School of Physiotherapy and Exercise Science

Determining diagnostic indicators for neuropathic pain in patients with osteoarthritis

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Declaration

To the best of my knowledge this thesis contains no material previously published by any person except where due acknowledgement has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

The research presented and reported in thesis was conducted in accordance with the National Health and Medical Council National Statement on Ethical Conduct in Human Research (2007) – updated March 2014. The proposed research study received human research ethics approval from Curtin University Human Research Ethics Committee (EC00262), Approval # 39/2016.

Farhat Bashir

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Abstract

Osteoarthritis (OA) is the most common joint disease, which affects millions of people worldwide. OA pain is heterogeneous in nature. Traditionally it was considered to occur as a result of peripheral nociceptive input. However, there is increasing evidence that some patients present with features of neuropathic pain (NP). At present there are no diagnostic tests available to make a definite diagnosis of neuropathic pain in patients with OA. Developing a better diagnostic procedure for OA patients who may be suffering neuropathic pain is important for early diagnosis and targeted treatment.

This research project aimed to determine if neuropathic pain and inflammatory pain can be distinctly demonstrated in patients with OA of the knee and help to develop diagnostic criteria for such patients.

The research included 99 participants with knee OA and 38 age matched pain free participants were recruited and clinically screened to ensure suitability for inclusion in the study. OA participants were comprehensively evaluated using questionnaires (PainDETECT, S-LANSS, PQAS, WOMAC, PCS, DASS, PSQI and CQ) related to pain and function, features of neuropathic pain, psychosocial state, sleep quality and comorbidity. Pain free participants also completed questionnaires related to psychosocial state, sleep quality and comorbidity. Participants with osteoarthritis digitally mapped their pain and additional sensations with their location around the knee. This was followed by a range of quantitative sensory tests (QST) which included sensory and pain function related to cold, heat, vibration, touch and pressure pain thresholds. In addition, measures of proprioceptive function, MRI and a range of biomarkers related to nerve injury and inflammation (CRP, IL1, IL6, TNF-α and NGF) were assessed.

OA participants were divided in to three groups. Those with PainDETECT score of < 13 were included in the inflammatory pain group. Participants with PainDETECT score of ≥ 13 and reporting additional sensations or demonstrating a sensory deficit were classified as possible neuropathic pain (PoNPG). Participants with a PainDETECT score of ≥ 19 who reported

additional sensations along with related sensory deficit in the same anatomical area (medial, lateral, or popliteal) were classified as the probable neuropathic pain group (PrNPG).

Results indicated that OA participants experience sensory and proprioceptive deficits and increased pain sensitivity relative to the control group. Almost half of the OA cohort showed vibration hypoesthesia and impaired proprioceptive function. OA participants suffered greater sleep disruption and psychological dysfunction. They also exhibited more comorbidities and had higher BMIs than the control group.

In the OA cohort, the membership of the IPG, PoNPG and PrNPG was 49%, 29% and 21%, respectively. PrNPG participants scored higher on the self-report NP questionnaires S-LANSS and PQAS. This group reported the greatest number of neuropathic symptoms on digital pain mapping. They also reported more severe pain, physical disability, compromised sleep quality and psychosocial measures. Assessment of sensory functions revealed the highest number of related sensory deficits in this group. Participants in the PoNPG also reported NP symptoms and had some sensory deficits. Most of the sensory and pain measures were not different between the three OA groups, except vibration and tactile threshold and cold pain threshold. There were more severe structural changes, cartilage defects, bone oedema, bone cysts, bone attrition, osteophytes, and meniscal lesions, on MRI in the neuropathic pain groups compared to the inflammatory pain group.

Logistic regression models were developed to find which variables were associated with the neuropathic pain group. Logistic regression was performed by combining the data from PoNPG and PrNPG (NPG).

Self-reporting measures, S-LANSS, pain severity, stiffness and functional disability, psychosocial characteristic and sleep disruption, digital mapping of burning sensation, electric shock sensation, and hypersensitivity, pressure pain thresholds on the medial the index knee, cold pain thresholds, vibration and cold detection threshold over the popliteal fossa, cartilage defects on the medial side of the knee, osteophytes on both the medial and lateral sides, lateral tibial bone oedema, lateral meniscal lesions (pathological features on MRI) were strongly associated with NPG in univariate logistic models.

The variables (multivariate logistic regression model) most strongly related to NP were vibration threshold at the popliteal fossa of the index knee, cold pain threshold on the lateral

aspect of the index knee, lateral osteophytes, lateral meniscus lesions and lateral burning and hypersensitivity. Most of the variables related to NP were identified on the lateral side of knee. Implementing a better diagnostic protocol for osteoarthritis pain will ultimately lead to rational therapeutic targets for OA.

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List of abbreviations

BE	Bone marrow oedema
BML	Bone marrow lesions
°C	Degrees celsius
CDT	Cold detection threshold
CPT	Cold pain threshold
DASS	Depression, anxiety and stress scale
HDT	Heat detection threshold
HPT	Heat pain threshold
IL	Interleukin
MRI	Magnetic resonance Imaging
NP	Neuropathic pain
NSAID	Non-steroidal anti-inflammatory drug
OA	Osteoarthritis
OR	Odds ratio
PCS	pain catastrophizing scale
PD	PainDETECT
PoNP	Possible neuropathic pain
PQAS	Pain quality assessment scale
PrNP	Probable neuropathic pain
PSQI	Pittsburgh sleep quality index
QST	Quantitative sensory testing
ROC	Receiver operating characteristic curve
S-LANSS	Leeds assessment of neuropathic symptoms and signs
VT	Vibration threshold
WOMAC	Western Ontario McMaster Arthritis Index

1. Literature review

1.1. Osteoarthritis

Osteoarthritis (OA) is a leading cause of joint pain and functional disability. Its incidence is directly related to age; about 50% of the affected people are aged over 65 years (Sofat et al., 2011). Osteoarthritis pain is a common reason to attend general practice (Peat et al., 2001). There were approximately 2.2 million (9.3%) Australians with OA in 2017-18 (Australian Institute Health and Welfare (AIHW)). There has been an approximately 38% increase in the rate of total knee replacements (TRK) over the last decade, as this treatment is used to treat severe OA cases (AIHW).

Patients with knee OA commonly present with symptoms and signs of pain, joint stiffness, tenderness, crepitus, muscle weakness, limitation of motion, impaired proprioception, disability and enlargement deformity (Tsauo et al., 2008).

Some OA patients present with pain that is difficult to manage and relatively insensitive to the recommended medications like non-steroidal anti-inflammatory drugs (NSAID) (Bjordal, Ljunggren, Klovning, & Slordal, 2004; Neame et al., 2004). A review of nine studies found that the incidence of neuropathic pain (based on responses to self-report questionnaires) is significant among knee and hip OA patients at 23% (95%CI: 10-39%) (French et al., 2017). Of the nine studies reported, only one study included hip OA patients whereas the rest of the studies only included knee OA patients. Therefore, as the aging population is growing rapidly worldwide (Pearson et al., 2021), it is important to determine accurate diagnostic criteria for people who may be experiencing neuropathic pain and to improve the management of patients suffering more severe pain as a consequence of OA.

At present, there are no tests available to make a definitive diagnosis of neuropathic pain in patients with OA. Currently, guidelines recommend that OA patients should be treated with standard analgesics and NSAIDs (ACR, 2000; Bjordal, Ljunggren, Klovning, & Slørdal, 2004). Often, the patient's management is based only on symptomatic responses. This study was designed to investigate diagnostic criteria which could possibly differentiate pain phenotypes among OA patients and determine a well-defined set of measures which would

assist in early diagnosis of neuropathic pain (NP). NP management requires different medication compared to conventional NSAIDs. OA patients can therefore benefit from more targeted pain management once their NP is diagnosed.

1.1.1. Pathophysiology of osteoarthritis

OA is a degenerative disease of joints. It affects the entire joint structure including articular cartilage, synovial membrane, subchondral bone, ligaments, joint capsule, neural structures and muscles (Figure 1) (Samuels et al., 2008; Yunus et al., 2020).

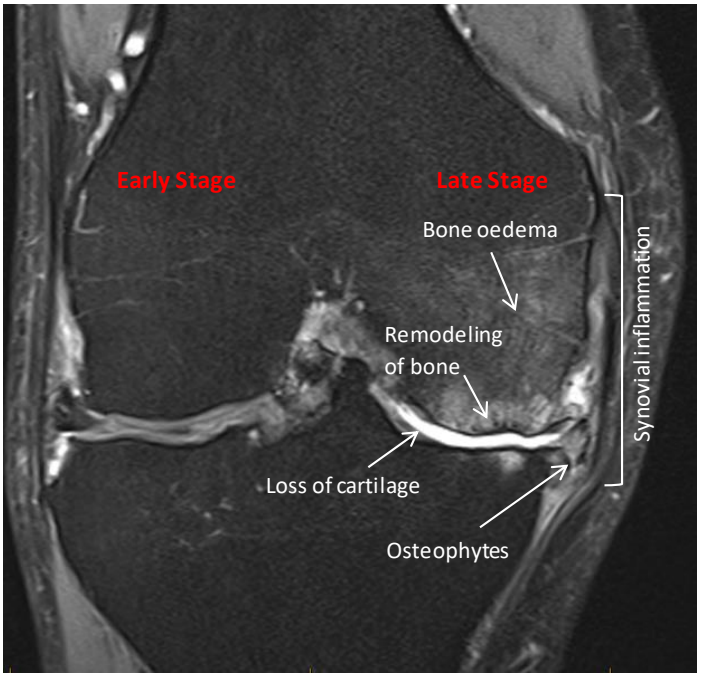


Figure 1: Description of degenerative changes in knee osteoarthritis.

In the normal joint, chondrocytes maintain equilibrium between synthesis and degeneration of extracellular matrix (ECM) (Sandell & Aigner, 2001). Osteoarthritic changes lead to disruption of the matrix equilibrium with progressive loss of cartilage, cellular expansion of chondrocytes, and induction of oxidative states in a stressful cellular environment, which leads to apoptosis of cells (Lane et al., 2011). This eventually leads to the destruction of the ECM and cartilage degradation. Clinically, degradation of ECM results in gradual impairment of articular cartilage, often accompanied by pain and physical disability (Goldring & Berenbaum, 2004; Sandell & Aigner, 2001). These changes stimulate remodelling in

subchondral bone in the form of sclerosis and osteophyte formation (Figure 2) (Abramson & Attur, 2009).

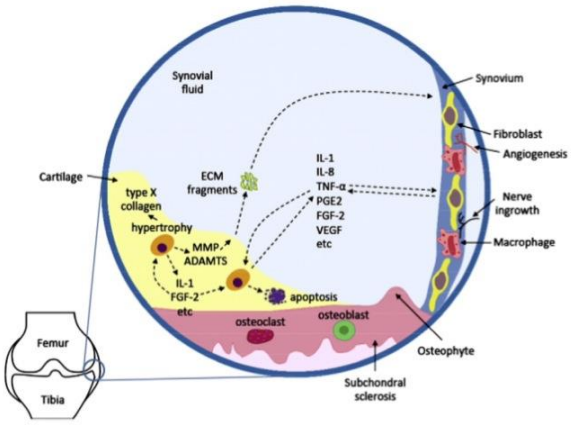


Figure 2: Complex cellular changes in an OA joint: activated chondrocytes produce ECM degrading proteases, pro-inflammatory cytokines and catabolic growth factors (Abramson & Attur, 2009).

Inflammatory and mechanical stimuli initiate the release of cytokines from chondrocytes, which then initiate a complex biochemical and mechanical interplay, resulting in the development of OA (Schaible et al., 2011). Pro-inflammatory cytokines from the interleukin family (IL-1, IL-6, IL-17), tumour necrosis factor alpha (TNF-α) and prostaglandin E2 initiate pain and hyperalgesia by a number of direct and indirect actions, including sensitisation of nociceptors for mechanical stimuli (Schaible, 2007). These pro-inflammatory mediators stimulate production of cartilage degrading proteases which also contributes to the development of peripheral sensitisation and pain in OA. Nerve growth factor (NGF) is a neurotrophic factor, which regulates a variety of metabolic pathways and organ functions. These include supporting the development of neurons in the central and peripheral nervous system, wound healing and immune suppression (McMahon et al., 2005). NGF is known to produce significant nociceptive input by binding to the specific receptors TrkA and p75NTR (Galoyan et al., 2003). Several chronic pain disorders like diabetic neuropathy, cystitis, pancreatitis and osteoarthritis pain are associated with nerve growth factor dysregulation (Eibl et al., 2012).

1.2. Pain

Pain is a protective mechanism to alert to an event which may lead to damage in the body tissues. It is a complex sensory and emotional experience, which is usually categorised as nociceptive/inflammatory or neuropathic (Lewin & Mendell, 1993). Spontaneous pain and mechanical hypersensitivity can develop as a result of sensitisation of primary afferents directly involved in the inflammatory process, but also following sensitisation of neurons in the spinal cord (central sensitisation) or higher centres. Persistent pain is the most common reason people with osteoarthritis seek medical advice and treatment (Riel et al., 2020). Inflammatory pain is linked to activation and sensitisation of peripheral nociceptors whereas pain originating from nerve damage (peripheral neuropathic pain) has been linked to other physiological phenomena such as ectopic discharge and axonal cross excitation, occurring as a result of damage to nerve tissue (Schaible et al., 2006).

The current American College of Rheumatology (ACR) and Osteoarthritis Research Society International (OARSI) guidelines for the pharmacological treatment of OA pain advise the prescription of acetaminophen/paracetamol (up to 4000 mg/day) as a standard initial oral analgesic (Hochberg et al., 2012; Zhang et al., 2008). In the absence of an adequate response, or in the presence of severe pain and/or inflammation, alternative pharmacologic therapy may then be considered (Zhang et al., 2008). This may include the use of NSAIDs although the use of neuropathic pain medication is not routinely considered. The concept that some patients with OA may exhibit features of neuropathic pain is gaining more widespread acceptance however (French et al., 2017; Hochman et al., 2011; Ohtori et al., 2012; Woolf, 2011) and so in some cases it may be appropriate to consider medications specifically targeting neuropathic pain.

1.3. Pain phenotypes

1.3.1. Inflammatory pain

Inflammatory pain is caused by local inflammation and tissue damage. This type of pain usually results from the activation of nociceptive afferent neurons by tissue-damaging stimuli and inflammatory mediators (Mease et al., 2011). Impulses are transmitted via the

peripheral nerves and spinal cord tracts to the brain (Schaible et al., 2006). Nociceptors are located throughout the joint tissues including joint capsule, ligaments, periosteum and subchondral bone (Buckwalter et al., 2004; Felson, 2005). A peripheral drive to pain occurs in approximately 60-80% of patients with OA (Ohtori et al., 2012). Intense prolonged input from nociceptors sensitises spinal cord pain transmitting neurons and leads to decreased activation thresholds, increased synaptic efficacy and decreased firing thresholds (central sensitisation) (Woolf, 2011).

1.3.2. Neuropathic pain

Neuropathic pain (NP) is defined as, 'pain arising as a direct consequence of a lesion or disease affecting the somatosensory system' (Treede et al., 2008). NP symptoms are often described using terms such as burning, shooting, tingling, stinging and numbness (Freynhagen et al., 2006). Patients also often report spreading or radiating pain and excessive sensitivity to various stimuli such as cold and light touch (Bennett et al., 2007). Patients with NP visit their doctor frequently due to substantial pain that interferes with their daily functioning despite receiving treatment (McDermott et al., 2006). Sensorimotor neuropathy affects large and small afferent nerve fibres to varying degrees resulting in mixed symptoms and sensory loss (Perkins et al., 2001; Vanik et al., 1986). Myelinated afferent nerve fibres transmit proprioception (limb location), touch, cold and vibration sensation (Gilman, 2002). Small unmyelinated afferent fibres are responsible for conducting nociceptive stimuli for touch and warm sensation (Vanik et al., 1986). Common conditions with NP include diabetes, shingles, spinal cord injury, stroke, multiple sclerosis, cancer, and HIV infection, as well as lumbar or cervical radiculopathies, and traumatic or postsurgical nerve injuries. Diabetic neuropathy is a well-recognised complication of type-1 and type-2 diabetes mellitus (DM). Approximately one third of patients with DM show evidence of peripheral neuropathy (Duby et al., 2004). The presence of NP is directly related to the duration of disease and glycaemic control.

Neurons triggered by nociceptive stimuli become hyper responsive to subsequent stimuli which results in sensitisation. Peripheral nociceptors may become sensitised from prolonged and intense input from cartilage and subchondral bone damage as well as synovial inflammation (Schaible, 2012). Nociceptive input from the OA knee joint may also lead to central sensitization, which may arise from chronic nociceptor stimulation and subsequent

modification of central pain transmitting neurons. It has been suggested that this may be associated with NP symptoms (Hochman et al., 2013; Hochman et al., 2011; Ohtori et al., 2012).

1.4. Innervation of the knee joint

The human knee joint receives innervation from a number of peripheral nerves. These are the femoral nerve (medially), the common peroneal nerve (laterally) and a plexus formed by the sciatic, tibial and obturator nerves (posteriorly) (Horner & Dellon, 1994). Each of these innervations combines articular, muscular, and cutaneous components (Figure 3).

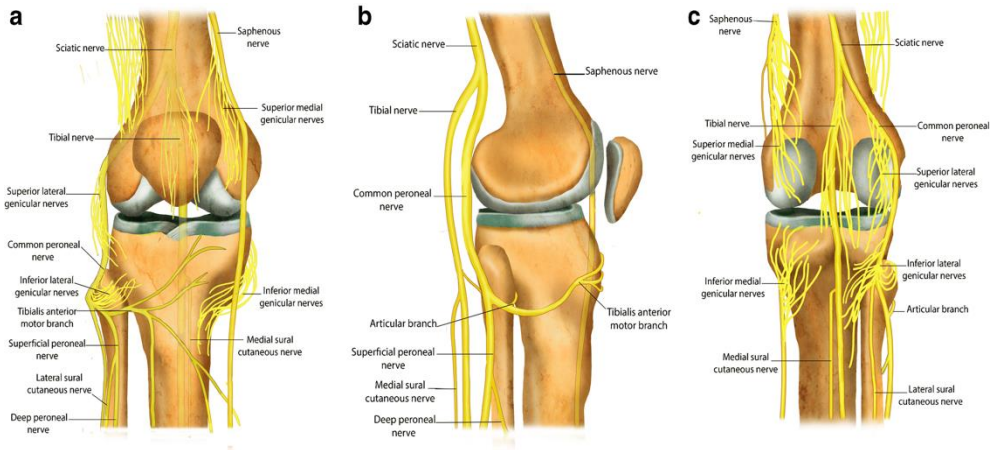


Figure 3: Nerve supply around the knee area. a. nerve distribution on the anterior aspect of the knee b. lateral distribution of nerves c. Nerve distribution on the posterior aspect of the knee. Femoral nerve (medial), the common peroneal nerve (lateral) and the posterior plexus, composing the tibial, common peroneal and superior medial geniculate nerve (Goldman et al., 2018).

The cutaneous innervations are not extensive and do not extend beyond the infrapatellar region. The medial femoral cutaneous nerve and medial reticular nerve are located over the medial aspect of the knee joint with a parapatellar and infrapatellar distribution. In addition, the infrapatellar branch of the saphenous nerve crosses the inferior knee from medial to lateral innervating both the skin below the patella and the anterior inferior knee capsule (Kennedy et al., 1982). The lateral reticular nerve and superior lateral genicular nerve innervate the lateral aspect of the knee. The posterior plexus innervates the central posterior region of the knee. This defines three areas of distinct innervation that will be important for

the purposes of this study. These are the anteromedial and lateral aspects of the knee and the popliteal fossa.

Considering the nerve distribution around the knee, it would appear that the medial, lateral and posterior aspects of the knee should be evaluated specifically in order to obtain information about any potential neurological deficits. Reporting of symptoms and sensory testing on these three sites can be carried out to investigate NP symptoms corresponding to the specific areas of innervation. Accurate diagnosis of pain is important as pharmacological therapy without proper diagnosis is ineffective and sometimes pain medication can pose major health risks due to drug interactions.

1.5. Self-reporting measures (questionnaires)

1.5.1. Neuropathic pain questionnaires

Several questionnaires have been used to distinguish neuropathic pain (NP) from other types of chronic pain. These questionnaires are based on verbal descriptors and pain qualities. These tools have been used in research as well as in some clinical settings. However, they do not provide sufficient information to establish a formal diagnosis of neuropathic pain.

The Leeds assessment of neuropathic symptoms and signs (LANSS) was the first questionnaire developed to identify NP (Bennett, 2001).. The S-LANSS has been used in some recent studies along with the PainDETECT questionnaire to identify NP (Hochman et al., 2013; Hochman et al., 2011; Moreton et al., 2015). In the absence of a gold standard these studies used both questionnaires to confirm the identification of participants with NP.

The PainDETECT questionnaire (PDQ) was developed and validated as an easy to use self-report questionnaire. It is a screening tool, initially developed to identify the presence of neuropathic pain in patients with chronic low back pain. It has been widely used in osteoarthritis patients to identify pain phenotypes among OA patients; higher sores > 19 suggest the presence of neuropathic pain (Moss et al., 2018; Moss et al., 2016; Ohtori et al., 2012; Wright et al., 2017). These studies demonstrated higher PainDETECT scores were related to wide-spread hyperalgesia to cold and pressure and hypoesthesia to warm, cold and touch; suggesting the presence of compromised sensory function.

The PainDETECT questionnaire has been frequently used in research studies and demonstrated different pain phenotypes among people with knee OA pain (Hochman et al., 2013; Moss et al., 2018; Valdes et al., 2014). These studies reported up to 32% of people scored positive for the NP category. DN4 is another questionnaire to evaluate neuropathic pain. Similar incidence of NP was reported by using S-LANSS and DN4, 30% and 29.4% respectively (Moreton et al., 2015; Oteo-Álvaro et al., 2015). These two questionnaires were used to identify a sub-group of the OA cohort who present with NP (Hochman et al., 2013; Moreton et al., 2015).

1.5.2. Pain and physical function assessment

Western Ontario and McMaster Universities Arthritis Index (WOMAC) is a hip and knee OA-specific self-report questionnaire which is widely used for the assessment of pain, stiffness and physical disability caused by knee OA. It has been widely used in research studies. Some studies have used the WOMAC to assess pain severity and physical function in OA patients along with the PainDETECT questionnaire (Hochman et al., 2013; Moss et al., 2018; Wright et al., 2017). The results of these studies have shown that people with OA who score high on PainDETECT had significantly greater pain and functional disability compared to those with low PainDETECT scores, linking a more severe impact of disease with evidence of neuropathic pain.

The Pain quality assessment scale (PQAS) questionnaire assesses the dimensions of spontaneous pain experienced by patients with a range of conditions including OA (Victor et al., 2008). The PQAS assessment is based on specific sub-scales, which reflect different pain domains: the paroxysmal sub-scale (shooting, sharp, electric, hot radiating) and the surface subscale (itchy, cold, numb, sensitive and tingling) seem to be related to NP pain and the deep subscale (aching, heavy, dull, cramping, throbbing, and tender) assesses inflammatory pain. Previously higher PQAS scores were related to the neuropathic pain group (based on PainDETECT) and higher cold pain threshold (CPT) values (Wright et al., 2017).

The pain catastrophizing scale (PCS) is a valid and reliable measure of pain catastrophizing in older adults, including those with OA. Higher scores indicate more pain catastrophizing (Osman et al., 1997). Higher PCS scores were reported previously among knee OA participants compared to controls (p= 0.0001) (Hochman et al., 2013). However, it is yet to

be explored whether more catastrophizing is related to the presence of NP among knee OA patients.

1.5.3. Psychosocial and sleep assessment

Higher depression and anxiety scores have been reported in people with chronic pain using different assessment tools (Bair et al., 2008). Poor sleep quality has also been demonstrated previously in knee OA participants with possible NP (Valdes et al., 2014). This is an area that requires more detailed investigation in the OA cohort to see if it relates to different pain phenotypes.

1.6. Pain mapping

Pain description indicating the location of pain and its distribution gives useful information to clinicians and researchers. Pain mapping has transitioned from paper-based mapping to digital pain mapping and digital pain mapping on 3D body schema (Figure 4).

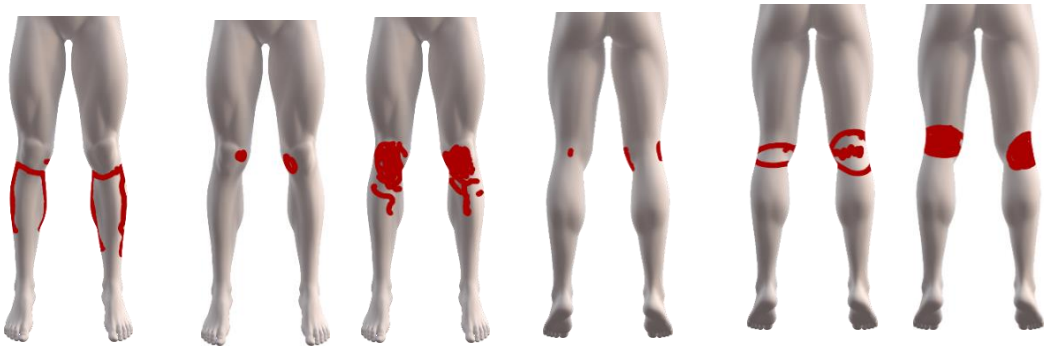


Figure 4: Pain mapping of study participants showing different pain patterns, on the anterior and posterior aspects of the knee on 3D lower limb body schema.

Mapping quantifies and assesses pain to help in diagnosis and follow-up i.e., treatment effect (Abbott et al., 2015; Wood et al., 2007). Pain mapping is a useful tool in research as well as in clinical settings to quantify pain and track changes over time, after treatment or for the progression of disease. Previous studies using pain mapping to examine pain location in knee OA, have suggested that the medial side is the most common site of pain (Creamer et al.,

1998; Wood et al., 2007). No particular pain pattern or location was found to be indicative of knee OA when pain mapping was used to evaluate pain pattern and location (Wood et al., 2007).

Pain mapping increases the analytic possibilities of pain description. Pain pattern, area and site were studied using digital pain mapping on a 3D body schema of the lower limb in participants with patellofemoral pain (Boudreau et al., 2017; Boudreau et al., 2018). Specific patterns of pain were recognised and bilateral pain was related to longer duration.

The area and site of symptoms of NP, like electric shock, burning, tingling and cold could help identify the area of NP symptoms and help to assess sensory deficit at a particular pain location by guiding testing for sensory detection threshold. Participants' photographic knee pain map results indicated 16% of the knee OA participants had isolated medial knee pain, whereas a diffuse pain pattern was most common (Van Ginckel et al., 2016). The same study also noted that diffuse knee pain reports were associated with severe pain and physical dysfunction indicated by higher WOMAC scores. Prevalence of neuropathic pain-like symptoms (high PainDETECT scores) were linked to diffuse and posterior-medial patterns rather than anterior-medial pain (Van Ginckel et al., 2016).

Pain mapping could be a good tool to assess the incidence and location of peripheral nociception and NP like symptoms in knee OA.

1.7. Quantitative sensory testing (QST)

Features of sensory deficits or hypersensitivity associated with NP have been identified using quantitative sensory testing (QST) in knee OA patients (Hochman et al., 2011; Wright et al., 2015). QST involves assessing the response to a range of evoked somatosensory stimuli which can be mechanical, chemical, electrical, and thermal (Wylde et al., 2011). Commonly cold, heat, tactile, vibration and pinprick perception thresholds and pressure, heat and cold pain thresholds are the measures used in research and sometimes in clinical settings. Somatosensory abnormalities like hypoesthesia, hyperalgesia, temporal summation and allodynia have been demonstrated in people with chronic knee pain using QST measures (Arendt-Nielsen et al., 2010; Harden et al., 2013; Hochman et al., 2013; Moss et al., 2018).

Some of these findings may be indicative of a potential role for QST in identifying somatosensory abnormalities associated with the development of neuropathic pain in knee OA. QST is a valuable tool to identify sensory deficit in clinical setting. It was suggested valuable for staging diabetic neuropathy (Hansson et al., 1991; Zaslansky & Yarnitsky, 1998) and evaluating drug efficacy (Schliessbach et al., 2018). This research project used a number of QST measures to evaluate sensory deficits and hyperalgesia among knee OA participants.

1.8. Proprioception

Proprioception is the sense of position and relative movement of different body parts i.e., limbs and joints in space (Kalaska, 1994). Proprioceptive sense plays an important role in muscle contraction and joint stabilisation. Patients with OA of the knee, when tested for partial weight bearing joint reposition sense (JRS) performed poorly compared to control subjects (Garsden & Bullock-Saxton, 1999). In individuals with knee OA proprioceptive impairments have been reported not only in the affected knee but also in the unaffected knee, in people with unilateral disease (Garsden & Bullock-Saxton, 1999). Compromised movement detection at the elbow and knees in patients with knee OA has also been reported, which supports the presence of generalised proprioceptive impairment. However, a more recent investigation of joint repositioning proprioceptive deficit found that it was localised to the affected knee joint (Shanahan et al., 2015). It is known that myelinated nerve fibres transmit proprioception and vibration sensation (Vinik et al., 2000). Proprioceptive deficits may reflect a sensorimotor neuropathy affecting myelinated afferent nerve fibres.

A longitudinal study assessed proprioceptive function using joint reposition error at baseline and at 30 months and reported reduced proprioceptive perception in OA participants. This was related to the presence and severity of knee pain and the associated functional disability (Felson et al., 2009). Impaired proprioceptive function was related to the presence and severity of knee pain but not with the presence of radiographic findings of OA. People with knee OA who had poor proprioceptive function also had worse physical functional ability and more severe pain over time compared to those with good proprioceptive function (Felson et al., 2009).

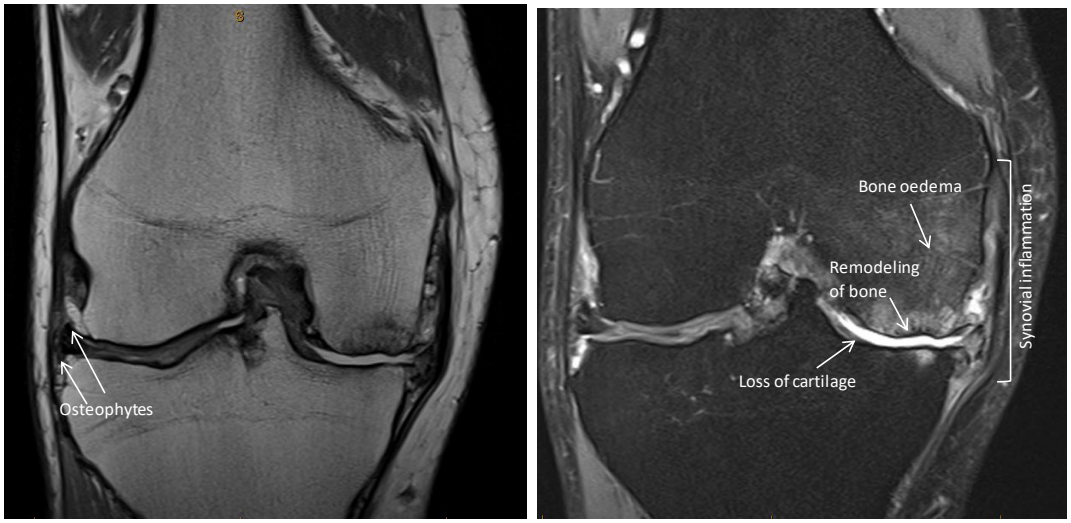
Proprioceptive impairment may lead to poor control of joint position and more mechanical load on the joint, which may lead to a higher risk of development and progression of OA (Salo et al., 2002). The posterior cruciate ligament (PCL) is a collagen tissue to provide structural support, but it also has neural elements (mono-receptors) which provide proprioceptive input to the body by mediating knee kinaesthesia, leading to dynamic stability (Rajgopal et al., 2014). Retaining the PCL in total knee replacement is a good option for better proprioception and stability, indicating the overall importance of proprioception (Rajgopal et al., 2014).

Proprioceptive performance may be adversely affected by damage to the joint receptors of OA knees. These receptors are important for the normal reflexive knee joint functions. It appears that these deficits may be overcome to some degree with knee replacement (Weiler et al., 2000). Impairment of joint position sense may occur as a result of OA pathophysiology and may also be a primary factor in initiation of joint damage (Barrett et al., 1991). Restoration of joint structure or joint replacement leads to improvement in joint position sense but does not completely restore proprioception to the levels of age matched controls (Barrett et al., 1991). Diseases associated with sensory loss like diabetes mellitus and/or leprosy may also lead to joint arthropathy (O'Connor et al., 1985). When protective muscular reflexes are abolished or their effectiveness in protecting articular and periarticular tissues is decreased this may lead to neuropathic arthropathy (a Charcot joint) (Finsterbush & Friedman, 1975; O'Connor et al., 1985). This might suggest that neuropathic deficits might also play a role in the aetiology of OA.

The focus of this study was to use joint reposition sense error to evaluate proprioceptive deficits among OA participants and to determine whether deficits of proprioceptive function help to identify pain phenotypes of OA participants.

1.9. Magnetic resonance imaging (MRI)

Magnetic resonance imaging (MRI) allows visualisation of the knee joint with excellent anatomical resolution and tissue contrast. MRI can be used to visualize all the structures of the joint including cartilage, bony structures, and tendons (Kim et al., 2016), Figure 5. Bursae are synovium lined structures, which cannot be detected on any other imaging method.



(a) (b)

Figure 5: (a) T1 weighted MRI image and (b) T2 weighted MRI image showing some structural changes of OA knee.

The diagnostic performance of MRI is found to be better compared to X-ray, arthroscopy and histological sections to look at cartilage, meniscus and synovium (Hunter et al., 2011). MRI has shown associations between specific synovial and bone abnormalities and pain intensity (Felson, 2005; Roubille et al., 2014). There was a strong association reported with the presence of pain in the OA knee and the presence of bone marrow lesions on MRI (Felson et al., 2010). MRI detects the whole joint such as synovium, subchondral bone, meniscus, cartilage, and cyst-like lesions to help diagnose numerous disease conditions. When knee structural changes were assessed on MRI in symptomatic OA patients based on PainDETECT score, the presence of meniscal extrusion and meniscal tears were strongly associated with higher PainDETECT scores (Roubille et al., 2014). Bilateral popliteal cysts, sub-gastrocnemius bursitis and Hoffa's fat pad ganglion cyst like lesions are also commonly observed in patients with chronic knee pain (Hayashi et al., 2010). MRI has emerged as a technique of choice to visualise such lesions. An increasing prevalence of sub-gastrocnemius bursitis is associated with increasing severity of both effusion and synovitis (Hayashi et al., 2010). Some pathological features like synovitis and cysts can be detected on MRI and diagnosis can be made before these signs appear on X-ray.

In this study knee MRIs were used to determine which structural changes in knee OA were pathognomonic of neuropathic pain.

1.10. Biomarkers in knee osteoarthritis

The pathological process in osteoarthritis leads to the development of chronic inflammation and the potential to damage the neural tissue. There are a number of biomarkers that are commonly evaluated as indicators of inflammation and nerve damage in research and clinical practice. Established measures of inflammation include the erythrocyte sedimentation rate (ESR), white blood cell (WBC) count and C-reactive protein levels (CRP). ESR and WBC have low sensitivity and specificity as diagnostic markers of joint pain. CRP and interleukin 6 (IL-6) are relatively newer markers and their diagnostic value is still unclear from the literature. Some recent studies have demonstrated that there is enhanced expression and release of pro-inflammatory cytokines like Tumour necrosis factor alpha (TNF-α), interleukin-1β (IL-1β) and IL-6 from dorsal root ganglion (DRG) cells following nerve injury (Dimitroulas et al., 2014). These markers are found to be important indicators of neuropathic pain in rodents (Leung & Cahill, 2010; Wells et al., 1992; Xu et al., 2006). TNF-α can stimulate sensory neuronal excitability and produce NP. Direct application of TNF-α in the periphery induces pain and can be blocked by anti-inflammatory medications. Anti TNF-α antibody application produces a prolonged reduction of pain symptoms in OA (Grunke & Schulze-Koops, 2006). It has also been reported that TNF-α is involved in the pathogenesis of diabetic neuropathy (DNP) in mice and inhibition of TNF-α improved DNP. In a mouse NP model with L5 spinal nerve transection, a gene therapy was used to relieve NP by silencing TNF-α expression in DRG. This led to significant improvement of NP (Ogawa et al., 2014).

In this study a number of biomarkers will be assessed to see if they demonstrate any association to the presence of neuropathic pain in the OA cohort.

1.11. Diagnosis of neuropathic pain

A number of studies over the last decade have begun to provide evidence that a sub-group of patients with knee OA have high scores on neuropathic pain questionnaires, suggesting that they may have clinical features of neuropathic pain (Dimitroulas et al., 2014; Hochman et al., 2011; Moss et al., 2018). Currently, however, there are no established criteria for formally diagnosing neuropathic pain in patients with OA. Treede proposed a revised definition of neuropathic pain and proposed a grading system of definite, probable and

possible neuropathic pain (Treede et al., 2008). To diagnose neuropathic pain evidence of sensory deficit and a relevant pathological process is required (Treede & Baron, 2008; Treede et al., 2008). Evidence of sensory deficit is yet to be systematically explored in knee OA. Further research is required in this area so that a diagnostic procedure can be established to confirm neuropathic pain in people with knee OA. That is the objective of this research project.

1.12. Aims

The main aim of the study was

- To develop a logistic regression model to determine which measures most clearly differentiate between each of the pain categories and determine a well-defined set of measures that can be used to classify a patient with knee osteoarthritis into a neuropathic or inflammatory pain category.

To achieve this following aims were adopted

- To evaluate sensory and pain thresholds using QST, joint reposition error (JRE), self-reporting psychosocial function, sleep quality and comorbidities and compare them between OA and pain free controls.
- To evaluate sensory deficit among OA participants in comparison to sensory data from pain free controls.
- To develop a standard methodology to differentiate different pain phenotypes
- To determine whether these phenotype groupings can be differentiated by using questionnaires based on a range of self-report measures.
- To digitally map the location of pain and other sensations/symptoms in participants with knee OA on a template representing the lower limb to identify pain and symptom location.
- To determine the relationship of the location of pain and symptoms related to neuropathic pain within different pain phenotype groups of knee OA participants.
- To determine if proprioceptive deficits, sensory deficits and pain thresholds are different between pain phenotype groups in people with knee OA.

- To determine if proprioceptive deficits, sensory deficits and pain thresholds are different between pain phenotype groups in people with knee OA.
- To determine whether pathological features present on MRI demonstrate differences based on pain phenotype groups.
- To measure serum biomarkers of CRP, IL-1, IL-6, TNF-α and NGF in OA participants to determine if any of these serum biomarkers can help predict inclusion into a neuropathic or inflammatory pain category.
- To determine cut-off values for the measures found significant on logistic regression and to determine the sensitivity and specificity of these measures to discriminate between the two groups.

1.13. Overview of the thesis

The thesis starts with an introduction (Chapter 1) to osteoarthritis (OA), neuropathic pain (NP) and the measures available to assess pain and sensory functions i.e., questionnaires, QST, proprioceptive function etc. Pain free participants were also recruited to collect normative data on psychosocial state, sleep quality and comorbidity using questionnaires. The QST measures which included sensory and pain function related to cold, heat, vibration, touch, pressure pain thresholds and measures of proprioceptive function, were assessed in pain free as well as in OA participants. The methodology used to measure these variables is described in Chapter 2. In the following Chapter (Chapter 3) the comparison of these measures between pain free participants and OA participants is presented. Questionnaire based measures were assessed, which included neuropathic pain descriptors, pain severity, stiffness, physical disability, anxiety, depression, and sleep quality. Results of these self-reporting measures are presented in Chapter 4. Chapter 5 describes the results of pain and NP related symptom mapping carried out electronically by OA participants. Assessment of QST and proprioceptive function among different OA groups is included in Chapter 6. Structural changes, because of knee OA, were assessed on knee MRIs of OA participants. MRI results are discussed in Chapter 7. Serum biomarkers related to nerve injury and inflammation (IL1, IL6, TNF-α and NGF) are discussed in Chapter 8. Logistic regression models are presented in Chapter 9 to determine which variables were associated with neuropathic pain. Finally, the overall summary of the results and conclusions is discussed in Chapter 10.

References are listed to acknowledge past researchers whose work helped provide an understanding of the different concepts of this study.

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2. Methods

2.1. Study participant recruitment

Participants for this study were recruited through advertisement on Curtin radio, adverts in gyms and retirement villages and through a research participant recruiting service (Trialfacts).

2.2. Ethical approval

Ethics application was prepared and submitted to obtain ethics approval. This research project was approved by Curtin University Human Research Ethics Committee (HREC). HREC project number was HRE 39/2016.

2.3. Power / sample size calculation

Cold pain threshold data were used as a representative QST measure from a previous study to calculate power and sample size for one-way ANOVA (alpha 0.05; beta 0.80): the largest difference between means = 9.4 and SD = 7.5; predicted sample size of 11. Similarly, joint reposition error data, for a representative proprioception measure, from a previous study, were used to calculate power and sample size for a one-way ANOVA: largest difference between means = 3.62; largest SD = 5.5; predicted sample size = 34. This suggests that, for a minimum sample (group) size of 34, it would be necessary to recruit at least 100 OA and 35 pain free control participants.

To perform the logistic regression analysis the widely accepted criterion of 10 outcomes per predictor variable and a maximum of 4 predictor variables were assumed. It was anticipated that the ratio of participants with inflammatory pain and neuropathic pain would be 2 to 1. It was anticipated that 4 predictor variables would be applied to each of the logistic regression models. This would therefore mean that an overall sample size of approximately 120 would be needed (40 participants in the PrNPG; 80 in the IPG). Based on these assumptions it was considered that 120 OA and 35 pain free participants were required for the study.

2.3.1. Inclusion criteria

Osteoarthritis participants

- Participants aged 50 years and over diagnosed with unilateral knee OA for at least six months.
- Participants diagnosed with OA according to the American College of Rheumatology (ACR) criteria for a diagnosis of osteoarthritis: pain ≥ 3, stiffness and the presence of crepitus/crackling.
- Participant's pain in the index knee ≥ 3 on a scale of 10 on most days of the last week.

Pain free participants

- Participants aged 50 years and over with no history of joint pain and no previous history of knee surgery.

2.3.2. Exclusion criteria (both groups)

- History of other inflammatory conditions (e.g., rheumatoid arthritis)
- History of a significant neurological condition (e.g., stroke, multiple sclerosis)
- Recent lower limb injury or surgery (last 6 months)
- Previous joint replacement
- Diabetes mellitus for more than 2 years
- History of significant lumbar and radicular pain (last 6 months)
- History of other chronic pain disorders (e.g., fibromyalgia).

2.3.3. Screening

All participants went through a comprehensive screening procedure to ensure suitability for inclusion in the study. All participants were first interviewed/screened over the phone for suitability. Suitable participants were then given the project information sheet and the consent form. Medical screening was done by an experienced rheumatologist to confirm the diagnosis of OA and exclude any other medical conditions described in the exclusion criteria.

Suitable OA participants had a further detailed clinical examination. In the OA participants' group this was focused on clearly describing the area and nature of their pain, assessing range of movement in both knees and a standard neurological examination of the lower limbs. As part

of this process, pain free (PF) volunteers were also screened to ensure that they had full normal pain free function of the lower limbs. The following diagram describes the stages of the testing process (Figure 1).

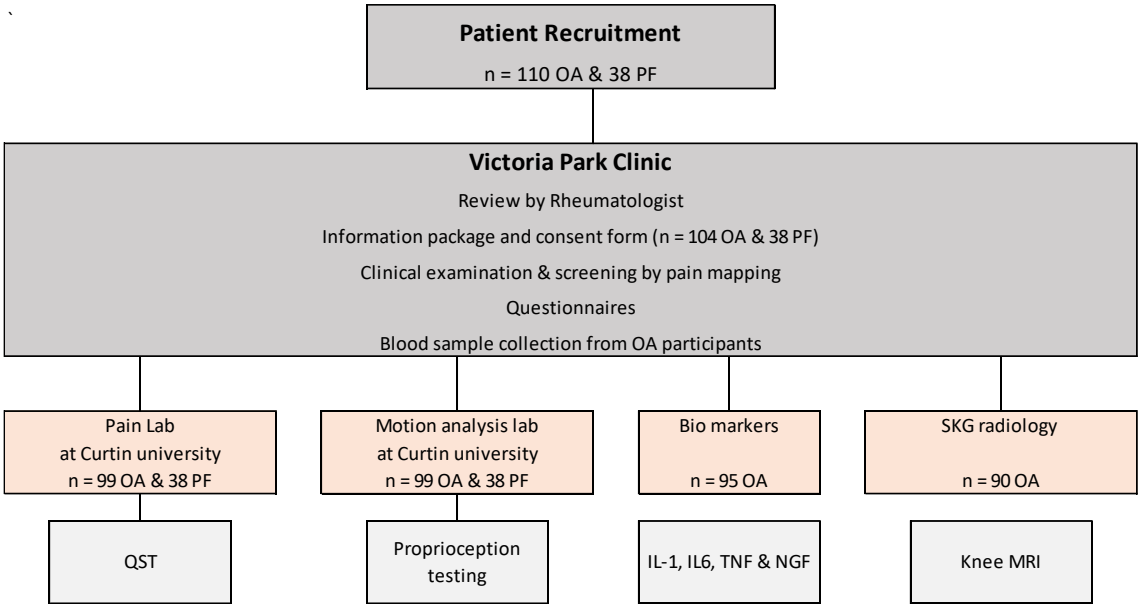


Figure 1: Flow diagram of study protocol.

2.4. Pain diagram

Pain mapping has advanced from paper drawings through to digital pain mapping. In this digital age, digital pain mapping is an easy and convenient method to explore the course of disease or follow up treatment. Digital pain mapping has been validated against paper drawings (Darnall et al., 2017). It is a good tool to assess pain pattern, area and site (Boudreau et al., 2016; Boudreau et al., 2017). OA participants completed a lower limb pain diagram on a high-resolution 3D body schema representing the leg and knees on a computer tablet (Samsung Galaxy Tab A, model SM-P350, Android 6.0.1, 2017 Edition) using the navigate pain app developed by Aalborg University, Denmark. Two different types of images were used to map pain and other sensations; a 3D image to describe the location and area of the superficial pain and dotted line images to describe deeper pain and other symptoms (explained later) participants may have. Participants drew around the knee indicating where they felt pain and any other sensations or unusual symptoms that they experienced in the lower limb. They were

asked to differentiate between the areas of superficial pain and sensitivity and deep pain (using different images available on the navigate pain app), Figure 2.

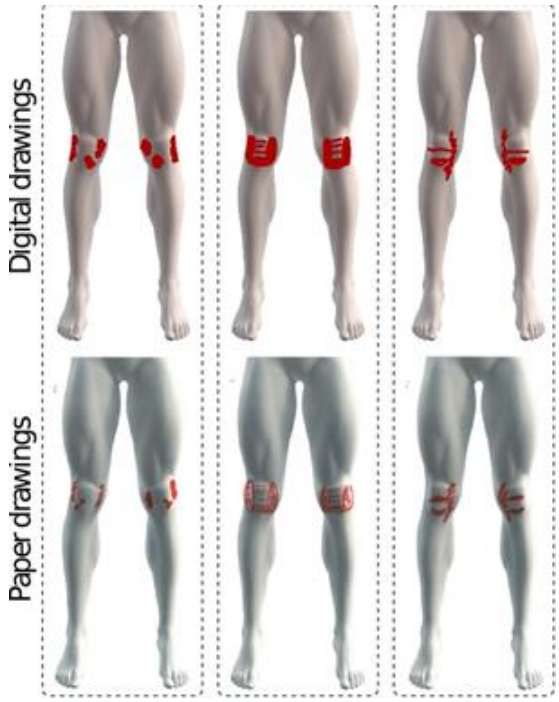


Figure 2: Pain mapping on digital and paper drawing (Matthews et al., 2018).

Different colours, as below, were used to represent pain, and other sensations like cold, electric shock, burning and decreased or increased sensitivity around the knee area.

Red: pain
Blue: cold
Black: electric shock
Grey: hot/burning
Green: increased sensation or allodynia

2.5. Questionnaires

All OA participants were asked to complete eight questionnaires. These included:

i. PainDETECT questionnaire
ii. Leeds Assessment of neuropathic symptoms and signs (S-LANSS)
iii. Western Ontario McMaster Osteoarthritis Index (WOMAC)
iv. Pain Quality Assessment Scale (PQAS)
v. Pain catastrophizing scale (PCS)
vi. Depression, anxiety, stress scale (DASS)
vii. Pittsburgh sleep quality index (PSQI)
viii. Comorbidity questionnaire

Control participants were asked to complete the Depression, anxiety and stress scale, the Pittsburgh sleep quality index, and the comorbidity questionnaire.

2.5.1. PainDETECT questionnaire

The PainDETECT questionnaire was developed and validated in adults with chronic low back pain (Freynhagen et al., 2006). It is available in English and widely used in research settings to identify NP. It is an easy to use self-report questionnaire with nine items, which do not require clinical examination. There are seven sensory descriptor items (answers range from never to very strong). It was validated and has sensitivity of 85% and a specificity of 80% to identify neuropathic pain features in a range of conditions (Freynhagen et al., 2006).

2.5.2. Leeds Assessment of Neuropathic Symptoms and Signs (S-LANSS)

Leeds assessment of neuropathic symptoms and signs consists of five symptom items to evaluate features of NP and two clinical examination items and can be easily used in the clinical setting. It has also been validated as a self-reporting tool, called S-LANSS (Bennett, 2001; Bennett et al., 2005). Positive scores identify NP. When compared with clinical assessment, S-LANSS was able to correctly identify 82% of patients with neuropathic pain, with 85% sensitivity and 80% specificity. The self-report version of the questionnaire (S-LANSS) was used in this study.

2.5.3. Western Ontario McMaster Osteoarthritis Index (WOMAC)

The Western Ontario and McMaster osteoarthritis index (WOMAC) is specific to hip and knee OA studies, and does not contain items about other joints. WOMAC is comprised of 24 questions. This OA-specific self-report scale has been commonly used to measure pain, stiffness and disability from knee OA. The WOMAC questionnaire demonstrates good internal validity and test-retest reliability. Good to excellent reliability has been shown for the pain and physical function sub-scales, > 0.80 for both (Jinks et al., 2002).

2.5.4. Pain Quality Assessment Scale (PQAS)

The pain quality assessment scale (PQAS) is a 20 item measure to evaluate a range of pain qualities. The first couple of questions are introductory. The rest of the items of PQAS are divided into 3 subscales; deep subscale (Q 4, 13, 15, 16, 17), surface subscale (Q 5, 6, 7, 8, 10, 12) and paroxysmal subscale (Q 3, 9, 11, 14). The deep subscale (aching, heavy, dull, cramping, throbbing and tender) is considered to reflect inflammatory pain. The paroxsysmal (shooting, sharp, electric, hot radiating) and surface subscales (itchy, cold, numb, sensitive and tingling) are considered to be reflective of neuropathic pain (Victor et al., 2008).

Participants responded to the severity of each descriptor by rating from 0 to 10. The sum of each of the subscales are included in the analysis. PQAS has shown excellent reliability and 85% sensitivity and 76% specificity to identify peripheral neuropathy (Gammaitoni et al., 2013).

2.5.5. Pain Catastrophizing Scale (PCS)

Sullivan et al. developed the pain catastrophizing scale (PCS). It was given to the participants to measure their feelings and thoughts about pain. Later it was translated and validated for clinical and research settings. PCS comprises of 13 questions, each answered on a 5-point scale: 0 being pain not felt at all and 5 being pain felt all the time (Sullivan et al., 1998). The total score is between 0 to 52. Higher scores indicate the presence of greater catastrophizing about pain.

A meta-analysis of PCS scores across studies found PCS to be a reliable measure. It has shown high internal reliability of 0.92 and test–retest reliability of 0.88 (95% confidence interval of 0.83-0.93) (Wheeler et al., 2019). PCS reliability data were reported to be good to excellent with high internal validity scores (coefficient alphas: 0.87-0.93, rumination 0.87-0.91, magnification 0.66-0.75, and helplessness 0.78-0.871 (Osman et al., 1997; Sullivan et al., 1995).

2.5.6. Depression, Anxiety and Stress Scale (DASS)

The depression, anxiety and stress scale (DASS) is a self-administered questionnaire designed to measure the magnitude of depression, anxiety and stress. It consists of 42 items, each item is measured on a 4-point scale in response to the experiences of the past week. The rating scale ranges from 0 to 3:

0 Did not apply to me at all
1 Applied to me to some degree, or some of the time
2 Applied to me to a considerable degree, or a good part of time
3 Applied to me very much, or most of the time.

Higher scores on each subscale indicate increasing severity of depression, anxiety, or stress as described in earlier research (Gloster et al., 2008). DASS has excellent internal consistency of 0.96 for depression, 0.89 for anxiety and 0.93 for stress in a clinical sample of people with mood disorders (Brown et al., 1997). Concurrent validity was examined via correlations with other depression and anxiety measures, including the Beck Depression Inventory, the Beck Anxiety Inventory and the State-Trait Anxiety Inventory- Trait Version, with results indicating moderately high correlations (Antony et al., 1998).

2.5.7. Pittsburgh Sleep Quality Index (PSQI)

The Pittsburgh sleep quality index (PSQI) is commonly used in clinical as well as research settings to assess sleep quality. PSQI is a self-rated questionnaire. It consists of 19 individual items which make seven “component” scores: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction. The sum of scores for these seven components yields one global score.

At present the PSQI is the only standardized clinical measure which covers a broad range of indicators relevant to sleep quality. In a review of studies, Cronbach's alphas ranged from 0.70 to 0.83, meeting the cut-point for a positive rating for within- and between-group comparisons (Mollayeva et al., 2016).

2.5.8. Comorbidity

The self-administered comorbidity questionnaire (SCQ) was used to assess comorbidities. This is a relatively new measure and an effective method to assess comorbid conditions in clinical

settings as well as clinical research. This questionnaire consists of a total of 12 defined medical problems and 3 optional medical conditions. An individual can score a maximum of 3 points for each medical condition: 1 point for the presence of the condition, another point if the individual receives treatment for it, and an additional point if the problem causes a limitation in functioning. A maximum total of 45 points can be scored.

The SCQ is a reproducible measure of comorbid conditions that has moderately strong associations with a standard medical record-based comorbidity measure (Sangha et al., 2003).

2.6. Quantitative sensory testing (QST)

QST measures can be applied at different anatomical sites (Rolke et al., 2006). An examiner systematically applies the mentioned stimuli to an anatomical site until the participant indicates sensation perception or sensation of pain. In particular, widespread mechanical and cold hyperalgesia have been found to be important clinical features of many neuropathic pain states (Maier et al., 2010). OA as well as PFG participants were tested with an extensive battery of quantitative sensory testing (QST) measures. Sensory detection thresholds as well as pain thresholds were recorded. The sensory measures recorded included heat detection, cold detection, vibration perception and tactile detection thresholds. Pain related measures included heat pain thresholds, cold pain thresholds and pressure pain thresholds. Testing was conducted with participants lying comfortably on a plinth. QST was carried out in three areas around each knee, the anteromedial aspect of the knee, the lateral aspect of the knee and the popliteal fossa, as these are three areas of distinct innervation (section 1.4), which was important for the purposes of this study. Control measures were obtained over the lateral aspect of the elbow joint at the extensor carpi radialis brevis muscle in the upper limb (Moss et al., 2018; Riek et al., 2000). Overall, QST was measured at seven different sites.

QST is a reliable method to assess sensory function and has been previously used in several studies on people with knee OA and identified a range of somatosensory abnormalities in that population (Hochman et al., 2013; Moss et al., 2018; Moss et al., 2016; Wright et al., 2015; Wylde et al., 2011).

2.6.1. Heat and cold detection

Heat or warmth detection threshold (HDT) was measured using a Peltier thermode (Medoc, Israel) and standard 'Method of Limits', which is widely reported in the literature (Rolke et al., 2006). A 30 x 30 mm contact probe was attached to the test site. When the device was activated the thermode temperature was increased at a rate of 1°C/sec. Participants were instructed to depress the hand-held switch when they first perceived an increase in warmth. The threshold temperature was recorded. For each of the test sites, one practice was followed by 3 tests. Each trial was separated by a randomly assigned pause of between 3 and 6 seconds. The mean HDT value was calculated and used for further analysis.

Cold detection thresholds (CDT) were also measured with the Medoc Peltier thermode using a similar method as for HDT (baseline 32°C to 1°C/s decrease). This method is found to be an effective means of assaying cold sensitivity (Allchorne et al., 2005). Participants were asked to press the control switch as soon as they perceived a change towards a decrease or colder temperature. Three measurements were obtained for CDT. An average of these three observations was used as CDT for that particular site.

HDT and CDT were obtained at the medial, lateral and posterior aspects of both knees and the forearm test site. Intra-rater reliability of these ranged between 0.78 and 0.97 (Rolke et al., 2006). Both have been used in OA participants before (Hochman et al., 2013; Moss et al., 2018; Moss et al., 2016).

2.6.2. Heat and cold pain thresholds

After heat and cold perception testing, heat and cold pain thresholds were recorded using the Medoc Thermode. Using the baseline temperature of 32°C the temperature was increased, and participants were asked to press the control switch as soon as they perceived the sensation changing from one of warmth to painful heat. This temperature was recorded as the heat pain threshold (HPT). Variable inter-stimulus intervals were used, and triplicate measures obtained. A maximum cut-off temperature of 50°C was used to avoid any risk of tissue damage.

Cold pain threshold (CPT) was recorded in a similar manner with temperature decreasing at a rate of 1°C/s. Participants were asked to activate the control switch when they perceived the sensation changing from one of cold to one of painful cold. Triplicate measures were obtained and the machine had a baseline cut-off temperature of 0°C.

2.6.3. Tactile detection threshold

Tactile detection threshold (TDT) was assessed using standard von Frey filaments (Aesthesio® USA) at each of the test sites (Figure 3).

For TDT the staircase method was applied; probe tips were applied in ascending and then descending order of magnitude. TDT is defined as the smallest tip (g) perceived as a touch sensation. A practice plus three readings at a standardized position at seven test sites were taken (three at each knee plus the forearm). The average of the three test readings was used for analysis.

Figure 3: Von frey filaments (Aesthesio® USA) used for tactile assessment.

2.6.4. Vibration threshold (VT)

Vibration threshold (VT) was assessed using a Vibrameter (Somedic AB, Sweden), Figure 4.

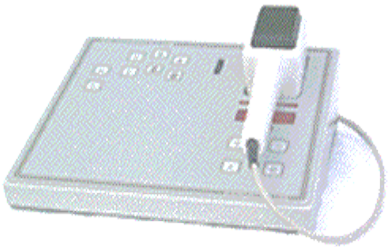


Figure 4: Digital Vibrameter (Somedic AB).

As for other QST measures, vibration threshold was measured at seven different sites.

Participants were asked to lie down on their back to test VT on extensor carpi radialis brevis (ECRB). They were then asked to move on their right side to measure VT on their right medial and left lateral knees and then on their left side to measure vibration on left medial and right lateral knees. Participants were then asked to lay on their tummy to test vibration on the right and left popliteal fossae. The vibrameter was applied at a standard pressure equivalent to the probe weight (650 g) and the vibration amplitude was gradually increased to the point where the participant could perceive vibration. Participants were instructed to indicate when they first perceived vibration by saying "now" which is recorded as vibration perception threshold (VPT). The device was then activated at a higher amplitude and was gradually decreased to the point where the vibration was no longer felt. This was recorded as vibration disappearance threshold (VDT). One practice and three trials were carried out at each site. VT was determined using the following formula, VT = [(VPT1+VPT2+VPT3)/3] + [(VDT1+VDT2+VDT3)/3]/2. This methodology has shown moderate to good reliability in the literature (ICC (2,1) 0.8) (O' Conaire et al., 2011)

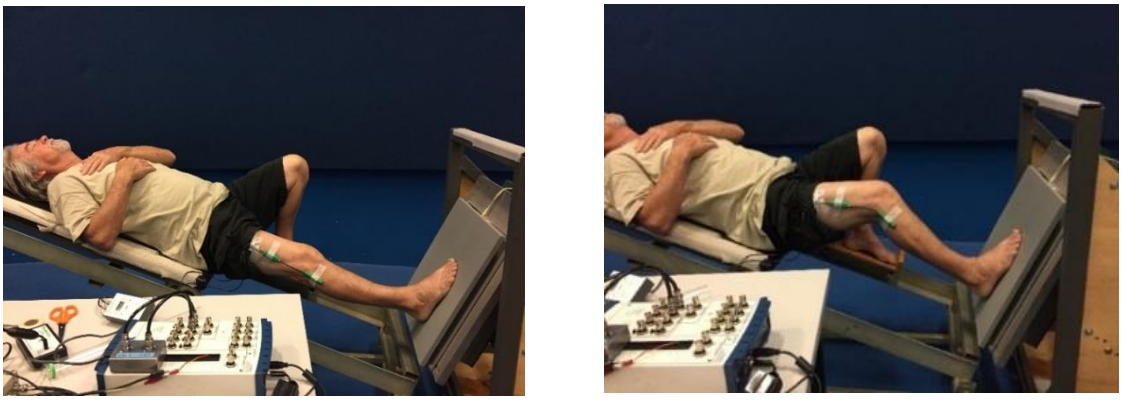
2.6.5. Pressure Pain Threshold

Pressure pain threshold (PPT) was measured using a digital pressure algometer (Somedic AB, Sweden). The 1-cm^2 algometer probe was applied at a 90^o angle to the skin at a ramp of 40 kPa/s. Participants were asked to depress the hand-held switch as soon as the pressure sensation became painful. The mean of three trials at each site was calculated and used for analysis. PPT was defined as the lowest stimulus intensity at which a subject perceives mechanical pain. The test has good to excellent test/retest reliability, ICC = 0.94-0.97 over four consecutive days (Jones et al., 2007), and ICC = 0.60-0.90 (Jakorinne et al., 2018).

2.6.6. Proprioceptive function

Participants were tested for proprioceptive function by using joint reposition sense. Knee joint angle was measured using an electro-goniometer (Penny and Giles Co, England), which was aligned with the tibia and femur of the knee being tested and attached with double sided adhesive tape. Both knees were tested with the OA subject's non-affected knee tested first. Participants were positioned comfortably with their back fully supported on a 30^o reclined sliding platform and with their non-test limb supported by another platform. Participants were given instructions to move down to a pre-determined angle. Participants were asked to focus

on the target angle for 5 seconds before they were passively elevated back to neutral by the researcher. Participants were then instructed to move back to the previous position and inform the researcher when they believed they had reached the target position, Figure 5.



(a) (b)

Figure 5: Joint reposition error assessment set up. (a) electro-goniometer attached to the right knee at neutral position, (b) at knee flexion.

The knee angle was recorded at that position by pressing a control switch. The participants were then asked to support their weight on both feet to avoid any memory of the previous target angle. The procedure was then repeated with a new target angle until a total of 12 trials were completed. A total of 6 measurements at two target angle ranges, between 15-20^{o} knee flexion and 35-40^{o} knee flexion were obtained. The order of testing was randomised by a computer program for each participant. Joint position sense was assessed as the accuracy with which subjects could reposition to a target angle (position). The difference between the target angle and the reposition angle is known as reposition error. The mean reposition error and standard deviation of reposition errors for 6 measures in each target range were calculated and used for analysis.

Proprioceptive testing and all QST measures were carried out on both osteoarthritis (OA) and pain free (PFG) participants. The following measures were carried out only on participants with knee OA.

2.7. MRI

Each OA participant was referred for an MRI to a cooperating SKG Radiology centre. The MRIs were viewed and scored for the presence of key pathological features using established protocols described in the literature (Kornaat et al., 2005).

SKG's InteleViewer software Version 4-6-1-P171 Mac OS X (Intelerad Inc. Montreal, Canada) was used for semi quantitative analysis of the following measures (Felson, 2005; Kornaat et al., 2005; Peterfy et al., 2006).

i. Cartilage morphology
ii. Bone marrow lesions (oedema)
iii. Bone cysts
iv. Subarticular bone attrition
v. Osteophytes
vi. Medial and lateral meniscal integrity
vii. Synovitis

To score the above lesions the knee joint was divided in the following compartments.

- Medial and lateral femoral
- Medial, central and lateral tibial
- Medial or lateral patella

A detailed description of the scoring methods is included in Chapter 7.

2.8. Biomarkers of inflammation and nerve related injury

Blood samples were obtained from all OA participants using standard phlebotomy techniques. Each sample was dated and labelled with each participant's code number. Samples were centrifuged and serum was collected with a pipette. This serum sample was then stored at -20^{o}C in a freezer at the School of Pharmacy and Biomedical Sciences. When all samples were collected at the end of recruitment of OA participants they were analysed. Samples were analysed for C Reactive Protein (CRP), Interleukin-1 (Il-1), Interleukin-6 (Il-6), nerve growth factor (NGF) and Tumour Necrosis Factor alpha (TNFα). All analyses for IL-1, IL-6, TNFα and NGF were carried out in the School of Pharmacy and Biomedical Sciences using standard enzyme-linked

immunosorbent assays (ELISA). ELISA is a commonly used analytical biochemistry assay. The assay uses a solid-phase enzyme immunoassay (EIA) to detect the presence of a protein in a liquid sample using antibodies directed against the protein to be measured.

CRP analysis was carried out at the R3Gen lab as per the Royal College of Pathology Australia (RCPA) requirements, using an Immunoassay method (Immuno turbidimetric analyses method) which is the main technique for performing routine protein tests. The analytical method used provided a full range of CRP readings covering a measuring range of 0.263-184 mg/L and was calibrated using a 6 point calibration method to ensure accuracy of analyses, and a precision test to ensure instrument stability.

2.9. Data Analysis

IBM® SPSS version 26 was used for analyses with alpha set at 0.05.

2.9.1. Normality test

The Shapiro-Wilk test as well as graphic assessment was performed to check for normality of all test data.

HPTM, HPTL, HPTPF, CPTM, CPTL, CPTPF, HDTM, HDTL, HDTPF, CDTM, CDTL, CDTPF, tests of the index and non-index knees and forearm showed normal distribution with low skewness values. Therefore, parametric analysis was applied to these dependent variables. However, WOMAC total score, WOMAC pain, WOMAC stiffness, WOMAC function, PQASP, PQASS, PQASD, JRE15°, JRE35°, PPTM, PPTL, PPTPF, VTM, VTL, VTPF, TM, TL, TPF of the index and non-index knees, VTECRB, PPT ECRB, CDTECRB(Arm), TFECRB, PCS, DASS, PSQI and Comorbidity data, were highly skewed, therefore, non-parametric statistics (Mann-Witney *U* test, Kruskal-Wallis test) were applied to these test data.

2.9.2. Index knee and non-index knee

For OA participants the painful knee or the knee with the worst pain was called the index knee. In control participants the knee with the worst scores for a measure was called the index knee and the knee with the better score was called the non-index knee.

2.9.3. Process to identify sensory deficits among OA patients

Data from the control group were used to determine sensory deficits in OA participants. Mean and standard deviation (SD) of sensory measures, heat, cold, tactile and vibration perception thresholds were used to calculate Z-scores. GEOMEAN of VT, Tactile, HDT and CDT was calculated from the original 3 values of each measure and then log transformed to achieve a secondary normal distribution. Mean and SD of the log transformed data were calculated which was then converted back to base$_{10}$ to convert it back to the original units.

$$Z\ score = \frac{(\mathrm{X, OA}\ participant - \mathrm{Mean}, pain\ free\ controls)}{\mathrm{SD}, pain\ free\ controls}$$

OA participants with mean values ≥ 1.96 Z-score (95%) different to the control group mean were identified as having deficit for that measure.

2.9.4. Process to identify pain phenotypes among OA patients

One of the objectives of this research project was to identify if two distinct pain phenotypes, neuropathic pain and inflammatory pain, can be demonstrated among people with OA of the knee. In light of Treede's grading system of definite, probable and possible neuropathic pain. Diagnosis of neuropathic pain requires evidence of sensory deficit and a relevant pathological process. Participants were assessed as possible neuropathic pain (PoNP) based on PD questionnaire score with report of symptoms electric shock, burning, hypersensitivity or sensory deficit and probable neuropathic pain (PrNP) based on PD questionnaire score, specific symptom location and a related sensory deficit determined by calculating Z-scores. OA participants on the pain mapping diagram reported electric shock, hypersensitivity, burning and cold/numbness, which are potential features of neuropathic pain. Sensory deficit for a particular measure of heat, cold, tactile and vibration thresholds was defined as ≥ 1.96 Z-score deviation from the mean of the control group.

Participants with OA were initially grouped based on PainDETECT questionnaire scores and findings from the clinical examination and pain mapping. Participants with a PainDETECT score of ≥ 13 and reporting superficial pain, cutaneous sensitivity, burning, electric shock or numbness over either the medial, lateral or posterior aspects of the knee joint were classified in the possible neuropathic pain group (PoNPG). All remaining OA participants were classified in the

inflammatory pain group (IPG). Participants in the PoNPG were further classified based on the findings from QST sensory testing. If their measures for any of the sensory tests were outside ± 1.96 Z-score from the mean of the pain free control group they were classified as having a sensory deficit. OA participants with deficit in any of the sensory measures and the presence of a related unusual symptom/sensation in the same area, along with a PainDETECT score of ≥ 19 were classified as probable neuropathic pain (PrNPG), Table 1.

Table 1: OA groups.

	PainDETECT score	Pain mapping report
IPG	PainDETECT score ≤ 12	
PoNPG	PainDETECT score ≥ 13	Either reported additional sensations or sensory deficit.
PrNPG	PainDETECT score ≥ 19	Cutaneous hypersensitivity, heat, cold and electric shock on pain mapping app along with sensory deficit.

2.9.5. Logistic regression models

Data from all the parameters were used to develop regression models to find which measures were most strongly related to inclusion in each of the diagnostic groups.

2.9.6. ROC curve analysis

A receiver operating characteristic curve, or ROC curve, was developed between sensitivity and 1-specificity to understand cut-off points at which a parameter can be classified as a point of interest (OA pain groups).

2.9.7. Sensitivity and specificity

Sensitivity and specificity were calculated to assess the ability of a binomial logistic regression model to correctly classify OA groups. All of these measures were calculated based on a cut-off point of 0.5 (50%), meaning that a participant had a predicted probability of being included in the IPG or the neuropathic pain group (NPG).

A Logistic regression model was developed for each independent category of tests which included self-report questionnaires, NP symptom report on pain mapping, sensory and pain QST measures, and MRI structural abnormalities (described elsewhere in this document). Groups of covariates that were significant in the final multivariable models previously described for diagnostic test predictors were entered into an overall multivariable logistic regression model to find the best diagnostic predictor outcomes of NP.

All the data collection and analysis was performed by myself except blood samples were analysed in the biomedical laboratory by one of the staff member in the lab.

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3. Proprioception, somatosensory sensation and pain sensitivity of knee osteoarthritis and pain free participants

3.1. Background

The IASP task force in 2018 defined pain as an unpleasant sensory and emotional experience associated with actual or potential tissue damage (Raja et al., 2020). Chronic pain is defined as when pain persists beyond the normal tissue healing time which is anticipated as when it exceeds 3 months (IASP, 1986).

Osteoarthritis (OA) is a leading cause of chronic joint pain and functional disability in older people (Tsang et al., 2008). OA pain is traditionally considered as an nociceptive pain due to local inflammation (Mease et al., 2011). However, recent studies suggest that up to 30% of OA patients present with features of neuropathic pain (NP), (Hochman et al., 2011; Moreton et al., 2015; Ohtori et al., 2012; Wright et al., 2017; Wright et al., 2015).

Quantitative sensory testing (QST) has been used for the assessment of sensory function in knee OA (Hochman et al., 2013; Wright et al., 2017). Sensory measures including cold, heat, vibration and tactile detection thresholds and pain thresholds for pressure, heat and cold are used in research and sometimes in clinical settings (Moss et al., 2018; Moss et al., 2016; Wright et al., 2015; Wylde et al., 2012).

Proprioceptive function is another measure to assess sensorimotor function. Deficits of proprioceptive function may reflect a neuropathy affecting myelinated afferent nerve fibres. It is known that myelinated nerve fibres contribute to both proprioception and vibration sensation (Gilman, 2002).

Hypotheses

1. OA participants will show greater deficits in joint reposition error (JRE) compared to the pain free control group (PFG).

2. OA participants will exhibit greater levels of hyperalgesia and hypoesthesia(based on QST measures) than the PFG.

3. OA participants will exhibit reduced levels of sleep quality and increased psychosocial distress compared to the PFG.

This study focusses on the following aims

Aims

- To evaluate sensory and pain thresholds using QST, joint reposition error (JRE), self-reporting psychosocial function, sleep quality and comorbidities and compare them between OA and pain free controls.
- To evaluate sensory deficit among OA participants using sensory data from pain free controls.

3.2. Methods

Potential volunteers were first screened over the phone. Participants were examined by the study Rheumatologist and the researcher and were asked to complete a series of questionnaires as detailed in chapter 2. After their first appointment at the clinic, participants attended two laboratory testing sessions. During the first session quantitative sensory testing (QST) was performed in the pain laboratory at Curtin University, which included a battery of tests for sensory function as well as measures of cold, heat and pressure pain thresholds. On the subsequent day proprioceptive testing was carried out in the motion analysis laboratory at Curtin University.

Sensory and pain thresholds, psychosocial measures, sleep and comorbidities were assessed in OA as well as in control (PFG) participants. The pain free group (PFG) participants were recruited to establish normal baseline data for QST and proprioception measures. Sensory measures (heat, cold, tactile, vibration detection) and pain thresholds (heat, cold and pressure pain) were tested at three sites (medial, lateral, and popliteal fossa) around each knee and at the forearm over the extensor carpi radialis brevis (ECRB) muscle, as a control site (section 1.4) (Moss et al., 2018).

Proprioception tested using knee joint reposition angle (JRE) in partial weight bearing was measured using an electro-goniometer at two ranges of knee flexion angles, between 15-20^{o} and 35-40^{o} (chapter 2.4.6).

Data from the control group were used to determine sensory deficits in OA participants. Mean and standard deviation (SD) of sensory measures, heat, cold, tactile and vibration perception thresholds were used to calculate Z-scores (detail section in 2.8.3). OA participants with ≥ 1.96 Z-score (95%) deviation from the control group mean were identified as having deficit for that measure.

3.2.1. Data analysis

IBM® SPSS version 26 was used for analyses.

Average of the three tests at each site was used for further analysis.

The Shapiro-Wilk test as well as graphic assessment was performed to check normality of the data. Heat and cold detection and pain threshold tests of the index and non-index knees and forearm showed normal distribution. Therefore, parametric analysis, independent sample t-test was applied to these variables. However, joint reposition errors, vibration threshold, tactile detection threshold, pressure pain thresholds, DASS, PSQI and Comorbidity data, were highly skewed, therefore, non-parametric statistics (Mann-Witney *U* test, Kruskal-Wallis test) were applied to these test data.

3.3. Results

3.3.1. Demographics

Through the recruitment process, a total of 240 people were screened via phone call interview, out of which 110 with osteoarthritis (OA) and 38 pain free group (PFG) participants were selected and were considered suitable according to the inclusion criteria and who agreed to participate in the study. Of the selected participants, 104 OA and 38 control participants attended the clinic for their first appointment. Two OA and three control participants were found not to be suitable following the clinical examination (according to the inclusion criteria) and three OA participants did not continue after their first session due to personal reasons.

Therefore, data from 99 OA and 35 control participants were available for analysis. Among OA participants 46 were male and 53 females, and in the PFG group there were 17 male and 18 female participants. Average age of OA participants was 64.3 ±8 years and of control participants 64.6 ±7 years. BMI in the OA group (28.9 ±6) was significantly higher (p = 0.02) compared to that in the PFG group (24.9 ±4) (Table 1).

Table 1: Demographics.

Participants	**Male**	**Female**	**Total**
Control	17 (49%)	18 (51%)	35
OA groups	46 (46%)	53 (54%)	99
Control/OA	17/46	18/53	134

	Control	**OA**	p-value
Age	64.3±8	64.6±7	0.52
BMI	24.9±4	28.9±6	0.02

3.3.2. Joint reposition error (JRE)

A total of 12 trials were performed, 6 measurements at each of the two target angles, in the ranges of 15-20° and 35-40° knee flexion. JRE was calculated as the difference between the target angle and the reposition angle.

Results indicated that JRE was significantly higher ($p < 0.001$) in OA participants compared to that in pain free participants (control). This was observed at both 15-20° and 35-40° knee flexion for index and non-index knees (Table 2, Figure 1).

Table 2: Joint reposition error (JRE) at 15-20° and 35-40° knee flexion of Control and OA.

	Control	**OA**	p-value
	Median (IQR) [min-max]		
15-20° index knee	2.0 (1.4-2.5) [0.7-7.3]	5.3 (3.7-7.0), [1.4-15.7]	< 0.001
35-40° index knee	1.5 (1.0-2.2) [0.5-5.7]	3.9 (2.7-5.1) [0.8-9.3]	< 0.001
15-20° non-index knee	1.9 (1.1-2.4) [0.7-4.4]	4.9 (3.3-6.7) [0.9-12.3]	< 0.001
35-40° non-index knee	1.4 (1.0-1.6) [0.5-4.2]	3.2 (2.8-4.3) [0.8-9.4]	< 0.001

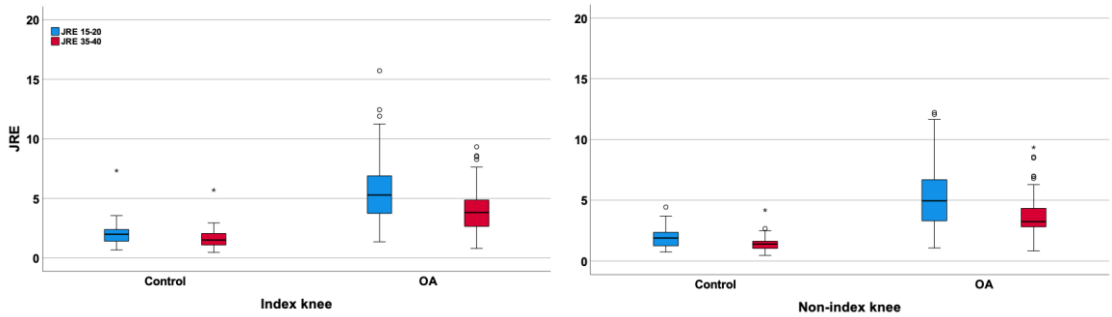

Figure 1: Comparison of JRE between control and OA at 15-20° and 35-40° knee flexion for index and non-index knee.

It was also noted (Figure 1) that JRE in the OA group and in the PFG was greater at 15-20° knee flexion (nearly straight) compared to that in mid-range flexion (35-40°).

3.3.3. Heat detection threshold (HDT)

In response to heat stimulation for both the index and non-index knees, OA participants had significantly higher detection thresholds than healthy participants at the knee as well as at the forearm ($p < 0.05$) (Table 3, Figure 2). This indicates generalised thermal hypoesthesia to warmth. There appeared to be more variability in OA data compared to the PFG data.

Table 3: Heat and cold detection threshold for Control and OA at medial (HDTM), lateral (HDTL) and popliteal fossa (HDTPF) of the index and non-index knee as well as forearm (HDT ECRB).

	Control	**OA**	p-value
	Mean (std) [min-max]		
HDTM index knee	34.6 (0.6) [33.6-36.4]	35.6 (1.9) [33.6-43.2]	0.003
HDTL index knee	35.6 (1.9) [33.7-44.8]	37.0 (2.1) [33.9-43.0]	0.001
HDTPF index knee	35.1 (0.8) [33.6-37.1]	36.1 (1.8) [33.5-47.1]	0.001
HDTM non-index knee	34.5 (0.8) [33.6-37.5]	35.3 (1.7) [32.5-42.8]	0.010
HDTL non-index knee	35.4 (1.7) [28.8-39.8]	36.9 (2.3) [34.1-46.2]	0.001
HDTPF non-index knee	35.2 (1.6) [33.4-42.3]	36.0 (1.5) [34.1-43.1]	0.014
HDT ECRB	34.7 (0.8) [33.3-36.8]	35.1 (1.0) [33.3-39.7]	0.039

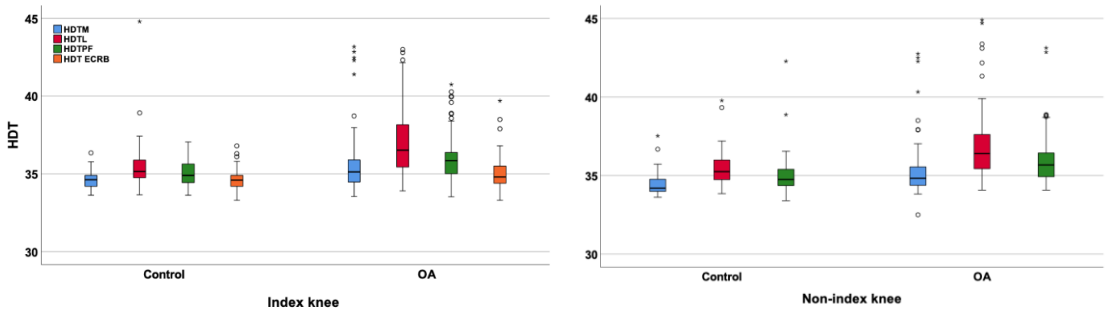

Figure 2: Heat and cold detection threshold between control and OA participants at medial (HDTM), lateral (HDTL) and popliteal fossa (HDTPF) of the index and non-index knee as well as forearm (HDT ECRB).

3.3.4. Cold detection threshold (CDT)

In response to cold stimulation, for both the index and non-index knees, OA participants had significantly lower detection thresholds than healthy participants at the knee as well as at the forearm ($p < 0.05$) (Table 4, Figure 3). This indicates generalised cold hypoesthesia. There appeared to be more variability in OA data compared to the PFG data.

Table 4: Cold detection threshold for control and OA participants at medial (HDTM), lateral (HDTL) and popliteal fossa (HDTPF) of the index and non-index knees as well as the forearm (HDT ECRB).

	Control	**IPG**	p-value
	Mean (std) [min-max]		
CDTM index knee	29.8 (0.8) [28.0-30.8]	29.4 (0.9) [26.1-31.1]	0.026
CDTL index knee	29.5 (1.0) [27.5-31.1]	29.1 (1.0) [26.5-30.7]	0.028
CDTPF index knee	29.8 (0.7) [28.3-30.9]	29.0 (1.0) [24.7-30.9]	< 0.001
CDTM non-index knee	30.0 (0.7) [28.2-31.0]	29.4 (0.9) [26.6-31.2]	< 0.001
CDTL non-index knee	29.5 (1.1) [26.9-31.2]	29.0 (1.1) [24.2-30.9]	0.036
CDTPF non-index knee	29.8 (1.0) [25.9-31.0]	29.1 (1.2) [23.2-31.4]	0.005
CDT ECRB	30.3 (0.7) [28.3-31.3]	29.5 (1.2) [23.4-31.3]	< 0.001

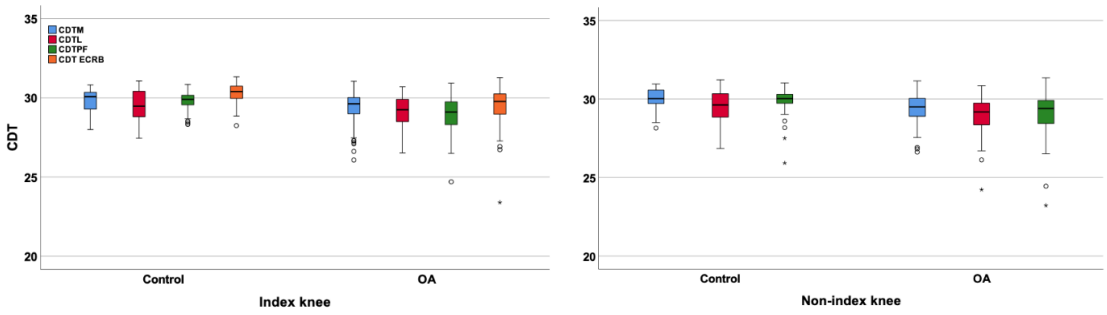

Figure 3: Comparison of cold detection threshold between control and OA participants at medial (HDTM), lateral (HDTL) and popliteal fossa (HDTPF) sites of the index and non-index knee as well as the forearm (HDT ECRB).

3.3.5. Vibration threshold (VT)

Vibration thresholds for both the index and non-index knees were significantly higher ($p < 0.001$) in the OA group at each of the medial, lateral, popliteal fossa and forearm sites compared to the control group (Table 5, Figure 4). This indicates local and distant vibration hypoesthesia.

Table 5: Vibration threshold for control and OA at medial (VTM), lateral (VTL) and popliteal fossa (VTPF) and forearm (VT ECRB) sites.

	Control	**OA**	p-value
	Median (IQR) [min-max]		
VTM index knee	5.5 (3.3-8.1) [1.4-16.2]	15.1 (10.2-21.7) [2.0-39.6]	< 0.001
VTL index knee	5.6 (3.7-9.2) [2.0-16.8]	15.6 (10.1-21.5) [1.3-37.4]	< 0.001
VTPF index knee	5.0 (3.9-8.5) [2.0-15.2]	12.7 (7.2-19.0) [1.4-42.9]	< 0.001
VTM non-index knee	5.5 (4.1-8.7) [1.4-16.5]	13.5 (8.1-21.9) [1.9-39.7]	< 0.001
VTL non-index knee	5.7 (3.8-9.3) [1.9-16.5]	14.1 (9.1-20.4) [1.7-38.2]	< 0.001
VTPF non-index knee	5.8 (3.4-8.6) [1.3-29.4]	10.7 (7.3-17.2) [1.1-37.0]	< 0.001
VT ECRB	4.1 (3.4-6.0) [1.9-15.0]	7.2 (5.7-11.9) [1.9-27.9]	< 0.001

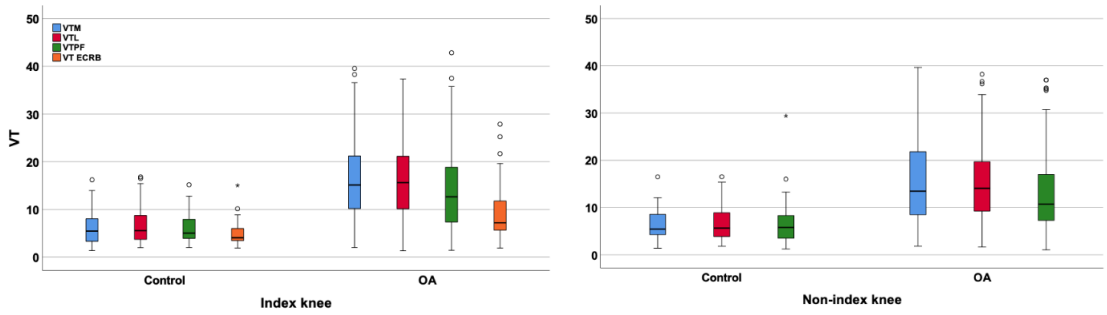

Figure 4: Comparison of vibration threshold between control and OA participants at medial (VTM), lateral (VTL) and popliteal fossa (VTPF) and forearm (VT ECRB) sites.

3.3.6. Tactile threshold

OA participants had significantly higher median light touch thresholds than healthy participants, at both knees ($p < 0.001$) and at the forearm site ($p = 0.008$), indicating local and distant tactile hypoesthesia (Table 6, Figure 5).

Table 6: Tactile detection threshold for control and OA participants at medial (TM), lateral (TL) and popliteal fossa (TPF) sites of the index and non-index knee and at forearm (TP ECRB).

	Control	**IPG**	p-value
	Median (IQR) [min-max]		
TM index knee	0.2 (0.1-0.3) [0.1-0.7]	0.4 (0.2-0.7) [0.1-19.6]	< 0.001
TL index knee	0.2 (0.2-0.4) [0.1-0.7]	0.7 (0.4-1.6) [0.1-39.2]	< 0.001
TPF index knee	0.2 (0.1-0.2) [0.1-0.5]	0.4 (0.2-0.7) [0.1-13.7]	< 0.001
TM non-index knee	0.2 (0.1-0.3) [0.1-0.8]	0.4 (0.2-0.7) [0.1-19.6]	< 0.001
TL non-index knee	0.2 (0.2-0.4) [0.1-0.7]	0.7 (0.2-0.7) [0.1-19.6]	< 0.001
TPF non-index knee	0.1 (0.1-0.2) [0.1-0.7]	0.4 (0.2-0.7) [0.1-19.6]	< 0.001
TP ECRB	0.1 (0.1-0.2) [0.1-0.4]	0.2 (0.1-0.2) [0.0-3.9]	0.008

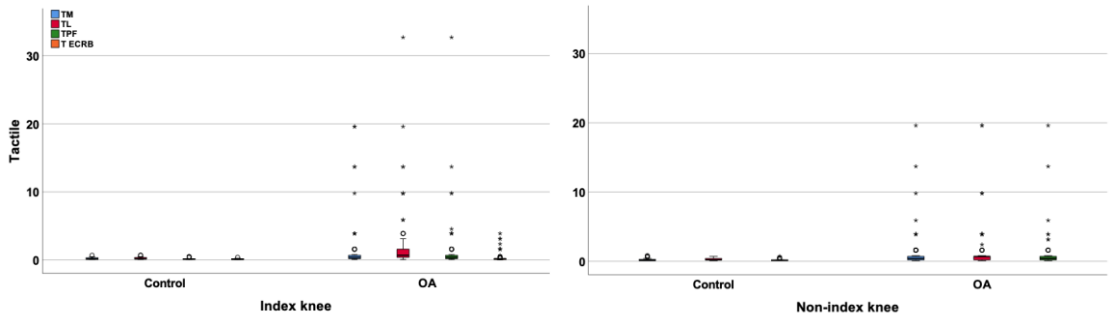

Figure 5: Comparison of tactile detection threshold between control and OA participants at medial (TM), lateral (TL) and popliteal fossa (TPF) sites of the index and non-index knee and at the forearm (TP ECRB).

3.3.7. Sensory deficit among OA patients

A large number of OA participants showed sensory deficits (Table 7). Z-score analysis revealed cold hypoesthesia was present in 15.0% of OA participants at the medial, 8.0% at the lateral and 18.0% at the posterior sites on the index knee. On the non-index knee cold hypoesthsia was present in 18.0% on the medial side, 12.0% on the lateral side and 18.0% at the posterior site.

Table 7: Participants (%) showing sensory deficit at medial, lateral and posterior of index and non-index knee for heat, cold, vibration, tactile and proprioception.

		% Z scores	
		Index Knee	Non-index Knee
Medial	Heat	22	19
	Cold	15	18
	Vibration	45	39
	Tectile	32	29
Lateral	Heat	26	13
	Cold	8	12
	Vibration	47	37
	Tectile	31	25
Posterior	Heat	11	13
	Cold	18	18
	Vibration	31	29
	Tectile	27	22
Proprioception	15-20^{O}	49	49
	35-40^{O}	51	34

Z-score deviation for heat was present in 22.0% of OA participants on the medial side, 26.0% on the lateral side and 11.0% at the posterior aspect of the index knee. On the non-index knee 1 Z-score heat hypoesthesia was present in 19.0% of participants on the medial side, 13.0% on the lateral side and 13.0% at the posterior site.

Z-score analysis revealed vibration hypoesthesia was present in 45.0% of OA participants at the medial, 47.0% at the lateral and 31.0% at the posterior sites on the index knee. On the

non-index knee cold hypoesthsia was present in 39.0% of participants on the medial side, 37.0% on the lateral side and 29.0% at the posterior site.

Z-score analysis revealed tactile hypoesthesia was present in 32.0% of OA participants at the medial, 31.0 % at the lateral and 27.0% at the posterior site on the index knee. On the non-index knee cold hypoesthsia was present in 29.0% on the medial side, 25.0% on the lateral side and 22.0% at the posterior site.

Z-score analysis revealed JRE deviation was present in 49.0% of OA participants at the index and non-index knee for 15-20^{o} knee flexion and 51.0% at the index knee and 34.0% at the non-index knee for 35-40^{o} knee flexion. Half of all OA participants showed compromised proprioceptive function.

3.3.8. Heat pain threshold (HPT)

No difference was found in heat pain thresholds (HPT) in OA participants and control participants at the medial, lateral, posterior and forearm sites at both the index and non-index knees ($p > 0.05$) (Table 8, Figure 6).

Table 8: Heat pain threshold for control and OA participants at medial (HPTM), lateral (HPTL) and popliteal fossa (HPTPF) sites of the index and non-index knee and at the forearm (HPT ECRB).

	Control	**IPG**	p-value
	Median (IQR) [min-max]		
HPTM index knee	44.8 (42.7-46.1) [39.4-49.2]	44.3 (42.4-46.5) [37.1-49.5]	0.57
HPTL index knee	44.56 (42.5-46.5) [39.0-49.6]	46.3 (44.6-47.6) [37.6-49.8]	0.99
HPTPF index knee	44.5 (42.1-46.4) [38.0-49.5]	44.8 (43.3-46.5) [37.7-49.6]	0.35
HPTM non-index knee	44.9 (41.6-46.9) [38.1-48.6]	43.9 (42.1-46.2) [35.7-49.4]	0.54
HPTL non-index knee	44.69 (43.2-45.9) [39.7-50.0]	46.3 (43.8-47.8) [29.7-50.0]	0.29
HPTPF non-index knee	45.1 (42.6-46.5) [38.4-49.5]	45.0 (43.0-46.6) [37.6-48.8]	0.99
HPT ECRB	46.4 (43.3-47.7) [37.8-49.9]	45.2 (43.6-47.6) [38.7-50.0]	0.87

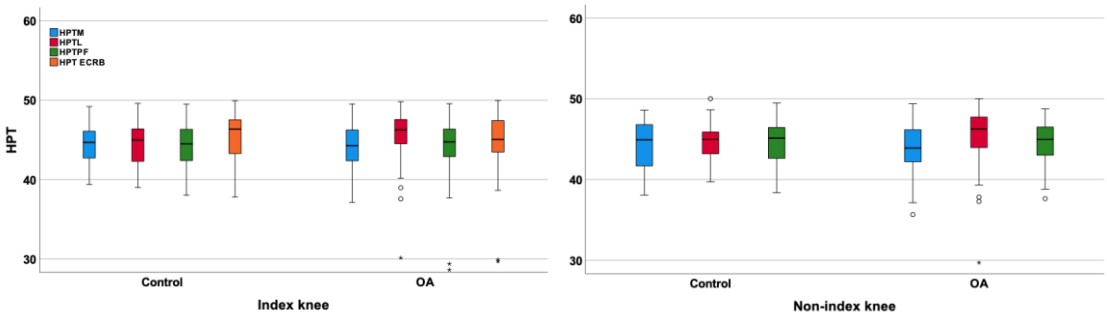

Figure 6: Comparison of heat pain threshold between control and OA participants at the medial (HPTM), lateral (HPTL) and popliteal fossa (HPTPF) sites of the index and non-index knees and at the forearm (HPT ECRB).

3.3.9. Cold pain threshold (CPT)

CPT of OA participants was significantly higher at the index and non-index knees as well as at the forearm ($p < 0.001$) (Table 9, Figure 7). Cold hyperalgesia among OA participants is evident in these results (Table 9).

Table 9: Cold pain threshold for control and OA participants at the medial (CPTM), lateral (CPTL) and popliteal fossa (CPTPF) sites of the index and non-index knee and the forearm (CPT ECRB).

	Control	**OA**	p-value
	Median (IQR) [min-max]		
CPTM index knee	13.3 (7.1-18.0) [0.0-24.1]	22.4 (18.5-24.2) [0.0-27.1]	< 0.001
CPTL index knee	15.3 (11.7-18.3) [1.0-30.6]	22.3 (19.7-23.9) [0.0-27.6]	< 0.001
CPTPF index knee	13.1 (6.0-17.8) [0.5-24.2]	21.1 (18.6-23.4) [0.0-27.4]	< 0.001
CPTM_non_indexknee	14.8 (8.6-17.4) [0.9-22.7]	21.7 (19.1-24.0) [0.0-27.0]	< 0.001
CPTL non-index knee	14.5 (8.7-17.9) [0.8-25.2]	21.5 (18.7-23.6) [0.0-27.3]	< 0.001
CPTPF non-index knee	13.2 (7.1-18.1) [0.5-23.6]	21.4 (17.0-23.7) [0.0-26.9]	< 0.001
CPT ECRB	10.7 (4.5-15.8) [0.0-23.7]	20.0 (16.8-23.4) [0.0-27.3]	< 0.001

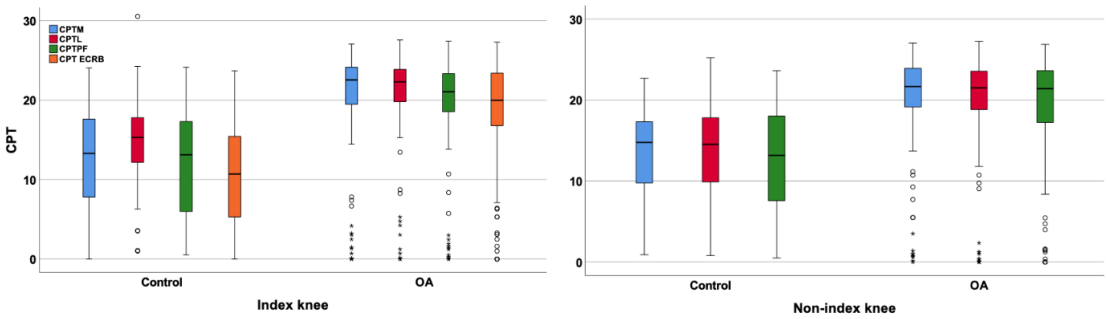

Figure 7: Comparison of cold pain threshold between control and OA participants at medial (CPTM), lateral (CPTL) and popliteal fossa (CPTPF) sites of the index and at non-index knee and the forearm (CPT ECRB).

3.3.10. Pressure pain threshold (PPT)

PPT comparison between control and OA participants for both the index and non-index knees as well as for the forearm was carried out. PPT was significantly lower ($p < 0.001$) in OA participants compared to PFG participants at all sites (medial, lateral and popliteal fossa) around the index and non-index knees as well as at the forearm (Table 10, Figure 8). PPT results indicate generalised pressure hyperalgesia (Figure 8).

Table 10: Pressure pain thresholds for control and OA participants at medial (PPTM), lateral (PPTL) and popliteal fossa (PPTPF) sites of the index and non-index knee and at the forearm (PPT ECRB).

	Control	**OA**	p-value
	Median (IQR) [min-max]		
PPTM index knee	571.3 (482.0-657.7) [227.3-1,058.0]	331.3 (229.0-429.3) [78.7-1,418.7]	< 0.001
PPTL index knee	573.3 (493.3-774.0) [303.7-1,007.3]	341.0 (240.7-492.0) [102.7-1,389.0]	< 0.001
PPTPF index knee	606.3 (532.7-807.0) [358.3-1,004.0]	383.3 (258.3-480.3) [72.0-1,371.3]	< 0.001
PPTM non-index knee	573.7 (409.7-655.7) [248.3-976.0]	354.0 (238.0-432.0) [80.3-1,266.7]	< 0.001
PPTL non-index knee	582.3 (468.0-698.7) [278.7-1,049.3]	376.0 (245.7-483.7) [81.7-1,471.7]	< 0.001
PPTPF non-index knee	590.7 (545.7-780.0) [407.0-1,002.0]	389.3 (292.7-508.3) [88.7-1,385.7]	< 0.001
PPT ECRB	453.3 (343.3-596.0) [253.0-840.7]	325.0 (250.7-420.3) [159.3-905.0]	< 0.001

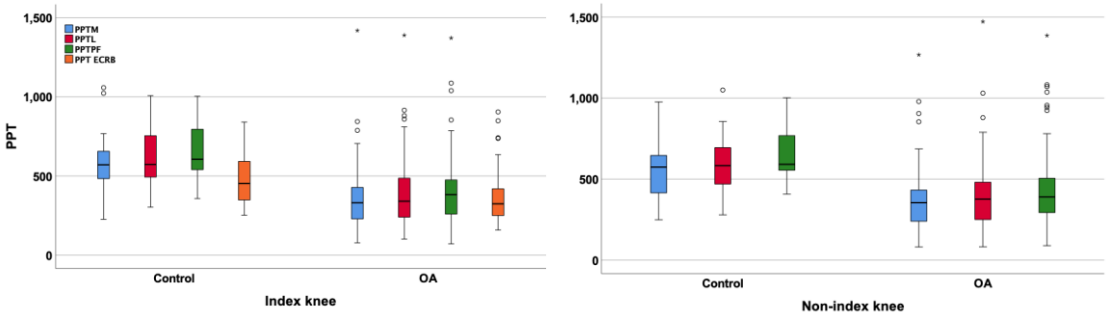

Figure 8: Comparison of pressure pain threshold between control and OA participants at the medial (PPTM), lateral (PPTL) and popliteal fossa (PPTPF) sites of the index and non-index knee and at the forearm (PPT ECRB).

It was also noted that for both the index and non-index knees there was a steeper and steady increase in PPT from PPTM to PPTL and to PPTPF in control participants compared to that in OA participants. PPT in the PFG suggests that the medial side of the knee is more sensitive to pressure.

3.4. Questionnaires

Three questionnaires were administered to both OA and control participants. OA participants scored significantly higher ($p < 0.001$) for depression and anxiety, sleep quality and comorbidities compared to the control participants (Table 11).

Table 11: DASS, PSQI and comorbidity questionnaire scores for Control and OA, median (IQR) [min-max].

	Control	**OA**	p-value
	Median (IQR) [min-max]		
DASS	2.0 (0.0-5.0) [0.0-40.0]	8.0 (4.0-25.0) [0.0-104.0]	< 0.001
PSQI	3.0 (2.0-6.0) [1.0-10.0]	6.0 (4.0-10.0) [0.0-17.0]	< 0.001
Comorbidity	0.0 (0.0-1.0) [0.0-4.0]	5.0 (3.0-7.0) [0.0-14.0]	< 0.001

3.5. Discussion

People with knee OA showed hypoesthesia to a range of somatosensory stimuli including; cold, vibration and touch. Pain thresholds were tested for heat, cold and pressure. OA participants had significantly higher pain thresholds for cold and pressure but not for heat. In line with the nerve distribution around the knee (section 1.4), QST measures were recorded at medial, lateral and popliteal fossa sites around the knee to evaluate sensory loss at the sites reported on pain mapping. QST results indicated generalized hypoesthesia and hyperalgesia (Tables 3 to 10) in the OA group. Results suggested that people with OA may have pain sensitivity and sensory changes as a result of the disease process. These results are in line with the previously reported literature (Moss et al., 2016; Wright et al., 2017; Wylde et al., 2012) and thus provide confidence in the integrity of the current study data.

QST has been extensively used to assess sensory function in the knee OA cohort. A meta-analysis of seven studies performed between OA participants and controls found that PPT was significantly lower in OA participants and had merit to further evaluate as a means to differentiate pain phenotypes among people with OA (Suokas et al., 2012). Results suggest lower PPTs in people with OA at the affected sites indicate peripheral and in the remote site central sensitisation. This study noted similar observations that generalized PPT hyperalgesia was present in OA participants (Table 10, Figure 8).

Proprioceptive function in OA participants was compromised. OA participants showed significantly higher JRE compared to that in the control group (Table 2, Figure 1). Vibratory sense is a separate yet closely related sensory pathway to proprioception and neuronal activity related to both of these measures is transmitted in parallel through the dorsal columns of the spinal cord (Gilman, 2002). Diminished proprioceptive function and higher JRE has been recognised in OA participants previously (Barrett et al., 1991). This study showed that both JRE as well as vibration threshold are compromised in OA participants, which shows the presence of a sensory deficit. Lack of proprioception sense is suggested as a factor that contributes to altered gait and imbalanced joint loading, which leads to progressive joint degeneration (Stauffer et al., 1977). In contrast to previous studies participants in this study underwent extensive and detailed assessment of somatosensory functions. There is a lack of available data on normative QST values, which in part was overcome by following strict selection criteria for the control as well as for OA participants

by excluding neurological disorders and history of limb injury or surgery in the past 6 months (section 2.1.2).

Data from the control group were used to determine sensory deficit in OA participants by calculating Z-scores (section 2.8.3). A large number of OA participants showed sensory deficits for all measures (Table 7). VT and proprioception was compromised in nearly 50% of the OA cohort. Sensory deficit in OA participants was presented previously for warm and cold perception with a similar number of OA participants showing cold and warm hypoesthesia as in this study (Table 7) (Wright et al., 2017)

Questionnaires were used to assess sleep quality, psychological function and comorbidity. Results of this study suggested sleep disruption, impairment of psychosocial function and higher comorbidities in OA participants compared to pain free participants. Previously it is known that chronic pain causes depression and anxiety (Bair et al., 2008). Depressed mood is also shown in OA participants (Gureje et al., 1998; Hochman et al., 2013; Ohayon & Schatzberg, 2003). Poor sleep quality is also related to chronic pain (Marty et al., 2008). This study confirmed previous findings of a review that chronic pain causes physical and psychological consequences and is associated with various comorbidities (Fine, 2011). The review was based on various chronic conditions i.e., OA, low back pain, spinal pain and fibromyalgia. This study demonstrated similar findings in OA patients.

In contrast to the research carried out in the past, this study included questionnaires, related sensory and pain measures (QST), along with proprioceptive functions in the same cohort. This study showed clear differences between the OA and PFG (control) cohorts in somatosensory function (Tables 2 – 10, Figures 1 – 8). The OA cohort have also shown compromised quality of life with poor sleep quality and psychosocial function and a greater number of comorbidities compared to the control group (Table 11). OA participants had greater psychosocial problems as they had higher depression, anxiety and stress scores compared to the pain free group. Psychosocial problems have also been reported before in this cohort using a different assessment questionnaire (Hochman et al., 2013).

Conclusions

Results are in line with the previous reports in the literature that OA participants have been found to experience sensory and proprioceptive deficits and increased pain sensitivity. Almost half of the OA cohort showed vibration hypoesthesia and impaired proprioceptive function. These results suggest that either prolonged inflammation or severe structural damage of the joint leads to damage to the innervation of the knee or significant central changes in the processing of sensory information. The peripheral nociceptors may be sensitized by, for example, inflamed synovium and damaged subchondral bone. It is also apparent that OA participants suffer chronic pain which may lead to sleep disruption and in turn cause psychological dysfunction or vice versa. Impaired sensory function is very common in the OA cohort.

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4. Comparison of different pain phenotypes in a knee OA cohort

4.1. Introduction

Pain is the most common symptom of osteoarthritis (OA) and forms the reason for many consultations in general practice and physiotherapy clinics among older adults. Osteoarthritis pain has traditionally been considered as nociceptive pain (Mease et al., 2011) but some OA patients present with more severe chronic pain that includes features of neuropathic pain (NP) (Hochman et al., 2011; Moreton et al., 2015; Ohtori et al., 2012).

Several neuropathic pain questionnaires have been developed based on self-report descriptors. These descriptors are related to pain qualities and sensory symptoms (e.g., burning, electric shocks, tingling, pricking, pins and needles, and painful light touch). These questionnaires have been used as a tool to distinguish NP from other types of chronic pain in clinical as well as research settings. Assessment questionnaires have been used to phenotype OA patients (Bouhassira & Attal, 2011). However, they do not provide sufficient information to establish a formal diagnosis of neuropathic pain which requires evidence of somatosensory deficit and relevant pathology (Treede et al., 2008).

PainDETECT and the Leeds assessment of neuropathic symptoms and signs (LANSS) are validated self-report questionnaires commonly used in both clinical as well as research settings (Hochman et al., 2013).

This study used PainDETECT and S-LANSS questionnaires to evaluate neuropathic pain symptoms. These two questionnaires were selected because these are self-report questionnaires and do not require clinical examination.

PainDETECT scores were used to initially group OA participants, S-LANSS scores were used to subsequently evaluate NP presentations amongst groups of OA participants. Based on the requirements identified by Treede et al (Treede et al., 2008), participants were further grouped based on data from pain and sensation mapping and detailed evaluation of sensory deficits. This process differentiated participants with knee OA into three pain phenotypes,

those with predominantly inflammatory pain, those with probable neuropathic pain and an intermediate grouping with possible neuropathic pain.

Following literature review and preliminary data from recent studies hypotheses and aims were developed, as below

Hypotheses

1. More than one pain phenotype can be identified using self-report neuropathic pain questionnaires and measures of sensory function among OA patients.
2. Neuropathic pain descriptors, pain severity, stiffness, physical disability, anxiety, depression and sleep quality will demonstrate differences between OA pain groups.

Aims

- To develop a standard methodology to differentiate different pain phenotypes
- To determine whether these phenotype groupings can be differentiated based on a range of self-report measures.

4.2. Methods

Self-report questionnaires were administered to OA participants. Questionnaires were given to the participants at the end of their first appointment to take home and complete at their leisure and bring back when they returned for their second appointment. Ninety nine (99) OA participants returned their questionnaires. The following questionnaires (Appendix 1) were given to the OA participants.

- PainDETECT
- S-LANSS
- PQAS
- WOMAC
- PCS
- PSQI
- DASS
- Comorbidity

Potential volunteers were first screened over the phone. Participants were examined by the study Rheumatologist and the researcher and were asked to complete a series of questionnaires as explained in chapter 2. After their first appointment at the clinic, participants attended two laboratory testing sessions. During the first session quantitative sensory testing (QST) was performed in the pain laboratory at Curtin University, which included a battery of tests for sensory function as well as measures of cold, heat and pressure pain thresholds. On the subsequent day proprioceptive testing was carried out in the motion analysis laboratory at Curtin University.

4.3. Data analysis

OA participants were divided into three groups. Participants were classified into the inflammatory pain group (IPG), possible neuropathic pain group (PoNPG) and probable neuropathic pain group (PrNPG) as follows (details in Chapter 2 sections 2.8.3 and 2.8.4).

- IPG PainDETECT score ≤ 12
- PoNPG PainDETECT score ≥ 13, and
  reporting of additional sensations or demonstrating a sensory deficit.
- PrNPG PainDETECT score ≥ 19, and
  Reporting additional sensations along with a related sensory deficit in the same anatomical area (medial, lateral, or popliteal).

4.3.1. Statistical analysis

IBM® SPSS version 26 was used for statistical analysis.

PainDETECT and S-LANSS data were compared using one way ANOVA and post-hoc Dunnett's test, to compare IPG (as control) to PoNPG and PrNPG. WOMAC, PQAS, PCS, DASS and comorbidity data did not meet requirements for normality (section 2.8.1) hence these were compared using the nonparametric independent samples Kruskal-Wallis test and the Mann-Witney U test. In all tests, significance level was set at ≤ 0.05.

Univariate and multivariate logistic regression was carried out to determine which questionnaires scores were associated with membership of the neuropathic pain group (NPG: combined PoNPG and PrNPG).

4.4. Results

Data from 99 OA participants were used for analysis. Among the participants, 46 were male and 53 females. Average age and BMI of the participants was 64.3 ±8 years and 28.9 ±6 respectively.

Results of questionnaires are presented as mean (SD) for PainDETECT and S-LANSS and median (IQR) for WOMAC, PQAS, DASS, PSQI, PCS, and comorbidity. Comparison of scores for the IPG and the PoNPG is shown in Table 1. Comparison of scores for the IPG and the PrNPG is presented in Table 2.

Table 1: Comparison of questionnaire responses between the IPG and the PoNPG; PainDETECT, Leeds Assessment of neuropathic symptoms and signs (S-LANSS), Western Ontario McMaster Osteoarthritis Index version VA 3.1 (WOMAC), the pain quality assessment scale (PQAS), the depression, anxiety and stress scale (DASS), the pain catastrophizing scale (PCS), Pittsburgh sleep quality index (PSQI) and the comorbidity questionnaire.

	IPG	**PoNPG**	p-value
	Mean (SD) [min-max]		
PainDETECT	10.8 (4.1) [2.0-18.0]	15.8 (2.0) [13.0-19.0]	< 0.001
S-LANSS	7.9 (4.1) [0.0-18.0]	11.1 (3.7) [2.0-19.0]	< 0.001
	Median (IQR) [min-max]		
WOMAC-T	85.5 (47.6-108) [0.0-185]	97.5 (78.9-143) [11.6-199]	< 0.001
WOMAC-Pain	15.9 (9.6-20.7) [0.0-34.6]	19.6 (14.4-28.5) [0.0-39.5]	0.024
WOMAC-Stiffness	10.0 (5.7-13.4) [0.0-16.5]	12.2 (8.2-13.8) [0.7-20.0]	0.077
WOMAC-Function	56.1 (28.2-75.9) [0.0-138]	64.5 (50.7-101) [2.4-141]	0.055
PQAS-P	6.0 (2.0-15.0) [0.0-27.0]	17.0 (11.0-23.0) [1.0-33.0]	< 0.001
PQAS-S	6.0 (1.5-11.0) [0.0-28.0]	11.0 (5.0-21.0) [0.0-32.0]	< 0.001
PQAS-D	16.0 (10.5-25.0) [0.0-34.0]	22.0 (11.0-32.5) [3.0-47.0]	< 0.001
DASS	7.0 (2.0-16.0) [0.0-45.0]	8.0 (4.5-26.5) [2.0-72.0]	0.110
PSQI	5.0 (3.5-7.5) [0.0-17.0]	8.0 (4.5-10.0) [0.0-15.0]	< 0.001
PCS	7.0 (2.5-10.0) [0.0-34.0]	7.0 (3.0-18.5) [0.0-40.0]	0.410
Comorbidity	5.0 (3.0-7.0) [0.0-12.0]	5.0 (3.0-8.0) [0.0-14.0]	0.730

Table 2: Comparison of questionnaire responses between the IPG and the PrNPG; PainDETECT, Leeds Assessment of neuropathic symptoms and signs (S-LANSS), Western Ontario McMaster Osteoarthritis Index version VA 3.1 (WOMAC), the pain quality assessment scale (PQAS), the depression, anxiety and stress scale (DASS), the pain catastrophizing scale (PCS), Pittsburgh sleep quality index (PSQI) and the comorbidity questionnaire.

	IPG	**PrNPG**	p-value
	Mean (SD) [min-max]		
PainDETECT	10.8 (4.1) [2.0-18.0]	21.4 (3.4) [19.0-33.0]	< 0.001
S-LANSS	7.9 (4.1) [0.0-18.0]	15.4 (3.2) [11.0-22.0]	< 0.001
	Median (IQR) [min-max]		
WOMAC-T	85.5 (47.6-108) [0.0-185]	145 (104-163) [68-210]	< 0.001
WOMAC-Pain	15.9 (9.6-20.7) [0.0-34.6]	27.7 (23.5-32.0) [15.8-44.3]	< 0.001
WOMAC-Stiffness	10.0 (5.7-13.4) [0.0-16.5]	12.7 (11.0-16.1) [7.9-19.6]	0.002
WOMAC-Function	56.1 (28.2-75.9) [0.0-138]	102.8 (69.9-120) [41.4-148]	< 0.001
PQAS-P	6.0 (2.0-15.0) [0.0-27.0]	24.0 (14.0-29.0) [9.0-34.0]	< 0.001
PQAS-S	6.0 (1.5-11.0) [0.0-28.0]	21.0 (13.5-34.0) [5.0-43.0]	< 0.001
PQAS-D	16.0 (10.5-25.0) [0.0-34.0]	31.0 (21.0-36.0) [11.0-41.0]	< 0.001
PCS	7.0 (2.0-16.0) [0.0-45.0]	15.0 (10.0-25.0) [3.0-42.0]	< 0.001
DASS	5.0 (3.5-7.5) [0.0-17.0]	24.0 (5.0-38.0) [0.0-104.0]	0.010
PSQI	7.0 (2.5-10.0) [0.0-34.0]	9.0 (6.0-10.5) [5.0-17.0]	0.001
Comorbidity	5.0 (3.0-7.0) [0.0-12.0]	6.0 (3.5-7.0) [3.0-12.0]	0.528

4.4.1. PainDETECT

Mean (SD) PainDETECT questionnaire scores for the IPG, PoNPG and PrNPG were 10.8 (4.1), 15.8 (2) and 21.4 (3.4) respectively (Table 1, Table 2). PainDETECT scores were significantly higher (Figure 1) in the PoNPG and the PrNPG compare to the IPG ($p < 0.001$) (Table 1, Table 2). PainDETECT scores ranged from 2-33. Thirty two participants scored 2-12, forty three participants 13-18, and twenty four OA participants scored 19-33. It was anticipated that there would be a clear differentiation between groups based on PainDETECT score since this score was used as part of the group selection process.

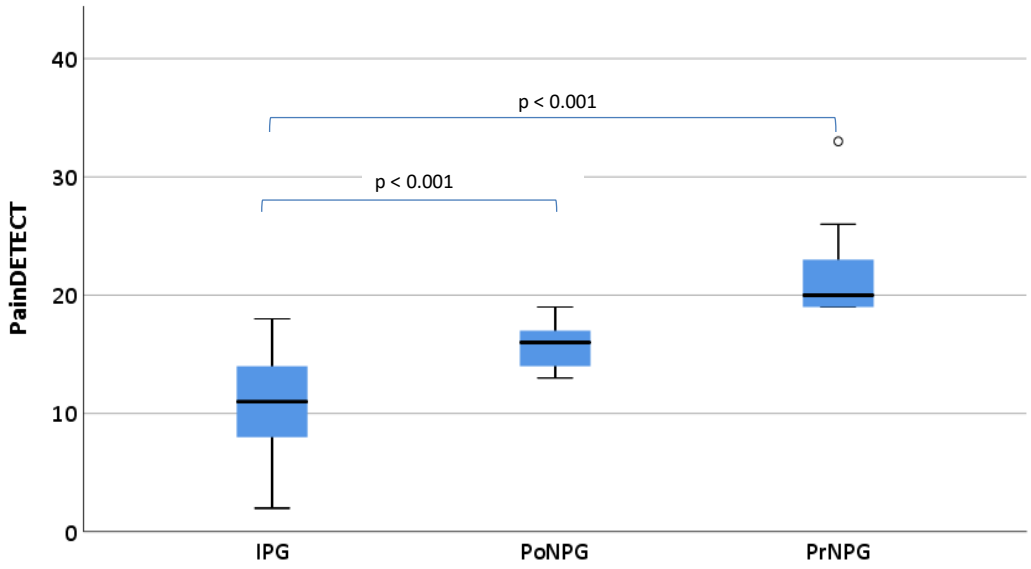

Figure 1: Comparison of PainDETECT scores between OA groups, IPG, PoNPG and PrNPG.

4.4.2. S-LANSS

Mean (SD) S-LANSS questionnaire scores for the IPG, PoNPG and PrNPG cohorts were 7.9 (4.0), 11.1(3.7) and 15.4 (3.2) respectively (Table 1, Table 2). Overall, S-LANSS scores showed an increasing trend (Figure 2) from IPG to PoNPG to PrNPG ($p < 0.001$). Between individual groups, scores were significantly higher in the PoNPG and the PrNPG compare to the IPG, returning a p-value of < 0.001 (Figure 2, Table 1, Table 2).

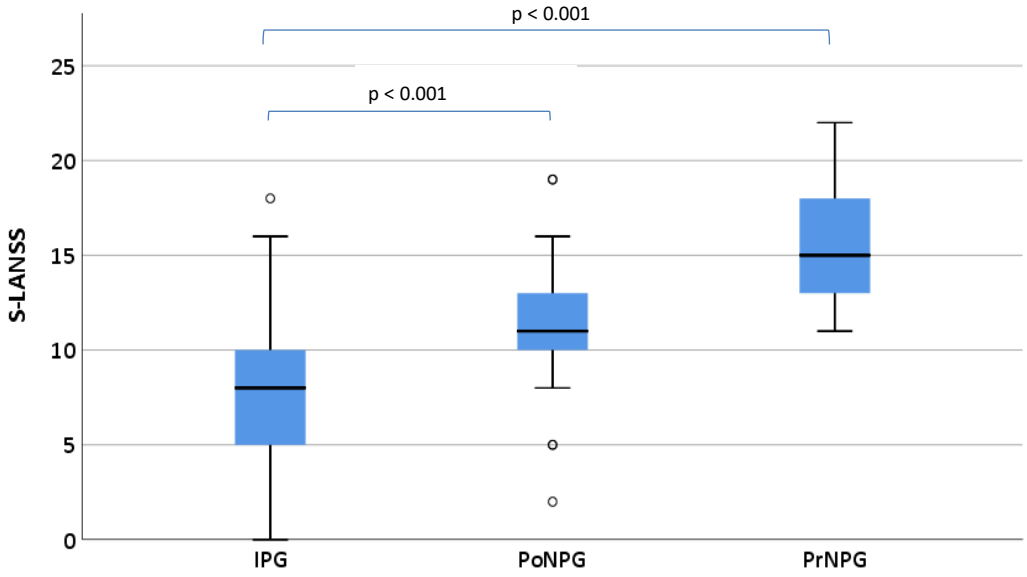

Figure 2: Comparison of S-LANSS scores between OA groups, IPG, PoNPG and PrNPG.

4.4.3. WOMAC

The median (IQR) WOMAC total scores for the IPG was 85.5 (47.6-107.7), PoNPG 97.5 (78.9-142.7) and the PrNPG 145 (104-163) (Table 1, Table 2). Overall, WOMAC total scores showed an increasing trend from IPG to PoNPG to PrNPG ($p < 0.001$) (Figure 3). Between individual groups, WOMAC total score was significantly higher in the PoNPG vs the IPG and the PrNPG vs the IPG ($p < 0.001$) (Table 1, Table 2). (Figure 3)

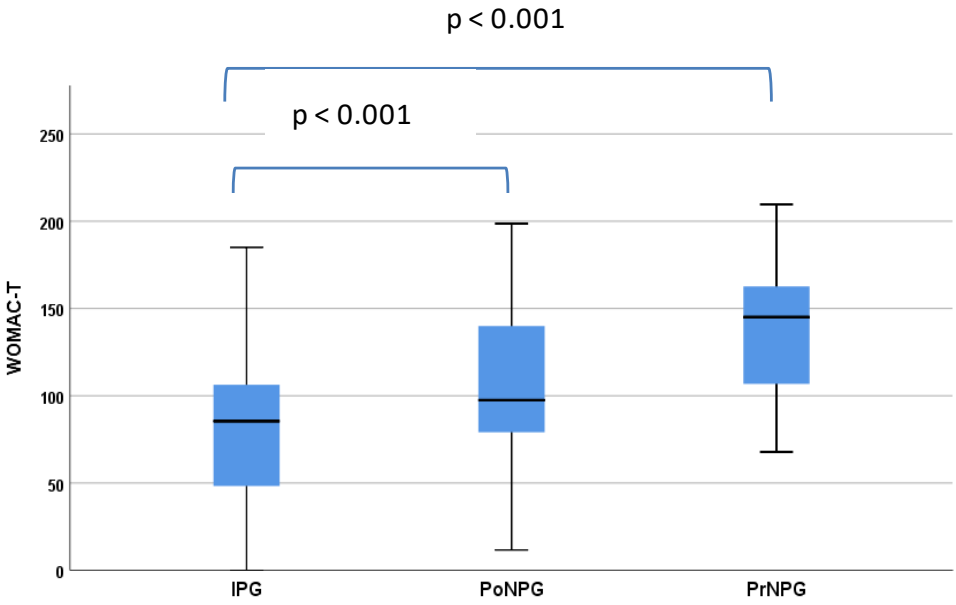

Figure 3: Comparison of WOMAC total scores between the OA groups, IPG, PoNPG and PrNPG.

The median (IQR) WOMAC pain scores for the IPG, PoNPG and PrNPG were 15.9 (9.6-20.7), 19.6 (14.4-28.5) and 27.7 (23.5-32.0), respectively (Table 1, Table 2). Overall, WOMAC pain scores showed an increasing trend from IPG to PoNPG to PrNPG ($p < 0.001$) (Figure 4-a). Between individual groups, WOMAC pain score was significantly higher in the PoNPG when compared to the IPG ($p < 0.024$) (Table 1) and the PrNPG compared to that in the IPG ($p < 0.001$) (Table 2).

The median (IQR) WOMAC functional disability scores for the IPG, PoNPG and PrNPG were 56.1 (28.2-76.0), 64.5 (50.7-101.2) and 103 (70-119.5), respectively (Table 1, Table 2). Overall, WOMAC dysfunction scores showed an increasing trend from IPG to PoNPG to PrNPG ($p < 0.001$) (Figure 4-b). Between individual groups, the WOMAC dysfunction score was significantly higher in PrNPG compared to that in the IPG ($p < 0.001$) but there was no significant difference between IPG and PoNPG.

The median (IQR) WOMAC stiffness scores for the IPG, PoNPG and PrNPG were 10.0 (5.7-13.4), 12.2 (8.2-13.8) and 12.7 (11.0-16.1), respectively (Table 1, Table 2). Overall, WOMAC stiffness scores showed an increasing trend from IPG to PoNPG to PrNPG ($p < 0.001$) (Figure 4-c). Between individual groups, WOMAC pain score was significantly higher in the PrNPG compared to that in the IPG ($p = 0.002$) but there was no significant difference between IPG and PoNPG.

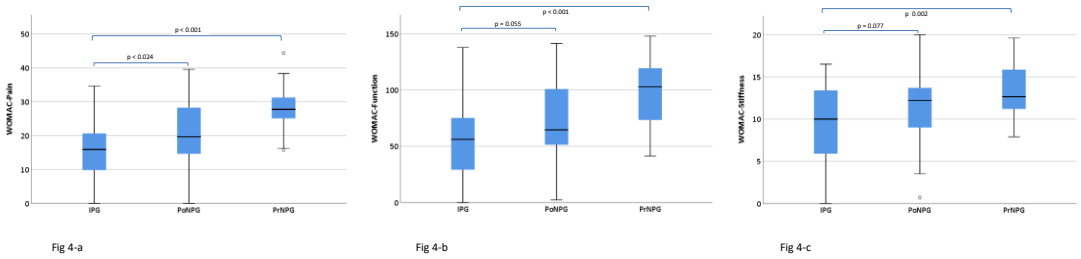

Figure 4: Comparison of WOMAC, pain (Fig 4-a), function (Fig 4-b) and stiffness (Fig 4-c) scores between the IPG, PoNPG and PrNPG groups.

4.4.4. PQAS

Three sub-groups of PQAS scores, paradoxical (PQAS-P), surface (PQAS-S) and deep (PQAS-D) were assessed. Each of these sub-groups showed an increasing trend from IPG to PoNPG to PrNPG ($p < 0.001$) (Figure 5-a, b, c). For all PQAS types (P, S and D), the PoNPG had significantly higher scores compared to those in the IPG ($p < 0.001$) (Table 1). Similarly, the PrNPG showed increased scores compared to the IPG ($p < 0.001$) (Table 2) (Figure 5-a,b,c).

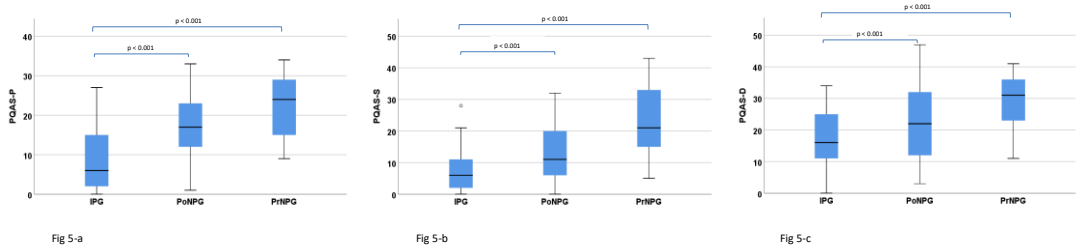

Figure 5: Comparison of PQAS, paradoxical (PQAS-P) (Fig 5-a), surface (PQAS-S) (Fig 5-b) and deep (PQAS-D) (Fig 5-c) scores between the IPG, PoNPG and PrNPG groups.

4.4.5. PCS

Scores for the pain catastrophizing scale (PCS) showed an increasing trend from IPG to PoNPG to PrNPG ($p < 0.001$) (Figure 6). Between the groups, PCS scores were significantly higher in the PrNPG compared to the IPG ($p < 0.001$) (Table 2). No significant difference in PCS was found between the IPG and the PoNPG ($p = 0.41$) (Table 1).

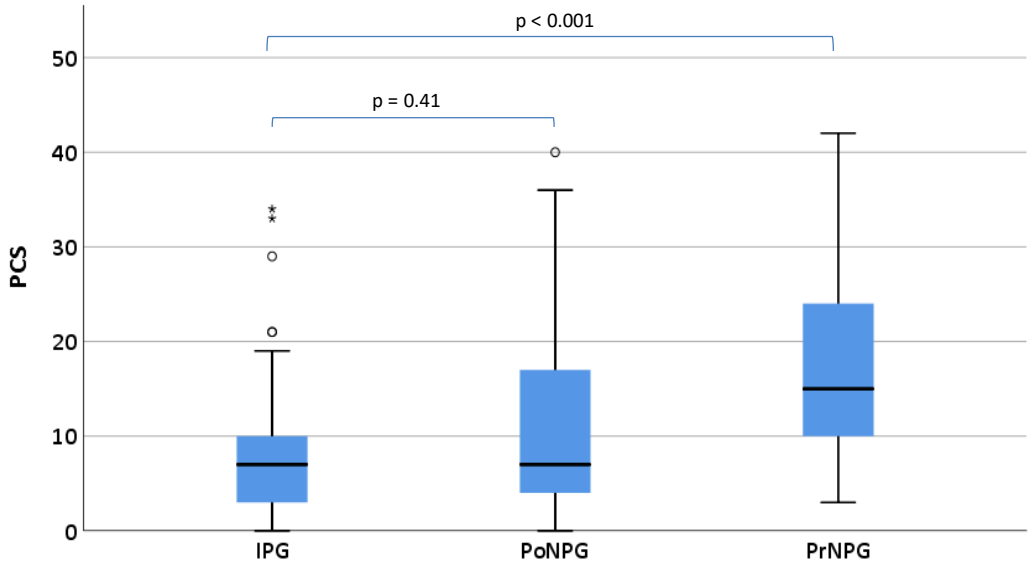

Figure 6: Comparison of PCS scores between the IPG, PoNPG and PrNPG cohorts.

4.4.6. PSQI

PSQI scores showed an increasing trend from IPG to PoNPG to PrNPG ($p < 0.001$) (Figure 7). Between the groups, PSQI scores were significantly higher in the PoNPG compared to the IPG ($p < 0.001$) (Table 1) and PrNPG ($p = 0.001$) (Table 2).

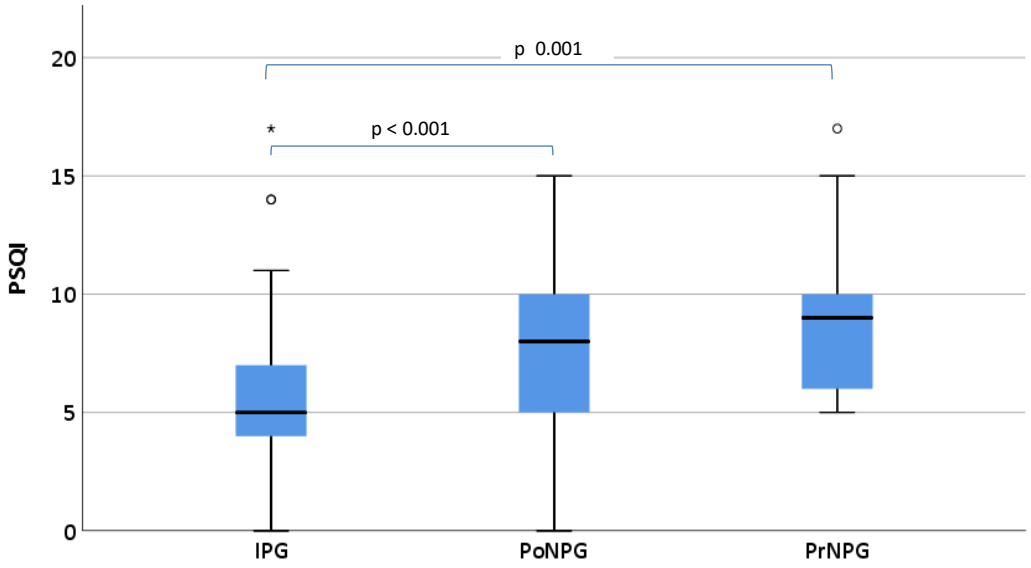

Figure 7: Comparison of PSQI scores between the IPG, PoNPG and PrNPG cohorts.

4.4.7. DASS

DASS scores showed an increasing trend between the IPG, PoNPG and PrNPG ($p < 0.001$) (Figure 8). The IPG and PrNPG showed a significant difference in DASS scores ($p = 0.010$), however, no statistically significant difference between the IPG and the PoNPG ($p = 0.110$) was noted.

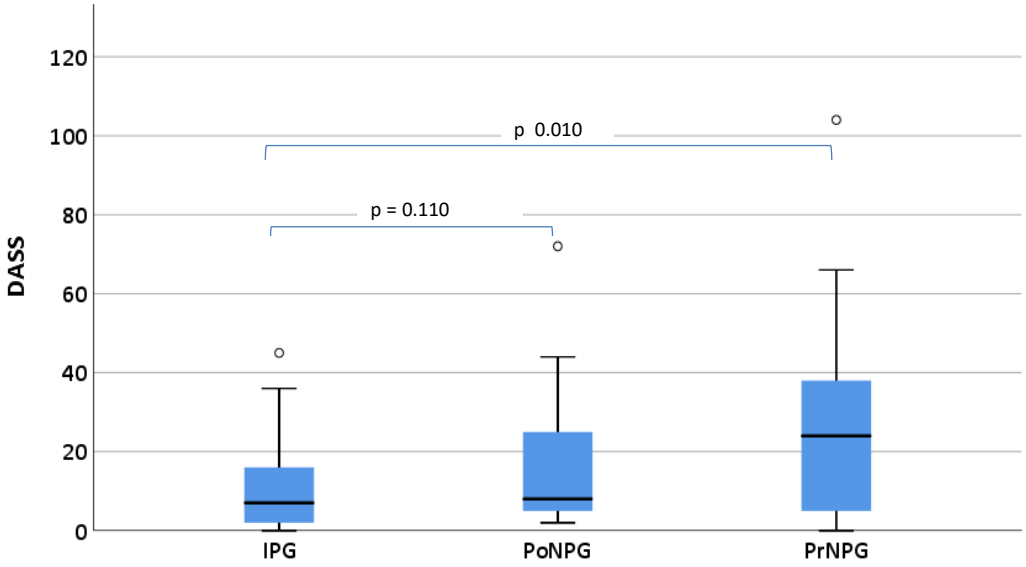

Figure 8: Comparison of DASS scores between the IPG, PoNPG and PrNPG cohorts.

4.4.8. Comorbidity

Comorbidities were compared between the three groups of OA participants; comorbidity scores did not show significant difference between IPG and PoNPG ($p = 0.730$) and between IPG and PrNPG ($p = 0.528$) (Figure 9, Table 1, Table 2).

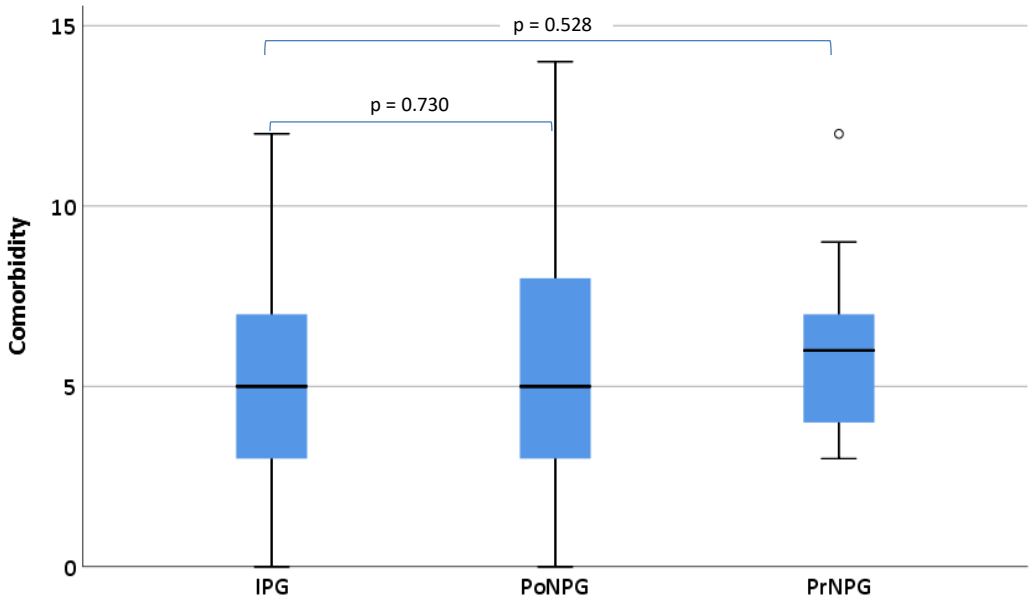

Figure 9: Comparison of Comorbidity scores between the IPG, PoNPG and PrNPG cohorts.

Neuropathic pain, pain intensity, pain quality, pain catastrophizing scale, sleep quality and psychological measures were significantly higher in the PoNPG and PrNPG relative to the IPG (Table 1, Table 2). These measures were further tested by combining participants from the PoNPG and PrNPG into a new grouping called the neuropathic pain group (NPG). Comparison of the NPG and IPG is presented in Table 3. S-LANSS, WOMAC total with all subscales, all PQAS sub scales, PCS, DASS and PSQI scores were significantly higher in the NPG ($p \leq 0.010$). The only exception was the comorbidity scores ($p = 0.683$). Therefore, logistic regression was performed between the IPG and the NPG.

Table 3: Comparison of questionnaire responses between the IPG and the NPG; PainDETECT, Leeds Assessment of neuropathic symptoms and signs (S-LANSS), Western Ontario McMaster Osteoarthritis Index version VA 3.1 (WOMAC), the pain quality assessment scale (PQAS), the depression, anxiety and stress scale (DASS), the pain catastrophizing scale (PCS), Pittsburgh sleep quality index (PSQI) and the comorbidity questionnaire.

	IPG	**NPG** *(PoNPG + PrNPG)*	p-value
	Mean (SD) [min-max]		
PainDETECT	10.8 (4.1) [2.0-18.0]	18.1 (3.9) [13.0-33.0]	< 0.001
S-LANSS	7.9 (4.1) [0.0-18.0]	12.9 (4.1) [2.0-22.0]	< 0.001
	Median (IQR) [min-max]		
WOMAC-T	85 (48-108) [0-185]	118 (88-152) [12-210]	< 0.001
WOMAC-Pain	15.9 (9.6-20.7) [0.0-34.6]	26.2 (16.3-30.1) [0.0-44.3]	< 0.001
WOMAC-Stiffness	10.0 (5.7-13.4) [0.0-16.5]	12.3 (10.1-14.6) [0.7-20.0]	0.004
WOMAC-Function	56.1 (28.2-75.9) [0.0-138]	77.1 (57.3-109) [2.4-148]	0.001
PQAS-P	6.0 (2.0-15.0) [0.0-27.0]	18.5 (12.8-24.3) [1.0-34.0]	< 0.001
PQAS-S	6.0 (1.5-11.0) [0.0-28.0]	16.0 (7.0-24.3) [0.0-43.0]	< 0.001
PQAS-D	16.0 (10.5-25.0) [0.0-34.0]	28.5 (17.0-35.3) [3.0-47.0]	< 0.001
PCS	7.0 (2.5-10.0) [0.0-34.0]	11.5 (5.0-21.3) [0.0-42.0]	0.006
DASS	7.0 (2.0-16.0) [0.0-45.0]	9.0 (5.0-35.5) [0.0-104]	0.010
PSQI	5.0 (3.5-7.5) [0.0-17.0]	8.0 (6.0-10.0) [0.0-17.0]	0.001
Comorbidity	5.0 (3.0-7.0) [0.0-12.0]	5.5 (3.0-7.3) [0.0-14.0]	0.683

4.4.9. Logistic regression analysis

Univariate and multivariate analyses were performed to test which self-reporting variables were associated with membership of the NPG.

Univariate logistic regression

Univariate logistic regression analysis suggested that the response variable, NPG, was independently and significantly associated ($p \leq 0.01$) with all predictor variables listed in Table 4. Table 4 shows significance levels and odds ratios for the respective questionnaires.

Table 4: Univariate model.

	OR	**95%CI OR**	p-value
S-LANSS	1.36	1.19-1.57	< 0.001
WOMAC-T	1.02	1.01-1.04	< 0.001
WOMAC-Pain	1.11	1.05-1.17	< 0.001
WOMAC-Stiffness	1.16	1.05-1.27	< 0.001
WOMAC-Function	1.03	1.01-1.04	< 0.001
PQAS-P	1.16	1.09-1.23	< 0.001
PQAS-S	1.12	1.06-1.19	< 0.001
PQAS-D	1.08	1.03-1.12	< 0.001
PCS	1.07	1.02-1.12	0.010
DASS	1.04	1.01-1.07	0.010
PSQI	1.20	1.06-1.36	< 0.001

Multivariate analysis

The questionnaires that were significant in the univariate regression models, described in Table 4, for diagnostic test predictors, were entered into an overall multivariable logistic regression model to find the model that provided the optimal predictors for inclusion in the NPG. A manual forward and backward selection strategy of variables with $p < 0.10$ was performed to select predictors for the final model. Predictors were deleted step by step from the model based on the highest p-value. Results of this multivariable model showed that the most strongly associated ($p < 0.05$) questionnaires were S-LANSS and PQAS-P, with OR of 1.26 and 1.12, respectively, for these measures (Table 5).

Table 5: Odds ratio confidence interval and p value for multiple regression.

	OR	**95%CI OR**	p value
S-LANSS	1.26	1.06-1.50	0.010
WOMAC-T	1.03	0.96-1.10	0.473
WOMAC-Pain	0.99	0.87-1.13	0.932
WOMAC-Stiffness	1.01	0.85-1.19	0.930
WOMAC-Function	0.98	0.92-1.05	0.622
PQAS-P	1.12	1.02-1.24	0.019
PQAS-S	1.03	0.94-1.13	0.464
PQAS-D	0.95	0.87-1.02	0.163
PCS	0.98	0.90-1.06	0.561
DASS	1.05	1.00-1.10	0.074
PSQI	1.11	0.93-1.32	0.261

4.5. Discussion

PainDETECT (PD) scores, pain and symptom report, and sensory deficits were used to initially group OA participants. The PainDETECT questionnaire is widely used in research settings to identify neuropathic pain (NP). PD score of ≤ 12 was scored by 32 OA participants who were categorised as the inflammatory pain group. PD scores from 13-18 (unsure) were scored by 43 participants and PD scores >19, likely NP, were scored by 24 participants. For this study, in addition to PD score, NP symptoms mapped on a lower limb diagram and corresponding sensory deficits were taken into account to differentiate participants into groups. The OA participants were divided into the inflammatory pain group, possible neuropathic pain and probable neuropathic pain group (section 4.3). PainDETECT has been used by several studies to evaluate characteristics of NP in OA patients (Hochman et al., 2011; Moss et al., 2018; Roubille et al., 2014). Previous studies have used PainDETECT scores to report NP in knee OA participants. Previous studies have also reported abnormal sensory and pain thresholds when participants were identified in NP group based on PainDETECT scores (Moss et al., 2018; Wright et al., 2017). In the absence of a gold standard, self-reporting tool for knee OA, S-LANSS and PQAS, two additional questionnaires were used to confirm the presence of NP and to provide supporting evidence in addition to PainDETECT scores. Two cut-off scores, 10 and 12, have been reported for S-LANSS (Bennett et al., 2005). In this study, 65% scored ≤ 10 on S-LANSS and 34% scored > 12. A PainDETECT score of ≥ 13 was scored by 68% and > 18 scored by 24% of OA participants PoNPG participants scored between 2 to 19 in the S-LANSS questionnaire and S-LANSS scores for some participants were not in agreement with the PainDETECT scores. Overall, 48% scored in the NP range on both questionnaires.

Results of this study indicated that S-LANSS and PQAS scores were higher in the PrNPG. All those participants who scored on PainDETECT in the NP range also scored on S-LANSS and PQAS sub scores related to NP (PQAS-P and PQAS-S), in the NP range. PainDETECT scores were used for initial grouping, but in this study, PainDETECT scores were not the only criteria used to pain phenotype OA participants. To diagnose NP evidence of somatosensory deficit is required (Treede et al., 2008). To find diagnostic indicators of NP, participants were extensively tested for sensory and pain thresholds and additional measures were also used to evaluate differences between the IPG, PoNPG and PrNPG (details in Chapter 2).

PQAS scores were lower in the IPG compared to the PoNPG and the PrNPG. PQAS has shown a steady increase with the severity of disease; similar findings were reported previously on two sub scales of PQAS-P and PQAS-S (Moss et al., 2018). This suggests that S-LANSS and PQAS-P are good indicators of suspected NP.

PainDETECT and S-LANSS have been previously used for pain phenotyping knee OA patients and found similar results (Hochman et al., 2011; Moreton et al., 2015). IPG and PoNPG participants' scores of S-LANSS, WOMAC, PQAS were more variable, with a wider range.

WOMAC scores in this study revealed that pain and functional disability increased from the IPG to the PoNPG and the PrNPG. Similar results were reported previously when OA participants were grouped based on PainDETECT scores (Hochman et al., 2013; Moss et al., 2018; Wright et al., 2017).

S-LANSS and PQAS scores show that even in IPG and PoNPG there were some participants who scored as high as to be included in NP. However, they did not show sensory loss in the same area where they reported NP symptoms. This suggests nerve involvement or central augmentation earlier in the disease process. WOMAC also showed these participants had higher pain and functional disability.

In addition, PCS, DASS, PSQI and comorbidity questionnaires were administered to assess pain severity and its effect on activity and pain catastrophization, psychosocial state, sleep quality and comorbidities in OA participants and the effect of NP. OA participants had significantly higher depression, anxiety, sleep disruption and comorbidities compared to the pain free control group (section 3.4).

These measures were compared between the three OA groups, IPG, PoNPG and PrNPG. Physical activity, pain catastrophization, psychosocial state, sleep quality and comorbidities were lowest in the IPG and highest in the PrNPG (Table 1, Table 2). These results indicate a more severe impact of disease in the PrNPG. Higher WOMAC scores for physical disability may be due to severe pain which may also be causing sleep disruption and psychological problems like depression and anxiety.

Logistic regression modelling was carried out by combining the PoNPG and PrNPG. One reason for this being that the sample size was not sufficient to allow us to perform logistic regression between the IPG and the PrNPG only. Secondly, most of the sensory and pain

measures were similar in PoNPG and PrNPG. Logistic regression between the IPG and NPG noted that S-LANSS and PQAS-P self-reporting measures had a strong association with the presence of NP (Table 4).

Previous studies have reported NP incidence of up to 22% using PainDETECT (Hochman et al., 2011; Moss et al., 2018). Following strict criteria to define NP groups, 29% of participants of this study were included in PoNPG and 21% in PrNPG. A meta-analysis of nine studies found a significant prevalence of NP at 23% among people with hip or knee OA (French et al., 2017). This is very close; 24% participants of this study scored positive NP based on only PD scores as was previously reported (Hochman et al., 2011; Roubille et al., 2014). Five of the nine studies used PainDETECT, two studies used a modified PainDETECT and DN4 and S-LANSS were used in two studies. Study participants of Hochman et al. reported that 28% had neuropathic symptoms but this was reduced to 19% when people with existing neurological conditions were excluded whereas this study recruited OA participants without any neurological condition and reported 21% of participants in the PrNPG. The PrNPG experienced greater pain, psychological distress, pain catastrophization and sleep disruption than people in the IPG. Similar results have been previously reported but different questionnaires were used (Hochman et al., 2011). This study recruited participants excluding all possible neuropathic conditions and adopted a more extensive approach to categorise the NPG. Hypothesis was accepted for S-LANSS, PQAS-S and PQAS-P and rejected for WOMAC, PQAS-, DASS and comorbidity.

Conclusions

Results of self-report questionnaires show a clear difference from IPG to PoNPG to PrNPG (Figures 1 to 8). PrNPG participants had higher NP symptoms reflected on S-LANSS, PQAS-S and PQAS-P. The PrNPG scored the highest on pain scores, functional disability, and psychosocial dysfunction. The PrNPG, results indicated that these OA patients suffer a more severe impact from their disease. These questionnaires, after finding accurate cut-off values, can be used in clinical as well as research settings to discriminate OA patients who should be further evaluated for the presence of NP. Multivariate logistic regression has shown that S-LANSS and PQAS-P are strongly associated with NP. Positive scores on these two questionnaires can assist to confirm the presence of NP.

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5. Neuropathic pain sensations and symptoms on digital pain mapping

5.1. Introduction

Osteoarthritis (OA) is a leading cause of joint pain and functional disability in older people (Cross et al., 2014). OA pain is heterogeneous in nature, both nociceptive and neuropathic components have been described for OA pain. OA pain is traditionally considered as pain caused by degenerative changes and local inflammation (Dimitroulas et al., 2014). However, recent studies suggest that up to 30% of patients present with neuropathic pain (NP) (Hochman et al., 2011; Ohtori et al., 2012).

PainDETECT and other questionnaires are used in research settings to identify NP but these are not diagnostic tools. Diagnosis of NP requires evidence of somatosensory deficit in addition to the report of neuropathic pain symptoms (Treede et al., 2008). In fact, at present, there are no tests available to make a definite diagnosis of neuropathic pain in patients with OA.

Information about intensity, area and location of pain is vital for clinicians to diagnose the cause of pain. Pain over the medial side of the knee in an older patient may indicate medial compartment tibiofemoral osteoarthritis. Location of pain helps to guide further investigations. For example, tibiofemoral joint pain may suggest the requirement for an MRI and may lead in some patients to diagnoses of meniscal tear (Riddle & Makowski, 2015). Pain drawing or pain mapping is an unbiased tool for describing pain location and intensity. Pain location has been described as heterogeneous, no particular pain pattern was found for symptomatic knee OA on pain mapping (Wood et al., 2007), although that study found that OA pain was more frequently reported on the medial side of knee.

Pain mapping has progressed from body manikins and paper drawings to digital mapping using a computer or tablet. The Navigate Pain App Ver. 1 (Aalborg University, Denmark), enables patients to digitally draw their pain area on a 3D body template. Digitally acquired pain maps have been validated against paper drawings (Jibb et al., 2020; Matthews et al., 2018) and are found to be comparable. This app was used by Boudrea and colleagues to assess knee pain in patients with patellofemoral pain, which demonstrated discrete patterns of pain location and area (Boudreau et al., 2018).

The same App (Navigate Pain App Ver. 1) was used for pain mapping for this study. OA participants in this study were asked to map pain and other symptoms so it can be used to identify the location and

severity of pain and other common symptoms that people with knee OA may experience and to identify symptoms that may be particularly associated with NP.

Hypotheses

1. The number (frequency) of digitally mapped pain and other sensations/symptoms, i.e., electric-shock, burning, hypersensitivity and cold will be greater in participants in PoNPG and PrNPG.

Aims

- To digitally map the location of pain and other sensations/symptoms in participants with knee OA on a template representing the lower limb to identify pain and symptom location.
- To determine the relationship of the location of pain and other neuropathic pain-like symptoms with different pain groups of knee OA participants.

5.2. Methods

5.2.1. Participant recruitment

People with knee Osteoarthritis were recruited by advertisement on Curtin radio, in gyms and in retirement villages and via Trialfacts (patient recruitment service). All participants were first interviewed over the phone for suitability to take part in the research by the researcher. They were then examined by an experienced rheumatologist on their first appointment to confirm the diagnosis of OA (section 2.3).

5.2.2. Pain mapping

After clinical examination, OA participants completed a lower limb pain diagram on a high-resolution 3D body schema representing the leg and knees on a computer tablet. OA participants were given a computer tablet (Samsung Galaxy Tab A, model SM-P350; Android 6.0.1, 2017 edition) to map pain area and location using the Navigate Pain App Ver. 1 (Aalborg University, Denmark). Two different types of images were used to map pain; the 3D image was used to describe location and area of superficial pain and the matching body outline 2D image to map deeper pain, located within the knee structure. Participants were asked to draw around the knee using a stylus (S pen) indicating where

they felt pain and any unusual sensation they experienced in the lower limb using various images available on the 'App'. Different colours were used to describe pain and other sensations like cold, heat, electric shock, burning and increased sensitivity around the knee area.

Colour representation

Red pain
Blue cold
Black electric shock
Grey hot/burning
Green increased sensation or allodynia

Participants drew around the knee area, describing pain and other symptoms on a lower limb chart for both right and left knees. These knee maps may reflect unilateral or bilateral knee pain.

5.3. Data analysis

The number of pain mapping reports on the medial side and the front of the knee were the same and considering the same nerve supply in both regions (section 1.4), in line with the rest of the testing, the pain drawing data for these regions were combined so data are presented in this study for the medial, lateral and popliteal fossa (PF) locations.

Participants were classified into the inflammatory pain group (IPG), possible neuropathic pain group (PoNPG) and probable neuropathic pain group (PrNPG) as follows

- IPG PainDETECT score ≤ 12
- PoNPG PainDETECT score ≥ 13, and
  reporting of additional sensations or demonstrating a sensory deficit.
- PrNPG PainDETECT score ≥ 19, and
  Reporting additional sensations along with a related sensory deficit in the same anatomical area (medial, lateral, or popliteal).

Based on the results of sensory measures and pain mapping, most of the measures were similar in the PoNPG and PrNPG. IPG and PoNPG, IPG and PrNPG were significantly different in most sensory and

pain mapping reports, therefore, for logistic analysis, the PoNPG and PrNPG were combined and then compared with IPG.

5.3.1. Statistical analysis

IBM® SPSS version 26 was used for analyses.

Cross tab analysis was performed for all pain mapping variables for frequency distribution and to test for statistically significant differences. A p-value of ≤ 0.05 was considered significant.

Univariate and multivariate logistic regression was carried out to determine which variables predict inclusion in the NPG.

5.4. *Results*

5.4.1. Demographics

Ninety nine osteoarthritis (OA) participants were recruited for this study; 46 male and 53 females. Average age of the participants was 64.7 ±7 years and the average BMI was 28.9 ±6.

5.4.2. Frequency of symptoms

82% of OA participants reported pain in both knees, on the pain drawing. 10% reported pain in only the right knee and 7% solely in the left knee.

The index knee for OA participants was defined as the 'painful knee' or the 'knee with the worst pain'. In the IPG, 51% reported right and 49% reported left as the worst affected knee. In the PoNP group, 45% reported right knee and 55% reported the left as the worst. Among the PrNPG participants, 67% reported right knee and 33% left knee as being the most painful.

Comparison of symptoms reported by OA participants is plotted in Figure 1.

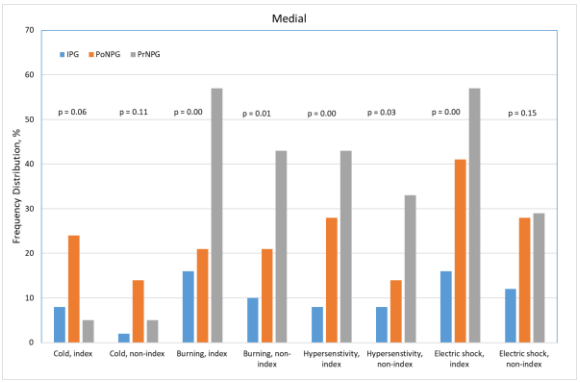


Fig-1 a

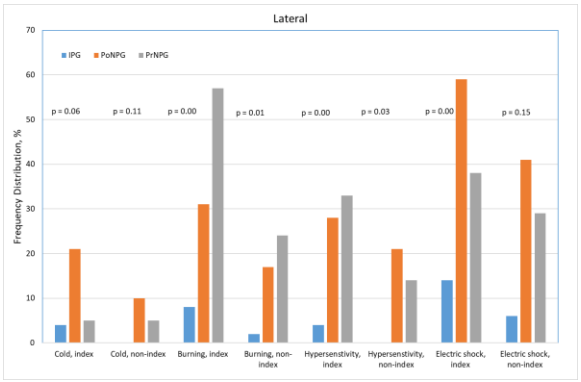


Fig-1 b

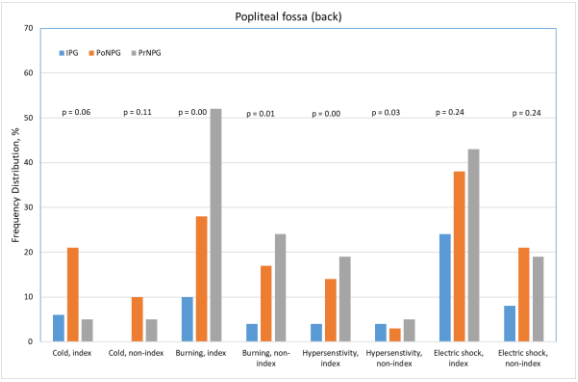


Fig-1 c

Figure 1: Frequency distribution of cold, burning, hypersensitivity and electric shock symptoms at the index and non-index knee between IPG, PoNPG and PrNPG.

Cold sensation was not reported by any participant from the IPG on the lateral aspect and back of the non-index knee. Hypersensitivity was also not reported on the lateral side of the non-index knee, Figure 1-b, c.

5.4.3. Location of pain

OA participants mapped their pain and additional symptoms, i.e., electric shock, burning, hypersensitivity and cold. In reference to innervation around the knee (section 1.4), pain mapping analysis was performed on the medial, lateral, and posterior (popliteal fossa) aspects of the knee.

Pain and other sensations were more commonly reported on the medial side of the knee except for electric-shock which was equally reported on the medial, lateral and posterior aspects of the knee (Table 1). The number of NP symptoms and sensations reported on the medial side was significantly higher compared to the lateral and posterior aspects of both the index and non-index knees. Pain

reports for both the index and non-index knees showed a decreasing trend from medial to lateral to popliteal fossa (Figure 2).

Table 1: Percent (%) of OA participants, reported sensations/symptoms around medial, lateral and popliteal fossa/posterior (PF) of the knee.

Pain/Sensations		Medial, %	Lateral, %	PF (back), %	p-value
Pain	index knee	73	62	57	0.081
Cold		12	9	10	0.006
Burning		26	25	24	
Hypersenstivity		21	17	10	
Electric shock		32	32	32	
Pain	non-index knee	54	43	32	0.007
Cold		6	4	4	0.003
Burning		20	11	12	
Hypersenstivity		15	9	4	
Electric shock		20	21	14	

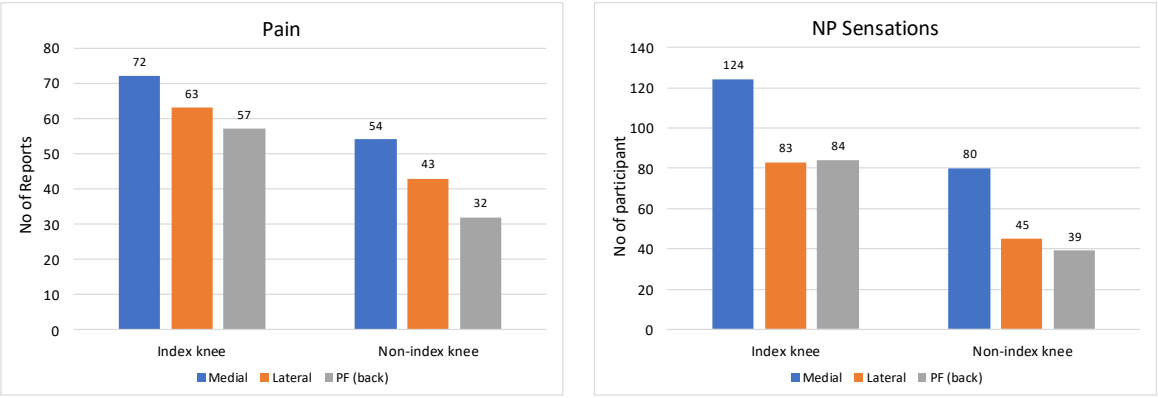
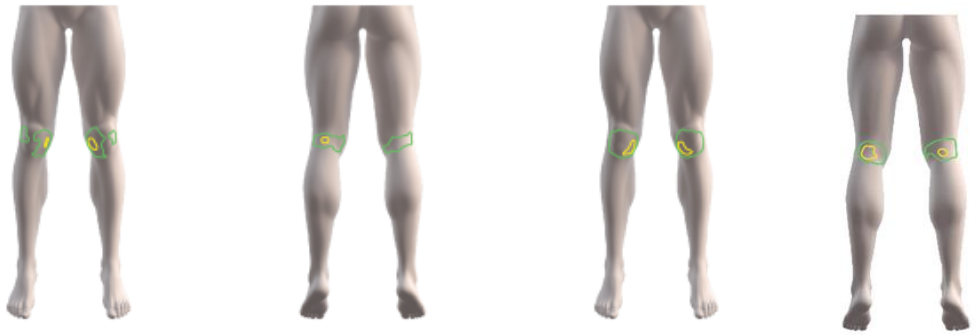


Figure 2: Comparison of number of reports of pain and NP sensations (cold, burning, hypersensitivity and electric-shock) at medial, lateral and popliteal fossa.

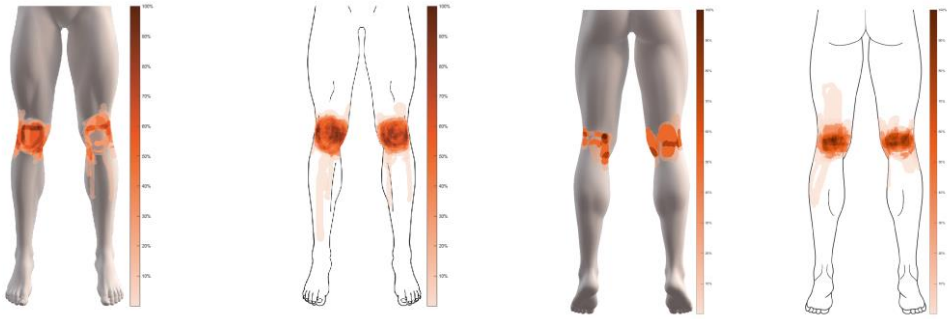
Data driven plots generated from digital pain mapping (Figure 3) are presented to reflect the location of the pain. The yellow boundary represents the common sites for 75% of participants and the green boundary represents common pain site for 50% participants (Figure 3).

Computer generated overlays from digital pain mapping were obtained for each symptom and sensation (Figure 4). The darkest colour represents the site where the maximum number of people drew for the particular sensation/symptom. Figure 4 (1) represents the area and site drawn by all participants for burning (n = 16) the darkest colour (max colour) represent 43.8% of total participants drawn at that site for burning and (Figure 4) (2) shows overlays of hypersensitivity drawings a: superficial n = 33, max 51.5% , c: back superficial n = 22, max 45.5%.



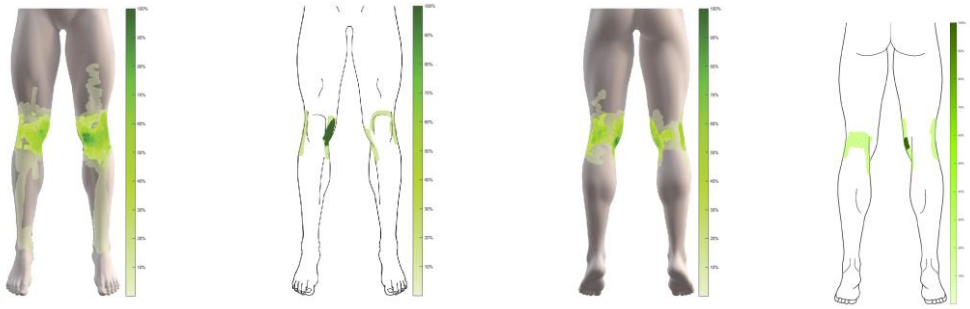
(a) (b) (c) (d)

Figure 3: Data driven pain plots, computer generated diagram from overall pain mapping data: green boundary 50% and yellow boundary 75%, of people having common site of pain.



(a) (b) (c) (d)

(1) Burning



(a) (b) (c) (d)

(2) Hypersensitivity

Figure 4: The distributions of location on superimposed overlays of the reconstructed pain maps with colour scales for highlighting the common regions of participants with knee OA; a: front superficial, b: front deep, c: back superficial, d: back deep. (1) burning (2) hypersensitivity.
Darkest (100%) colour represents max number of participants drawn at a particular location.

The number of sensations/symptoms, other than pain were significantly higher in the PoNPG and PrNPG compared to the IPG ($p < 0.05$), Table 2.

Table 2: Frequency of symptoms other than pain reported by OA participants.

	IPG	PoNPG	PrNPG	p-value	p-value
				IPG vs PoNPG	IPG vs PrNPG
Index knee, medial	35	42	40	0.001	< 0.001
Index knee, lateral	15	40	28	< 0.001	< 0.001
Index knee, PF	31	27	26	0.125	0.001
Non-index knee, medial	17	27	35	0.003	< 0.001
Non-index knee, lateral	4	26	15	< 0.001	< 0.001
Non-index knee, PF	10	18	11	0.015	0.075

Frequency distribution of pain and additional sensations were similar in the PoNPG and PrNPG, although some symptoms were reported by more participants in the PoNPG. Logistic regression was performed by combining the PoNPG and PrNPG (NPG, n = 50) and then comparing to the IPG (n = 49) (Table 3). A Significantly higher percentage of people in the NPG reported electric-shock, burning and hypersensitivity compared to the IPG at most sites. Pain and cold did not show a statistically significant difference but there was a trend for more people to report pain and cold sensation in the NPG compared to the IPG (Table 3).

Table 3: Frequency distribution (%) and comparison of pain, cold, burning, hypersensitivity, electric shock symptoms at medial, lateral and popliteal fossa of the index and non-index knee in IPG and in the combined NPG (PoNPG + PrNPG) as reported on the pain mapping diagram.

	Medial			Lateral			PF (Back)		
	IPG 1 (n=49)	PoNPG+PRNPG (n=50)	p-value	IPG 1 (n=49)	PoNPG+PRNPG (n=50)	p-value	IPG 1 (n=49)	PoNPG+PRNPG (n=50)	p-value
Pain, index knee	67%	80%	0.357	57%	68%	0.300	6%	14%	0.130
Pain, non-index knee	51%	58%	0.550	35%	52%	0.110	33%	32%	1.000
Cold, index knee	8%	16%	0.357	4%	14%	0.090	6%	14%	0.320
Cold, non-index knee	2%	10%	0.204	0%	8%	0.160	0%	8%	0.120
Burning, index knee	16%	36%	0.023	8%	42%	< 0.001	10%	38%	< 0.001
Burning, non-index knee	10%	30%	0.160	2%	20%	0.010	4%	20%	0.030
Hypersenstivity, index knee	8%	34%	< 0.001	4%	30%	< 0.001	4%	16%	0.090
Hypersenstivity, non-index knee	8%	22%	0.090	0%	18%	< 0.001	4%	4%	1.000
Electric shock, index knee	12%	28%	0.080	14%	50%	< 0.001	25%	40%	0.020
Electric shock, non-index knee	12%	28%	0.000	6%	36%	0.230	8%	20%	0.140

5.4.4. Logistic regression

Univariate logistic regression analysis results are shown in Table 4 as odds ratios and their significance levels for respective sensory measures. The three most sensitive sensory measures were burning lateral, hypersensitivity lateral and electric shock lateral with odds ratios in the order of 12, 10 and 9 for these measures, respectively (Table 4).

Table 4: Univariate logistic regression.

Univariate models, NPG (PoNPG+PrNPG)	OR	95% CI	p-value
Burning index knee, medial	2.88	1.11-7.47	0.029
Hypersenstivity index knee, medial	5.80	1.78-18.83	0.004
Electric shock index knee, medial	4.73	1.85-12.10	0.001
Burning index knee, lateral	8.15	2.54-26.16	< 0.001
Hypersenstivity index knee, lateral	9.86	2.11-45.96	0.004
Electric shock indexknee, lateral	6.00	2.27-15.88	< 0.001
Burning non-index knee, lateral	12.00	1.47-97.80	0.020
Electric shock non-index knee, lateral	8.62	2.34-31.74	0.001
Burning index knee, PF	5.39	1.82-15.99	0.002
Burning non-index knee, PF	5.87	1.22-28.40	0.028

Given all sensory measures, independently, had strong association with the NPG, multivariate regression analysis was carried out to develop an overall model relationship between predictor and response variables. Outcomes of the multivariable model variables are shown in Table 5.

Table 5: Multivariate logistic regression.

Multivariate models, NPG (PoNPG+PrNPG)	OR	95% CI	p-value
Burning, index knee, lateral	7.18	1.63-23.32	0.007
Hypersenstivity index knee, medial	4.24	1.07-17.23	0.040
Electric shock index knee, medial	3.96	1.02-10.65	0.047

It is interesting to note that where there was a strong association between all variables, when independently tested and NPG (Table 4: Univariate logistic regression.), however, only burning on the lateral side of the index knee and hypersensitivity and electric shock on the medial side of the index knee were significantly associated with NPG in the multivariate model (Table 5: Multivariate logistic regression.).

5.5. Discussion

Pain and other symptoms were mostly bilateral (82%) in this OA cohort; significantly more than was reported on verbal interview and symptoms were commonly reported on the medial side of the joint.

Pain was more commonly reported on the medial side (73%) than on the lateral side (62%). NP symptoms, electric shock and burning, were commonly reported while hypersensitivity and cold were less common. The NP symptoms and sensations were significantly higher ($p < 0.05$) on the medial side compared to the lateral and posterior aspects of the knee, either on the index or non-index knee (Table 1). This is in line with the previous studies indicating the medial side as a common pain site. However, generally, OA participants reported more than one region as being painful, using pain drawing (Creamer et al., 1998; Thompson et al., 2009; Van Ginckel et al., 2016). These studies

investigated pain locations and the number of painful sites in OA participants. Mapping of other symptoms among OA participants was done for the first time in this study.

One of the main findings of this study was that OA participants could comfortably digitally map pain and NP symptoms for a specific location and area. This is the first time that digital pain mapping of NP symptoms (hypersensitivity, heat, cold and electric-shock) has been investigated in knee OA. Participants drew around the different knee regions (medial, lateral, and posterior), which allowed us to evaluate relationships with quantitative sensory and pain measures in the same area. The objective of this study was to find out if pain mapping can be used to help differentiate pain phenotypes among OA participants. This study has shown that pain mapping is an effective tool for clinical differentiation between IPG and NPG. The hypothesis that participants who qualify for the NPG will report a greater number of symptoms and sites of pain was tested (

Table *2*). PoNPG and PrNPG participants reported a significantly higher number of sites and number of symptoms on the medial and lateral sides compared to the IPG ($p < 0.001$), which supported the hypothesis frequency of digitally mapped pain and other sensations/symptoms, i.e., electric-shock, burning, hypersensitivity and cold were greater in participants in NPG.

These results indicate digital pain mapping is a useful tool to be used as first line of screening for OA patients with possible neuropathic pain. As logistic regression analysis indicated inclusion of OA participants in the NPG was associated with reports of electric shock, burning and hypersensitivity symptoms (Table 4, Table 5).

Electric shock was the most widely reported symptom among all OA participants, even in the IPG and was reported on more sites by the participants who scored 13 or above on the PD questionnaire. In this study, NP like symptoms were reported by all OA participants. Participants in the IPG group reported at either the medial or lateral sites but for participants in the PoNPG and PrNPG, these symptoms were more widely reported, bilateral and medial, lateral as well as posterior.

Conclusions

Pain mapping is a useful tool to assess type and location of pain and other sensations. Digital mapping reports of symptoms and sensations can guide clinicians to further investigate and diagnose the type of pain, which may result in early diagnosis and management of more difficult to manage pain. Hypersensitivity and electric shock were suggestive of neuropathic pain. Digital pain mapping can be useful in research as well as in clinical settings. Further progress towards an online completion of pain

mapping can provide useful information to clinicians to diagnose and in follow up consultations in person as well as on e-consultations. Pain mapping can also be used as a tool to follow up efficacy of any intervention/treatment.

5.6. References

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6. Somatosensory assessment of participants with knee osteoarthritis

6.1. Introduction

Somatosensory function can be assessed using quantitative sensory testing (QST) (Cardoso et al., 2016; Frey-Law et al., 2016; Suokas et al., 2012), which may assist in identifying features of sensory deficits or hypersensitivity associated with neuropathic pain. QST assesses the response induced by a range of somatosensory stimuli, which can be mechanical, electrical and/or thermal. Commonly cold, heat, tactile, vibration and pinprick perception thresholds and pressure, heat and cold pain thresholds are the measures used in research and sometimes in clinical settings. QST measures can be applied at different anatomical sites. An examiner systematically applies the stimulus in a graded manner to an anatomical site until the participant indicates perception of sensation or pain. In particular, generalised mechanical and cold hyperalgesia have been found to be important clinical features of many neuropathic pain states (Maier et al., 2010) and people with these conditions often exhibit hypoesthesia to a range of stimuli including vibration and tactile sensitivity (Harden et al., 2013; Jakorinne et al., 2018). Previous studies have also identified somatosensory abnormalities like hypoesthesia, hyperalgesia, temporal summation and allodynia in people with osteoarthritis and chronic knee pain using QST measures, suggesting that people with this condition may exhibit some features of neuropathic pain (Arendt-Nielsen et al., 2015; Hochman et al., 2013; Moss et al., 2016).

Proprioception is the sense of position and relative movement of different body parts (i.e., limbs and joints) in space (Kalaska, 1994). This perception is derived from neural inputs which arise from mechanoreceptors in the joints and muscles. Proprioceptive sense plays an important role in muscle contraction and joint stabilisation. It has been reported that joint position sense progressively declines with age in people with normal knees and that those with osteoarthritic knees have impaired joint position sense at all ages (Barrett et al., 1991). It has been suggested that proprioceptive impairment may be an important factor in initiating or advancing degeneration of the knee in elderly individuals with osteoarthritis (Barrett et al., 1991). This is an interesting concept because it suggests that nervous system impairment may be an important factor in the aetiology of osteoarthritis (OA).

People with knee OA performed poorly compared to control participants when joint reposition sense was assessed in partial weight bearing (Garsden & Bullock-Saxton, 1999). In individuals with knee OA, proprioceptive impairments have been reported not only in the affected knee but also in the unaffected knee in individuals with unilateral disease (Garsden & Bullock-Saxton, 1999). Lund et al also reported compromised movement detection at the elbow in people with knee OA, supporting a generalised impairment that may be linked to the aetiology of knee OA (Lund et al., 2008). However, a more recent investigation found that proprioceptive deficits were localised to the affected knee joint (Shanahan et al., 2015). It is possible that proprioceptive deficits may reflect a sensorimotor neuropathy affecting myelinated afferent nerve fibres. It is known that myelinated afferent neurons contribute to proprioception and vibration sensation (Vinik et al., 2000).

In this study, joint reposition error was used to evaluate deficits in proprioceptive function and a range of QST measures to evaluate sensory function in OA participants. Within the knee OA group comparisons were made between groups identified as having inflammatory pain (IPG), possible neuropathic pain (PoNPG) and probable neuropathic pain (PrNPG) (detail in section 2.8.4).

Hypotheses

1. Participants in the probable neuropathic pain group (PrNPG) will show greater deficits in joint reposition error compared to participants in the IPG and PoNPG.
2. Participants in PrNPG will exhibit greater levels of hypoesthesia (based on QST measures) than participants in the IPG and PoNPG.
3. Participants in PrNPG will exhibit greater levels of hyperalgesia (based on QST measures) than participants in the IPG and PoNPG.
4. A range of QST and proprioception measures (which may include sensory measures and measures of pain threshold) will predict inclusion in the NPG.

Aims

- To determine if proprioceptive deficits, sensory deficits and pain thresholds are different between pain phenotype groups in people with knee OA.

6.2. Methods

Data from 99 participants with knee osteoarthritis were included in this study (recruitment details in section 2.3).

Potential volunteers were first interviewed over phone. Suitable participants attended an initial clinic appointment. They were examined by the Rheumatologist and the researcher and were asked to complete a series of questionnaires (detailed in section 2.5). After their first appointment at the clinic, participants attended two laboratory testing sessions. During the first session quantitative sensory testing (QST) was performed in the pain laboratory at Curtin University, which included a battery of tests for sensory function as well as measures of cold, heat and pressure pain thresholds. On the subsequent day proprioceptive testing was carried out in the motion analysis laboratory at Curtin University.

All variables were measured at the index and non-index knees and at a site on the forearm (control site). The index knee was defined as the painful knee or the knee with the worst pain in the case of bilateral knee pain. Sensory measures (heat, cold, tactile, vibration detection) and pain thresholds (heat, cold and pressure pain) were tested at three sites (medial, lateral, and popliteal fossa) around each knee and at the forearm (ECRB), as a control site (section 1.4, 2.6).

Proprioception was tested using knee joint reposition error (JRE) in partial weight bearing and was measured using an electro-goniometer. A total of 12 trials were performed, 6 measurements at each at two target angle ranges between 15-20^{o} and 35-40^{o} knee flexion (Chapter 2.4.6).

Heat and cold detection threshold (HDT and CDT) as well as heat and cold pain thresholds (HPT and CPT) were measured using a Peltier thermode (Medoc, Israel). Pressure pain threshold (PPT) was measured using a digital pressure algometer (Somedic AB, Sweden). (detailed QST method in Chapter 2, section 2.6)

The order of testing was randomized between QST modalities and between test sites.

6.3. Data analysis

6.3.1. OA phenotypes

Sensory data from a pain free (control) group were used to calculate Z-scores. Mean and standard deviation values of sensory perception measures for heat cold, vibration and tactile were calculated and used to calculate Z-scores. If the measures for any of the sensory tests in participants with knee OA were outside ± 1.96 Z-score from mean of the pain free control group, they were classified as having a sensory deficit for that area (details in section 2.8.3).

Participants with knee OA were grouped based on scores for PainDETECT questionnaire, Z-score deviation from the pain free control group scores for the sensory measures and reporting of neuropathic pain like symptoms during pain mapping (superficial pain, cutaneous sensitivity, cold, electric shock and burning).

Participants were classified into the inflammatory pain group (IPG), possible neuropathic pain group (PoNPG) and probable neuropathic pain group (PrNPG) as follows:

- IPG PainDETECT score ≤ 13
- PoNPG PainDETECT score ≥ 13, and
  reporting additional sensations or demonstrating a sensory deficit.
- PrNPG PainDETECT score ≥19, and
  reporting additional sensations along with related sensory deficit in the same anatomical area (medial, lateral or popliteal).

6.3.2. Statistical analysis

IBM® SPSS version 26 was used for statistical analyses.

JRE was calculated as the difference between target angle and reposition angle. Mean and standard deviation values of reposition errors for 6 measures in each target range were calculated and used for analysis.

Three tests were performed at each site for heat, cold, vibration and tactile perception thresholds, and heat, cold and pressure pain thresholds. The mean and standard deviation of three observations of each QST measure was used for further analysis.

Analysis of Variance (ANOVA) was used to compare sensory thresholds, pain thresholds and joint reposition error (proprioceptive function) between the IPG, PoNPG and PrNPG.

Data from PoNPG and PrNPG were combined and designated as the NPG for logistic regression models. Univariate and multivariable logistic regression analyses were carried out to determine which measures predict inclusion in the NPG.

6.4. Results

Results from a total of 99 OA participants are presented in this section.

6.4.1. Demographics

Demographic variables for each group are presented in Table 1. There was no significant difference in age and BMI between OA pain phenotype groups, p = 0.177 and 0.116, respectively.

Table 1: Demographics; Mean (SD) [min-max], age and BMI of IPG, PoNPG and PrNPG. p - ANOVA between three OA (IPG, PoNPG and PrNPG) groups.

	IPG	**PoNPG**	**PrNPG**	p-value
	Mean (SD) [min-max]			
Age	65.3 (8.6) [50.0-88.0]	65.3 (7.6) [50.0-84.0]	61.7 (6.5) [51.0-73.0]	0.177
BMI	27.8 (5.0) [17.1-39.7]	29.9 (5.4) [21.6 -45.8]	30.4 (7.0) [21.0-54.9]	0.116

Gender

Within the OA participants, in the IPG male to female ratio was 43/57, in the PoNPG 45/55 and in the PrNPG it was 57/43 (Table 2).

Table 2: Gender distribution of study participants.

Participants	**Male**	**Female**	**Total**
OA groups			
IPG	21(43%)	28(57%)	49
PoNPG	13(45%)	16(55%)	29
PrNPG	12(57%)	9(43%)	21

6.4.2. OA pain phenotypes

Among OA participants, 49% were allocated to the IPG, 29% to the PoNPG and 21% to the PrNPG. Comparison of joint reposition error, sensory and pain measures was done between these three pain groups.

6.4.3. Joint reposition error (JRE)

ANOVA for JRE at 15-20° flexion between the three OA groups showed no significant difference (p = 0.874 for index and 0.840 for non-index knees) whereas at 35-40° flexion, JRE was significantly different for the index knee but not for the non-index knee (p = 0.002 and 0.068 respectively). At 35-40° flexion, JRE of the IPG was higher compare to the PoNPG and PrNPG (Table 3, Figure 1, d).

Table 3: Joint reposition error (JRE); Median (IQR) [min-max] at 15-20° and 35-40° knee flexion at index and non-index knee. ANOVA between three OA (IPG, PoNPG and PrNPG) groups.

	IPG	PoNPG	PrNPG	p-value
	Median (IQR) [min-max]			
15-20° index knee	5.0 (3.5-6.5) [1.7-15.7]	4.9 (4.2-6.9) [1.4-12.4]	6.0 (3.9-7.6) [2.0-10.8]	0.874
35-40° index knee	4.7 (3.1-6.2) [1.1-9.3]	3.1 (1.8-4.4) [0.8-5.6]	3.8 (2.6-4.8) [1.4-8.5]	0.002
15-20° non-index knee	4.7 (3.3-6.3) [1.1-12.1]	5.0 (3.3-7.5) [0.9-12.2]	5.3 (3.0-7.9) [1.9-9.8]	0.840
35-40° non-index knee	3.3 (2.9-4.4) [1.7-9.4]	3.2 (2.8-4.3) [0.8-5.1]	2.9 (2.3-4.5) [1.8-5.6]	0.068

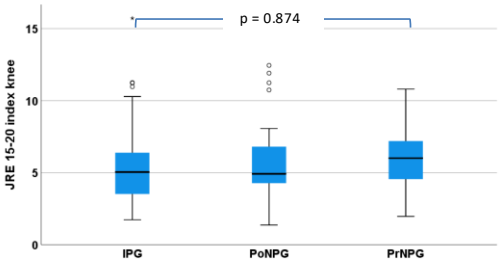


Fig-1a

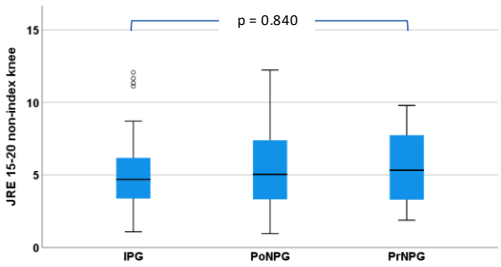


Fig-1b

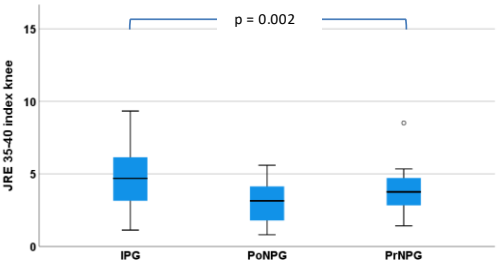


Fig-1c

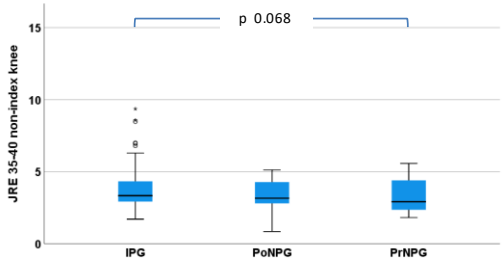


Fig-1d

Figure 1: JRE comparison between IPG, PoNPG and PrNPG at 15-20° (Fig-1a & 1b) and 35-40° (Fig-1c & 1d) knee flexion for index and non-index knee.

6.4.4. Heat detection threshold (HDT)

Heat detection thresholds (HDT) were compared between the three OA groups (IPG, PoNPG and PrNPG). HDT values between the three OA groups were not significantly different at the medial, lateral, and posterior aspects of the index and non-index knees. HDT at the forearm was significantly different between groups (Table 4, Figure 2, g).

Table 4: Heat detection threshold for IPG, PoNPG and PrNPG at medial (HDTM), lateral (HDTL) and popliteal fossa (HDTPF) of index and non-index knee as well as at forearm (HDT ECRB). p - ANOVA within three OA (IPG, PoNPG and PrNPG) groups.

	IPG	**PoNPG**	**PrNPG**	p-value
	Mean (SD) [min-max]			
HDTM index knee	35.6 (2.2) [33.6-42.9]	35.6 (1.8) [34.0-43.2]	35.6 (1.0) [34.2-38.7]	0.995
HDTL index knee	37.0 (2.4) [34.2-42.8]	36.8 (1.8) [34.0-43.0]	37.5 (2.0) [33.9-41.3]	0.439
HDTPF index knee	35.8 (1.2) [33.5-40.8]	36.6 (2.6) [34.2-47.1]	36.3 (1.5) [34.1-39.6]	0.164
HDTM non-index knee	35.2 (1.8) [33.9-42.8]	35.5 (1.8) [33.8-42.5]	35.3 (1.5) [32.5-40.3]	0.678
HDTL non-index knee	36.8 (2.4) [34.4-46.2]	36.7 (1.7) [34.1-43.1]	37.4 (2.6) [35.0-44.7]	0.968
HDTPF non-index knee	35.7 (1.4) [34.2-43.1]	35.9 (1.4) [34.1-38.9]	36.6 (1.8) [34.6-42.9]	0.480
HDT ECRB	34.8 (0.8) [33.3-36.8]	35.2 (1.1) [34.2-38.5]	35.6 (1.2) [34.0-39.7]	0.006

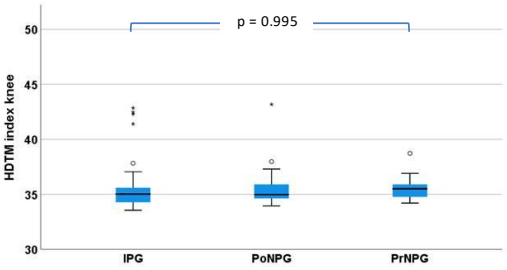


Fig-2a

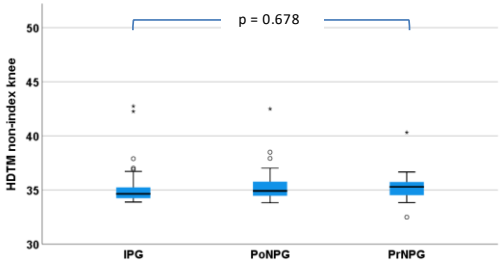


Fig-2b

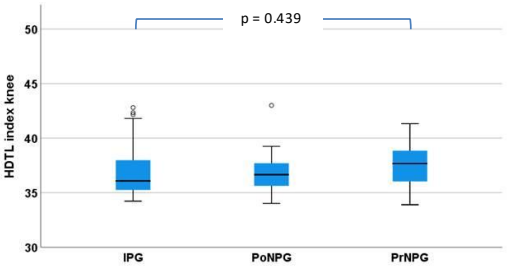


Fig-2c

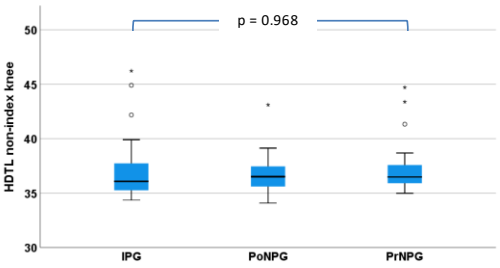


Fig-2d

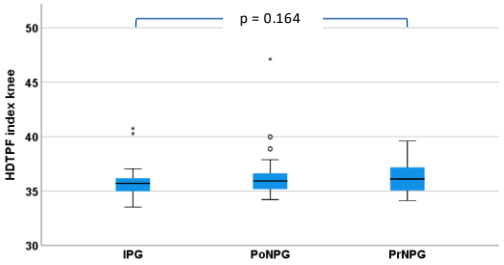


Fig-2e

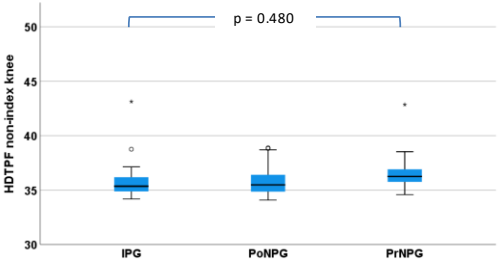


Fig-2f

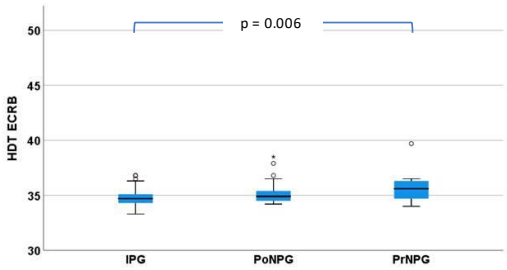


Fig-2g

Figure 2: Heat detection threshold for IPG, PoNPG and PrNPG at medial (HDTM, Fig-2a & 2b), lateral (HDTL, Fig-2c & 2d) and popliteal fossa sites (HDTPF, Fig-2e & 2f) of the index and non-index knees as well as at the forearm (HDT ECRB, Fig-2g).

6.4.5. Cold detection threshold (CDT)

Cold detection threshold (CDT) was compared between the three OA groups (IPG, PoNPG, PrNPG). ANOVA showed no significant difference in CDT at the medial, lateral and posterior aspects of the index and non-index knees as well as at the forearm (Table 5, Figure 3, a-f).

Table 5: Cold detection threshold for the IPG, PoNPG and PrNPG at the medial (CDTM), lateral (CDTL) and popliteal fossa sites (CDTPF) of the index and non-index knees as well as at the forearm (CDT ECRB). p - ANOVA within three OA (IPG, PoNPG and PrNPG) groups.

	IPG	**PoNPG**	**PrNPG**	p-value
	Mean (SD) [min-max]			
CDTM index knee	29.5 (1.0) [26.1-30.7]	29.3 (0.7) [27.3-30.4]	29.3 (1.1) [26.6-31.1]	0.877
CDTL index knee	29.1 (1.0) [26.7-30.7]	29.1 (0.9) [26.5-30.2]	29.2 (1.1) [26.6-30.4]	0.935
CDTPF index knee	29.2 (1.0) [26.5-30.9]	28.9 (0.8) [26.9-30.4]	28.7 (1.3) [24.7-30.7]	0.080
CDTM non-index knee	29.4 (1.0) [26.6-31.2]	29.4 (0.9) [26.8-30.8]	29.4 (0.8) [27.9-30.9]	0.950
CDTL non-index knee	29.1 (1.2) [24.2-30.9]	29.0 (0.9) [27.2-30.8]	28.8 (1.0) [26.7-30.4]	0.413
CDTPF non-index knee	29.3 (1.2) [24.5-31.4]	28.9 (1.0) [26.5-30.6]	28.9 (1.6) [23.2-30.9]	0.321
CDT ECRB	29.5 (1.4) [23.4-31.3]	29.5 (1.0) [26.9-30.5]	29.3 (0.9) [27.6-31.1]	0.820

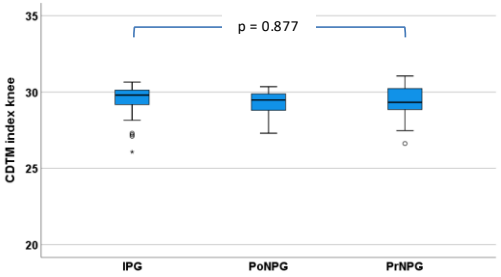


Fig-3a

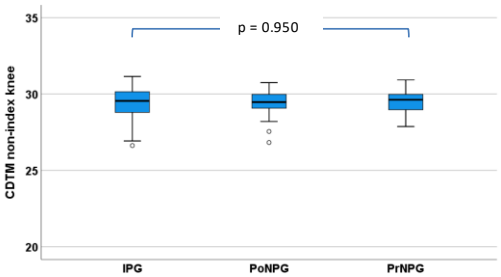


Fig-3b

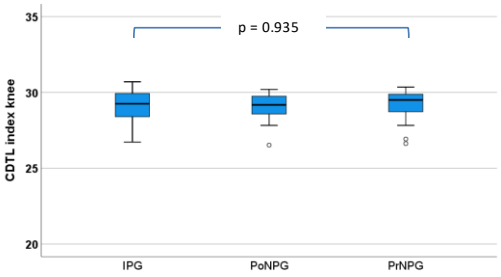


Fig-3c

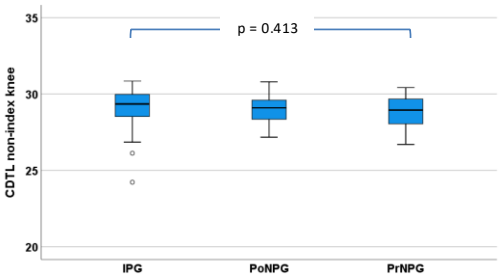


Fig-3d

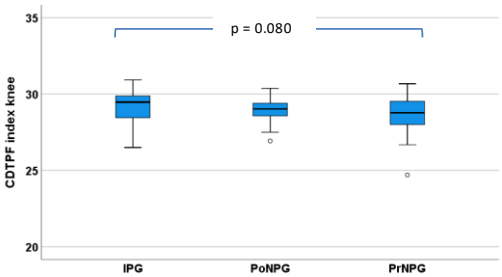


Fig-3e

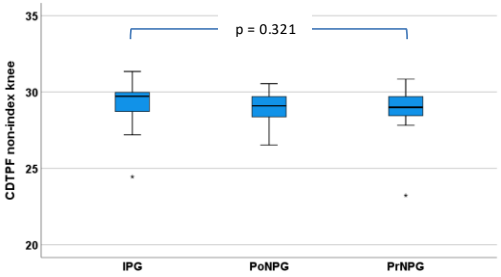


Fig-3f

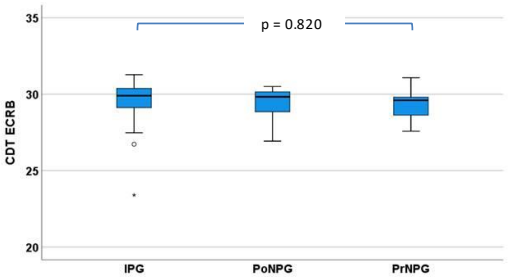


Fig-3g

Figure 3: Comparison of cold detection threshold between the IPG, PoNPG and PrNPG at the medial (CDTM, Fig-3a & 3b), lateral (CDTL, Fig-3c & 3d) and popliteal fossa sites (CDTPF, Fig-3e & 3f) of the index and non-index knees as well as at the forearm (CDT ECRB, Fig-3g).

6.4.6. Vibration threshold (VT)

A comparison of vibration threshold (VT) indicated there was no statistically significant difference in VT between the three OA groups (IPG, PoNPG and PrNPG) at the non-index knee (p = 0.078, 0.138, 0.185), however, at the index knee there was a statistically significant difference (p = 0.027, 0.040, 0.014). ANOVA of VT results between the three OA groups at the forearm site was also significantly different (p = 0.005). VT was highest in the PoNPG (Table 6, Figure 4).

Table 6: Vibration threshold for IPG, PoNPG and PrNPG at medial (VTM), lateral (VTL) and popliteal fossa (VTPF) of index and non-index knee as well as at forearm (VT ECRB). p - ANOVA within three OA (IPG, PoNPG and PrNPG) groups.

	IPG	PoNPG	PrNPG	p-value
	Median (IQR) [min-max]			
VTM index knee	13.5 (8.8-19.0) [2.0-39.6]	18.2 (13.7-26.2) [4.7-38.3]	11.2 (7.9-19.9) [4.2-36.1]	0.027
VTL index knee	14.9 (8.3-19.8) [1.3-37.4]	18.3 (12.8-28.8) [7.1-37.2]	15.4 (9.9-18.7) [4.1-35.9]	0.040
VTPF index knee	9.7 (5.9-15.1) [1.4-37.5]	17.4 (10.2-24.3) [5.5-42.9]	13.3 (8.1-20.2) [3.8-34.1]	0.014
VTM non-index knee	12.4 (7.4-19.5) [1.9-39.7]	17.7 (12.5-25.1) [5.8-36.2]	12.9 (7.8-23.0) [3.8-33.1]	0.078
VTL non-index knee	13.7 (7.1-18.5) [1.7-38.2]	16.4 (12.2-25.7) [3.3-33.9]	13.5 (7.8-20.3) [4.3-32.1]	0.138
VTPF non-index knee	10.2 (7.2-15.6) [1.1-37.0]	13.5 (8.7-20.5) [5.0-35.3]	9.9 (6.8-16.9) [4.1-34.8]	0.185
VT ECRB	6.6 (4.4-9.6) [1.9-27.9]	11.1 (7.0-14.2) [4.2-17.5]	6.8 (5.7-11.3) [4.4-21.7]	0.005

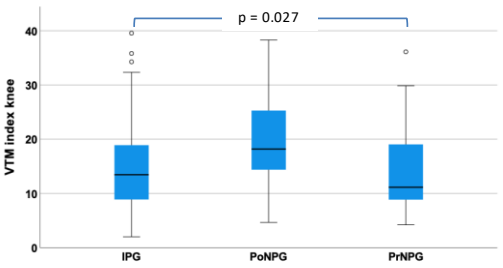


Fig-4a

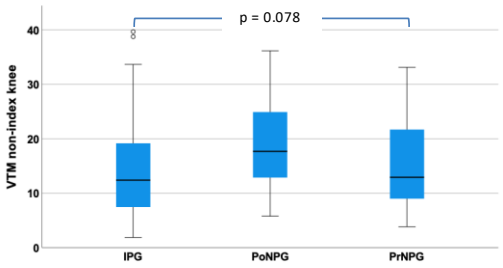


Fig-4b

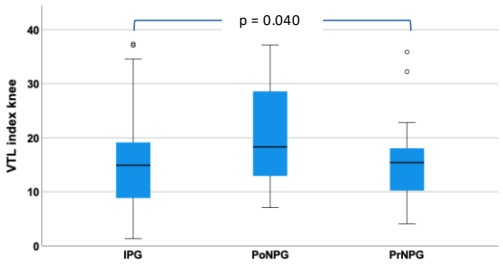


Fig-4c

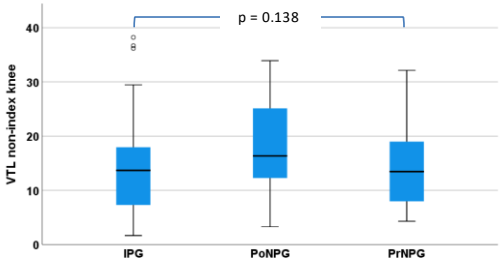
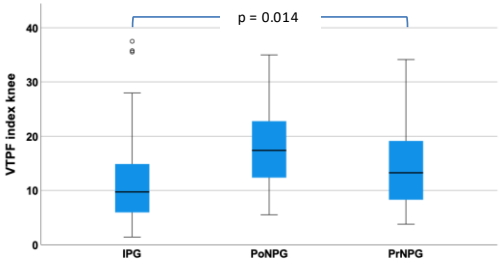


Fig-4d



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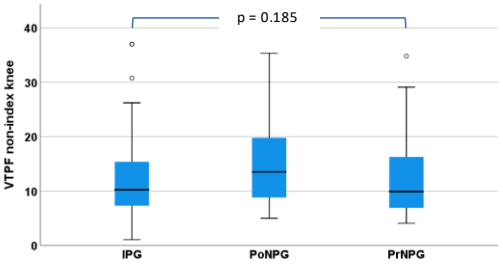

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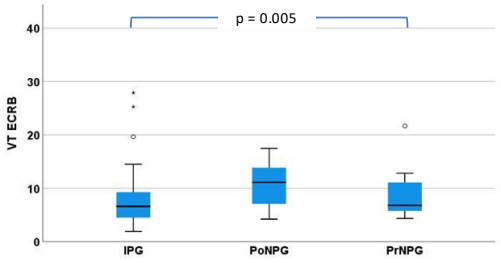


Fig-4g

Figure 4: Comparison of vibration detection threshold between the IPG, PoNPG and PrNPG at the medial (VTM, Fig-4a & 4b), lateral (VTL, Fig-4c & 4d) and popliteal fossa sites (VTPF, Fig-4e & 4f) of the index and non-index knees as well as at the forearm (VT ECRB, Fig-4g).

6.4.7. Tactile threshold (TT)

Light touch or tactile threshold (TT) was compared between the OA groups (IPG, PoNPG, PrNPG) and with the exception of the medial side of the non-index knee ($p = 0.024$), no statistically significant difference was found. TT was the higher in the PoNPG (Table 7, Figure 5).

Table 7: Tactile threshold for the IPG, PoNPG and PrNPG at the medial (TM), lateral (TL) and popliteal fossa sites (TPF) of the index and non-index knees as well as at the forearm (TP ECRB). p - ANOVA within three OA (IPG, PoNPG and PrNPG) groups.

	IPG	**PoNPG**	**PrNPG**	p-value
	Median (IQR) [min-max]			
TM index knee	0.4 (0.2-0.7) [0.1-13.7]	0.4 (0.4-1.2) [0.1-19.6]	0.4 (0.2-0.7) [0.1-3.9]	0.303
TL index knee	0.7 (0.3-1.6) [0.1-13.7]	0.7 (0.4-1.6) [0.2-39.2]	0.7 (0.4-1.2) [0.2-9.8]	0.932
TPF index knee	0.4 (0.2-0.7) [0.1-13.7]	0.4 (0.2-0.7) [0.2-9.8]	0.4 (0.2-0.7) [0.1-3.9]	0.271
TM non-index knee	0.4 (0.2-0.7) [0.1-13.7]	0.4 (0.4-1.6) [0.2-19.6]	0.4 (0.2-0.4) [0.1-1.6]	0.024
TL non-index knee	0.4 (0.2-0.7) [0.2-9.8]	0.7 (0.4-0.8) [0.1-19.6]	0.4 (0.3-0.7) [0.2-19.6]	0.633
TPF non-index knee	0.2 (0.2-0.7) [0.1-13.7]	0.4 (0.2-0.7) [0.2-19.6]	0.4 (0.2-0.7) [0.1-3.9]	0.288
T ECRB	0.2 (0.1-0.2) [0.0-3.9]	0.2 (0.1-0.4) [0.0-3.9]	0.2 (0.1-0.3) [0.1-1.6]	0.591

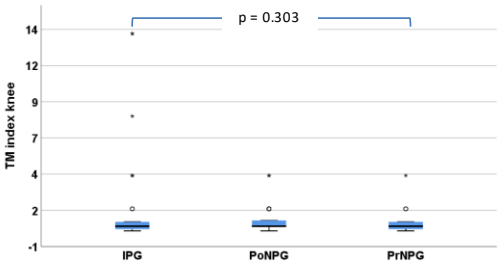


Fig-5a

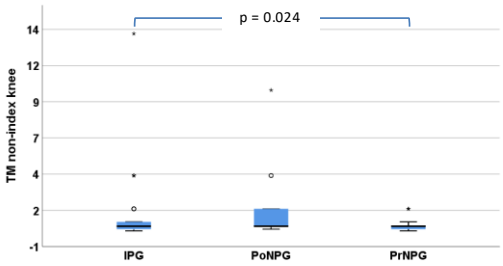


Fig-5b

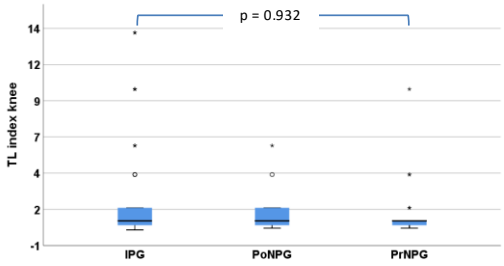


Fig-5c

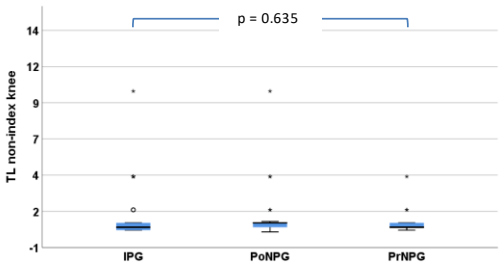
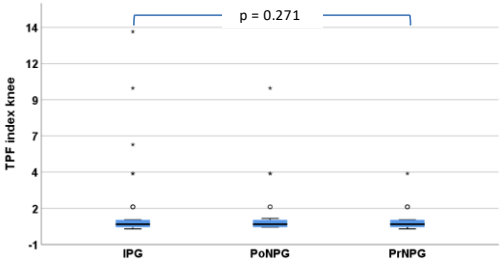


Fig-5d



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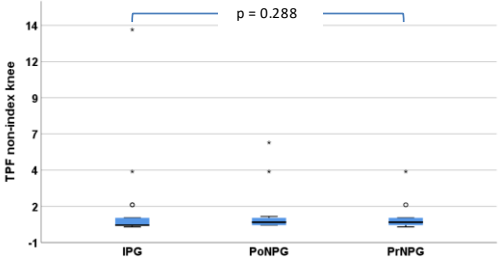

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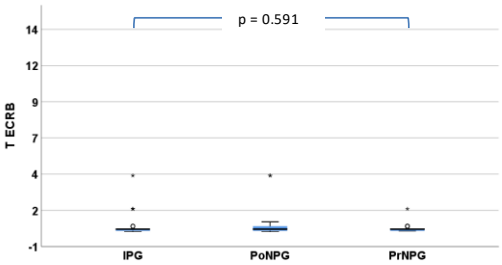


Fig-5g

Figure 5: Comparison of tactile threshold between the IPG, PoNPG and PrNPG at the medial (TM, Fig-5a & 5b), lateral (TL, Fig-5c & 5d) and popliteal fossa sites (TPF, Fig-5e & 5f) of the index and non-index knees as well as at the forearm (T ECRB, Fig-5g).

6.4.8. Heat pain threshold (HPT)

There was no statistically significant difference in HPT between OA groups at all knee locations for both index and non-index knees (Table 8, Figure 6).

Table 8: Heat pain threshold for the IPG, PoNPG and PrNPG at the medial (HPTM), lateral (HPTL) and popliteal fossa sites (HPTPF) of the index and non-index knees as well as at the forearm (HPT ECRB). p - ANOVA within three OA (IPG, PoNPG and PrNPG) groups.

	IPG	**PoNPG**	**PrNPG**	p-value
	Median (IQR) [min-max]			
HPTM index knee	44.5 (42.7-46.6) [37.1-49.5]	43.5 (42.0-45.4) [39.1-49.1]	44.7 (42.3-46.9) [38.8-49.0]	0.606
HPTL index knee	46.5 (44.7-47.7) [39.0-49.8]	45.4 (42.8-47.1) [30.2-48.8]	46.3 (45.5-47.6) [40.6-48.8]	0.070
HPTPF index knee	45.4 (43.4-46.8) [37.7-49.3]	44.1 (41.6-45.4) [28.7-49.6]	44.8 (42.4-46.0) [39.3-49.1]	0.106
HPTM non-index knee	44.1 (41.7-46.8) [35.7-49.0]	43.4 (42.2-45.7) [38.0-48.0]	44.1 (42.6-46.0) [39.9-49.4]	0.773
HPTL non-index knee	46.7 (44.6-47.8) [37.3-50.0]	45.0 (43.7-46.7) [37.8-48.9]	46.0 (42.9-48.0) [29.7-50.0]	0.356
HPTPF non-index knee	45.3 (43.0-46.5) [37.6-48.7]	44.4 (42.3-46.7) [40.3-48.6]	45.3 (43.3-46.6) [40.1-48.8]	0.920
HPT ECRB	45.9 (43.4-48.0) [39.1-50.0]	44.1 (42.7-45.6) [29.7-48.8]	45.0 (43.9-47.3) [27.8-49.6]	0.054

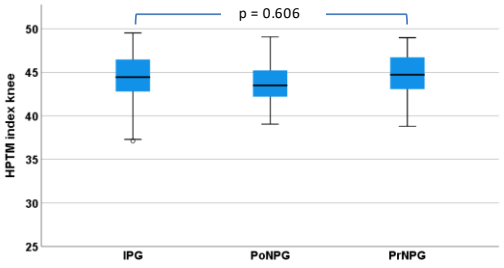


Fig-6a

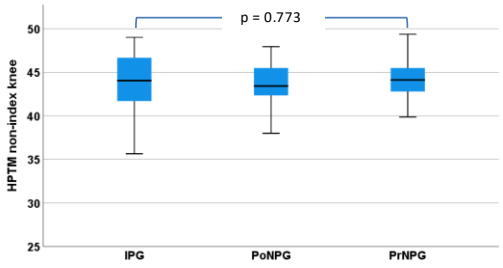


Fig-6b

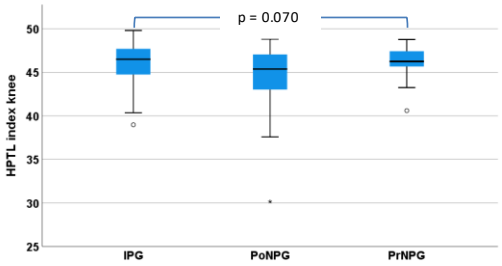


Fig-6c

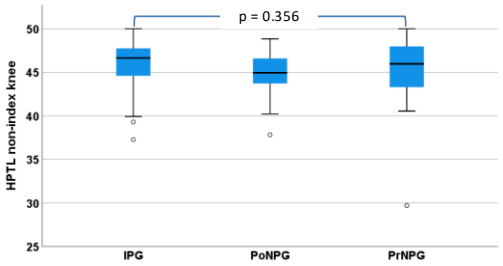


Fig-6d

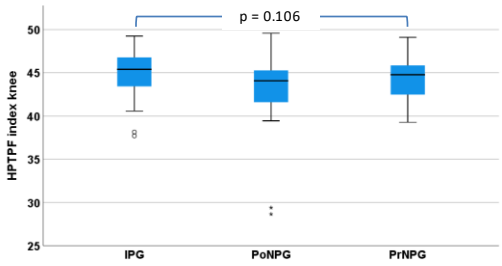


Fig-6e

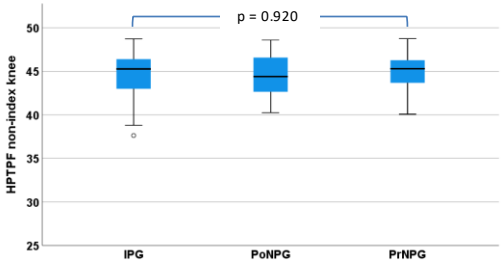


Fig-6f

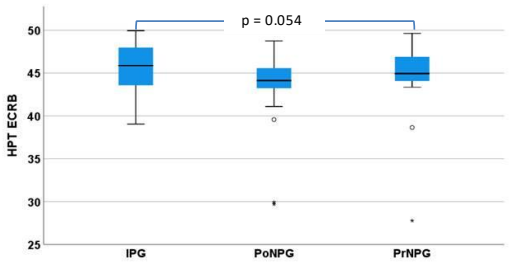


Fig-6g

Figure 6: Comparison of heat pain threshold between the IPG, PoNPG and PrNPG at the medial (HPTM, Fig-6a & 6b), lateral (HPTL, Fig-6c & 6d) and popliteal fossa sites (HPTPF, Fig-6e & 6f) of the index and non-index knee as well as at the forearm (HPT ECRB, Fig-6g).

6.4.9. Cold pain threshold (CPT)

Comparison of cold pain threshold (CPT) between the OA groups (IPG, PoNPG, PrNPG) showed no statistical difference at the medial of index knee (p = 0.068), there was significant difference of CPT at the medial of non - index knee (p = 0.001). ANOVA results for CPT showed significant difference between OA groups at the lateral and popliteal sites for the index (p = 0.012, 0.007) and for the non-index knees (p = 0.001, 0.042, 0.021) as well as at the forearm (p = 0.010). No statistically significant difference was found when CPT was compared between the IPG and PoNPG (p = 0.107) and PoNPG and PrNPG (p = 0.671). CPT was greater in the PrNPG compared to IPG (p = 0.055). Table 9, Figure 7.

Table 9: Cold pain threshold for the IPG, PoNPG and PrNPG at the medial (CPTM), lateral (CPTL) and popliteal fossa sites (CPTPF) of the index and non-index knees as well as at the forearm (CPT ECRB). p - ANOVA within three OA (IPG, PoNPG and PrNPG) groups.

	IPG	**PoNPG**	**PrNPG**	p-value
	Median (IQR) [min-max]			
CPTM index knee	20.7 (16.4-23.5) [0.0-27.1]	22.9 (19.0-24.7) [0.1-26.0]	23.3 (20.0-24.9) [7.8-25.9]	0.068
CPTL index knee	20.4 (16.2-23.4) [0.0-26.6]	23.0 (21.0-24.4) [4.8-25.8]	23.3 (20.0-25.0) [1.3-27.6]	0.012
CPTPF index knee	20.0 (14.2-22.4) [0.0-26.3]	22.2 (20.3-24.0) [1.6-26.0]	21.6 (19.8-23.6) [5.8-27.4]	0.007
CPTM_non_indexknee	20.5 (10.9-23.2) [0.0-27.0]	22.3 (19.8-23.6) [0.8-26.6]	24.0 (21.9-24.9) [19.2-26.6]	0.001
CPTL non-index knee	20.7 (11.3-23.5) [0.0-27.3]	22.8 (19.1-23.9) [1.0-26.6]	22.0 (20.2-24.3) [0.4-25.4]	0.042
CPTPF non-index knee	20.0 (11.6-23.2) [0.0-25.8]	22.7 (20.2-24.5) [1.5-26.0]	21.6 (19.7-24.2) [12.5-26.9]	0.021
CPT ECRB	18.8 (11.1-23.3) [0.0-27.3]	20.7 (19.0-23.7) [1.6-26.3]	21.5 (18.0-23.5) [16.1-27.0]	0.010

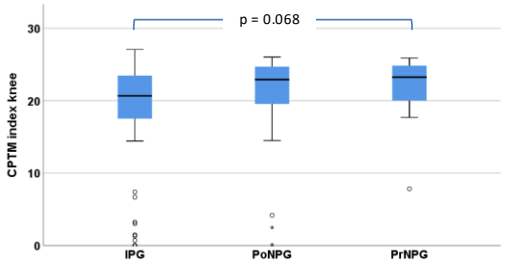


Fig-7a

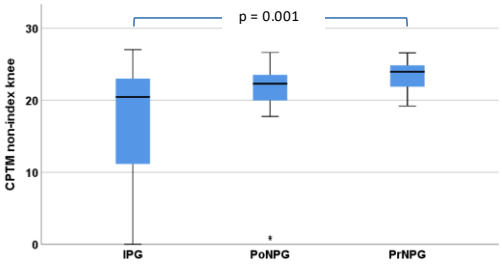


Fig-7b

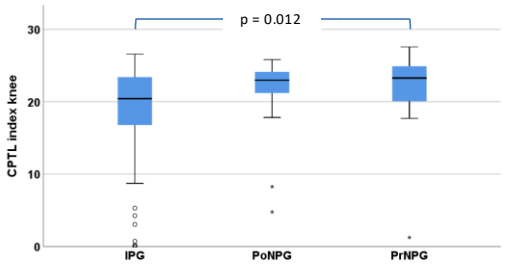


Fig-7c

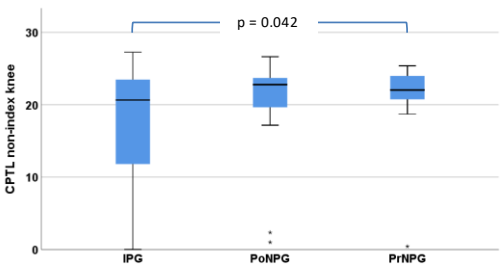


Fig-7d

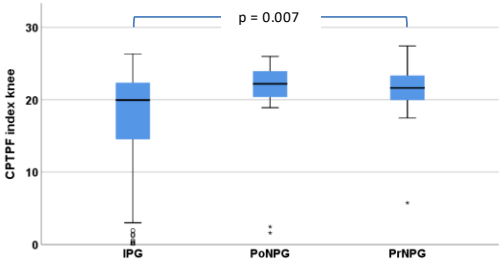
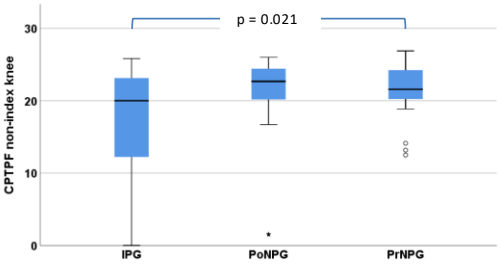


Fig-7e



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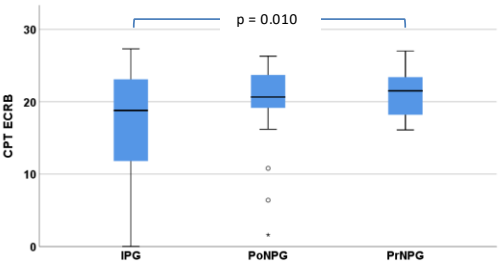


Fig-7g

Figure 7: Comparison of cold pain threshold between the IPG, PoNPG and PrNPG at the medial (CPTM, Fig-7a & 7b), lateral (CPTL, Fig-7c & 7d) and popliteal fossa sites (CPTPF, Fig-7e & 7f) of the index and non-index knees as well as at the forearm (CPT ECRB, Fig-7g).

6.4.10. Pressure pain threshold (PPT)

Although there was no statistically significant difference when the three OA groups were compared ($p > 0.05$), PPT values showed a decreasing trend from IPG to PrNPG. Table 10, Figure 8.

Table 10: Pressure pain threshold for the IPG, PoNPG and PrNPG at the medial (PPTM), lateral (PPTL) and popliteal fossa sites (PPTPF) of the index and non-index knees as well as at the forearm (PPT ECRB). p - ANOVA within three OA (IPG, PoNPG and PrNPG) groups.

	IPG	**PoNPG**	**PrNPG**	p-value
	Median (IQR) [min-max]			
PPTM index knee	389 (227-511) [127-1,419]	325 (234-402) [79-844]	254 (219-411) [137-535]	0.101
PPTL index knee	387 (228-577) [103-1,389]	341 (271-442) [172-741]	324 (213-500) [127-647]	0.547
PPTPF index knee	428 (277-562) [72-1,371]	324 (249-436) [93-1,087]	388 (245-465) [145-676]	0.145
PPTM non-index knee	381 (260-463) [80-1,267]	366 (236-407) [151-904]	257 (216-369) [117-517]	0.068
PPTL non-index knee	418 (238-565) [82-1,472]	350 (251-468) [145-879]	345 (237-452) [122-700]	0.346
PPTPF non-index knee	430 (301-578) [89-1,386]	382 (279-480) [115-1,070]	349 (255-438) [131-678]	0.171
PPT ECRB	332 (262-436) [159-905]	307 (245-407) [166-742]	310 (240-423) [182-495]	0.490

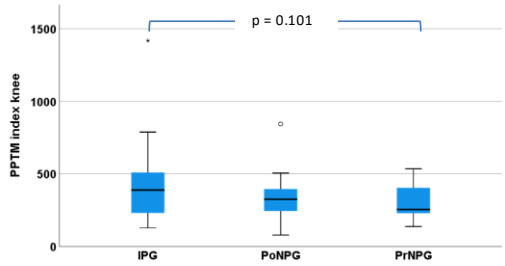
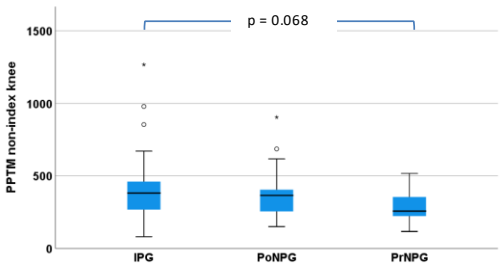


Fig-8a



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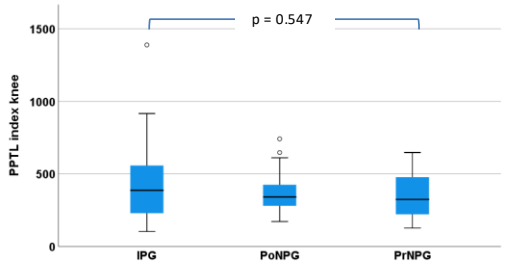

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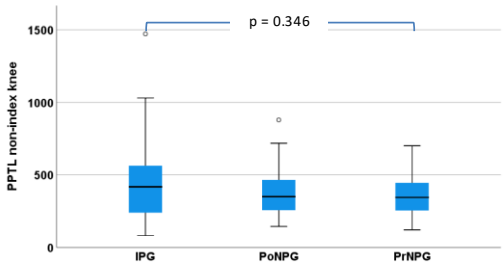

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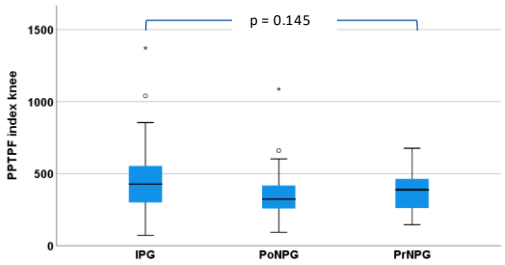

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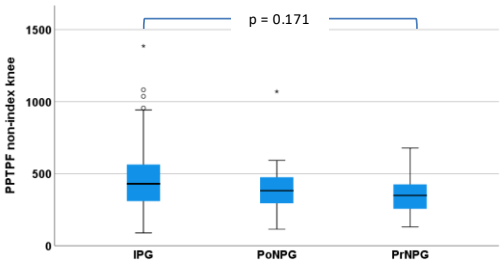

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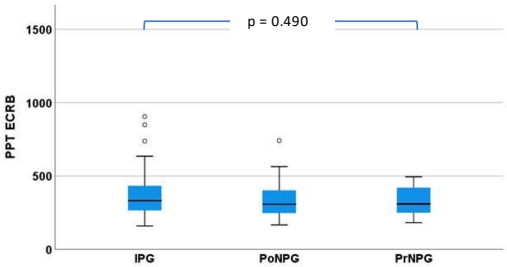

Fig-8g

Figure 8: Comparison of pressure pain threshold between the IPG, PoNPG and PrNPG at the medial (PPTM, Fig-8a & 8b), lateral (PPTL, Fig-8c & 8d) and popliteal fossa sites (PPTPF, Fig-8e & 8f) of the index and non-index knees as well as at the forearm (PPT ECRB, Fig-8g).

6.4.11. Logistic regression

Univariate regression model

As most of the variables studied showed no significant difference between PoNPG and PrNPG, univariate logistic regression analysis was carried out between the IPG and the combined PoNPG and PrNPG (designated as the neuropathic pain group, NPG) to determine which variables predict inclusion in the NPG and IPG groups.

Table 11 shows the significance levels and odds ratios for the respective sensory measures. The most sensitive sensory measures were PPTM, CPTM, CPTL, and VTPF of the index knee, and CPTM, CPTL, CPTPF of the non-index knee.

Table 11: Univariate logistic regression models of sensory and pain measures.

Variables	OR	95%CI OR	p-value
PPTM index knee	1.00	0.99-1.00	0.02
CPTM index knee	1.07	1.01-1.14	0.02
CPTL index knee	1.12	1.03-1.20	0.02
CPTM non-index knee	1.13	1.05-1.22	0.02
CPTL non-index knee	1.08	1.02-1.15	0.02
CPTPF non-index knee	1.07	1.02-1.13	0.02
JRE35 index knee	0.67	0.52-0.86	0.02
VTPF index knee	1.06	1.01-1.11	0.02
CDTPF index knee	0.80	0.56-1.13	0.20
CDTPF non-index knee	0.89	0.70-1.14	0.35

Multivariate regression model

Variables, PPTM, VTPF, CPTM and CPTL of the index knee, and CPTM, CPTL, CPTPF of the non-index knee were entered into a multivariable logistic regression model. The variables PPTM, VTPF of index knee were significant with OR of 1.00 and 1.08, Table 12.

Table 12: Multivariable logistic regression models of sensory and pain measures.

Variables	**OR**	**95%CI OR**	**p-value**
PPTM index knee	1.00	0.99-1.00	0.017
CPTM index knee	0.94	0.81-1.09	0.423
CPTL index knee	1.12	0.97-1.28	0.122
CPTM non-index knee	1.11	0.98-1.27	0.111
CPTL non-index knee	0.96	0.84-1.09	0.533
VTPF index knee	1.08	1.01-1.14	0.018

6.5. Discussion

Results of this study demonstrated that proprioceptive deficits are higher in the inner knee flexion range (15-20^{o}). JRE was not different between the index and non-index knees for both ranges of knee flexion, 15-20^{o} or 35-40^{o}, suggesting a fairly generalized change.

JRE was measured to assess if it helps to differentiate pain phenotypes among OA patients. This is the first study to assess proprioceptive function in different pain phenotype groups in people with OA. There was no difference of JRE between the IPG, PoNPG and PrNPG at 15-20^{o} flexion ($p = 0.840$ at index knee and $p = 0.874$ at non-index knee). IPG participants had higher JRE and greater variability compared to the PoNPG and PrNPG at 35-40^{o} knee flexion at the index knee ($p = 0.002$). This difference was opposite to what was hypothesized, higher JRE for PoNPG and PrNPG. Hence, this hypothesis was not supported by the results of this study.

These results indicate that the presence of NP may not be linked to a higher JRE or proprioceptive impairment. Previous research has suggested that after pain treatment and knee replacement, proprioceptive function improves in people with knee OA (Barrett et al., 1991; Shakoor et al., 2012). This highlights the importance of treating OA pain promptly as if left untreated proprioceptive impairment may make the person more susceptible to further degenerative changes.

Index and non-index knees were evaluated separately for JRE as well as QST. Results of all measures were very similar in the index and non-index knee as well as at the forearm. These results indicate that altered sensory and pain thresholds are generalized phenomena.

The hypotheses of this study that participants in the PrNPG will exhibit greater levels of hypoesthesia and hyperalgesia (based on QST measures) compared to the participants in the IPG and PoNPG were only supported for some of the sensory and pain measures.

QST is considered a sophisticated method to assess somatosensory function (Arendt-Nielsen et al., 2015).When compared between the OA groups (IPG, PrNPG, PoNPG), sensory thresholds for heat, cold and tactile measures were not significantly different between the OA groups. The vibration threshold at the index knee and forearm showed significant differences. The PoNPG had higher vibration thresholds compared to the PrNPG and the IPG. Diminished vibration detection threshold associated with knee OA may be indicative of compromised sensory function, secondary to presynaptic inhibition (Geber et al., 2008).

Altered sensory detection threshold at the painful knee has been reported previously as hypoesthesia among OA participants (Hochman et al., 2013; Wright et al., 2015). The Z-score scores for this study's participants showed a large number of people with sensory deficit (section 3.3.7). Participants in the IPG also showed sensory deficits, suggesting neurological damage starts early in the disease process and is a very widespread phenomenon amongst OA sufferers.

QST has been widely used to assess somatosensory function in OA patients (Hochman et al., 2013; Moss et al., 2016; Wright et al., 2017). This study used QST measures to differentiate pain phenotypes in the OA population. In general, there is a worsening trend from the IPG to the PrNPG for pain thresholds (heat, cold and pressure pain thresholds).

Although ANOVA for PPT did not show a significant difference between OA groups ($p > 0.05$), there was a trend of higher PPT in the IPG relative to the PoNPG and PrNPG. PPT values were similar in the PoNPG and PrNPG. The CPT values were variable and had a broader range at all sites. CPT in the PoNPG and PrNPG were similar and no difference was found in CPT between the PoNPG and PrNPG ($p > 0.05$). In the light of these results multiple logistic regression models were developed between the IPG and an overall NPG (PoNPG and PrNPG combined).

Previous studies either compared pain and sensory measures to control participants or between OA participants who were divided based on self-report PainDETECT questionnaire scores. This study took into account participants' sensory deficit and report of NP symptoms as well as PainDETECT scores to assign them to the different pain phenotype groups. As such, this is a first account of a more comprehensive neurological assessment of knee OA participants.

Conclusions

Although sensory, pain measures and proprioceptive functions were significantly compromised in OA participants when compared pain free participants (Chapter 3). Sensory deficit was evident for vibration and tactile sensation in almost 50% of OA participants (Chapter 3). Most of these measures when compared between OA groups showed no significant difference. Pressure pain threshold on medial, cold pain threshold on medial and lateral, cold detection on medial and vibration threshold on the popliteal fossa of the index knee, cold pain threshold on medial and lateral and popliteal fossa of the non-index knee, all showed significant differences. A step wise multivariate logistic regression model provided support for the inclusion of vibration threshold on the popliteal fossa and pressure algometry

on the medial side of the index knee to differentiate pain phenotypes. However further investigation is required to establish cut-offs for these measures to confirm inclusion into NPG or IPG.

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7. MRI features to identify pain phenotypes in knee osteoarthritis

7.1. Introduction

Osteoarthritis (OA) is the most common joint disease worldwide (Bijlsma et al., 2011). Loss of cartilage was considered to be the hallmark of OA but studies have also shown the combined role of bone and synovial tissue (Sellam & Berenbaum, 2010) in the pathophysiology of OA. Pathological changes, bone oedema, bone cysts and osteophytes are features of the OA joint that are known to be potential sources of pain (Felson et al., 2001). Structural changes involve the whole joint and cause pain and functional disability (Roubille et al., 2014).

It has been reported that a number of people with knee OA experience persistent pain even after surgery (Wright et al., 2015). This persistent pain was associated with widespread pressure hyperalgesia, cold hyperalgesia, and greater neuropathic-type pain (Wright et al., 2015). The true source of OA pain is not clear. Pain in OA has been conventionally attributed to joint structural damage. However, radiological features do not always match with the severity of symptoms such as pain and physical disability in OA patients (Mease et al., 2011; Roubille et al., 2014). This suggests that factors other than the joint pathology itself may also contribute to the pain. Pharmacological management is primarily aimed at treating the pain, but it is not effective for everyone (Wang et al., 2020). The heterogeneous nature of pain phenotypes among knee OA patients has been emphasised (Knoop et al., 2011). OA patients may experience both nociceptive and neuropathic pain (NP) to varying degrees (Schaible, 2012). Studies over the last decade have reported that a percentage of people with OA may experience neuropathic symptoms (Hochman et al., 2011; Ohtori et al., 2012). However, there are no specific tools available to confirm a NP diagnosis. There is therefore a need to explore which measures are associated with NP so a proper diagnostic process can be made available to clinicians to diagnose and treat patients with NP accordingly.

MRI is the best non-invasive method of making a comprehensive assessment of joint morphology. The knee joint of OA patients has been assessed using MRI for several OA features (Eckstein et al., 2006; Felson et al., 2001; Garnero et al., 2005; Roubille et al., 2014). These features include thinning of cartilage, bone marrow oedema, osteophytes, bone

attrition and synovitis in several studies. Bone marrow lesions (BMLs) in young adults are associated with knee symptoms and knee structural lesions (Antony et al., 2016). The presence of bone marrow lesions on MRI was associated with presence of pain (Felson et al., 2001). Meniscal lesions were also suggested to progress OA and a recent study found lateral meniscal lesions were associated with NP (Englund et al., 2012; Roubille et al., 2014).

The focus of this study was to investigate whether any of these OA pathological features relate to NP and can help in making a diagnosis of NP.

Hypothesis

Structural changes found on knee MRI in people with OA, including osteophytes, bone oedema, bone cysts, bone attrition areas and synovitis will be more marked in participants in the PrNPG (probable neuropathic pain group) compared to the PoNPG (possible neuropathic pain group) and the IPG (inflamatory pain group).

Aims

- To determine whether pathological features present on MRI demonstrate differences based on pain phenotype categories.
- To develop regression models to determine which measures are most clearly associated with membership of the neuropathic pain group (NPG).

7.2. Methods

OA participants completed a series of self-report questionnaire related to neuropathic pain, pain quality, physical function, psycho-social assessment, sleep quality and comorbidity. They were also tested for a range of sensory and pain related measures using quantitative sensory testing (QST) and assessment of proprioceptive function (detailed methodology described in Chapter 2). All OA participants attended three appointments; the first for clinical assessment, the second for QST and the third for proprioceptive testing. At the end of the first session participants were given a referral for knee MRI.

Knee MRIs were performed using a 1.5T whole body MR unit (GE Healthcare, Buckinghamshire, UK) using a dedicated 8-channel knee coil. All MRIs were taken at SKG Radiology. MRIs were programmed to proton density-weighted T2 fat saturation, 3D fast spin

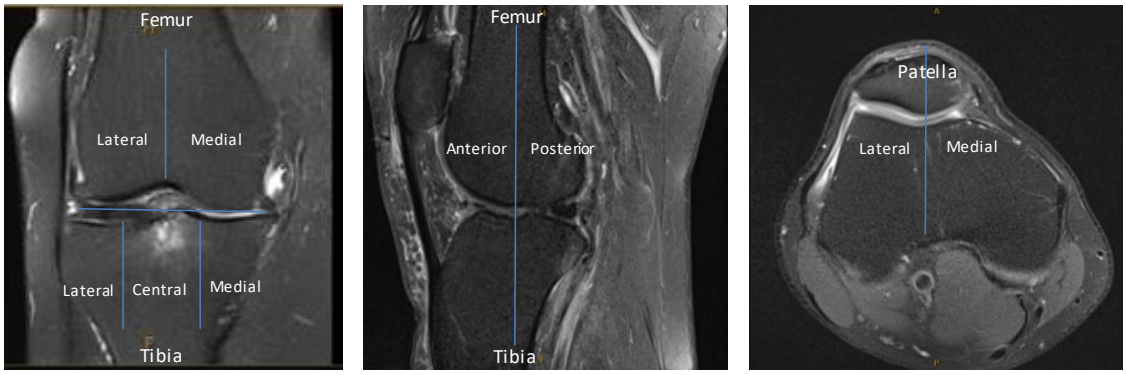
echo sequence in the axial, sagittal and coronal planes, fat sat images. Coronal Images were sliced to 2.5 mm, Sagittal 2.3 mm and axial images 4 mm.

SKG's InteleViewer software Version 4-6-1-P171 Mac OS X (Intelerad Inc., Montreal, Canada) was used for semi-quantitative analysis of the following features (Felson, 2005; Kornaat, Watt, et al., 2005; Peterfy et al., 2006).

i. Cartilage morphology
ii. Bone marrow lesions (oedema)
iii. Bone cysts
iv. Subarticular bone attrition
v. Osteophytes
vi. Medial and lateral meniscal integrity
vii. Effusion

To score the above lesions the knee joint was divided in the following compartments (Figure 1).

- Medial and lateral femoral
- Medial, central, and lateral tibial
- Medial and lateral patella



(a) (b) (c)

Figure 1: Knee joint compartments for scoring; (a) coronal, (b) sagittal, (c) axial images adopted from MRI feature analysis described by Felson et al, Konaat et al and Peterfy et al (Felson et al., 2001; Kornaat, Ceulemans, et al., 2005; Peterfy et al., 2006).

7.2.1. Cartilage morphology

To assess cartilage defects a semi quantitative method was adopted from the Knee Osteoarthritis Scoring System (KOSS) (Kornaat, Ceulemans, et al., 2005) (Figure 2). Cartilage defects were scored on T2 Fat Saturated Coronal, axial & sagittal images.

Coronal and sagittal images were used to assess tibio-femoral and axial images for patello-femoral cartilage. Cartilage defects were scored in relation to the height/depth of adjacent cartilage whether focal or diffuse and graded as (Kornaat, Ceulemans, et al., 2005),

0 Normal thickness and signal.
1 Normal thickness but increased signal on T2-weighted images.
2 Partial or full-thickness focal defect < 1 cm in greatest width.
3 Multiple areas of partial-thickness (Grade 2) defects intermixed with areas of normal thickness, or a Grade 2 defect wider than 1 cm but < 75% of the region.
4 Diffuse (≥ 75% of the region) partial-thickness loss.
5 Multiple areas of full-thickness loss (Grade 2) or a Grade 2 lesion wider than 1 cm but < 75% of the region.
6 Diffuse (≥ 75% of the region) full-thickness loss.

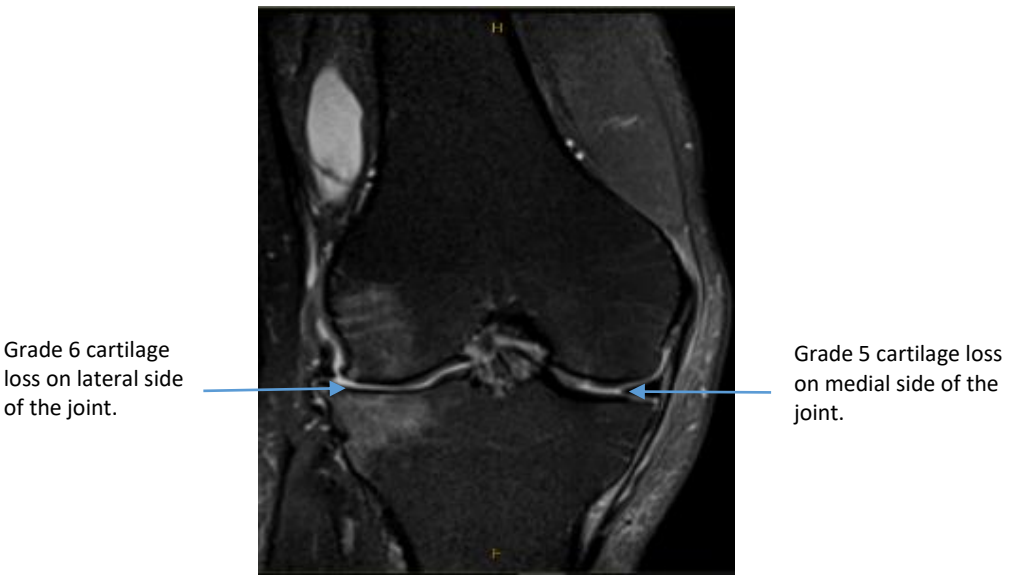


Figure 2: Grade 6 cartilage loss on lateral and Grade 5 cartilage loss on the medial side of joint, MRI image of a study participant. Scoring protocol adopted from (Kornaat, Ceulemans, et al., 2005).

7.2.2. Bone oedema

Bone marrow lesions (BML) were defined as areas of increased signal intensity adjacent to subchondral bone in either the distal femur or the proximal tibia (Felson, 2005). The presence of BML was defined as any increase in intensity of signal from baseline. The presence and extent of BMLs was classified based on the following grades adopted from Felson et al and Wang et al (Felson, 2005; Wang et al., 2015) ,

0 Absent.

1 A lesion was identified as being definitely present if it appeared on 2 or more adjacent slices. The lesion was classified as Grade 1 if it covered less than one-fourth of the width of any compartment of femur, tibia or patella (small lesions).

2 A bone marrow lesion, the same size as Grade 1 but present in three adjacent slices was scored Grade 2.

3 When a bone marrow lesion covered at least one-fourth of the width of the medial or lateral compartment and appeared on 3 or more slices it was classified as Grade 3 (Felson, 2005; Wang et al., 2015) (Figure 3).

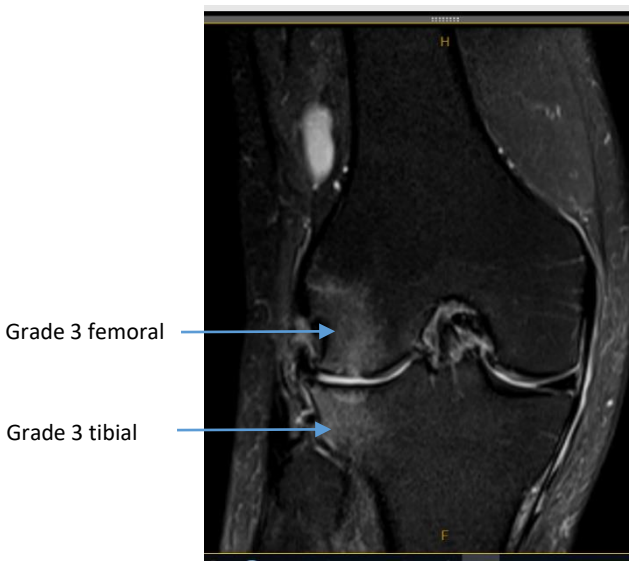


Figure 3: Grade 3 bone oedema in the lateral femur and tibia on coronal image (MRI image of a study participant).

The scoring system was adopted from a previously described methodology that exhibited good inter and intra reader reliability, ($k = 0.88$, $P < 0.001$) (Wang et al., 2015).

7.2.3. Bone cysts

Bone cysts are visualised on MRI as distinctly increased signal in the subarticular bone. Cysts are defined as increased signal with sharp, round margins and no evidence of internal marrow tissue or trabecular bone. Cysts were defined (scored) using T2 weighted fat sat images (Figure 4). Subchondral cysts were defined as well-defined foci of high signal intensity on T2-weighted images, in the cancellous bone underlying the joint cartilage.

Bone cysts were scored by measuring the largest dimension. Cysts were graded as follows as described by Peterfy et al (Peterfy et al., 2006),

0 absent
1 < 3 mm (small)
2 3-5 mm (medium)
3 5 mm (large)

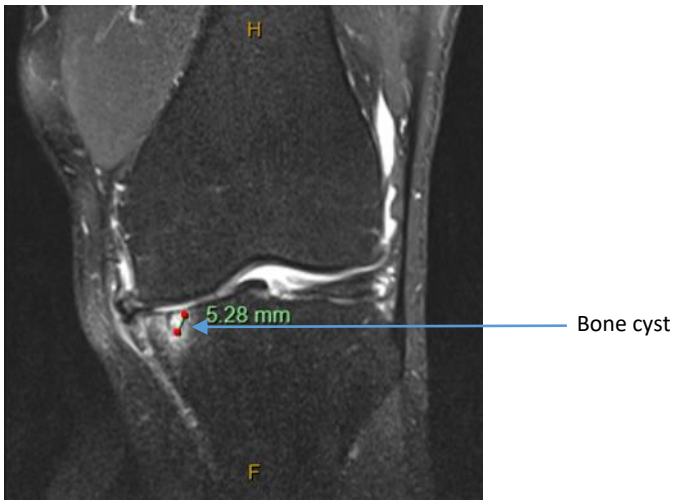


Figure 4: Subchondral medium cyst in the central tibia on T2 weighted coronal image (MRI image of a study participant).

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7.2.4. Subarticular bone attrition

Bone attrition is described as remodelling of the bone. Bone attrition results in flattening or depression of the articular surfaces and is a common feature of OA. Bone attrition was graded 0 to 3 based on deviation from normal bone contour,

0 normal
1 mild
2 moderate
3 severe

Relative flattening of the medial and lateral articular surfaces of the femur, tibia and patella was used for grading (Peterfy et al., 2004) (Figure 5). Coronal T2 weighted images were used to grade bone attrition.

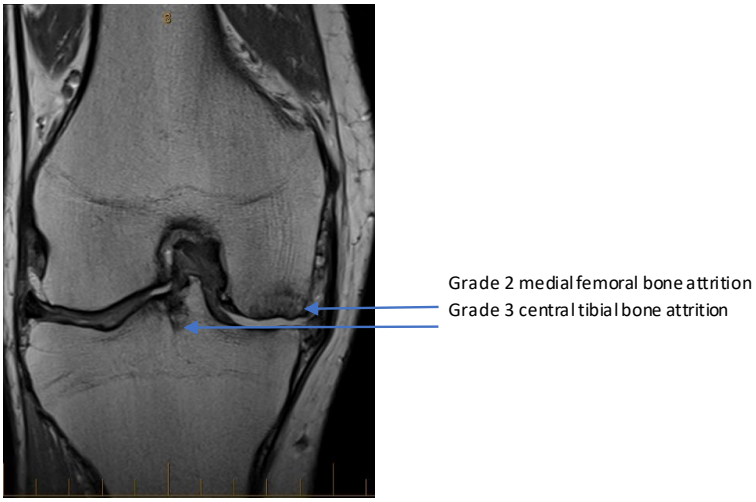

Figure 5: Grade 2 medial femoral and Grade 3 central tibial attrition visualised on coronal image (MRI image of a study participant).

7.2.5. Osteophytes

Osteophytes are fibro-cartilaginous bony outgrowths formed as a result of new bone formation in joints with OA. The following grading system was used for osteophyte scoring (Peterfy et al., 2006).

0 no osteophyte detected
1 small osteophyte

2 medium osteophyte

3 large osteophyte.

T1 weighted images were used to grade osteophytes at the margins of the medial and lateral aspects of the femur, tibia, and patella on coronal and axial images and anterior and posterior on sagittal images (Figure 6).

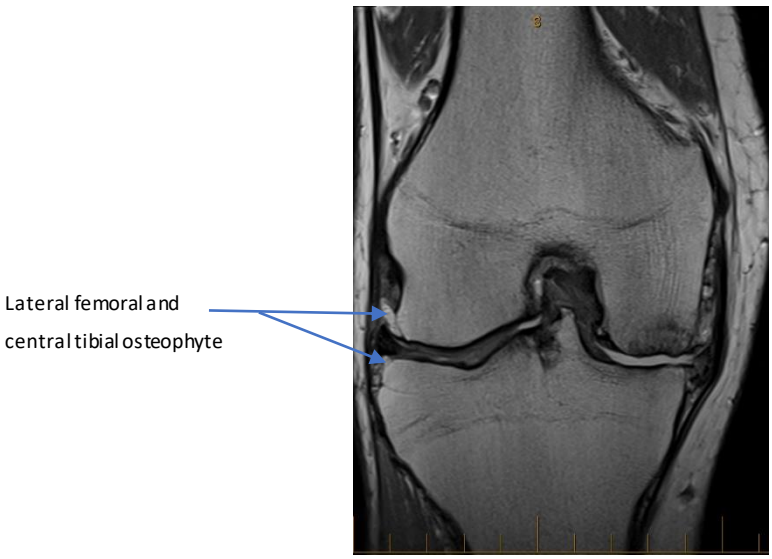

Figure 6: Coronal T1 weighted MRI image showing lateral and central osteophytes (MRI image of a study participant).

7.2.6. Medial and lateral meniscal integrity

The anterior horn, body segment and posterior horn of the medial and lateral menisci were graded separately from 0 to 4 based on both the sagittal and coronal images (Peterfy et al., 2004) (Figure 7),

0 Intact

1 minor radial tear or fan shaped tear

2 non-displaced tear or prior surgical repair

3 displaced tear or partial resection

4 complete maceration or complete resection

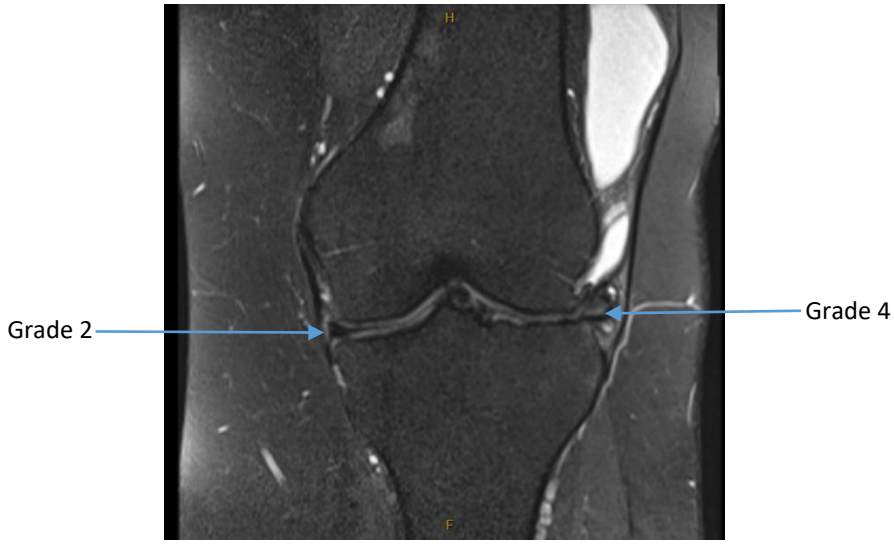

Figure 7: Grade 4 lateral meniscal lesion and Grade 2 medial meniscal lesion on coronal (MRI image of a study participant).

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7.2.7. Effusion

Effusion was given a grade based on T2 Fat Saturated axial and sagittal images (Figure 8). Grades for both the popliteal and suprapatellar regions were given. The grading system was adopted from the knee osteoarthritis scoring system (Kornaat, Ceulemans, et al., 2005). The grading system was as follows,

0 no effusion evident
1 mild effusion present
2 moderate effusion present
3 severe effusion present

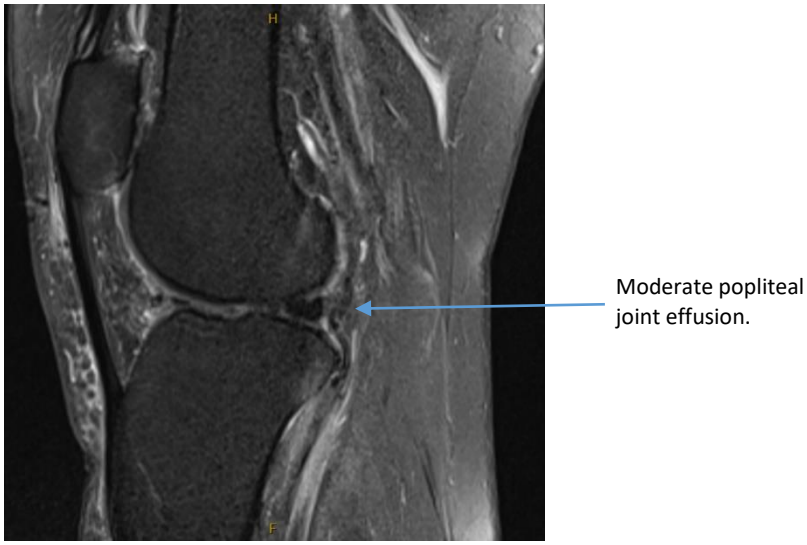

Figure 8: Sagittal T2 weighted MRI of a study participant showing moderate joint effusion (MRI image of a study participant).

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7.3. Data Analysis

IBM® SPSS version 26 was used for statistical analyses.

Mean and standard deviation (SD) of sensory measures; heat, cold, tactile and vibration perception thresholds were used to calculate Z-scores. OA participants with a PainDETECT score ≤ 12 were included in the inflammatory pain group (IPG), participants with PainDETECT scores of ≥ 13 and reporting superficial pain or cutaneous sensitivity were classified in the possible neuropathic pain group (PoNPG). Participants in the PoNPG were further classified based on findings from the QST sensory testing. If their measures for any of the sensory tests were outside ± 1.96 Z-score from the mean of the pain free control group, they were classified as having a sensory deficit. OA participants in the PoNPG with a sensory deficit and related neuropathic symptoms in the same region, were classified as probable neuropathic pain (PrNPG).

MRI features were assessed by the researcher, blinded to questionnaire scores and sensory and pain measures. All MRI pathological measures were analysed for frequency distribution in each grade in the whole OA cohort as well as the separate incidence of these features in the IPG, PoNPG and PrNPG. Cross tabulation was performed to find differences in OA groups.

The cohort was also divided based on the IPG and the NPG, where data from PoNPG and PrNPG was combined.

Logistic regression analysis was carried out to predict association of MRI variables with membership of the NPG group.

7.4. Results

7.4.1. Demographics

A total of Ninety (90) OA participants attended radiology for MRI scans. 9 participants could not attend radiology due to personal reasons.

Amongst the 90 OA participants, 41 were in the inflammatory pain group (IPG), 28 in the possible neuropathic pain group (PoNPG) and 21 in the probable neuropathic pain group (PrNPG). Demographic variables for each group are presented in Table 1.

Table 1: Demographic distribution of participants.

Participants	Age	BMI	Gender
IPG	65 ±8	28 ±5	17 M, 24 F
PoNPG	65 ±7	30 ±5	13 M, 15 F
PrNPG	62 ±7	30 ±7	12 M, 9 F

The age range was similar in the three (IPG, PoNPG and PrNPG) groups and there was no significant difference in BMI between the respective groups. Male to female ratio did vary slightly between groups with a slight preponderance of males in the PrNPG.

7.4.2. MRI features

MRIs were analysed for cartilage morphology, bone oedema, bone cysts, bone attrition, osteophytes, meniscal lesions, and joint effusion. Incidence of these pathologies are presented as a percentage in Table 2.

Table 2: Frequency (%) distribution of pathological features of MRI in various grades.

		Grades						
		0	1	2	3	4	5	6
Tibio-femoral joint								
Cartilage defects								
	Anterior	1.2		2.3	4.7	29.1	45.3	17.4
	Posterior			3.5	4.7	18.6	51.2	22.1
Femur	Medial			4.5	5.7	14.8	38.6	36.4
	Lateral		1.1	6.8	13.6	37.5	31.8	9.1
Tibia	Medial		1.1	1.1	9.1	15.9	43.2	29.5
	Lateral		1.1	4.5	12.5	33.0	42.0	6.8
Bone oedema								
Femur	Anterior	31.4	26.7	20.9	20.9			
	Posterior	18.6	26.7	27.9	26.7			
	Medial	14.0	34.9	20.9	30.2			
	Lateral	29.1	39.5	25.6	5.8			
Tibia	Anterior	15.1	30.2	29.1	25.6			
	Posterior	23.3	33.7	22.1	20.9			
	Medial	23.3	24.4	18.6	33.7			
	Central	22.1	27.9	36.0	14.0			
	Lateral	34.1	37.6	18.8	9.4			
Bone cysts								
Femur	Medial	61.9	14.3	9.5	14.3			
	Lateral	70.2	8.3	13.1	8.3			
Tibia	Medial	79.0	6.2	4.9	9.9			
	Central	59.0	1.2	22.9	16.9			
	Lateral	63.4	8.5	15.9	11.0			
Bone attrition								
Femur	Medial	19.5	43.7	25.3	11.5			
	Lateral	48.3	41.4	9.2	1.1			
Tibia	Medial	2.3	35.6	40.2	21.8			
	Central	2.3	27.6	62.1	8.0			
	Lateral	16.3	53.5	26.7	3.5			
Osteophytes								
	Medial		5.7	31.8	62.5			
	Lateral		5.7	36.4	58.0			
	Posterior	1.1	9.2	31.0	58.6			
	Anterior		4.6	48.3	47.1			
Meniscus								
	Medial		16.5	11.8	36.5	35.3		
	Lateral	1.2	38.1	32.1	16.7	11.9		
Effusion								
		1.3	61.3	36.3	1.3			
Patello-femoral joint								
Cartilage	Medial		1.1	3.4	9.1	10.2	61.4	14.8
	Lateral			3.4	13.6	26.1	43.2	13.6
Cyst		69.8	26.7	2.3	1.2			
BE		13.6	22.7	42.0	21.6			
Effusion		2.3	67.0	28.4	2.3			

7.4.3. Cartilage Morphology

Cartilage morphology was assessed by grading cartilage defects from 0 to 6 (mild to severe). The frequency distribution is shown in Table 2. Anterior and posterior cartilage defects were graded using sagittal images which did not yield significant results ($p < 0.05$) for either IPG, PoNPG and PrNPG or IPG compared to NPG. Femoral and tibial cartilage were assessed separately on the medial and lateral side of the joint using coronal MRI images. There was no significant difference in the cartilage defects ($p < 0.05$) either between IPG, PoNPG and PrNPG or IPG compared to NPG cohorts except for the medial femoral cartilage, $p = 0.042$ (IPG, PoNPG and PrNPG) and $p = 0.006$ (IPG vs NPG). Medial femoral cartilage defects were higher in the neuropathic pain groups compared to the IPG. There was also a trend that a higher % of grades 5 and 6 cartilage defects were present in the PoNPG and the PrNPG (Figure 9, Table 3).

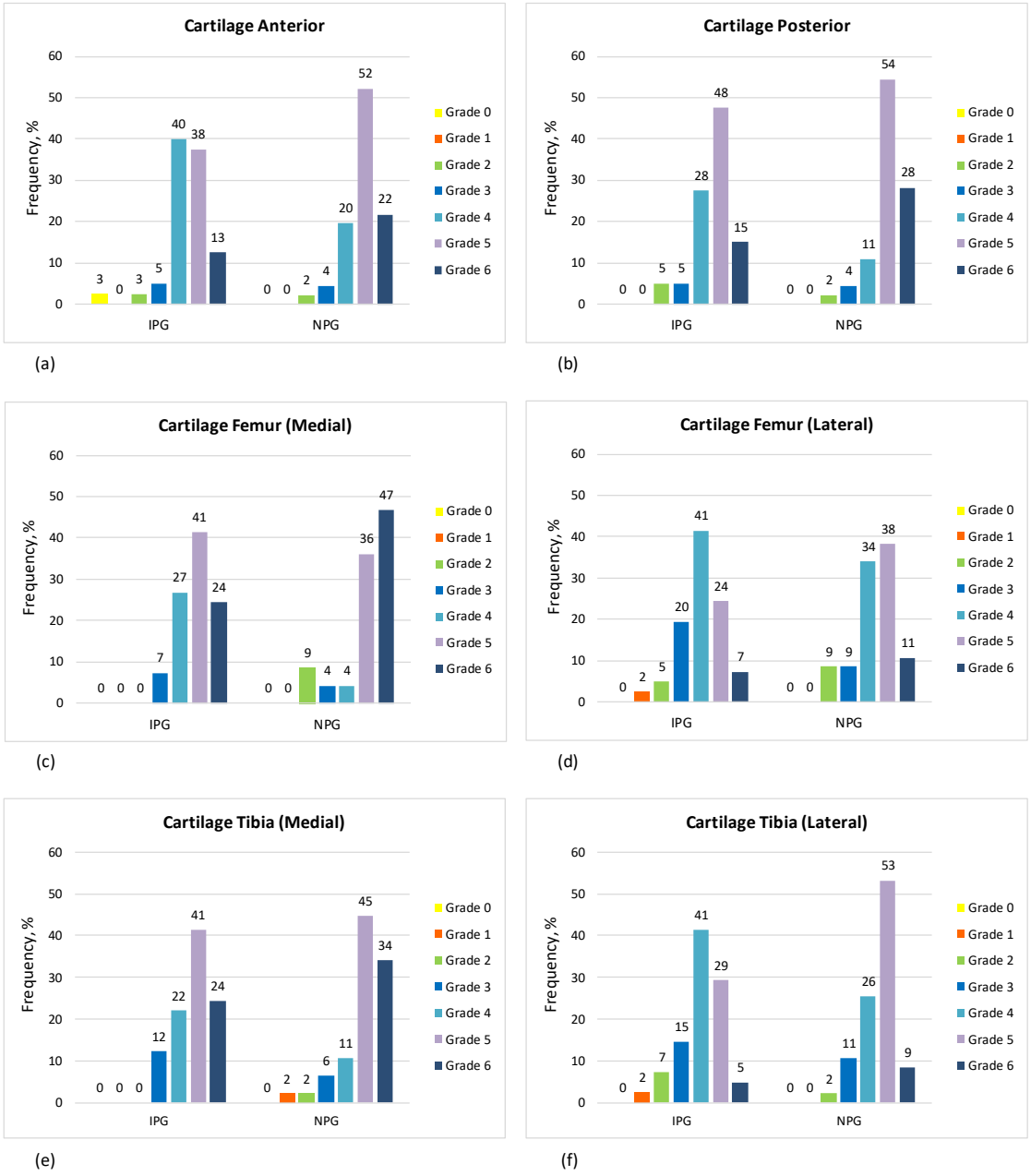

Figure 9: Cartilage defects compared between the IPG and the NPG; (a) anterior, (b) posterior cartilage on sagittal MRI images, (c) medial, (d) lateral femoral cartilage on coronal images, (e) medial, (f) lateral tibial cartilage on coronal MRI images.

Table 3: Frequency (%) distribution of cartilage defects as grades 0 to 6 in the IPG, PoNPG PrNPG and NPG at anterior and posterior aspects of the joint on sagittal images and femoral and tibial cartilage on coronal images; p* = comparison of IPG, PoNPG and PrNPG and p** = IPG compared to NPG.

			Grades							p-value	
			0	1	2	3	4	5	6	*	**
Cartilage											
	Anterior	IPG	2.5		2.5	5.0	40.0	37.5	12.5		
		NPG			2.2	4.3	19.6	52.2	21.7	0.492	0.277
		PoNPG				7.7	15.4	53.8	23.1		
		PrNPG			5.0		25.0	50.0	20.0		
	Poterior	IPG			5.0	5.0	27.5	47.5	15.0		
		PoNPG				7.7	7.7	53.8	30.8	0.401	0.232
		PrNPG			5.0		15.0	55.0	25.0		
		NPG			2.2	4.3	10.9	54.3	28.3		
Femur	Medial	IPG			0.0	7.3	26.8	41.5	24.4		
		PoNPG			7.4	7.4	3.7	33.3	48.1	0.042	0.006
		PrNPG			10.0		5.0	40.0	45.0		
		NPG			8.5	4.3	4.3	36.2	46.8		
	Lateral	IPG		2.4	4.9	19.5	41.5	24.4	7.3		
		PoNPG			7.4	7.4	29.6	40.7	14.8	0.700	0.365
		PrNPG			10.0	10.0	40.0	35.0	5.0		
Tibia		NPG			8.5	8.5	34.0	38.3	10.6		
	Medial	IPG			0.0	12.2	22.0	41.5	24.4		
		PoNPG		3.7	3.7	7.4	11.1	40.7	33.3	0.613	0.408
		PrNPG				5.0	10.0	50.0	35.0		
		NPG		2.1	2.1	6.4	10.6	44.7	34.0		
	Lateral	IPG		2.4	7.3	14.6	41.5	29.3	4.9		
		PoNPG				7.4	22.2	59.3	11.1	0.408	0.167
		PrNPG			5.0	15.0	30.0	45.0	5.0		
		NPG			2.1	10.6	25.5	53.2	8.5		

7.4.4. Bone oedema

Bone oedema (BE) was assessed by grading 0 to 3 (mild to severe) and the frequency distribution is shown in Table 4 and Figure 10. Anterior and posterior bone oedema was graded using sagittal images with no significant differences ($p < 0.05$) either between the IPG, PoNPG and PrNPG or the IPG compared to the NPG. Femoral and tibial bone oedema was assessed separately on the medial and lateral sides of the joint using coronal MRI images. There was no significant difference in the bone oedema ($p < 0.05$) either between the IPG, PoNPG and PrNPG or the IPG compared to the NPG cohorts except on the lateral aspect of the tibia. Lateral tibial bone oedema was worse in the neuropathic pain groups compared to the IPG, $p = 0.020$ (IPG, PoNPG and PrNPG) and $p = 0.006$ (IPG vs NPG).

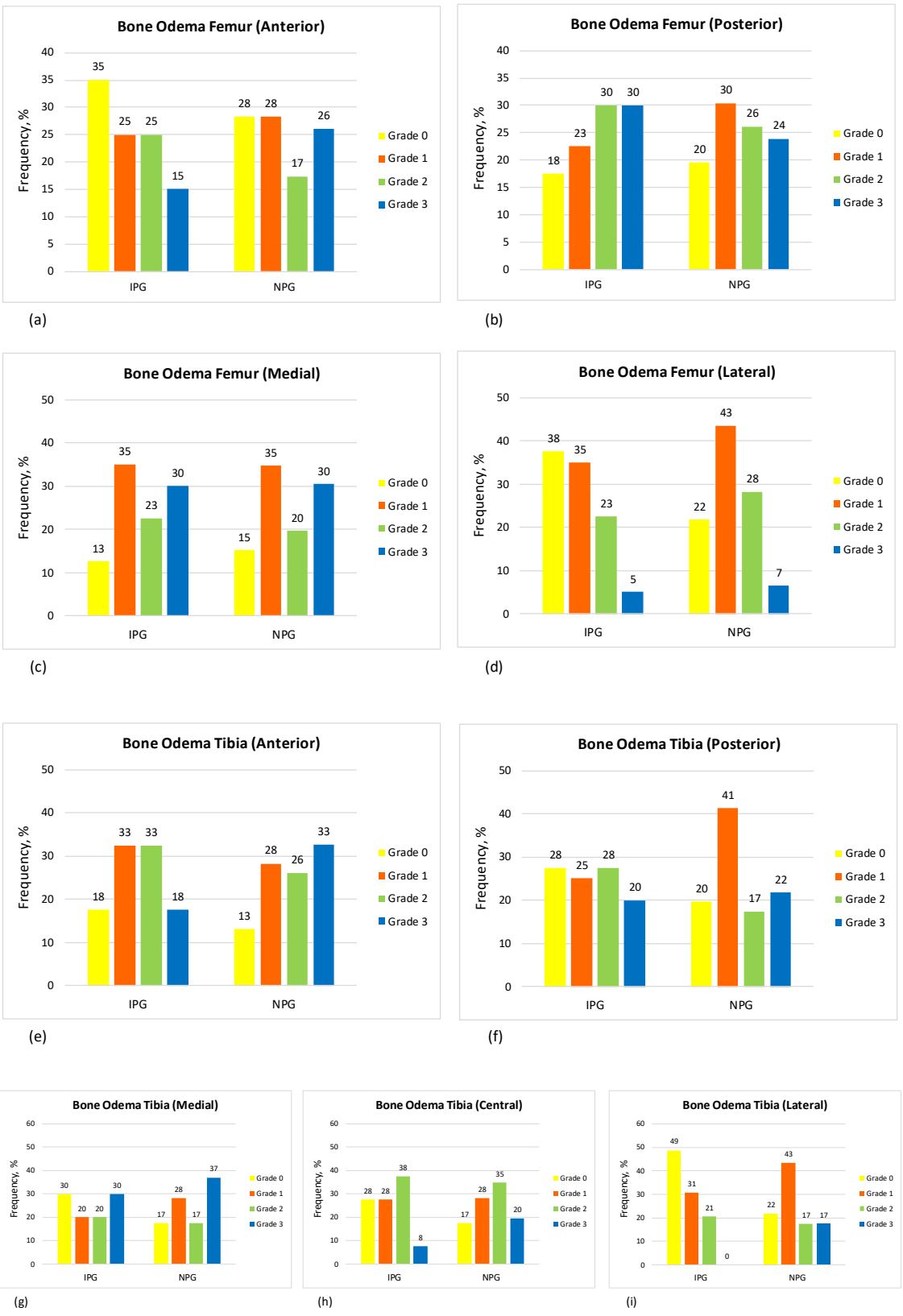

Figure 10: Bone oedema compared between the IPG and NPG; (a) anterior femur, (b) posterior femur, (c) medial femur, (d) lateral femur, (e) anterior tibia, (f) central tibia, (g) posterior tibia (h) medial tibia, (i) lateral tibia.

Table 4: Bone oedema frequency (%) distribution of grades 0 to 3 in the IPG, PoNPG PrNPG and NPG at the anterior and posterior aspects of the joint on sagittal images and femoral and tibial BE, scored on coronal images; p* = comparison of IPG, PoNPG and PrNPG and p** = IPG compared to NPG.

			Grades				p-value	
			0	1	2	3	*	**
Bone Oedema								
	Anterior	IPG	35.0	25.0	25.0	15.0	0.737	0.524
		PoNPG	26.9	34.6	15.4	23.1		
		PrNPG	30.0	20.0	20.0	30.0		
		NPG	28.3	28.3	17.4	26.1		
	Posterior	IPG	17.5	22.5	30.0	30.0	0.947	0.809
		PoNPG	23.1	26.9	26.9	23.1		
		PrNPG	15.0	35.0	25.0	25.0		
		NPG	19.6	30.4	26.1	23.9		
Femur								
	Medial	IPG	12.5	35.0	22.5	30.0	0.132	0.977
		PoNPG	11.5	42.3	30.8	15.4		
		PrNPG	20.0	25.0	5.0	50.0		
		NPG	15.2	34.8	19.6	30.4		
	Lateral	IPG	37.5	35.0	22.5	5.0	0.653	0.461
		PoNPG	19.2	38.5	34.6	7.7		
		PrNPG	25.0	50.0	20.0	5.0		
		NPG	21.7	43.5	28.3	6.5		
	Anterior	IPG	17.5	32.5	32.5	17.5	0.060	0.454
		PoNPG	15.4	34.6	34.6	15.4		
		PrNPG	10.0	20.0	15.0	55.0		
		NPG	13.0	28.3	26.1	32.6		
	Central	IPG	27.5	27.5	37.5	7.5	0.210	0.352
		PoNPG	11.5	34.6	26.9	26.9		
		PrNPG	25.0	20.0	45.0	10.0		
		NPG	17.4	28.3	34.8	19.6		
	Posterior	IPG	27.5	25.0	27.5	20.0	0.118	0.350
		PoNPG	19.2	26.9	23.1	30.8		
		PrNPG	20.0	60.0	10.0	10.0		
		NPG	19.6	41.3	17.4	21.7		
Tibia								
	Medial	IPG	30.0	20.0	20.0	30.0	0.145	0.485
		PoNPG	15.4	38.5	23.1	23.1		
		PrNPG	20.0	15.0	10.0	55.0		
		NPG	17.4	28.3	17.4	37.0		
	Lateral	IPG	48.7	30.8	20.5	0.0	0.020	0.006
		PoNPG	19.2	38.5	19.2	23.1		
		PrNPG	25.0	50.0	15.0	10.0		
		NPG	21.7	43.5	17.4	17.4		

7.4.5. Bone cysts

Bone cysts were not common compared to other pathological features among the OA groups. There were no significant differences in bone cysts presence ($p < 0.05$) either between the IPG, PoNPG and PrNPG or the IPG compared to the NPG cohorts (Table 5, Figure 11). Grade 3 cysts were more commonly present on the medial side of the femur and at the centre of the tibia, 12 and 14% respectively.

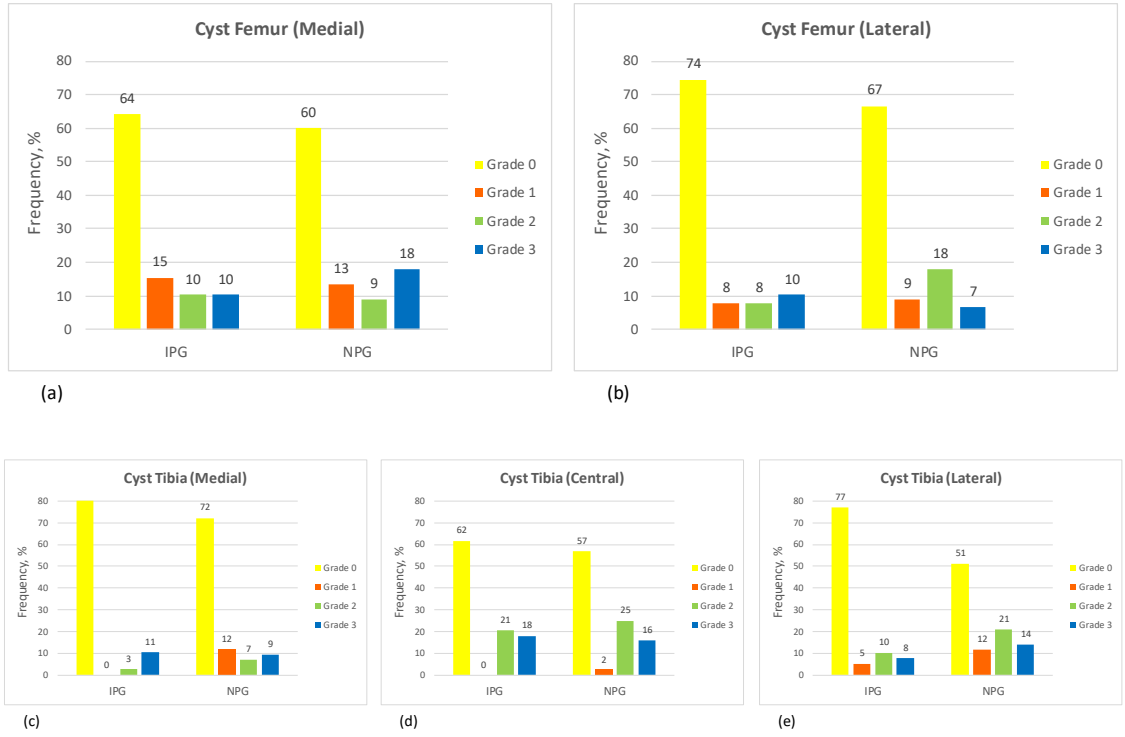

Figure 11: Comparison of bone cysts between the IPG and NPG; (a) medial femur (b) lateral, (c) medial tibia, (d) central tibia, (e) lateral tibia.

Table 5: Frequency (%) of bone cyst grades among the IPG, PoNPG, PrNPG and NPG; p* = comparison of IPG, PoNPG and PrNPG and p** = IPG compared to NPG.

			Grades						p-value	
			0	1	2	3	4	5	*	**
Bone cysts										
Femur	Medial	IPG	64.1	15.4	10.3	10.3				
		PoNPG	56.0	16.0	8.0	20.0			0.949	0.804
		PrNPG	65.0	10.0	10.0	15.0				
		NPG	60.0	13.3	8.9	17.8				
	Lateral	IPG	74.4	7.7	7.7	10.3				
		PoNPG	68.0	12.0	12.0	8.0			0.625	0.540
		PrNPG	65.0	5.0	25.0	5.0				
		NPG	66.7	8.9	17.8	6.7				
Tibia	Medial	IPG	86.8		2.6	10.5				
		PoNPG	75.0	16.7	4.2	4.2			0.109	0.123
		PrNPG	68.4	5.3	10.5	15.8				
		NPG	72.1	11.6	7.0	9.3				
	Central	IPG	61.5	0.0	20.5	17.9				
		PoNPG	60.0	0.0	20.0	20.0			0.546	0.754
		PrNPG	52.6	5.3	31.6	10.5				
		NPG	56.8	2.3	25.0	15.9				
	Lateral	IPG	76.9	5.1	10.3	7.7				
		PoNPG	50.0	12.5	20.8	12.5		4.2	0.440	0.181
		PrNPG	52.6	10.5	21.1	15.8				
		NPG	51.2	11.6	20.9	14.0		2.3		

7.4.6. Bone attrition

Bone attrition was graded 0 to 3. There were no significant differences in bone attrition ($p < 0.05$) either between the IPG, PoNPG and PrNPG or the IPG compared to the NPG cohorts except at the medial femur. Grade 3 bone attrition was most prevalent in the PrNPG on the medial side of the femur and tibia, 40 and 50% respectively, whereas Grade 2 attrition was commonly present at the central tibia, 75% in the PrNPG (Table 6, Figure 12).

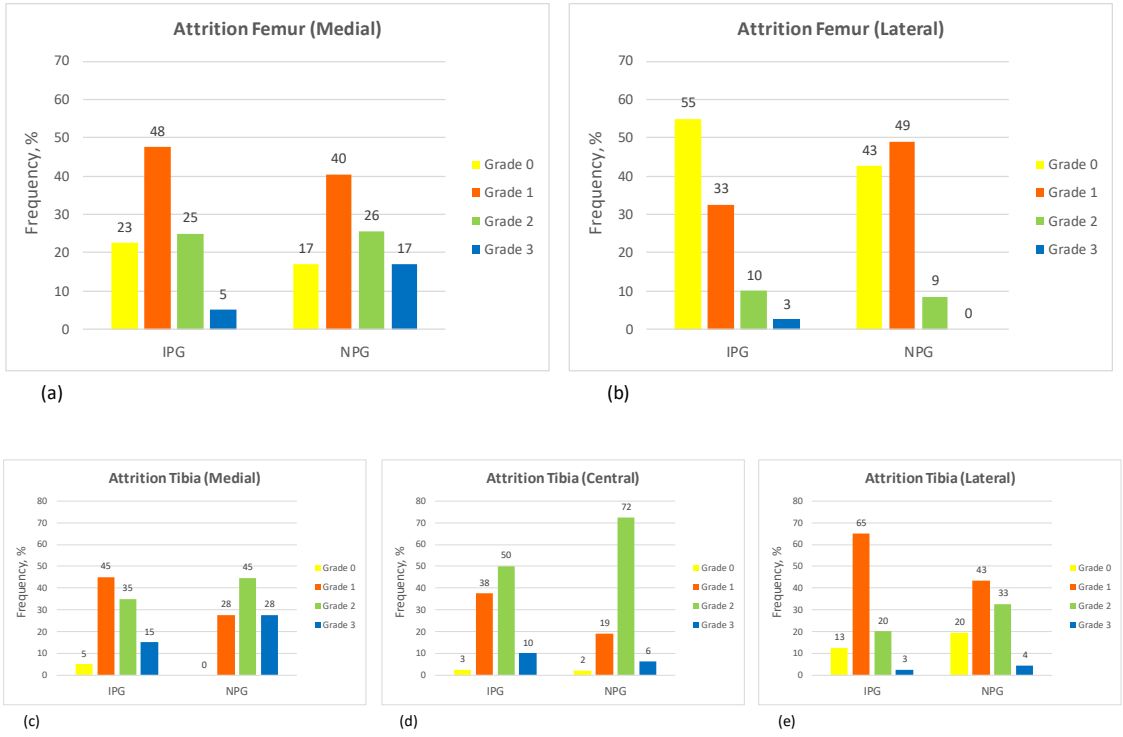

Figure 12: Comparison of bone attrition among the IPG and NPG; (a) medial femur, (b) lateral femur, (c) medial tibia, (d) central tibia, (e) lateral tibia.

Table 6: Frequency (%) of bone attrition grades among the IPG, PoNPG, PrNPG and NPG; p* = comparison of IPG, PoNPG, PrNPG and p** = IPG compared to NPG.

			Grades				p-value	
			0	1	2	3	*	**
Bone attrition								
Femur	Medial	IPG	22.5	47.5	25.0	5.0	0.017	0.348
		PoNPG	22.2	55.6	14.8	7.4		
		PrNPG	10.0	20.0	40.0	30.0		
		NPG	17.0	40.4	25.5	17.0		
	Lateral	IPG	55.0	32.5	10.0	2.5	0.325	0.343
		PoNPG	37.0	59.3	3.7	0.0		
		PrNPG	50.0	35.0	15.0	0.0		
		NPG	42.6	48.9	8.5	0.0		
Tibia	Medial	IPG	5.0	45.0	35.0	15.0	0.062	0.100
		PoNPG	0.0	40.7	40.7	18.5		
		PrNPG	0.0	10.0	50.0	40.0		
		NPG	0.0	27.7	44.7	27.7		
	Central	IPG	2.5	37.5	50.0	10.0	0.265	0.192
		PoNPG	0.0	25.9	70.4	3.7		
		PrNPG	5.0	10.0	75.0	10.0		
		NPG	2.1	19.1	72.3	6.4		
	Lateral	IPG	12.5	65.0	20.0	2.5	0.597	0.263
		PoNPG	18.5	48.1	29.6	3.7		
		PrNPG	21.1	36.8	36.8	5.3		
		NPG	19.6	43.5	32.6	4.3		

7.4.7. Osteophytes

Osteophytes (OP) were scored from grade 0 to Grade 3. Frequencies of the presence of OP are shown in Figure 13, Table 7. A higher % of Grade 2 and 3 osteophytes were present in the PoNPG and PrNPG compared to the IPG on the medial, lateral, anterior and posterior aspects of the knee. A statistically significant difference was found on the medial side between the IPG and the NPG ($p = 0.048$) and on the lateral side between the IPG, PoNPG and PrNPG ($p = 0.021$) and between the IPG and the NPG ($p = 0.003$).

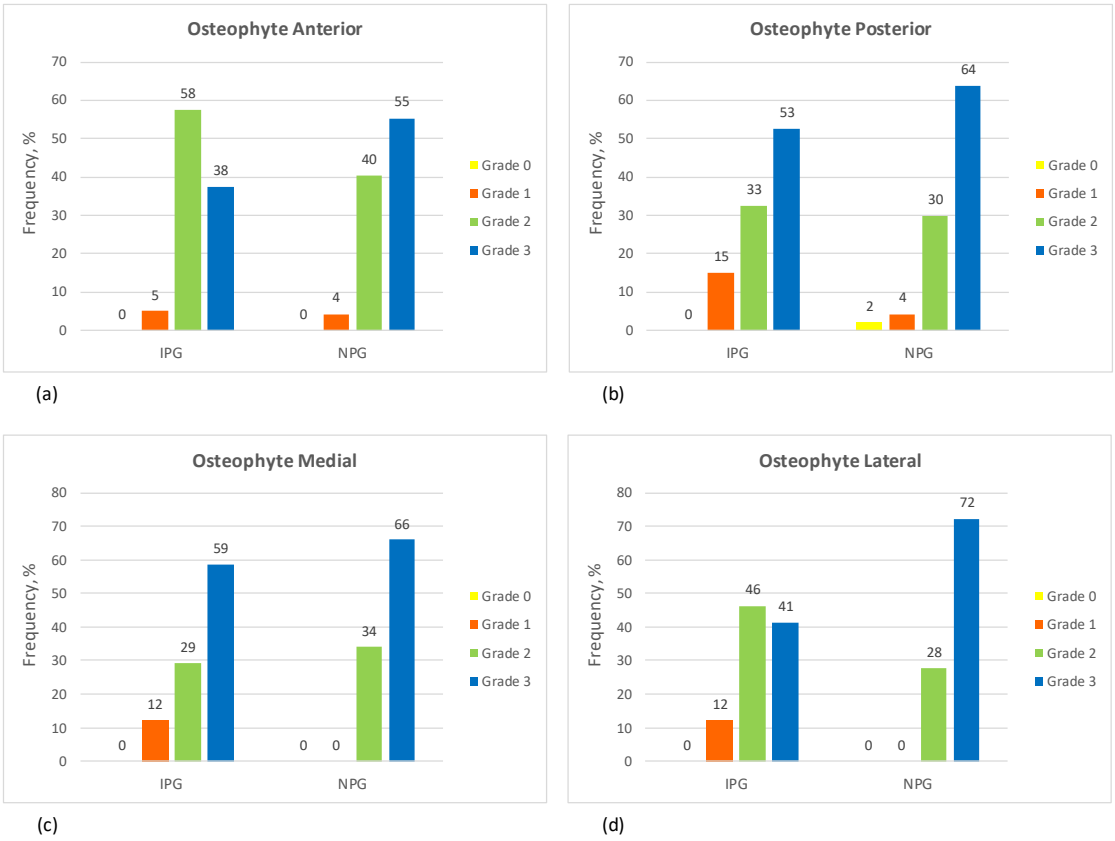

Figure 13: Comparison of osteophytes between the IPG and NPG; (a) anterior, (b) posterior, (c) medial, (d) lateral.

Table 7: Frequency (%) of osteophytes among the IPG, PoNPG, PrNPG and NPG participants; p* = comparison of IPG, PoNPG and PrNPG and p** = IPG compared to NPG.

			Grades				p-value	
			0	1	2	3	*	**
Osteophytes								
	Anterior	IPG		5.0	57.5	37.5	0.354	0.248
		PoNPG		7.4	40.7	51.9		
		PrNPG			40.0	60.0		
		NPG		4.3	40.4	55.3		
	Poterior	IPG		15.0	32.5	52.5	0.374	0.252
		PoNPG	3.7	7.4	25.9	63.0		
		PrNPG			35.0	65.0		
		NPG	2.1	4.3	29.8	63.8		
	Medial	IPG		12.2	29.3	58.5	0.115	0.048
		PoNPG			40.7	59.3		
		PrNPG			25.0	75.0		
		NPG			34.0	66.0		
	Lateral	IPG		12.2	46.3	41.5	0.021	0.003
		PoNPG			29.6	70.4		
		PrNPG			25.0	75.0		
		NPG			27.7	72.3		

7.4.8. Meniscal lesions

Meniscal lesions were graded from 0 to 4 as described in the methods section on coronal images. A higher frequency (%) of Grade 3 and 4 lesions was observed in the PoNPG and PrNPG compared to the IPG. A statistically significant difference was shown only for the lateral meniscus between the IPG and NPG (p = 0.024), Figure 14, Table 8.

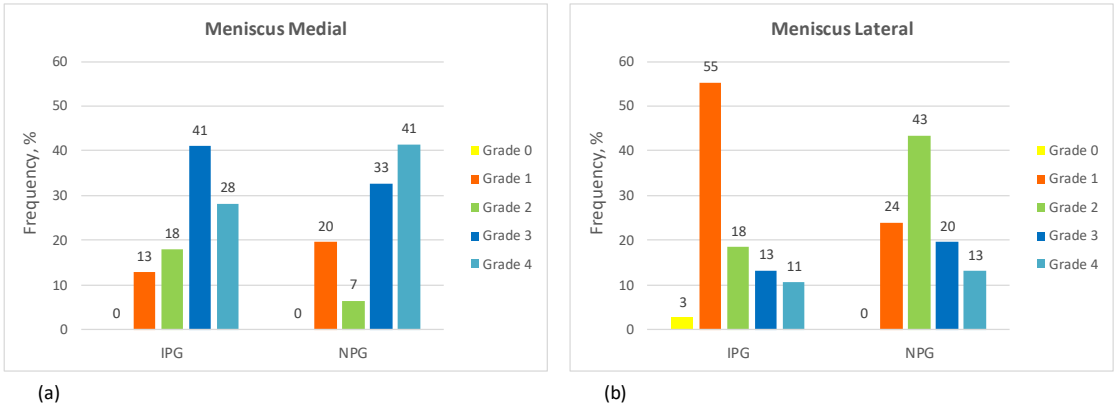

Figure 14: Comparison of meniscal lesions among the IPG and NPG; (a) medial, (b) lateral.

Table 8: Frequency (%) of meniscal lesions in the IPG, PoNPG, PrNPG and NPG cohorts; p* = comparison of IPG, PoNPG, PrNPG and p** = IPG compared to NPG.

			Grades					p-value	
			0	1	2	3	4	*	**
Meniscus									
	Medial	IPG		12.8	17.9	41.0	28.2	0.214	0.225
		PoNPG		26.9	7.7	34.6	30.8		
		PrNPG		10.0	5.0	30.0	55.0		
		NPG		19.6	6.5	32.6	41.3		
	Lateral	IPG	2.6	55.3	18.4	13.2	10.5	0.172	0.024
		PoNPG		26.9	42.3	19.2	11.5		
		PrNPG		20.0	45.0	20.0	15.0		
		NPG		23.9	43.5	19.6	13.0		

7.4.9. Effusion

Mild to moderate effusion was present in both the IPG and NPG groups. No difference was found between the two groups. Severe effusion was only reported in the IPG group and the incidence was 3%, Figure 15.

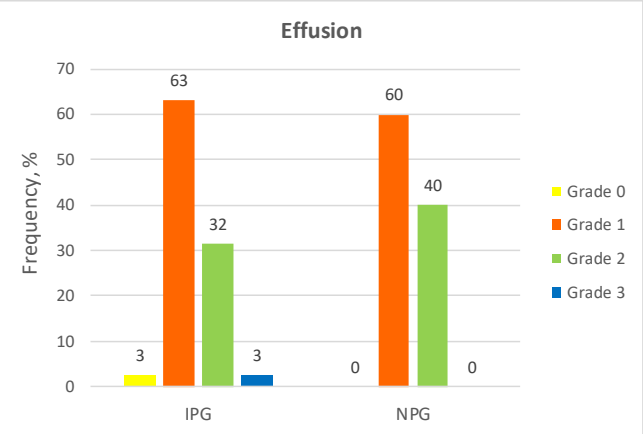

Figure 15: Comparison of presence of effusion among IPG and NPG.

7.4.10. Logistic regression

Univariate logistic regression analyses (IPG: NPG) were performed on the variables shown to be significant on cross tabulation (Table 9).

Table 9: Univariate logistic regression analysis

	OR	95%CI OR	p-value
Femoral medial cartilage	0.09	0.01-0.70	0.022
Lateral osteophytes	3.67	1.46-9.24	0.006
Medial osteophytes	1.32	0.56-3.12	0.531
Lateral tibial BE	2.92	1.04-8.24	0.042
Lateral meniscus	5.71	1.86-17.59	0.002

Lateral tibial oedema, lateral meniscal lesions and lateral and medial osteophytes were initially entered into the univariate models (Table 9). Femoral medial cartilage defects, medial and lateral osteophytes, lateral tibial bone oedema and lateral meniscal lesions (significant at $p < 0.1$) were entered into multivariable models. P-values < 0.05 were considered statistically significant (Table 10). Lateral osteophytes and lateral meniscus lesions were identified as pathological changes significantly associated with membership of the NPG.

Table 10: Multivariable logistic regression model of structural changes on MRI.

	OR	95% CI OR	p value
Lateral osteophytes	5.47	1.53 - 19.52	0.009
Lateral meniscus lesions	6.4	1.56 - 26.73	0.010

7.5. Discussion

The MRIs of OA patients were explored to assess the relationship between structural changes and the presence of NP.

Overall MRI assessment revealed that joint structural damage was moderate to severe in the PrNPG group. However, statistically significant differences were not present for most of the features. The logistic regression model indicated that the presence of osteophytes, tibial bone oedema and meniscal lesions on the lateral side were most strongly associated with membership of the NPG. Similar structural changes were also present on the medial side but the presence of these structural changes on the lateral side was most strongly associated with NP. Results also showed Grade 3 osteophytes (OP) were associated with the presence of NP. Previous research has indicated that OP on MRI were commonly detected in older adults and were likely to progress over time (Zhu et al., 2018) and the presence of large OPs was associated with more pain and lower physical activity (Hakky et al., 2015; Sowers et al., 2003). A higher number of OPs have been related to having three times greater risk of progressing to joint arthroplasty (Liu et al., 2017). Results of this study that osteophytes were related to severe pain and functional disability and that osteophytes were more prevalent in the NPG as shown in Table 7, were supported by the findings from previous research (Hakky et al., 2015; Sowers et al., 2003).

A longitudinal study previously found that the majority of cysts developed from pre-existing bone oedema (Carrino et al., 2006). Bone cysts were less common in this OA cohort compared to other structural pathologies. Small to medium cysts were present in higher numbers in the NPG but this difference was not significant.

No significant difference of cartilage loss was found between OA participants in the IPG, PoNPG and PrNPG except for the medial femoral cartilage ($p = 0.006$) but in that region moderate to severe cartilage loss (Grade 4 to 6) was more prominent in the PrNPG. Cartilage morphology of OA participants has been previously studied to investigate any association between NP and cartilage loss by dividing on the basis of PainDETECT scores, however, no link was found between NP and cartilage loss (Roubille et al., 2014).

Bone marrow lesions were also assessed in OA participants and comparison was made between the IPG, PoNPG and PrNPG. Results of this study indicated that there was no statistical difference in the presence of BMLs between the IPG and NPGs except for the

presence of BMLs on the lateral side of the tibia. Overall, the number of bone marrow lesions was greater in the NPG. These results were in line with previous reports in which bone marrow lesions were shown to be correlated to the severity of pain. Significant correlation was shown with intensity of pain and size of BMLs (Sansone et al., 2019). In the present study univariate regression showed strong association of lateral tibial bone marrow lesions with membership of the NPG and similar findings have been reported previously (Roubille et al., 2014). Therefore, presence of lateral bone marrow oedema may be considered as indicator of neuropathic pain.

Severe meniscal lesions were associated with NP and were more prevalent in the NPG (Figure 14 and Table 8). This finding has also been reported previously by (Roubille et al., 2014). However, Roubille et al. used only PainDETECT score to assign participants to their NP group. The present study used a more structured process to identify pain phenotypes. Nevertheless, a similar association with severe meniscal lesions in the lateral compartment was identified.

The results of this study partly support the hypothesis that the presence of osteophytes and lateral meniscal lesions are associated with the presence of NP.

Conclusions

The results of this study suggest that the presence of osteophytes and lateral meniscal lesions are associated with the presence of NP. The presence of these lesions should be considered when examining patients who present with features of NP.

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8. Biomarkers and pain phenotype in knee osteoarthritis

8.1 Introduction

Osteoarthritis is a degenerative disease, characterized by deterioration of the main joint structures; cartilage, bone, and synovium (Berenbaum, 2013). Changes in the joint structure may lead to the development of chronic inflammation and potentially damage to neural tissue (Baron et al., 2017; Sandell & Aigner, 2001). Cartilage degradation is facilitated largely by pro-inflammatory cytokines, most notably interleukin 1 (IL-1) and tumour necrosis factor alpha (TNFα) (Fernandes et al., 2002). These cytokines contribute to tissue destruction by disrupting the balance of the catabolic and anabolic activities of chondrocytes. Cytokines such as IL-1 and tumour necrosis factor alpha (TNF-α) produced by activated synoviocytes, mononuclear cells or by articular cartilage, significantly up-regulate metalloproteinase (MMP) gene expression which results in the degradation of the extracellular matrix (Fernandes et al., 2002; He et al., 2002). Increased innervation of synovium was found in symptomatic hip OA participants and it was the likely source of expression of sensory nerve related proteins and TNF-α (Takeshita et al., 2012). Both increased innervation and cytokines may be a source of pain in OA. Increased expression of TNF-α in synovial fluid of knee OA patients was associated with increased pain and physical disability based on WOMAC scores and numerical pain rating scores (Leung et al., 2017; Liles & Van Voorhis, 1995).

IL-1 is a cytokine which plays a role in the regulation of inflammation. In the immune system IL-1 is produced in response to inflammation but its effect is not limited to inflammation and it also has a role in bone formation and remodelling of bone (Kusano et al., 1998). TNF-α and IL-1 are also released by macrophages in neural tissues after nerve injury (Carman-Krzan et al., 1991). Cytokines such as IL-1, interleukin-6 (IL-6), and TNF-α, under various disease conditions and following injury to neural tissue, induce pain hypersensitivity (Kawasaki et al., 2008). Synovitis is a significant confounding variable in relation to biomarkers and neuropathic features in OA patients (Radojčić et al., 2017).

Local inflammation is thought to be one of the causes of pain due to the OA joint and can be detected systemically (Filková et al., 2009; Toncheva et al., 2009). C-reactive protein (CRP) has been found to be modestly higher in OA patients compared to controls and CRP levels were

associated with pain and decreased physical function (Jin et al., 2015). Nerve injury also leads to the release of inflammatory cytokines, including TNFα which causes nociceptor sensitisation, similar to inflammatory response.

Nerve growth factor (NGF) is a neurotropic factor. It is causes pain and hypersensitivity (Sørensen et al., 2019). The administration of a NGF antagonist has been suggested as an effective therapeutic approach in many pain states (Mantyh et al., 2011). Treatment with a NGF inhibitor was viewed as an acceptable alternative to NSAIDs or opioids (Turk et al., 2020). The heterogeneous nature of OA pain requires differentiation of pain types and it is likely that different pathophysiological processes may be contributing to pain perception in different patients.

There are a number of biomarkers that are commonly evaluated as indicators of inflammation and nerve damage in research and clinical practice. Established measures of inflammation include the erythrocyte sedimentation rate (ESR), white blood cell (WBC) count and C-reactive protein levels. ESR and WBC have low sensitivity and specificity as diagnostic markers of joint pain. It is therefore important to evaluate a range of markers to understand which might be most closely related to the presence of inflammatory or neuropathic pain.

Hypothesis

Biomarker levels, including CRP, IL-1, IL-6, TNFα and NGF will help to differentiate between pain phenotype groups in people with knee osteoarthritis.

Aims

The aim of this study was to measure serum biomarkers of CRP, IL-1, IL-6, TNF-α and NGF in OA participants to determine if any of these serum biomarkers can help predict inclusion into a neuropathic or inflammatory pain category.

8.2 Methods

A total of 99 OA participants were recruited for this study. 96 participants' blood samples were available for analysis (details section 2.3).

Participants were comprehensively assessed for sensory and proprioceptive function (Chapters 4 to 8).

Blood samples were obtained from the participants using standard phlebotomy technique. Samples were collected in a gold or serum separator vacutainer which contained polymer gel. Each sample was dated and labelled with each participant's code number. Samples were centrifuged and the serum was collected with a pipette. This serum sample was then stored at -20 ^{o}C in a freezer at the School of Pharmacy and Biomedical Sciences. When all samples had been collected at the end of recruitment of all OA participants, they were analysed for CRP, IL-1, IL-6, NGF and TNF-α.

Analyses for IL-1, IL-6, NGF and TNf-α were carried out in the School of Pharmacy and Biomedical Sciences using standard enzyme-linked immunosorbent assays (ELISA) (R&DSYSTEMS, a biotechne brand). ELISA is a commonly used analytical biochemistry assay, developed in the 1970s (Lequin, 2005). The assay uses a solid-phase enzyme immunoassay (EIA) to detect the presence of a specific protein in a liquid sample using antibodies directed against the protein to be measured. Each ELISA kit comes with its testing protocol (Appendix 3), which was followed for the serum detection of IL-1, IL-6, NGF and TNF-α.

CRP analysis was carried out at the R3Gen lab as per the Royal College of Pathology Australia (RCPA) requirements using an immunoassay (immune-turbidimetric analysis method), which is the main technique for performing routine protein tests. The turbidimetric immunoassay system operates in the antibody excess zone. It keeps the concentration of antibody constant and the amount of antigen–antibody complex formed depends directly on the concentration of antigen in the mixture. The analytical method used provided a full range of CRP readings covering a measuring range of 0.263 - 184 mg/L and was calibrated using a 6 point calibration method to ensure accuracy of tests, and a precision in tests to ensure instrument stability.

8.3 Data analysis

OA participants were divided in to three groups on the basis of PainDETECT questionnaire scores, neuropathic symptoms reported on pain mapping and sensory deficit (based on Z-score deviation) (details in Chapter 2 sections 2.8.3 and 2.8.4).

IBM® SPSS version 26 was used for analyses with alpha set at 0.05.

The Shapiro-Wilk test as well as graphical assessment was performed to check for the normality of the test data. IL-1, IL-6 and CRP, TNF-α and NGF data were not normally distributed therefore nonparametric tests were used to compare these variables between IPG, PoNPG, PrNPG. Data were expressed as medians, interquartile range (IQR, 3^{rd} – 1^{st} quartiles) and range (min-max). Significance (p-value) level of ≤ 0.05 was used in the comparisons.

8.4 Results

Blood samples of 95 OA participants, 45 males, 50 females with an average age of 64.5 ±8 years and an average BMI of 28.9 ±6 were tested and analysed.

Data obtained for TNF-α and NGF showed that only 5% of the participants exhibited a measurable level of TNF-α and only 8% of participants exhibited a measurable level of NGF. It was therefore decided not to undertake any further statistical analysis of these measures.

Comparisons between the IPG, PoNPG and PrNPG results of CRP, IL-1 and IL-6 are presented in Table 1 and Figure 1,Figure *2* and Figure *3*.

There was no significant difference (p = 0.109) when CRP was compared between IPG, PoNPG and PrNPG (Table 1, Figure 1). The serum level of IL-1 was the highest in the IPG and lowest in the PrNPG (Table 1, Figure 2) with significant difference (p = 0.001) between IPG, PoNPG and PrNPG. There was no difference (p = 0.383) in the serum IL-6 levels between the IPG, PoNPG and PrNPG (Table 1, Figure 3).

Table 1: Comparison of CRP, IL-1 and IL-6 (median (IQR) [min-max]) between IPG, PoNPG and PrNPG.

	IPG	**PoNPG**	**PrNPG**	p-value
	Median (IQR) [min-max]			
CRP	1.2 (0.6-3.4) [0.3-7.0]	2.4 (1.0-5.8) [0.4-12.2]	2.6 (1.1-3.8) [0.5-7.0]	0.109
IL 1	181.1 (149.4-269.4) [26.1-665.6]	113.0 (41.6-161.6) [15.1-489.3]	65.7 (41.4-114.7) [18.6-431.5]	0.001
IL 6	15.7 (10.8-18.7) [9.6-69.6]	14.5 (10.3-18.2) [8.2-23.7]	15.5 (12.7-19.5) [9.1-77.3]	0.383

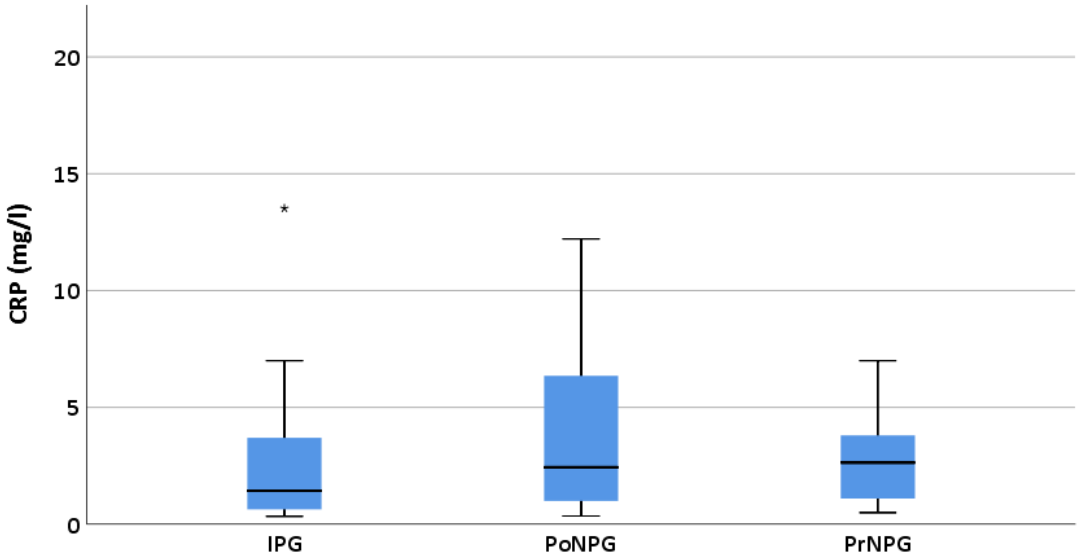

Figure 1: Comparison of CRP between the IPG, PoNPG and PrNPG.

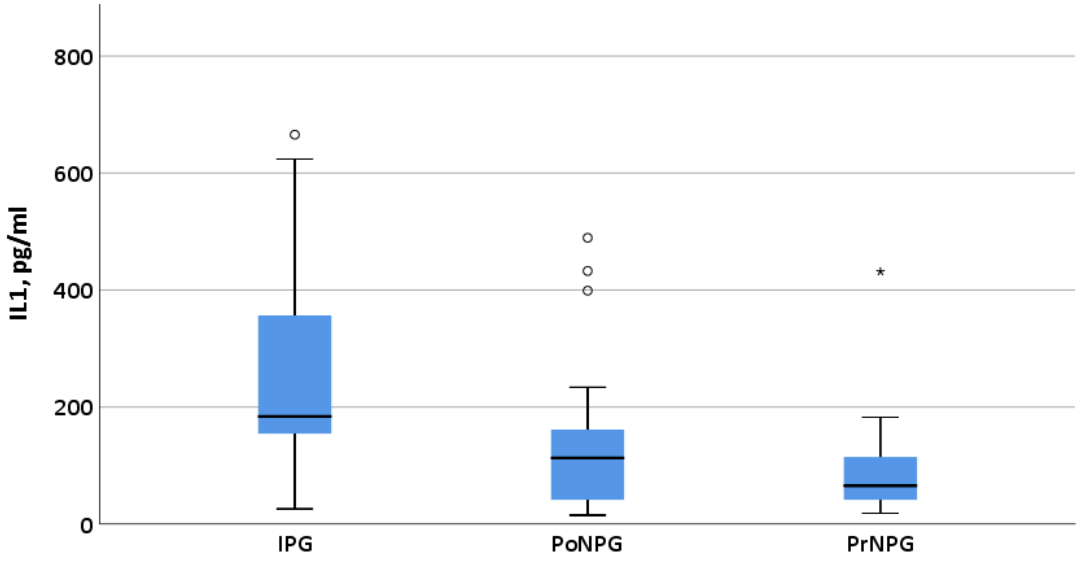

Figure 2: Comparison of IL-1 between the IPG, PoNPG and PrNPG.

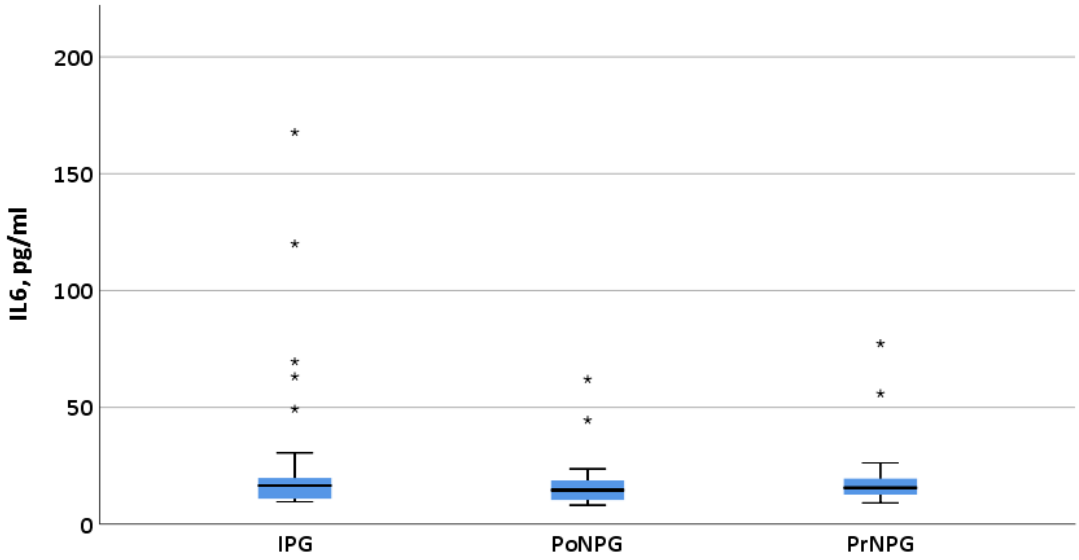

Figure 3: Comparison of IL-6 between the IPG, PoNPG and PrNPG.

Logistic regression was carried out to establish any association between biomarker measures and membership of the IPG or NPG (PoNPG and PrNPG groups combined).

Table 2: Logistic regression.

RF	OR	95% CI OR	p
IL-1	0.989	o.97 - 0.99	0.03

Logistic regression results showed an odds ratio (OR) of 0.99 with p = 0.036. Although the model is significant, an OR of nearly 1 suggests IL-1 was not a clear differentiating variable between the two pain groups. It is noted that observations of IL-1 in the IPG are widely scattered compared to those in the PoNPG and PrNPG, which could be a possible reason for IL-1 being not a clear differentiating variable.

8.5 Discussion

Results indicated that there was an increasing trend for serum CRP levels from IPG to PoNPG to PrNPG, though no statistically significant difference was found in the levels between the OA groups ($p = 0.109$). The results of this study are in line with the findings of a previous study where no association was found between ESR and CRP values and PainDETECT scores (Roubille et al., 2014). Royal College of Pathology Australia recommends values of < 1 mg/l as normal, 1-3 mg/l as low grade risk and > 5 mg/l as high risk (referenced in lab test reports). The serum CRP level of OA participants of this study ranged from 0.3 to 12.2 mg/l, which is similar to the previously reported figures by Roubille et al. (Roubille et al., 2014). Within this range, 25% of the OA participants had CRP level > 3 and 22% had values > 5. 22% of the OA participants were in the high risk group. Higher CRP reflects the presence of inflammation in the body.

IL-1 and IL-6 are pro-inflammatory cytokines and have been found in the synovial fluid of OA participants and related to the pathophysiology of OA. Serum levels of IL-1 in the participants in the IPG were significantly higher compared to the PoNPG and PrNPG. The lowest serum IL-1 was detected in the participants of the PrNPG (Table 1). No previous data is available for serum IL-1 in people with knee OA, although low grade synovial inflammation and IL-1 and NGF were found in synovial membrane samples (Smith et al., 1997; Towle et al., 1997).

No difference was found in serum levels of IL-6 between the IPG, PoNPG and PrNPG (Table 1). Serum levels of IL-6 are markedly raised in inflammatory conditions like rheumatoid arthritis (Boyapati et al., 2020). Osteoarthritis disease is initiated by joint cartilage erosion and structural changes in the joint, which leads to inflammation. Until recently, osteoarthritis was considered as a degenerative disease due to aging (Hadler, 1992; Sokolove & Lepus, 2013). However the heterogeneous nature of OA pain has now been clearly demonstrated. OA pain may be inflammatory (Jin et al., 2015) but it may include a neuropathic pain component in some OA participants (Hochman et al., 2013; Ohtori et al., 2012). So far, only mild systemic inflammation has been reported in OA participants through CRP results (Jin et al., 2015). Studies on CRP levels are not conclusive to diagnose inflammatory pain. Similarly, results of this study were not conclusive. Results of serum biomarker levels, including CRP, IL-1, IL-6, TNFα and NGF, does not support the hypothesis that these biomarkers will help differentiate between pain phenotype groups in people with knee osteoarthritis.

Conclusions

Results indicate serum biomarkers IL-1, IL-6 and CRP results were not able to differentiate pain phenotype. NGF was detected only in 5% and TNF-α detected in only 8% of OA participants. It is suggested that further research should be carried out to develop a better understanding of the serum biomarker levels in relation to pain phenotypes.

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9. Predictive indicators of neuropathic pain in patients with knee osteoarthritis

9.1 Introduction

Osteoarthritis (OA) pain is traditionally considered to be caused by local nociceptive afferent stimulation caused by structural changes in the joint (Schaible, 2012). OA pain is of heterogeneous origin. It has been reported that OA pain is both nociceptive and neuropathic in origin (Hochman et al., 2011; Mease et al., 2011; Ohtori et al., 2012). Neuropathic pain (NP) is a result of a lesion or dysfunction of the nervous system and a direct consequence of a disease or injury affecting the somatosensory system (Treede et al., 2008). It is important to be able to clinically distinguish the type of pain a person is experiencing in order to provide appropriate management. Currently there are no diagnostic tools available to make a definite diagnosis of NP in osteoarthritis. Treede along with other prominent researchers and practitioners in neurology and pain science, developed a grading system of definite, possible and probable NP (Treede et al., 2008). The probable and definite pain-grades require confirmatory evidence from a neurologic examination. The site of neuropathic pain should conform to the innervation regions of peripheral nerves, branches of the brachial or lumbar plexus, or spinal segments. The distribution of pain or hyperalgesia should be in a distribution that is typical for the underlying somatosensory disorder (Treede et al., 2008).

This research project had two main objectives; firstly, to determine whether two distinct pain phenotypes, neuropathic and inflammatory pain can be demonstrated among patients with knee OA and secondly, to find a well-defined set of measures which can help clinicians to diagnose NP.

Hypothesis

> Questionnaire based measures (which may include anxiety, depression, sleep quality, and pain related measures), NP symptoms, quantitative sensory and pain measures and structural changes on MRI, which were significant on univariate analysis, are predictive of inclusion in the neuropathic pain group (NPG).

Aims

- To develop a logistic regression model to determine which measures most clearly differentiate between each of the pain categories and determine a well-defined set of measures that can be used to classify a patient with knee osteoarthritis into a neuropathic or inflammatory pain category.
- To determine cut-off values for the measures found significant on logistic regression and to determine the sensitivity and specificity of these measures to discriminate between the two groups.

9.2 Methods

Participants were comprehensively assessed for sensory and proprioceptive function. OA participants completed self-report measures explained previously related to pain quality, severity, psychosocial state, sleep quality and comorbidities (Chapters 4 to 7).

9.3 Data analysis

Univariate logistic regression analyses were performed to evaluate the association between each test variable, which included self-report questionnaire scores, symptom reports on pain mapping, quantitative sensory and pain measures and MRI structural abnormalities associated with NP (Chapters 4 to 7). Groups of measures that were significant in the regression models previously described for diagnostic test predictors were entered into an overall multivariable, logistic regression model to find the model that provided the optimal diagnostic predictors for the presence of NP. A manual forward and backward selection strategy of variables with $p < 0.10$ was adopted to select predictors for the final model. Predictors were deleted step by step from the model based on the highest p-value. Selected predictors were used to evaluate the discriminative power of the model, expressed by the area under the receiver operating characteristics curve (ROC). Results are summarised in Table 1 as odds ratio (OR) and respective statistical significance.

9.4 Results

Results from self-report measures and sensory assessment were used to divide study participants in three groups, the inflammatory pain group (IPG) and possible and probable neuropathic pain groups (PoNPG and PrNPG), details in Chapters 2 and 6. Comparison of IPG, PoNPG and PrNPG results showed significantly lower or higher thresholds for some quantitative sensory measures in the IPG compared to the PoNPG and PrNPG (Chapters 4 to 7). There were limited differences between the PoNPG and the PrNPG for a number of measures and so logistic regression was performed between the IPG and the NPG (a combined PoNPG and PrNPG).

It was noted that S-LANSS, PQAS-P, vibration threshold at the popliteal fossa (PFVT) of the index knee, cold pain threshold (CPT) on the lateral aspect of the index knee, lateral osteophytes, lateral meniscus lesions and lateral hypersensitivity were significant at the $p < 0.05$ level (Table 1). These variables have higher than 1 odds ratio (OR) which suggests their significant role in discriminating NPG. The OR of burning sensation on the lateral aspect of the knee was also quite high (12.88), therefore, although the significance level was slightly low ($p = 0.06$) this measure was retained in the model.

Table 1: Multivariate logistic regression model NPG.

RF	OR	95%CI OR	p-value
S-LANSS	1.11	1.03-1.20	0.006
PQAS-P	1.17	1.06-1.29	0.002
PFVT index knee	1.32	1.12-1.56	0.001
Lateral CPT index knee	1.15	1.06-1.25	0.001
Lateral osteophytes	6.36	1.29-31.53	0.026
Lateral meniscus	17.08	2.52-115.32	0.004
Lateral Burning index knee	12.88	0.88-188.67	0.062
Lateral Hypersensitivity index knee	21.21	1.58-285.36	0.021

ROC curve

PainDETECT on the ROC curve showed excellent discrimination power with an area under the curve (AUC) of 0.92 with 90% sensitivity and 71% specificity (Figure 1).

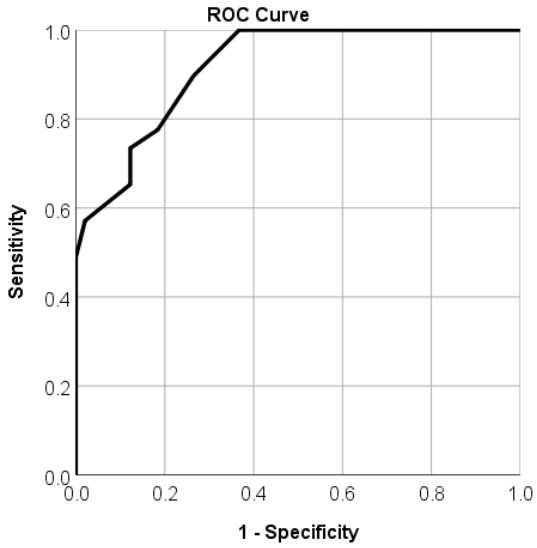

Figure 1: Receiver Operating Characteristic (ROC) curve for PainDETECT.

The area under the ROC curves for S-LANSS and PQAS-P questionnaires, vibration threshold at popliteal fossa (PFVT, sensory variable), cold pain threshold on the lateral aspect of the index knee (CPT, pain measure), lateral osteophytes and the presence of lateral meniscal lesions on MRI and pain mapping of burning sensation and hypersensitivity on the lateral side are shown in Figure 2, Table 1.

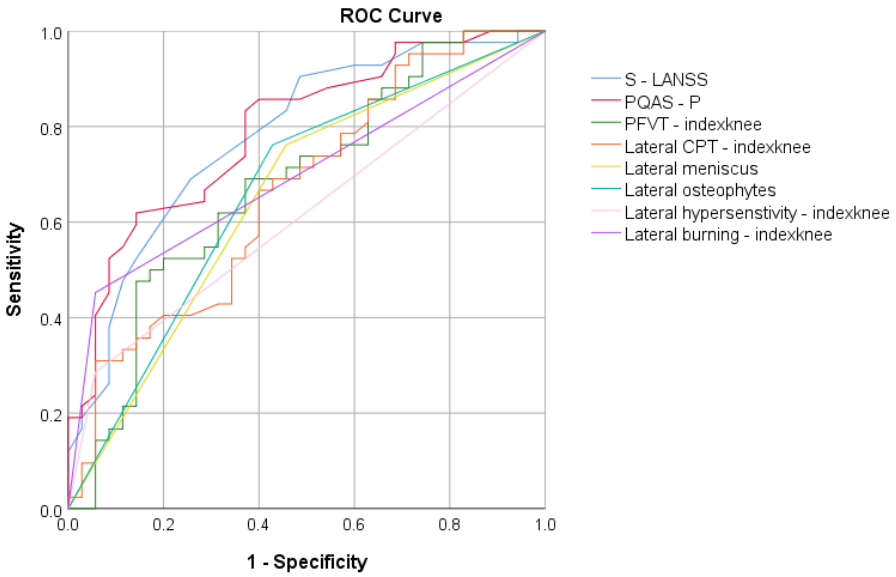

Figure 2: ROC curve from all variables in multiple logistic regression model.

Table 2: Diagnostic performance of variables.

Variables	AUC	Cut-off	Sensitivity	Specificity
S-LANSS	0.79	9	87%	57%
PQAS-P	0.80	11	79%	65%
PFVT index knee	0.67	12	67%	62%
Lateral CPT index knee	0.67	21	67%	58%
Lateral osteophytes	0.63	2.5	74%	53%
Lateral meniscus	0.63	1.5	76%	53%
Lateral Hypersenstivity index knee	0.61			
Lateral Burning index knee	0.70			

9.5 Discussion

The PainDETECT questionnaire was used for initial grouping of OA participants. Several previous studies have used PainDETECT scores to identify NP among OA participants (Hochman et al., 2011; Roubille et al., 2014; Wright et al., 2017). In this study a more comprehensive approach was adopted to identify NP. In addition to PainDETECT other neuropathic questionnaires, S-LANSS and PQAS were also used to confirm NP. Participants also reported the location of neuropathic pain symptom on pain maps and quantitative sensory testing was carried out to confirm coresponding sensory deficits.

Results of this study support the hypothesis that NP questionnaires (PainDETECT, S-LANSS and PQAS-P) are adequate tools to identify people with knee OA who present with NP. PainDETECT emerged as the strongest indicator of NP. However, this needs to be considered in the context that PainDETECT was used as part of the initial categorisation process of OA participants. This may bias the discriminative power shown in these results.Two other NP questionnaire, S-LANSS and PQAS-P showed acceptably high discrimination power with 87% and 79% sensitivity and 57% and 65% specificity, respectively. This suggested that these questionnaires can be conveniently used in clinical settings as a first line process in the assessment of people with OA pain to determine whether they might be presenting with features of neuropathic pain. Higher scores on these questionnaires were associated with greater odds of having NP. The cut-off score for the S-LANSS was 9, which is comparable to the previously reported cut-off score of 10 for this questionnaire (Bennett et al., 2005). The cut-off score for PQAS-P was 11. These results support the results of previous studies (Hochman et al., 2013; Roubille et al., 2014).

Reporting of NP symptoms on pain mapping can be useful to guide further examination and diagnosis. The presence of burning sensation or hypersensitivity over the lateral aspect of the index knee were identified as features of pain mapping that were associated with the presence of neuropathic pain.

The study identified vibration threshold at the popliteal fossa as a key sensory measure and cold pain threshold over the lateral aspect of the knee as a key indicator of hyperalgesia. The cut-off values for these measures were 12μ and 21°C respectively.

The presence of MRI features of severe meniscal lesions and osteophytes on the lateral side of the knee joint were also relatively discriminative measures for identifying people with

features of NP. These observations are suggestive of NP being associated with lateral meniscal extrusion as previously reported (Roubille et al., 2014). This is the first report to indicate that the presence of grade 3 (larger and more prominent) osteophytes may also be related to the development of NP.

This study has identified a number of measures which can helpful in the diagnosis of NP. This supports the hypothesis that a number of measures based on questionnaires, sensory, pain measures and MRI findings will help to determine inclusion of OA participants in the NPG. Importantly, these measures cover a number of different domains including symptom report, sensory deficits, augmented sensations and pathological features, which relate to each of the key domains identified by Treede and colleagues as being important for the diagnosis of NP (Treede et al., 2008). It therefore appears that it should be possible to use a number of these measures to arrive at a definitive diagnosis of NP in at least some people who present with painful knee OA.

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10. Summary and conclusions

The main objective of this study was to develop a set of diagnostic criteria to identify patients with osteoarthritis (OA) who may be experiencing neuropathic pain. In clinical settings this may help in distinguishing patients with neuropathic pain so they can receive more targeted treatment and pain can be managed efficiently. To achieve this objective, the aims were to determine whether two distinct pain phenotypes, inflammatory and neuropathic, can be demonstrated among participants with knee OA and to find the relative incidence of participants with possible (PoNP) and probable (PrNP) neuropathic pain within the overall neuropathic pain group (NPG).

To achieve these aims, this study was designed to comprehensively assess sensory and proprioceptive functions in participants with knee OA and to compare these functions with age matched participants without joint pain. OA participants were evaluated using various questionnaires, sensory and pain thresholds, MRI and blood tests for biomarkers related to nerve injury and inflammation. Results of the study demonstrated that some sensory functions which include proprioception, vibration, cold and tactile threshold were compromised in the OA group compared to the control group ($p < 0.001$). These results are in agreement with the previous literature which indicated that people with OA had significant proprioceptive deficits, other sensory deficits and increased pain sensitivity (hyperalgesia) compared to pain free control participants (Garsden & Bullock-Saxton, 1999; Hochman et al., 2013; Wright et al., 2017).

Following strict criterion, as explained in Chapter 2 (Methods), OA participants were divided into an inflammatory pain group (IPG), a possible neuropathic pain group (PoNPG) and a probable neuropathic pain group (PrNPG). The criteria developed were based on an expert consensus statement developed by Treede et al (Treede et al., 2008). In the whole OA cohort, the incidence of IPG, PoNPG and PrNPG was 49%, 29% and 21%, respectively. More than half of the OA participants had a neuropathic pain component (PoNPG and PrNPG combined). This was a larger percentage than originally anticipated, although it must be acknowledged that the PoNPG (29%) did not fulfil the criteria to have sensory deficit and reports of NP symptoms in the same area. Nevertheless, a large number of PoNPG participants showed sensory deficits when they were compared to the control participants. A number of

participants in PoNPG also scored high on PainDETECT and S-LANSS and reported severe pain, stiffness and functional disability. Some of the sensory measures such as vibration threshold and proprioception functions were more compromised in the PoNPG compared to the PrNPG, however, most of the sensory and pain thresholds were not different between these two groups. Results of this study also demonstrated that participants in the PoNPG and PrNPG experienced greater pain, hyperalgesia, and sleep disruption than the participants with predominantly nociceptive (non-neuropathic) pain.

Proprioceptive function was not found to be different between the three OA groups. In fact, participants in the inflammatory pain group (IPG) showed greater joint reposition error (JRE) compared to the PrNPG. These results may support the concept that nervous system impairment may be an important factor in the aetiology of OA. It has been previously suggested that proprioceptive impairment can be an important factor in initiating or advancing degeneration of the knee in elderly individuals with osteoarthritis (Barrett et al., 1991). Our data suggest that neurological impairment, particularly in terms of impaired proprioceptive function, is an extremely common feature in people with knee osteoarthritis.

PrNPG participants scored high on self-report NP questionnaires (S-LANSS and PQAS-P). This group also reported the greatest number of neuropathic symptoms on pain mapping. They also reported severe pain, physical disability, compromised sleep quality and increased psychosocial distress when assessed using a range of measures. Participants in the PoNPG also reported NP symptoms and had sensory deficits but did not qualify to be included in PrNPG according to the strict criteria, as described in Chapter 2.

Structural changes on MRI demonstrated that the presence of osteophytes and lateral meniscal lesions, bone oedema and medial femoral cartilage defects were significantly higher in the NPG compared to the IPG. These findings are of interest because when they are considered together, they suggest that neuropathic pain may emerge as the pathology of OA worsens and particularly if it spreads to encompass the lateral compartment of the knee.

Inflammatory marker serum CRP has been found to be higher in OA patients compared to controls and elevated CRP levels suggest that mild systemic inflammation may play a role in symptom presentation. High CRP were associated with pain and decreased physical function (Jin et al., 2015). Serum biomarkers of CRP, IL-1, IL-6, TNF-α and NGF were measured in OA participants, in this study to determine if any of these serum biomarkers can help predict inclusion into a neuropathic or inflammatory pain category. Results of this study have shown

no difference in these biomarkers between neuropathic pain phenotype groups. Further research is warranted in a larger cohort especially for CRP.

Univariate logistic regression models indicated neuropathic pain descriptors on S-LANSS, pain severity, stiffness and functional disability, psychosocial characteristics, sleep disruption, digital mapping of burning, electric shock, and hypersensitivity, pressure pain threshold on the medial side of the index knee, cold pain threshold, vibration and cold detection threshold over the popliteal fossa, cartilage defects on the medial side, osteophytes on both the medial and lateral side, lateral tibial bone oedema, lateral meniscal lesions (pathological features on MRI) were all strongly associated with membership of the NPG.

The strongest variables related to NP were vibration threshold at the popliteal fossa of the index knee, cold pain threshold on the lateral aspect of the index knee, the presence of lateral osteophytes, lateral meniscus lesions and lateral burning and hypersensitivity sensations.

Identification of these variables provides considerable promise that it might be possible to develop a limited set of variables that could be assessed in the clinical setting in order to be able to diagnose the presence of neuropathic pain with good sensitivity and specificity.

Strengths

Strengths of this study included a non-biased community-based sample selection of participants. Participants in the control and OA cohorts were age matched with an even gender distribution. The BMI of the OA participants was higher which is reflective of the average BMI in the Australian population and similar to what is reported in the literature for OA patients. There was no difference in BMI between the three OA groups. Until the current study, OA participants had not been extensively assessed for sensory and pain measures along with MRI and biomarkers in the same cohort. This study demonstrated that it is possible to undertake detailed assessment of sensory function in regions that align with the anatomical innervation of the knee.

Limitations

One of the limitations was the relatively small sample size. We were unable to recruit sufficient participants to meet our original projected sample size, although the sample size

achieved was large enough to perform most of the statistical analyses we aimed to carry out. It is also the case that the proportion of participants with features of neuropathic pain was larger than we originally anticipated. However, the sample size of the inflammatory pain group and the probable neuropathic pain group, did not allow use to perform logistic regression using those groups alone. It is therefore possible that some important relationships may have been missed.

Although sensory deficit was reported, OA participants with ≥ 1.96 Z-score (95%) deviation from the control group mean were identified as having deficit for that measure. Direct evidence of nerve lesions could be confirmed by performing nerve conduction (NC) studies of lower limb. Confirmation of nerve lesion using conduction studies and then relating the results to PainDETECT and SLANSS scores would be an appropriate area for further research.

Clinical and research implications

Osteoarthritis (OA) is the most common joint cause of pain in older people (Hunter et al., 2014). This is especially important as there is an increased aging population in western society. OA management requires costly pharmacological and surgical interventions which poses a huge economic burden on health services. Current OA management is symptom oriented and surgery is required in severe cases. There is still no effective, disease modifying, medical treatment available for this complex and heterogeneous disease. Effective management of pain is therefore a key element of current treatment.

Our results strongly support previous research that PainDETECT is an excellent tool to identify patients with knee OA who may be experiencing neuropathic pain. It can be conveniently used in clinical settings. Previous work from our research group has demonstrated that a sub group of OA patients who score high on PainDETECT exhibit significant cold and pressure pain hyperalgesia (Moss et al., 2016; Wright et al., 2015). Similar results demonstrated by Hochman et al (Hochman et al., 2011). The value of the PainDETECT questionnaire is further supported by the finding that persistent pain after total knee arthroplasty was associated with widespread pressure and cold hyperalgesia, and greater neuropathic-pain symptoms as measured by PainDETECT. High preoperative PainDETECT scores (patients with knee OA NP symptoms) independently predict postoperative pain (Wright et al., 2015).

PainDETECT scores in conjunction with reporting of NP symptoms on digital pain mapping could be used as a first line of evaluation in general practice and rheumatology clinics. This can then further guide management of knee osteoarthritis pain. If these measures are indicative of neuropathic pain then it should be further investigated by evaluating vibration thresholds and cold pain thresholds and MRI findings of lateral osteophytes and lateral meniscal lesions. Vibration threshold assessment and cold pain threshold assessment are not commonly carried out in Rheumatology clinics but with further development of diagnostic criteria this could become feasible. By assessing symptom distribution using pain mapping, key sensory deficits and the presence of key pathological features on MRI it appears to be possible to follow the key criteria that Treede and colleagues have identified as being necessary to make a definitive diagnosis of neuropathic pain in this group of patients.

Early detection and diagnosis of OA patients who experience neuropathic pain will allow not only less time of suffering with pain for OA patients but also it would also reduce the burden on the health system. Satisfactory treatment of neuropathic pain might help to reduce the number of surgeries being carried our for OA pain. Past research has demonstrated that OA pain persists even after total knee replacement and this cohort had features of neuropathic pain (Valdes et al., 2014; Wright et al., 2015). As the aging population is growing, such studies are of clinical importance cosidering OA is associated with substantial morbidity.

Another strong point of this study is that identification of neuropathic pain (NP) was supported by three NP questionnaires, PainDETECT, S-LANSS and PQAS-P, which provides practical tools to be used in clinics or posted to patients before consultation. These questionnaires should also be useful for future research studies.

Future directions

Future research can now focus on a more limited set of measures to confirm the findings of this research. There would be considerable value in undertaking a prospective study using pain mapping, vibration testing and MRI findings and to evaluate how successfully people could be assigned to neuropathic and inflammatory pain groups, with blinded data from neuropathic pain questionnaires to provide confirmation. It would also be useful to use these criteria to evaluate people prospectively and to then evaluate drug management based on pain phenotype to see of mechanism based treatment can result in more effective pain management than is the case for current practice.

Results of this study indicated that neuropathic pain is associated with severe disease, but it's not known whether disease progresses, in some people, faster than the others. Longitudinal studies may provide a better understanding of the cause of the pain and progression of the disease. This sort of study would be very useful in order to evaluate whether neuropathic pain emerges at a particular stage in the pathological process.

Conclusions

Self-reporting tools PainDETECT, SLANSS and PQAS and digital mapping of neuropathic pain sensations, burning and electric shock are strongly associated with neuropathic pain. These measures are recommended for use in clinics and in research investigating pain phenotypes in OA patients. QST measures, including elevated vibration threshold at the back of the painful knee and elevated cold pain threshold on the lateral aspect of the index knee, associated with lateral burning sensation and hypersensitivity can help to provide clinical confirmation of neuropathic pain. The presence of MRI features of lateral osteophytes and lateral meniscus lesions also provide important pathological indicators of the likely presence of neuropathic pain. There is considerably more research required but it does appear to be possible to develop diagnostic criteria that might be successfully used to diagnose the presence of neuropathic pain in people suffering from knee OA.

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Research Support, Non-U.S. Gov't

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Appendix 1

painDETECT™

PAIN QUESTIONNAIRE

Date: Patient: Last name: First name:

How would you assess your pain **now**, at this moment?

0 1 2 3 4 5 6 7 8 9 10

none max.

How strong was the **strongest** pain during the past 4 weeks?

0 1 2 3 4 5 6 7 8 9 10

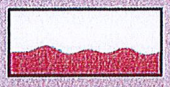
none max.

How strong was the pain during the past 4 weeks **on average**?

0 1 2 3 4 5 6 7 8 9 10

none max.

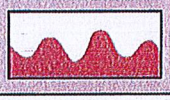
Mark the picture that best describes the course of your pain:



Persistent pain with slight fluctuations ☐

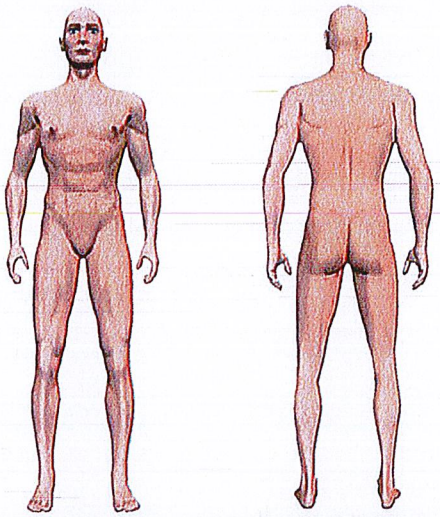
Persistent pain with pain attacks ☐

Pain attacks without pain between them ☐



Pain attacks with pain between them ☐

Please mark your **main area of pain**



Does your pain radiate to other regions of your body? yes ☐ no ☐

If yes, please draw the direction in which the pain radiates.

Do you suffer from a burning sensation (e.g., stinging nettles) in the marked areas?

never ☐ hardly noticed ☐ slightly ☐ moderately ☐ strongly ☐ very strongly ☐

Do you have a tingling or prickling sensation in the area of your pain (like crawling ants or electrical tingling)?

never ☐ hardly noticed ☐ slightly ☐ moderately ☐ strongly ☐ very strongly ☐

Is light touching (clothing, a blanket) in this area painful?

never ☐ hardly noticed ☐ slightly ☐ moderately ☐ strongly ☐ very strongly ☐

Do you have sudden pain attacks in the area of your pain, like electric shocks?

never ☐ hardly noticed ☐ slightly ☐ moderately ☐ strongly ☐ very strongly ☐

Is cold or heat (bath water) in this area occasionally painful?

never ☐ hardly noticed ☐ slightly ☐ moderately ☐ strongly ☐ very strongly ☐

Do you suffer from a sensation of numbness in the areas that you marked?

never ☐ hardly noticed ☐ slightly ☐ moderately ☐ strongly ☐ very strongly ☐

Does slight pressure in this area, e.g., with a finger, trigger pain?

never ☐ hardly noticed ☐ slightly ☐ moderately ☐ strongly ☐ very strongly ☐

(To be filled out by the physician)

never	hardly noticed	slightly	moderately	strongly	very strongly
☐ x 0 = 0	☐ x 1 = ☐	☐ x 2 = ☐☐	☐ x 3 = ☐☐	☐ x 4 = ☐☐	☐ x 5 = ☐☐

Total score ☐☐ **out of 35**

R. Freynhagen, R. Baron, U. Gockel, T.R. Tölle, Current Medical Research and Opinion vol 22 (10) 2006; 1911-1920

painDETECT™

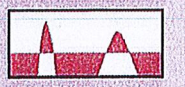
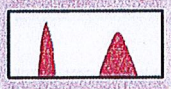
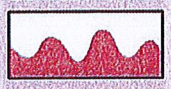
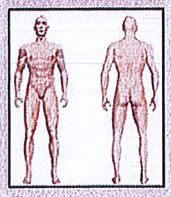
SCORING OF PAIN QUESTIONNAIRE

Date: _____ Patient: Last name: _____ First name: _____

Please transfer the total score from the pain questionnaire:

Total score ☐☐

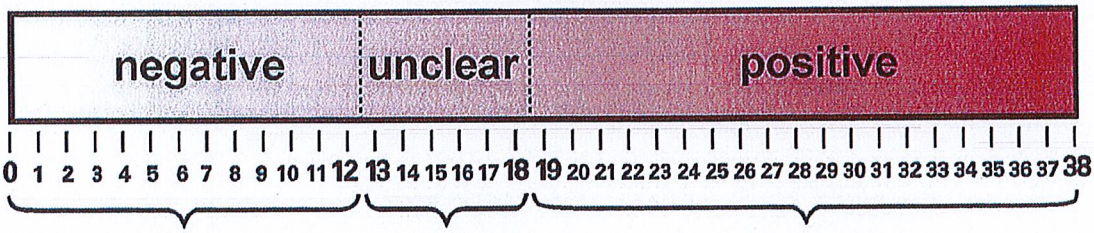
Please add up the following numbers, depending on the marked pain behavior pattern and the pain radiation. Then total up the final score:

	Persistent pain with slight fluctuations	0	
	Persistent pain with pain attacks	- 1	if marked, or
	Pain attacks without pain between them	+1	if marked, or
	Pain attacks with pain between them	+1	if marked
	Radiating pain?	+2	if yes

Final score ☐☐

Screening Result

Final score



A neuropathic pain component is unlikely (< 15%)	Result is ambiguous, however a neuropathic pain component can be present	A neuropathic pain component is likely (> 90%)

This sheet does not replace medical diagnostics.
It is used for screening the presence of a neuropathic pain component.

DFNS

pain

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APPENDIX

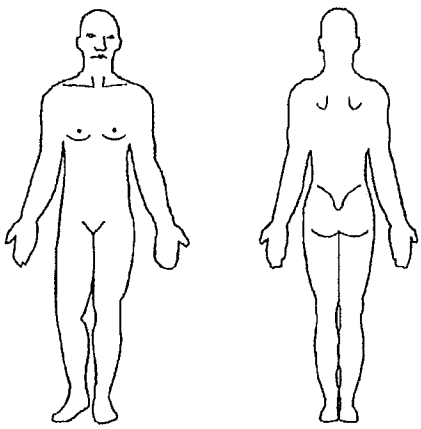
THE S-LANSS PAIN SCORE

Leeds Assessment of Neuropathic Symptoms and Signs (self-complete)

NAME_____ DATE_____

- This questionnaire can tell us about the type of pain that you may be experiencing. This can help in deciding how best to treat it.

- Please draw on the diagram below where you feel your pain. If you have pain in more than one area, **only shade in the one main area where your worst pain is.**



- On the scale below, please indicate how bad your pain (that you have shown on the above diagram) has been in the last week where:
'0' means no pain and '10' means pain as severe as it could be.

NONE 0 1 2 3 4 5 6 7 8 9 10 **SEVERE PAIN**

- On the other side of the page are 7 questions about your pain (the one in the diagram).

- Think about how your pain that you showed in the diagram has felt **over the last week.** Please circle the descriptions that best match your pain. These descriptions may, or may not, match your pain no matter how severe it feels.

- Only circle the responses that describe your pain. **Please turn over.**

S-LANSS

1. In the area where you have pain, do you also have 'pins and needles', tingling or prickling sensations?

a) NO – I don't get these sensations (0)

b) YES – I get these sensations often (5)

2. Does the painful area change colour (perhaps looks mottled or more red) when the pain is particularly bad?

a) NO – The pain does not affect the colour of my skin (0)

b) YES – I have noticed that the pain does make my skin look different from normal (5)

3. Does your pain make the affected skin abnormally sensitive to touch? Getting unpleasant sensations or pain when lightly stroking the skin might describe this.

a) NO – The pain does not make my skin in that area abnormally sensitive to touch (0)

b) YES – My skin in that area is particularly sensitive to touch (3)

4. Does your pain come on suddenly and in bursts for no apparent reason when you are completely still? Words like 'electric shocks', jumping and bursting might describe this.

a) NO – My pain doesn't really feel like this (0)

b) YES – I get these sensations often (2)

5. In the area where you have pain, does your skin feel unusually hot like a burning pain?

a) NO – I don't have burning pain (0)

b) YES – I get burning pain often (1)

6. Gently <u>rub</u> the painful area with your index finger and then rub a non-painful area (for example, an area of skin further away or on the opposite side from the painful area). How does this rubbing feel in the painful area?

a) The painful area feels no different from the non-painful area (0)

b) I feel discomfort, like pins and needles, tingling or burning in the painful area that is different from the non-painful area (5)

7. Gently <u>press</u> on the painful area with your finger tip then gently press in the same way onto a non-painful area (the same non-painful area that you chose in the last question). How does this feel in the painful area?

a) The painful area does not feel different from the non-painful area (0)

b) I feel numbness or tenderness in the painful area that is different from the non-painful area (3)

Scoring: a score of 12 or more suggests pain of predominantly neuropathic origin

WOMAC OSTEOARTHRITIS INDEX VERSION VA3.1

INSTRUCTIONS TO PATIENTS

In Sections A, B, and C questions will be asked in the following format. You should give your answers by putting an " ✗ " on the horizontal line.

EXAMPLES:

1. If you put your " ✗ " at the left of the line as shown below, then you are indicating that you have **no** pain.

2. If you put your " ✗ " at the right end of the line as shown below, then you are indicating that your pain is **extreme.**

3. Please note:
   a) that the further to the right you place your " ✗ " the **more** pain you are experiencing.
   b) that the further to the left you place your " ✗ " the **less** pain you are experiencing.
   c) **please do not** place your " ✗ " **past the end of the line.**

You will be asked to indicate on this type of scale the amount of pain, stiffness or disability you have experienced in the last 48 hours.

Think about your _____ (study joint) when answering the questionnaire. Indicate the severity of your pain, stiffness and physical disability that you feel is caused by arthritis in your _____ (study joint).

Your study joint has been identified for you by your health care professional. If you are unsure which joint is your study joint, please ask before completing the questionnaire.

WOMAC VA3.1 QUESTIONNAIRE **WOMA**

Section A

PAIN

Think about the pain you felt in your _____ (study joint) due to your arthritis during the last 48 hours.

(Please mark your answers with an "✗" on the horizontal line.)

QUESTION: **How much pain do you have?**

1. Walking on a flat, even surface.

No Pain ——— Extreme Pain

2. Going up or down stairs.

No Pain ——— Extreme Pain

3. At night while in bed, i.e., pain that disturbs your sleep.

No Pain ——— Extreme Pain

4. Sitting or lying awake in bed.

No Pain ——— Extreme Pain

5. Standing upright (but not moving).

No Pain ——— Extreme Pain

Study Coordinator Use Only	
PAIN1	
PAIN2	
PAIN3	
PAIN4	
PAIN5	

Section B

STIFFNESS

Think about the stiffness (not pain) you felt in your_____ (study joint) due to your arthritis during the last 48 hours.

Stiffness is a sensation of **decreased** ease in moving your joint.

(Please mark your answers with an " ✗ " on the horizontal line.)

6. How **severe** is your stiffness **after first awakening** in the morning?

No Stiffness |——————————————————| Extreme Stiffness

7. How **severe** is your stiffness immediately after sitting, lying or resting **later in the day**?

No Stiffness |——————————————————| Extreme Stiffness

Study Coordinator Use Only

STIFF6 ____

STIFF7 ____

Section C

DIFFICULTY PERFORMING DAILY ACTIVITIES

Think about the difficulty you had in doing the following daily physical activities due to arthritis in your _____ (study joint) during the last 48 hours. By this we mean **your ability to move around and to look after yourself.** (Please mark your answers with an " X " on the horizontal line.)

QUESTION: **What degree of difficulty do you have?**

		Study Coordinator Use Only
8. Going down stairs.	No Difficulty — Extreme Difficulty	PFTN8 _____
9. Going up stairs.	No Difficulty — Extreme Difficulty	PFTN9 _____
10. Standing up after sitting.	No Difficulty — Extreme Difficulty	PFTN10 _____
11. Standing (in one position).	No Difficulty — Extreme Difficulty	PFTN11 _____
12. Bending to the floor, i.e., to pick something up.	No Difficulty — Extreme Difficulty	PFTN12 _____
13. Walking on a flat, even surface.	No Difficulty — Extreme Difficulty	PFTN13 _____

DIFFICULTY PERFORMING DAILY ACTIVITIES

Think about the difficulty you had in doing the following daily physical activities due to arthritis in your _____ (study joint) during the last 48 hours. By this we mean **your ability to move around and to look after yourself.** (Please mark your answers with an " ✗ " on the horizontal line.)

QUESTION: **What degree of difficulty do you have?**

		Study Coordinator Use Only
14. Getting in or out of a car, or getting on or off a bus.	No Difficulty — Extreme Difficulty	PFTN14 ____
15. Going shopping.	No Difficulty — Extreme Difficulty	PFTN15 ____
16. Putting on your socks or stockings.	No Difficulty — Extreme Difficulty	PFTN16 ____
17. Getting out of bed.	No Difficulty — Extreme Difficulty	PFTN17 ____
18. Taking off your socks or stockings.	No Difficulty — Extreme Difficulty	PFTN18 ____
19. Lying and turning in bed.	No Difficulty — Extreme Difficulty	PFTN19 ____

DIFFICULTY PERFORMING DAILY ACTIVITIES

Think about the difficulty you had in doing the following daily physical activities due to arthritis in your _____ (study joint) during the last 48 hours. By this we mean **your ability to move around and to look after yourself.** (Please mark your answers with an " ✗ " on the horizontal line.)

QUESTION: **What degree of difficulty do you have?**

		Study Coordinator Use Only
20. Getting in or out of the bath.	No Difficulty — Extreme Difficulty	PFTN20 _____
21. Sitting.	No Difficulty — Extreme Difficulty	PFTN21 _____
22. Getting on or off the toilet.	No Difficulty — Extreme Difficulty	PFTN22 _____
23. Performing heavy domestic duties.	No Difficulty — Extreme Difficulty	PFTN23 _____
24. Performing light domestic duties.	No Difficulty — Extreme Difficulty	PFTN24 _____

Pain Quality Assessment Scale (PQAS-R)

Instructions: There are different aspects and types of pain that patients experience and that we are interested in measuring. Pain can feel sharp, hot, and achy. Some pains may feel like they are very superficial (at skin-level), or they may feel like they are from deep inside your body. Some people can feel these different types of pain at the same time.

The Pain Quality Assessment Scale helps us measure these and other different aspects of your pain. For one patient, a pain might feel extremely hot and burning, but not at all achy, while another patient may not experience any burning pain, but feel like their pain is very achy. Therefore, we expect that you may rate higher on some of the scales and lower on others.

We are asking you to rate the intensity or severity of the different types of pain you have felt over the past week **ON AVERAGE**, using a question like the one below. We realize that it can be difficult to make these estimates for some of the items, but please give us your best estimate.

Place an "X" through the number that best describes your pain. For example:

0	1	2	3	4	X	6	7	8	9	10

And remember, we want you to rate how severe each pain type has been **ON AVERAGE** during the past week.

1. Please use the scale below to tell us how **intense** your pain is

No Pain

0	1	2	3	4	4	6	7	8	9	10

The most **intense** pain sensation imaginable

2. Please use the scale below to tell us how **sharp** your pain feels. Words used to describe "sharp" feelings include "like a knife", "like a spike", "jabbing", or "like jolts".

Not Sharp

0	1	2	3	4	4	6	7	8	9	10

The most **sharp** sensation imaginable ("like a knife")

3. Please use the scale below to tell us how **hot** your pain feels. Words used to describe very hot pain include "burning" and "on fire".

Not Hot

0	1	2	3	4	4	6	7	8	9	10

The most **hot** sensation imaginable ("on fire")

4. Please use the scale below to tell us how **dull** your pain feels. Words used to describe very dull pain include "like a dull toothache", "dull pain", and "like a bruise".

Not Dull

0	1	2	3	4	4	6	7	8	9	10

The most **dull** sensation imaginable

*Remember to rate how severe each pain type has been **ON AVERAGE** during the past week.*

---

5. Please use the scale below to tell us how **cold** your pain feels. Words used to describe very cold pain include "like ice" and "freezing".

Not Cold | 0 | 1 | 2 | 3 | 4 | 4 | 6 | 7 | 8 | 9 | 10 | The most **cold** sensation imaginable ("freezing")

---

6. Please use the scale below to tell us how **sensitive** your skin is to light touch or clothing. Words used to describe sensitive skin include "like sunburned skin", and "raw skin".

Not Sensitive | 0 | 1 | 2 | 3 | 4 | 4 | 6 | 7 | 8 | 9 | 10 | The most **sensitive** sensation imaginable ("raw skin")

---

7. Please use the scale below to tell us how **tender** your pain is when something has pressed against it over the past week. Another word used to describe tender pain is "like a bruise."

Not Tender | 0 | 1 | 2 | 3 | 4 | 4 | 6 | 7 | 8 | 9 | 10 | The most **tender** sensation imaginable ("like a bruise")

---

8. Please use the scale below to tell us how **itchy** your pain feels. Words used to describe itchy pain include "like poison oak" and "like a mosquito bite".

Not Itchy | 0 | 1 | 2 | 3 | 4 | 4 | 6 | 7 | 8 | 9 | 10 | The most **itchy** sensation imaginable ("like poison oak")

---

9. Please use the scale below to tell us how much your pain has felt like it has been **shooting** over the past week. Another word used to describe shooting pain is "zapping."

Not Shooting | 0 | 1 | 2 | 3 | 4 | 4 | 6 | 7 | 8 | 9 | 10 | The most **shooting** sensation imaginable ("zapping")

---

10. Please use the scale below to tell us how **numb** your pain has felt over the past week. A phrase that can be used to describe numb pain is "like it is asleep."

Not Numb | 0 | 1 | 2 | 3 | 4 | 4 | 6 | 7 | 8 | 9 | 10 | The most **numb** sensation imaginable ("asleep")

---

11. Please use the scale below to tell us how much your pain sensations have felt **electrical** over the past week. Words used to describe electrical pain include "shocks," "lightning," and "sparking."

Not Electrical | 0 | 1 | 2 | 3 | 4 | 4 | 6 | 7 | 8 | 9 | 10 | The most **electrical** sensation imaginable ("shocks")

---

12. Please use the scale below to tell us how **tingling** your pain has felt over the past week. Words used to describe tingling pain include "like pins and needles" and "prickling."

Not Tingling | 0 | 1 | 2 | 3 | 4 | 4 | 6 | 7 | 8 | 9 | 10 | The most **tingling** sensation imaginable

("pins and needles")

*Remember to rate how severe each pain type has been **ON AVERAGE** during the past week.*

---

13. Please use the scale below to tell us how **cramping** your pain has felt over the past week. Words used to describe cramping pain include "squeezing" and "tight."

Not Cramping	0	1	2	3	4	4	6	7	8	9	10	The most **cramping** sensation imaginable ("squeezing")

---

14. Please use the scale below to tell us how **radiating** your pain has felt over the past week. Another word used to describe radiating pain is "spreading."

Not Radiating	0	1	2	3	4	4	6	7	8	9	10	The most **radiating** sensation imaginable ("spreading")

---

15. Please use the scale below to tell us how **throbbing** your pain has felt over the past week. Another word used to describe throbbing pain is "pounding."

Not Throbbing	0	1	2	3	4	4	6	7	8	9	10	The most **throbbing** sensation imaginable ("pounding")

---

16. Please use the scale below to tell us how **aching** your pain has felt over the past week. Another word used to describe aching pain is "like a toothache."

Not Aching	0	1	2	3	4	4	6	7	8	9	10	The most **aching** sensation imaginable ("like a toothache")

---

17. Please use the scale below to tell us how **heavy** your pain has felt over the past week. Other words used to describe heavy pain are "pressure" and "weighted down."

Not heavy	0	1	2	3	4	4	6	7	8	9	10	The most **heavy** sensation imaginable ("weighted down")

---

18. Now that you have told us the different types of pain sensations you have felt, we want you to tell us overall how **unpleasant** your pain has been to you over the past week. Words used to describe very unpleasant pain include "annoying," "bothersome," "miserable," and "intolerable." Remember, pain can have a low intensity but still feel extremely unpleasant, and some kinds of pain can have a high intensity but be very tolerable. With this scale, please tell us how **unpleasant** your pain feels.

Not Unpleasant	0	1	2	3	4	4	6	7	8	9	10	The most **unpleasant** sensation imaginable ("intolerable")

*Remember to rate how severe each pain type has been **ON AVERAGE** during the past week.*

19. Finally, we want you to give us an estimate of the severity of your deep versus surface pain over the past week. We want you to rate each location of pain separately. We realize that it can be difficult to make these estimates, and most likely it will be a "best guess," but please give us your best estimate.

HOW INTENSE IS YOUR *DEEP* PAIN?

No **Deep** pain

0	1	2	3	4	4	6	7	8	9	10

The most **intense deep** pain sensation imaginable

HOW INTENSE IS YOUR *SURFACE* PAIN?

No **Surface** pain

0	1	2	3	4	4	6	7	8	9	10

The most **intense surface** pain sensation imaginable

20. Pain can also have different time qualities. For some people, the pain comes and goes and so they have some moments that are completely without pain; in other words the pain "comes and goes". This is called **intermittent** pain. Others are never pain free, but their pain types and pain severity can vary from one moment to the next. This is called **variable** pain. For these people, the increases can be severe, so that they feel they have moments of very intense pain ("breakthrough" pain), but at other times they can feel lower levels of pain ("background" pain). Still, they are never pain free. Other people have pain that really does not change that much from one moment to another. This is called **stable** pain. Which of these best describes the time pattern of your pain (please select only one):

( ) I have **intermittent** pain (I feel pain sometimes but I am pain-free at other times).
( ) I have **variable** pain ("background" pain all the time, but also moments of more pain, or even severe "breakthrough pain or varying types of pain).
( ) I have **stable** pain (constant pain that does not change very much from one moment to another, and no pain-free periods).

DASS

Name: *Date:*

Please read each statement and circle a number 0, 1, 2 or 3 which indicates how much the statement applied to you *over the past week*. There are no right or wrong answers. Do not spend too much time on any statement.

The rating scale is as follows:

0 Did not apply to me at all
1 Applied to me to some degree, or some of the time
2 Applied to me to a considerable degree, or a good part of time
3 Applied to me very much, or most of the time

1	I found myself getting upset by quite trivial things	0	1	2	3
2	I was aware of dryness of my mouth	0	1	2	3
3	I couldn't seem to experience any positive feeling at all	0	1	2	3
4	I experienced breathing difficulty (eg, excessively rapid breathing, breathlessness in the absence of physical exertion)	0	1	2	3
5	I just couldn't seem to get going	0	1	2	3
6	I tended to over-react to situations	0	1	2	3
7	I had a feeling of shakiness (eg, legs going to give way)	0	1	2	3
8	I found it difficult to relax	0	1	2	3
9	I found myself in situations that made me so anxious I was most relieved when they ended	0	1	2	3
10	I felt that I had nothing to look forward to	0	1	2	3
11	I found myself getting upset rather easily	0	1	2	3
12	I felt that I was using a lot of nervous energy	0	1	2	3
13	I felt sad and depressed	0	1	2	3
14	I found myself getting impatient when I was delayed in any way (eg, lifts, traffic lights, being kept waiting)	0	1	2	3
15	I had a feeling of faintness	0	1	2	3
16	I felt that I had lost interest in just about everything	0	1	2	3
17	I felt I wasn't worth much as a person	0	1	2	3
18	I felt that I was rather touchy	0	1	2	3
19	I perspired noticeably (eg, hands sweaty) in the absence of high temperatures or physical exertion	0	1	2	3
20	I felt scared without any good reason	0	1	2	3
21	I felt that life wasn't worthwhile	0	1	2	3

Please turn the page ☞

Reminder of rating scale:

0 Did not apply to me at all
1 Applied to me to some degree, or some of the time
2 Applied to me to a considerable degree, or a good part of time
3 Applied to me very much, or most of the time

22	I found it hard to wind down	0	1	2	3
23	I had difficulty in swallowing	0	1	2	3
24	I couldn't seem to get any enjoyment out of the things I did	0	1	2	3
25	I was aware of the action of my heart in the absence of physical exertion (eg, sense of heart rate increase, heart missing a beat)	0	1	2	3
26	I felt down-hearted and blue	0	1	2	3
27	I found that I was very irritable	0	1	2	3
28	I felt I was close to panic	0	1	2	3
29	I found it hard to calm down after something upset me	0	1	2	3
30	I feared that I would be "thrown" by some trivial but unfamiliar task	0	1	2	3
31	I was unable to become enthusiastic about anything	0	1	2	3
32	I found it difficult to tolerate interruptions to what I was doing	0	1	2	3
33	I was in a state of nervous tension	0	1	2	3
34	I felt I was pretty worthless	0	1	2	3
35	I was intolerant of anything that kept me from getting on with what I was doing	0	1	2	3
36	I felt terrified	0	1	2	3
37	I could see nothing in the future to be hopeful about	0	1	2	3
38	I felt that life was meaningless	0	1	2	3
39	I found myself getting agitated	0	1	2	3
40	I was worried about situations in which I might panic and make a fool of myself	0	1	2	3
41	I experienced trembling (eg, in the hands)	0	1	2	3
42	I found it difficult to work up the initiative to do things	0	1	2	3

PCS-CF

Nom: _____ Age: _____ Sexe: _____ Date: _____

Chacun d'entre nous aura à subir des expériences douloureuses. Cela peut être la douleur associée aux maux de tête, à un mal de dent, ou encore la douleur musculaire ou aux articulations. Il nous arrive souvent d'avoir à subir des expériences douloureuses telles que la maladie, une blessure, un traitement dentaire ou une intervention chirurgicale.

Dans le présent questionnaire, nous vous demandons de décrire le genre de pensées et d'émotions que vous avez quand vous avez de la douleur. Vous trouverez ci-dessous treize énoncés décrivant différentes pensées et émotions qui peuvent être associées à la douleur. Veuillez indiquer à quel point vous avez ces pensées et émotions, selon l'échelle ci-dessous, quand vous avez de la douleur.

0 – pas du tout **1** – quelque peu **2** – de façon modéré **3** – beaucoup **4** – tout le temps

Quand j'ai de la douleur ...

1. ☐ j'ai peur qu'il n'y aura pas de fin à la douleur.
2. ☐ je sens que je ne peux pas continuer.
3. ☐ c'est terrible et je pense que ça ne s'améliorera jamais.
4. ☐ c'est affreux et je sens que c'est plus fort que moi.
5. ☐ je sens que je ne peux plus supporter la douleur.
6. ☐ j'ai peur que la douleur s'empire.
7. ☐ je ne fais que penser à d'autres expériences douloureuses.
8. ☐ avec inquiétude, je souhaite que la douleur disparaisse.
9. ☐ je ne peux m'empêcher d'y penser.
10. ☐ je ne fais que penser à quel point ça fait mal.
11. ☐ je ne fais que penser à quel point je veux que la douleur disparaisse.
12. ☐ il n'y a rien que je puisse faire pour réduire l'intensité de la douleur.
13. ☐ je me demande si quelque chose de grave va se produire.

... Total

PCS

Client No.: _____ Age: _____ Sex: M(__) F(__) Date: _____

Everyone experiences painful situations at some point in their lives. Such experiences may include headaches, tooth pain, joint or muscle pain. People are often exposed to situations that may cause pain such as illness, injury, dental procedures or surgery.

We are interested in the types of thoughts and feelings that you have when you are in pain. Listed below are thirteen statements describing different thoughts and feelings that may be associated with pain. Using the following scale, please indicate the degree to which you have these thoughts and feelings when you are experiencing pain.

0 – not at all **1** – to a slight degree **2** – to a moderate degree **3** – to a great degree **4** – all the time

When I'm in pain ...

1. ☐ I worry all the time about whether the pain will end.
2. ☐ I feel I can't go on.
3. ☐ It's terrible and I think it's never going to get any better.
4. ☐ It's awful and I feel that it overwhelms me.
5. ☐ I feel I can't stand it anymore.
6. ☐ I become afraid that the pain will get worse.
7. ☐ I keep thinking of other painful events.
8. ☐ I anxiously want the pain to go away.
9. ☐ I can't seem to keep it out of my mind.
10. ☐ I keep thinking about how much it hurts.
11. ☐ I keep thinking about how badly I want the pain to stop.
12. ☐ There's nothing I can do to reduce the intensity of the pain.
13. ☐ I wonder whether something serious may happen.

... *Total*

Welstein, L.; Dement, W.C.; Redington, D.; and Guilleminault, C. Insomnia in the San Francisco Bay Area: A telephone survey. *Sleep/Wake Disorders: Natural History, Epidemiology, and Long-Term Evolution.* New York: Raven Press, 1983. pp. 73-85.

Appendix. Pittsburgh Sleep Quality Index (PSQI)

Name _____ ID # _____ Date _____ Age _____

Instructions:

The following questions relate to your usual sleep habits during the past month *only*. Your answers should indicate the most accurate reply for the *majority* of days and nights in the past month. Please answer all questions.

1. During the past month, when have you usually gone to bed at night?
   USUAL BED TIME _____
2. During the past month, how long (in minutes) has it usually take you to fall asleep each night?
   NUMBER OF MINUTES _____
3. During the past month, when have you usually gotten up in the morning?
   USUAL GETTING UP TIME _____
4. During the past month, how many hours of *actual sleep* did you get at night? (This may be different than the number of hours you spend in bed.)
   HOURS OF SLEEP PER NIGHT _____

For each of the remaining questions, check the one best response. Please answer *all* questions.

5. During the past month, how often have you had trouble sleeping because you...

(a) Cannot get to sleep within 30 minutes

Not during the past month ____	Less than once a week ____	Once or twice a week ____	Three or more times a week ____

(b) Wake up in the middle of the night or early morning

Not during the past month ____	Less than once a week ____	Once or twice a week ____	Three or more times a week ____

(c) Have to get up to use the bathroom

Not during the past month ____	Less than once a week ____	Once or twice a week ____	Three or more times a week ____

(d) Cannot breathe comfortably

Not during the past month ____	Less than once a week ____	Once or twice a week ____	Three or more times a week ____

(e) Cough or snore loudly

Not during the past month ____	Less than once a week ____	Once or twice a week ____	Three or more times a week ____

(f) Feel too cold

Not during the past month ____	Less than once a week ____	Once or twice a week ____	Three or more times a week ____

(g) Feel too hot

Not during the past month ____	Less than once a week ____	Once or twice a week ____	Three or more times a week ____

(h) Had bad dreams

Not during the past month ____	Less than once a week ____	Once or twice a week ____	Three or more times a week ____

(i) Have pain

Not during the past month ____	Less than once a week ____	Once or twice a week ____	Three or more times a week ____

(j) Other reason(s), please describe _____

_____

How often during the past month have you had trouble sleeping because of this?

Not during the past month ____	Less than once a week ____	Once or twice a week ____	Three or more times a week ____

6. During the past month, how would you rate your sleep quality overall?

Very good _____
Fairly good _____
Fairly bad _____
Very bad _____

7. During the past month, how often have you taken medicine (prescribed or "over the counter") to help you sleep?

Not during the past month ____	Less than once a week ____	Once or twice a week ____	Three or more times a week ____

8. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?

Not during the past month ____	Less than once a week ____	Once or twice a week ____	Three or more times a week ____

9. During the past month, how much of a problem has it been for you to keep up enough enthusiasm to get things done?

No problem at all _____
Only a very slight problem _____
Somewhat of a problem _____
A very big problem _____

10. Do you have a bed partner or roommate?

No bed partner or roommate _____
Partner/roommate in other room _____
Partner in same room, but not same bed _____
Partner in same bed _____

If you have a roommate or bed partner, ask him/her how often in the past month you have had...

(a) Loud snoring

Not during the past month ____	Less than once a week ____	Once or twice a week ____	Three or more times a week ____

(b) Long pauses between breaths while asleep

Not during the past month ____	Less than once a week ____	Once or twice a week ____	Three or more times a week ____

(c) Legs twitching or jerking while you sleep

Not during the past month ____	Less than once a week ____	Once or twice a week ____	Three or more times a week ____

(d) Episodes of disorientation or confusion during sleep

Not during the past month ____	Less than once a week ____	Once or twice a week ____	Three or more times a week ____

(e) Other restlessness while you sleep; please describe _____

_____

Not during the past month ____	Less than once a week ____	Once or twice a week ____	Three or more times a week ____

Scoring Instructions for the Pittsburgh Sleep Quality Index

The Pittsburgh Sleep Quality Index (PSQI) contains 19 self-rated questions and 5 questions rated by the bed partner or roommate (if one is available). Only self-rated questions are included in the scoring. The 19 self-rated items are combined to form seven "component" scores, each of which has a range of 0-3 points. In all cases, a score of "0" indicates no difficulty, while a score of "3" indicates severe difficulty. The seven component scores are then added to yield one "global" score, with a range of 0-21 points, "0" indicating no difficulty and "21" indicating severe difficulties in all areas.

Scoring proceeds as follows:

Component 1: Subjective sleep quality

Examine question #6, and assign scores as follows:

Response	Component 1 score
"Very good"	0
"Fairly good"	1
"Fairly bad"	2
"Very bad"	3

Component 1 score: _____

Component 2: Sleep latency

1. Examine question #2, and assign scores as follows:

Response	Score
≤ 15 minutes	0
16-30 minutes	1
31-60 minutes	2
> 60 minutes	3

Question #2 score: _____

2. Examine question #5a, and assign scores as follows:

Response	Score
Not during the past month	0
Less than once a week	1
Once or twice a week	2
Three or more times a week	3

Question #5a score: _____

3. Add #2 score and #5a score

Sum of #2 and #5a: _____

4. Assign component 2 score as follows:

Sum of #2 and #5a	Component 2 score
0	0
1-2	1
3-4	2
5-6	3

Component 2 score: _____

Component 3: Sleep duration

Examine question #4, and assign scores as follows:

Response	Component 3 score
> 7 hours	0
6-7 hours	1
5-6 hours	2
< 5 hours	3

Component 3 score: _____

Component 4: Habitual sleep efficiency

(1) Write the number of hours slept (question # 4) here: _____

(2) Calculate the number of hours spent in bed:

Getting up time (question # 3): _____

– Bedtime (question # 1): _____

Number of hours spent in bed: _____

(3) Calculate habitual sleep efficiency as follows:

(Number of hours slept/Number of hours spent in bed) × 100 = Habitual sleep efficiency (%)

(_____/_____) × 100 = _____%

(4) Assign component 4 score as follows:

Habitual sleep efficiency %	Component 4 score
> 85%	0
75-84%	1
65-74%	2
< 65%	3

Component 4 score: _____

Component 5: Sleep disturbances

(1) Examine questions # 5b-5j, and assign scores for *each* question as follows:

Response	Score
Not during the past month	0
Less than once a week	1
Once or twice a week	2
Three or more times a week	3

#5b score _____

c score _____

d score _____

e score _____

f score _____

g score _____

h score _____

i score _____

j score _____

(2) Add the scores for questions # 5b-5j:

Sum of # 5b-5j: _____

(3) Assign component 5 score as follows:

Sum of # 5b-5j	Component 5 score
0	0
1-9	1
10-18	2
19-27	3

Component 5 score: _____

Component 6: Use of sleeping medication

Examine question # 7 and assign scores as follows:

Response	Component 6 score
Not during the past month	0
Less than once a week	1
Once or twice a week	2
Three or more times a week	3

Component 6 score: _____

Instructions:

The following is a list of common problems. Please indicate if you currently have the problem in the first column. If you do not have the problem, skip to next problem.

If you do have the problem, please indicate in the second column if you receive medications or some other type of treatment for the problem.

In the third column indicate if the problem limits any of your activities.

Finally, indicate all medical conditions that are not listed under "other medical problems" at the end of the page.

	Do you have the problem?		Do you receive treatment for it?		Does it limit your activities?	
PROBLEM	No (0)	Yes→ (1)	No (0)	Yes (1)	No (0)	Yes (1)
Heart disease	N	Y	N	Y	N	Y
High blood pressure	N	Y	N	Y	N	Y
Lung disease	N	Y	N	Y	N	Y
Diabetes	N	Y	N	Y	N	Y
Ulcer or stomach disease	N	Y	N	Y	N	Y
Kidney disease	N	Y	N	Y	N	Y
Liver disease	N	Y	N	Y	N	Y
Anemia or other blood disease	N	Y	N	Y	N	Y
Cancer	N	Y	N	Y	N	Y
Depression	N	Y	N	Y	N	Y
Osteoarthritis, degenerative arthritis	N	Y	N	Y	N	Y
Back pain	N	Y	N	Y	N	Y
Rheumatoid arthritis	N	Y	N	Y	N	Y
Other medical problems (please write in)						
	N	Y	N	Y	N	Y
	N	Y	N	Y	N	Y

Appendix 2

Security Classification:

Neuropathic Pain indicators in Osteoarthritis

PARTICIPANT INFORMATION STATEMENT

HREC Project Number:	*39/2016*
Project Title:	Determining diagnostic indicators for neuropathic pain in patients with osteoarthritis
Principal Investigator:	*Prof Tony Wright*
Student researcher:	*Farhat Bashir*
Version Number:	*2*
Version Date:	*1 March 2016*

What is the Project About?

Osteoarthritis (OA) pain is traditionally considered to occur as a result of inflammation of joints. Recently studies have shown that 20-30% of people with knee OA also experience neuropathic pain rather than the common nociceptive pain. Neuropathic pain (NP) is generally described as a sharp, stinging or burning pain. NP occurs when nerves within the knee are damaged, severed or irritated. Nociceptive pain is generally caused by damage to the knee joint and is often described as an aching or throbbing pain. At present there are no diagnostic tests available to make a definite diagnosis of neuropathic pain in patients with OA. Developing better processes to identify patients with OA who may be experiencing neuropathic pain is important so they can be distinguished from patients with predominantly nociceptive pain and can be treated accordingly. This study aims to develop diagnostic criteria for such patients.

Who is doing the Research?

The project is being conducted by Farhat Bashir as part of her PhD research project at Curtin University.

Costs of being involved in the project and any payment

There will be no cost to you for taking part in this research. You will be offered a $25 gift card to compensate for travel costs.

Why am I being asked to take part and what will I have to do?

You are asked to take part in this study because you are diagnosed with osteoarthritis of the knee. This information sheet explains the study and describes what will be involved should you decide to take part in the study. This study aims to assess the presence of neuropathic pain using sensory testing and self-report questionnaires.

If you choose to participate, you will be assessed by a Rheumatologist at a Rheumatology Clinic in Victoria Park, for suitability to take part in this research project. If you are suitable to take part in the study and appointment will be made for your first assessment session. You will undergo a detailed

clinical examination which consists of a standard neurological examination performed by researcher who is also medical practitioner. As part of this process you will complete a lower limb pain diagram using a tablet based application, clearly indicating where you feel pain and any other sensations or unusual symptoms in the lower limb.

During the same session you will be asked to complete a number of self-report questionnaires. These include the PainDETECT questionnaire (Appendix 1), Leeds Assessment of neuropathic symptoms and signs (Appendix 2), Western Ontario McMaster Osteoarthritis Index version VA 3.1 (appendix 3), the Pain Quality Assessment Scale (Appendix 4), the Depression, Anxiety and Stress Scale (appendix 5), the pain catastrophizing scale (Appendix 6), Pittsburgh sleep quality index (Appendix 7) and the comorbidity questionnaire (Appendix 8). These questionnaires will take about 45 minutes to complete.

At the end of first assessment session you will be asked to make three more appointments. These include a sensory testing session at the Rheumatology clinic in Victoria Park, a testing session at the motion analysis lab in Curtin University, Bentley campus, and MRI test at SKG radiology at Kelmscott. You will be assisted to make these appointments.

Sensory testing will be performed to assess your sensitivity to heat, cold, pressure and vibration stimuli. The heat and cold sensitivity will be measured by using a thermode pressed against your skin, which will slowly decrease or increase in temperature. We will ask you to press a switch when you feel a change in temperature as well as when you feel the temperature change to a "painful" sensation. Similar tests will be conducted to measure pressure and vibration sensitivity.

Proprioceptive test means testing your awareness of joint position. You will be positioned comfortably on a 30° reclined sliding platform with your back fully supported and your other leg supported by another platform. The test procedure will be explained to you. You will be asked to focus on a particular knee position for 5 seconds. You will then be moved back to a neutral position by the researcher. You will then be instructed to move back to the original knee position and inform the researcher when you believe you reached the target position. The researcher will record the knee angle in that position by pressing a control switch. A total of 6 measurements at two knee positions will be obtained.

You will receive a referral for MRI to be performed at SKG radiology. MRI images will be analysed in detail to find any differences found between the neuropathic pain group and nociceptive pain group.

Benefits to being in the research project?

Participation in this study may have no direct benefit for you, but may help with better management of those patients who suffer from neuropathic pain with osteoarthritis of the knee in the future.

Are there any risks, side-effects, discomforts or inconveniences from being in the research project?

There are no foreseeable major risks associated with participation in this research project.

Blood sample collection can cause mild discomfort, bruising and sometimes light headedness; to minimise this the sample will be collected by researcher with phlebotomy training. You will be able to sit/lie down during the procedure.

During the research project we may find out new information about the risks and benefits of this study. If this happens we will tell you the new information and what it means to you. It may be that this new information means that you can no longer be in the study or you may choose to keep going or to leave the study. You might be asked to sign a new consent form to let us know you understand any new information we have told you.

Who will have access to my information?

The information collected in this research will be re-identifiable (coded). The information we collect will be treated as confidential and used only in the project. We can let others know this information only after your permission or if the law says we need to.

All information will be stored securely in a locked cupboard at the Rheumatology Clinic in Victoria Park and the School of Physiotherapy and Exercise Science, Curtin University. Digital data will be stored on Curtin University R drive and it will be password protected. The research team will have access to the information we collect in this research. The Curtin Ethics Office may access the data for audit purposes. This information will be stored for seven years.

The results of this research may be presented at conferences or published in professional journals. You will not be identified in any results that are published or presented.

Will you tell me the results of the research?

A summary of the project's overall results will be sent to you at the end of the research (in about 18 months) to let you know the results of the research. Results will not be individual but based on all the information we collect and review as part of the research.

Do I have to take part in the research project?

Participation in this study is entirely voluntary. You do not have to participate if you do not want to and your decision to participate or not will in no way affect your current or future care at Dr Will's private rooms. You are also free to withdraw from the study anytime without reason or justification. You may request that any data that has been collected from you be withdrawn.

What happens next and who can I contact about the research?

If you decide to take part in this research we will ask you to sign the consent form. Signing the consent indicates that you agree to be in the research project and understand what has been discussed.

If you have questions about this study, please contact Farhat Bashir on 0422 313 846 (private number).

Curtin University Human Research Ethics Committee (HREC) has approved this study (HREC number 39/2016). Should you wish to discuss the study with someone not directly involved, in particular, any matters concerning the conduct of the study or your rights as a participant, or you wish to make a confidential complaint, you may contact the Ethics Officer on (08) 9266 9223 or the Manager, Research Integrity on (08) 9266 7093 or email hrec@curtin.edu.au.

CONSENT FORM

HREC Project Number:	*39/2016*
Project Title:	*Diagnostic indicators of Neuropathic pain in osteoarthritis*
Principal Investigator:	*Prof Tony Wright*
Student researcher:	*Farhat Bashir*
Version Number:	*2*
Version Date:	*1 March 2016*

- I have read the information statement version listed above and I understand its contents.
- I believe I understand the purpose, extent and possible risks of my involvement in this project.
- I voluntarily consent to take part in this research project.
- I have had an opportunity to ask questions and I am satisfied with the answers I have received.
- I understand that this project has been approved by Curtin University Human Research Ethics Committee and will be carried out in line with the National Statement on Ethical Conduct in Human Research (2007) – updated March 2014.
- I understand I will receive a copy of this Information Statement and Consent Form.

Participant Name	
Participant Signature	
Date	

Declaration by researcher: I have supplied an Information Letter and Consent Form to the participant who has signed above, and believe that they understand the purpose, extent and possible risks of their involvement in this project.

Researcher Name	
Researcher Signature	
Date	

Note: All parties signing the Consent Form must date their own signature.

Appendix 3

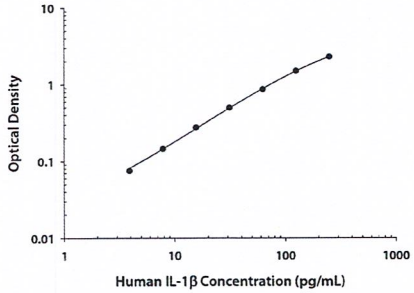
CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human IL-1β concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is only for demonstration purposes. A standard curve should be generated for each set of samples assayed.



SPECIFICITY

The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference.

Recombinant human:
IL-1α

Recombinant rat:
IL-1α

Recombinant mouse:
IL-1α
IL-1β

Recombinant porcine:
IL-1α
IL-1β

A sample containing 6250 pg/mL of recombinant rat IL-1β reads as 240 pg/mL (3.8% cross-reactivity).

A sample containing 125 pg/mL of recombinant human IL-1β precursor (aa 1-269) reads as 8 pg/mL (6.3% cross-reactivity).

TECHNICAL HINTS & LIMITATIONS

- We recommend the use of R&D Systems' Reagent Diluent Concentrate 2 (Catalog # DY995) to prepare Reagent Diluent for use in this assay.
- The use of high quality Bovine Serum Albumin (BSA) for the Reagent Diluent is crucial for the optimum performance of the DuoSet ELISA Development kit. Impurities such as proteases, binding proteins, soluble receptors or other interfering substances can be found to varying degrees in virtually all BSA preparations and can inhibit or interfere with the detection of certain analytes. If the standard curve appears suppressed, consider evaluating a different preparation of BSA.
- It is suggested to start Reagent Diluent optimization for serum and plasma samples by using PBS supplemented with 10-50% animal serum. Do not use buffers with animal serum to reconstitute or dilute the Detection Antibody or Streptavidin-HRP.
- It is important that the Reagent Diluent selected for dilution of the standard reflects the environment of the samples being measured.
- Avoid microbial contamination of reagents and buffers.
- A thorough and consistent wash technique is essential for proper assay performance. Wash Buffer should be dispensed forcefully and removed completely from the wells by aspiration or decanting. Remove any remaining Wash Buffer by inverting the plate and blotting it against clean paper towels.
- Individual results may vary due to differences in technique, plasticware and water sources.
- It is recommended that all standards and samples be assayed in duplicate.
- The use of PBS from tablets may interfere in this assay.

TROUBLESHOOTING

Note: *For more detailed troubleshooting, please visit:*
www.RnDSystems.com/ELISADevelopment

Poor Standard Curve

- Impure BSA used for Reagent Diluent preparation.
- Improper reconstitution and/or storage of standard.
- Improper dilution of highest standard and standard curve.
- Incomplete washing and/or aspiration of wells.
- Unequal volumes added to wells/pipetting error.
- Incorrect incubation times or temperatures.

Poor Precision

- Unequal volumes added to wells/pipetting error.
- Incomplete washing and/or aspiration of wells.
- Unequal mixing of reagents.

Low or No Color Development

- Inadequate volume of substrate added to wells.
- Incorrect incubation times or temperatures.
- Impure BSA used for Reagent Diluent preparation.

DuoSet® ELISA
DEVELOPMENT SYSTEM

Human IL-1β/IL-1F2

Catalog Numbers: DY201-05 (5 plates)
DY201 (15 plates)

INTENDED USE

For the development of sandwich ELISAs to measure natural and recombinant human Interleukin 1 beta (IL-1β). The Reagent Diluent recommended may be suitable for most cell culture supernate, serum, and plasma samples. The Reagent Diluent selected for use can alter the performance of an immunoassay. Reagent Diluent optimization for samples with complex matrices such as serum and plasma, may improve their performance in this assay.

This kit contains sufficient materials to run ELISAs on at least five 96 well plates, provided the following conditions are met:

- The reagents are prepared as described in this package insert.
- The assay is run as described in the General ELISA Protocol.
- The recommended microplates, buffers, diluents, substrates, and solutions are used.

This package insert must be read in its entirety before using this product.
Refer to the Certificate of Analysis for component concentrations as they may vary.
For research use only. Not for use in diagnostic procedures.

MANUFACTURED AND DISTRIBUTED BY:
USA & Canada | R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:
UK & Europe | R&D Systems Europe, Ltd.
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

China | R&D Systems China Co., Ltd.
24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

OTHER MATERIALS & SOLUTIONS REQUIRED

DuoSet Ancillary Reagent Kit 2 (5 plates):
(R&D Systems, Catalog # DY008) containing 96 well microplates, plate sealers, substrate solution, stop solution, plate coating buffer (PBS), wash buffer, and Reagent Diluent Concentrate 2.

The components listed above may be purchased separately:

96 well microplates: (R&D Systems, Catalog # DY990).

Plate Sealers: (R&D Systems, Catalog # DY992).

PBS: 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.2-7.4, 0.2 µm filtered (R&D Systems, Catalog # DY006).

Wash Buffer: 0.05% Tween® 20 in PBS, pH 7.2-7.4 (R&D Systems, Catalog # WA126).

Reagent Diluent: 1% BSA in PBS, pH 7.2-7.4, 0.2 µm filtered (R&D Systems, Catalog # DY995).
Quality of BSA is critical (see Technical Hints).

Substrate Solution: 1:1 mixture of Color Reagent A (H_2O_2) and Color Reagent B (Tetramethylbenzidine) (R&D Systems, Catalog # DY999).

Stop Solution: 2 N H_2SO_4 (R&D Systems, Catalog # DY994).

PRECAUTIONS

Some components in this kit contain ProClin® which may cause an allergic skin reaction. Avoid breathing mist.

The Stop Solution suggested for use with this kit is an acid solution.

The Color Reagent B suggested for use with this kit may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

CALIBRATION

This DuoSet is calibrated against a highly purified *E. coli*-expressed recombinant human IL-1β produced at R&D Systems.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

DESCRIPTION	PART #	CATALOG # DY201-05	CATALOG # DY201	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human IL-1β Capture Antibody†	840168	1 vial	3 vials	Refer to the lot-specific Certificate of Analysis (C of A) for storage conditions.
Human IL-1β Detection Antibody†	840169	1 vial	3 vials	
Human IL-1β Standard†	840170	1 vial	3 vials	
Streptavidin-HRP	893975	1 vial	3 vials	

†This product is covered by one or more of the following U.S. patents.: 5,681,933, 5,474,899.

REAGENT PREPARATION

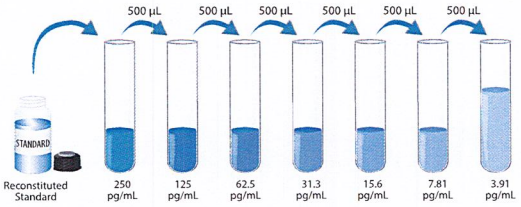
Bring all reagents to room temperature before use. Allow all components to sit for a minimum of 15 minutes with gentle agitation after initial reconstitution. Working dilutions should be prepared and used immediately.

Streptavidin-HRP: Each vial contains 2.0 mL of streptavidin conjugated to horseradish-peroxidase. Dilute to the working concentration specified on the vial label using Reagent Diluent.

Mouse Anti-Human IL-1β Capture Antibody: Refer to the lot-specific C of A for amount supplied. Reconstitute each vial with 0.5 mL of PBS. Dilute in PBS without carrier protein to the working concentration indicated on the C of A.

Biotinylated Goat Anti-Human IL-1β Detection Antibody: Refer to the lot-specific C of A for amount supplied. Reconstitute each vial with 1.0 mL of Reagent Diluent. Dilute in Reagent Diluent to the working concentration indicated on the C of A.

Recombinant Human IL-1β Standard: Refer to the lot-specific C of A for amount supplied. Reconstitute each vial with 0.5 mL of deionized or distilled water. A seven point standard curve using 2-fold serial dilutions in Reagent Diluent is recommended. Prepare 1000 µL of high standard per plate assayed at the concentration indicated on the C of A.



GENERAL ELISA PROTOCOL

Plate Preparation

1. Dilute the Capture Antibody to the working concentration in PBS without carrier protein. Immediately coat a 96-well microplate with 100 µL per well of the diluted Capture Antibody. Seal the plate and incubate overnight at room temperature.
2. Aspirate each well and wash with Wash Buffer, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
3. Block plates by adding 300 µL of Reagent Diluent to each well. Incubate at room temperature for a minimum of 1 hour.
4. Repeat the aspiration/wash as in step 2. The plates are now ready for sample addition.

Assay Procedure

1. Add 100 µL of sample or standards in Reagent Diluent, or an appropriate diluent, per well. Cover with an adhesive strip and incubate 2 hours at room temperature.
2. Repeat the aspiration/wash as in step 2 of Plate Preparation.
3. Add 100 µL of the Detection Antibody, diluted in Reagent Diluent, to each well. Cover with a new adhesive strip and incubate 2 hours at room temperature.
4. Repeat the aspiration/wash as in step 2 of Plate Preparation.
5. Add 100 µL of the working dilution of Streptavidin-HRP to each well. Cover the plate and incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
6. Repeat the aspiration/wash as in step 2.
7. Add 100 µL of Substrate Solution to each well. Incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
8. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well immediately, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

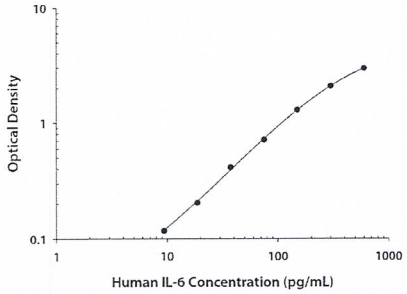
CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human IL-6 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is only for demonstration purposes. A standard curve should be generated for each set of samples assayed.



SPECIFICITY

The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference.

Recombinant human:
CNTF
G-CSF
gp130
IL-6 R
IL-11
IL-12
LIF
LIF R
OSM

Recombinant mouse:
IL-6
IL-11
IL-12

Recombinant rat:
CNTF

TECHNICAL HINTS & LIMITATIONS

- We recommend the use of R&D Systems' Reagent Diluent Concentrate 2 (Catalog # DY995) to prepare Reagent Diluent for use in this assay.
- The use of high quality Bovine Serum Albumin (BSA) for the Reagent Diluent is crucial for the optimum performance of the DuoSet ELISA Development kit. Impurities such as proteases, binding proteins, soluble receptors or other interfering substances can be found to varying degrees in virtually all BSA preparations and can inhibit or interfere with the detection of certain analytes. If the standard curve appears suppressed, consider evaluating a different preparation of BSA.
- It is suggested to start Reagent Diluent optimization for serum and plasma samples by using PBS supplemented with 10-50% animal serum. Do not use buffers with animal serum to reconstitute or dilute the Detection Antibody or Streptavidin-HRP.
- It is important that the Reagent Diluent selected for dilution of the standard reflects the environment of the samples being measured.
- Avoid microbial contamination of reagents and buffers.
- A thorough and consistent wash technique is essential for proper assay performance. Wash Buffer should be dispensed forcefully and removed completely from the wells by aspiration or decanting. Remove any remaining Wash Buffer by inverting the plate and blotting it against clean paper towels.
- Individual results may vary due to differences in technique, plasticware and water sources.
- It is recommended that all standards and samples be assayed in duplicate.
- The use of PBS from tablets may interfere in this assay.

TROUBLESHOOTING

Note: *For more detailed troubleshooting, please visit:*
www.RnDSystems.com/ELISADevelopment

Poor Standard Curve
- Impure BSA used for Reagent Diluent preparation.
- Improper reconstitution and/or storage of standard.
- Improper dilution of highest standard and standard curve.
- Incomplete washing and/or aspiration of wells.
- Unequal volumes added to wells/pipetting error.
- Incorrect incubation times or temperatures.

Poor Precision
- Unequal volumes added to wells/pipetting error.
- Incomplete washing and/or aspiration of wells.
- Unequal mixing of reagents.

Low or No Color Development
- Inadequate volume of substrate added to wells.
- Incorrect incubation times or temperatures.
- Impure BSA used for Reagent Diluent preparation.

DuoSet® ELISA DEVELOPMENT SYSTEM

Human IL-6

Catalog Numbers: DY206-05 (5 plates)
DY206 (15 plates)

INTENDED USE

For the development of sandwich ELISAs to measure natural and recombinant human Interleukin 6 (IL-6). The Reagent Diluent recommended may be suitable for most cell culture supernate, serum, and plasma samples. The Reagent Diluent selected for use can alter the performance of an immunoassay. Reagent Diluent optimization for samples with complex matrices such as serum and plasma, may improve their performance in this assay.

This kit contains sufficient materials to run ELISAs on at least five 96 well plates, provided the following conditions are met:

- The reagents are prepared as described in this package insert.
- The assay is run as described in the General ELISA Protocol.
- The recommended microplates, buffers, diluents, substrates, and solutions are used.

This package insert must be read in its entirety before using this product.
Refer to the Certificate of Analysis for component concentrations as they may vary.
For research use only. Not for use in diagnostic procedures.

MANUFACTURED AND DISTRIBUTED BY:
USA & Canada | R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:
UK & Europe | R&D Systems Europe, Ltd.
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

China | R&D Systems China Co., Ltd.
24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

R&D SYSTEMS™ a biotechne brand

OTHER MATERIALS & SOLUTIONS REQUIRED

DuoSet Ancillary Reagent Pack 2 (5 plates):
(R&D Systems, Catalog # DY008) containing 96 well microplates, plate sealers, substrate solution, stop solution, plate coating buffer (PBS), wash buffer, and Reagent Diluent 2.

The components listed above may be purchased separately:

96 well microplates: (R&D Systems, Catalog # DY990).

Plate sealers: (R&D Systems, Catalog # DY992).

PBS: 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.2-7.4, 0.2 µm filtered (R&D Systems, Catalog # DY006).

Wash Buffer: 0.05% Tween® 20 in PBS, pH 7.2-7.4 (R&D Systems, Catalog # WA126).

Reagent Diluent: 1% BSA in PBS, pH 7.2-7.4, 0.2 µm filtered (R&D Systems, Catalog # DY995).
Quality of BSA is critical (see Technical Hints).

Substrate Solution: 1:1 mixture of Color Reagent A (H_2O_2) and Color Reagent B (Tetramethylbenzidine) (R&D Systems, Catalog # DY999).

Stop Solution: 2 N H_2SO_4 (R&D Systems, Catalog # DY994).

PRECAUTIONS

Some components in this kit contain ProClin® which may cause an allergic skin reaction. Avoid breathing mist.

The Stop Solution suggested for use with this kit is an acid solution.

The Color Reagent B suggested for use with this kit may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

CALIBRATION

This DuoSet is calibrated against a highly purified *E. coli*-expressed recombinant human IL-6 produced at R&D Systems.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

DESCRIPTION	PART #	CATALOG # DY206-05	CATALOG # DY206	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human IL-6 Capture Antibody	840113	1 vial	3 vials	Refer to the lot-specific Certificate of Analysis (C of A) for storage conditions.
Human IL-6 Detection Antibody	840114	1 vial	3 vials	
Human IL-6 Standard	840115	1 vial	3 vials	
Streptavidin-HRP	893975	1 vial	3 vials	

REAGENT PREPARATION

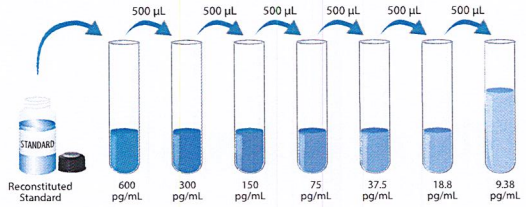
Bring all reagents to room temperature before use. Allow all components to sit for a minimum of 15 minutes with gentle agitation after initial reconstitution. Working dilutions should be prepared and used immediately.

Streptavidin-HRP: Each vial contains 2.0 mL of streptavidin conjugated to horseradish-peroxidase. Dilute to the working concentration specified on the vial label using Reagent Diluent.

Mouse Anti-Human IL-6 Capture Antibody: Refer to the lot-specific C of A for amount supplied. Reconstitute each vial with 0.5 mL of PBS. Dilute in PBS without carrier protein to the working concentration indicated on the C of A.

Biotinylated Goat Anti-Human IL-6 Detection Antibody: Refer to the lot-specific C of A for amount supplied. Reconstitute each vial with 1.0 mL of Reagent Diluent. Dilute in Reagent Diluent to the working concentration indicated on the C of A.

Recombinant Human IL-6 Standard: Refer to the lot-specific C of A for amount supplied. Reconstitute each vial with 0.5 mL of deionized or distilled water. A seven point standard curve using 2-fold serial dilutions in Reagent Diluent is recommended. Prepare 1000 µL of high standard per plate assayed at the concentration indicated on the C of A.



GENERAL ELISA PROTOCOL

Plate Preparation

1. Dilute the Capture Antibody to the working concentration in PBS without carrier protein. Immediately coat a 96-well microplate with 100 µL per well of the diluted Capture Antibody. Seal the plate and incubate overnight at room temperature.
2. Aspirate each well and wash with Wash Buffer, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
3. Block plates by adding 300 µL of Reagent Diluent to each well. Incubate at room temperature for a minimum of 1 hour.
4. Repeat the aspiration/wash as in step 2. The plates are now ready for sample addition.

Assay Procedure

1. Add 100 µL of sample or standards in Reagent Diluent, or an appropriate diluent, per well. Cover with an adhesive strip and incubate 2 hours at room temperature.
2. Repeat the aspiration/wash as in step 2 of Plate Preparation.
3. Add 100 µL of the Detection Antibody, diluted in Reagent Diluent, to each well. Cover with a new adhesive strip and incubate 2 hours at room temperature.
4. Repeat the aspiration/wash as in step 2 of Plate Preparation.
5. Add 100 µL of the working dilution of Streptavidin-HRP to each well. Cover the plate and incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
6. Repeat the aspiration/wash as in step 2.
7. Add 100 µL of Substrate Solution to each well. Incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
8. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well immediately, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

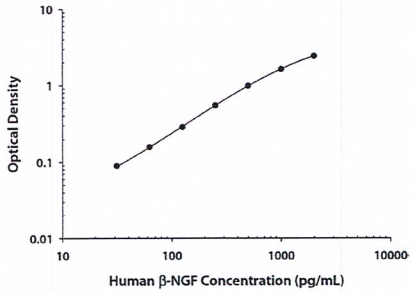
CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human β-NGF concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is only for demonstration purposes. A standard curve should be generated for each set of samples assayed.



SPECIFICITY

The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference.

Recombinant human:
BDNF
CNTF
GDNF
NT-3
NT-4

Recombinant rat:
CNTF
GDNF
GDNF Rα

A sample containing 1.56 ng/mL of recombinant mouse β-NGF reads as 506 pg/mL (32.4% cross-reactivity).

A sample containing 12.5 ng/mL of recombinant rat β-NGF reads as 340 pg/mL (2.7% cross-reactivity).

TECHNICAL HINTS & LIMITATIONS

- We recommend the use of R&D Systems' Reagent Diluent Concentrate 2 (Catalog # DY995) to prepare Reagent Diluent for use in this assay.
- The use of high quality Bovine Serum Albumin (BSA) for the Reagent Diluent is crucial for the optimum performance of the DuoSet ELISA Development kit. Impurities such as proteases, binding proteins, soluble receptors or other interfering substances can be found to varying degrees in virtually all BSA preparations and can inhibit or interfere with the detection of certain analytes. If the standard curve appears suppressed, consider evaluating a different preparation of BSA.
- It is suggested to start Reagent Diluent optimization for serum and plasma samples by using PBS supplemented with 10-50% animal serum. Do not use buffers with animal serum to reconstitute or dilute the Detection Antibody or Streptavidin-HRP.
- It is important that the Reagent Diluent selected for dilution of the standard reflects the environment of the samples being measured.
- Avoid microbial contamination of reagents and buffers.
- A thorough and consistent wash technique is essential for proper assay performance. Wash Buffer should be dispensed forcefully and removed completely from the wells by aspiration or decanting. Remove any remaining Wash Buffer by inverting the plate and blotting it against clean paper towels.
- Individual results may vary due to differences in technique, plasticware and water sources.
- It is recommended that all standards and samples be assayed in duplicate.
- The use of PBS from tablets may interfere in this assay.

TROUBLESHOOTING

Note: *For more detailed troubleshooting, please visit:*
www.RnDSystems.com/ELISADevelopment

Poor Standard Curve

- Impure BSA used for Reagent Diluent preparation.
- Improper reconstitution and/or storage of standard.
- Improper dilution of highest standard and standard curve.
- Incomplete washing and/or aspiration of wells.
- Unequal volumes added to wells/pipetting error.
- Incorrect incubation times or temperatures.

Poor Precision

- Unequal volumes added to wells/pipetting error.
- Incomplete washing and/or aspiration of wells.
- Unequal mixing of reagents.

Low or No Color Development

- Inadequate volume of substrate added to wells.
- Incorrect incubation times or temperatures.
- Impure BSA used for Reagent Diluent preparation.

DuoSet® ELISA
DEVELOPMENT SYSTEM

Human β-NGF

Catalog Numbers: DY256-05 (5 plates)
DY256 (15 plates)

INTENDED USE

For the development of sandwich ELISAs to measure natural and recombinant human beta Nerve Growth Factor (β-NGF). The Reagent Diluent recommended may be suitable for most cell culture supernate, serum, and plasma samples. The Reagent Diluent selected for use can alter the performance of an immunoassay. Reagent Diluent optimization for samples with complex matrices such as serum and plasma, may improve their performance in this assay.

This kit contains sufficient materials to run ELISAs on at least five 96 well plates, provided the following conditions are met:

- The reagents are prepared as described in this package insert.
- The assay is run as described in the General ELISA Protocol.
- The recommended microplates, buffers, diluents, substrates, and solutions are used.

This package insert must be read in its entirety before using this product.
Refer to the Certificate of Analysis for component concentrations as they may vary.
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TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

OTHER MATERIALS & SOLUTIONS REQUIRED

DuoSet Ancillary Reagent Kit 2 (5 plates):
(R&D Systems, Catalog # DY008) containing 96 well microplates, plate sealers, substrate solution, stop solution, plate coating buffer (PBS), wash buffer, and Reagent Diluent Concentrate 2.

The components listed above may be purchased separately:

96 well microplates: (R&D Systems, Catalog # DY990).

Plate Sealers: (R&D Systems, Catalog # DY992).

PBS: 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.2-7.4, 0.2 µm filtered (R&D Systems, Catalog # DY006).

Wash Buffer: 0.05% Tween® 20 in PBS, pH 7.2-7.4 (R&D Systems, Catalog # WA126).

Reagent Diluent: 1% BSA in PBS, pH 7.2-7.4, 0.2 µm filtered (R&D Systems, Catalog # DY995).
Quality of BSA is critical (see Technical Hints).

Substrate Solution: 1:1 mixture of Color Reagent A (H_2O_2) and Color Reagent B (Tetramethylbenzidine) (R&D Systems, Catalog # DY999).

Stop Solution: 2 N H_2SO_4 (R&D Systems, Catalog # DY994).

PRECAUTIONS

Some components in this kit contain ProClin® which may cause an allergic skin reaction. Avoid breathing mist.

The Stop Solution suggested for use with this kit is an acid solution.

The Color Reagent B suggested for use with this kit may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

CALIBRATION

This DuoSet is calibrated against a highly purified NS0-expressed recombinant human β-NGF produced at R&D Systems.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

DESCRIPTION	PART #	CATALOG # DY256-05	CATALOG # DY256	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human β-NGF Capture Antibody	840366	1 vial	3 vials	Refer to the lot-specific Certificate of Analysis (C of A) for storage conditions.
Human β-NGF Detection Antibody	840367	1 vial	3 vials	
Human β-NGF Standard	840368	1 vial	3 vials	
Streptavidin-HRP	893975	1 vial	3 vials	

REAGENT PREPARATION

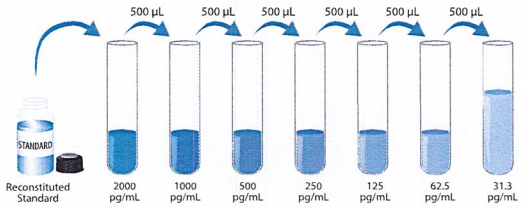
Bring all reagents to room temperature before use. Allow all components to sit for a minimum of 15 minutes with gentle agitation after initial reconstitution. Working dilutions should be prepared and used immediately.

Streptavidin-HRP: Each vial contains 2.0 mL of streptavidin conjugated to horseradish-peroxidase. Dilute to the working concentration specified on the vial label using Reagent Diluent.

Mouse Anti-Human β-NGF Capture Antibody: Refer to the lot-specific C of A for amount supplied. Reconstitute each vial with 0.5 mL of PBS. Dilute in PBS without carrier protein to the working concentration indicated on the C of A.

Biotinylated Goat Anti-Human β-NGF Detection Antibody: Refer to the lot-specific C of A for amount supplied. Reconstitute each vial with 1.0 mL of Reagent Diluent. Dilute in Reagent Diluent to the working concentration indicated on the C of A.

Recombinant Human β-NGF Standard: Refer to the lot-specific C of A for amount supplied. Reconstitute each vial with 0.5 mL of Reagent Diluent. A seven point standard curve using 2-fold serial dilutions in Reagent Diluent is recommended. Prepare 1000 µL of high standard per plate assayed at the concentration indicated on the C of A.



GENERAL ELISA PROTOCOL

Plate Preparation

1. Dilute the Capture Antibody to the working concentration in PBS without carrier protein. Immediately coat a 96-well microplate with 100 µL per well of the diluted Capture Antibody. Seal the plate and incubate overnight at room temperature.
2. Aspirate each well and wash with Wash Buffer, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
3. Block plates by adding 300 µL of Reagent Diluent to each well. Incubate at room temperature for a minimum of 1 hour.
4. Repeat the aspiration/wash as in step 2. The plates are now ready for sample addition.

Assay Procedure

1. Add 100 µL of sample or standards in Reagent Diluent, or an appropriate diluent, per well. Cover with an adhesive strip and incubate 2 hours at room temperature.
2. Repeat the aspiration/wash as in step 2 of Plate Preparation.
3. Add 100 µL of the Detection Antibody, diluted in Reagent Diluent, to each well. Cover with a new adhesive strip and incubate 2 hours at room temperature.
4. Repeat the aspiration/wash as in step 2 of Plate Preparation.
5. Add 100 µL of the working dilution of Streptavidin-HRP to each well. Cover the plate and incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
6. Repeat the aspiration/wash as in step 2.
7. Add 100 µL of Substrate Solution to each well. Incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
8. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well immediately, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

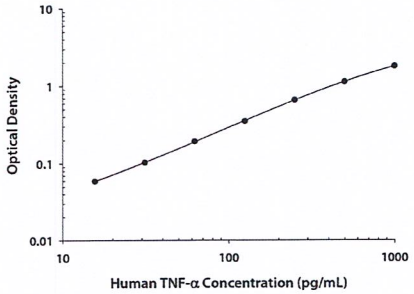
CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human TNF-α concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is only for demonstration purposes. A standard curve should be generated for each set of samples assayed.



SPECIFICITY

The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference.

Recombinant human:
TNF-β
TNF RI
TNF RII

Recombinant mouse:
TNF-α
TNF RI
TNF RII

Other recombinants:
porcine TNF-α
rat TNF-α

TECHNICAL HINTS & LIMITATIONS

- We recommend the use of R&D Systems' Reagent Diluent Concentrate 2 (Catalog # DY995) to prepare Reagent Diluent for use in this assay.
- The use of high quality Bovine Serum Albumin (BSA) for the Reagent Diluent is crucial for the optimum performance of the DuoSet ELISA Development kit. Impurities such as proteases, binding proteins, soluble receptors or other interfering substances can be found to varying degrees in virtually all BSA preparations and can inhibit or interfere with the detection of certain analytes. If the standard curve appears suppressed, consider evaluating a different preparation of BSA.
- It is suggested to start Reagent Diluent optimization for serum and plasma samples by using PBS supplemented with 10-50% animal serum. Do not use buffers with animal serum to reconstitute or dilute the Detection Antibody or Streptavidin-HRP.
- It is important that the Reagent Diluent selected for reconstitution and dilution of the standard reflects the environment of the samples being measured.
- Avoid microbial contamination of reagents and buffers.
- A thorough and consistent wash technique is essential for proper assay performance. Wash Buffer should be dispensed forcefully and removed completely from the wells by aspiration or decanting. Remove any remaining Wash Buffer by inverting the plate and blotting it against clean paper towels.
- Individual results may vary due to differences in technique, plasticware and water sources.
- It is recommended that all standards and samples be assayed in duplicate.
- The use of PBS from tablets may interfere in this assay.

TROUBLESHOOTING

Note: *For more detailed troubleshooting, please visit:*
www.RnDSystems.com/ELISADevelopment

Poor Standard Curve
- Impure BSA used for Reagent Diluent preparation.
- Improper reconstitution and/or storage of standard.
- Improper dilution of highest standard and standard curve.
- Incomplete washing and/or aspiration of wells.
- Unequal volumes added to wells/pipetting error.
- Incorrect incubation times or temperatures.

Poor Precision
- Unequal volumes added to wells/pipetting error.
- Incomplete washing and/or aspiration of wells.
- Unequal mixing of reagents.

Low or No Color Development
- Inadequate volume of substrate added to wells.
- Incorrect incubation times or temperatures.
- Impure BSA used for Reagent Diluent preparation.

DuoSet® ELISA DEVELOPMENT SYSTEM

Human TNF-α

Catalog Numbers: DY210-05 (5 plates)
DY210 (15 plates)

INTENDED USE

For the development of sandwich ELISAs to measure natural and recombinant human Tumor Necrosis Factor alpha (TNF-α). The Reagent Diluent recommended may be suitable for most cell culture supernate, serum, and plasma samples. The Reagent Diluent selected for use can alter the performance of an immunoassay. Reagent Diluent optimization for samples with complex matrices such as serum and plasma, may improve their performance in this assay.

This kit contains sufficient materials to run ELISAs on at least five 96 well plates, provided the following conditions are met:

- The reagents are prepared as described in this package insert.
- The assay is run as described in the General ELISA Protocol.
- The recommended microplates, buffers, diluents, substrates, and solutions are used.

This package insert must be read in its entirety before using this product.
Refer to the Certificate of Analysis for component concentrations as they may vary.
For research use only. Not for use in diagnostic procedures.

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OTHER MATERIALS & SOLUTIONS REQUIRED

DuoSet Ancillary Reagent Kit 2 (5 plates):
(R&D Systems, Catalog # DY008) containing 96 well microplates, plate sealers, substrate solution, stop solution, plate coating buffer (PBS), wash buffer, and Reagent Diluent Concentrate 2.

The components listed above may be purchased separately:

96 well microplates: (R&D Systems, Catalog # DY990).

Plate Sealers: (R&D Systems, Catalog # DY992).

PBS: 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.2-7.4, 0.2 µm filtered (R&D Systems, Catalog # DY006).

Wash Buffer: 0.05% Tween® 20 in PBS, pH 7.2-7.4 (R&D Systems, Catalog # WA126).

Reagent Diluent: 1% BSA in PBS, pH 7.2-7.4, 0.2 µm filtered (R&D Systems, Catalog # DY995).
Quality of BSA is critical (see Technical Hints).

Substrate Solution: 1:1 mixture of Color Reagent A (H_2O_2) and Color Reagent B (Tetramethylbenzidine) (R&D Systems, Catalog # DY999).

Stop Solution: 2 N H_2SO_4 (R&D Systems, Catalog # DY994).

PRECAUTIONS

Some components in this kit contain ProClin® which may cause an allergic skin reaction. Avoid breathing mist.

The Stop Solution suggested for use with this kit is an acid solution.

The Color Reagent B suggested for use with this kit may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

CALIBRATION

This DuoSet is calibrated against a highly purified *E. coli*-expressed recombinant human TNF-α produced at R&D Systems.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

DESCRIPTION	PART #	CATALOG # DY210-05	CATALOG # DY210	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human TNF-α Capture Antibody	840119	1 vial	3 vials	Refer to the lot-specific Certificate of Analysis (C of A) for storage conditions.
Human TNF-α Detection Antibody	840120	1 vial	3 vials	
Human TNF-α Standard	840121	1 vial	3 vials	
Streptavidin-HRP	893975	1 vial	3 vials	

REAGENT PREPARATION

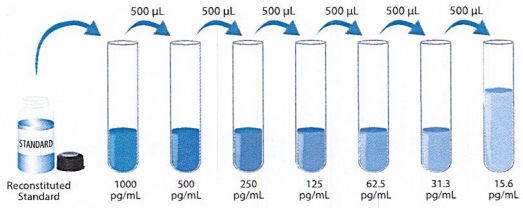
Bring all reagents to room temperature before use. Allow all components to sit for a minimum of 15 minutes with gentle agitation after initial reconstitution. Working dilutions should be prepared and used immediately.

Streptavidin-HRP: Each vial contains 2.0 mL of streptavidin conjugated to horseradish-peroxidase. Dilute to the working concentration specified on the vial label using Reagent Diluent.

Mouse Anti-Human TNF-α Capture Antibody: Refer to the lot-specific C of A for amount supplied. Reconstitute each vial with 0.5 mL of PBS. Dilute in PBS without carrier protein to the working concentration indicated on the C of A.

Biotinylated Goat Anti-Human TNF-α Detection Antibody: Refer to the lot-specific C of A for amount supplied. Reconstitute each vial with 1.0 mL of Reagent Diluent. Dilute in Reagent Diluent to the working concentration indicated on the C of A.

Recombinant Human TNF-α Standard: Refer to the lot-specific C of A for amount supplied. Reconstitute each vial with 0.5 mL of Reagent Diluent. A seven point standard curve using 2-fold serial dilutions in Reagent Diluent is recommended. Prepare 1000 µL of high standard per plate assayed at the concentration indicated on the C of A.



GENERAL ELISA PROTOCOL

Plate Preparation

1. Dilute the Capture Antibody to the working concentration in PBS without carrier protein. Immediately coat a 96-well microplate with 100 µL per well of the diluted Capture Antibody. Seal the plate and incubate overnight at room temperature.
2. Aspirate each well and wash with Wash Buffer, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
3. Block plates by adding 300 µL of Reagent Diluent to each well. Incubate at room temperature for a minimum of 1 hour.
4. Repeat the aspiration/wash as in step 2. The plates are now ready for sample addition.

Assay Procedure

1. Add 100 µL of sample or standards in Reagent Diluent, or an appropriate diluent, per well. Cover with an adhesive strip and incubate 2 hours at room temperature.
2. Repeat the aspiration/wash as in step 2 of Plate Preparation.
3. Add 100 µL of the Detection Antibody, diluted in Reagent Diluent, to each well. Cover with a new adhesive strip and incubate 2 hours at room temperature.
4. Repeat the aspiration/wash as in step 2 of Plate Preparation.
5. Add 100 µL of the working dilution of Streptavidin-HRP to each well. Cover the plate and incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
6. Repeat the aspiration/wash as in step 2.
7. Add 100 µL of Substrate Solution to each well. Incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
8. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well immediately, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

Appendix 4

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