)	<0.05
Other only eg co-codamol/ tramadol	3	(3.3%)	5	(8.5%)	1	(3.1%)	6	(9.7%)	<0.05
Combination paracetamol/ NSAID oral/topical /Other	0	(0%)	15	(23.7%)	2	(6.2%)	19	(27.4%)	<0.05

Figure 3-18 Mean pain visual analogue scale (VAS) scores for each comparison group.

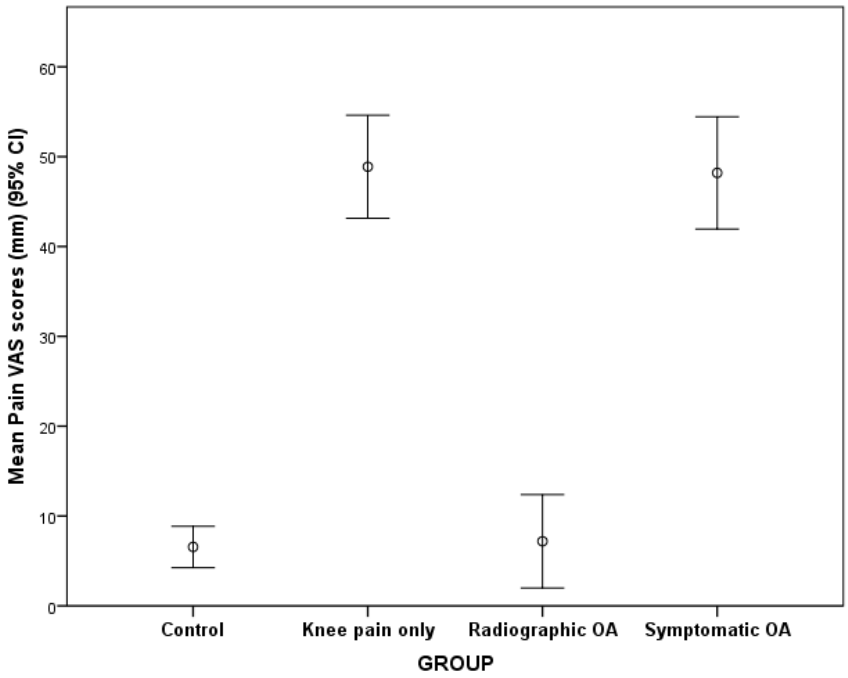


Figure 3-19 Mean WOMAC Pain subscale scores for each comparison group.

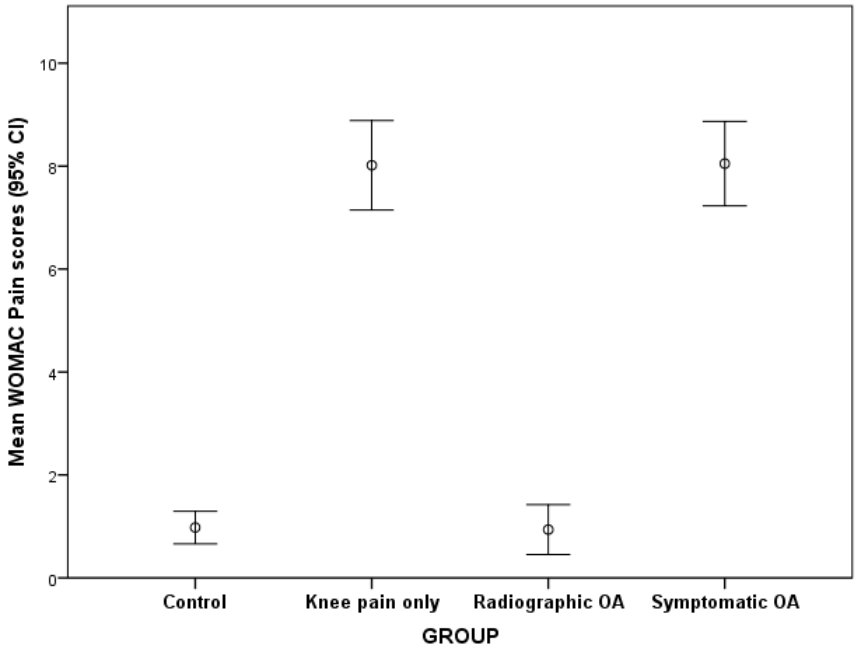


Figure 3-20 Mean ICOAP Constant subscale score for each comparison group

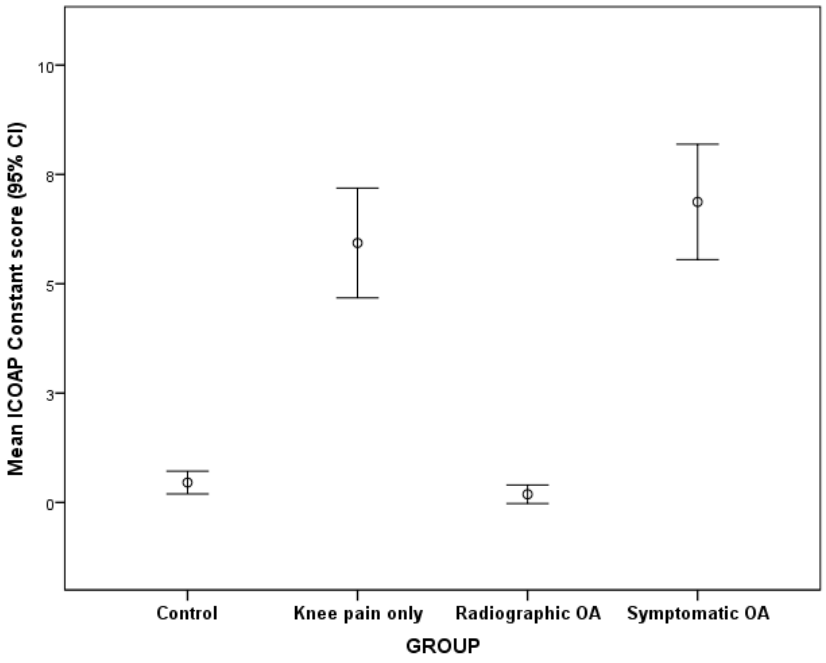
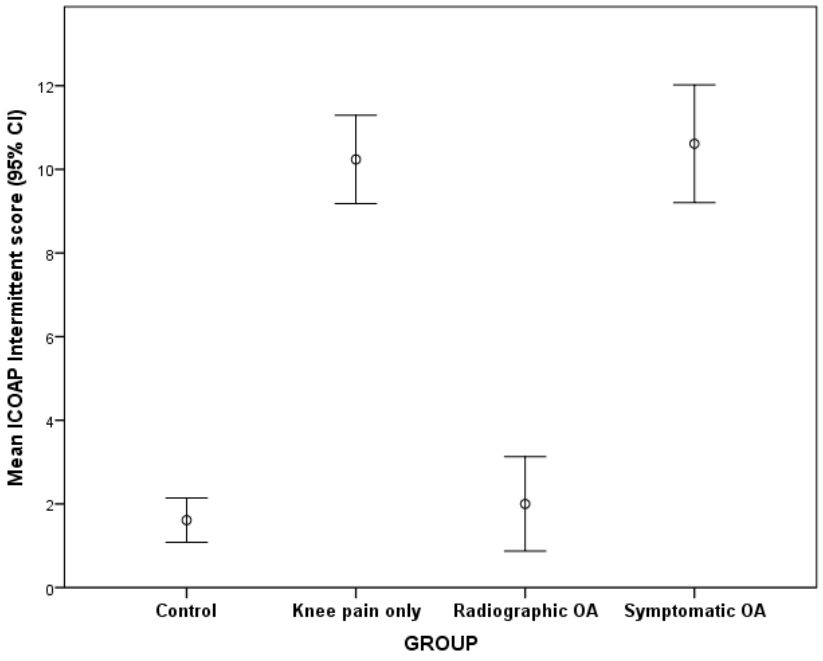


Figure 3-21 Mean ICOAP Intermittent subscale score for each comparison group



3.5.3 US evaluation – comparison between groups

3.5.3.1 Index knee

Frequency data for US features in the index knee are presented in Table 3-12 and are presented graphically in Figure 3-22. Descriptive data for continuous measures of depth of effusion, thickness of synovial hypertrophy and depth of popliteal cysts are presented in Table 3-13.

Table 3-13. There was no statistical difference in the observed frequency of US features or measures between control participants and those with KP for all features.

Table 3-12 Index knee: frequency data for US features – comparison between groups

Group	**Controls**	**KP**	**ROA**	**SOA**	
n	**90**	**59**	**32**	**62**	**p**
	n (%)	n (%)	n (%)	n (%)	
Effusion	26 (28.9)	19 (32.2)	26 (81.3)	57 (91.9)	<0.001
Synovial hypertrophy	7 (7.8)	7 (11.9)	13 (40.6)	51 (82.3)	<0.001
Popliteal cysts	11 (12.4)	5 (8.6)	7 (21.9)	23 (39.2)	<0.001
Infra-pat bursitis	3 (3.3)	4 (6.8)	0 (0)	5 (8.1)	=0.28
Pes- Anserine Bursitis	0 (0)	0 (0)	0 (0)	4 (6.5)	
PD signal	2 (2.2)	2 (3.4)	2 (6.3)	10 (16.2)	=0.005
Grade 1	2 (2.2)	1 (1.7)	2 (6.3)	5 (8.1)	
Grade 2	0 (0)	0 (0)	0 (0)	5 (8.1)	
Grade 3	0 (0)	1 (1.7)	0 (0)	0 (0)	
Chondrocalcinosis	2 (2.2)	1 (1.7)	0 (0)	7 (11.3)	=0.01

(KP= knee pain only; ROA= radiographic OA; SOA= symptomatic OA; PD = power Doppler; CC= chondrocalcinosis)

Figure 3-22 Index knee: frequency of US features – comparison between groups (KP= knee pain only; ROA= radiographic OA; SOA= symptomatic OA)

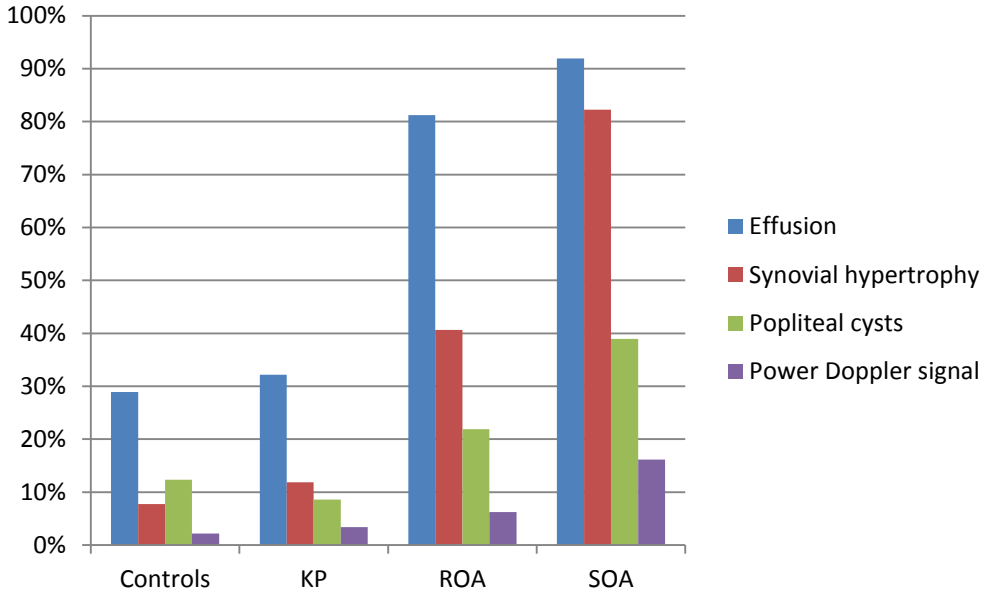


Table 3-13 Index knee: descriptive data of continuous US measures for each comparison group

	Controls	KP	ROA	SOA	p
	90	59	32	62	
Effusion					
Mean (SD)	2.6 (2.7)	3.4 (3.2)	6.0 (2.6)	8.1 (4.0)	<0.001
Median (range)	2.6 (0-9)	3.1 (0-13.6)	5.6 (1.6-13.7)	8.0 (0-21.8)	
Synovial Hypertrophy					
Mean (SD)	0.7 (1.5)	1.0 (1.9)	3.9 (3.9)	6.7 (3.3)	<0.001
Median (range)	0 (0-6.7)	0 (0-8.1)	3.10 (0-12.9)	6.90 (0-12.9)	
Popliteal cysts					
Mean (SD)	1.0 (2.6)	0.8 (2.2)	1.8 (3.6)	3.5 (4.7)	=0.001
Median (range	0 (0-12.5)	0 (0-11.7)	0 (0-12.4)	0 (0-14.3)	

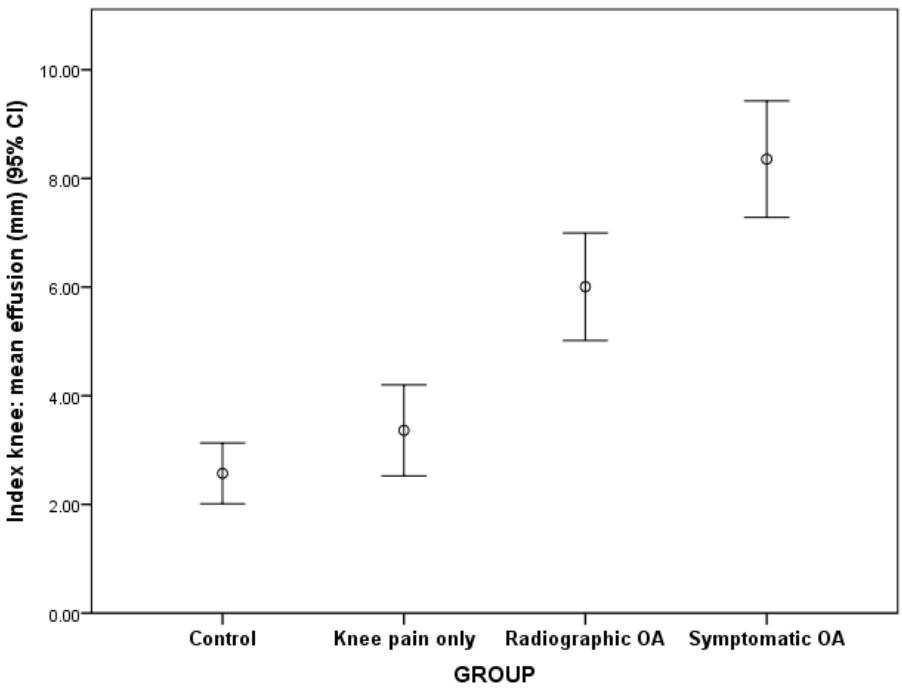
(KP= knee pain only; ROA= radiographic OA; SOA= symptomatic OA)

3.5.3.1.1 Effusion

Effusions on ultrasound were observed in 28.9% of control participants and 32.2% of those with knee pain. The frequency was significantly higher in those with ROA (81.3%; $p<0.05$) and was higher again in those with SOA (91.9%) but not significantly so.

The mean depth of effusion showed a trend increasing in size in those with KP, ROA and SOA compared to controls (Figure 3-23). Post-hoc comparisons revealed no difference between the control group and the KP group but significant differences between all other groups ($p<0.05$).

Figure 3-23 Index knee: mean US measures of effusion (mm) (95% CI) for each comparison group

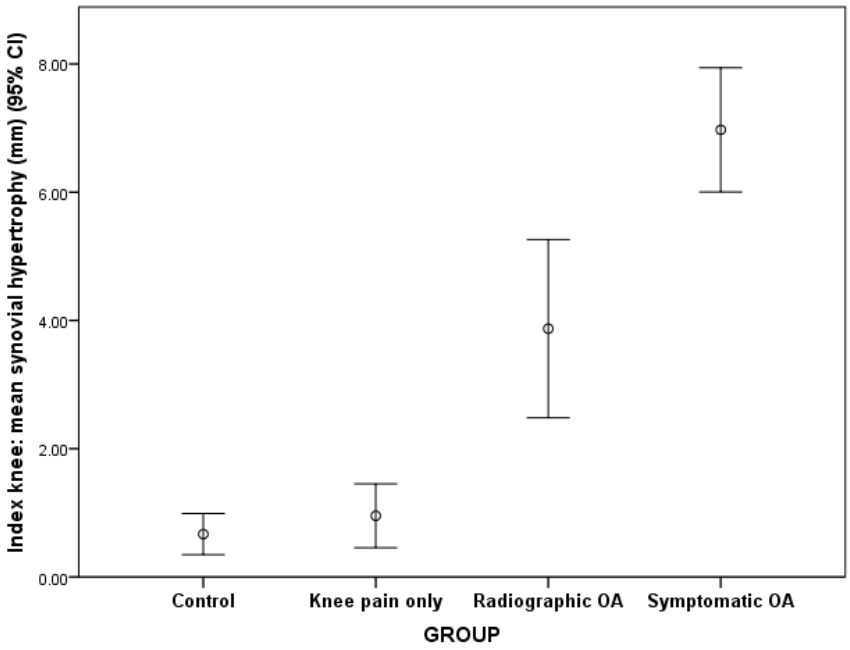


3.5.3.1.2 Synovial Hypertrophy

The frequency of synovial hypertrophy was significantly different between groups ($p<0.001$). The frequency was less than 8% in controls and 12% in those with KP but was significantly higher in those with ROA (40.6%) ($p<0.05$) and higher again in SOA (82.3%) ($p<0.05$).

The depth of synovial hypertrophy also differed significantly between groups (Figure 3-24). Post hoc analysis revealed no significant difference between the control and KP groups. The ROA and SOA groups differed significantly from the control group and from each other (both $p<0.05$).

Figure 3-24 Index knee: mean US measures of synovial hypertrophy (mm) (95%CI) for each comparison group

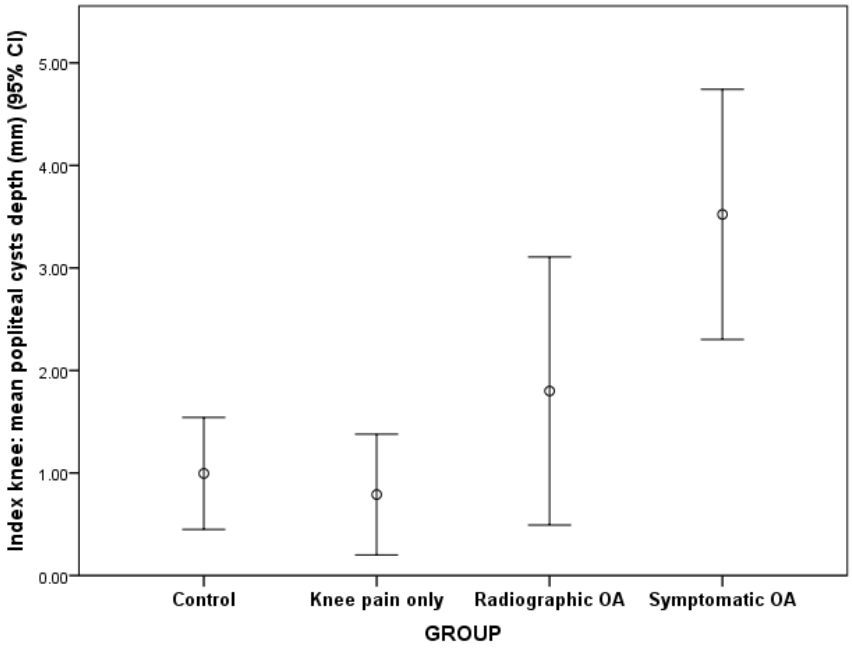


3.5.3.1.3 Popliteal cyst

Popliteal cysts were observed in 12.4% of controls which was not significantly different from the KP group where they were observed less frequently (8.6%). A higher occurrence was found in the ROA (21.9%, $p<0.05$) and SOA (39%, $p<0.05$) groups which was significantly different from the control and KP groups but were not significantly different to each other.

The size of popliteal cyst also differed significantly between groups ($p=0.001$). Post-hoc tests showed that popliteal cysts were significantly larger in the SOA group compared to both the control and KP groups ($p<0.05$) but not significantly different to the ROA group ($p=0.11$).

Figure 3-25 Index knee: mean US measures of popliteal cyst (mm) (95% CI) for each comparison group



3.5.3.1.4 Power Doppler Signal

The frequency of PD signal in the SOA group (16.2%) was significantly higher than in the control (2.2%, $p<0.05$) and KP groups (3.4%, $p<0.05$) but not significantly different to the ROA group (6.3%). The grade of PD signal was not subject to analysis due to the low frequency observed.

3.5.3.1.5 Chondrocalcinosis

US-detected chondrocalcinosis was significantly higher in the SOA compared to all other groups ($p=0.01$).

3.5.3.2 Contra-lateral knee

US features in the contra-lateral knee are presented in Table 3-14 and are presented graphically in Figure 3-26. Descriptive data for continuous US measures are presented in Table 3-15. No significant differences were observed in the contra-lateral knees of controls and knee pain participants for any US measure.

In the SOA group, contra-lateral knees had a significantly higher frequency of US effusion, hypertrophy and popliteal cysts compared to the contra-lateral knees of all other groups ($p<0.05$). Size of effusion and synovial hypertrophy were also significantly higher ($p<0.05$) when compared to all other groups (Figure 3-27, Figure 3-28). Popliteal cyst size was only significantly greater compared to controls and knee pain only participants ($p<0.05$) (Figure 3-29).

Frequency of Power Doppler activity was only significantly different between controls and SOA participants ($p<0.05$), There was no significant difference between groups for US detected chondrocalcinosis in the contra-lateral knee ($p=0.07$)

Table 3-14 Contra-lateral knee: frequency data for US features in each comparison group

Group	Controls	KP	ROA	SOA	
n	**90**	**59**	**32**	**62**	**p**
	n (%)	n (%)	n (%)	n (%)	
Effusion	17 (18.9)	21 (35.6)	14 (43.8)	46 (74.2)	<0.001
Synovial hypertrophy	6 (3.7)	6 (10.2)	10 (31.3)	35 (36.5)	<0.001
Popliteal cysts	8 (9)	4 (6.9)	2 (6.3)	20 (33.9)	<0.001
Infra-pat bursitis	0 (0)	2 (3.4)	1 (3.1)	2 (3.2)	=0.39
Pes- Anserine Bursitis	0 (0)	0 (0)	0 (0)	2 (3.2)	=0.12
PD signal	0 (0)	4 (6.8)	2 (6.3%)	8 (12.9)	=0.01
Grade 1	0 (0)	4 (6.8)	2 (6.3%)	5 (8.1)	
Grade 2	0 (0)	0 (0)	0 (0)	3 (4.8)	
Grade 3	0 (0)	0 (0)	0 (0)	0 (0)	
Chondrocalcinosis	2 (2.2)	2 (3.4)	0 (0)	4 (6.5)	=0.07

(KP= knee pain only; ROA= radiographic OA; SOA= symptomatic OA; PD = power Doppler; CC= chondrocalcinosis)

Figure 3-26 Contra-lateral knee: frequency of US features in each comparison group

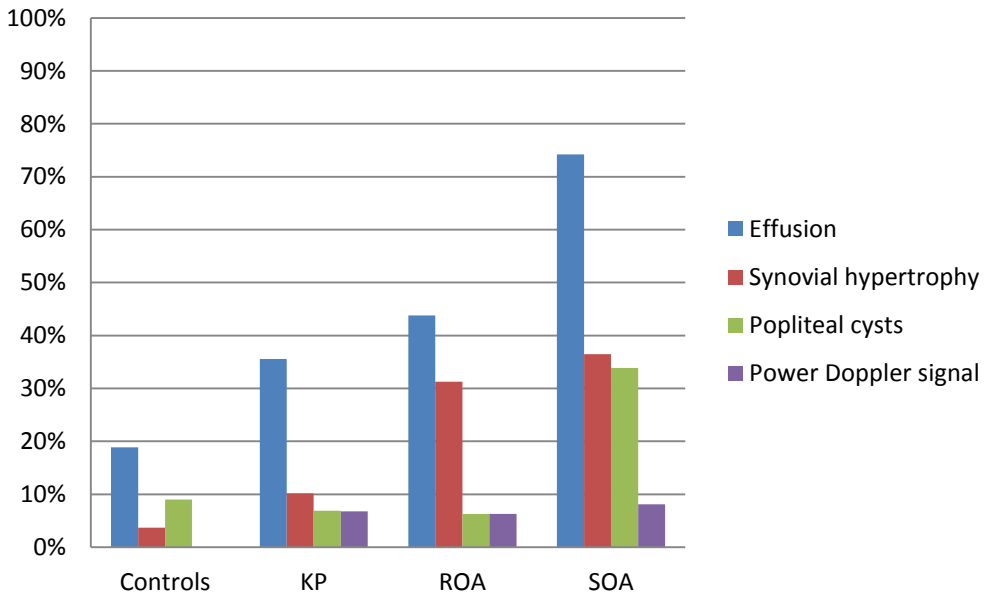


Table 3-15 Contra-lateral knee: descriptive data of continuous US measures in each comparison group

	Controls		KP		ROA		SOA		p
	90		59		32		62		
Effusion									
Mean (SD)	2.2	(2.4)	2.8	(2.6)	3.8	(2.9)	6.4	(4.1)	
Median (range)	1.7	(0-9.3)	3.1	(0-10.4)	3.4	(0-9.4)	5.3	(0-14.6)	<0.001
Synovial Hypertrophy									
Mean (SD)	0.6	(1.6)	1.1	(2.5)	2.5	(3.1)	5.0	(4.2)	
Median (range)	0	(0-8.7)	0	(0-13.1)	0.8	(0-9.4)	4.1	(0-13.8)	<0.001
Popliteal cysts									
Mean (SD)	0.8	(2.3)	0.6	(1.8)	0.7	(1.5)	2.9	(4.7)	
Median (range)	0	(0-13.5)	0	(0-9.0)	0	(0-4.7)	0	(0-20.2)	<0.001

(KP= knee pain only; ROA= radiographic OA; SOA= symptomatic OA)

Figure 3-27 Contra-lateral knee: mean US measures of effusion (mm) (95% CI) in each comparison group

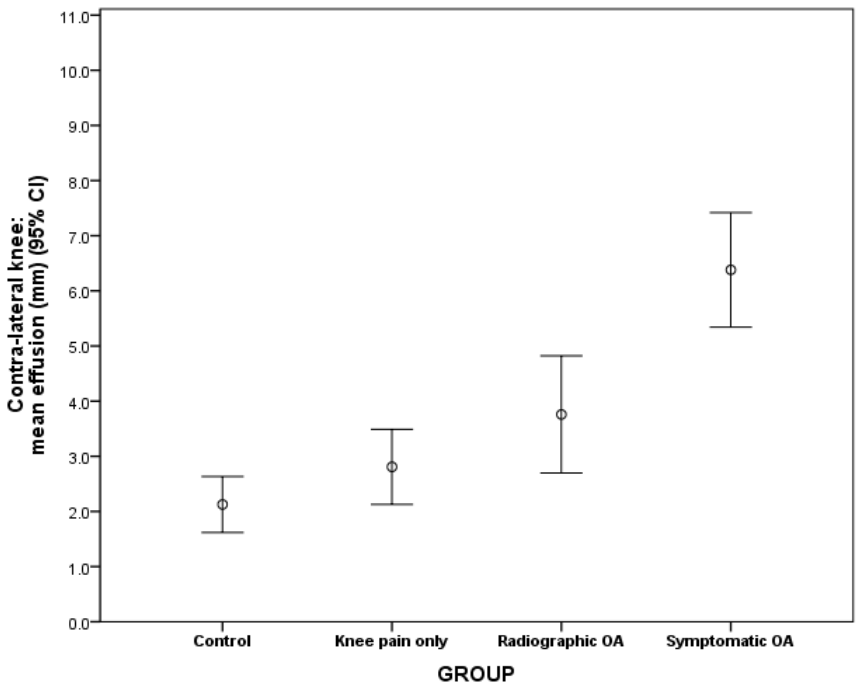


Figure 3-28 Contra-lateral knee: mean US measures of synovial hypertrophy (mm) (95% CI) for each comparison group

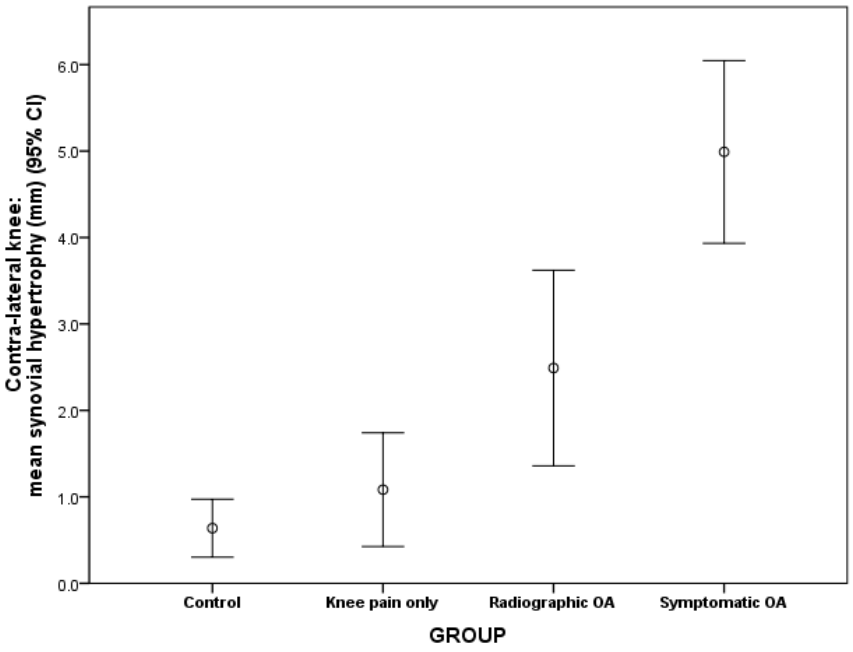
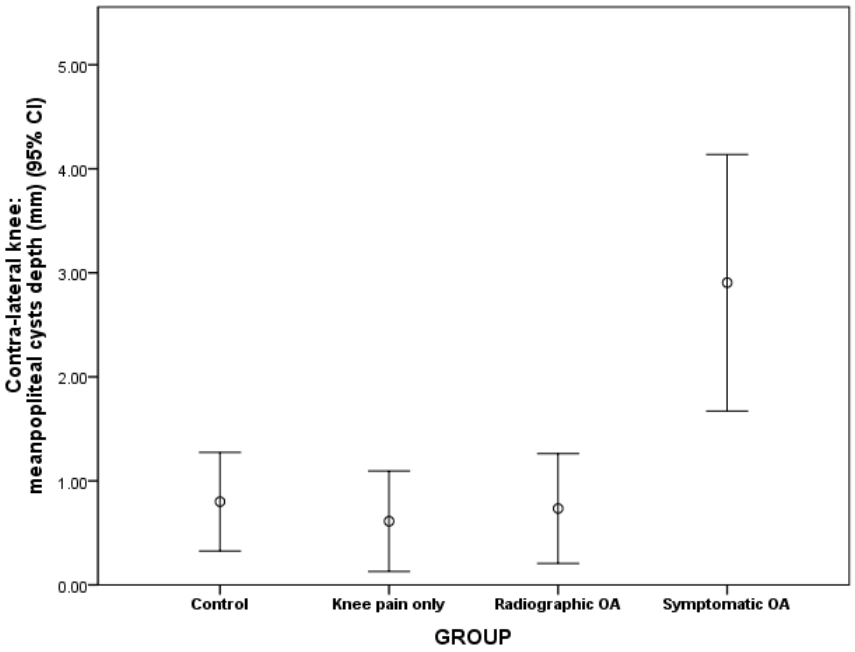


Figure 3-29 Contra-lateral knee: mean US measures of popliteal cyst size (mm) 95% CI) for each comparison group



3.5.3.3 Relationships between US features

US measures of effusion and synovial hypertrophy were strongly associated with each index and non-index knee joints (Table 3-16). Popliteal cysts measures showed only a weak correlation with both effusion and synovial hypertrophy.

Table 3-16 Relationship between continuous US measures for Index and Non-index knees

Knee			Effusion (mm)	Synovial Hypertrophy (mm)	Popliteal cyst (mm)
Index	Effusion (mm)	r	1.00		
	Hypertrophy(mm)	r	0.79**	1.00	
	Popliteal cyst (mm)	r	0.22**	0.35**	1.00
Non-Index	Effusion (mm)	r	1.00		
	Hypertrophy (mm)	r	0.61**	1.00	
	Popliteal cyst (mm)	r	0.26**	0.38**	1.00

*** Correlation is significant at the 0.05 level**
**** Correlation is significant at the 0.01 level**
mm = millimetres

When correlations were examined within the SOA group (data not shown), the strength of the relationship between effusion and hypertrophy was reduced but remained significant for both index and non-index knees ($r=0.55$, $p<0.01$ and $r= 0.53$, $p<0.01$ respectively)) and remained similar for synovial hypertrophy and popliteal cyst ($r=0.30$, $p<0.05$, and $r= 0.37$ $p<0.01$ respectively).

3.5.4 Clinical evaluation (Index knee) and function– comparison between groups

Clinical evaluation and functional assessment included clinically detected knee effusion, range of movement, quadriceps and hamstring muscle strength, timed get up and go tests and a biomechanical assessment of stiffness and damping co-efficients. The WOMAC questionnaire was also used to evaluate self-reported stiffness and function. Table 3-17 shows the descriptive data for clinical and functional measures.

3.5.4.1 Clinical Effusion

For the index knee, clinical effusions were detected in just 2.2% of control knees and 3.4% of knee pain only knees. A significantly higher number of effusions were observed in ROA knees (15.6%) and higher again (50%) in SOA knees ($p<0.001$).

3.5.4.2 Stiffness

Measures of knee joint stiffness included self-reported morning stiffness of greater than 30 minutes duration, the stiffness subscale from the WOMAC index, and the damping and stiffness co-efficient calculated from the passive pendulum knee test.

Self-reported stiffness that was at least moderate in intensity was reported more frequently in participants with knee pain (69.5%) and SOA (82.3%) compared to those with ROA (6.3%) and control participants (12.2%).

Table 3-17 Clinical and functional measures – comparison between groups

	Controls		KP		ROA		SOA		
	90		59		32		62		p
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
ROM (degrees)	141	(8.2)	136	(13.2)	135	(8.4)	121	(20.5)	<0.001
Quadriceps St (kg)	16.7	(4.2)	16.6	(5.0)	16.6	(4.4)	12.8	(4.1)	<0.001
Hamstring St (kg)	7.4	(1.6)	8.0	(2.1)	7.9	(2.0)	6.9	(1.8)	=0.038
Timed GUG 3m (s)	7.7	(1.8)	8.9	(2.3)	8.5	(1.9)	11.9	(5.3)	<0.001
Timed GUG 50ft (s)	10.7	(2.1)	12.0	(2.6)	11.8	(2.4)	15.8	(6.9)	<0.001
WOMAC sub-scale:									
Stiffness (0-8)	.8	(1.1)	3.4	(1.6)	0.9	(0.9)	4.0	(1.8)	<0.001
Function (0-68)	4.2	(6.5)	25.8	(12.3)	5.5	(6.6)	29.6	(11.8)	<0.001
Damping co-eff (N/m/rad/sec)	0.46	(0.15)	0.49	(0.19)	0.47	(0.11)	0.50	(0.18)	=0.70
Stiffness co-eff (N/m/rad)	15.25	(4.41)	17.21	(4.99)	18.17	(3.37)	18.16	(5.52)	=0.009
Clinical Effusion n (%)	2	(2.2%)	2	(3.4%)	5	(15.6%)	31	(50%)	<0.001
Valid Stiffness test n (%)	63	(70%)	34	(57.6%)	21	(65.5%)	36	(58.1%)	=0.34
Moderate Stiffness (WOMAC) n (%)	11	(12.2%)	41	(69.5%)	2	(6.3%)	51	(82.3%)	<0.001
AM Stiffness >30mins n (%)	0	(0%)	20	(37.7%)	0	(0%)	15	(27.3%)	<0.001

(KP= knee pain only; ROA= radiographic OA; SOA= symptomatic OA)

Morning stiffness lasting 30 minutes or longer was reported only by participants with knee pain regardless of radiographic OA, which was highly significant ($p<0.001$).

WOMAC scores for stiffness reflected the same pattern and showed differences between groups that were significant for KP and SOA compared to controls and ROA ($p<0.001$) (Figure 3-30).There was no significant difference between the KP group and SOA group for self-reported stiffness.

The calculation of stiffness and damping co-efficients were dependent on the performance of a valid pendulum test. Not all participants were able to complete the passive pendulum test. Successful tests were performed by 154 participants (63%). A higher proportion of control participants (70%) were able to complete the pendulum test compared to other groups but this was not statistically significant ($p=0.34$).

Mean stiffness co-efficients were significantly higher in the SOA group (mean difference = 2.91 Nm/rad (95% CI 0.07-5.75),$p=0.04$) compared to controls (Figure 3-31). There were no other significant differences between groups. Mean damping co-efficients did not show any differences between groups ($p=0.70$) (Figure 3-32).

Figure 3-30 Mean WOMAC Stiffness subscale (95% CI) for each comparison group

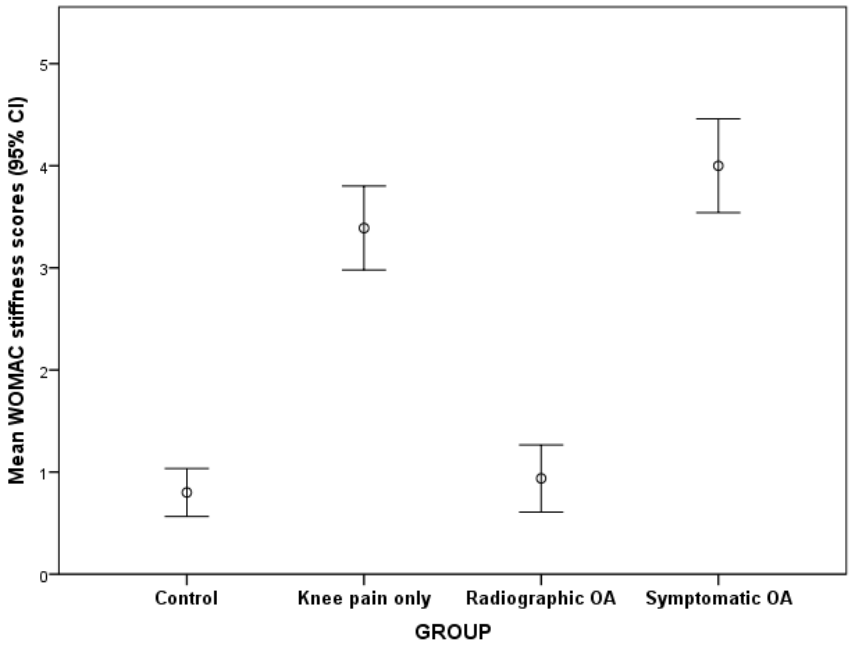


Figure 3-31 Index knee: mean stiffness co-efficient (95% CI) for each comparison groups.

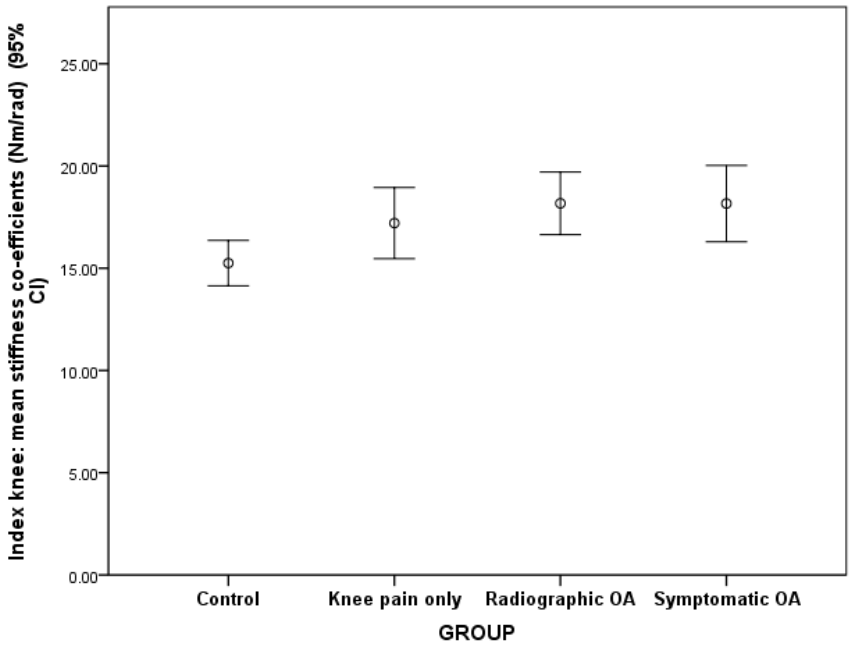
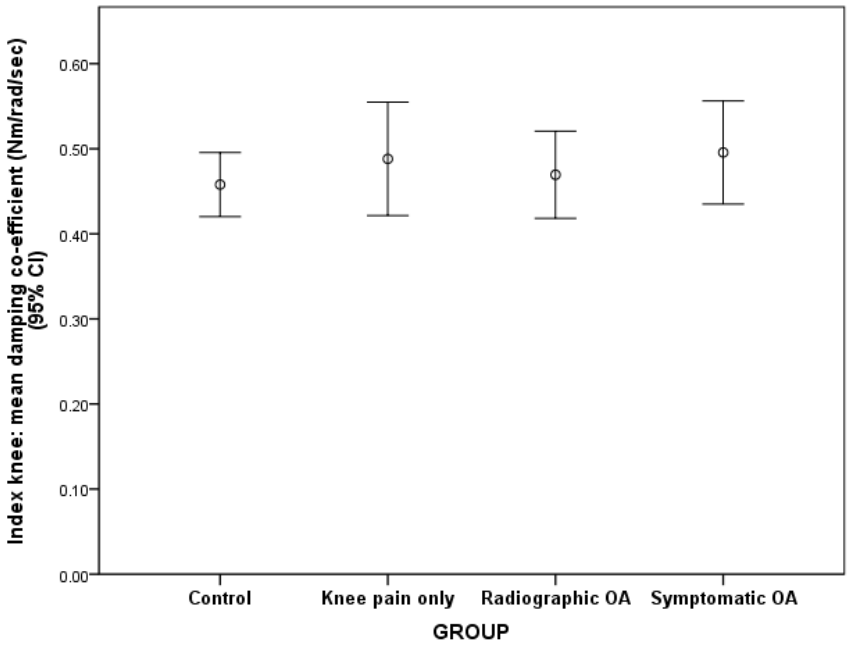


Figure 3-32 Index knee: mean damping co-efficient (95% CI) for each comparison groups



3.5.4.3 Range of movement

Significant differences between groups were observed for ROM, $p<0.001$ (Figure 3-33). Those with SOA had significantly less ROM compared to all other groups ($p<0.001$). Those with KP or ROA also has significantly less ROM compared to controls ($p<0.01$) but were not significantly different to each other.

3.5.4.4 Muscle strength

Quadriceps strength was significant lower in the SOA group compared to other groups ($p<0.001$). Other groups did not differ from each other (Figure 3-34). No significant differences were found for hamstring strength ($p=0.07$) (Figure 3-35).

Figure 3-33 Index knee: mean range of movement (ROM) (95% CI) for each comparison group

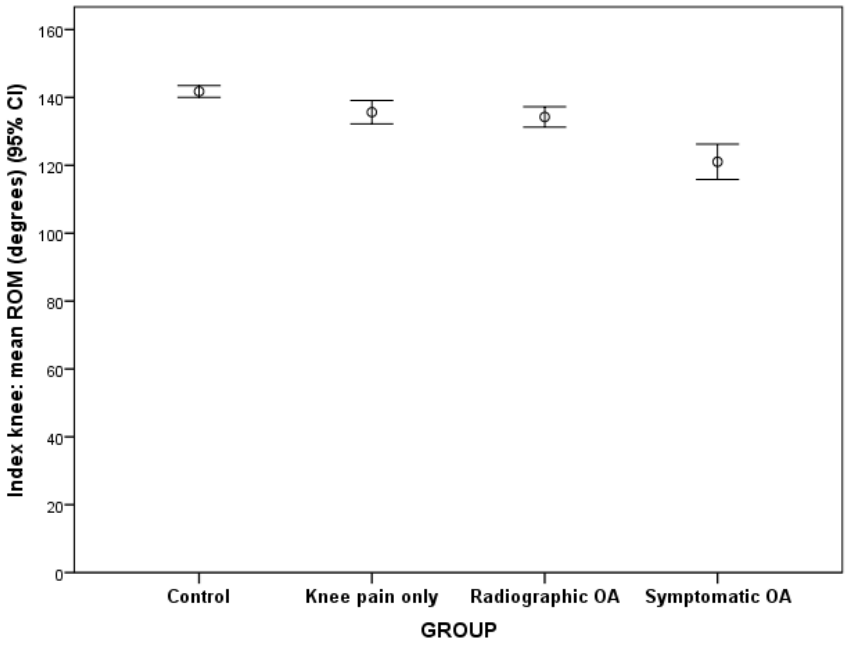


Figure 3-34 Index knee: mean Quadriceps strength (kg) (95% CI) for each comparison group

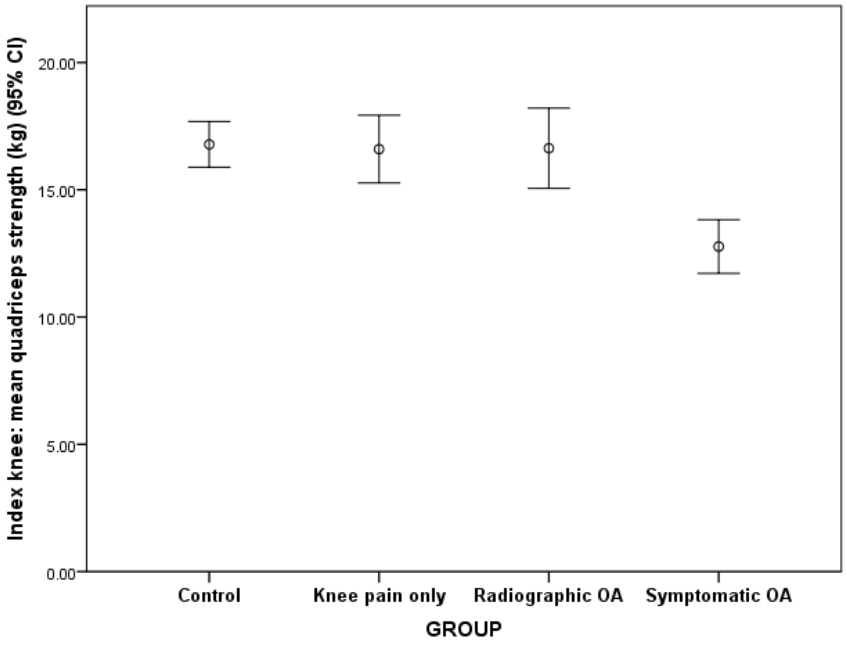
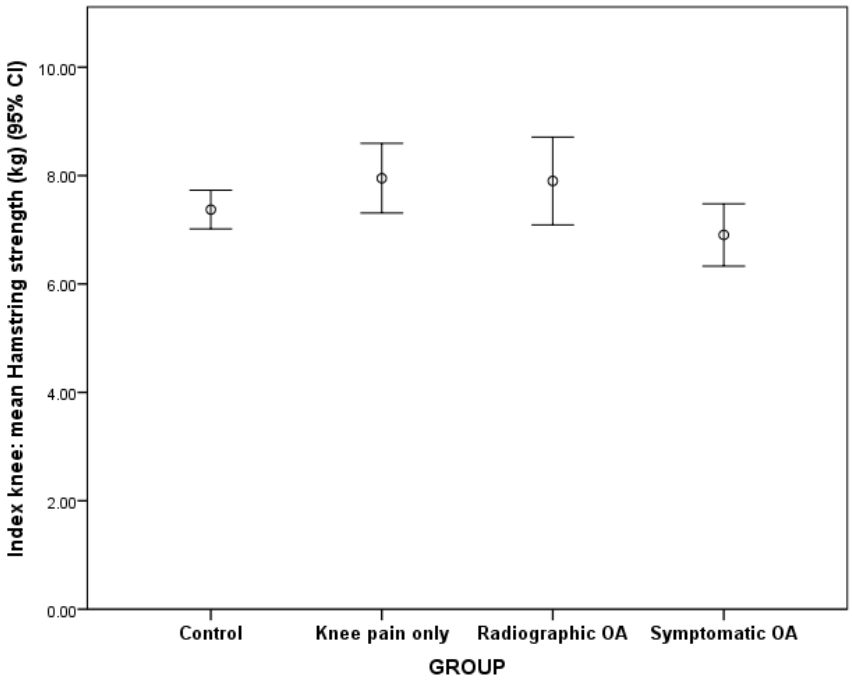


Figure 3-35 Index knee: mean Hamstring strength (kg) (95%CI) for each comparison group



3.5.4.5 Get Up and Go tests

Those in the SOA group were significantly slower than all other groups ($p<0.001$) (Figure 3-36).Those with KP were slower compared to controls ($p=0.007$) but not significantly different to those with ROA. The same observations were also significant in the GUG (50 feet) test ($p<0.001$).

3.5.4.6 WOMAC Function subscale

Differences between groups were observed for WOMAC function subscale ($p<0.001$) (Figure 3-37). Those in the KP and SOA groups has significantly higher WOMAC scores (denoting worse function) compared to controls and ROA ($p<0.001$). There was no difference in self-reported function between ROA and controls.

Figure 3-36 Mean time GUG 3m (seconds) (95% CI) for each comparison group

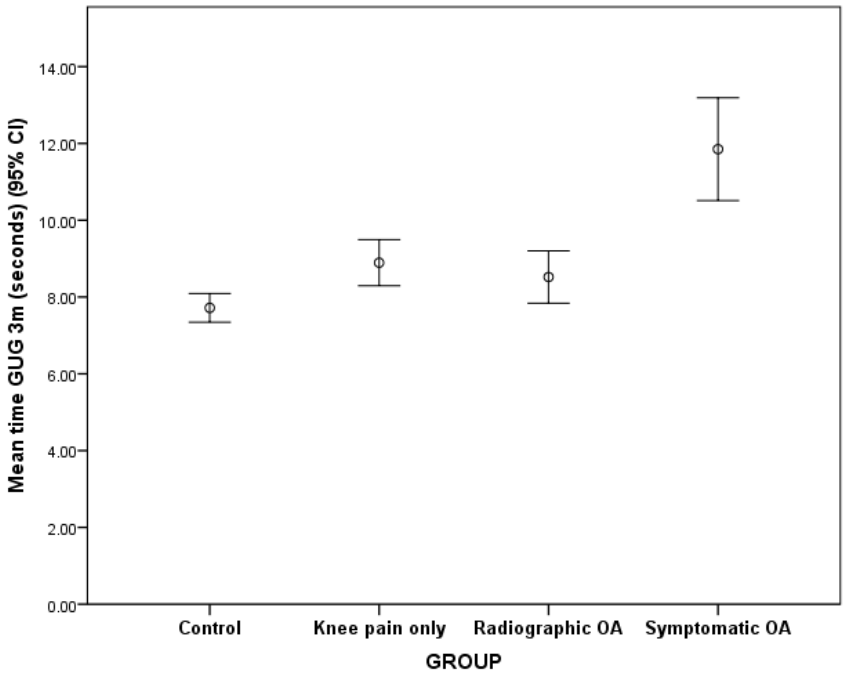
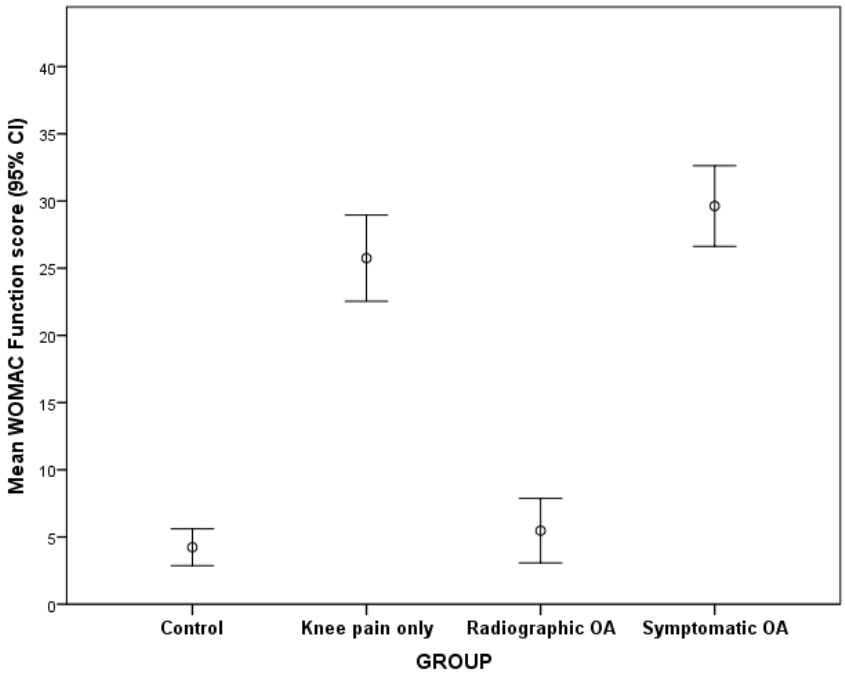


Figure 3-37 Mean WOMAC Function scores (95%CI) for each comparison group



3.5.4.7 Clinical evaluation in the Contra-lateral knee

Joint specific clinical evaluations included the presence of clinically detected effusion, range of movement, muscle strength of quadriceps and hamstrings, and stiffness and damping co-efficients Table 3-18.

Table 3-18 Contra-lateral knee: Clinical and functional measures in each comparison group

	Controls		KP		ROA		SOA		
	90		59		32		62		p
	n	(%)	n	(%)	n	(%)	n	(%)	
Clinical Effusion	0	(0%)	1	(1.7%)	2	(6.3%)	16	(25.8%)	<0.001
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	
ROM (degree)	142.2	(8.3)	136.6	(12.4)	134.4	(8.1)	126.9	(19.7)	<0.001
Quadriceps St (kg)	16.9	(4.1)	17.2	(4.9)	17.1	(4.7)	14.9	(4.6)	<0.001
Hamstring St (kg)	7.4	(1.7)	8.2	(2.0)	7.8	(1.6)	7.2	(2.5)	=0.076
Damping co-eff: (N/m/rad/sec)	0.44	(0.17)	0.47	(0.18)	0.51	(0.17)	0.49	(0.18)	=0.26
Stiffness co-eff: (N/m/rad)	15.28	(4.42)	17.30	(4.90)	18.43	(3.66)	18.03	(5.07)	=0.003

Effusions in the contra-lateral knee were only significantly higher in the SOA group ($p<0.05$). Kruskal-Wallis tests identified differences between groups for contra-lateral ROM, quadriceps strength and stiffness co-efficients. Post hoc Mann-Whitney U tests identified that ROM was significantly reduced in SOA participants compared to controls ($p<0.05$)(Figure 3-38), that contra-lateral quadriceps strength was significantly lower in SOA participants compared to all other groups ($p<0.05$)(Figure 3-39), and that stiffness co-efficients were significantly higher in SOA and ROA participants compared to controls ($p<0.05$)(Figure 3-40).

Figure 3-38 Contra-lateral knee: mean range of movement (ROM) (degrees) (95% CI)

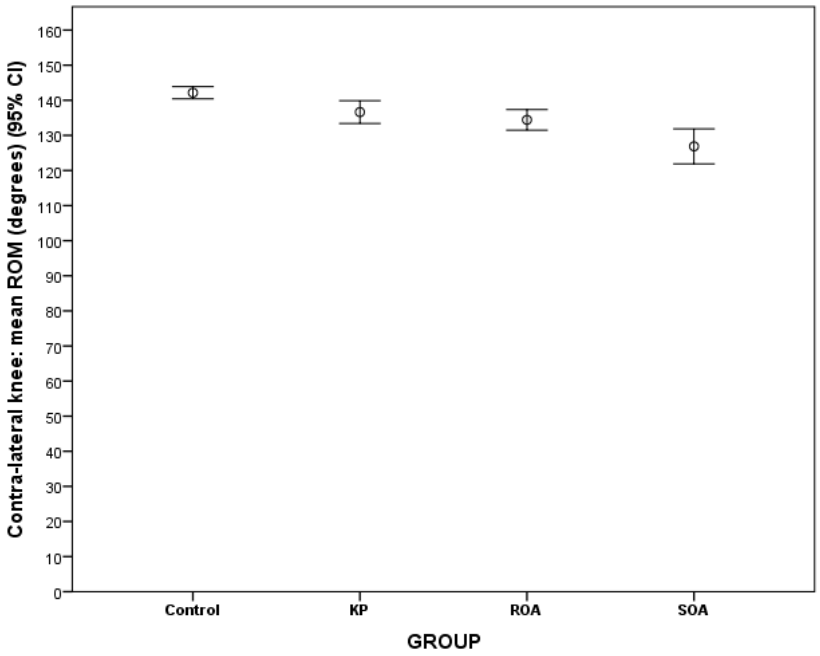


Figure 3-39 Contra-lateral knee:mean quadriceps strength (kg) (95% CI)

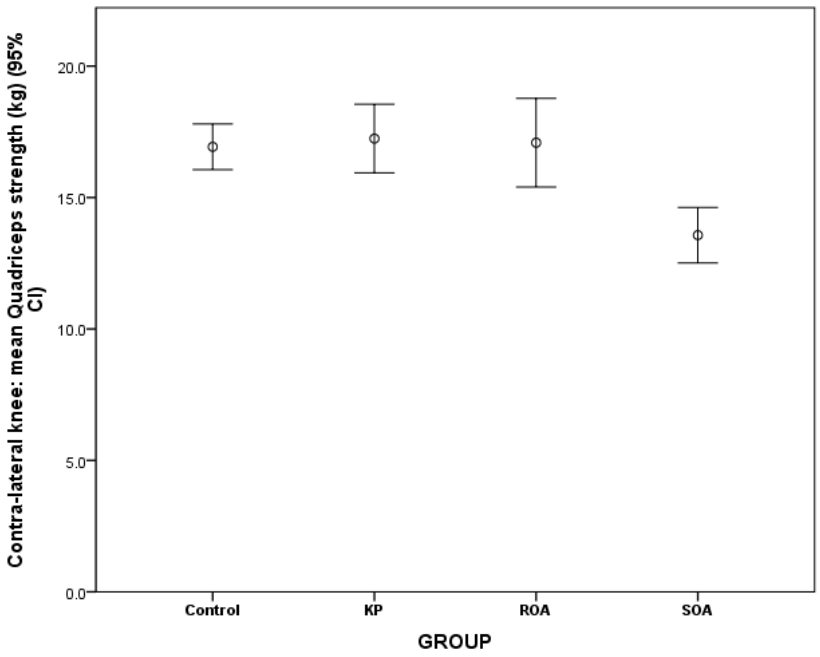
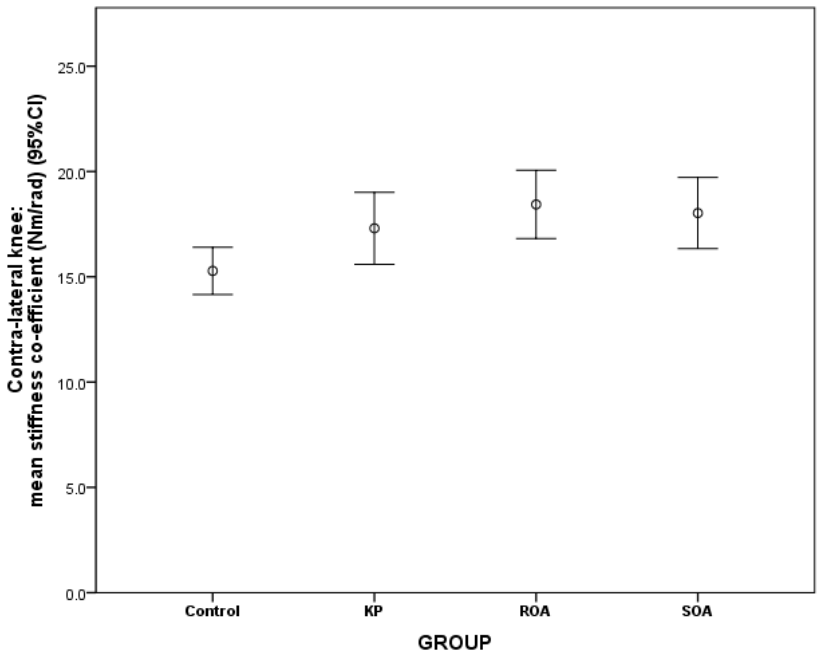


Figure 3-40 Contra-lateral knee: mean stiffness co-efficient (Nm/rad) (95% CI)



3.6 Secondary analysis

3.6.1 Associations between knee pain and US features

Knee pain was determined from the pain subscale of the WOMAC questionnaire. Of 243 participants, 121 (49.8%) were classified as knee pain positive and 122 (50.2%) as pain negative. Knee pain was examined in relation to the presence US features (Table 3-19). After adjusting for age, sex, BMI and ROA, only synovial hypertrophy was independently associated with knee pain (aOR=6.56, 95%CI 2.85, 15.11).

Table 3-19 Association between US features and knee pain

US features	Knee pain		Odds ratio (95% confidence interval)		
	no	yes	Crude OR	Adjusted[1]	Adjusted[2]
Effusion					
no	70	45	1	1	1
yes	52	76	**2.27 (1.36, 3.80)**	2.61 (1.48, 4.57)	1.54 (0.80, 2.95)
Hypertrophy					
no	102	63	1	1	1
yes	20	58	**4.70 (2.58, 8.53)**	**8.87(4.22,18.64)**	**6.56 (2.85,15.11)**
Popliteal cyst					
no	103	89	1	1	1
yes	18	28	1.80 (0.93, 3.47)	**2.40 (1.18, 4.86)**	1.82 (0.87, 3.84)
PD signal					
no	118	109	1	1	1
yes	4	12	**3.25(1.02,10.37)**	**4.31(1.25,14.82)**	3.43 (0.96, 12.30)

(KP= knee pain only; ROA= radiographic OA; SOA= symptomatic OA)

Knee pain is defined as the reporting of at least moderate knee pain in the previous week on WOMAC index.
Adjusted[1] is adjusted for age, sex and BMI
Adjusted[2] is adjusted for age, sex, BMI and ROA

Significant associations are highlighted in bold

3.6.1.1 Night Pain

Night pain of at least moderate intensity was reported by 61 (25.1%) participants. Synovial hypertrophy was associated with night pain and remained so after adjusting for co-variates, (aOR=4.93, 95%CI 2.28, 10.63). The presence of US detected effusion, popliteal cysts and PD signal were not associated with night pain (Table 3-20).

Table 3-20 Association between US features and night pain

US features	Night pain		Odds ratio (95% confidence interval)		
	no	yes	Crude OR	Adjusted[1]	Adjusted[2]
Effusion					
no	92	23	1	1	1
yes	90	38	1.69 (0.93, 3.06)	1.80 (0.96, 3.41)	1.80 (0.94, 3.57)
Hypertrophy					
no	135	30	1	1	1
yes	47	31	**2.97 (1.63, 5.42)**	**4.47 (2.17, 9.21)**	**4.93(2.28, 10.63)**
Popliteal cyst					
no	147	45	1	1	1
yes	32	14	1.43 (0.70, 2.91)	1.74 (0.81, 3.75)	1.69 (0.78, 3.70)
PD signal					
no	170	57	1	1	1
yes	12	4	0.99 (0.31, 3.21)	1.17 (0.34, 4.01)	1.13 (0.33, 3.92)

(KP= knee pain only; ROA= radiographic OA; SOA= symptomatic OA)

Night pain is defined as the reporting of at least moderate night pain in the previous week on WOMAC index.
Adjusted[1] is adjusted for age, sex and BMI
Adjusted[2] is adjusted for age, sex, BMI and ROA

Significant associations are highlighted in bold

3.6.1.2 Intermittent and Constant knee pain

Intermittent knee pain of at least moderate intensity was reported in 102 (42%) and constant knee pain in 58 (23.9%) of all participants. Associations between US features and intermittent and constant knee pain are presented in **Table 3-21** and **Table 3-22**.

The presence of moderate intermittent knee pain was associated with US detected effusion and synovial hypertrophy. This remained significant after adjusting for age, sex, BMI and ROA (aOR=2.67, 95% CI 1.46, 4.92 and aOR =6.30, 95% CI 3.03, 13.08 respectively).

Constant knee pain was not associated with the presence of effusion. The presence of synovial hypertrophy had an increased risk of constant knee pain which remained significant after adjusting for age, sex, BMI and ROA (aOR =8.34, 95%CI 3.56, 19.52).

The presence of a popliteal cyst was also associated with constant KP after adjusting for co-variates (aOR=2.88, 95% CI 1.28, 6.45).

PD signal was neither associated with intermittent or constant knee pain as derived from the ICOAP.

Table 3-21 Association between US features and intermittent knee pain

US features	Intermittent knee pain		Odds ratio (95% confidence interval)		
	no	yes	Crude OR	Adjusted[1]	Adjusted[2]
Effusion					
no	77	38	1	1	1
yes	64	64	**2.03 (1.20,3.41)**	**2.36 (1.34,4.18)**	**2.67 (1.46, 4.92)**
Hypertrophy					
no	110	55	1	1	1
yes	31	47	**3.03 (1.74,5.29)**	**5.12(2.59,10.15)**	**6.30(3.03,13.08)**
Popliteal cyst					
no	115	77	1	1	1
yes	24	22	1.37 (.71, 2.61)	1.83 (.91, 3.68)	1.90 (.93, 3.87)
PD signal					
no	134	93	1	1	1
yes	7	9	1.85 (.67, 5.15)	2.46 (.82, 7.42)	2.57 (.85,7.80)

(KP= knee pain only; ROA= radiographic OA; SOA= symptomatic OA)

Intermittent knee pain is defined as the reporting of at least moderate intensity intermittent pain in the previous week as reported in the ICOAP questionnaire.
Adjusted[1] is adjusted for age, sex and BMI
Adjusted[2] is adjusted for age, sex, BMI and ROA

Significant associations are highlighted in bold

Table 3-22 Association between US features and Constant knee pain

US features	Constant knee pain		Odds ratio (95% confidence interval)		
	no	yes	Crude OR	Adjusted[1]	Adjusted[2]
Effusion					
no	93	22	1	1	1
yes	92	36	1.65 (.95, 3.03)	1.84 (.96, 3.53)	1.89 (.86, 3.75)
Hypertrophy					
no	139	26	1	1	1
yes	46	32	**3.72 (2.01, 6.83)**	**6.92(3.18,15.06)**	**8.34 (3.56,19.52)**
Popliteal cyst					
no	153	39	1	1	1
yes	31	15	1.89 (0.93, 3.86)	**2.88 (1.31, 6.34)**	**2.88 (1.28, 6.45)**
PD signal					
no	175	52	1	1	1
yes	10	6	2.01 (0.70, 5.82)	2.52 (0.81, 7.90)	2.51 (0.79, 2.06)

(KP= knee pain only; ROA= radiographic OA; SOA= symptomatic OA)

Constant knee pain is defined as the reporting of at least moderate intensity constant pain in the previous week as reported in the ICOAP questionnaire.
Adjusted[1] is adjusted for age, sex and BMI
Adjusted[2] is adjusted for age, sex, BMI and ROA

Significant associations are highlighted in bold

3.6.2 Correlations between pain and US features

Correlations between pain measures and continuous measures of US detected effusion, synovial hypertrophy and popliteal cyst were explored and are presented in Table 3-23.

Table 3-23 Correlation matrix between pain measures and US features (mm)

		Pain VAS (mm)	WOMAC Pain score	Pain at night in bed (WOMAC)	Intermittent pain score	Constant pain score
Pain VAS (mm)	r	1.00				
WOMAC Pain score	r	0.90**	1.00			
At night in bed (WOMAC)	r	0.72**	0.85**	1.00		
Intermittent pain score	r	0.90**	0.89**	0.75**	1.00	
Constant pain score	r	0.79**	0.81**	0.71**	0.81**	1.00
Effusion (mm)	r	0.29**	0.29**	0.20**	0.30**	0.31**
Synovial hypertrophy (mm)	r	0.31**	0.32**	0.20**	0.33**	0.31**
Popliteal cyst (mm)	r	0.09	0.10	0.04	0.11	0.08

**** Correlation is significant at the 0.01 level**

All pain measures were strongly correlated with each other. The correlation between pain measures and US features were weak but significant for effusion (r=0.29, p=0.001 for pain VAS; Figure 3-41) and synovial hypertrophy (r=0.3, p<0.001 for pain VAS; Figure 3-42). No correlation was found between popliteal cyst size and pain severity.

These relationships were also explored within the SOA group (data not shown). The correlation between pain severity and effusion remained

significant (r=0.28, p=0.01) but was non-significant for synovial hypertrophy (r=0.20, p=0.06).

Figure 3-41 Scatterplot of relationship between effusion (mm) and pain visual analogue scale (VAS) score (mm).

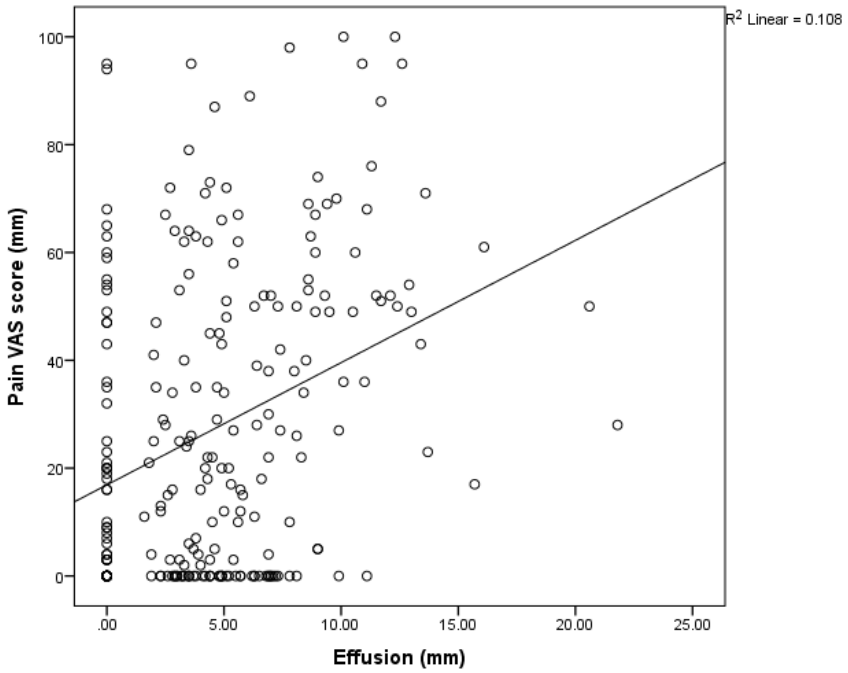
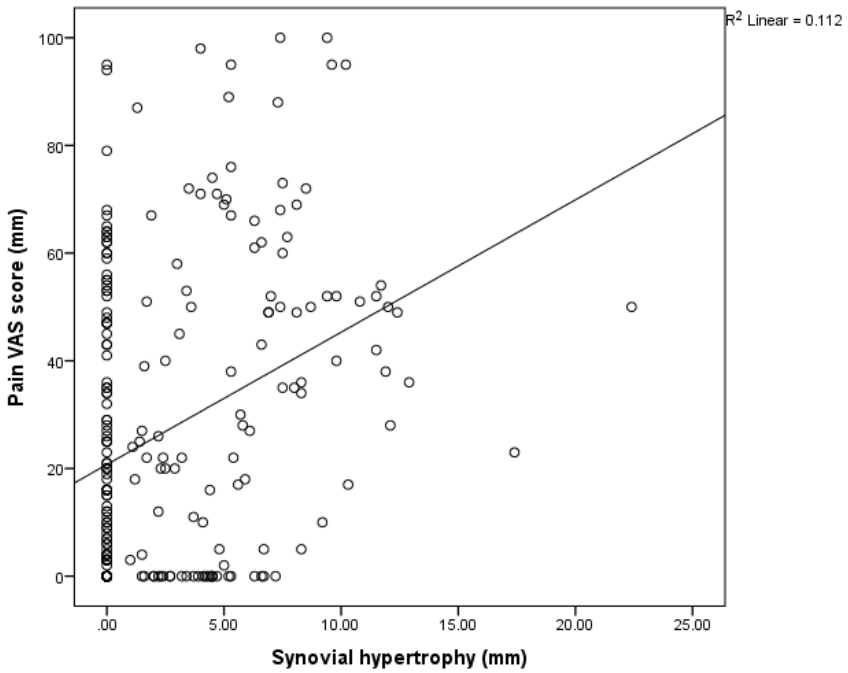


Figure 3-42 Scatterplot of relationship between synovial hypertrophy (mm) and pain visual analogue scale (VAS) score (mm).



3.6.3 Associations between ROA and US features of inflammation

Associations between the presence of ROA (K&L ≥ grade 2) and the US features of inflammation were examined using logistic regression (Table 3-24). The presence of radiographic OA (K&L grade 2 or above) was strongly associated with US detected effusion, synovial hypertrophy and popliteal cyst after adjusting for age, sex, BMI and the presence of at least moderate knee pain.

PD signal was observed in 12 participants (12.8%) with ROA compared to 4 (2.7%) without ROA. This was highly significant (p=.005). However the association became insignificant after adjusting for the presence of knee pain.

Table 3-24 Association between Radiographic OA (ROA) and Ultrasound features in Index knee

		ROA		Odds ratio (OR)		
US Features		**No**	**Yes**	**(95% confidence interval)**		
				Crude OR	Adjusted[1]	Adjusted[2]
Effusion	**No**	104	11	1	1	1
	Yes	45	83	**17.44**	**16.09**	**13.39**
				(8.49, 35.81)	**(7.41, 34.96)**	**(6.14, 29.02)**
Synovial						
hypertrophy	**No**	135	30	1	1	1
	Yes	14	64	**20.57**	**17.80**	**14.39**
				(10.21, 41.45)	**(8.07, 39.27)**	**(6.28, 32.94)**
Popliteal cyst	**No**	131	61	1	1	1
	Yes	16	30	**4.03**	**3.65**	**3.19**
				(2.04, 7.94)	**(1.70, 7.84)**	**(1.42, 7.17)**
PD signal	**No**	145	82	1	1	1
	Yes	4	12	**5.31**	**3.92**	2.83
				(1.66, 16.98)	**(1.10, 13.90)**	(0.76, 10.43)

ROA as defined by K&L ≥Grade 2
Adjusted[1] is adjusted for age, sex and BMI
Adjusted[2] is adjusted for age, sex, BMI and knee pain (≥moderate on WOMAC)

Significant associations are highlighted in bold

Chondrocalcinosis (CC) was also examined as an individual radiographic feature (Table 3-25). Grey scale features were not associated with radiographic CC. However, PD signal was found to be independently associated with CC (p=0.007). In index knees, PD signal was detected in almost 30% of participants with CC compared to 5% without. The aOR of CC in those with PD signal was 6.6 (95% CI 1.67, 26.05) after adjusting for co-variates. PD signal was also found to be independently associated when left and right knees were examined (aOR = 4.98, 95% CI 1.17, 21.10 for left knees and aOR = 4.90, 95% CI 1.15, 20.79 for right knees). Additional adjustment for the presence of ROA (K&L ≥ Grade 2) did not appreciably alter the aOR.

Table 3-25 Association between the presence of chondrocalcinosis (on x-ray) and US features in Index knees

		Chondro-calcinosis		Odds ratio (95% confidence interval)		
US Features		**No**	**Yes**	Crude OR	Adjusted[1]	Adjusted[2]
Effusion	**No**	109	6	1	1	1
	Yes	117	11	1.71	1.31	1.02
				(0.61, 4.77)	(0.44, 3.91)	(0.31, 3.23)
Synovial Hypertrophy	**No**	156	9	1	1	1
	Yes	70	8	1.98	1.33	0.91
				(0.73, 5.35)	(0.43, 4.10)	(0.26, 3.27)
Popliteal cyst	**No**	183	9	1	1	1
	Yes	40	6	**3.05**	2.28	2.10
				(1.03, 9.06)	(0.73, 7.12)	(0.66,6.71)
PD signal	**No**	215	12	1	1	1
	Yes	11	5	**8.14**	**7.57**	**6.57**
				(2.44, 27.12)	**(1.96, 29.24)**	**(1.66, 25.94**

Adjusted[1] is adjusted for age, sex and BMI
Adjusted[2] is adjusted for age, sex, BMI and knee pain (≥moderate on WOMAC)

Significant associations are highlighted in bold

Though US detected chondrocalcinosis was not a primary outcome measure for this study, agreement between US and radiographic chondrocalcinosis was examined (Table 3-26). A poor level of agreement (kappa =0.26, $p<0.001$) was found between the two and consequently US detected chondrocalcinosis was not used in any further exploratory analysis. Reasons for this are explored in the discussion.

Table 3-26 Cross-tabulation of chondrocalcinosis detected on radiographs and by US

		Radiographic detected Chondro-calcinosis	
		No	**Yes**
US- detected Chondrocalcinosis	**No**	220 (90.5%)	13 (5.3%)
	Yes	6 (2.5%)	4 (1.7%)

3.6.4 Correlations between radiographic scores and US features

Radiographic OA and US features were further examined using a correlation matrix to identify relationships between global x-ray scores, individual radiographic features and compartments, and US features (Table 3-27).

Table 3-27 Correlations between summated radiographic scores and continuous US measures.

	Effusion (mm)	Synovial Hypertrophy (mm)	Popliteal cyst (mm)
Index knee			
Global score (0-60)	0.57**	0.71**	0.33**
Osteophytes (0-40)	0.58**	0.73**	0.32**
Joint space narrowing (0-20)	0.51**	0.65**	0.30**
Tibio-fem joint (0-30)	0.54**	0.64**	0.32**
Pat-fem joint (0-30)	0.50**	0.67**	0.30**

*** Correlation is significant at the 0.05**
**** Correlation is significant at the 0.01 level**
mm = millimeters

All radiographic scores were positively correlated with US features, for the index knee ($p<0.01$). Effusion showed a moderate relationship with all features. For individual features this was strongest for osteophytes ($r=0.58$, $p<0.01$) and for compartments this was strongest for the tibio-femoral joint score ($r=0.54$, $p<0.01$). Synovial hypertrophy demonstrated the strongest correlation with radiographic scores. For individual features this was strongest for osteophyte scores ($r=0.73$, $p<0.01$) and for compartments this was strongest for the patello-femoral joint ($r= 0.67$, $p<0.001$). Correlations between popliteal cyst size and radiographic features were weaker but remained significant ($r=0.33$, $p<0.01$ for global x-ray score).

Scatterplots showing the relationship between global radiographic scores and US measures of effusion, synovial hypertrophy and popliteal cysts size are shown for the index knees (Figure 3-43- Figure 3-45).

Figure 3-43 Scatterplot showing relationship between global x-ray score and US measured effusion (millimetres) for Index knee

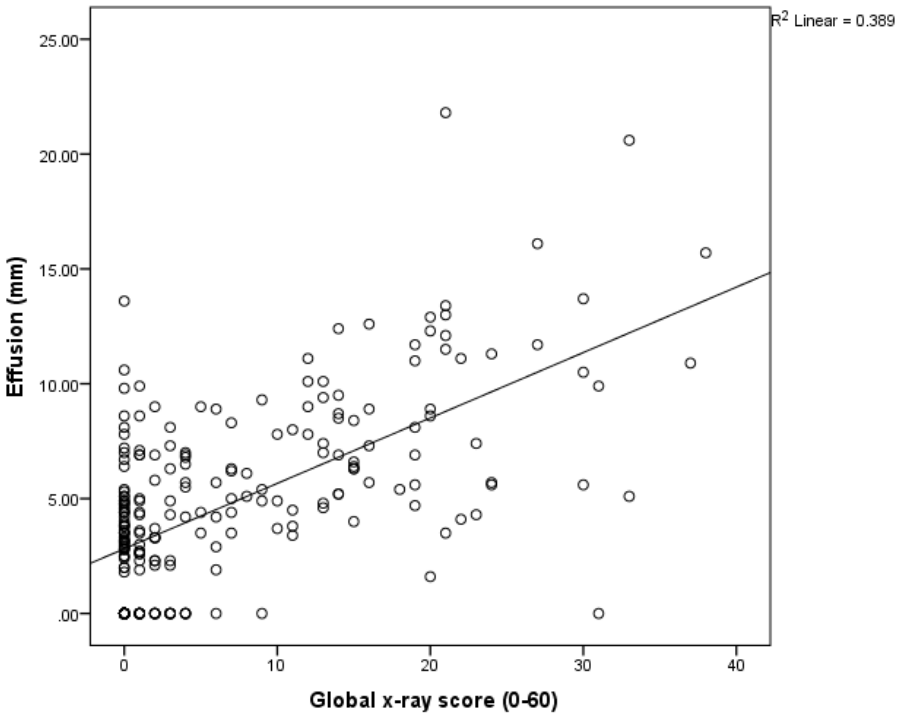


Figure 3-44 Scatterplot showing relationship between global x-ray score and US measured synovial hypertrophy (millimetres) for Index knee

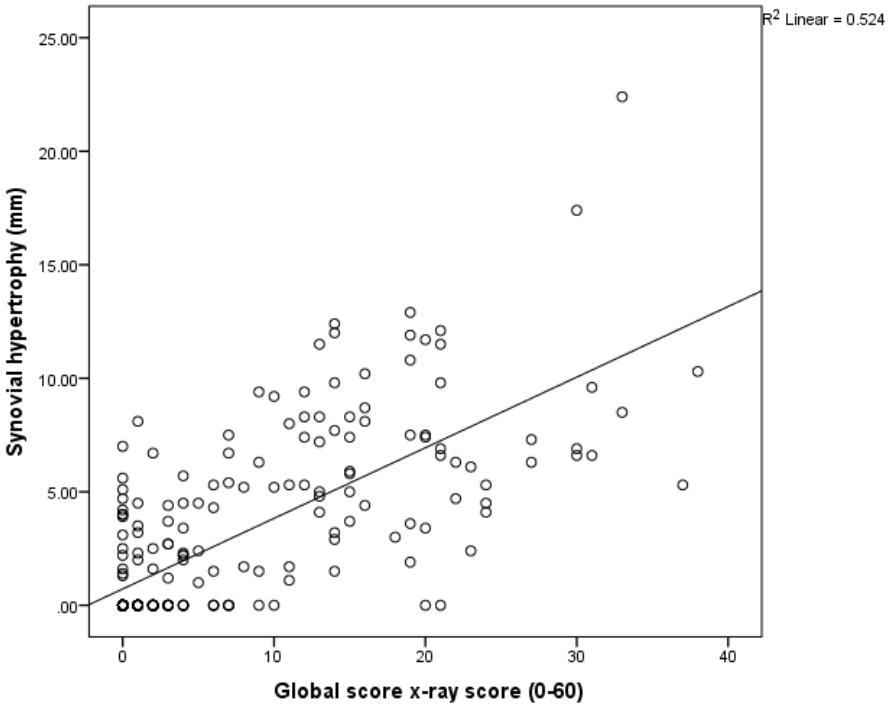
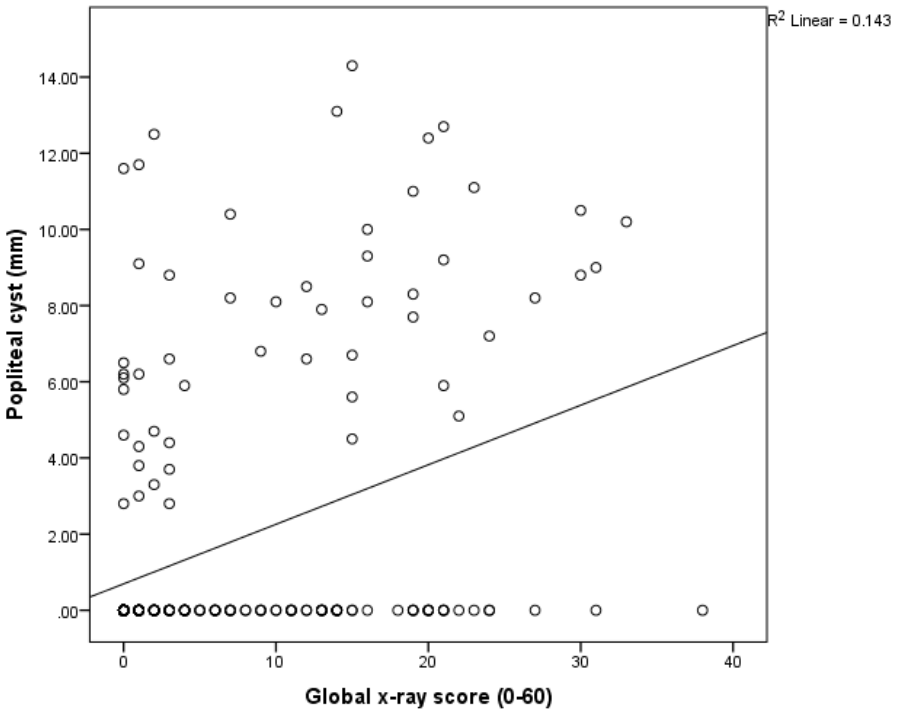


Figure 3-45 Scatterplot showing relationship between global x-ray score and US measure of popliteal cyst (millimetres) for Index knee



3.6.5 Associations between knee pain and clinical features

Associations between knee pain and clinical features were explored using logistic regression. All ORs were adjusted for age, sex, BMI and ROA (Table 3-28).

The presence of a clinical effusion was associated with more than a six-fold risk for knee pain, which remained significant after adjusting for co-variables (aOR = 5.75, 95% CI 2.15, 15.34).

After adjusting for covariates restricted ROM was significant for those in the third tertile (the most restricted range) (aOR 6.56, 95% CI 2.83, 15.25). The OR for those in the middle tertile was not significant after adjusting for ROA.

Table 3-28 Associations between knee pain and clinical features

Clinical features	Knee pain		Odds ratio (95% confidence interval)		
	No	Yes	Crude OR	Adjusted[1]	Adjusted[2]
Clinical effusion					
absent	115	88	1	1	1
present	7	33	**6.16 (2.60,14.58)**	**8.53 (3.36,21.64)**	**5.75 (2.15,15.34)**
Range of Movement					
Tertile 1 ≥141°	55	24	1	1	1
Tertile 2 ≥133-140°	45	38	**1.94 (1.02, 3.69)**	**2.12 (1.05, 4.28)**	1.86 (0.90, 3.84)
Tertile 3 ≤132°	22	59	**6.15 (3.10, 12.20)**	**8.81 (3.89,19.94)**	**6.56 (2.83, 15.25)**
Quadriceps strength					
Tertile strongest	49	30	1	1	1
middle	41	39	1.55 (0.83, 2.92)	**2.22 (1.10, 4.50)**	1.99 (0.97, 4.10)
weakest	30	49	**2.67 (1.40, 5.07)**	**5.56 (2.47,12.51)**	**4.26 (1.83, 9.88)**
GUG 3 m					
time <10s	108	70	1	1	1
time >10s	13	50	**5.93 (3.01,11.72)**	**10.46 (4.67,23.43)**	**9.31 (4.06, 21.37)**
Moderate stiffness (WOMAC)					
absent	109	29	1	1	1
present	13	92	**26.60 (13.07,54.14)**	**27.14 (12.88,57.18)**	**25.88 (12.04,55.66)**
AM stiffness > 30 min					
absent	121	79	1	1	1
present	0	35	2.47 p=.99	2.23 p=.99	2.23 p=.99
Damping co-efficient					
Tertile lowest	23	23	1	1	1
middle	37	18	0.49 (0.22, 1.09)	**0.28 (0.11, 0.69)**	**0.25 (0.9, 0.65)**
highest	24	29	1.21 (0.54, 2.67)	0.42 (0.14, 1.28)	0.40 (0.12, 1.28)
Stiffness co-efficient					
Tertile lowest	31	20	1	1	1
middle	28	23	1.23 (0.56, 2.69)	0.63 (0.24, 1.60)	0.63 (0.23, 1.69)
highest	24	27	1.74 (0.79, 3.83)	0.32 (0.08, 1.28)	0.29 (0.07, 1.22)

Knee pain is defined as the reporting of at least moderate knee pain in the previous week on WOMAC index.
Adjusted[1] is adjusted for age, sex and BMI
Adjusted[2] is adjusted for age, sex, BMI and radiographic OA
Significant associations are highlighted in bold

An association between muscle strength and knee pain was only significant for those with the weakest quadriceps strength. These had an aOR of 4.26 (95% CI 1.83, 9.88) compared to those with the strongest quadriceps.

Fifty participants with knee pain (41.6%) took over 10 seconds to complete the GUG 3m test compared to 10.7% of participants without knee pain. This conferred over a nine-fold risk for knee pain after adjusting for co-variables (aOR 9.31, 95% CI 4.03, 21.37).

Moderate self-reported stiffness reported on the WOMAC index strongly associated with knee pain (aOR 25.88, 95% CI 12.04, 55.66). Morning stiffness lasting more than 30 minutes duration was reported by 35 participants all of whom had knee pain.

Biomechanical assessments of joint stiffness found no association between stiffness co-efficient and knee pain. Damping co-efficient derived from the same test showed a negative association for those in the middle tertile where there was a 75% reduction in odds for knee pain after adjusting for co-variates (aOR 0.25, 95% CI 0.9, 0.65). The OR for those in the stiffest third was not significant.

3.6.6 Association between clinical signs and symptoms and US features

3.6.6.1 Clinical Effusion

All US features were associated with the presence of a clinical effusion although the association with PD signal became insignificant after adjusting for co-variates Table 3-29.

Table 3-29 Odds ratios for US features in the presence of clinical effusion.

Presence of US Features	Clinical Effusion		Odds ratio (95% confidence interval)			
	No	**Yes**	Crude OR	Adjusted[1]	Adjusted[2]	Adjusted[3]
Effusion						
No	114	1	1	1	1	1
Yes	89	39	**49.95**	**35.75**	**24.51**	**11.81**
			(6.73, 370.67)	**(4.75, 269.41)**	**(3.2, 188.03)**	**(1.44, 97.10)**
Synovial Hypertrophy						
No	162	3	1	1	1	1
Yes	41	37	**48.73**	**36.14**	**24.56**	**13.55**
			(14.31, 165.97)	**(10.12,129.04)**	**(6,58, 91.7)**	**(3.26, 56.46)**
Popliteal cyst						
No	173	19	1	1	1	1
Yes	27	19	**6.41**	**5.81**	**4.77**	**3.46**
			(3.01, 13.62)	**(2.50, 13.54)**	**(1.93, 11.81)**	**(1.33, 8.97)**
PD signal						
No	196	31	1	1	1	1
Yes	7	9	**8.13**	**5.91**	**4.39**	3.35
			(2.82, 23.41)	**(1.84, 18.99)**	**(1.30, 14.75)**	(0.88, 12.75)

Adjusted[1] is adjusted for age, sex and BMI
Adjusted[2] is adjusted for age, sex, BMI and knee pain
Adjusted[3] is adjusted for age, sex, BMI, knee pain and radiographic OA
Significant associations are highlighted in bold

3.6.6.2 Self-reported stiffness

US features were not associated with self-reported stiffness after adjusting for co-variates (Table 3-30).

Table 3-30 Associations between the presence of US features and self-reported stiffness.

Presence of US Features	Self-reported Stiffness (>mod WOMAC)		Odds ratio (95% confidence interval)			
	No	**Yes**	Crude OR	Adjusted[1]	Adjusted[2]	Adjusted[3]
Effusion						
No	114	1	1	1	1	1
Yes	89	39	**1.81**	**1.88**	0.97	0.86
			(1.08, 3.02)	**(1.06, 3.31)**	(0.45, 2.09)	(0.35, 2.07)
Synovial Hypertrophy						
No	162	3	1	**1**	1	1
Yes	41	37	**2.58**	**3.47**	0.89	0.75
			(1.49, 4.49)	**(1.79, 9.72)**	(0.71, 4.88)	(0.27, 2.09)
Popliteal cyst						
No	173	19	1	**1**	1	1
Yes	27	19	1.82	**2.50**	1.86	1.83
			(0.95, 3.48)	**(1.22, 5.14)**	(0.71, 4.88)	(0.69, 4.92)
PD signal						
No	196	31	1	1	1	1
Yes	7	9	2.32	3.08	1.44	1.41
			(0.81, 6.59)	(0.98, 9.69)	(0.33, 6.28)	(0.32, 6.20))

Adjusted[1] is adjusted for age, sex and BMI
Adjusted[2] is adjusted for age, sex, BMI and knee pain
Adjusted[3] is adjusted for age, sex, BMI, knee pain and radiographic OA
Significant associations are highlighted in bold

3.6.6.3 Biomechanical measures of stiffness

Table 3-31 shows the correlations between biomechanical assessed stiffness and damping co-efficients and continuous US measures. Modest relationships were observed between stiffness co-efficients and US measures of effusion (r=0.33, p<0.01; Figure 3-46) and synovial hypertrophy (r=0.32, p<0.01; Figure 3-47). Relationships between damping co-efficients and effusion, and synovial hypertrophy were weaker but remained significant (r=0.25, p<.01 and r=0.18, p<0.05 respectively). Popliteal cyst size was not correlated with either stiffness or damping co-efficients.

Table 3-31 Relationship between biomechanical measures of stiffness and measures of US features in index knee

		Damping co-efficient	Stiffness co-efficient
Stiffness co-efficient	r	0.74**	
Effusion (mm)	r	0.25**	0.33**
Synovial hypertrophy (mm)	r	0.18*	0.33**
Popliteal cyst (mm)	r	-0.09	-0.08

*** Correlation is significant at the 0.05**
**** Correlation is significant at the 0.01 level**
mm = millimetres

Figure 3-46 Scatterplot of relationship between effusion and stiffness co-efficient in index knee

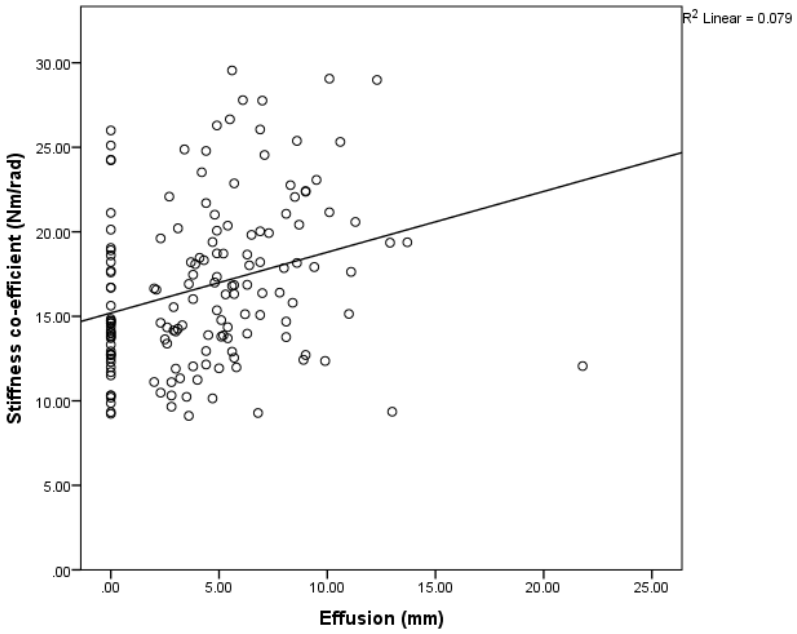
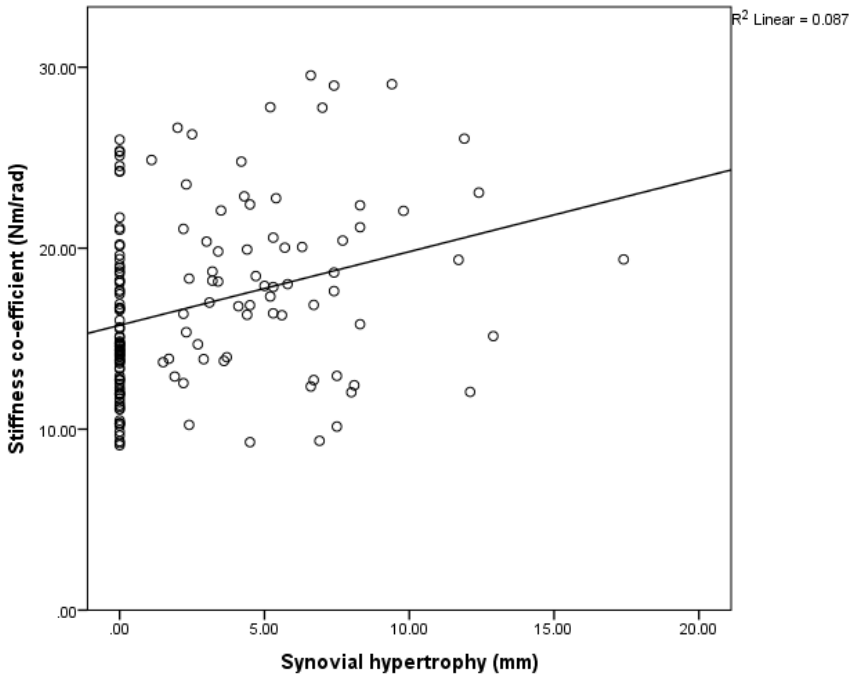


Figure 3-47 Scatterplot of relationship between US measured synovial hypertrophy (m) and stiffness co-efficient in index knee



3.7 Exploratory analysis of biomechanically assessed stiffness

Exploratory analysis was undertaken to see if the biomechanical assesed stiffness and damping co-efficients were correlated with symptoms and other clinical and radiographic features of OA.

3.7.1 Self-reported morning stiffness and biomechanically assessed stiffness

The relationship between self-reported stiffness on the WOMAC index and the biomechanical measures were examined using correlation analysis (Table 3-32). Biomechanically assessed stiffness and damping were strongly associated with each other ($r = 0.74$, $p < 0.001$) (Figure 3-48). A weak but significant correlation was found between self-reported stiffness and the stiffness co-efficient ($r = 0.29$, $p < 0.001$, Table 3-32). This was stronger when examined in the SOA group ($r = 0.39$, $p < 0.05$; data not shown, Figure 3-50). The weak relationship between self-reported stiffness and damping co-efficients ($r = 0.14$, $p = 0.04$) was not found within the SOA group.

Table 3-32 Correlation between WOMAC stiffness scores and stiffness and damping co-efficients

	WOMAC Stiffness score	Damping co-efficient	Stiffness co-efficient
WOMAC Stiffness score	1.00		
Damping co-efficient	0.14*	1.00	
Stiffness co-efficient	0.29**	0.74**	1.00

*** Correlation is significant at the 0.05 level (1-tailed)**
**** Correlation is significant at the 0.01 level (1-tailed)**

Figure 3-48 Scatterplot showing relationship between Stiffness and Damping co-efficients for the index knee

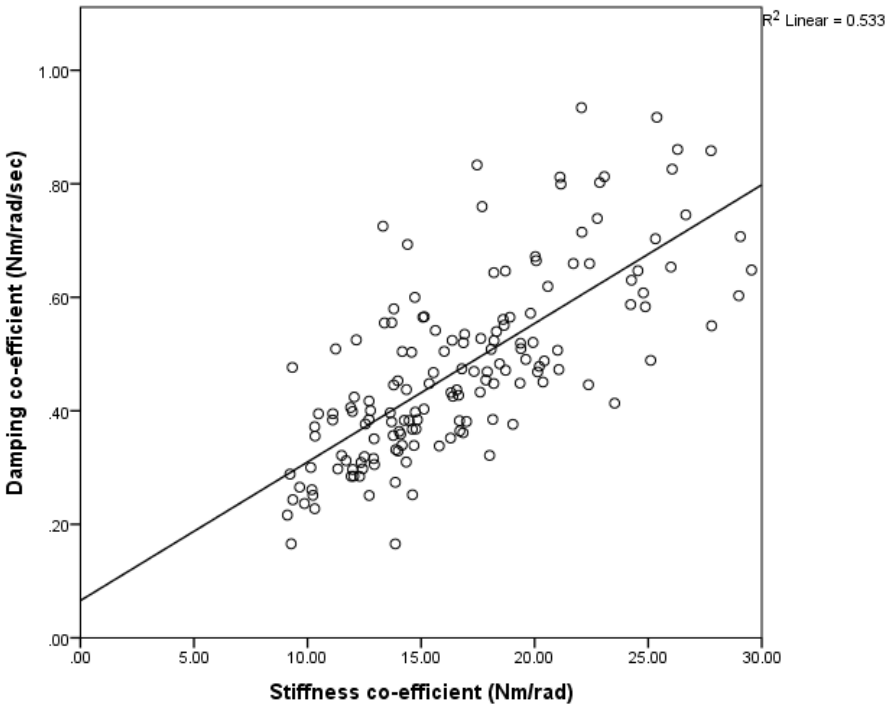


Figure 3-49 Scatterplot showing relationship between self-reported stiffness and stiffness co-efficients for the index knee

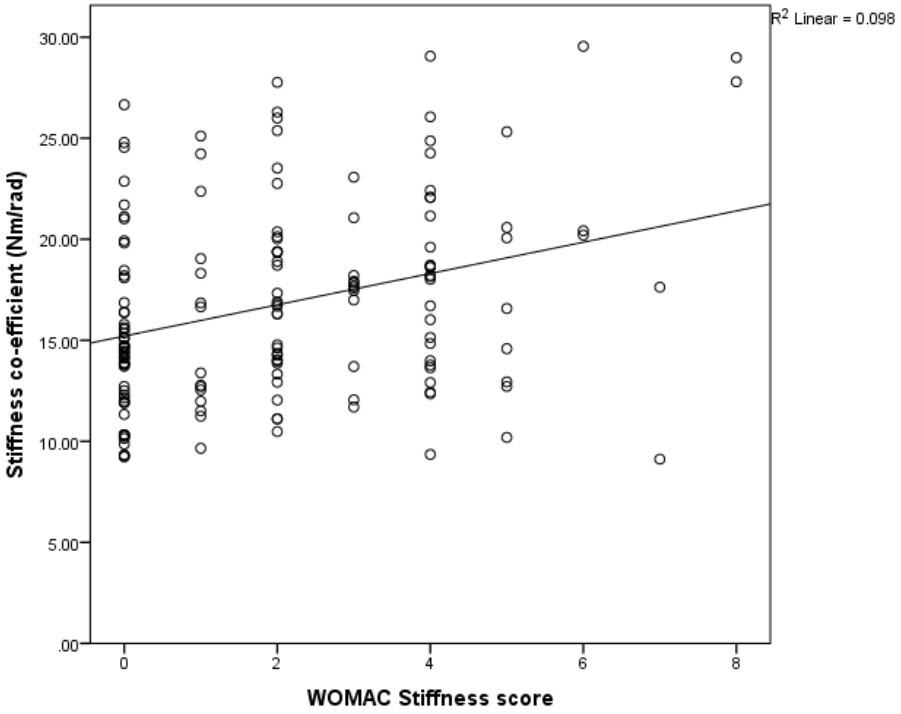
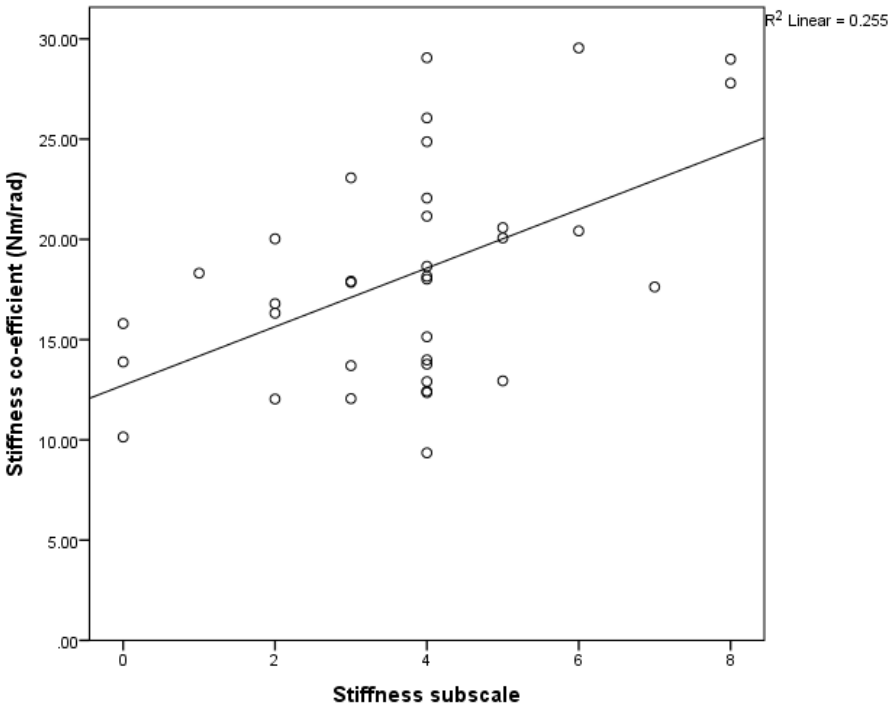


Figure 3-50 Scatterplot showing relationship between self-reported stiffness and stiffness co-efficients for the index knee in the SOA group



3.7.2 Pain and biomechanically assessed stiffness

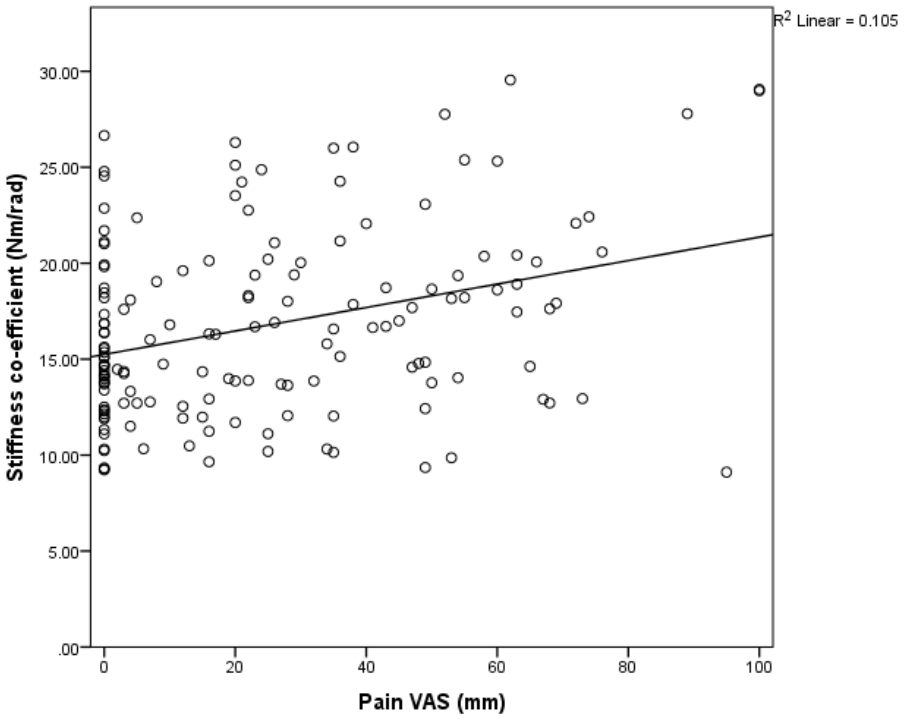
Knee pain was not correlated with the damping co-efficient (Table 3-33). A weak but significant correlation was found between the stiffness co-efficient and pain VAS (r=0.28, p=0.001) (Figure 3-51) and WOMAC pain score (r=.22, p=0.006). WOMAC stiffness scores were significantly correlated with scores from the WOMAC pain scores (r=0.81, p<0.001).

Table 3-33 Relationships between measures of joint stiffness and pain measures

		WOMAC Stiffness score	Damping co-efficient	Stiffness co-efficient
Pain VAS (mm)	r	0.80**	0.08	0.28**
WOMAC Pain score	r	0.81**	0.05	0.22**

**** Correlation is significant at the 0.01**

Figure 3-51 Scatterplot of relationship between pain VAS score and stiffness co-efficient in index knee



3.7.3 Correlations between biomechanical stiffness and radiographic severity.

Stiffness co-efficients showed weak but significant correlations with the global x-ray score (r=0.19, p=0.02). Individual x-ray features and joint compartments also showed weak but significant relationship with all features, with no single feature showing a stronger relationship. These relationships disappeared when sub-groups were examined (data not shown). The WOMAC stiffness scores also showed a weak correlation with radiographic scores, (r=0.23, p<0.001 for global score). No relationships were found between damping co-efficients and radiographic scores (Table 3-34).

Table 3-34 Relationships between radiographic scores and measures of knee joint stiffness

		WOMAC Stiffness subscale	Damping co-eff	Stiffness co-eff
Global x-ray score	r	0.23**	0.05	0.19*
Osteophytes	r	0.25**	0.05	0.20*
Joint space narrowing	r	0.22**	0.05	0.19*
Tib-femoral joint	r	0.24**	0.09	0.18
Pat-fem joint	r	0.24**	0.001	0.18*

*** Correlation is significant at the 0.05 level**
**** Correlation is significant at the 0.01 level**

3.7.4 Correlations between biomechanical stiffness and clinical measures

Other clinical features which may contribute to joint stiffness and damping co-efficients were explored (Table 3-35). These were chosen on the basis of previously published findings (Oatis 1993; Oatis, Wolff et al. 2006; Valle, Casabona et al. 2006).

Table 3-35 Correlation matrix for measures of joint stiffness and clinical features

		WOMAC Stiffness subscale	Damping co-eff	Stiffness co-eff
Age	r	-0.10	-0.20**	-0.19**
BMI	r	0.32**	0.48**	0.71**
Range of Movement	r	-0.40**	-0.21**	-0.40**
Quadriceps strength	r	-0.30**	0.31**	0.23**
Hamstring strength	r	-0.09	0.37**	0.37**

*** Correlation is significant at the 0.05 level**
**** Correlation is significant at the 0.01 level**

Increasing age had a negative relationship with both the damping and stiffness co-efficient. The strength of relationship was weak but significant for both measures ($r= -0.20$, $p<.01$ and $r=-0.19$, $p<0.01$ for damping and stiffness co-efficients respectively).

BMI had a strong correlation with stiffness co-efficient ($r=0.71$, $p<0.001$) and a moderate correlation with damping ($r=0.48$, $p<0.008$) (Figure 3-52). BMI and self-reported stiffness showed a weak relationship between the two ($r= 0.32$, $p<0.001$).

Maximum range of movement was inversely correlated with measures of stiffness. The relationship was modest for both self-reported stiffness ($r= -0.40$, $p<0.001$) and for the stiffness co-efficient ($r= -0.40$, $p<0.001$;Figure 3-53). The relationship with the damping co-efficient was weaker but still significant ($r= -0.21$, $p<0.01$).

Quadriceps strength was correlated weakly with the stiffness co-efficient ($r= 0.23$, $p<0.01$) and modestly with the damping co-efficients ($r=0.31$, $p<0.001$, Figure 3-54). A negative correlation was found with quadriceps strength and self-reported stiffness ($r= -0.30$, $p<0.001$).

Hamstring strength was associated with both biomechanical measures ($r=0.37$, $p<0.001$ for both stiffness and damping co-efficients) but was not associated with self-reported stiffness.

Figure 3-52 Scatterplot showing the relationship between Body Mass Index and stiffness co-efficient in index knee

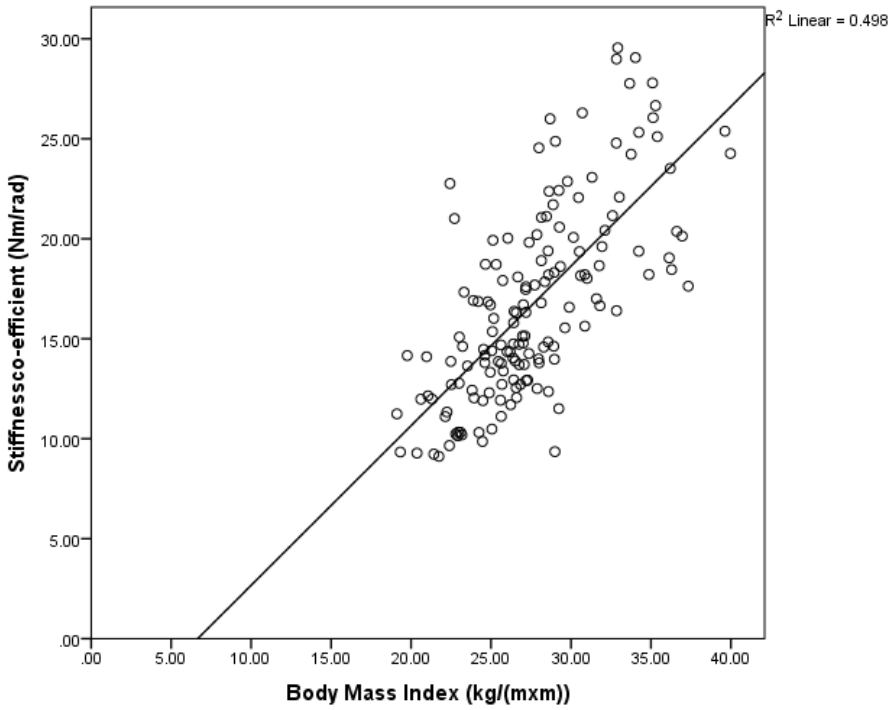


Figure 3-53 Scatterplot showing relationship between range of movement and stiffness co-efficient in index knee

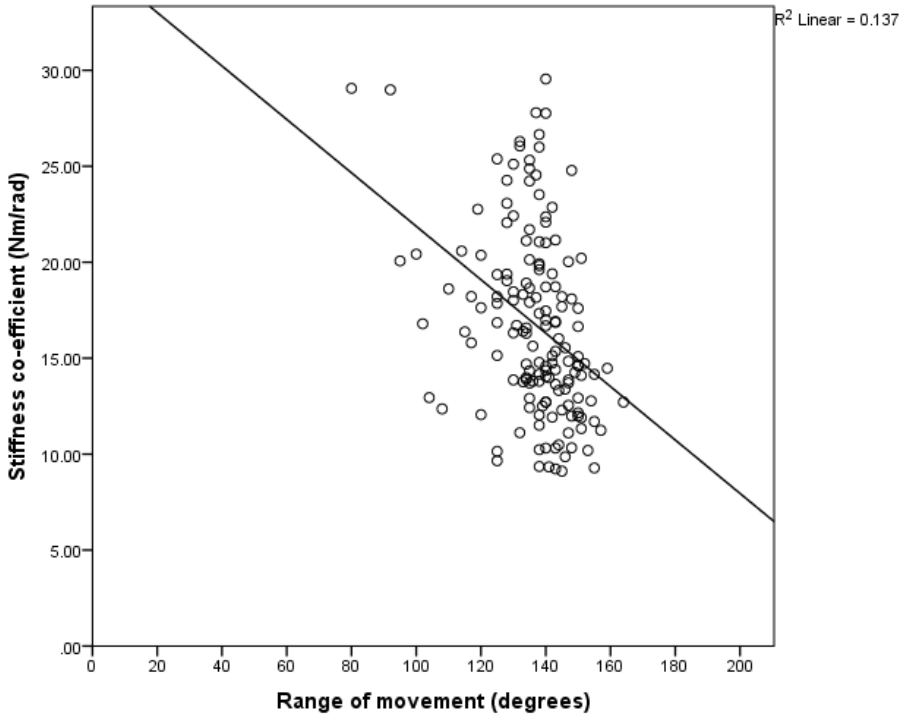
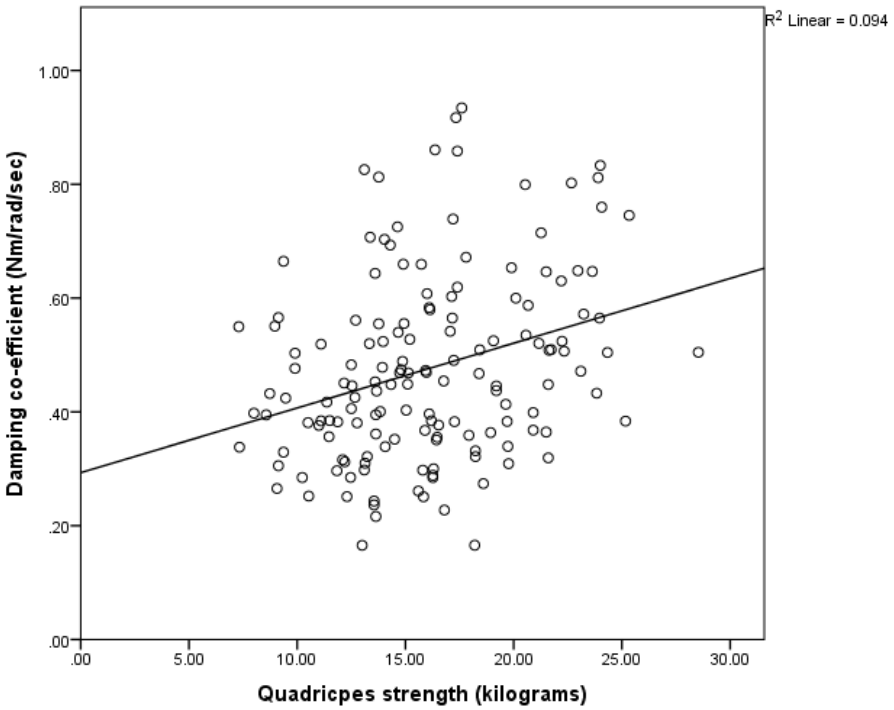


Figure 3-54 Scatterplot showing relationship between Quadriceps strength and damping co-efficient in index knee



3.7.5 Multiple linear regression

Multiple linear regression was used to explore how clinical, pain, radiographic and US variables contributed to the overall variance in biomechanically assessed stiffness co-efficients. Age, gender, BMI, pain VAS score, global x-ray score and synovial hypertrophy and range of movement were examined as these had significant correlations with the stiffness co-efficient.

All of the variables were entered together (Table 3-36). The model had an R of 0.85, R^2 of 0.72 and adjusted R^2 of 0.71; thus this model accounted for 71% of the variance. The model as a whole had a significant fit to the data ($p<0.001$). Of the 7 predictor variables, 4 contributed significantly to the variance in stiffness co-efficient, age

(p=0.04), gender (p<0.001), BMI (p<0.001) and synovial hypertrophy (p=0.03).

Table 3-36 Multiple linear regression analyses (forced entry method) for stiffness co-efficient and clinical variables

Model	Unstandardized Coefficients		Standardized Coefficients	Sig.	95% CI for B
	B	Standard Error	Beta		
(Constant)	13.40	4.85		<0.01	(3.82, 22.98)
Age	-0.06	0.03	-0.11	0.04	(-0.12, -0.004)
Gender	-4.37	0.45	-0.43	<0.01	(-5.26, -0.49)
BMI	0.71	0.06	0.63	<0.01	(0.59, 0.82)
Pain VAS (mm)	-0.01	0.01	-0.03	0.61	(-0.03, 0.02)
Global x-ray score	-0.07	0.04	-0.12	0.08	(-0.16, 0.01)
Synovial hypertrophy (mm)	0.24	0.09	0.17	0.01	(0.06, 0.42)
ROM (degrees)	-0.03	0.02	-0.09	0.12	(-0.08, 0.01)

ROM = range of movement

Significant variables were then re-entered into the equation using a hierarchical model to examine the individual contribution of each variables (Table 3-37). BMI accounted for almost 50% of the total variance in biomechanically assessed stiffness. Age did not significantly change the variance. Gender explained a further 20% to the variance whereby females had a lower stiffness co-efficient by 4.52 (Nm/rad) when other variables were held constant. Synovial hypertrophy exerted a small (1.1% of variance) but significant influence on stiffness whereby an increase of 1mm of synovial hypertrophy increases stiffness by 0.16 (Nm/rad/) when other variables are held constant. Finally pain severity and global x-ray scores were added to

the model to see if they contributed but did not demonstrate an independent effect on stiffness co-efficients.

Table 3-37 Multiple linear regression (hierarchial entry) for biomechanical stiffness co-efficient and clinical variables (continued next page)

Model		Unstandardized Coefficients		Standardized Coefficients	Sig.	95% CI for B	Change statistics			
		B	Std. Error	Beta			R^2	Adjusted R^2	Change in R^2	Sig for R^2 change
1	(Constant)	-5.32	1.82		< 0.01	(-8.91,-1.72)	0.50	0.49	0.49	<0.01
	BMI	0.80	0.07	0.71	<0.01	(0.67, 0.93)				
2	(Constant)	-1.36	3.21		0.13	(-0.85, 6.33)	0.50	0.50	0.01.	0.14
	BMI	0.78	0.07	0.69	<0.01	(0.65, 0.91)				
	Age	-0.05	0.03	-0.09	0.14	(-0.12,0.02)				
3	(Constant)	5.62	2.59		0.03	0.49, 10.74)	0.70	0.70	.20	<0.01
	BMI	0.77	0.05	0.68	<0.01	(0.67, 0.87)				
	Age	-0.04	0.03	-0.07	0.14	(-0.09, 0.01)				
	Gender	-4.52	0.45	-0.44	<0.01	(-5.41, -3.62)				
4	(Constant)	7.94	2.73		<0.01	2.54, 13.33)	0.71	0.71	.01	0.02
	BMI	0.72	0.05	0.64	<0.01	(0.62, 0.83)				
	Age	-0.06	0.03	-0.11	0.03	(-0.11, -0.01)				
	Gender	-4.44	0.45	-0.44	<0.01	(-5.32, -3.56)				
	Synovial hypertrophy (mm)	0.16	0.07	0.12	0.02	(0.03, 0.29)				

Table 3-36 continued… Multiple linear regression (hierarchial entry) for biomechanical stiffness co-efficient and clinical variables

	Model	Unstandardized Coefficients		Standardized Coefficients	Sig.	95% CI for B	Change statistics			
		B	Std. Error	Beta			R^2	Adjusted R^2	Change in R^2	Sig for R^2 change
5	(Constant)	7.97	2.78		<0010	(2.49, 13.46)	0.71	0.70	0.00	0.93
	BMI	0.72	0.06	0.64	<0.01	(0.61, 0.83)				
	Age	-0.06	0.03	-0.11	0.03	(0.12, -0.01)				
	Gender	-4.40	0.45	-0.44	<0.01	(-5.33, 3.55)				
	Synovial hypertrophy(mm)	0.16	0.07	0.12	0.02	(0.02, 0.31)				
	Pain VAS (mm)	0.00	0.01	0.00	0.93	(-0.02, 0.02				
6	(Constant)	7.20	2.82		0.01	(1.64, 12.77)	0.72	0.71	.01	0.15
	BMI	0.73	0.06	0.65	<0.01	(0.62, 0.84)				
	Age	-.05	0.03	-0.09	0.07	(-0.11, 0.00)				
	Gender	-4.40	0.45	-0.43	<0.01	(5.29, -3.52)				
	Synovial hypertrophy (mm)	0.25	0.09	0.18	0.01	(0.07, 0.43)				
	Pain VAS (mm)	0.00	0.01	0.03	0.98	(-0.02, 0.02)				
	Global x-ray score	-0.06	0.04	-0.1	0.15	(-0.14, -0.02)				

3.8 Results: Follow-up evaluation

3.8.1 Participants

A total of 116 participants were followed up after their baseline assessments, 3 participants who self-reported changes in symptoms and 113 who were followed up by arrangement. Table 3-38 shows the descriptive data of follow-up participants and their original group allocation (as per index knee). The mean length of time between baseline and follow-up assessment was 99 days (SD 24).

As the preliminary analysis showed no significant differences between the control and KP group for US features, these groups were combined for the follow-up analysis and referred to as controls to increase the power of this analysis. The ROA and SOA groups were also combined and are referred to as the OA group.

Table 3-38 Descriptive data for follow-up participants

Group	**Control**	**KP**	**ROA**	**SOA**
n	**57**	**8**	**6**	**45**
Gender				
male n(%)	21 (36.8%)	5 (62.5%)	0 (0%)	14 (31.1%)
female n(%)	36 (63.2%)	3 (37.5%)	6 (100%)	31 (68.9%)
Age (yrs)				
mean (SD)	70.9 (8.0)	68.3 (8.8)	69.2 (3.4)	73.0 (7.8)
median (range)	70 (53-90)	68.5 (52-80)	69 (65-75)	73 (56-89)

(KP= knee pain only; ROA= radiographic OA; SOA= symptomatic OA)

3.8.2 Changes in knee pain over time

Changes in knee pain were defined as an increase or decrease in the worst item score reported in the WOMAC Pain subscale and are shown in Table 3-39. Eighty one participants (69.8%) did not change their maximal rating on the index, 10 participants from each group reported lower knee pain scores and 5 controls and 10 with OA reported higher knee pain scores. There was no significant difference between the two groups for change in pain (in either direction).

Table 3-39 Change in knee pain at follow-up

	Control		OA	
Change in WOMAC Worst item score (pain subscale)	**65**		**51**	
	n	(%)	n	(%)
No change	50	(76.9%)	31	(57.4%)
Pain better	10	(15.4%)	10	(19.6%)
Pain worse	5	(7.7%)	10	(19.6%)

3.8.3 Association between change in knee pain and change in US features

Change in the presence or absence of US effusion, synovial hypertrophy, popliteal cyst and power Doppler signal was examined in relation to change in pain at follow up. Chi square analysis found no association between change in knee pain and change in US features for either the control or OA groups (Table 3-40).

Table 3-40 Association between change in knee pain and change in presence of US features at follow-up

Change in presence of US features		Control group			OA group		
		Pain changed at follow-up		p	Pain changed at follow-up		p
		No	Yes		No	Yes	
Effusion	**No**	41	12	0.86	26	17	0.91
	Yes	9	3		5	3	
Synovial hypertrophy	**No**	47	12	0.10	25	18	0.37
	Yes	3	3		6	2	
Popliteal cyst	**No**	44	16	0.89	25	20	0.13
	Yes	6	2		3	0	
Power Doppler signal	**No**	50	15		30	19	0.75
	Yes	0	0		1	1	

(Pain change = Change in worst item score of WOMAC pain subscale)

3.8.4 Correlations between changing pain and US features

Correlations between the change in pain VAS scores and the change in continuous measures of effusion, synovial hypertrophy and popliteal cysts were explored for controls and OA groups (Table 3-41).

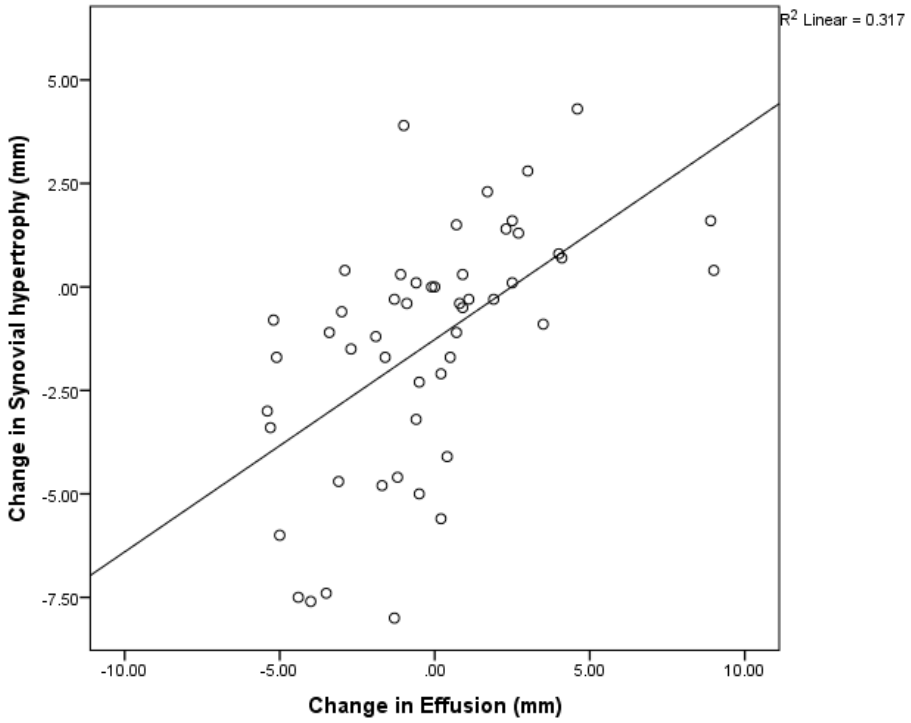
Change in VAS pain scores showed no relationship with change in US measures. Change in depth of effusion was correlated with change in depth of synovial hypertrophy for both groups. The strength of the relationship was moderate within the OA group ($r=0.66$, $p<0.01$) (Figure 3-55) and weak within the control group ($r=0.34$, $p<0.01$).

Table 3-41 Correlation matrix for change in pain and US measures in control and OA groups

Change		Pain VAS (mm)	Effusion (mm)	Synovial hypertrophy (mm)	Popliteal cysts (mm)
Control Group					
Pain VAS (mm)	r	1.0			
Effusion (mm)	r	-0.1	1.0		
Synovial hypertrophy (mm)	r	-0.1	0.3**	1.00	
Popliteal cysts (mm)	r	-0.1	-0.02	0.2	1.0
OA Group					
Pain VAS (mm)	r	1.0			
Effusion (mm)	r	-0.2	1.00		
Synovial hypertrophy (mm)	r	-0.01	0 .7**	1.0	
Popliteal cysts (mm)	r	0.01	0.2	0.1	1.0

*** Correlation is significant at the 0.05 level**
**** Correlation is significant at the 0.01 level**
mm = millimetres

Figure 3-55 Scatterplot showing relationship between change in depth of effusion and depth of synovial hypertrophy in OA group



3.8.5 Change in biomechanical stiffness and damping co-efficients with changing pain

Baseline and follow-up stiffness and damping co-efficients were available for 62 (53.4%) of the 116 participants that were followed up (35 controls and 27 OA).

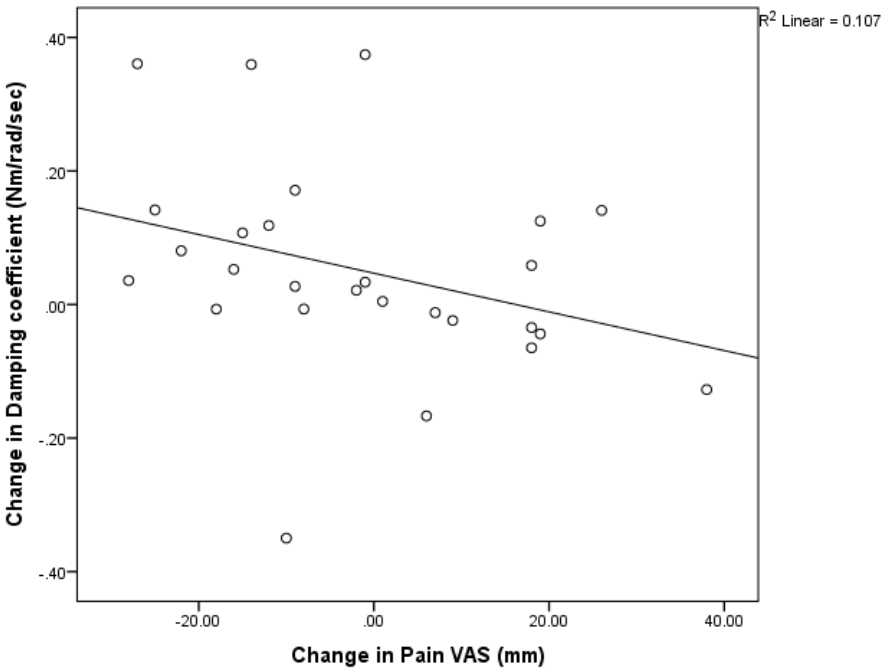
Changes in stiffness and damping co-efficients were explored to see if they correlated with changing pain (VAS score) or changing US features (Table 3-42). No correlations were observed in the control group. Increase in pain severity on the OA group was negatively correlated to change in damping co-efficients (r=-4.1, p<0.5) (Figure 3-56).

Table 3-42 Correlation matrix for change in pain, US measures and stiffness and damping co-efficients

		Change in Damping co-efficient	Change in Stiffness co-efficient
Control Group			
Change Pain VAS (mm)	r	0.02	-0.01
Change Effusion (mm)	r	-0.2	-0.1
Change Synovial hypertrophy (mm)	r	0.2	-0.3
Change Popliteal cysts (mm)	r	0.1	.06
OA Group			
Change Pain VAS (mm)	r	-0.4*	.3
Change Effusion (mm)	r	0.1	-0.03
Change Synovial hypertrophy (mm)	r	0.2	0.003
Change Popliteal cysts (mm)	r	0.01	-0.02

*** Correlation is significant at the 0.05 (1-tailed)**
**** Correlation is significant at the 0.01 level (1-tailed)**
mm = millimetres

Figure 3-56 Scatterplot showing relationship between change in pain VAS (mm) and change in damping co-efficient in OA group.

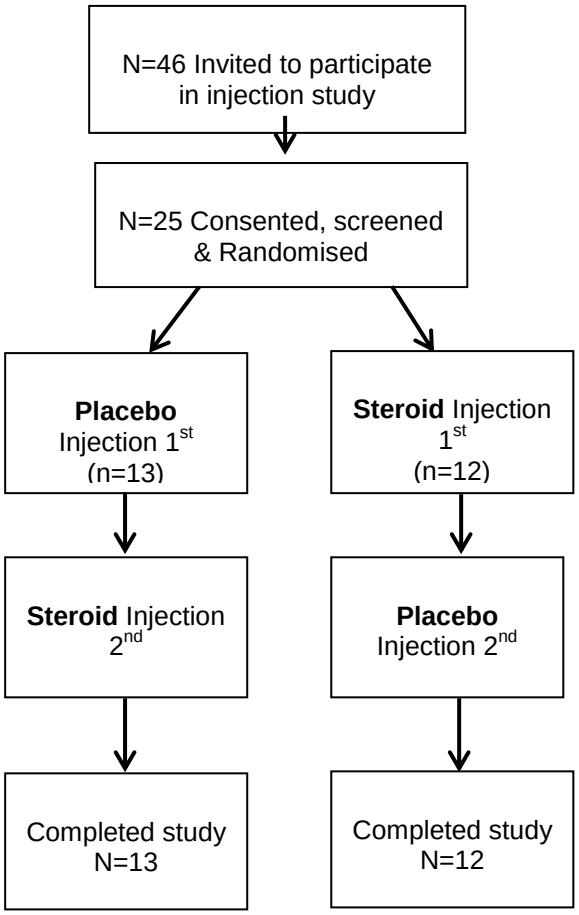


3.9 Intervention study

3.9.1 Participants

Of the 46 participants approached for the intervention study, 25 participants consented to take part. Two subjects had undergone a total knee replacement since their participation in the cross-sectional study but were included as they had SOA in the contra-lateral knee. One participant in the study had inconclusive OA (G0) on x-ray but was symptomatic and had positive US findings including PD signal. Thirteen participants were randomised to receive the placebo injection first and twelve to receive the steroid injection first (Figure 3-57). There were no withdrawals from the study.

Figure 3-57 Flow chart of Injection study



3.9.2 Baseline characteristics

Groups were balanced at baseline with no significant differences in age, gender, BMI, radiographic severity, presence of clinical effusion, and the number of days between injections (Table 3-43).

Table 3-43 Participant characteristics according to treatment order

		Group A Placebo first n=13	Group B Steroid first n=12	p
Age years	**mean (SD)**	71.2 (10.4)	73 (3.8)	0.57
BMI (kg/m^2)	**mean (SD)**	30.5 (5.3)	29.9 (3.8)	0.73
Gender	**male n (%)**	7 (54%)	3 (24%)	0.23
Knee injected	**right n (%)**	8 (61.5%)	6 (50%)	0.70
K&L Grade Injected knee	**n (%)**			
	Grade 0	1 (7.7%)	0	
	Grade 1	0	0	
	Grade 2	0	1 (8%)	
	Grade 3	5 (38.5%)	6 (50%)	
	Grade 4	7 (53.8%)	5 (42%)	0.75
Clinical effusion,	**n (%)**			
	absent	2 (15.4%)	3 (25.0%)	
	small	3 (23.1%)	4 (33.3%)	
	large	8 (61.5%)	5 (41.7%)	0.61
Time between 1st and 2nd injection, days	**mean (SD)**	81 (47)	95 (65)	0.81

3.9.3 Response following injections

The study was designed to ensure no carryover effect following the first injection by waiting until participant's knee pain had returned to its pre-injection severity before administering the second injection. Independent t-tests showed no order effect for pain response following the steroid injection (mean difference = -1.5, p=0.87) or placebo injection (mean difference = - 4.0, p=0.72).

Baseline measures and differences at 1 week are presented in Table 3-44 and visually in Figure 3-58 to Figure 3-61. Paired tests showed no significant difference in mean pain or US variables at baseline, or for change at 1 week, between placebo and steroid injections.

Significant improvements in pain VAS scores were observed following both placebo and steroid injection (mean difference for steroid = -17.4, SD 26.8, p=0.003; mean difference for placebo = -13.4, SD 22.4, p=0.006) but these were not statistically different from each other. Synovial hypertrophy showed a significant difference between baseline measures and measures at 1 week following steroid injection (mean difference 0.94 (SD 2.18, p=0.04) but was not different from placebo.

Table 3-44 Baseline characteristics and change in outcome measures at 1 week following injection.

	Baseline				Change at 1 week		
	Placebo N=25	**Steroid N=25**	**p**		**Placebo N=25**	**Steroid N=25**	p
Frequency of US features	**n (%)**	**n (%)**					
Effusion	21 (84%)	21 (84%)	1.0				
Synovial hypertrophy	20 (80%)	18 (72%)	0.51				
Popliteal cyst	8 (32%)	7 (28%)	0.75				
PD signal	7 (28%)	6 (24%)	0.75				
				Change in PDS No change	19	19	
				Change in PDS Increase	4	4	
				Change in PDS Decrease	2	2	0.59
	Mean (SD)	**Mean (SD)**	**Paired p**		**Mean (SD)**	**Mean (SD)**	**Paired p**
Pain VAS (mm)	61.8 (20.5)	61.4 (22.2)	0.95		-13.4 (22. 4)	-17.4 (26.8)	0.59
US Features Effusion (mm)	7.3 (3.6)	7.0 (3.9)	0.76		-0.6 (2.5)	-0.06 (2.1)	0.40
Synovial hypertrophy (mm)	7.7 (4.5)	6.9 (3.6)	0.50		-1.0 (3.7)	-0.94 (2.2)	0.91
Popliteal cyst (mm)	2.9 (4.4)	3.12 (4.4)	0.70		0.3 (1.8)	-0.72 (2.5)	0.12
ICOAP Intermittent (0- 24)	11.1 (4.2)	11.4 (3.5)	0.74		-0.4 (3.2)	-2.48 (5.4)	0.12
Constant (0-20)	7.5 (4.4)	8.0 (4.3)	0.72		-0.7 (3.2)	-1.96 (4.7)	0.28
WOMAC Pain (0-20)	9.2 (3.5)	9.4 (2.7)	0.82		-0.8 (2.4)	-1.96 (2.7)	0.13
Stiffness (0-8)	3.9 (1.7)	3.9 (1.2)	1.00		-0.3 (1.3)	-0.60 (1.5)	0.43
Function (0-58)	33.4 (11.2)	32.3 (10.3)	0.70		-1.0 (6.6)	-2.60 (8.4)	0.61

Paired p = significance values for paired test for variables between placebo and steroid injections

Figure 3-58 Mean change in VAS pain score (mm) following steroid and placebo injection

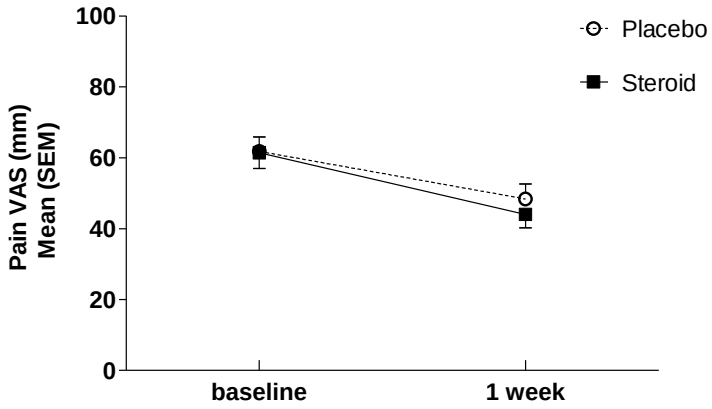


Figure 3-59 Mean change in US effusion (mm) following steroid and placebo injection

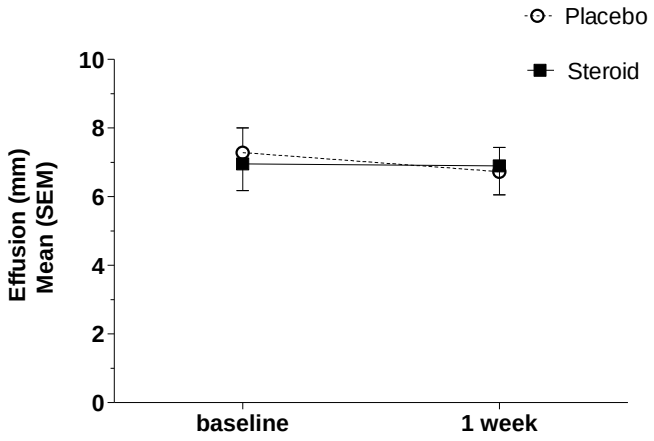


Figure 3-60 Mean change in synovial hypertrophy (mm) following steroid andplacebo injection

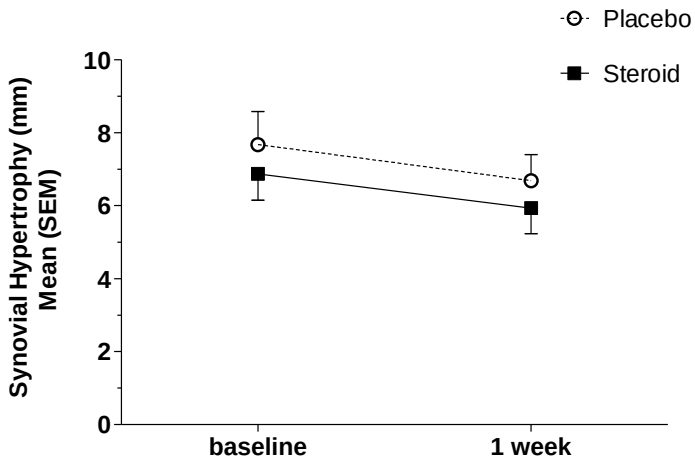
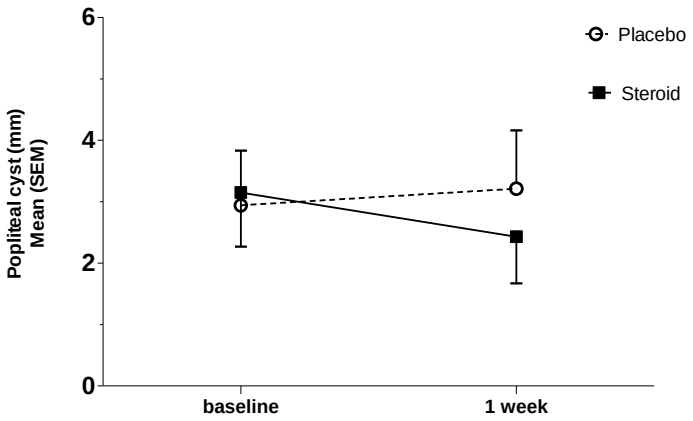


Figure 3-61 Mean change in popliteal cyst (mm) following steroid and placebo injection



3.9.4 Responders to steroid injection

The association between response following steroid injection and the presence of US features was examined using logistic regression. No statistically significant predictors of response were found Table 3-45 .

Table 3-45 Crudes odds ratios for predictors for patient response to steroid injection

US features at Baseline	Responder to steroid injection		Crude Odds ratio (95% CI)	p
	no	yes		
Effusion				
no	1	3	1	
yes	9	12	0.44 (0.04, 5.01)	0.51
Synovial Hypertrophy				
no	4	3	1	
yes	6	12	2.67 (0.46, 15.96)	0.28
Popliteal cyst				
no	8	10	1	
yes	2	5	2.00 (0.30, 13.17	0.47
Power doppler signal				
no	8	11	1	
yes	2	4	1.46 (0.21, 9.98)	0.70

Individual features were then examined for the 15 participants identified as responders (decrease in pain VAS ≥ 15 mm) following steroid injection. Continuous US measures for these participants following the steroid injection were examined (Table 3-46).

Correlation analysis found no statistically significant relationships between change in pain VAS and change in US measures after steroid injection in steroid responders (Table 3-47). Changes in effusion (mm) were strongly correlated with changes in synovial hypertrophy ($r=0.77$, $p<0.01$) (Figure 3-62).

Table 3-46 Pain responders to steroid: Ultrasound responses 1 week following steroid injection

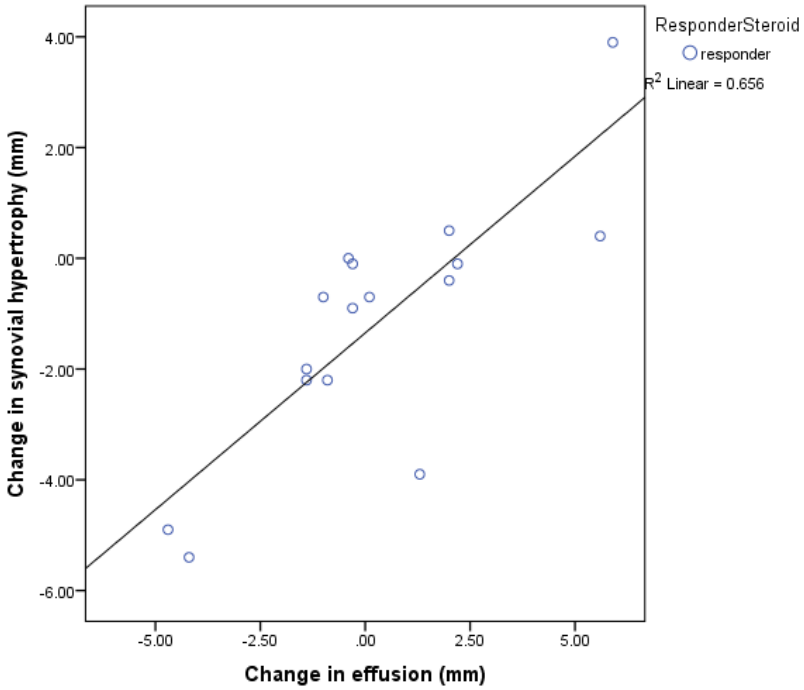
	Responses (change) following steroid injection at 1 week			
Participant id	VAS (mm)	Effusion (mm)	Synovial Hypertrophy (mm)	Popliteal Cyst (mm)
2	-26	-0.4	0	0.6
3	-20	2.0	-0.4	-0.9
12	-20	-1.4	-2.0	0
15	-66	-0.3	-0.1	-9.8
32	-63	0.1	-0.7	0
35	-21	-1.0	-0.7	0
36	-22	-0.3	-0.9	0
51	-32	-4.7	-4.9	-4.2
54	-24	-1.4	-2.2	0
61	-34	5.6	0.4	0.3
89	-36	5.9	3.9	4.4
96	-44	-0.9	-2.2	-4.3
114	-43	2.2	-0.1	-1.6
136	-36	1.3	-3.9	0
154	-38	2.0	0.5	0

Table 3-47 Correlation matrix for differences in Pain and US measures at 1 week in responders to steroid injection

Differences in		VAS (mm)	Effusion (mm)	Synovial Hypertrophy (mm)	Popliteal cyst (mm)
VAS	r	1.00			
Effusion	r	-0.43	1.00		
Synovial hypertrophy	r	-0.35	0.77**	1.00	
Popliteal cysts	r	0.28	0.27	0.39	1.00

*** Correlation is significant at the 0.05 level**
**** Correlation is significant at the 0.01 level**
mm = millimetres

Figure 3-62 Scatterplot showing relationship between change in effusion (mm) and change in synovial hypertrophy in participants responding to steroid injection



Individual pain and US response were then examined for responders to steroid injection (Figure 3-63 to Figure 3-66). Of the 15 participants who showed a positive pain response following steroid injection more than half (n=9) showed a reduction in effusion depth which increased again

when pain returned to pre-injection level (Figure 3-64). Ten participants showed a reduction in synovial hypertrophy depth, 8 of which also increased when pain returned (Figure 3-65). Popliteal cysts were present in 8 of the participants, 5 of whom showed a reduction in size, with 2 completely resolving. All showed increased size when pain returned apart from the two who had completely resolved (Figure 3-66).

PD signal was detected in 4 steroid responders at baseline (Table 3-48). One week after the steroid injection this had resolved in 3 and remained unchanged in 1 of the 4. However, new PD signal activity was recorded in 2 other participants. At follow-up when pain had returned to its pre-injection level, PD signal persisted in 2 participants and appeared in 1 new participant.

Figure 3-63 Pain responders to steroid: Change in pain VAS scores (mm) after steroid injection

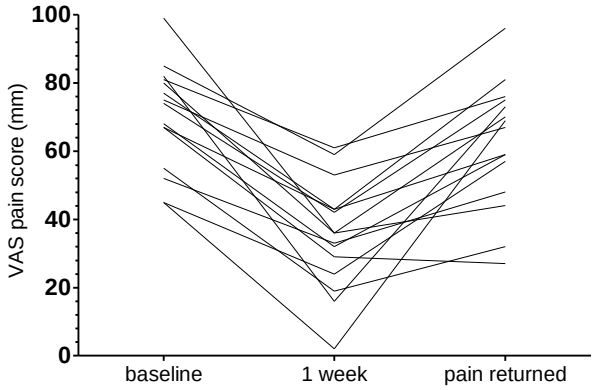


Figure 3-64 Pain responders to steroid: Change in Effusion after steroid injection

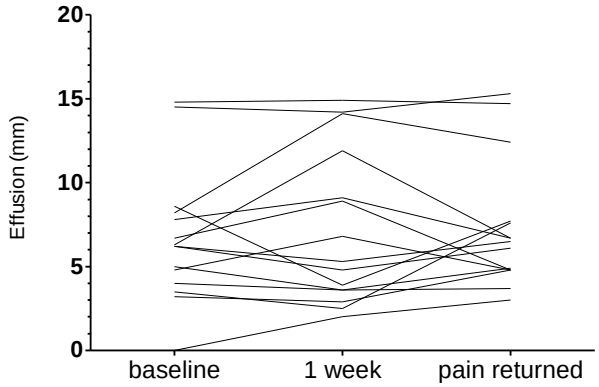


Figure 3-65 Pain responders to steroid: Change in synovial hypertrophy after steroid injection

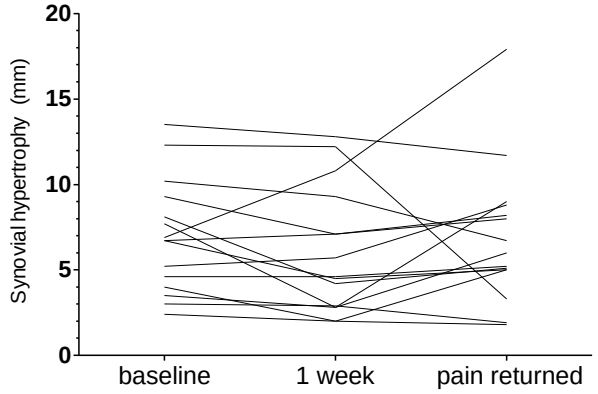


Figure 3-66 Pain responders to steroid: Change in popliteal cyst after steroid injection

Table 3-48 Observed PD signal in pain responders following steroid injection

	Observed PD signal		
Participant Id	Baseline	Week 1	Follow-up (pain returned)
2	0	**1**	0
3	0	0	**1**
12	0	0	0
15	0	0	0
32	0	**1**	**1**
35	0	0	0
36	**1**	0	0
51	**1**	0	0
54	0	0	0
61	0	0	0
89	0	0	0
96	**1**	0	0
114	0	0	0
136	0	0	0
154	**1**	**1**	**1**

(**1**= PD signal observed, 0= PD signal not observed)

3.9.5 Pain responders to placebo injection

Eleven participants were identified as pain responders following placebo injection (Table 3-49). US measures were explored in these individuals. No association was found between response following placebo injection and the presence of US features (data not shown).

Correlation analysis between change in pain VAS scores and continuous US measures found no significant relationships in placebo responders (Table 3-50). However as with responders following steroid injection changes in effusion were correlated with changes in synovial hypertrophy (r= 0.71, p=0.02).

Table 3-49 Pain responders to placebo: Ultrasound responses 1 week following placebo injection

	Responses (change) following placebo injection at 1 week			
Participant id	VAS (mm)	Effusion (mm)	Synovial Hypertrophy (mm)	Popliteal Cyst (mm)
2	-21	-0.1	1.2	1
6	-31	2.1	4.5	2
15	-27	3.5	1.3	0
24	-61	-1.7	0.2	0
30	-35	0.6	1.9	4.7
36	-18	-1.9	-4.2	0
38	-29	-4.2	-4.9	0
51	-48	2.5	3.8	0
61	-45	1.9	-0.7	-0.2
114	-31	4.1	6.1	-3.1
164	-38	-1.1	4.0	0

Table 3-50 Correlation matrix for differences in Pain and US measures at 1 week in responders to placebo injection

Differences in		VAS (mm)	Effusion (mm)	Synovial Hypertrophy (mm)	Popliteal cyst (mm)
VAS	r	1.00			
Effusion	r	-0.13	1.00		
Synovial hypertrophy	r	-0.23	0.71*	1.00	
Popliteal cysts	r	0.19	-0.20	0.08	1.00

*** Correlation is significant at the 0.05 level**
mm = millimetres

Individual responses were examined in participants who responded to placebo injection. Pain responses are shown graphically in Figure 3-67. Five participants showed improved measures of effusion though these were small (only one of these was an improvement greater than 2mm, Figure 3-68). Three showed a reduced depth of synovial hypertrophy, with two showing improvements greater than 2mm (Figure 3-69). Popliteal cysts size only improved significantly in 1 of 3 participants with popliteal cysts (Figure 3-70). PD signal was detected in 2 placebo responders at baseline (Table 3-51).One week following the placebo injection PD was not detected in the same participants but was observed in another participant. At follow-up when pain had returned to its pre-injection severity, PD signal could still be observed in that same participant and a further two others.

Figure 3-67 Pain responders to Placebo: change in Pain VAS scores (mm) after placebo injection

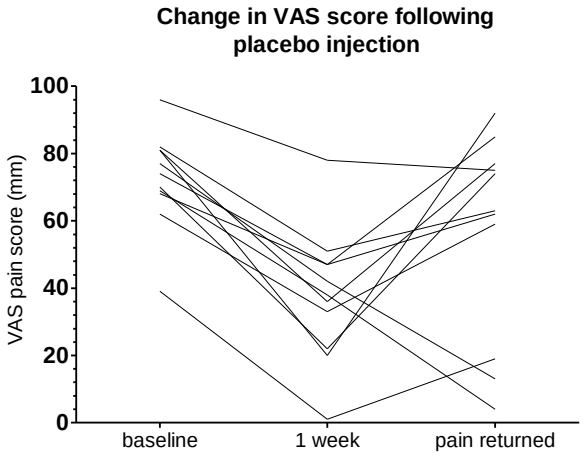


Figure 3-68 Pain responders to Placebo: change in effusion (mm) after placebo

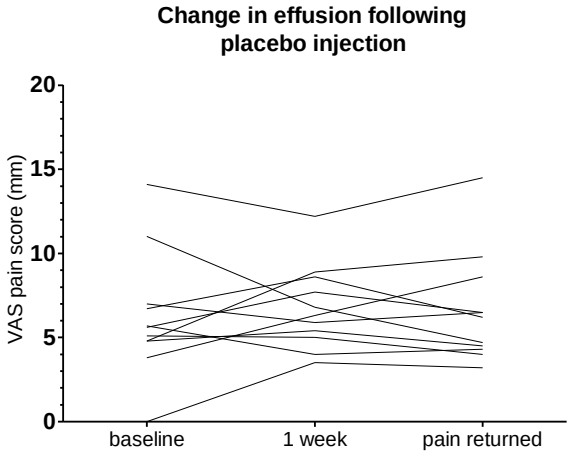


Figure 3-69 Pain responders to Placebo: change in synovial hypertrophy (mm) after placebo injection

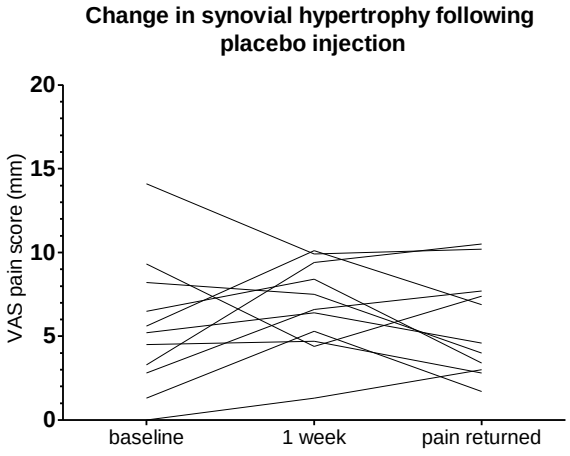


Figure 3-70 Pain responders to Placebo: change in popliteal cysts (mm) after placebo injection

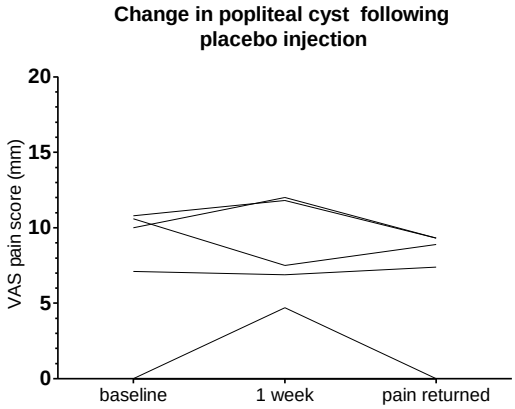


Table 3-51 Observed PD signal in pain responders following placebo injection

	Observed PD Signal		
Participant id	Baseline	Week 1	Follow-up (pain returned)
2	0	0	0
6	0	**1**	**1**
15	0	0	0
24	**1**	0	0
30	0	0	0
36	0	0	**1**
38	0	0	0
51	0	0	**1**
61	0	0	0
114	0	0	0
164	**1**	0	0

(**1**= PD signal observed, 0= PD signal not observed

4 Discussion

This chapter revisits the objectives of the thesis and the original hypotheses are accepted or rejected in light of the study findings. Study findings are discussed in the context of previous work and novel findings are explored. Caveats of the study are addressed and finally the thesis is brought to a close by suggesting directions for future research and drawing final conclusions.

4.1 Study objectives & Hypotheses

The primary objectives of this thesis were to firstly determine the frequency of US features suggestive of joint inflammation (effusion, synovial hypertrophy and PD signal) in normal knees, painful knees, radiographic OA knees and symptomatic OA knees. Secondly, to determine if US features were associated with knee pain, radiographic features, and clinical symptoms and signs associated with inflammation rest pain, clinical effusion and stiffness) and function. These objectives were achieved by conducting a cross-sectional multiple group comparison study in the community. The hypotheses related to this study were:

> **Hypothesis 1**. US features of inflammation will be more common in knees with radiographic changes, irrespective of pain status, and will be more pronounced in people with knee pain.
>
> **Hypothesis 2.** US features of inflammation will be independently associated with an increased risk for knee pain.

Grey-scale US features (effusion, synovial hypertrophy and popliteal cysts) were observed more frequently in participants with radiographic OA (regardless of the presence of symptoms) compared to those without. Those features were also more pronounced, and for effusion and synovial hypertrophy were significantly more pronounced in those with SOA compared to ROA. PD signal was only more common in those with SOA compared to those without radiographic changes. As a result, hypothesis 1 can be accepted. Secondary analysis found synovial hypertrophy to be the only US feature that was consistently and independently associated with knee pain, and so hypothesis 2 can be partly accepted for this feature.

Secondary objectives for the study were to determine if US features of inflammation changed in tandem with fluctuations in knee pain in those with symptomatic OA. The hypothesis related to this was:

> **Hypothesis 3.** Changes in US features of inflammation in SOA knees will correlate with changes in reported knee pain over time and also following an intervention for knee pain.

Follow-up observation of control and SOA participants was conducted after a period of three months. Only 39% of participants with SOA reported a change in pain severity (increased or decreased) and no correlation was found between change in pain severity and change in US measures. A small intervention study subsequently set out to examine US response following pain reduction from an intra-articular steroid injection and a placebo saline injection. Pain severity decreased

following both the cortico-steroid and placebo injection but no correlation was found between change in pain severity and US measures. While these findings do not initially support the hypothesis, the small number of participants for whom pain changed at follow-up and the small sample size of the intervention study suggest that this hypothesis should not be rejected outright at this stage. Rather, additional work that is adequately powered should be undertaken to answer this question definitively.

4.2 Study findings

4.2.1 Reliability

When considering the use of an outcome measure there are several characteristic to bear in mind. Validity and reliability determine the confidence that can be placed in the inferences drawn from their use, sensitivity and specificity are important for diagnostic purposes, and responsiveness is important for monitoring disease progression or treatment. Additionally, an outcome measure should be clinically meaningful and easy to use.

The criterion validity of ultrasound in detecting synovial pathology in knee OA has been demonstrated by the comparison with arthroscopy findings and histopathological evaluation (Karim, Wakefield et al. 2004) whereas construct validity has been demonstrated more widely against

clinical and laboratory findings and other imaging techniques such as radiographs, bone scans and MRI imaging

The issue of reliability remains an important consideration for any study using ultrasonography. Though reliability and validity are separate issues in terms of metric properties, the validity of an outcome measure is dependent on how reliable it is. Issues in the reliability in US assessment can be further divided into the acquisition and reading of scans. The potential for variation is greater in the acquisition of scans due to the nature of the scanning process. Even with a standardised scanning protocol, the choice of image and the optimising of US settings remains at the discretion and skill of the sonographer.

This study has demonstrated good reliability in determining the presence or absence, and magnitude of US features and is comparable with other studies (Karim, Wakefield et al. 2004; Abraham, Goff et al. 2011; Iagnocco, Perricone et al. 2012; Wu, Shao et al. 2012). For intra-observer reliability, kappa values between 0.7 and 0.8 ($p \leq 0.03$) were reached for detecting the presence or absence of grey-scale features of inflammation in 28 knees. Intra class correlation co-efficients of between 0.82 and 0.95 ($p < .001$) were found for continuous US measures demonstrating substantial to excellent reliability.

Inter-observer reliability was tested in ten knee joints and demonstrated against an experienced consultant musculoskeletal radiologist. Reliability was good for both the presence US features (kappa = 0.62 to 0.78, $p \leq 0.02$) and continuous measures of US features (ICC = 0.8 to

0.9, p<.001). Similar reliability was also reported for the dichotomous scoring of grey-scale features in a comparable study of 17 OA knee joints where inter-observer reliability was tested between a senior and a junior sonographer (Iagnocco, Perricone et al. 2012).

However the low number of participants (due to the availability of the 2nd sonographer (KL)) and the low prevalence of US findings are likely to contribute to the high values. Bland-Altman plots did show a small but systematic bias within the reading where by the main study sonographer (MH) had higher reading compared to the second sonographer (KL) which needs to be borne in mind when interpreting the results.

Reliability for PD signal was statistically also high for both intra and inter-observer agreement (kappa = 1.0 and 0.76 respectively) However it is worth noting that the kappa statistic is affected by the prevalence of the feature under consideration and for rare findings such as PD signal low values may not necessarily reflect low agreement and vice versa. Sample sizes for future reliability studies should address this issue.

Structural US features of osteophytes and depth of femoral articular cartilage were not the primary outcome measures of interest in this study. However, they were also included in a subset of 10 knees which underwent testing for intra- and inter-observer reliability. Intra-observer reliability was higher than inter-observer for osteophytes (kappa = 0.80 and 0.55, respectively). Inter-observer agreement was less than other published studies (Abraham, Goff et al. 2011; Iagnocco, Perricone et al.

2012). For depth of femoral articular cartilage in the transverse view the ICC for intra and inter-observer reliability was 0.71 and 0.52 respectively, and was comparable to reliability reported by Abraham et al (2011).

The presence of chondrocalcinosis (CC) was not examined in terms of reliability as it was not observed in any of the reliability participants. It was also not used as a primary outcome measure for this study. Poor agreement (kappa = 0.28, $p<0.001$) was found between US and radiographic detected chondrocalcinosis in this study and there are several reasons why this might have occurred.

Firstly, CC was not observed throughout the training period. Therefore it cannot be assured that the presence of CC was not missed, particularly during the early phases of data collection. Secondly, the detection of CC was limited to the appearance within the femoral articular cartilage which may lead to an under-reporting of the true US prevalence. Thirdly, the assessment of the femoral articular cartilage was standardised to 90° knee flexion which may not be the optimal for assessing CC.

The diagnosis of CC is mainly based in radiographic and detection of calcium pyrophosphate dihydrate (CPPD) crystals within the joint fluid. CPPD crystal deposits in the knee joint can also be observed by US as thin hyper-echoic bands within the articular cartilage, as hyper-echoic spots within the menisci and fibrocartilage and as hyper-echoic aggregates within the synovial fluid and popliteal cysts (Dufauret-

Lombard, Vergne-Salle et al. 2010). Only a few small studies have assessed the agreement between radiography and US in detecting CC. In 28 knees with chondrocalcinosis and 46 normal controls, no difference was reported between US and radiography in detecting CC within the articular cartilage, but US was less sensitive for detecting meniscal calcification (Coari, Iagnocco et al. 1995). A small study of 11 patients with CPPD and 13 patients with mild OA but without CPPD found that plain radiographs confirmed the presence of the US CC in all cases. Two cases of CC were identified by US where standard radiographs did not confirm the diagnosis, highlighting both sensitivity and specificity of US, albeit in a very small group (Frediani, Filippou et al. 2005). Further work on the validity and reliability of US in the diagnosis of chondrocalcinosis is required but is beyond the scope of this current body of work.

Although good reliability was demonstrated, other technical aspects of scanning may also have affected the overall reliability. Assessment of the knee joint is largely superficial but scanning was more difficult in obese participants. Attenuation of US energy occurs as waves pass through the tissues and results in echoes from deeper structures being displayed less intensively. While the depth of penetration can be altered this is often at the sacrifice of frequency and image resolution. The detection of blood flow using Doppler in these participants was also more technically challenging.

4.2.2 Diurnal variation

A potential source of variation in US scanning is the time of day that is performed. Diurnal variations (daily patterns) in joint pain and stiffness are well recognised in inflammatory conditions and have been also demonstrated in knee OA in addition to variations in serum levels of hyaluronan and other biomarkers of inflammation (Bellamy, Sothern et al. 1990; Criscione, Elliott et al. 2005; Kong, Stabler et al. 2006). A recent study of hand joints with active RA demonstrated US variations in PD signal and semi-quantitative scoring over a 24 hour period, where higher scores were awarded during morning scans compared to the afternoon and evening (Semerano, Gutierrez et al. 2011). It is plausible therefore, that US features of inflammation may also exhibit diurnal variations in knee OA.

This current study found no such diurnal variation within a sample of participants with bilateral symptomatic OA. Furthermore pain severity showed no significant differences across the three weeks reflecting a chronic but stable pain state. Participants with more unpredictable pain may show greater variation in US measures and this should be considered in future studies. PD signal was not detected in any participants and so it is unknown whether diurnal variation may occur in this feature in OA.

4.2.3 Cross-sectional multiple group comparison study

4.2.3.1 Primary findings

The primary objective for this study was to firstly determine the frequency of US features of joint inflammation in a community sample with normal knees (controls), painful knees (KP), radiographic OA knees (ROA) and symptomatic OA knees (SOA), and secondly, to determine if US features are associated with pain, structural change or clinical symptoms and signs of inflammation.

US features representative of joint inflammation were not restricted to symptomatic OA. Within the control group, a background presence of US effusion was observed in 29% and synovial hypertrophy in 8%. The size of effusions were small (mean depth <3mm) but some knees exhibited effusions up to 10.3mm. Synovial thickening was generally minor (mean depth <1mm) but was observed to be as thick as 6.7mm. The presence of PD signal was low at around 2%. Popliteal cysts were detected in 12.4% in control knees and were mainly small (mean <1 mm) although the range extended up to 13.5mm. Pes anserine bursae were not observed within controls and patellar bursae were infrequent (< 3.3% in control knees).

Previous studies have reported US detected effusions in between 0-35% of normal and control participants (Tarhan and Unlu 2003; Naredo, Cabero et al. 2005; de Miguel Mendieta, Cobo Ibáñez et al. 2006). Different cut-off measures used to define effusions can account for

some variation in the findings but differences in scanning protocols may also yield different findings.

No previous studies have reported the frequency of US features for people with knee pain without structural change. This study found that US features were more common in the KP group compared to controls but were not statistically different. Effusions were observed in 33% of the KP group, synovial hypertrophy in 12%, popliteal cysts in 9% and PD signal in around 3.5%. Frequency of popliteal cysts and PD signal were also not significantly different from the control group. The presence of US effusion and synovial hypertrophy in control and KP groups may reflect pre-clinical and pre-radiographic changes that have been observed histologically (Benito, Veale et al. 2005).

A clear increase in grey-scale features was observed in the ROA group compared to control and KP groups. Prevalence of effusions rose to 81% within index knees, Synovial hypertrophy was observed in 41% and PDS in 6% of the group. Popliteal cysts were between 8 and 10 times more common in ROA (between 17-22%) compared to controls ($p<.05$), and twice as common as those with knee pain alone (though not statistically different).

De Miguel et al (2006) are the only other authors to have reported US findings in patients with radiographic OA with and without symptoms as independent groups. The frequency of US features in the SOA group, were similar to the current findings of this study but there were different observations in the ROA group. Effusions were less frequently

observed (35%) whereas popliteal cysts and infra-patellar bursitis were more common (40% and 15% respectively). The disparity for infra-patellar bursitis can be explained by the lower cut-off value for defining bursitis (>2mm), but is somewhat surprising for US effusion which had the same definition. Scanning protocol could account for this as effusions were measured in the midline of the supra-patellar pouch and therefore may have underestimated the true frequency. Schmidt et al (2004) reported fluid not only in the midline longitudinal plane of the supra-patellar pouch but also in the lateral recess of the pouch. Criteria for popliteal cysts were very similar for both studies (>4mm and ≥4mm) and while the observed frequency of popliteal cysts was similar in the SOA group for both studies, differences in the ROA may be a reflection of the sample sizes of the ROA groups.

US features were most frequently observed in the SOA group**.** Effusions were found in over 90% participants, synovial hypertrophy in 82% and popliteal cysts in 39%. Mean depth of effusion, synovial hypertrophy and size of popliteal cyst were significantly greater than in all other groups. PD signal was observed in 16% of SOA group which was significantly higher than in control and KP groups but not compared to the ROA group.

Many studies have reported similar observations for US effusion with frequencies around 69-86% (Tarhan and Unlu 2003; de Miguel Mendieta, Cobo Ibáñez et al. 2006; Kristoffersen, Torp-Pedersen et al. 2006; Pendleton, Millar et al. 2008; Mermerci, Garip et al. 2011). Others

though have reported considerably lower prevalence rates but aggregate around 43-47% (D'Agostino, Conaghan et al. 2005; Naredo, Cabero et al. 2005; Iagnocco, Meenagh et al. 2010) which is suggestive of a systematic difference between these studies. Observations of synovial hypertrophy from other studies are more varied and range from of 16.7% to 100% which makes interpretation difficult (D'Agostino, Conaghan et al. 2005)(Kristoffersen, Torp-Pedersen et al. 2006). The presence of popliteal cysts showed good consensus with the other studies (Tarhan and Unlu 2003; de Miguel Mendieta, Cobo Ibáñez et al. 2006; Pendleton, Millar et al. 2008).

PD signal is not a common observation in knee OA, and this is agreed by most studies where it has been investigated. Pendleton et al (2008) reported PD activity in 5 of 86 (6%) patients with SOA, while Iagnocco et al (2010) reported occurrence in only 2 of 82 (3%) and Mermerci et al (2011) reported no PD activity in any of 143 patients. However there are some studies who have reported it to be more frequent, Kristoffersen et al (2006) reported colour Doppler in 51 of 71 participants with SOA, while Song et al (2008) reported PD in 63% of cases. Reviewing the criteria and methodology for each study there appears to be no obvious reason was to why such a wide discordance showed be observed. The main inclusion criteria for all studies was knee joint OA according to American College of Rheumatology criteria (Altman, Asch et al. 1986). Radiographic OA was defined according Kellgren & Lawrence (Kellgren and Lawrence 1957), though Mermerci et al (2011) included some participants with grade 1 changes.

Participants with possible inflammatory pathologies were excluded with the possible exception of Song et al (2006) who did not report specific exclusion criteria.

Observed PD signal in this study was 16% and this may reflect differences in scanning protocol. Doppler activity was assessed in all areas where synovial hypertrophy was found, including the supra-patellar recesses, medial and lateral joint margins, and within popliteal cysts. Studies of hand OA show similar disparity in terms of frequency of PD signal, with reported prevalence ranging from 7% (Keen, Wakefield et al. 2008) to 86% (Kortekaas, Kwok et al. 2010). While it is not the intention to directly compare data from hand and knee studies here, it does highlight the major difficulty in using PD for clinical and epidemiological studies.

The largest study of symptomatic OA knees with which to compare these findings was carried out in 600 secondary-care patients (D'Agostino, Conaghan et al. 2005). Frequency of US features was lower with joint effusion observed in 43.6% of patients and synovial hypertrophy in just 16.7%. Mean size and depth of features were also smaller. However there are some distinct differences between the studies which may account for the disparity. A very conservative approach to assessing and defining synovitis was used with descriptions of synovitis to be excluded, for example fibrous synovitis and “normal” hypertrophic synovium. This is at odds with previous research where it has been shown that grey-scale US cannot

differentiate between synovial hypertrophy and synovitis (Schmidt, Volker et al. 2000; Walther, Harms et al. 2001). Tissue debris, blood clots and fibrosis are known to mimic some US features of synovial proliferation but these feature do not exhibit PD signal (Fiocco, Cozzi et al. 1996) and it is unfortunate that PD signal was not utilised in the EULAR study. Observation was also restricted to its appearance in the supra-patellar region which can result in hypertrophy being missed from the medial and lateral recess of the supra-patellar pouch and joint margins (Song, Burmester et al. 2008; Hayashi, Roemer et al. 2011). Wu et al reported a higher prevalence of synovial hypertrophy (63% in ROA and 93% in SOA patients) in a study where the joint was scanned not just the median longitudinal plane of the suprapatellar pouch but also the medial and lateral recesses, where focal synovitis has been previously observed on arthroscopy (Ayral, Pickering et al. 2005).

Further possible explanations for the wide variation reported are the study populations. The EULAR population was derived from hospital out-patient clinics and although they were younger and had less severe x-ray changes they reported more severe pain. This probably reflects differences between those referred to secondary centres because of their knee pain and community participants who are more successfully managing their symptoms. Secondly, it has been shown that prescribed stable doses of non-steroidal anti-inflammatory drugs can have a significant effect on the detection and grading of ultra-sound detected synovitis in the hands of patients with rheumatoid arthritis (Zayat, Conaghan et al. 2011). Whilst it has not been demonstrated whether

this is also true for osteoarthritic joints, this study had a washout period for NSAIDs of 48 hours prior to ultrasound assessment which is a sufficient period to ensure the washout of most short-life NSAIDs such as ibuprofen and diclofenac. Other studies of knee OA did not specify whether a wash-out period for NSAIDs (D'Agostino, Conaghan et al. 2005; Naredo, Cabero et al. 2005; Mermerci, Garip et al. 2011) or specified that drugs were not altered for the study (de Miguel Mendieta, Cobo Ibáñez et al. 2006) which may have led to an under-estimating of frequency and severity of US features. Finally, reliability is always a consideration for large multi-centre studies. Whilst training was undertaken for the EULAR study to ensure standardisation, no formal assessment of inter-reliability was performed.

4.2.3.2 Secondary findings

This study found that synovial hypertrophy was the only US feature independently associated with knee pain. The adjusted risk for knee pain was 6.6 times greater in those with US detected synovial hypertrophy than in those without.

Pain at rest (at night) or pain of a constant nature is commonly thought to reflect the pain of inflammation. Synovial hypertrophy was associated with both though the association was stronger for constant knee pain (aOR 8.34 (95% CI 3.56, 19.52)) compared to night pain (aOR 4.93 (95% CI 2.28, 10.63)). Intermittent knee pain which can be more mechanical in nature was associated with both US detected effusion

(aOR 2.67 (95% CI 1.46, 4.92)) and synovial hypertrophy (aOR 6.30 (95%CI 3.03, 13.08)).

Wu et al (2012) found a dose dependent relationship between synovial hypertrophy (mm) and pain severity on motion (mm) (β=5.47 95%CI (31.08, 76.70), pain at rest (β= 3.05 95%CI (12.26, 46.34)) and between effusion and pain on motion (β= 2.21 95% CI (6.14, 39.92)), but not for popliteal cysts. Linear regression modelling was not performed in this study but we found that pain severity was weakly correlated continuous US measures (r = 0.3 for effusion and synovial hypertrophy) and remained weak or insignificant even when the relationship was examined in the SOA group. Popliteal cysts were not found to be independently associated with knee pain or correlate with pain severity.

US features were themselves were related. A strong correlation was found between depth effusion and synovial thickness (r=0.79, p<0.01), though this was not as strong within the SOA group (r= 0.55, p<0.01). This finding is similar to that reported by the EULAR study (r= 0.51) (D'Agostino, Conaghan et al. 2005). Popliteal cyst size was also correlated with synovial thickening (r=0.35, p<0.01) but there was no relationship with effusion.

The association between US features of inflammation and ROA and was very strong. Even after adjusting for age, sex, BMI and knee pain, the risk for ROA was high (aOR 14.39 (95% CI 6.28, 32.94) for synovial hypertrophy, 13.39 (95% CI 6.14, 29.02) for effusion and 3.19

(95% CI 1.42, 7.17) for popliteal cyst). PD signal was not independently associated with ROA after adjusting for knee pain.

Significant relationships were also observed between severity of radiographic OA using summated scores from the Nottingham LDA and continuous measures of US features. The strongest relationship observed was between synovial hypertrophy and the global score ($r=0.71$, $p<0.01$) whereas US effusion showed a more moderate relationship ($r=0.57$, $p<0.01$). Popliteal cyst size was weakly correlated but remained highly significant ($r= 0.33$, $p<0.01$). Though direct correlations with radiographic severity have not been previously reported D'Agostino et al (2005) reported that more severe radiographic scores (K&L ≥ grade 3) was associated with a 2.2-fold increased probability of synovitis. These findings demonstrate that although there is a clear association between synovial hypertrophy and the presence of knee pain, US features are more strongly associated with radiographic OA.

4.2.4 Follow-up and intervention studies

Secondary objectives for the study were concerned with the responsiveness of US measures. The ability to demonstrate change and the extent to which change correlates with other outcome measures, is a fundamental consideration if US is to be employed as a clinical outcome measure but this has not yet been fully demonstrated (Keen, Mease et al. 2011).

No longitudinal studies of US features in knee OA over time have been reported. This study carried out follow-up evaluations in participants who self-reported a change in symptoms (increase or decrease in knee pain) and routinely after 3 months in control and symptomatic OA participants. Follow-up analysis was carried out in 116 participants, only 3 of whom self-reported a change in knee pain.

Pain severity remained stable for most people and did not change for over 60% of participants with OA and 77% of controls. Change in knee pain was not associated with a gross change in the presence or absence of US features. Additionally, change in pain VAS scores did not correlate with changes in measure of effusion, hypertrophy or popliteal cyst size. Change in the depth of effusion was correlated with change in synovial hypertrophy and was stronger for those with OA ($r=0.66$, $p<.01$), reinforcing the relationship between these two features that was previously observed in the cross-sectional study.

The relative stability of knee-pain and symptoms observed during the follow-up evaluations prompted the inclusion of an intervention study to observe the effects of pain relief following a cortico-steroid injection and a placebo injection on US features. Corticosteroid injections are a safe and effective intervention in knee OA. They reduce the symptoms of pain within a few days and the effects may last several weeks and in some case several months. Though not firmly established, the mechanism of action is thought to be mediated in part by an anti-inflammatory effect on the synovium which may be detected by US

examination (Creamer 1999). Corticosteroid injections are a well-established treatment for pain in OA and are recommended in all current guidelines including those by NICE (NICE 2008). Although many may not expect an objective change following placebo, there is good evidence that placebo produces a marked improvement in pain though this is not generally thought to relate to a direct biological effect on the synovium (Doherty and Dieppe 2009; Zhang, Nuki et al. 2010). However it has been theorised that changes to the synovium may occur via a central effect whereby increased endogenous glucocorticoids are released via the hypothalamic-pituitary-adrenal (HPA) axis), resulting in a reduction of local inflammation (Guess, Kleinman et al. 2002).

At present there is a paucity of research to demonstrate change in US features following therapeutic interventions in knee OA. A degree of responsiveness has been demonstrated for measures of synovial hypertrophy and effusion (Jan, Chai et al. 2006) and popliteal cyst size (Acebes, Sánchez-Pernaute et al. 2006) though the measures used were somewhat atypical. Song et al (2009) reported no significant change in grey-scale US, PD signal, contrast enhanced US or MRI measures following a series of intra-articular injections of Icatibant (a bradykinin receptor 2 antagonist) despite a significant pain response. The authors suggest that this may be due to Icatibant having an analgesic mechanism of action as opposed to an anti-inflammatory mechanism.

In this current study, significant pain improvements were demonstrated following both cortico-steroid and placebo injections. A response criterion for the study was set at an absolute change of ≥15mm on a 100mm VAS. Although this is more generous that the treatment response defined by the Osteoarthritis Research Society International criteria (Pham, van der Heijde et al. 2004) retrospective examination of the data showed that this amounted to only one additional participant being classified as responder.

Synovial hypertrophy was the only US feature to show a statistical significant difference from baseline but this was not statistically different to change following the placebo injection. As was observed in the follow-up evaluation at 3 months, change in pain did not correlate with change in US measures, though once again change in effusion was strongly correlated with change in synovial hypertrophy ($r=0.77$, $p<.01$). The presence of US features at baseline was not found to predict response following injection. However, it is important to recognise that though the size of the sample used was sufficient to detect significant change in pain, it may not have been adequately powered to detect significant changes in US measures.

Individual examination of responders to the steroid injection showed that more than half showed improved measures of effusion, hypertrophy and popliteal cysts size most of which deteriorated again when pain returned, indicating some degree of internal responsiveness.

When this was examined in the eleven responders to the placebo injection, US response was not as evident. Less than half showed an improvement in effusion size, around 1 quarter showed an improvement in synovial hypertrophy and only 1 out of 3 popliteal cysts improved.

Change in PD signal was not significant for steroid or placebo responders and when it was examined within individuals it had an unpredictable occurrence.

Though it was not the intention of this study to evaluate the efficacy of the steroid over the placebo injection, it was of interest to find no significant difference in pain response between them. Clinical response to placebo is well recognised for patient-reported subjective outcome such as OA pain (Zhang, Robertson et al. 2008; Doherty and Dieppe 2009). Determinants of response to placebo include blinding, effect size of the active treatment and invasive route of delivery all of which are relevant to this study. Pooled effect size of intra-articular cortico-steroid injection on pain in knee OA is 0.58 (95% CO 0.34, 0.75) and for a placebo comparison is 0.39 (0.18, 0.59). However there are other contextual considerations which may account for the high placebo response observed in this study.

Response expectancy has demonstrated physiological changes even to the point of reversing the pharmacological effect of a drug (Doherty and Dieppe 2009). Participants in this study were told that they could expect an improvement in their symptoms with both the placebo and steroid injection which may last for several weeks or months, added to this,

most if not all of the participants were naive to intra-articular injection which may well have enhanced their expectancy further.

In addition to pain response, the length of maintained response was also considerably greater in this study. Effect sizes for pain response following intra-articular steroid injections in published trials mainly peak at 1-2 weeks after injection and decrease considerably after four weeks (Zhang, Robertson et al. 2008). In this study, however, mean time from injection to pain returning was in the order of 10-12 weeks with no significant difference between placebo and steroid.

The on-going relationship between the participants and the researcher is also likely to have a contextual effect on the outcome. Positive consultations where patients are given a confident diagnosis following a thorough examination, where clinicians are positive about treatment outcomes and where outcomes are monitored are all proposed to improve response (Doherty and Dieppe 2009). The same is likely to apply to research participants, especially when seen in a less hurried research setting.

4.3 Novel findings

As an adjunct to the analysis of radiographic OA, the presence of chondrocalcinosis (CC) was examined as a separate radiological feature. CC is usually due to calcium pyrophosphate (CPP) crystal deposition (CPPD) within the cartilage and most commonly affects the knee joint. Acute inflammatory synovitis secondary to CPP crystal shedding from cartilage is well recognised but lower degrees of

persistent inflammation may also associate with CC and CPPD. The community prevalence of CC is estimated at 4.5% after adjusting for age, knee pain and sex (Neame, Carr et al. 2003). For this study, CC was most commonly observed in knees with SOA and was found in 13% of left and index knees but as many as 18.5% of right knees. CC was observed around in 5% of controls knees with no statistical difference between controls, knee pain and ROA groups.

The presence of grey-scale US features were not associated with CC but the presence of PD signal conferred a more than 6-fold increase in the risk of CC on x-ray (aOR 6.57, 95% CI 1.66, 25.94) after adjusting for age, sex, BMI and knee pain. Additional adjustment for the presence of ROA did not appreciably alter the risk.

CPPD can lead to acute CPP crystal synovitis in 10-20% of cases. CPPD is typically diagnosed from radiographs but can also be visualised using grey-scale US though the accuracy and sensitivity of this has not been established. The association observed here between PD signal and CC on radiographs suggests that US may be useful in identifying not only CC but also the associated inflammation in some patients.

A novel aspect of this thesis was the consideration of knee joint stiffness (self-reported as a symptom and as objective biomechanical measurements). This is the first to look at associations between self-reported stiffness, biomechanically assessed stiffness, radiographic OA and knee pain. Self-reported stiffness of at least moderate intensity was

reported by over 80% of participants with SOA, though prolonged stiffness (>30 minutes) was less common (27.3%). Self-reported stiffness in ROA (6.3%) was not significantly different to control participants (12.2%). Of interest though was how frequently stiffness was reported in those with knee pain without structural change (69.5%). This was not significantly different to those with symptomatic OA and would suggest that self-reported stiffness is more associated with the presence of knee pain than radiographic OA. The same patterns were also observed for the WOMAC subscale for stiffness.

Correlations between self-reported stiffness and pain severity showed a strong relationship ($r=0.80$, $p<.001$). The association was confirmed by logistic regression which showed that the odds of knee pain were increased 25 fold in those self-reporting moderate knee joint stiffness compared to those reporting none or mild stiffness, after adjusting for age, sex, BMI and radiographic OA.

A passive pendulum test was used to calculate 2 biomechanical measures of joint stiffness: a stiffness co-efficient and a damping co-efficient. However a large number of participants were unable to perform the passive pendulum test (n=89, 36.6%). Valid testing was most successful in control participants where 70% were able to complete the test. Smaller numbers of participants with knee pain (n=34, 58%), ROA (n=21, 66%) and SOA (n=36, 58%) completed the testing successfully but this was not statistically different. Where valid testing was conducted the reliability of the testing was shown to be very

high (ICC >0.85) and only those participants were included in the analysis.

Stiffness co-efficients were significantly higher in the SOA group compared to controls, but there were no other statistical differences between groups. Damping and stiffness co-efficients were not associated with knee pain, though pain severity had a modest correlation with biomechanical stiffness in the SOA group ($r=0.39$, $p=.02$). Change in pain VAS scores over time did not however correlate with change in stiffness co-efficients.

Radiographic severity was weakly correlated with self-reported stiffness ($r= 0.23$, $p<0.01$) and stiffness co-efficients ($r= 0.19$, $p<0.05$). Additionally correlations between self-reported stiffness and stiffness and damping co-efficients were weak indicating that they may not be measuring the same thing.

This poses an interesting question, that if biomechanical measures of joint stiffness show only a weak relationship with knee pain and radiographic OA at best, what are the factors which contribute to biomechanically measures of joint stiffness? US and clinical features were explored using correlations to identify possible explanatory variables.

The variance observed in the stiffness co-efficients was largely explained by BMI and gender, local factors such as radiographic severity and knee pain did not contribute significantly. Synovial hypertrophy contributed to about 1% of the overall variance in line with

previous estimates by Wright and Johns (1960). Caution must be applied when interpreting these results, given the number of participants who were unable to successfully complete the test, its validity as a clinically useful test is questionable.

4.4 Study caveats

There are several caveats to this study which need to be considered when interpreting the reported findings.

Recruitment to the study was not random; participants were purposefully recruited to each study group with the aim of comparing four groups with a balanced number of 50 participants. Participants were drawn from previous community studies of knee pain for whom a variable amount of time (between 3 and 10 years) had passed between participation in the original and current study (Limer, Tosh et al. 2009; Doherty, Hawkey et al. 2011; Ingham, Zhang et al. 2011). As such, previous radiographic and pain status could not predict current x-ray and pain status i.e. it would be expected that there would an incidence of new radiographic OA and new knee pain, as well as knee pain having resolved in others. Consequently, recruitment to each group was unbalanced, with the ROA group under represented and SOA group over represented in the final analysis. Estimates suggest that the prevalence of ROA lies between 27-44% of the population (Felson, Naimark et al. 1987; Dillon, Rasch et al. 2006; Jordan, Helmick et al. 2007) but identifying those participants is inherently difficult as they are

asymptomatic and require radiographs to confirm their status. Recruitment was ceased after all previously identified ROA participants had been approached, and 183 participants with and without ROA were screened for knee pain, in order to prevent further over recruiting of control and SOA participants.

Recruited participants were largely representative of their original studies in terms of age though slightly more females than males were recruited, and participants in the KP only group were younger than other groups by around 15 years.

The population for the study was drawn from individuals from the community which limited the comparison that could be made with hospital-based studies. This was most obvious when comparing the results to the findings of D'Agostino et al (2005) where the hospital-based sample was younger, has less severe radiographic changes but reported higher levels of pain.

Comparison with other studies is also limited by variations in definitions of US pathology and protocols for scanning and scoring US features. This study used the definitions developed by the OMERACT ultrasonography and current EULAR guidance which is leading the way in terms of developing US as an imaging tool that is useful for both clinical trials and clinical practice. It is hoped that in utilising these definitions and guidelines that direct comparison with future studies will be easier.

The allocation of study participants to the four comparison groups was not without problems, notably the Kellgren & Lawrence grading to classify participants as controls or cases. It has been suggested that studies should classify K&L grade 1 as a case rather than a control as it has been shown that progression to definite OA (K&L ≥Grade 2) occurs in 62% of women graded K&L 1 at baseline compared with only 22% of controls with K&L Grade 0 (Hart and Spector 2003). Because this study was concerned with the prevalence and association of US features with current symptoms and radiographic features participants with K&L grade 1 were allocated to the control group.

The allocation to study groups was also challenging with regards to the presence of knee pain. The issue of screening for pain in knee OA is old but one that still causes difficulties for researchers especially when severity of pain is also considered. Most studies of knee pain require symptoms on most days of the previous month and a VAS score of ≥3cm on a 10 scale. Initial screening of patients in this study found that participants would describe infrequent episodes of moderate knee pain (usually related to specific activities) or regular pain (on most days of the last month) but of very low severity when scored on the VAS. Not wanting to exclude these participants from the analysis, classification of knee pain used a method previously reported in an MRI study of synovitis and knee pain (Baker, Grainger et al. 2010). Those reporting at least moderate knee pain in any of the five questions within the pain subscale were classified as pain positive. Those reporting none or mild pain were classified as pain negative.

The consequence of this is that the comparison groups are not completely “clean” and that some control and ROA participants have mild or infrequent knee pain and the control and KP groups have some participants with K&L grade 1 structural change.

Outcomes from the secondary analysis should be interpreted with caution as the analysis was undertaken irrespective of the study design. For example, the association between pain and US features in all 243 participants was assembled from 4 different groups: control, knee pain, radiographic knee OA and symptomatic knee OA. The association from such mixed groups may not be representative to the true association in the general population.

A further limitation of the analysis of this study was the use of an overall pain VAS score which could only be attributed to the index knee. A discrete pain score for each knee would have allowed a separate analysis of US features with respect to pain to be carried out in each knee.

4.5 Future research & Conclusions

This study represents a significant contribution to the current research on the prevalence of US features of inflammation and its association with pain and radiographic features. Within the community, US detected effusion and synovial hypertrophy were significantly more common and profuse in those with knee pain, ROA and SOA. Popliteal cysts were more frequent and larger in those with OA regardles of pain.

The findings confirm that US detected synovial hypertrophy is an independent predictor of knee pain, conferring over a six-fold increase in the risk of pain but highlights the stronger associations between greyscale features and the presence of ROA. PD signal which was most common observed in SOA knees was not found to be associated with pain. These findings may be a reflection of the community nature of the participants who have not yet sought secondary care for their knee symptoms and may differ in hospital population.

However, the nature of these findings question the general assumption that US features are “inflammatory” in nature and are responsible for driving pain in OA, and suggest rather that they are part of the overall structural pathology.

Further research still needs to be carried out before ultrasound findings can be used as a primary outcome to measure clinical response or guide clinical decisions.

Longitudinal observational studies are required to demonstrate temporal changes and associations between US features and the development and progression of radiographic OA and symptoms. This has been carried out with some success in MRI studies (Felson 2011) and the incorporation of US imaging into a sub-group within an MRI study would have the additional benefits of demonstrating external validity and allow bony MRI lesions (which strongly associate with symptoms) to be considered in the analysis. Additional analysis of biochemical biomarkers of inflammation may help substantiate or refute any association between US features and inflammation.

Whether US measures are sensitive to flares in pain is yet to be determined. MRI studies have shown that effusion and synovial hypertrophy may be sensitive to increasing, but not decreasing knee pain (Zhang, Nevitt et al. 2011). The follow-up period for this study was limited to 3 months due to time restrictions on the study. Replication in a larger sample over a longer time period may show greater fluctuations of pain over time which can address this question.

The prognostic value of US in knee OA remains uncertain. US features were not found to be predictors of response to intra-articular steroid injection by this and previous studies (Pendleton, Millar et al. 2008) and for longer term-prognosis only effusion was found to be an independent predictor of total knee replacement (Conaghan, D'Agostino et al. 2009). These results have been based on the dichotomous presence or absence of US findings (themselves based on arbitrary cut-off

measures). The development and validation of joint based scoring systems may better facilitate this and other studies. An adequately powered study, which also addresses optimal doses of cortico-steroid would help clarify if US features exhibit change following a cortico-steroid or are prognostic indicator to response.

In conclusion, this cross-sectional study has shown that grey-scale US features which are considered of be suggestive of inflammation are not unique to those with painful knee OA but are also frequently found in those with ROA. PD signal which is generally regarded as a surrogate for inflammation was significantly higher in SOA but was not independently associated with pain. US detected synovial hypertrophy is an important predictive factor of knee and longitudinal studies are required to better understand its contribution to the pain in OA.

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6 APPENDIX 1

Participant information sheets

Consent forms

Ethical approval letters

Ultrasound detected inflammation in knee joints

Chief Investigator: Prof Michael Doherty,
Michelle Hall, Dr Weiya Zhang ,

Participant Information Sheet

Version 5.0 24/11/2010

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish. Ask us if there is anything that is not clear or if you would like further information. Take time to decide whether or not you wish to take part.

Background to the study

Knee pain and knee OA are both very common in older people and often associate with disability. X-rays have been the most common method of assessing the knee joints in people with OA. However x-rays only show changes to the bony structure of the joint, and changes to soft tissues of the joint (such as the joint lining or "synovium") may also contribute to knee pain, stiffness and the loss of physical function.

Ultrasound scanning is a relatively new way of examining the soft tissues of the joint. It allows the measurement of increased joint fluid or "effusion", thickening of the joint lining, and increased blood flow within the synovium – all of which reflect joint inflammation. Ultrasound also can show changes in ligaments and tendons, the thickness of cartilage and the presence of joint osteophytes (bony outgrowths).

Inflammation of the joint lining has been found in about half the people with painful OA who are under hospital care for their knee OA. However only people with marked symptoms and severe OA tend to get referred to hospital clinics, and we have no information on how common inflammation is present in the broader spectrum of knee pain and OA present in the community.

Also, it is not clear whether inflammation only occurs in knees with OA and whether this inflammation contributes directly to pain, stiffness or impaired function of the knee. Nevertheless, it has been suggested that people who show inflammation may develop more severe and progressive OA and experience greater pain and disability. The presence of inflammation therefore could be important in terms of diagnosis and management of knee pain and OA.

In this current study we will be using Ultrasound to measure features of inflammation in the knees of 200 people some of whom will have OA

and or Knee pain and some without to compare groups and re-testing some groups and individuals to see how these features change.
We hope that the results of this study will lead to a better understanding of role of inflammation in people with knee pain and knee OA, and how useful Ultrasound is in detecting signs of inflammation.

Why have I been chosen?
We are asking you to take part because we know from the investigations that were undertaken on you in the past that you are suitable for inclusion into this new study, and that you have agreed to for us to keep your details on a database and contact you for this reason. We will need to recruit approximately 200 people in total.

However if any of the following apply to you, then you may not be suitable for the study.

- You have been diagnosed with an inflammatory arthritis e.g. rheumatoid arthritis
- You have been diagnosed with osteoarthritis in the Hip
- You have had a steroid injection in either of your knees in the past 3 months
- You have had a knee joint replacement
- You have a neurological condition eg a Stroke or Parkinsons disease
- You have widespread body pain
- You are confined to a wheel chair

If you are unsure if you are suitable for the study for any other reason, please ring us so we can discuss it with you.

Do I have to take part?
You should only take part in this study if you want to. It is entirely up to you whether or not you wish to take part. If you do, you will be given this information sheet to keep and be asked to sign a consent form. You are free to withdraw at any time, without having to give a reason. This in no way will influence the medical care you receive at the Nottingham University Hospitals NHS Trust.

What does the study involve?
If you return your reply slip indicating that you are happy to take part in this study, you will be contacted by telephone by a researcher to arrange an appointment to see you. You will be given an initial appointment to see Michelle Hall in Academic Rheumatology at the City Hospital which will last around 3.5 hours. We can arrange for prepaid taxi transport to bring you to the City Hospital or we can reimburse your travel expenses incurred as a result of participating.

You will be asked to **not** take any anti-inflammatory medication e.g. “Neurofen”, “Ibuprofen” or “Asprin” for 48 hours prior to attending. You make take Paracetamol if needed the night before your appointment,

but not on the morning itself. You should bring a list of your current medication with you.

You will have a chance to further discuss the study and before you are asked to sign and date a consent form and this will be stored in a secure office within Academic Rheumatology. You will also receive a copy of the consent form and the information sheet for your own records.

During your visit you will have the following assessments taken:

- X-rays of your both your knees (unless you have had previous x-rays taken in the past year)
- You will be asked about any knee pain or symptoms that you might have, and complete 2 questionnaires about the pain that you may have in your knee(s) and your ability to carry out day to day activities.
- Your knees will be examined for swelling and stability of the ligaments, the strength of your thigh muscles will be measured and your height and weight measured.
- An Ultrasound scan of both your knees will be taken
- Knee joint stiffness will be measured by a special camera which can track the movement of your knee during a swinging motion. This is a system of cameras which track the movement of infra-red markers attached to the individual's leg using tape. The system does not video or take images of the individual themselves. You will need to wear loose fitting shorts for this.
- You will carry out a physical performance test where you will be timed while you get up from a chair and walk 50 feet, and while you get up from a chair, walk 3 meters, turn around, walk back and sit down.

Depending on the outcome of these tests you may also be asked to attend for a 3 months later (this repeat assessment will not include x-rays). In addition if you experience a significant change in your knee symptoms, either an increase or decrease in your knee pain that lasts 3 days or more, in the next 3 months then we would like you to contact us so we can repeat the Ultrasound scan and tests on your knees.

As Ultrasound is a relatively new way of assessing OA we need to evaluate how reliable it is. To do this some participants will undergo repeat ultrasound testing in the afternoon following their initial assessment. This will be repeated (morning and afternoon) on a further 2 days, one week apart.
Some participants will also be asked to return at 3 days where they will have a repeat ultrasound scan carried out by an expert radiologist.

What are the side effects of any treatment or procedures received when taking part?
There are no side-effects associated with this study.

This study involves radiation exposure from a knee x-ray. As part of everyday living, everyone is exposed to small amounts of background radiation that comes from soil, rocks and outer space (3mSv each year). The absorbed radiation dose you will receive in this study (0.003mSv) is about the amount you receive in less than one day from background radiation. This risk from this dose is small. The radiation exposure is not necessary for your medical care but is necessary to obtain the research information required.

What are the possible disadvantages and risks of taking part?
There are no disadvantages or risks to taking part in this study.

What are the potential benefits of taking part in this study?
There is no direct benefit to you in taking part in this study. However, the information we obtain might help improve the treatment of people with knee OA and knee pain in the future.

Will my taking part in this study be kept confidential?
Your GP will be informed of your participation in this study and the results of any x-rays taken.
Your details will be kept strictly confidential and you will be given a unique identification number if you agree to participate in this study. Your personal and medical details will be linked to this number, but this link will be held in a secure file within Academic Rheumatology and will only be accessed by study personnel. The x-rays of your knees will be stored electronically on the City Hospital's system (PACS). These images would be available for review for clinical purposes in the future by other doctors and clinicians based at the City Hospital and QMC Campus.

What will happen if I don't want to carry on with the study?
Your participation in the study is voluntary and you are free to withdraw at any time, without giving reason, and without your legal rights being affected. If you withdraw the information collected so far cannot be erased and this information may still be used in the project analysis.

What will happen to my information once the study has finished?
All data will remain confidential and only be linked by your unique ID number. It will be stored within Academic Rheumatology, Clinical Sciences Building, Nottingham City Hospital. Only study personnel will have access to this information.

Who has reviewed the study?
All medical research is looked at by an independent group of people called a Research Ethics Committee to protect your interests. This study has been reviewed and given a favourable opinion by Derbyshire Research Ethics Committee.

Will I be paid for participation in this study?
Participation in this study is entirely voluntary. You will receive no payment for your participation. However we will reimburse the travel expenses you incur as a result of visiting the City Hospital to participate in the study.

Who is organising and funding the research?
This study is being organized by Academic Rheumatology, who are a department of the University of Nottingham and who are based at the City Hospital.
The study is being funded by the Arthritis Research Campaign UK, a charity who gives grants for research into arthritis.

What will happen to the results of the study?
We hope that the results of this study will lead to a better understanding of role of inflammation in people with knee pain and knee OA, and how useful Ultrasound is in detecting signs of inflammation. Results from the study will be published in scientific and medical journals.

Contact for Further Information
If you have any concerns or questions about any aspect of the study, you should ask to speak to Michelle Hall, who will do her best to answer your questions (telephone number 0115-8231761).

What if there is a problem?
If you are unhappy or wish to complain about any aspect of this study, you should ask to speak to Professor Michael Doherty, Chief Investigator, who will do his best to answer your questions.

University of Nottingham,
Division of Academic Rheumatology
Clinical Sciences Building
Nottingham City Hospital
Nottingham
NG5 1PB
Tel 0115 8231756

If you remain unhappy, and wish to complain formally, the Patient Advice and Liaison Service provide a confidential service and can advise you regarding the NHS Complaints Procedure.

NUH NHS Trust
c/o PALS
Freepost
NEA 14614
Nottingham
NG7 1BR
Freephone: 0800 183 0204

Thank you for taking the time to read this information sheet.

The University of Nottingham

CONSENT FORM
(Version 5.0 24/11/2010)

Ultrasound detected inflammation in knee joints

Chief Investigator: Prof Michael Doherty Academic Rheumatology
Michelle Hall, Prof Weiya Zhang

REC ref: 09/H0401/83

Please initial box

Name of Participant:

1. I confirm that I have read and understand the information sheet *Version 5.0 dated 24/11/2010* for the above study and have had the opportunity to ask questions. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, and without my medical care or legal rights being affected. I understand that should I withdraw then the information collected so far cannot be erased and that this information may still be used in the project analysis. ☐

3. I understand that relevant sections of my medical notes and data collected in the study may be looked at by authorised individuals from the University of Nottingham, the research group and regulatory authorities where it is relevant to my taking part in this study. I give permission for these individuals to have access to these records and to collect, store, analyse and publish information obtained from my participation in this study. I understand that my personal details will be kept confidential. ☐

4. I understand and agree that an x-ray of both my knees at Nottingham University Hospital NHS Trust (City Hospital) will be taken to determine the severity of osteoarthritis in the joints. ☐

5. I understand and agree that an Ultrasound scan of both my knees will be taken to evaluate inflammation in the joints. ☐

6. I understand that my details will be kept on a database in Academic Rheumatology so that I may be contacted for future studies (optional). ☐

7. I agree to take part in the above study. ☐

Name of Participant	**Date**	**Signature**
Name of Person taking consent	**Date**	**Signature**

NHS
National Research Ethics Service
Derbyshire Research Ethics Committee
The Old Chapel
Royal Standard Place
Nottingham
NG1 6FS

Tel: 0115 8839435
Fax: 0115 9123300

26 November 2010

Professor Michael Doherty
Professor of Academic Rheumatology
Academic Rheumatology
Clinical sciences Building
Nottingham City Hospital
NG5 1PB

Dear Professor Doherty

Study title:	**Ultrasound detected inflammation in knees of community-derived individuals, and its possible relationship to radiographic knee osteoarthritis (ROA), knee pain (KP), joint stiffness and physical function.**
REC reference:	**09/H0401/83**
Amendment number:	**4**
Amendment date:	**18 October 2010**

The above amendment was reviewed at the meeting of the Sub-Committee held on 16 November 2010.

Ethical opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

Document	Version	Date
Participant Consent Form	5.0	24 November 2010
Participant Information Sheet	5.0	24 November 2010
Protocol	4.0	18 October 2010
Notice of Substantial Amendment (non-CTIMPs)	4	18 October 2010
Covering Letter		20 October 2010

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

R&D approval

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval of the research.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

09/H0401/83:	Please quote this number on all correspondence

Yours sincerely

pp Lisa Gregory

Mr Phil Hopkinson
Chair

E-mail: lisa.gregory@nottspct.nhs.uk

Enclosures: *List of names and professions of members who took part in the review*

Copy to: *Mr Paul Cartledge, University of Nottingham*
R&D office for NHS care organisation at lead site - NUH

Response of knee pain and Ultrasound findings following intra-articular injection in painful knee osteoarthritis.

Chief Investigator: Prof Michael Doherty,
Sally Doherty, Michelle Hall, Dr Weiya Zhang .

Participant Information Sheet
Version 6.0 10/05/2011

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish. Ask us if there is anything that is not clear or if you would like further information. Take time to decide whether or not you wish to take part.

Background to the study
Painful knee osteoarthritis (OA) is a very common and disabling condition. X-rays are the usual way of assessing the knee joints in OA but the pain people experience is not always matched by the severity of the disease on x-ray. We know from previous studies and our ongoing Ultrasound study, which you kindly participated in, that people with painful knee OA often have swelling and synovitis (inflammation of synovium or joint lining), which may contribute to the severity of knee pain and disability experienced. For the next phase of this study we are looking at the effects of two different intra-articular injections on knee pain and ultrasound findings in people with painful knee osteoarthritis.

Intra-articular injections are injections into a joint. Corticosteroid injections are commonly given to reduce inflammation, swelling and pain within a joint. The injection should give relief within a few days and may last for 2 months or longer. Corticosteroids are thought to work by acting on synovium so may reduce the swelling and synovitis seen on Ultrasound. Saline (placebo) injections can also significantly relieve pain in knee OA but they do not contain an active ingredient and so are unlikely to have an effect on the synovium.

The study is a randomised cross-over study which means that you will receive both the corticosteroid injection and the saline injection in turn, but in a random order. Neither you nor the ultrasound assessor will know which order you receive the injections (though we can find out if we need to). This is because sometimes if participants or the research team know the order of the treatments it may affect the results.

Why have I been chosen?
We are asking you to take part because we know from the investigations in the Ultrasound study that you have painful knee OA and are suitable, and that you have agreed to for us to contact you to

take part in further research. However if any of the following apply to you, then you may not be suitable for this part of the study.

- You have had a steroid injection in either of your knees in the past 3 months
- You have had any knee joint surgery in the past 3 months
- You have had a knee injury in the past 3 months

If you are unsure if you are suitable for the study for any other reason, please ring us so we can discuss it with you.

Do I have to take part?
You should only take part in this study if you want to. It is entirely up to you whether or not you wish to take part. If you do, you will be given this information sheet to keep and be asked to sign a consent form. You are free to withdraw at any time, without having to give a reason. This in no way will influence the medical care you receive at the Nottingham University Hospitals NHS Trust.

What does the study involve?
If you return your reply slip indicating that you are happy to take part in this study, you will be contacted by telephone to arrange an appointment with the research team in Academic Rheumatology at the City Hospital.

You will have a chance to further discuss the study before you are asked to sign and date a consent form. This will be stored in a secure office within Academic Rheumatology. You will also receive a copy of the consent form and the information sheet for your own records.

During your initial visit you will be asked about your current knee symptoms, pain severity and current medication. You will be asked to identify which knee is the most painful. We will then ask you to complete two questionnaires about your knee pain and an Ultrasound examination of both your knees will be carried out.

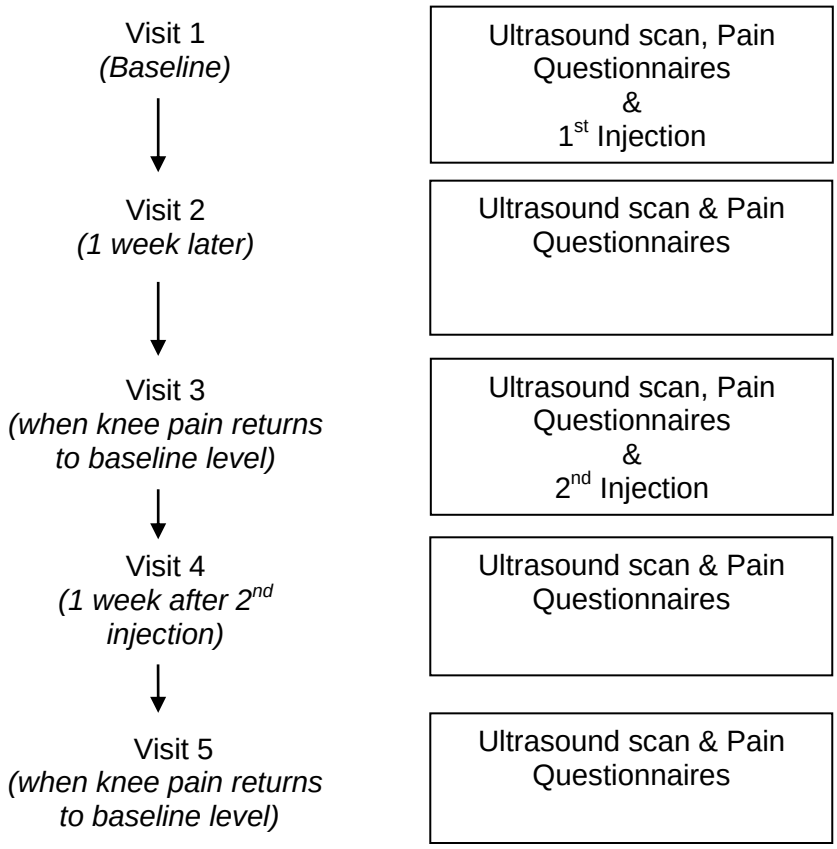
You will then receive an injection to your most painful knee from Prof Doherty. If neither knee is more painful than the other, a knee will be randomly chosen (this is like tossing a coin). The injection you receive will either contain 1ml (40mg) of a corticosteroid called methyl prednisolone or 1ml of 0.9% saline. The order of the injections will be randomised and you will not know which injection you receive and neither will the person scanning your knee.

You will return for a follow-up visit one week after the initial injection and again when your pain severity returns close to the level before your injection. This may be several weeks later so we will arrange to telephone you on a weekly basis to monitor this.

You will then receive a second injection, and return for a follow-up visit one week after the injection and again when your pain severity returns close to your baseline level.

Each visit should last no longer than 1½ hours. There are five visits in total which may be spread over three to six months depending on how long your pain-relief lasts.

The following flow chart shows the pathway of the study.



Following each injection you can continue with your normal day to day activities. You may continue to take your usual knee pain medication if required and you should continue with any other usual medication.

You will be asked to **not** take any anti-inflammatory medication e.g. “Neurofen”, “Ibuprofen” or “Aspirin” for 48 hours before each assessment. You may take Paracetamol if needed the night before your appointment, but not on the morning itself.

What are the side effects of any treatment or procedures received when taking part?
Side-effects are very unlikely but occasionally people notice a worsening in their knee pain within the first 24 hours of the injection. This usually settles with a couple of days without treatment.

Facial flushing may occur in small number of people.
Some people can feel faint when having an injection, but these will be given on a reclined couch by a trained medical doctor.
There is a negligible (very rare) risk of infection being introduced to the joint at the time of an injection. This is the same risk as giving a blood sample. The injection will be given using an aseptic (sterile) technique to minimise this risk.
There are no contra-indications for either the cortico-steroid or saline injections. You may take other medicines along with both.

What are the possible disadvantages and risks of taking part?
There are no disadvantages or risks to taking part in this study.

What are the potential benefits of taking part in this study?
Both the steroid and saline injection are likely to improve your knee pain.

Will my taking part in this study be kept confidential?
Your GP will be informed of your participation in this study.
Your details will be kept strictly confidential and you will be given a unique identification number if you agree to participate in this study. Your personal and medical details will be linked to this number, but this link will be held in a secure file within Academic Rheumatology and will only be accessed by study personnel.

What will happen if I don't want to carry on with the study?
Your participation in the study is voluntary and you are free to withdraw at any time, without giving reason, and without your legal rights being affected. If you withdraw the information collected so far cannot be erased and this information may still be used in the project analysis.

What will happen to my information once the study has finished?
All data will remain confidential and only be linked by your unique ID number. It will be stored within Academic Rheumatology, Clinical Sciences Building, Nottingham City Hospital. Only study personnel will have access to this information.

Who has reviewed the study?
All medical research is looked at by an independent group of people called a Research Ethics Committee to protect your interests. This study has been reviewed and given a favourable opinion by Derbyshire Research Ethics Committee.

Will I be paid for participation in this study?
Participation in this study is entirely voluntary. You will receive no payment for your participation. However we will reimburse the travel expenses you incur as a result of visiting the City Hospital to participate in the study.

Who is organising and funding the research?
This study is being organised by Academic Rheumatology, who are a department of the University of Nottingham and who are based at the City Hospital.
The study is being funded by Arthritis Research UK, a charity who gives grants for research into arthritis.

What will happen to the results of the study?
We hope that the results of this study will lead to a better understanding of role of inflammation in people with knee pain and knee OA, and how useful Ultrasound is in monitoring signs of inflammation after intra-articular injections. Results from the study will be published in scientific and medical journals.

Contact for Further Information

If you have any concerns or questions about any aspect of the study, you should ask to speak to Michelle Hall, who will do her best to answer your questions (telephone number 0115-8231761).

What if there is a problem?
If you are unhappy or wish to complain about any aspect of this study, you should ask to speak to Professor Michael Doherty, Chief Investigator, who will do his best to answer your questions.

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Division of Academic Rheumatology
Clinical Sciences Building
Nottingham City Hospital
Nottingham
NG5 1PB
Tel 0115 8231756

If you remain unhappy, and wish to complain formally, the Patient Advice and Liaison Service provide a confidential service and can advise you regarding the NHS Complaints Procedure.

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NEA 14614
Nottingham
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Freephone: 0800 183 0204

Thank you for taking the time to read this information sheet.

The University of Nottingham

CONSENT FORM
(Version 6.0 10/05/2011)

Response of knee pain and Ultrasound findings following intra-articular injection in painful knee osteoarthritis
REC ref: 09/H0401/83

Chief Investigator: Prof Michael Doherty,
Sally Doherty, Michelle Hall, Dr Weiya Zhang .

Name of Participant: **Please initial box**

1. I confirm that I have read and understand the information sheet *Version 6.0 dated 10/05/2011* for the above study and have had the opportunity to ask questions. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, and without my medical care or legal rights being affected. I understand that should I withdraw then the information collected so far cannot be erased and that this information may still be used in the project analysis. ☐

3. I understand that relevant sections of my medical notes and data collected in the study may be looked at by authorised individuals from the University of Nottingham, the research group and regulatory authorities where it is relevant to my taking part in this study. I give permission for these individuals to have access to these records and to collect, store, analyse and publish information obtained from my participation in this study. I understand that my personal details will be kept confidential. ☐

4. I understand and agree that an Ultrasound scan of both my knees will be taken to evaluate inflammation in the joints. ☐

5. I understand and agree that I will receive two separate intra-articular injections of a corticosteroid and a saline placebo in a random order. ☐

6. I understand that my details will be kept on a database in Academic Rheumatology so that I may be contacted for future studies (optional). ☐

7. I agree to take part in the above study. ☐

Name of Participant **Date** **Signature**

Name of Person taking consent **Date** **Signature**

NHS
National Research Ethics Service

NRES Committee East Midlands - Derby 2
The Old Chapel
Royal Standard Place
Nottingham
NG1 6FS

Tel: 0115 8839435
Fax: 0115 9123300

16 May 2011

Professor Michael Doherty
Professor of Academic Rheumatology
University of Nottingham
Academic Rheumatology
Clinical sciences Building
Nottingham City Hospital
NG5 1PB

Dear Professor Doherty

Study title:	**Ultrasound detected inflammation in knees of community-derived individuals, and its possible relationship to radiographic knee osteoarthritis (ROA), knee pain (KP), joint stiffness and physical function.**
REC reference:	**09/H0401/83**
Protocol number:	**09070**
Amendment date:	**11 May 2011**

The above amendment was reviewed by the Sub-Committee in correspondence.

Ethical opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

Document	Version	Date
Additional participant Reminder Letter	2.0	10 May 2011
Additional Participant Letter	2.0	10 May 2011
Additional Consent Form	6.0	10 May 2011
Additional Participant Information Sheet	6.0	10 May 2011
List of Changes - added text		10 May 2011
Protocol	5.0	10 May 2011
Notice of Substantial Amendment (non-CTIMPs)		

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

R&D approval

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval of the research.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

09/H0401/83:	Please quote this number on all correspondence

Yours sincerely

pp. JR Lea

Mr Phil Hopkinson
Chair

E-mail: jennifer.lea@nottspct.nhs.uk

Enclosures: *List of names and professions of members who took part in the review*

Copy to:

Mrs Michelle Hall
PhD Student
University of Nottingham
Academic Rheumatology
Clinical sciences Building
Nottingham City Hospital
NG5 1PB

Mrs Sheila O'Malley
Lead R&D Manager
Nottingham University Hospital NHS Trust
C12 Curie Court, QMC Campus
Derby Road, Nottingham NG7 2UH

Mr Paul Cartledge, Nottingham University

7 APPENDIX 2

Study questionnaires

WOMAC OSTEOARTHRITIS INDEX

SECTION 1: PAIN

The following questions concern the amount of pain you have experienced in your knees over the last week. *(Please tick one box for each item)*

How much pain do you have?

		None	Mild	Moderate	Severe	Extreme
1	Walking on a flat surface	☐	☐	☐	☐	☐
2	Going up or down stairs	☐	☐	☐	☐	☐
3	At night while in bed	☐	☐	☐	☐	☐
4	Sitting or Lying	☐	☐	☐	☐	☐
5	Standing upright	☐	☐	☐	☐	☐

SECTION 2: STIFFNESS

The following questions concern the amount of stiffness (not pain) you have experienced in your knees over the last week. Stiffness is a sensation of restriction or slowness in the ease with which you move your joints. *(Please tick one box for each item)*

6. How severe is your stiffness **after first waking** in the morning?

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

7. How severe is your stiffness after sitting, lying or resting **later in the day**?

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

SECTION 3: PHYSICAL FUNCTION

The following questions concern your physical function. By this we mean your ability to move around and look after your self. For each of the following activities, please indicate the degree of difficulty you have experienced over the last week due to problems with your knees. *(Please tick one box for each item)*

What degree of difficulty do you have with:

		None	Mild	Moderate	Severe	Extreme
8.	Descending stairs	☐	☐	☐	☐	☐
9.	Ascending stairs	☐	☐	☐	☐	☐
10.	Rising from sitting	☐	☐	☐	☐	☐
11.	Standing	☐	☐	☐	☐	☐
12.	Bending to the floor	☐	☐	☐	☐	☐
13.	Walking on the flat	☐	☐	☐	☐	☐
14.	Getting in/out of car	☐	☐	☐	☐	☐
15.	Going shopping	☐	☐	☐	☐	☐
16.	Putting on socks/stockings	☐	☐	☐	☐	☐
17.	Rising from bed	☐	☐	☐	☐	☐
18.	Taking off socks/stockings	☐	☐	☐	☐	☐
19.	Lying in bed	☐	☐	☐	☐	☐
20.	Getting in/out bath	☐	☐	☐	☐	☐

	None	Mild	Moderate	Severe	Extreme
21. Sitting	☐	☐	☐	☐	☐
22. Getting on/off toilet	☐	☐	☐	☐	☐
23. Heavy domestic duties	☐	☐	☐	☐	☐
24. Light domestic duties	☐	☐	☐	☐	☐

A Measure of Intermittent and Constant Osteoarthritis Pain, ICOAP: KNEE Version

People have told us that they experience different kinds of pain (including aching or discomfort) in their knee. To get a better sense of the different types of knee pain you may experience, we would like to ask you about any "constant pain" (pain you have all the time) separately from any pain that you may experience less often, that is, "pain that comes and goes". The following questions will ask you about the pain that you have experienced in your knee in the PAST WEEK. Please answer ALL questions.

A) CONSTANT PAIN

For each of the following questions, please select the response that best describes, on average, your *constant knee pain* in the PAST WEEK.

1. **In the past week, how intense has your *constant knee pain* been?**

☐0	☐1	☐2	☐3	☐4
Not at all/ No constant knee pain	Mildly	Moderately	Severely	Extremely

2. **In the past week, how much has your *constant knee pain* affected your sleep?**

☐0	☐1	☐2	☐3	☐4
Not at all/ No constant knee pain	Mildly	Moderately	Severely	Extremely

3. **In the past week, how much has your *constant knee pain* affected your overall quality of life?**

☐0	☐1	☐2	☐3	☐4
Not at all/ No constant knee pain	Mildly	Moderately	Severely	Extremely

4. **In the past week, how frustrated or annoyed have you been by your *constant knee pain?***

☐0	☐1	☐2	☐3	☐4
Not at all/ No constant knee pain	Mildly	Moderately	Severely	Extremely

5. **In the past week, how upset or worried have you been by your *constant knee pain*?**

☐0	☐1	☐2	☐3	☐4
Not at all/ No constant knee pain	Mildly	Moderately	Severely	Extremely

B) PAIN THAT COMES AND GOES

For each of the following questions, please select the response that best describes your *knee pain that comes and goes*, *on average*, in the PAST WEEK.

6. In the past week, how intense has your most severe *knee pain that comes and goes* been?

☐0	☐1	☐2	☐3	☐4
Not at all/ No knee pain that comes and goes	Mildly	Moderately	Severely	Extremely

7. In the past week, how frequently has this *knee pain that comes and goes* occurred?

☐0	☐1	☐2	☐3	☐4
Never/ No knee pain that comes and goes	Rarely	Sometimes	Often	Very Often

8. In the past week, how much has your *knee pain that comes and goes* affected your sleep?

☐0	☐1	☐2	☐3	☐4
Not at all/ No knee pain that comes and goes	Mildly	Moderately	Severely	Extremely

9. In the past week, how much has your *knee pain that comes and goes* affected your overall quality of life?

☐0	☐1	☐2	☐3	☐4
Not at all/ No knee pain that comes and goes	Mildly	Moderately	Severely	Extremely

10. In the past week, how frustrated or annoyed have you been by your *knee pain that comes and goes*?

☐0	☐1	☐2	☐3	☐4
Not at all/ No knee pain that comes and goes	Mildly	Moderately	Severely	Extremely

11. In the past week, how upset or worried have you been by your *knee pain that comes and goes*?

☐0	☐1	☐2	☐3	☐4
Not at all/ No knee pain that comes and goes	Mildly	Moderately	Severely	Extremely

THANK YOU!

Clinical History

Current Knee Pain status

"Have you ever had pain in or around the knee on most days for at least a month? **Y / N**

If so have you experienced any pain during the last year?" **Y / N**

Most symptomatic joint **L / R / Neither**

Knee Joint Stiffness

Do you experience knee joint stiffness first thing in the morning? **Y / N**

If so how long does it usually last? ___ minutes

Do you experience knee joint stiffness after rest during the day? **Y / N**

If so how long does it usually last? ___ minutes

Drugs History

What medication do you usually take?

How do you take medication for pain relief?

Every day	
When pain gets too bad	
Depends on how bad pain is	
Every day when having a bad patch, otherwise only as needed	
Before bed	
Before exercise	

When did you last take any NSAIDs drugs? _____

When did you last take any Paracetamol? _____

Clinical Examination

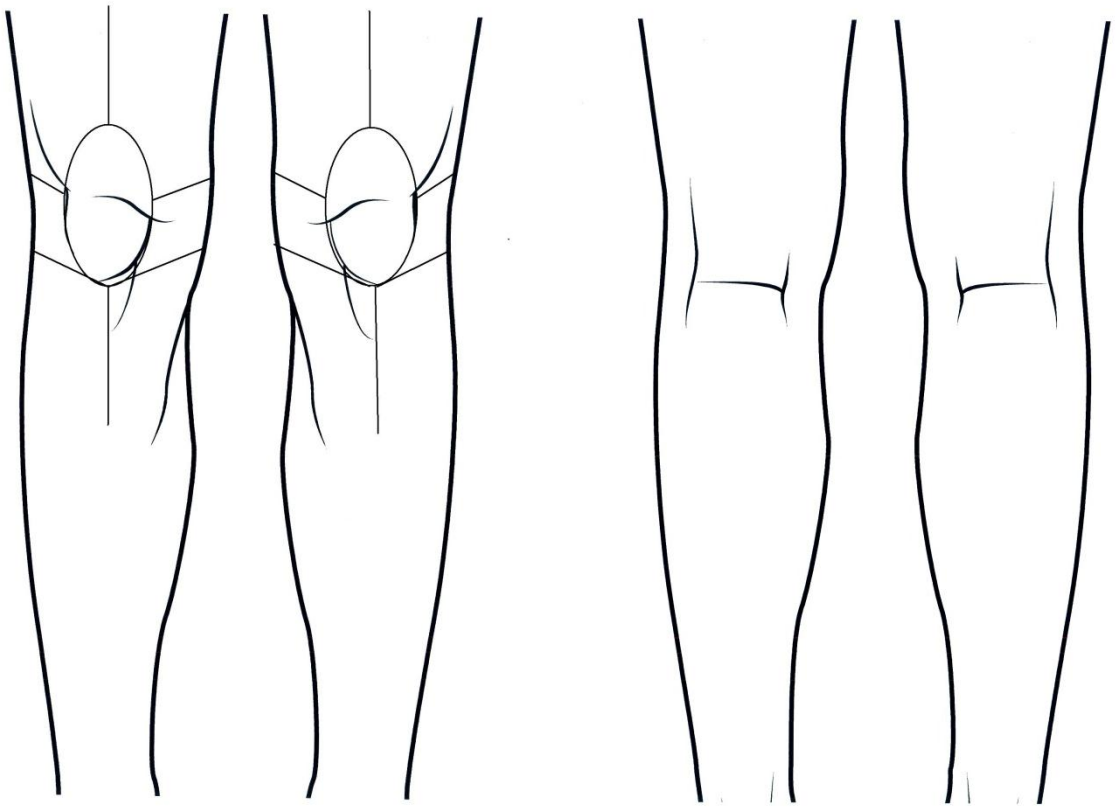
Height cm			
Weight kg			
		Left	Right
Joint effusion	zero/trace/1/2/3		
Warmth Y/N			
Clinical instability Y/N	ACL		
	PCL		
	MCL		
	LCL		
Deformity	Very bowlegged/bow legged/normal/knock-knee/very knock-knee		
ROM (degrees)	Ext		
	Flex		
Muscle strength (kg)	Quads		
	H/S		
Lower leg length		mm	mm

Functional performance

Get Up and Go (GUG) tests	On the command “Go” from a chair without arm rests, ask participant to stand up and walk as fast as they can to a mark **50 feet** from the chair. On the command “Go” from a chair without arm rests, ask participant to stand up and walk as to a mark **3m** from the chair, turn and return to the chair and sit down.	Secs Secs

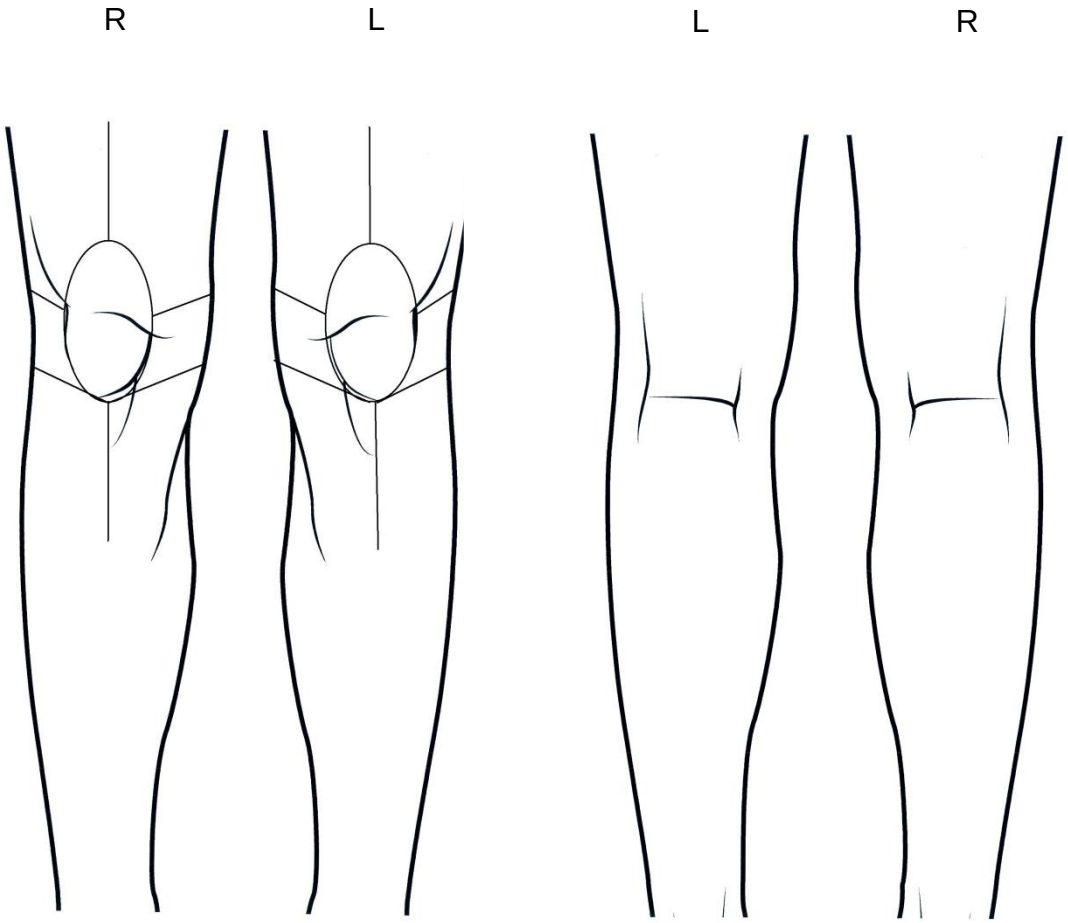
Knee Pain Map

R L L R



Pain	Right knee	Left knee
Localised Use of 1 or 2 fingers to point (patellar, superior-medial, inferior-medial, medial joint line, superior-lateral, inferior-lateral, lateral joint line, or back of knee).		
Regional Use of all fingers/hand to show(medial, lateral, patellar, or back of the knee).		
Diffuse unable to identify pain as localized or regional in nature. Pain all over.		

Knee Joint Tenderness



Pain VAS

Thinking about the pain you experience in your knee(s) over the past 48 hours, put an X on the line below where you think the severity of your pain lies.

If you put an X at the left hand end of the line you are indicating that you have no pain.

If you put an X at the right hand end of the line you are indicating that your pain is extreme.

NO PAIN EXTREME PAIN

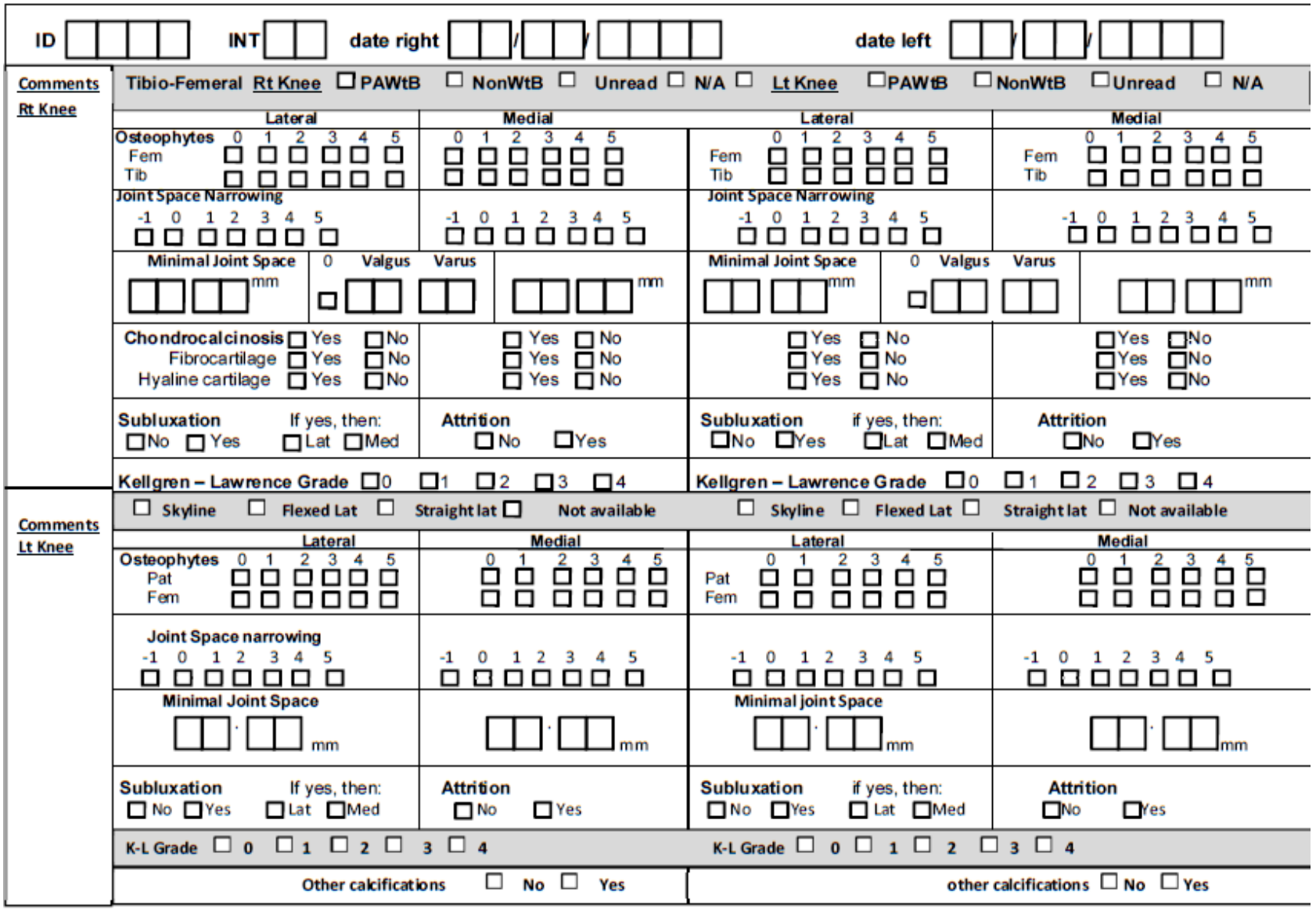
8 APPENDIX 3

Ultrasound Scanning proforma

Radiographic scoring sheet

US Scanning Proforma

	LEFT KNEE			RIGHT KNEE		
Suprapatellar						
Effusion	Absent <4mm	Present >4mm	mm	Absent <4mm	Present >4mm	mm
Synovial hypertrophy	Absent <4mm	Present >4mm	mm	Absent <4mm	Present >4mm	mm
Doppler	Absent 0 Mild 1 Moderate 2 Marked 3			Absent 0 Mild 1 Moderate 2 Marked 3		
Comments						
Medial joint line						
Osteophytes Femoral	Absent	Present	mm	Absent	Present	mm
Tibial	Absent	Present	mm	Absent	Present	mm
MCL thickness	mm			mm		
Doppler signal	Absent 0 Mild 1 Moderate 2 Marked 3			Absent 0 Mild 1 Moderate 2 Marked 3		
Comments						
Lateral joint Line						
Osteophytes Femoral	Absent	Present	mm	Absent	Present	mm
Tibial	Absent	Present	mm	Absent	Present	mm
LCL thickness	mm			mm		
Doppler signal	Absent 0 Mild 1 Moderate 2 Marked 3			Absent 0 Mild 1 Moderate 2 Marked 3		
Comments						
Infrapatellar						
Pat tendon thickness	mm			mm		
Bursitis	Absent	Present	mm	Absent	Present	mm
Pes Ans Bursitis	Absent	Present	mm	Absent	Present	mm
Doppler signal	Absent 0 Mild 1 Moderate 2 Marked 3			Absent 0 Mild 1 Moderate 2 Marked 3		
Comments						
Fem Cartilage						
Transverse view	medial mm		lateral mm	medial mm		lateral mm
Longitudinal view	medial mm		lateral mm	medial mm		lateral mm
Comments						
Posterior						
Popliteal cyst	Absent <4mm	Present >4mm	mm	Absent <4mm	Present >4mm	mm
Doppler signal	Absent 0 Mild 1 Moderate 2 Marked 3			Absent 0 Mild 1 Moderate 2 Marked 3		
Comments						


ID
INT
date right
date left
Comments Rt Knee
Tibio-Femeral Rt Knee PAWtB NonWtB Unread N/A Lt Knee PAWtB NonWtB Unread N/A
Lateral
Medial
Osteophytes 0 1 2 3 4 5
Fem
Tib
Joint Space Narrowing -1 0 1 2 3 4 5
Minimal Joint Space mm
0 Valgus Varus
Chondrocalcinosis Yes No
Fibrocartilage Yes No
Hyaline cartilage Yes No
Subluxation If yes, then: No Yes Lat Med
Attrition No Yes
Kellgren – Lawrence Grade 0 1 2 3 4
Skyline Flexed Lat Straight lat Not available
Comments Lt Knee
Pat
Fem
Joint Space narrowing
Minimal joint Space
K-L Grade 0 1 2 3 4
Other calcifications No Yes
other calcifications No Yes