Characterisation of the neurobiological phenotype of pain in psoriatic arthritis

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Dedication

I dedicate this thesis to Ester, my grandmother.

Thanks for your stubborn generosity, insatiable curiosity,
honesty, and contagious laugh.

Abstract

Psoriatic arthritis (PsA) is a prevalent immune-mediated inflammatory arthritis marked by chronic inflammation in both articular and periarticular regions. Advances in understanding the immunopathogenesis have paved the way for the development of advanced immunotherapies, effectively controlling inflammation and the associated tissue damage linked with PsA. Nevertheless, the chronic pain remains a significant issue for individuals with PsA. Chronic pain, frequently linked with musculoskeletal conditions, represents a substantial burden on those affected, leading to diminished quality of life and increased mortality.

The classical pain mechanisms involve damage to peripheral tissues (nociceptive) or peripheral nerves (neuropathic), causing pain. In PsA, nociceptive pain mechanisms are classically considered to prevail, primarily due to peripheral inflammation. Recently, however, a novel pain mechanism, described as nociplastic pain, characterised by dysfunctional nociception processes within the central nervous system (CNS) has been identified. This type of pain lacks evidence of peripheral damage. Fibromyalgia serves as a prototype for nociplastic pain. Specific neurobiological features are identified in fibromyalgia through functional neuroimaging and quantitative sensory testing (QST). Clinically, fibromyalgia (nociplastic pain) appears to co-exist in PsA, however there is no objective evidence to support this observation yet.

This thesis's primary hypothesis is that chronic pain in PsA manifests as a mixed pain state in individuals with a substantial pain burden, potentially explaining the high rates of chronic pain in PsA. To test this hypothesis, this study examines nociplastic pain features and their neurobiological correlations within a well-characterised cohort of 50 individuals with PsA with active disease and employing QST and functional MRI to objectively assess nociplastic pain.

The study's evidenced a heightened pressure pain sensitivity at articular and entheseal sites among participants experiencing pronounced nociplastic pain, indicating peripheral sensitisation where inflammation prevails. Observations also unveil altered functional connectivity in subjects with PsA with substantial nociplastic pain, particularly within the insula and DMN regions. Intriguingly,

distinct features in the parahippocampal and visual areas predominate within this subgroup, reflecting the complexities of pain perception. This individual and condition-specific diversity defines a distinctive “pain signature”. These findings present an opportunity to pinpoint specific neurobiological markers in PsA.

Despite available evidence suggesting the role of inflammation, the mechanisms sustaining the interaction between the nervous and immune systems remain elusive. Chronic inflammation in rheumatoid arthritis relates to altered connectivity in the inferior parietal lobule (IPL), a similar phenomenon is identified within this study participants with PsA. However, peripheral circulating pro-inflammatory cytokines did not exhibit significant associations with the nociplastic pain neurobiological features investigated in this study.

To date, this study represents the first exploration into the neurobiological features of nociplastic pain in PsA, employing advanced neuroimaging techniques alongside an extensive QST protocol. The findings suggest a distinct pain signature of PsA, sharing characteristics with fibromyalgia and rheumatoid arthritis. To confirm these findings and gain further insights into the role of inflammation in nervous system sensitisation, additional studies are needed. Ultimately, a better understanding of pain mechanisms in PsA will translate into improved patient management and a better quality of life for those affected by this challenging disease.

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Author's Declaration

I declare that the work described herein this thesis is my own work and does not consist of work submitted as part of a thesis submitted or pending submission within The University of Glasgow, or elsewhere. Appropriate acknowledgements have been made where any necessary support has been provided by another individual.

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Flavia Sunzini

Definitions/Abbreviations

5-HT - 5-Hydroxytryptamine or Serotonin

ACC - Anterior Cingulate Cortex

ACR - American College of Rheumatology

antIC - Anterior Insula Cortex

APC - Antigen Presenting Cells

AS – Ankylosing Spondylitis

ASAS - Assessment of Spondyloarthritis International Society

BASDAI - Bath Ankylosing Spondylitis Disease Activity Index

BMI - Body Mass Index

BOLD - Blood Oxygenation Level Dependent

BSA - Body Surface Area

CASPAR - Classification Criteria for Psoriatic Arthritis

CBD - Cannabidiol

CBT - Cognitive Behavioural Therapy

CDAI - Clinical Disease Activity Index

CNS - Central Nervous System

CPM - Conditioned Pain Modulation

CRF - Clinical Research Form

CRP - C-Reactive Protein

CSF - Cerebrospinal Fluid

CSI - Central Sensitisation Inventory

DAN - Dorsal Attention Network

DAPSA - Disease Activity in Psoriatic Arthritis

DAS - Disease Activity Score

deoxHb - Deoxygenated Haemoglobin

DIP - Distal Interphalangeal Joint

dlPFC - Dorsolateral Prefrontal Cortex

DMARDs - Disease-Modifying Anti-Rheumatic Drugs

DMN - Default Mode Network

DRG - Dorsal Root Ganglia

ESR - Erythrocyte Sedimentation Rate

EULAR - European Alliance For Associations For Rheumatology

FLS - Fibroblast-Like Synoviocytes

FM - Fibromyalgia

FMness - Fibromyalgianess

fMRI - Functional Magnetic Resonance Imaging

GABA - γ-Aminobutyric Acid

GRAPPA - Group for Research And Assessment of Psoriasis And Psoriatic Arthritis

GIFT - Group ICA of Fmri Toolbox

GWAS - Genome-Wide Association Studies

HDA - High Disease Activity

HIV - Human Immunodeficiency Virus

HLA - The Human Leukocyte Antigen

HRF - Hemodynamic Response Function

IASP - International Association for The Study Of Pain

IBD - Inflammatory Bowel Disease

IC - Insula Cortex

ICA - Independent Component Analysis

ICD-11 - International Classification of Diseases 11th Revision

ICN - Intrinsic Connectivity Networks

IL - Interleukin

ILC - Innate Lymphoid Cells

IQR - Interquartile Range

IPL - Inferior Parietal Lobule

JAKs - Janus Kinases

LDA - Low Disease Activity

LEI - Leeds Enthesitis Index

LTi - Lymphoid Tissue-Inducer

MAIT - Mucosal-Associated Invariant T

MASES - Maastricht Ankylosing Spondylitis Enthesitis Score

MDA - Minimal Disease Activity

MDA - Moderate Disease Activity

MHC - Major Histocompatibility Complex

midIC – Middle Insula Cortex

MNI - Montreal Neurological Institute

MOR - Opioid Receptor

mPFC - Medial Prefrontal Cortex

MRI - Magnetic Resonance Imaging

MTX - Methotrexate

MVN - Medial Visual Network

NA - Noradrenaline

NGF - Neurotrophic Growth Factor

NICE - National Institute for Health And Care Excellence

NRS - Numeric Rating Scales

NSAIDs - Non-Steroidal Anti-Inflammatory Drugs

OA - Osteoarthritis

OMERACT - Outcome Measures in Rheumatoid Arthritis Clinical Trials

oxHb - Oxygenated Haemoglobin

PASAT - Paced Auditory Serial Additional Test

PASI - Psoriasis Area and Severity Index

PHC - Parahippocampal Gyrus

PBMCs - Peripheral Blood Mononuclear Cells

PCC - Posterior Cingulate Cortex

PDE4 - Phosphodiesterase-4

PDQ – Pain Detect Questionnaire

PFC - Prefrontal Cortex

PGA - Patient Global Assessment

PhGA - Physician Global Assessment

pIC - Posterior Insula Cortex

PPT - Pressure Pain Threshold

PPTol - Pressure Pain Tolerance

PROMIS - Patient-Reported Outcomes Measurement Information System

PROs - Patient-Reported Outcomes

PsA - Psoriatic Arthritis

PsARC - Psoriatic Arthritis Response Criteria

PsD - Psoriatic Disease

PsO - Psoriasis

QST - Quantitative Sensory Testing

RA - Rheumatoid Arthritis

REM - Remission

RF - Rheumatoid Factor

RFP -Radio Frequency Pulse

ROI - Region of Interest

rs-fMRI - Resting State Functional Magnetic Resonance Imaging

SCDH - Spinal Cord Dorsal Horn

SD - Standard Deviation

SI - Primary Somatosensory Cortex

SII - Secondary Somatosensory Cortex

SJC - Swollen Joints Count

SLN - Salience Network

SMN - Sensory Motor Network

SNPs - Single-Nucleotide Polymorphisms

SNRIs - Serotonin–Norepinephrine Reuptake Inhibitors

SpA- Spondyloarthritis

SPARCC - Spondyloarthritis Research Consortium of Canada

SSS - Symptoms Severity Score

SSZ - Sulfasalazine

T - Tesla

T1 - Time Constant 1

T2 - Time Constant 2

T2* - Time Constant 2 Star

Tc17 - Cytotoxic CD8+ T Cells Expressing IL-17

TCR - T-Cell Receptor

Th - T Helper Cells

THC - Tetrahydrocannabinol

TJC - Tender Joints Count

TNF - Tumor Necrosis Factor-α

TOL - Tolerance

TS - Temporal Summation

VAS - Visual Analogue Scales

WPI - Widespread Pain Index

WUR - Wind-Up Ratio

α7nAChR - Cholinergic Receptor Nicotinic A7 Acetylcholine Receptor

Chapter 1 Introduction

1.1 Psoriatic Arthritis

1.1.1 Definition, epidemiology and classification

Psoriatic arthritis (PsA) is a chronic inflammatory immune-mediated disease that affects articular and peri-articular structures, and it is typically associated with a personal or family history of psoriasis (PsO). It is a progressive condition characterised by joint inflammation, chronic pain, and articular bony changes, including erosions and bone formation. It carries a significant burden on physical functioning and quality of life for the individuals affected, as well as high socio-economic costs. PsA is included in the larger group of inflammatory arthritis, known as Spondyloarthritis (SpA), that includes: ankylosing spondylitis (AS), axial SpA, reactive arthritis, PsA, arthritis/spondylitis associated with inflammatory bowel disease (IBD), and juvenile SpA; SpA share common clinical features, pathogenesis, and radiographic changes[1]. The clinical phenotypes are highly heterogeneous varying from peripheral arthritis to axial involvement, and variable association with extra-articular manifestations, such as enthesitis, uveitis, IBD, PsO and onychopathy; SpA are characterised by axial inflammation, association with the human leukocyte antigen (HLA)-B27 and negativity for the rheumatoid factor (RF)[2].

Epidemiologic studies of PsA have been challenging because of the clinical heterogeneity (covered in more detail in Section 1.1.3) and the variable definitions of PsA due to the lack of diagnostic criteria, before the widely accepted CASPAR classification criteria (ClASsification criteria for Psoriatic ARthritis) developed in 2006[3,4]. The disease onset is commonly between the third and fifth decades and affects men and women equally. Estimates for PsA prevalence range between 0.1 and 1% in the general population with a notable variability observed between different countries and continents, with a significantly higher prevalence in Western compared to Middle East countries[5,6]. The geographical differences may be associated to genetic and/or lifestyle factors, in addition to the application of non-standardised diagnostic criteria

across different countries[3]. The prevalence of PsA increases to more than 20% in people with a diagnosis of PsO[7]. It is estimated that up to 10-30% of patients with PsO will develop PsA within 7-12 years from the diagnosis[8]. The skin disease more commonly (60-80%) precedes the joint involvement[9], however, the onset of PsA can occur concomitantly with PsO (15%), but rarely precedes it[5,10-12]. Articular and skin involvement in PsA are strictly intertwined, sharing strong similarities in genetic and environmental risk factors, pathogenesis, as well as effective treatments, hence they are often considered as a continuum of a single disease entity, termed psoriatic disease (PsD)[13].

The first classification of PsA clinical manifestations was described by Moll and Wright in 1973. This identified five main patterns of joint involvement[14]:

- predominant distal interphalangeal joint (DIP) involvement;
- arthritis mutilans, characterised by osteolysis and deformities;
- symmetrical arthritis, similar to rheumatoid arthritis (RA), the second most frequent presentation;
- asymmetrical oligo- (or mono-) arthritis, in up to 70% of patients;
- predominant axial involvement with or without peripheral arthritis (5%).

These definitions are not rigid, and in fact, the initial clinical pattern often evolves in a different one[15], with the accumulation of joint involvement over time commonly leading to symmetrical polyarthritis[16].

In 2006, the CASPAR classification criteria were developed, and shown to perform well with high sensitivity and specificity, 98.7% e 91.4%, respectively (table 1.1.1).

Table 1.1.1 CASPAR criteria for PsA

To meet the CASPAR criteria, a patient must have the indispensable criterion with at least 3 points from the following categories:

Indispensable criterion: inflammatory articular disease: peripheral joints, spine, or entheseal involvement	
1. Evidence of current psoriasis (a, b, and/or c)	a) Current psoriasis: skin or scalp disease present today as judged by a rheumatologist or dermatologist*, b) A personal history of PsO: obtained from a patient or a qualified health care provider, c) A family history of PsO: a first- or second-degree relative according to patient report.
2. Typical psoriatic nail dystrophy	onycholysis, pitting, and hyperkeratosis observed on current physical examination.
3. Absence of rheumatoid factor	By any method except latex, preferably by enzyme-linked immunosorbent assay or nephelometry, according to the local laboratory reference range.
4. dactylitis (a or b)	d) current dactylitis, defined as swelling of an entire digit, or e) a history of dactylitis recorded by a rheumatologist.
5. Radiographic changes	Radiographic evidence of juxta-articular new bone formation, appearing as ill-defined ossification near joint margins (but excluding osteophyte formation) on plain radiographs of the hand or foot.

*current PsO is assigned a score of 2; all other features are assigned a score of 1[17].

1.1.2 Pathogenesis

The heterogeneity of the clinical presentations in PsA reflects the complexity of the underlying immune-mediated pathogenesis, which remains not completely understood to date. The interleukin (IL)-23/IL-17 pathway is considered the main driver of the disease, acting in synergy with other pro-inflammatory cytokines, including tumor necrosis factor-α (TNF), IL-6, and IL-22[18,19]. It is currently accepted that in genetically predisposed individuals, different environmental triggers induce auto-inflammatory and auto-immune responses leading to clinical manifestations[20]. The entheseal inflammation associated with mechanical stress is distinctive of PsA and can precede articular involvement by many years[21]. The joint inflammation is characterised by increased vascularisation and infiltration of immune cells in the synovium and activation of resident cells, i.e. fibroblast-like synoviocytes (FLS)[22]. The disruption of the cartilage allows the invasion of the subchondral bone by FLS, immune cells, and pro-inflammatory cytokines.

Over time, inflammation leads to bone erosions, deformities, and loss of function[23].

1.1.2.1 *Genetic and epigenetic factor*

An important role of genetic predisposition in PsA pathogenesis is suggested by the strong heritability characteristic and the concordance between twins[24-29]. Several genes within the HLA and non-HLA groups have been associated with both PsO and PsA. HLA genes constitute the major histocompatibility complex (MHC), which is essential in the development and function of the immune system. Single-nucleotide polymorphisms (SNPs) in HLA class I genes (HLA-A, -B and -C), which present intracellular antigens to CD8+ lymphocytes, have been associated with PsO and PsA. The HLA-Cw6 locus is associated with skin involvement and later onset of articular symptoms following the onset of PsO[30]. HLA-C06 has also been linked with the skin involvement, more strongly associated with PsO compared to PsA. Other HLA genes are more specifically associated with PsA and specific clinical phenotypes, for example HLA-B27, -B38, -B39, and -B08 [31-36]. In particular, HLA-B27 is associated with axial and entheseal involvement; while HLA-B08 seems to predispose to more aggressive disease with dactylitis and asymmetrical sacroiliitis[33,37]. Individuals with HLA-DR17[38] appear particularly predisposed to developing enthesitis. One proposed hypothesis is of a common antigen shared by enthesis, synovium and skin able to activate the adaptive immune response[39], however, further investigations are needed to corroborate this hypothesis. The genetic susceptibility associated with non-HLA genes has been investigated in genome-wide association studies (GWAS) that identified PsA-specific genetic risk factors, distinct from PsO[40]. Of particular interest are the genes involved in immune and barrier function, including TNF, IL-23/IL-17 and NF-κB pathways[41]. Gene locus variant and SNPs of IL-23 and its receptor, IL23R, are associated with IL-17-dependent articular and periarticular inflammation in PsD[42-44]. Other genes involved in the differentiation and function of T lymphocytes were found to be associated with PsA[45]. Variants of the transcriptional factors NF-κB and TNFAIP3 may alter TNF signalling, a key cytokine in systemic and peripheral joint inflammation[46]. Additionally, a link between IL-13 and PsA was found, especially in non-smokers[47-49]. Overall, IL-23R,

IL-23A (p19), IL-12B (p40), TYK2 (a Janus Kinase protein involved in intracellular signalling), TRAF3IP2 have been consistently associated with PsA onset[50,51].

Higher prevalence and earlier onset of PsA in the offspring of fathers with PsD suggests the involvement of epigenetic imprinting in the transmission of the disease[52–54]. Different studies investigating DNA methylation and histone modifications in peripheral blood mononuclear cells (PBMCs), sperm cells, and other cells lines from PsD individuals confirm that specific epigenetic changes have an impact on disease heritability, onset, and progression[36]. However, further studies are ongoing to understand these epigenetic factors and their interactions with environmental factors in PsA.

1.1.2.2 E*nvironmental risk factors and their impact on PsA development*

Environmental factors can trigger unbalanced or inappropriate immune responses leading to PsA onset[55]. The environmental factors that have been reported related to PsA pathogenesis include mechanical trauma, infections, obesity, smoking, psychosocial factors, and gut dysbiosis[56,57].

In PsO, the Koebner phenomenon is characterised by the onset or worsening of skin lesions after mechanical trauma of the skin. Similarly, PsA onset can follow traumatic lesions or biomechanical stress at joints or tendons; this has been hypothesized to represent a "deep Koebner phenomenon"[58–61]. Different studies have related the onset of PsA to a precedent physical trauma[62,63]. Entheseal biomechanical stress can initiate local auto-inflammatory processes leading to chronic inflammation and excessive tissue repair mechanisms; both can extend to involve the adjacent tissues, i.e. bone, synovia and nails[56,64–67].

Infections might also trigger PsA onset. For example, anti-streptococcal antibodies have been found in serum and synovial fluid of PsA subjects, suggesting a potential association with streptococcal infection[68–70]. Moreover, the incidence of PsD is increased in regions where human immunodeficiency virus (HIV) is endemic[71]; HIV can exacerbate or induce PsA and PsO, especially when associated with a depletion of $CD4^+$ T cells and a predominance of $CD8^+$ T cells[72,73].

Several evidence support an association between obesity and PsD. Obesity increases the risk to develop PsO in the general population and to develop PsA in individuals with PsO[74–77]. Mendelian randomisation indicates that obesity SNPs predispose to developing PsO, while PsO SNPs cause slight increase in risk obesity; these data suggest a bidirectional relationship[78]. Similarly, obesity and PsA appear to have a bidirectional relationship. Being overweight is a putative risk factor for developing PsA in predisposed individual for two main hypotheses: 1) obesity increase the mechanical stress at the level of periarticular and articular structures, i.e., enthesis; 2) the adipose tissue secretes pro-inflammatory cytokines, e.g. TNF, IL-6 and IL-17, which can trigger the disease in susceptible individuals. On the other hand, reduced mobility due to pain and reduced physical function can induce weight gain and perpetuating the vicious cycle between the two conditions. Furthermore, obesity is associated with reduced response to treatment[79], while weight loss with improved response to treatment[80].

Smoking increases the risk of developing PsA in healthy subjects, but a protective role is suggested in individuals with PsO (the smoking paradox); these phenomena have been correlated with IL-13 polymorphisms in a limited number of patients[81].

Psychological stress has also been suggested to be associated with the onset of PsA[82–84]. Interestingly, depression, anxiety, and suicidal behaviours are themselves more common in people with PsD. Depression seems to be a risk factor to develop PsA[85]. Recently, pro-inflammatory cytokines key in the pathogenesis of PsA have been shown to have a role in the development of depression; in particular, IL-17 is associated with psychological outcomes and PsA onset, disease activity, pain, and response to treatment[86–88].

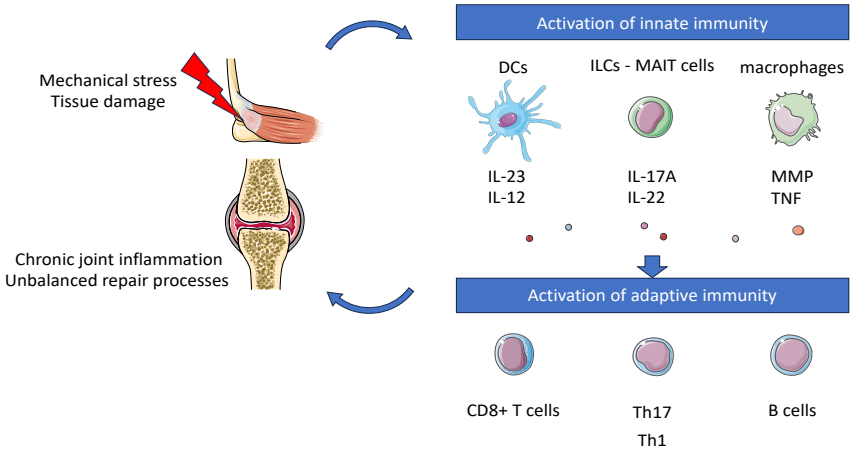
The interest in the role of gut microbiota in immune-mediated disease is growing, but remains poorly understood. In PsA, it has been demonstrated that subclinical intestinal inflammation is frequent, which has been linked to alteration of gut microbiota and disease activity[89,90]. Some species of faecal bacteria differ significantly in comparison with healthy donors[91,92]. Alteration in the gut microbiota can alter the gut-epithelial barrier, initiating local innate

immune responses and the production of several pro-inflammatory cytokines relevant in the PsA pathogenesis, in particular IL-23. Most studies focus on SpA and IBD, thus, further studies are needed to confirm the pathogenetic role of gut dysbiosis in PsD, while alterations in the skin microbiome may also be implicated.

1.1.2.3 *Immunopathogenesis*

PsA has an immune-mediated pathogenesis involving complex and intertwined autoinflammatory and autoimmunity mechanisms[20]. The *primum movens* in the disease onset has postulated to be the local tissue damage, associated with biomechanical stress alongside other risk factors, which can induce regional innate immune responses[93]. The local immune response has the aim of reacting to the damage quickly and inducing tissue repair. The activation of the innate immune cells leads to the expression of several pro-inflammatory cytokines, including TNF and IL-23. Due to the combination of genetic and environmental risk factors, the local inflammation triggers adaptive immune responses with local and systemic release of pro-inflammatory cytokines leading to chronic inflammation[94,95]. Persistent and unbalanced inflammation leads to tissue damage and tissue remodelling responsible for the disease clinical manifestation and pain (figure 1.1.1).

Figure 1.1.1 Summary of psoriatic arthritis immunopathogenesis



DCs -dendritic cells; ILCs – innate lymphoid cells; MAIT – mucosal-associated invariant; T MMP- matrix metalloproteinases; Th – T helper cells; TNF- tumor necrosis factor-α.

Innate immunity

Resident innate cells at the level articular and periarticular structures, including dendritic cells, monocytes/macrophages, and innate lymphoid cells (ILC), respond to local tissue damage by initiating a pro-inflammatory cascade with the aim to respond to the insult and induce tissue repair.

Dendritic cells are specialised antigen presenting cells (APC) and express key pro-inflammatory cytokine in the PsA pathogenesis[96], including IL-23 and IL-12. These pro-inflammatory mediators are in turn able to induce the differentiation of T helper (Th) 17 and Th1 cells, key cell populations in psoriatic inflammation[97]. In PsA synovial fluid, high levels of immature myeloid dendritic cells have been found, contributing to the pro-inflammatory environment[95]. Some subsets of dendritic cells are able to closely interact with $CD8^+$ T cells and are reduced after anti-TNF therapy, indicating a putative role in the pathogenesis of PsA[98].

Monocytes and macrophages also contribute to PsA inflammation, as well as other forms of inflammatory arthritis, by expressing pro-inflammatory cytokines and chemokines that induce leucocyte recruitment and activation, angiogenesis, the production of metalloproteinases, and by acting as APCs to directly activate T and B cells. Resident and monocyte-derived macrophages are plastic cells able to differentiate in two main subset M1, with a pro-inflammatory profile, and M2 which have an anti-inflammatory profile able to induce tissue repair[99–101]. A recent multiomics analysis has indicated that macrophages are highly represented in PsA synovial fluid and that they contribute to sustain the articular pro-inflammatory response[102]. Macrophages are an important source of TNF, key pathogenic cytokine for articular inflammation and damage[103]. However, the presence of synovial pro-inflammatory macrophages has been demonstrated to be lower in SpA and PsA compared to RA[103–105] and they tend to persist at a higher percentage after anti-TNF treatment in PsA[106]. This suggests a pathogenetic contribution to PsA disease independently of TNF pathway. Additionally, monocytes and macrophages can differentiate into osteoclasts responsible for bone erosion[101,107–109].

ILCs are effector cells of the innate immunity resident in the tissue where they serve as “sentinels” responding promptly to inflammatory, and damage, signals[110]. ILCs have been classified into five subsets based on their transcription factors and cytokine expression: NK cells,ILC1s, ILC2s, ILC3s and lymphoid tissue-inducer (LTi) cells[111]. ILCs contribute to the chronic inflammation by releasing several pro-inflammatory cytokines and amplifying adaptive immune response[112]. The main cytokine effectors of ILC3s and LTis are IL-17A and IL-22, highly relevant in the pathogenesis of PsD, as well as SpA in general and IBD. These cells are present in the enthesis, skin and gut, and appear to be able to migrate into the blood stream, synovial tissue and bone where they produce IL-17A in response to IL-23[44,113,114]. IL-17A producing ILC3s are found in PsA synovial fluid at higher frequencies than in RA, and the expression of the chemokine receptor CCR6 seems to facilitate their migration into the joints[115]. In the blood stream, ILC3s have been found to be increased in PsA donors, when compared to healthy, in contrast to reduced ILC2s (which produce IL-4, IL-5, IL-9, and IL-13); the ratio of LC3s:ILC2s positively correlated with disease activity and bone erosions[116]. Another type of cell at the crossover between innate and adaptive immunity are the mucosal-associated invariant T (MAIT) cells characterised by expression of CD8, CD161^{hi}, CCR6 and RORγt, and a peculiar T-cell receptor (TCR) restricted to TCR Vα7.2. MAIT cells mature in the gut and circulate in the peripheral blood and are able to express IL-17A and TNF, key pro-inflammatory cytokines in the PsD pathogenesis[117–119]. MAIT cells have been found to be increased in PsA synovium compared to RA and non-inflammatory arthritis, such as osteoarthritis (OA), and to correlate with disease activity in PsA[120]. Moreover, MAIT cells express IL-23R, hence are responsive to IL-23, and contribute to the production of IL-17A in PsA[121].

IL-17-dependent recruitment of neutrophils in the skin (Munro’s micro-abscesses) and nail disease is characteristic of PsO and can predispose to development of PsA[122]. Moreover, in PsA synovium, neutrophil NETosis was demonstrated and positively correlated with disease activity[123]. Treatment of PsA with IL-17 inhibitors reduces neutrophil infiltration in the PsA synovium[124]. However, the exact role of neutrophils in PsD pathogenesis remains elusive. Neutrophils are also present in the enthesis in SpA animal models which have suggested they can

mediate entheseal inflammation[125] and have been recently identified a source of IL-23[126].

Mast cells can promote synovial angiogenesis and immune cell recruitment and activation by the release of tryptase and IL-17A[127]; for this reason, a possible role of mast cells in the pathogenesis of PsA and PsO has been proposed, however more studies are needed to confirm the presence and impact of these cells in articular disease in PsA[128].

Adaptive immunity

Infiltration of lymphocytes into the synovium and its association with disease activity and severity are among the data that suggest the involvement of adaptive immune responses in the PsA pathogenesis[129,130]. The synovial fluid of inflamed PsA joint is rich in T-cell derived cytokines, including TNF, IL-17A, IFN-γ, and granulocyte-macrophage colony-stimulating factor[131]. The expression of NF-κB induced by TNF was found increased in skin and synovium of individuals with PsO or PsA[65,132]. IL-17A in the joint has a synergic pro-inflammatory effect with TNF and IL-1β, with activation effects on the surrounding stromal and immune cells, which in turn induce the maturation of osteoclasts and release of matrix metalloproteinases responsible for local joint damage[133–136]. Conventional $CD4^+$ T cells were the first cells discovered to produce IL-17 induced by IL-23; these cells were distinct from the established Th1 and Th2 cells subsets, leading to the identification of Th17 cells, defined by the presence of the transcription factor RORγt. Th17 cells have subsequently been implicated in the pathogenesis of a range of autoimmune diseases[137]. Th17 cells are elevated in the circulation and synovial fluid of PsA donors[120,138]. Moreover, cytotoxic $CD8^+$ T cells expressing IL-17 (Tc17) were found in the PsA synovium and in the skin of individuals diagnosed with PsO[139–141]. The genetic association with MHC-I, the infiltration of $CD8^+$ T cells, and their clonal expansion in the PsA synovium led to the hypothesis that these cells may have a key role in PsA pathogenesis[142]. Both Th17 and Tc17 cells have been positively correlated with disease activity and articular damage in different studies[120,143], underlying the complex pathogenesis involving different immune cells which may lead to overlapping clinical phenotypes[144].

Aggregates of B cells and ectopic lymphoid neogenesis have also been demonstrated in PsA synovium, with their absence associated with disease remission[145]. The role of B cells in the pathogenesis of PsA is still unclear. Interestingly, regulatory B cells secreting the anti-inflammatory cytokine IL-10 are decreased in PsA and seems to have a protective effect by suppressing the activity of pathogenic Th17 cells[146]. Although PsA is classified as a seronegative inflammatory arthritis, the presence of autoantibodies targeting articular citrullinated and carbamylated proteins have been demonstrated in PsA[147–150] and seem to be positively correlated with an aggressive disease[148,151].

1.1.2.4 *Musculoskeletal pathogenesis*

Psoriatic peripheral arthritis is characterised by a proliferative synovitis and angiogenesis, associated with entheseal and periosteal inflammation, and bony erosions[152,153]. Synovial angiogenesis is an early alteration in PsA synovium and is characterised by endothelial swelling, cell infiltration, and tortuous vessels[154–159]. The new vessels are dysfunctional resulting in hypoxic synovial membrane and secondary oxidative damage. The metabolic response to hypoxia in immune and synovial cells may contribute to perpetuation of the inflammatory loop in the PsA synovium. The articular inflammation and pro-inflammatory microenvironment gradually lead to cartilage fibrillation and damage, exposing the subchondral bone to the pro-inflammatory environment rich in cytokines and catalytic enzymes. This process leads to the typical PsA periosteal bone erosions[160–162]. PsA is also characterised by new bone formation as result of the attempt to repair the tissue damaged by the inflammatory insult, with bone morphogenetic proteins, Wnt and the Wnt antagonist Dickkopf-1 implicated in this process[163,164]. Radiographic changes, including erosions and characteristic bone formation at the level of the enthesis, can be found in the 47% of patients within 2 years from the disease onset[165]. FLS and synovium resident macrophages are the main effectors of articular damage[166]. In fact, FLS have a tumour-like behaviour in PsA joint, it is characterised increased proliferation and invasiveness, resistance to apoptosis, and active production of pro-inflammatory cytokines and matrix-degrading enzymes[167]. In addition, FLS can induce osteoclasts differentiation through the expression of the receptor activator of nuclear factor kappa-B ligand, TNF, and IL-17[168,169].

1.1.2.5 *Neuro-immune interactions in joint pathology*

Recently, the interest in the immunomodulatory role of the nervous system in inflammatory arthritis is growing. In synovial tissue from RA and PsA, several cells, predominantly FLS and macrophages, actively express the cholinergic receptor nicotinic α7 acetylcholine receptor (α7nAChR), when compared to healthy[170]. Agonist of α7nAChR reduced the FLS production of pro-inflammatory cytokines[171]. This evidence suggests that the cholinergic signals, mediated by the vagus nerve, might have an anti-inflammatory effect in inflammatory arthritis. Conversely, the neurotrophic growth factor (NGF) has also been demonstrated to be increased in the synovium, circulation and cerebrospinal fluid (CSF) in patient with rheumatic disease and can contribute to local neurogenetic inflammation in PsA mediating the immune response directly[172]. In fact, several immune cells, including T and B cells, express the NGF receptor and, in vitro, NGF can induce their differentiation and activation[173].

1.1.3 Clinical manifestations and clinimetric assessment

The clinical manifestations of PsA can be divided into five domains which may coexist to varying degrees over time in the same individual: peripheral arthritis (joints), axial disease (spine), enthesitis, dactylitis, and skin and nail disease (onychopathy)[174]. The diagnosis is usually based on the medical history and clinical presentation, with no PsA-specific diagnostic laboratory tests currently available. Circulating markers of peripheral inflammation, i.e., erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), are rarely raised and overall poorly related to disease severity. Because of the heterogeneous and multiple clinical manifestation, the diagnosis, assessment of disease activity and the evaluation of treatment response can be challenging. Disease activity scores designed for RA have been commonly used in PsA, however these do not incorporate all the different manifestations of the disease[175]. For this reason, PsA-specific scores and outcome measures have been proposed which include the different domains of the disease, either as composite scores or a set of domain-specific scores[176]. The most common parameters evaluated are the presence and severity of synovitis, psoriasis, dactylitis, enthesitis, nail disease, and bone erosions. In addition, health care professionals make a global

assessment as part of the physician global assessment (PhGA), while patient-reported outcomes (PROs) allow patients to directly report their global assessment (PGA), intensity of pain and fatigue, impacts on physical function, and other health-related quality of life parameters, using simple visual analogue scales (VAS), numeric rating scales (NRS), or validated questionnaires. Of note, while composite measures attempt to capture the multiple potential domains involved in PsA, this often comes at the loss of granularity in individual domains and ultimately no single outcome measure can capture the totality of the disease and its impacts[177].

1.1.3.1 *Peripheral arthritis*

Synovitis is clinically characterised by joint swelling, pain and tenderness, associated with inactivity and morning stiffness that improves with movement. The most frequently involved joints in PsA are the feet and hands, followed by the knees, wrists, ankles, and shoulders. Oligoarticular presentation is more likely to occur in early stages, and represents a good prognostic factor for remission[178], unless small joints of the hands are affected[179]. Polyarticular presentation may be associated with a more rapid disease progression. The joint involvement is usually asymmetric[180], which also tend to be less swollen, and therefore difficult to detect clinically, than in RA[181]. More rarely, DIP joint involvement is the only clinical presentation (5-10%). When the peripheral arthritis is rapidly progressive and destructive, it is called arthritis mutilans. This pattern appears to be rare and distinct from the other phenotypes of the diseases and is characterised by joint fusion and intense osteolysis[182,183]. Clinical assessment of joints is supplemented and supported by radiological examination with x-rays, ultrasound and magnetic resonance imaging (MRI) scans[184]. The radiographic changes in PsA can be located in the joint and the surrounding bone and soft tissues, and compromise bone destructive and osteo-proliferative lesions, such as, erosions, osteolysis, periostitis, calcification and ankyloses.

The clinical measures used to assess disease activity and evaluate treatment efficacy on peripheral arthritis are based on those used for assessment of joints in RA. The disease activity score for 28 or 44 joints (DAS28 or DAS 44) is widely used in clinical practice for RA and also often used to assess the response in PsA.

However, PsA can affect joints not usually involved in RA, most notably the DIPs, which are not included in these assessments, therefore, DAS28/44 do not comprehensively assess the extent of disease activity in peripheral joints in PsA[185]. For this reason, the group for research and assessment of psoriasis and psoriatic arthritis (GRAPPA) and the Outcome Measures in Rheumatoid Arthritis Clinical Trials (OMERACT) group recommended extended 66 swollen and 68 tender joint counts for use in both clinical practice and research[176,186]. The 66/68 joint count only provides information on the number of joints that are swollen and tender, respectively. Additional specific scores and response criteria have been developed for use in clinical trials and the assessment of treatment response. The guidance by the National Institute for health and Care Excellence (NICE) in the UK recommends using the Psoriatic Arthritis Response Criteria (PsARC) to assess response to targeted therapies used to treat PsA. The PsARC assesses response in swollen and tender joint counts (SJC and TJC), PGA and PhGA[187]. The Disease activity in Psoriatic Arthritis (DAPSA) a composite measure of peripheral joint disease activity, calculated by a simple sum of the PGA, patient reported pain, 66 swollen and 68 tender joints counts and CRP[188]. To note, PsARC and DAPSA do not include or assess enthesitis, dactylitis, axial disease, or skin manifestations[189]. Clinical trials continue to use the American College of Rheumatology (ACR) response criteria, adopted from RA as key efficacy outcomes. In addition to improvements in swollen and tender joints, these also incorporate improvements in PhGA, PGA, patient pain and disability assessments and acute phase reactants (ESR/CRP)[190]. However, the ACR response criteria were not designed for or appropriate for use in clinical settings.

1.1.3.2 *Axial disease*

Inflammatory back pain is the most frequent symptom of spondylitis and/or sacroiliitis. The pain is usually more intense during the night, and is associated with a morning stiffness of at least 30 minutes, which improves with activity, as described in the Assessment of SpondyloArthritis international Society (ASAS) classification criteria for axial SpA[191]. The prevalence of axial involvement is estimated to be between 25 and 70% of patients with PsA (often termed axial PsA) but is unclear due to a lack of agreed definition for axial PsA which is recognised as having differences from the axial SpA defined by the ASAS

criteria[192,193]. The axial involvement in PsA is usually associated with a peripheral arthritis, and in 2-4% of cases, it is the only clinical manifestation[194]. X-rays and MRI scans indicate that all the segments of spine can be affected and the sacroiliitis is usually asymmetrical in PsA, in contrast to the symmetrical picture in AS or axial SpA[193].

The diagnosis and assessment of spondylitis and sacroiliitis can be challenging, as other disease can present with similar symptoms and radiographic imaging. Most notably, degenerative disc disease and non-specific ("mechanical") low back pain can be difficult to distinguish, which has implications for treatment as these are not responsive the therapies used to treat the inflammatory disease in PsA. Furthermore, the outcome measures frequently used in clinical settings to evaluate axial inflammatory symptoms and functional impairment, namely the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI)[195], Bath Ankylosing Spondylitis Functional Index[196] and Bath Ankylosing Spondylitis Metrology Index[197] have not been validated in PsA[198,199]. The BASDAI, in particular, is widely used due to its easy administration; however, it is non-specific, as it assesses the intensity of spinal and peripheral pain, fatigue and the duration of morning stiffness, all of which can be influenced by non-PsA related issues such as disc disease and fibromyalgia (FM).

1.1.3.3 *Enthesitis*

The inflammation at the insertion of tendons and ligaments to the bones is known as enthesitis. Enthesitis is a classic manifestation of PsA; however, it can occur in other forms of inflammatory arthritis, more frequently SpA, but also RA, and associated with biomechanics in otherwise healthy individuals, especially in athletes[200,201]. Symptoms are characterised by pain and stiffness at the entheseal site, with or without swelling. Around 60% of individuals with PsA will experience enthesitis during the course of their disease[202]. Entheseal inflammation can be the only manifestation of PsA and occur even without peripheral joints or axial involvement[203]. A patient with isolated enthesitis can still be classified as having PsA, according with the CASPAR criteria. The entheseal inflammation is considered the *primum movens* in the natural history of PsA [93]. Ultrasounds studies in individuals with PsO without musculoskeletal symptoms revealed a

higher prevalence of enthesitis in comparison to healthy controls[204]. Moreover, the presence of enthesitis in PsO is associated with a higher risk of developing arthritis within 2 years, confirming that the enthesis can be a predictive factor of the development of PsA[205]. Enthesitis can be difficult to diagnose and evaluate due to non-specific and often subtle clinical features, lack of specific biomarkers and poor correlation of imaging with symptoms and clinical features. Moreover non-inflammatory conditions, such a high body mass index (BMI) and mechanical stress (e.g. sport, repetitive movements), can present with similar symptoms and findings[206]. When present at multiple sites, enthesis can cause widespread pain which can mimic other chronic pain conditions, such as FM (covered in more detail in section 1.2.2), posing diagnostic challenges, particularly where these conditions co-exist[207]. Despite recent advances, the underlying pathogenic mechanisms are yet to be completely understood, probably due to difficulties in tissue sampling and the relatively low number of enthesis resident cells[67], but likely involve an exaggerated inflammatory response and impaired repair, as outlined in Section 1.1.2. Therefore, diagnosis of enthesitis remains mainly clinical, supported by imaging. On plain radiographs, erosions (representing damage) and spurs (representing new bone formation as part of repair) may be visible, particularly relating to the Achilles tendons and plantar fasciae[208]. Ultrasonography and MRI studies are more sensitive for the detection of active enthesitis in early PsA[209]. Different clinical scores have been proposed to assess the presence of enthesitis and its progression over time. The main scores used for the assessment of enthesitis, are the following: the Leeds Enthesitis Index (LEI)[210], the Spondyloarthritis Research Consortium of Canada (SPARCC) enthesitis index[211], the Maastricht Ankylosing Spondylitis Enthesitis Score (MASES)[212], and the 4-points enthesitis measure[213]. The characteristic and site explored in these scores are represented in table 1.1.2. There are also a number of radiographic scores for the assessment of enthesitis, but these are mainly used in specific radiographic research studies and are not relevant to this work, so are not considered further here.

Table 1.1.2 Sites assessed in enthesitis scores.

Site	LEI	SPARCC	MASES	4-points
First costochondral joint			X	
Seventh costochondral joint			X	
Supraspinatus insertion		X		
Lateral epicondyle of humerus	X	X		
Medial epicondyle of humerus		X		
Posterior superior iliac spine			X	
Anterior superior iliac spine			X	
Iliac crest			X	
Fifth lumbar spinous process			X	
Achilles insertion into calcaneum	X	X	X	X
Greater trochanter of femur		X		
Medial condyle of distal femur	X			
Insertion of plantar fascia		X		X
Quadriceps insertion into patella		X		
Inferior pole of patella		X		
Tibial tubercle		X		
Total number of sites evaluated	6	18	13	4

LEI – Leeds Enthesitis Index; SPARCC - Spondyloarthritis Research Consortium of Canada; MASES - Maastricht Ankylosing Spondylitis Enthesitis Score; 4-points – 4-points enthesitis measure. Mod. from Mease PJ et al. Semin Arthritis Rheum. 2018[214].

1.1.3.4 *Dactylitis*

Dactylitis, also known as "sausage digit", is characterised by inflammation of an entire finger or toes, involving tendon sheaths, joints and surrounding soft tissues[215]. The diagnosis is based on physical examination: dactylitis is usually defined as a fully swollen digit associated with tenderness on palpation not limited to the joint line. Clinically, the digit is swollen, painful and red with a limited range of motion. Chronic dactylitis tends to be less painful, but an important loss of function may persist. It is estimated that approximately 30% of patients with PsA have dactylitis at the disease onset and up to 48% of the patients will have at least one episode over the course of their disease[216]. There is evidence that radiographic damage may be more severe in digits affected by dactylitis, which is generally considered to be a bad prognostic feature[217]. MRI

studies have confirmed the primary involvement of tendons sheaths in dactylitis, which is not always associated with concomitant synovitis[218].

Several methods are used to assess dactylitis clinically: 1) Simple dactylitis score: the number of digits with dactylitis (present/absent) is counted to give a score from 0-20; 2) Dactylitis Severity Score: digits are scored from 0 (no dactylitis) to 3 (severe dactylitis) based on the severity; 3) the Leeds Dactylitis Index (LDI): is usually only used in specialist research studies to assess tenderness and measure digit circumference using a dactylometer[219].

1.1.3.5 *Nail and skin disease*

Skin manifestations usually precede the onset of PsA in approximately the 60-80% of patients. In 15% of patients, skin and joint disease onset occur at the same time, while rarely the PsA develop before the onset of the skin disease[220]. Severity of skin PsO has been proposed as a risk factor for the onset of PsA in some studies[221], although this has not been consistently confirmed[222,223]. The most frequent presentation of skin involvement in PsA patients is plaque psoriasis, followed by guttate, flexural, and palmoplantar phenotypes. Some areas affected by PsO appear associated with a higher risk to develop a PsA, particularly scalp, intergluteal and perianal localisation, and nail dystrophy[221]. Nail disease is considered as a risk factor for developing PsA in patients with PsO. These lesions include nail pitting, onycholysis, hyperkeratosis, and nail bed crumbling. The prevalence of nail disease, also called onychopathy, in PsA patients has been estimated to be between 50-87%[224]. A pathogenic link between nail disease and enthesitis has been proposed, supported by anatomic similarities between the two structures and ultrasound and MRI findings[225,226].

The most commonly used methods to assess skin disease severity and extent in PsA are the Psoriasis Area and Severity Index (PASI) and the body surface area (BSA)[227]. PASI is the most used in dermatology settings and considered as the gold standard for the assessment of PsO[228], although this is more time consuming and technically more complex than the BSA, so the latter is more often used in rheumatology settings. The most widely used clinimetric scores for nail disease is the Nail Psoriasis Severity Index and its modified version[229,230]. Several

domains, such as pitting, onycholysis, dyschromia, crumbling of the nail plate, and leukonychia, are assessed in these scores, making them relatively time-consuming, so simple recording of the presence or absence of nail disease is often used in PsA settings.

1.1.3.6 *Disease activity scores assessing multiple domains*

Additional validated composite scores of disease activity incorporating multiple domains include the Psoriatic Arthritis Disease Activity Score and the GRACE Index[213,231]. However, these are not easy to use or calculate in normal clinical practice. In contrast, the "minimal disease activity" (MDA) has become widely used as a target state in PsA patients[232]. The MDA criteria include assessment, with cut-offs, for peripheral arthritis (66 swollen and 68 tender joint counts), patient self-reported pain, PtGA, enthesitis (LEI), functional ability and skin involvement (PASI/BSA)[233]. To reach the definition of MDA, 5 out of 7 criteria must be satisfied. MDA has been shown to be more accurate than DAS28 as treatment target in PsA[234], and relatively easy to use in clinical settings. The Composite Psoriatic Disease Activity Index[235] assesses all the five domains of the clinical PsA spectrum, namely peripheral arthritis, enthesitis, dactylitis, spinal disease and skin involvement[236] but is not currently widely used.

1.1.4 Treatment of Psoriatic arthritis

Treatment of PsA is based on non-pharmacological and pharmacological interventions. Pharmacological treatments range from intra-articular injections, topical treatment (for PsO) and non-steroidal anti-inflammatory drugs (NSAIDs) to conventional synthetic disease-modifying anti-rheumatic drugs (csDMARDs), biological DMARDs (bDMARDs), and targeted synthetic DMARDs (tsDMARDs). Due to the heterogeneity of the clinical presentation, it is important to identify and consider the prevalent clinical manifestations and involved domains, as well as common comorbidities, which requires a multidisciplinary approach including a range of different healthcare professionals, e.g. rheumatologist, dermatologist and physiotherapists. The principles guiding the treatment of patients with PsA are collected into guidelines and recommendations, published and updated by the European Alliance of Associations for Rheumatology (EULAR)[237] and by

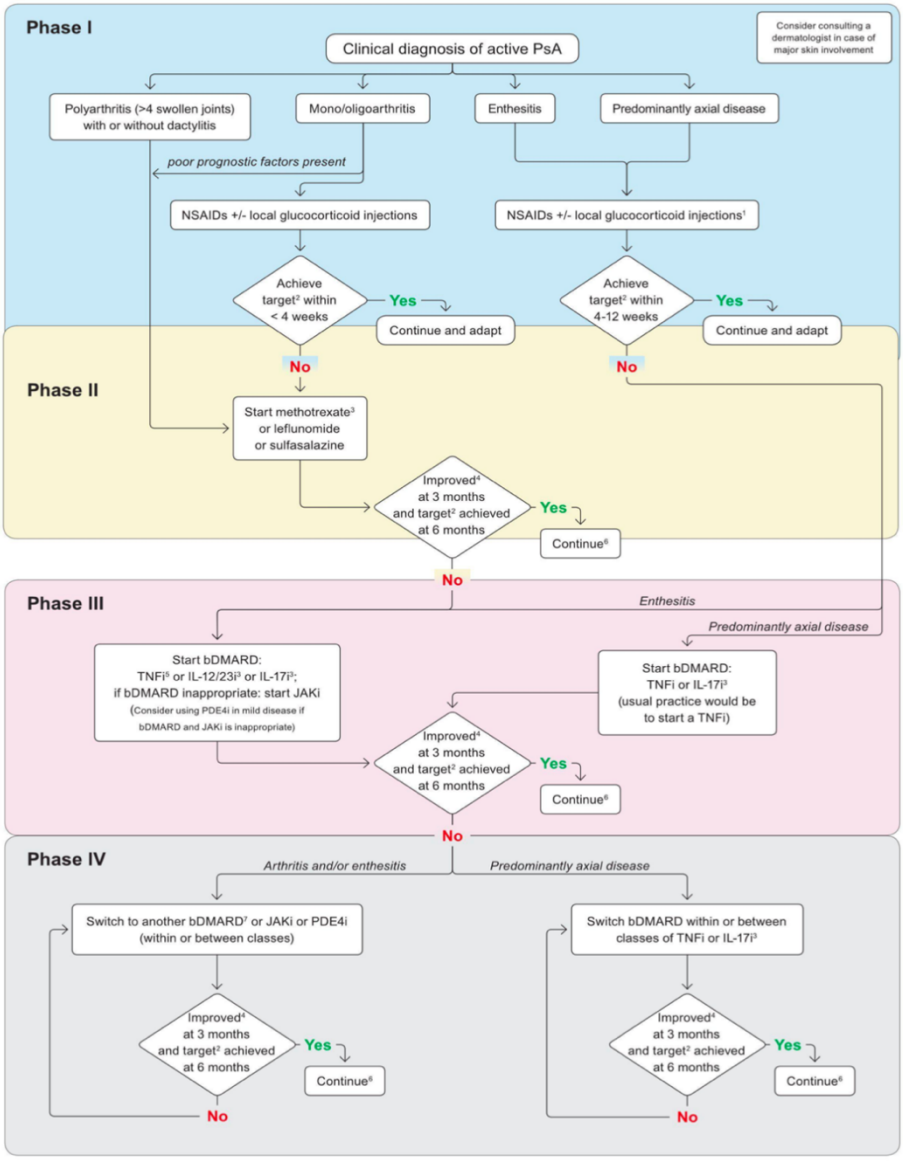
GRAPPA[238,239],-while in the UK there is also national guidance from the British Society for Rheumatology and NICE[240]. As indicated, overall, three major classes of DMARDs can be identified: 1) csDMARDs, including methotrexate, sulfasalazine, leflunomide, and cyclosporine A; 2) bDMARDs, selectively targeting a pro-inflammatory cytokine; 3) tsDMARDs, inhibiting intracellular inflammatory pathways, i.e. phosphodiesterase-4 (PDE4) or Janus kinases (JAKs). Recently, the inclusion of effective patient-centred self-management strategies in individuals with inflammatory arthritis has been recommended by EULAR, which include, patient education, promoting physical activity and lifestyles changes aiming to improve general wellbeing and reduce cardiovascular risk, as well as increasing awareness of mental health issues such as depression and anxiety associated with PsA[241]. Therefore, patient education/self-management strategies, physical exercise, and NSAIDs are commonly the first line treatment in patients with musculoskeletal symptoms from PsA. When the disease activity is severe, especially in presence of peripheral arthritis, structural damage, high inflammatory markers, and/or relevant extra-articular manifestations, the use of a csDMARDs is recommended. In association, local injection or systemic (oral or intramuscular) glucocorticoids may be considered. The guidelines recommend that only after at least one csDMARDs failure, or when there is a contraindication to their use, should bDMARDs be considered. TNF inhibitors are frequently used as first-line treatment because their efficacy has been widely demonstrated in long term studies and several biosimilars are available, helping to reduce the costs relative to other bDMARDs[242]. However, if anti-TNF agents are contraindicated, or severe skin involvement is present, bDMARDs targeting IL-23/IL-17 pathways may be preferred due to their superior efficacy for skin psoriasis. JAKs inhibitors are recommended in predominantly peripheral arthritis, after failure of at least one bDMARDs. When bDMARDs or JAKs inhibitors are not appropriate, the phosphodiesterase 4-inhibitor apremilast can be considered.

While the EULAR treatment recommendations follow the more hierarchical structure outlined above, the GRAPPA recommendations focus on the different clinical presentations, with the treatment choice based on the involvement of six domains, figure 1.1.2 For example, while for peripheral arthritis the pathway is similar to that described above with csDMARDs before bDMARDs, for axial

disease, where csDMARDs are not effective, direct progression from NSAIDs to bDMARDs is recommended.

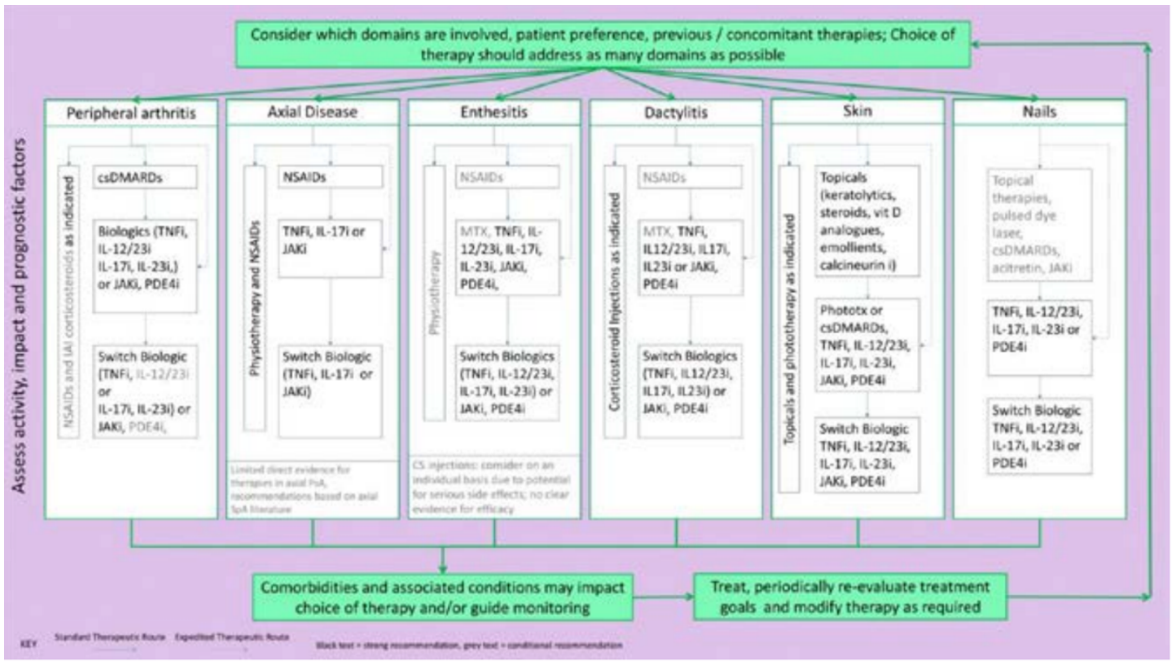
Defining remission and the treatment target in PsA can be controversial with several disease activity scores have been validated[214]. The use of DAPSA or MDA scores are recommended by EULAR guidelines[237], figure 1.1.3. However, there is accumulating evidence that tight control with assessment at regular visits and escalation of therapy based on the activity scores yields better disease outcomes[243].

Figure 1.1.2 The 2019 EULAR algorithm for the treatment of PsA.



From Gossec L et al. Ann Rheum Dis. 2020[237].

Figure 1.1.3 The 2021 GRAPPA algorithm for the treatment of PsA based.



From Coates LC et al. Ann Rheum Dis. 2021[244].

1.1.4.1 *Non-steroidal anti-inflammatory drugs and corticosteroids*

NSAIDs are recommended as first-line drugs in PsA patients, especially when axial involvement and enthesitis are present[245]. NSAIDs are effective in pain and stiffness relief in up to 60–70% of the patients[246]. Despite their known effect on inflammation, NSAID efficacy in reducing or arresting radiographic progression has not been demonstrated.

Corticosteroids are analogues of the hormone cortisol and leverage the physiologic anti-inflammatory effect of this adrenal gland hormone, and as such have potent anti-inflammatory effects. However, when used in long-term, corticosteroids often lead to potentially severe side effects, mainly metabolic disorders and diabetes, increased risk of infections, osteoporosis, cataracts or glaucoma, hypertension, depression, and/or gastrointestinal bleeding. Therefore, the general principle is to use local injections of glucocorticoids in limited disease (e.g. 1-2 joints) and to use systemic glucocorticoids with caution at the lowest effective dose for as short as possible.

1.1.4.2 *Conventional synthetic disease modifying anti-rheumatic drugs*

As recommended by guidelines, when there is severe peripheral involvement in PsA, early treatment with csDMARDs should be started.

Methotrexate

Methotrexate (MTX) is most frequently used as first-line treatment for PsA, especially in presence of a peripheral arthritis and skin involvement, at a dosage of 10-25 mg/week, either alone and in combination with other csDMARDs or bDMARDs. MTX is widely used despite limited efficacy[247,248]. However, MTX demonstrated efficacy in controlling PsA clinical course and progression in longitudinal and clinical trial[249,250], but MTX did not appear to reduce radiographic changes at 24 months in treated PsA patients[251]. Potential side effects of MTX include liver, lungs, and bone marrow toxicity, while the teratogenic effect of MTX makes this drug contraindicated in pregnancy[252].

Sulfasalazine

The mechanism of action of sulfasalazine (SSZ) is not clearly understood in PsA, but is most probably mediated by the 5-aminosalicylic acid and the inhibition of the 5-lipoxygenase pathway[253]. There is limited evidence to support SSZ use and no data regarding effect on structural damage[254-258]. The main side effects of SSZ include gastrointestinal intolerance, altered liver function tests, oligospermia, bone marrow suppression, and allergic reactions.

Leflunomide

Leflunomide, by inhibiting the synthesis of pyrimidine, exerts an immunosuppressive action directly on T cells[259]. Leflunomide has demonstrated superiority to placebo in the treatment of PsA[242,260]. The TOPAS study confirmed the efficacy of leflunomide on psoriasis in a PsA cohort[261]. The main side effects include diarrhoea and nausea, increasing of liver enzymes, infections, and reduced circulating leukocytes[252].

Cyclosporine A

Cyclosporine A is an immunosuppressant that has various mechanisms of action; the most important is thought to be to inhibition of the calcineurin[262]. Several clinical trials have confirmed the efficacy of Cyclosporine A on severe PsO, while only three studies have demonstrated its effectiveness also in PsA[263–265]. However, use is limited by significant side effects, particularly are related to nephrotoxicity and hypertension[252].

1.1.4.3 *Biologic disease modifying anti-rheumatic drugs*

TNF inhibitors

TNF inhibitors have demonstrated superior efficacy in treating both PsA and PsO compared with csDMARDs. The treatment with anti-TNF agents is a cornerstone for PsA management. Multiple anti-TNF drugs have been approved for PsA treatment; namely, infliximab, etanercept, adalimumab, golimumab, and certolizumab pegol[237].

Infliximab is a chimeric monoclonal antibody (25% murine, 75% human) that is administered classically intravenously, but also subcutaneously. It demonstrated efficacy in the treatment of both PsA and PsO, with sustained remission and reduction of radiographic progression at 1 year[266,267]. Etanercept is a fusion protein that fuses the TNF receptor with the Fc IgG1 antibody, which demonstrated its efficacy in controlling PsA[268] and PsO[269] symptoms and radiographic progression. The first fully human monoclonal antibody directed against TNF was adalimumab, which similarly demonstrated efficacy in controlling clinical symptoms in PsA and in significantly reducing radiographic progression in a long-term study[270,271]. Golimumab is a human monoclonal antibody that demonstrated efficacy in the treatment of PsA, and indirectly of PsO, in a randomized, placebo-controlled study, with sustained response after 1 year of treatment[272,273]. The last anti-TNF to be approved was certolizumab pegol. It has peculiar characteristics, because it is a fragment of an anti TNF monoclonal antibody conjugated with polyethylene glycol which prevents the drug crossing the placenta or entering breast milk[274]. Certolizumab efficacy was

demonstrated in the treatment of both PsA[275] and PsO[276], and it is the TNF inhibitor of choice during pregnancy.

Anti-IL-17 drugs

The IL-23/IL-17 pathway plays a key pathogenic role in the PsD pathogenesis. Inhibition of IL-17 has proven efficacy in ameliorating joint symptoms and preventing damage. Secukinumab, an anti-IL17A fully human antibody, was the first IL-17A inhibitor approved to treat severe PsO and PsA[277–279]. The efficacy in PsA was confirmed in patients both naïve and refractory to anti-TNF treatment, with efficacy in controlling peripheral arthritis, enthesitis, dactylitis, skin, and nail disease sustained to 2 years[277–279]. Ixekizumab is a recombinant IgG4κ monoclonal antibody binding IL-17A. Ixekizumab was demonstrated to be effective in patients naïve to biologics or non-responders to TNF inhibitors, and superior to adalimumab in controlling the skin involvement, and to placebo for peripheral arthritis, quality of life scores, as well as enthesitis and dactylitis[280–284].

Double inhibition of both IL-17A and IL-17F with the monoclonal humanized antibody, bimekizumab, has recently been approved to treat PsO. In PsA reported promising results, but still undergoing regulatory review at the time of writing[285–287].

Targeting the IL-17 receptor A (IL-17RA) with the human IgG2 antibody brodalumab was also investigated. Binding the common IL-17 receptors, brodalumab exerts concomitant inhibition of IL-17A, IL-17F, and IL-17E (IL-25). Brodalumab was successful in improving joint inflammation, PsO, dactylitis and enthesitis compared to placebo in patients with insufficient control of the disease with csDMARDs and anti-TNF inhibitors[288]. Of note, during the initial clinical trials, an elevated rate of suicide was reported, raising safety concerns and causing a delay in the introduction of brodalumab for PsA, although it is licensed for use in PsO. This finding highlights the potential effect of the IL-17 family on the central nervous system (CNS), with plausible differential effects between by different cytokines within the same cytokine family[289].

Intriguingly, the blockade of IL-17 may lead to apparently paradoxical exacerbation or new onset of IBD, limiting the use of this class of drugs when IBD or risk factors are present. This phenomenon appears to be explained by the IL-23-independent role of IL-17 in the repair and maintenance of the integrity the gut-epithelial barrier[290,291]. Interestingly, IL-23 inhibition appears to prevent the maturation of pathogenic Th17 immunity, without impairing the IL-17 protective homeostatic effect on intestinal epithelium, so this effect is not seen with IL-17 inhibitors[292].

IL-23 inhibitors

Ustekinumab is an anti-IL12/IL-23 fully human monoclonal antibody directed against the p40subunit shared by IL-12 and IL-23. IL-23 blockade inhibits the maturation and differentiation of pathogenic Th17 cells. In PsA, ustekinumab showed significant improvement in different domains, including arthritis, psoriasis, enthesitis, dactylitis, and radiographic progression. Both patients naïve to biologics or after anti-TNF failure showed similar improvement[293–295]. Ustekinumab is superior to TNF inhibitors in controlling entheseal and skin inflammation, highlighting the predominant role of this pathway in these clinical manifestations[296].

There are several agents specifically targeting the IL-23 p19 subunit in various stages of development for PsA. Guselkumab, a human IgG1λ antibody, was recently approved to treat PsA. Guselkumab demonstrated significant improvement in arthritis, PsO, enthesitis, dactylitis, as well as fatigue, physical function and quality of life's scores in bDMARD naïve and TNF inadequate responder patients[297–299] with indirect non-inferiority to IL-17 and TNF inhibitors[299]. Similarly, risankizumab and tildrakizumab selectively block IL-23 by binding the p19 subunit and have showed efficacy in PsO with PsA studies ongoing[300–302].

Overall, these agents appear to have an acceptable safety profile, although their long-term safety in PsA remains to be established.

1.1.4.4 *Targeted synthetic disease modifying anti-rheumatic drugs*

Apremilast

Apremilast is a small molecule inhibitor of PDE4, which, in contrast to the bDMARDs, is administered orally. The isoform 4 of PDEs is abundantly expressed on immune cells, such as monocytes, T cells and neutrophils[303]. The inhibition of PDE4 confers immunomodulatory properties, which can be used effectively to treat PsA and PsO. Apremilast demonstrated efficacy as monotherapy for the treatment of peripheral arthritis, dactylitis, and enthesitis in patients naïve to bDMARDs[304]. The efficacy of apremilast was sustained for over 52 weeks but does not reach the same levels as seen with TNF inhibitors or other bDMARDs[305]. The safety profile of apremilast is generally good, although gastrointestinal symptoms and headaches are common[306].

JAKs inhibitors

JAKs inhibitors have been designed to inhibit intracellular signals mediated by JAK-STAT family. The surface receptors using the JAKs signalling bind several key pro-inflammatory cytokines vital for onset and perpetuation of inflammation in PsO, PsA, and other immune-mediate diseases. Tofacitinib, an inhibitor of JAK1, JAK3 and, to a lesser extent, JAK2, has been demonstrated to be effective for the treatment of PsA in patients with PsA with an inadequate response to csDMARDs[307] and to anti-TNF[308]. Recently, a double-blind, placebo-controlled clinical trial demonstrated a comparable efficacy of tofacitinib in comparison with adalimumab in improving articular, skin and patient reported outcomes in PsA[309,310]. However, use of tofacitinib has been limited by unresolved safety concerns[311].

Selective inhibition of JAK1 with upadacitinib and filgotinib has also demonstrated superiority to placebo and comparable efficacy with adalimumab in patients with inadequate response to csDMARDs and bDMARDs[312–315]. Studies comparing the effect on important domains of PsD, including PsO, enthesitis, dactylitis and axial involvement of JAKs inhibitors in comparison with anti-IL-17 and IL-23 agents are still lacking.

1.1.5 Psoriatic arthritis prognosis

CsDMARDs are often used early in PsA cases with severe peripheral involvement. Despite their ability to control the clinical course and progression, they do not significantly reduce radiographic changes, and limited data are available on the overall efficacy of csDMARDs. Recent progresses in understanding the immune pathogenesis of PsA have led to the development of advanced immunotherapies, including biologics and targeted DMARDs that are effective in controlling both PsA and PsO and in preventing joints damage. While biologics and targeted synthetic DMARDs offer improved outcomes for individuals with PsA, the unmet need remains in addressing pain and optimizing treatment for specific manifestations of the disease[316,317].

Chronic pain is a defining feature of PsA. It is the main driver of functional and work disability, negatively impacting patients' quality of life. In patients' assessments, pain control ranks at the top of their priorities, higher than controlling the progression of their joint disease[318]. Despite therapeutic advancements in controlling disease activity in the past decades, patients with well-managed peripheral inflammation continue to experience significant pain[319,320]. The disconnect between the control of peripheral inflammation and the reported pain suggests the involvement of additional pain mechanisms beyond the classic inflammatory nociceptive pathways. The inflammatory processes involved in the pathogenesis of the disease are primarily responsible for the pain experienced due to the direct activation of peripheral nociceptors. However, chronic inflammation can sensitise the nervous system, inducing plastic changes that sustain pain perception even after the resolution of inflammation (detailed in section 1.2.3). The mechanisms of nervous system sensitisation by inflammatory processes are not yet completely understood. Understanding these neurobiological mechanisms is crucial for effective pain management and improving the quality of life for individuals with PsA. The next sections describe pain neurobiological mechanisms, pain persistence in PsA, and the associated clinical challenges.

1.2 Chronic pain

1.2.1 Pain pathophysiology

1.2.1.1 *Introduction*

Pain has been defined by the International Association for the Study of Pain (IASP) as "*an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage*."[321]. The IASP definition highlights the contribution of both sensory and affective/cognitive dimensions of pain; moreover, it acknowledges the potential disconnect between the actual tissue damage and the perception of pain. Actually, pain is a subjective experience in which peripheral sensory input are integrated with affective and cognitive dimensions to determine the conscient perception of pain[322]. Ultimately, pain is determined by the functional interactions between different areas of the brain ("neuromatrix") influenced by genetic, sensory, and cognitive (stress/emotions) factors[323]. The balance between these factors constitutes a unique neurobiological pain signature that can predispose an individual to develop a maladaptive chronic pain condition.

Acute pain is a physiologic response to external harmful stimuli and is essential to protect and preserve the organism as demonstrated by rare genetic disease: genetic disorders characterised by increased threshold or insensitivity to pain lead to premature death of individuals affected[324]. On the other hand, chronic pain, defined as persistent or recurrent pain for more than 3 months, often reflects a maladaptive response, persisting despite the resolution of the initial noxious stimulus. The International Classification of Diseases for Chronic pain (ICD)-11 has been recently updated by World Health Organisation in collaboration with the IASP [325]. The ICD-11 classifies chronic pain into 2 groups: "chronic primary pain" and "chronic secondary pain". Chronic primary pain is defined as pain not imputable to another chronic pain condition, with a negative impact on the activities of daily living of the individuals affected (discuss in section 1.2.2). The chronic secondary pain group implies that an underlying chronic disease, including inflammatory arthritis, is responsible for pain. The chronic conditions may lead to persistence of the peripheral noxious stimulation,

and therefore to pain; however, the recent ICD-11 classification stresses the concept that chronic pain should be considered as a symptom *per se*, independently from the primary diagnosis. In fact, chronic pain may endure following successful treatments for the primary medical state[325]. Different underlying medical conditions are commonly associated with chronic pain. Musculoskeletal diseases are often comorbid in patients with chronic pain: chronic low back pain, OA and inflammatory arthritis are frequent diagnosis in individuals with persistent pain[322]. Overall, chronic pain is a major issue and a global health burden affecting up to a third of the general world population. The presence of chronic pain is associated to reduced quality of life, disability, and reduced life expectancy for the individuals affected[326]. Chronic pain carries a global economic and social burden independently from the underlying diagnosis. Pain management is a current challenge for health care professionals due to the heterogeneous mechanisms that can generate pain which are often difficult to distinguish. Thus, an optimal treatment approach for each individual is difficult to establish, so current pain management strategies are often ineffective with more than the half of patients reporting minimal or no benefit from therapies[327]. In this context, understanding the pathophysiology of pain and the unravelling the mechanisms is vital for health care professionals across different clinical specialties to improve patient care and, ultimately, their quality of life.

The IASP has classified pain, based on the underlying mechanisms, into three groups: nociceptive, neuropathic, and nociplastic[321]. Nociceptive pain is generated by the activation of peripheral nociceptors by a noxious stimulus, while neuropathic pain is consequent to a *"lesion or disease affecting the somatosensory system"*. In contrast, nociplastic pain lacks evidence of nociceptors activation or nervous system damage, and it is characterised by dysfunctional nociception processes often uncoupled from the intensity, or even the presence, of the peripheral stimuli. The nociplastic pain definition adapts nicely to the ICD-11 definition of chronic pain where, as symptoms on its own, do not require the evidence of tissue or somatosensory system pathology [328]. Clinically, chronic pain in general is characterised by an increase sensory sensitivity to noxious stimuli, i.e. hyperalgesia, and/or response to a stimulus that does usually provokes pain, i.e. allodynia[321]. In addition, factors such as

sleep disturbances, fatigue, mood disorders, and increased sensitivity to other sensory stimuli (visual, odour, and sound) are often present in nociplastic pain states.

Chemical and molecular modifications in the nervous system, known as neural plasticity, lead to structural and functional changes in the peripheral and CNS in response to external stimulation. For example, extensive tissue damage or repeated stimuli can induce the expression and upregulation of specific neurotransmitter receptors leading to altered responsiveness and the signal transmission of neurons. Neural plasticity is an intrinsic characteristic of the nervous system, and it has an important physiological adaptive role[329]. However, neural plasticity can also contribute to dysfunctional pain perception, when maladaptive nervous system changes lead to nociplastic or other pain states[330,331].

Characterising the mechanisms of pain leading to symptoms is highly relevant because the efficacy of different treatment strategies varies depending on this. However, different pain mechanisms often coexist, results in mixed pain states. Therefore, distinguishing pain mechanisms is a current clinical challenge and area of unmet need as lack of pain characterisation can lead to ineffective treatments and poor patients' outcomes. Current evidence suggests that nociplastic pain frequently follows a pre-existing nociceptive and/or neuropathic pain states, which probably contributes to enhance or develop nociplastic pain in predisposed subjects[332]. However, the development of nociceptive or neuropathic pain conditions can also occur in individuals with an already established nociplastic pain state[333].

1.2.1.2 *Somatosensory biology*

In order to distinguish between pain phenotypes, it is important to understand the biology of pain perception. Pain perception involves different steps starting from the periphery, where noxious stimuli are detected by nociceptors that transmit electrical signals to the CNS. The peripheral nervous system and CNS are in constant communication via ascending and descending pathways to modulate the pain sensory information. In the brain, sensory and psychosocial information are integrated by pain-processing areas to finally form the conscious pain perception[334].

Peripheral sensory inputs from the periphery

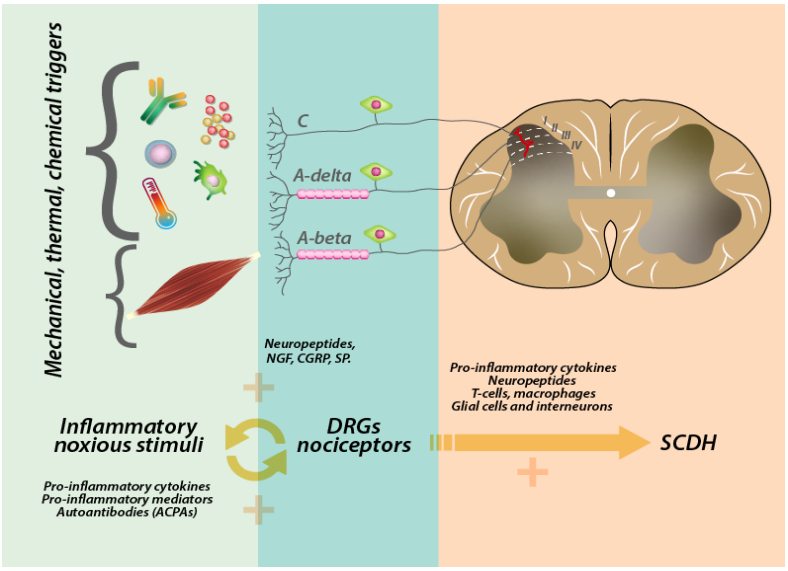
External noxious or potentially harmful stimuli are detected by nociceptors. Nociceptors are sensory neurons activated by stimuli of different modalities, such as mechanical, thermal, and chemical. Different nociceptors can be classified as myelinated or unmyelinated fibers, based on the presence or not, of a lipid-rich cellular sheath surrounding the axon, known as myelin, which is formed by specialised glial cells. The myelin sheath insulates the fibers, increasing the rapidity of electrical impulses transmission. The peripheral processes of nociceptors have free endings in the innervated tissues and include the C-fibers, A-δ and A-β fibers (table 1.2.1). C-fibers are unmyelinated and detect low intensity multimodal stimuli either mechanical, thermal, or chemical. C-fibers signals are perceived as dull pain in poorly defined large areas[335]. A-δ and A-β fibers are myelinated fast conducting fibers that respond to stimuli with different modalities. A-δ are activated by high intensity mechanical and thermal stimuli, resulting in sharp-pain sensation in well localised areas[336]. A-β fibers usually detect non-painful mechanical stimuli at low intensity, allowing perception of touch and stretching. In specific conditions, for example after the repeated activation of C-fibers, A-β fibers can also transmit nociceptive signals. The recruitment of A-β fibers also contributes to the wind-up phenomenon characterised by increased sensitivity to repeated sensory stimuli with the analogous intensities. The wind-up phenomenon itself is usually short lasting; however, it can initiate long-term neuronal plasticity and contribute to pain sensitisation (peripheral sensitisation)[330]. The cellular bodies of the primary sensory neurons are situated in the dorsal root ganglia (DRG) of the spinal nerves (figure 1.2.1). The peripheral stimuli reach the DRG and are then transmitted to the spinal cord dorsal horn (SCDH). In the SCDH, the second-order sensory neurons modulate the peripheral inputs by neurons-neurons and neurons-interneurons interactions integrating both the peripheral and CNS information. Finally, the second-order sensory neurons send the sensory information to the brain via different ascending pathways. The spinothalamic tract, formed by the axons of secondary sensory neurons, is the main ascending pathway for sensory inputs[337].

Table 1.2.1 Peripheral nociceptors

Nociceptor	Kind of stimulation	Intensity stimulation	Transmission of input	Target in SCDH	Perception
C fiber	Polymodal (thermal, Chemical, mechanical)	Low intensity stimuli	Unmyelinated, slow conducting	Lamina I	Dull, pressure, large areas
A-delta fiber	Mechanical, thermal	High intensity stimuli	Myelinated, fast conducting	Lamina II	Sharp pain in well-localised areas
A-beta fiber	Mechanical	Low intensity stimuli	Myelinated, fast conducting	Lamina III-IV	Touch, stretching, mechanical.

From Sunzini et al. Arthritis Rheumatol. 2023[334].

Figure 1.2.1 Role of dorsal root ganglia in pain processing

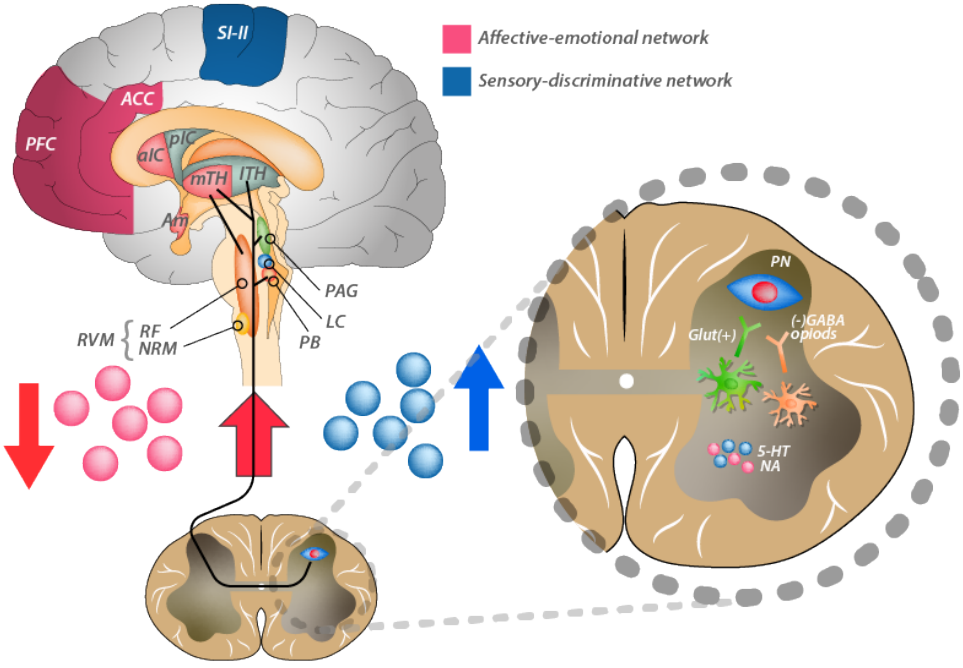


DRGs - dorsal root gangla; SCDH – spinal cord dorsal horn; NGF – nerve growth fact; CGRP-calcitonin gene-related peptide; SP – substance P.
From Sunzini et al. Arthritis Rheumatol. 2023;75:650-660[334].

From the spinal cord the spinothalamic tract reaches the thalamus in the brain (figure 1.2.2). The thalamus is considered as the sensory gate, where the majority of peripheral signals arrive and are then sent to different brain areas involved in pain processing. Similarly to the spinothalamic tract, other ascending

pathways, namely the spinoreticular and spinomesencephalic tracts, reach specific mesencephalic regions, i.e. the periaqueductal grey matter, parabrachial area, reticular formation, and locus coeruleus (figure 1.2.2). These mesencephalic regions project sensory information and receive electrical signals from the limbic system and brain areas encoding for affective and stress information[338]. Importantly, from the descending pathways, which originate from the mesencephalic regions, are able to finely modulate the sensory signals via inhibitory and excitatory signals directly to the secondary sensory neurons. Although the biology of descending pain pathways is not completely understood, their dysfunction appears to significantly contribute to chronic pain pathology[339]. Many drugs effective in acute and chronic pain conditions appear to modulate descending pain pathways, for example gabapentinoids and serotonin-norepinephrine reuptake inhibitors (SNRIs) (detailed in section 1.2.6.3).

Figure 1.2.2 Pain ascending pathways



5-HT - 5-Hydroxytryptamine or Serotonin; ACC – Anterior Cingulate Cortex; aIC – Anterior Insula Cortex; Am – Amigdala; GABA - γ-Aminobutyric Acid; Glut – Glutamate; LC - Locus Coeruleus; lTH - Lateral Thalamus; mTH – Medial Thalamus; NA – Noradrenaline; NRM – Nucleus Raphe Magnus; PAG - Periaqueductal Grey Matter; PB - Parabrachial Area; PFC – Prefrontal Cortex; pIC – Posterior Insula Cortex; PN – Projecting Neurons; RF - Reticular Formation; RVM – Rostral Ventromedial Medulla; SI-II – Somatosensory Cortex I and II.
From Sunzini et al. Arthritis Rheumatol. 2023;75:650-660[334].

Central pain processing

The sensation of pain is generated by the integration of nociceptive sensory inputs from the periphery with higher-order dimensions, including affective-emotional, cognitive and stress. Melzack was the first to describe first the "pain neuromatrix" or ''pain matrix'' pointing towards the hypothesis that the activation of anatomically defined brain areas generates the final pain perception[340]. The anatomical cortical brain areas "processing pain" can be simplistically divided into two networks; namely, the lateral sensory-discriminative network, encoding for pain intensity and localisation of stimuli, and the medial affective-cognitive network, integrating more complex dimensions of pain resulting in different degree of unpleasantness, partly independent of the original intensity of the stimulation[341]. The lateral network includes the lateral nuclei of the thalamus that relay to the primary and secondary somatosensory cortex (SI and SII, respectively), and the posterior nuclei of the thalamus which project sensory information to the posterior insula cortex (pIC)[342]. The SI allows localisation of the peripheral stimuli in the body, while the SII and pIC determine the intensity of stimuli and integrate cognitive components of pain. The medial network includes the medial thalamic nuclei, the anterior insula cortex (antIC), anterior cingulate cortex (ACC), and the amygdala. The regions forming the medial network modulate the degree of unpleasantness, emotional learning and reward, motor response (ACC) and integrate symptoms of depression and anxiety, when present[343,344]. The medial nuclei of the thalamus also transmit inputs to the prefrontal cortex (PFC); at this level, cognitive dimensions, such as attention and emotion, are integrated to modulate to final pain perception[345]. The amygdala, medial PFC (mPFC), and insula cortices can be defined as limbic areas important brain areas in chronic pain. These regions variably integrate attention (or salience), cognitive and affective components, as well stress[346]. Salience can be defined as the capacity to select among different stimuli the ones that deserve attention over others. The PFC is a highly developed region of the brain in humans compared to other animal species. The PFC has an important role in executive functions, guiding actions and emotions in actual or hypothetical context[347]. The PFC anatomically covers a large area of the brain anatomically and is functionally divided into mPFC, the orbitofrontal cortex, the ventrolateral PFC, the dorsolateral PFC

(dlPFC), and the caudal PFC. Not surprisingly, the role of the PFC, especially the dlPFC and mPFC, in pain perception is critical to integrate higher cognitive dimension in pain perception. Nonetheless, the PFC seems to have a dual pro-nociceptive and anti-nociceptive effects on pain, depending on the area activated and connectivity with other pain-processing regions reflecting the complex function associated to this area[348]. The insula is also a key hub in pain perception, as demonstrated by the invariable activation of the insula cortex (IC) in neuroimaging studies in both acute and chronic pain[341,349]. Despite its small overall area, the insula is a complex region of the brain where function and cytoarchitecture gradually vary from anterior to posterior[350]. Evidence suggests that the pIC encodes for stimuli intensity and laterality and modulates response processes; on the other hand, the antIC is part of the medial pathway encodes for aversion and unpleasantness integrating interoception, affective-emotions, learning and salience[351,352]. The connection between the antIC with the limbic system, e.g. the amygdala, is essential to complete its integrative function. Additionally, the hippocampus and nucleus accumbens, which are also included within the limbic areas and are allegedly involved in salience, memory and reward[353]. The presence of psychological comorbidities in individuals with chronic pain is associated in greater activation of the limbic regions, highlighting their important role in pain perception[338].

Importantly, the anatomy of the pain matrix does not completely reflect the heterogeneity of pain perception and variable activations of the same areas in different individuals to generate distinctive perceptions[354]. Beyond the primary nociceptive signals and anatomical distinction, complex interactions of genetic, history, mood, stress, and other factors finally results in a unique “pain signature”, nowadays considered more accurate than the pain matrix[352,354].

In summary, somatosensory biology unveils the intricate processes of pain perception. It begins with nociceptors detecting harmful stimuli, triggering a journey through the CNS. The spinal cord's dorsal horn modulates this input before sending ascending signals, via the thalamus, to several brain regions. Here, sensory and emotional aspects converge to form conscious pain perception. Two brain networks shape this perception: the sensory-discriminative network for intensity and location, and the affective-cognitive

network for emotional complexity, defining the complex and subjective pain perception in humans.

1.2.2 Fibromyalgia the prototype of nociplastic pain

The nociplastic pain mechanism is distinct from: 1) nociceptive pain- associated with inflammation or tissue damage, and 2) neuropathic pain - associated with somatosensory system damage. In pure nociplastic pain conditions, evidence of tissue damage, disease or altered integrity of the somatosensory system is not detectable. As yet, the pathologic mechanisms are not completely understood. Recent advances suggest a dysfunctional central or peripheral somatosensory system characterised by amplified activation or reduced inhibition, ultimately leading to increased sensitivity and altered pain perception[355]. Nociplastic pain is the underlying mechanistic descriptor of chronic primary pain conditions that have been grouped into 5 categories by IAPS: chronic widespread pain (including FM), complex regional pain syndrome, chronic primary headache or orofacial pain, chronic primary visceral pain, and chronic primary musculoskeletal pain[328]. FM is part of the chronic widespread pain syndromes, and it is currently one of the most investigated primary nociplastic pain disorders. Studies and findings in FM have helped understanding of the neurobiological mechanisms of nociplastic pain conditions in general; FM is considered the prototypic disease, the knowledge of which is leveraged to better understand other nociplastic pain syndromes. Moreover, characterisation of FM has shed a light on mixed pain states which have often been mistaken for refractory nociceptive (e.g. in RA, PsA) and/or neuropathic (chronic low back pain) diseases, instead of a continuum of overlapping pain mechanisms that require different managements[356]. Therefore, FM aids the investigation and understanding of classical nociplastic pain syndromes.

1.2.2.1 *Definition, epidemiology, and classification*

FM is a chronic primary pain syndrome characterised by chronic widespread pain in association with CNS symptoms, such as fatigue, sleep impairment,

psychological and cognitive difficulties, and mood disturbances[357]. FM is a frequent chronic pain condition worldwide with a prevalence estimated to be 2-4%, depending on the country and classification criteria used[358]. In the United States, FM is the third most prevalent cause of chronic pain, following OA and low back pain[359]. Individuals at any age can be affected, however, FM is more frequent in the 50's[358]. The prevalence is higher in the female sex with a ratio of 3:1[358,360]. The socioeconomic burden of this syndrome is significant, and it is mainly due to frequent clinical visits and investigations, as well as the negative impact on work productivity[361]. FM can occur in isolation or during the course of other chronic pain conditions, such as OA, inflammatory arthritis, including RA and PsA, or mechanical back pain[362,363].

Due to the lack of reliable biomarkers and the poor reproducibility of physical examination, the diagnosis of FM is difficult, and misdiagnosis remains common[364]. Different classification and diagnostic criteria are currently available to assist clinicians in identifying FM in both clinical practice and research contexts (table 1.2.2). The first classification criteria were developed in 1990 by the ACR; this set of criteria combines the presence of widespread pain with tender points, at least 11 out 18 points tender to pressure as applied by the examiner[365]. The weakness of the 1990 criteria is that tender points examination is not easily reproducible and is potentially misleading; moreover, the central symptoms characteristic of FM, including fatigue, sleep and mood disturbances, and cognitive difficulties, are not taken into account. The 2010 ACR diagnostic criteria and its modified version of 2011[366,367] acknowledged the key CNS symptoms by including in the symptoms severity score (SSS) questionnaire. Widespread pain remains an essential feature, evaluated with a body map, used to compile the widespread pain index (WPI). The combination of specific cut off values for the WPI and SSS is used to determine the diagnosis (figure 1.2.3). To minimise possible confounding diagnoses, a criterium excluding other underlying causes of pain was added. In 2016, the ACR updated the FM criteria by removing the latter exclusion criteria and adding the requisite of the pain to be spread across multiple regions of the body, a limitation of the previous criteria[368]. For example, a patient with sciatica would have had a high WPI by ticking all body areas in a single leg.

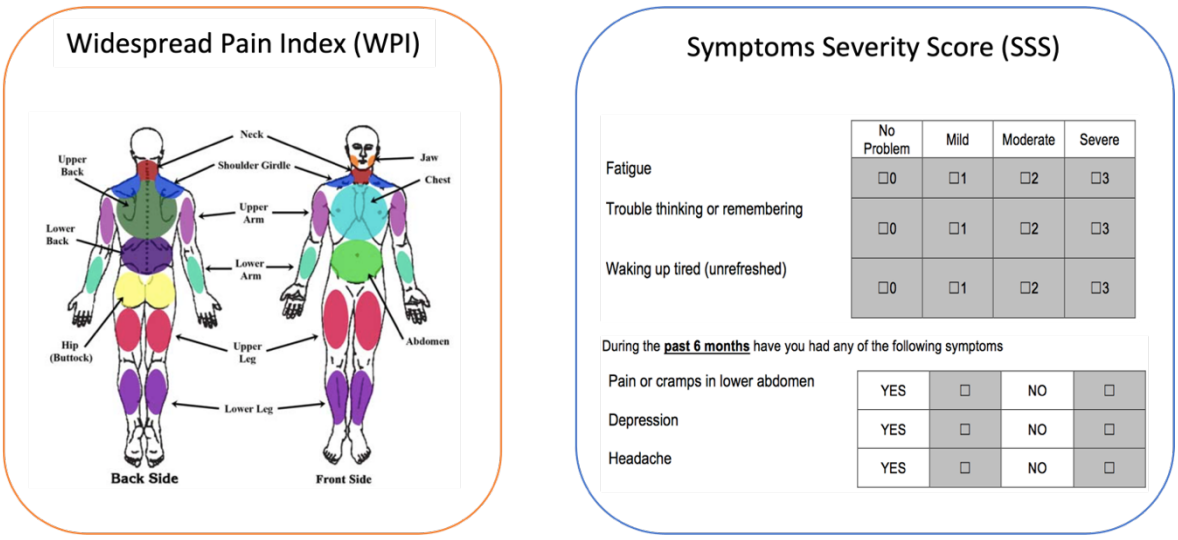
Table 1.2.2 ACR classification and diagnostic criteria for fibromyalgia.

To meet all the ACR criteria, symptoms must have been present at a similar level for at least 3 months.

ACR criteria	1990	2010	2011	2016
Widespread pain	History of widespread pain + Pain, on digital palpation, at 11 or more of the 18 specific tender points	WPI: number areas, out of 19, in which the patient has had pain over the last week	WPI: number areas, out of 19, in which the patient has had pain over the last week	WPI + Pain in at least 4 of 5 regions within the WPI
CNS symptoms	Not included	SSS: presence and severity of 3 CNS symptoms (fatigue, waking unrefreshed, cognitive symptoms) and somatic symptoms (evaluated by examiner)	SSS: presence and severity of 3 CNS symptoms (fatigue, waking unrefreshed, cognitive symptoms) and 3 self-reported somatic symptoms (headache, pain or cramps in lower abdomen, and depression)	SSS: presence and severity of 3 CNS symptoms (fatigue, waking unrefreshed, cognitive symptoms) and 3 self-reported somatic symptoms (headache, pain or cramps in lower abdomen, and depression)
Comorbidities	Concomitant radiographic or laboratory abnormalities are not evaluated	Subjects must not have a disorder that would otherwise explain the pain	Subjects must not have a disorder that would otherwise explain the pain	A diagnosis of FM does not exclude the presence of other clinically important illnesses.
Characteristics	Not considering CNS and somatic symptoms	Introducing SSS and elimination of tender points	Epidemiological studies advantages: introducing FM symptom scale (WPI and SSS, **c**ut-off 13 out of 31); no need of an examiner.	Excluded the absence of other diseases as exclusion criteria; improved assessment of WPI.

The 2016 ACR diagnostic criteria are now the most used in clinical practice. However, sensitivity and specificity of the 2016 and 2011 ACR FM criteria are similar. The 2011 ACR FM criteria are still commonly used in research settings, due to the practical advantage that participants can complete them independently without the direct supervision of an examiner/researcher, as required for the 2016 criteria[367]. The ACR FM criteria are summarised in table 1.2.2. It is important to note that individuals who do not satisfy the ACR FM criteria can still present a degree of nociplastic pain since this construct is a continuous spectrum of symptoms. Wolfe et al. introduced the concept of "fibromyalgianess" (FMness) which considers nociplastic pain as a condition with different degrees of severity rather than a simplicistic "all or nothing" symptom[369]. FMness can be measured in different ways, the most accredited one is the FM symptoms scale (0-31) derived from the sum of WPI and SSS; the FMness scale is particularly useful in research studies trying to understand nociplastic pain[367]. In some studies, the same FMness scale has been defined as polysymptomatic distress scale[370].

Figure 1.2.3 Widespread pain index and symptoms severity Score



Widespread pain index (WPI) and symptoms severity Score (SSS) are scores used to calculate the 2016 and 2011 ACR FM criteria. WPI ranges from 0 to 19, while SSS range is 0-12. To meet the criteria an individual should have a WPI ≥ 7 and SSS ≥ 5, or WPI 3-6 and SSS ≥ 9. The FM score is the sum of WPI and SSS, is a measure of fibromyalgianess which is a measure of degree of nociplastic pain present; FM score is commonly used in research studies.

1.2.2.2 *Pathogenesis of fibromyalgia*

The pathogenesis of FM is not completely understood, however augmented pain processing and/or reduced inhibitory activity are considered the main mechanisms. These phenomena occur within the nervous system at different levels of the somatosensory system, ranging from those involving peripheral nociceptors to the brain. Although the pain perceived does not reflect tissue damage, increased sensitivity has been often reported after acute injuries and chronic inflammation. Hyperalgesia and allodynia are examples of increased sensitivity following noxious and non-noxious stimuli. Neuroplasticity can explain this phenomenon, commonly defined as central sensitisation[371,372]. Central sensitisation may account for pain expanding to areas of the body that are not directly damaged. Therefore, a peripheral stressor can induce transitory central sensitisation which has the potential to persist in some individuals, leading to FM onset. Both nociceptive, such as inflammation, and neuropathic stimuli, including small fibers neuropathy, have been implicated in FM onset. For example, musculoskeletal diseases such as OA, low back pain and inflammatory arthritis are often associated to FM[373]. Moreover, the inflammatory response can directly and indirectly activate the nervous system in a process known as neuroinflammation, which may ultimately lead to peripheral or central sensitisation[374]. Evidence of reduced dermal nerve fibers diameter has been shown in subjects with FM[375]. This might suggest an additional peripheral trigger to develop nociplastic pain. However, small fibers neuropathy involvement in the FM pathogenesis of remains controversial, because it is strongly associated with metabolic disorders and other chronic painful musculoskeletal condition, frequently comorbid with FM[376]. Pain experts interpret the skin small fibers pathology more as an epiphenomenon of neuroplasticity, rather than a pathogenic trigger of the disease[377].

An initial tissue damage or trauma can facilitate the onset of nociplastic pain syndromes, including FM; however, it does not seem to be necessary for the development of FM. In addition to the “bottom-up” pathogenic hypothesis for FM (peripheral triggers inducing sensitisation), there is also evidence that a “top-down” mechanism is relevant[378,379]. Dysfunctional CNS processes can cause pain independently from peripheral inflammation or tissue damage. In fact, this

mechanism can explain the incongruence between peripheral damage and level of inflammation with reported pain in different rheumatological conditions. These two mechanisms can coexist within the same individual, with distinct prevalence rates. In recent years, advances in neuroimaging techniques have helped to better characterise the neurobiological features of FM and nociplastic pain in general. An increased activation of pain processing areas was initially demonstrated[380,381], followed by altered functional connectivity of specific brain areas, such as hyperconnectivity between IC and the default mode network (DMN), detailed in section 1.2.5[382]. Moreover, increased excitatory (Glutamate) and lowered inhibitory (γ-Aminobutyric Acid or GABA) neurotransmitters have been correlated with pain levels or response to treatment in the same brain regions[383,384]. A dysfunctional pain modulatory system has been shown in FM[385], characterised by an impaired endogenous opioid tone[386,387], reduced Noradrenaline (NA) and Serotonin (5-HT) in the CSF[388], and an altered dopamine response to pain[389]. Since pain perception is a complex phenomenon, it is important to remember that cognitive, psychosocial, mood and autonomic characteristics may all have an impact on FM onset and symptomatology[390–393]. Genetic polymorphisms and epigenetic changes in genes coding for proteins involved in pain perception have been associated with FM and are suggested as predisposing factors[394]. For example, epigenetic studies in FM have revealed an altered methylation profile (a key epigenetic modification) compared to healthy controls in several genes involved in DNA repair, immune system regulation, and nervous system development. Intriguingly, the methylation profile correlated with neurophysiological measures of cortical excitability[395,396]. However, there are no reliable and clinically relevant biomarkers to date. Having a family member with a chronic pain condition is a risk factor for developing FM (recurrence risk ratio 13.6[397,398]) and family clusters suggesting the role of genetic predisposition[399–402]. However, environmental factors (emotional and physical trauma) and behavioural traits shared with families can also contribute to this phenomenon[403,404]; for example, a diagnosis of FM is associated with an OR 2.52 of having had previous traumatic events[403], and childhood trauma was associated with increased pain sensitivity and self-assessment of nociplastic pain in individuals with knee OA[405]. Ultimately, FM is a complex, multifactorial

syndrome where different factors can contribute to its development; to date a single causal factor has not been found.

1.2.2.3 *Clinical manifestations and assessments*

The lack of straightforward pathological causes of primary FM is reflected in the difficulties of diagnosis. Often patients consult multiple different healthcare professionals and the lack of explanation of the origin of their symptoms may lead to patients' distress. Symptoms commonly suggest CNS involvement and are characterised by widespread pain potentially affecting any part of the body, along with increased pain sensitivity on peripheral physical examination. The most commonly described pain features are mixed between nociceptive, dull and sharp, and neuropathic pain, electrical and/or pins and needles sensation[406]. Pain distribution can change over time, and may be aggravated by different mechanical, environmental, and psychological stressors. CNS symptoms are usually reported in association with the widespread pain and include fatigue, sleep impairment, and memory problems, as well as hypersensitivity to external stimuli (i.e. increased sensitivity to light, odours, sounds); the severity of these symptoms varies in in each individual[332,407,408]. Other common symptoms include cognitive dysfunction ("fibro-fog"), regional pain (headaches, bowel and genitourinary tract dysfunctions), autonomic dysfunction, and restless leg syndrome[409–411]. Psychological and psychiatric comorbidities are also frequent in FM[412]; anxiety and depression are more frequent in FM than the general population and can either precede or follow FM onset (antecedent versus consequent hypotheses)[413]. A bidirectional influence is likely to be present considering that inflammatory pathways, neurobiological alterations, and psychosocial factors are commonly shared between the diseases[414]. The assessment of fibromyalgia, as well as the diagnosis, is mainly driven by patient reported symptoms, past medical history, and clinical judgement. However, validated questionnaire can help the health care professionals to assess FM symptoms. The 2011 and 2016 ACR FM score have some clinical validity to be used both as diagnostic tools and symptom monitoring[367,368]. Other comprehensive scores have been validated for the assessment of FM symptoms and impact on quality of life: the FM impact questionnaire, the FM Assessment Status, or the Patient Health Questionnaire, are examples of those[415–417] .

Another questionnaire which has been validated to detect central sensitisation features and assess symptoms severity is the central sensitisation inventory (CSI); CSI is mainly employed in research, but it can be used in clinical practice as screening tool for central symptoms[418,419], also in presence of other chronic pain disorders[420]. Similar to the CSI, the McGill pain questionnaire is useful to provide subjective measure of the pain experienced by an individual[421], however both are more commonly used in research settings. Otherwise, individual symptom domains associated with nociplastic pain, including sleep disturbance, depression, anxiety, and fatigue, can be evaluated with validated scores for each domain. A useful set of validated questionnaires, known as Patient-Reported Outcomes Measurement Information System (PROMIS), is available to assess different qualitative symptoms together or individually. Specifically, the PROMIS sleep disturbance and sleep-related impairment, PROMIS Fatigue, the Multidimensional Inventory of Subjective Cognitive Impairment (MISCI) and PROMIS emotional distress (depression and anxiety) have been validated in FM cohorts and can assess the symptoms severity in clinical practice, and, more commonly, in research studies[422–425]. While the efficacy of assessing individual manifestations is acknowledged, the various scores are not inherently designed to comprehensively capture distinct facets of nociplastic pain syndromes, for example the widespread pain.

1.2.2.4 *Treatment*

The multifaceted pathology of FM, where complex neurobiology is intertwined with emotional-cognitive factors, as well as social and personal experiences, necessitates a comprehensive and multidisciplinary approach for better management of FM[360]. Acknowledgment of patients' symptoms and diagnosis is the first step, followed by the overall target of attenuating, rather than eradicating the pain, which should be discussed with the individual affected, giving priorities to the function for daily living activities and patients' priorities, as suggested in the revised EULAR and NICE recommendations[426 427]. A graduated approach is recommended with emphasis on a multidisciplinary approach prioritising non-pharmacological strategies, successively integrated with pharmacological treatments when required. Patients' education is cardinal where healthy lifestyle should be encouraged as part of a "self-management"

approach, where the individual is empowered to manage their own symptoms in order to improve day to day quality of life. Self-management strategies, including sleep hygiene, relaxation techniques, weight management and regular physical activity should be encouraged to tackle common symptoms associated with pain. Both aerobic and strengthening exercises have been demonstrated to be effective in reducing pain and improve physical function[428]. Cognitive behavioural therapy (CBT) along with other mindful and meditative strategies (mindfulness) and physical therapies (such as acupuncture) have also demonstrated some degree of efficacy in improving pain and function, although conclusive results are lacking[426,429]. If reported pain is severe and reduced quality of life persists, pharmacological therapies should be considered. Antidepressants, e.g. SNRI and amitriptyline, are the most effective options available to date. Evidence of the efficacy of gabapentinoids is limited, however, systematic reviews and meta-analyses as well as the ability to revert altered functional connectivity, support their use off-label in selected cases (discussed in section 1.2.6.3). Other class of drugs, including NSAIDs, corticosteroids or opioids, expose patients to more side effects without evidence of efficacy for FM and therefore discourage their use, unless other underlying diseases would indicate and justify their use.

1.2.3 Psoriatic arthritis pain phenotype

A narrative review approach was carried out to select the current published studies focused on pain and PsA. The keywords used in the search were: psoriatic arthritis, nociplastic pain, chronic pain, fibromyalgia. A total of 367 publications were obtained using the NIH National Library Database, PubMed. After an abstract selection 39 were included, ranging from 2007 to 2023, and discussed in the following sections. Additional published material is integrated in the paragraph on the role of inflammation in chronic pain (1.2.3.2).

1.2.3.1 *Pain persistence in PsA*

Chronic pain is a hallmark symptom in PsA. When asked, patients rate pain as the top priority to improve their quality of life[318,320]. Indeed, reducing or, ideally, halting the chronic inflammatory processes at the joints and periarticular structures may improve pain. In fact, peripheral inflammation represents a direct nociceptive noxious stimulus that can justify the acute and chronic pain experienced by patients. Currently, several therapeutic options are available to control the disease. However, different studies highlighted that around 30% of individuals with good control of the peripheral inflammation continue to report significant pain with a negative impact on quality of life and physical function[317,430,431]. Counterintuitively, the presence and severity of erosive (and degenerative) joint damage is not linearly associated with the reported pain which seems independent from it[432]. This evidence points towards the simultaneous presence of different pain mechanisms, other than nociceptive. Indeed, both neuropathic and nociplastic pain can contribute to perceived pain in PsA. Despite neuropathic pain might have a role in individuals with specific comorbidities (diabetes, spondylosis with associated neuropathy, etc.), the frequency of neuropathic pain does not seem accountable for the inflammation-pain disconnect in PsA[433]. In the DANBIO registry, neuropathic pain was investigated using the PainDETECT Questionnaire (PDQ) in 1,180 subjects with PsA. PDQ was associated with disease activity scores and VAS pain, but not with markers of peripheral inflammation, namely CRP and SJC[434]. However, it is important to consider that neuropathic pain features, as captured by this questionnaire, are often present in nociplastic pain. In different studies the presence of neuropathic pain (PDQ) showed a strong overlap with the presence of FM in PsA (around 28% of all participants), more frequently than in other inflammatory arthritis[434–436]. Therefore, the PDQ can detect overlapping nociplastic pain and overestimate neuropathic symptoms, if FMness is present. These data suggest that neuropathic pain features may represent a predisposing factor to develop central sensitisation and, consequently, an overlapping nociplastic pain state. The lack of evidence for nerve damage or CNS-related symptoms can assist in the complex differentiation of the two pain mechanisms.

On the other hand, nociplastic pain is a better candidate to explain pain persistence in PsA. This hypothesis is supported by several strands of evidence. Classically, nociplastic pain is characterised by disconnect from peripheral inflammation and peripheral tissue damage. FM is more frequently diagnosed (using different ACR FM criteria) in individuals with PsA compared to the general population (estimated around 1.78%)[437]; the frequency estimated ranges from 9.3% to 38.3% in different cohorts; overall, the incidence is higher in the female sex and in the polyarticular phenotype[438–443]. In general, FM is also present in the 10-20% of patients with SpA, including PsA[373,442,444]. In a recent study, central sensitisation was evaluated with CSI in a PsA cohort, showing a slightly higher frequency (up to 42.9%) of nociplastic pain and a negative impact on functional disability and measures of disease activity[445]. Recently, it was demonstrated that pain reported by individuals with PsA on the WPI body map extends beyond the involved joints in 20-35% of patients, similarly to a FM control group; the widespreadness of pain was significantly correlated with disease activity and disability scores[446]. Although the presence of pain itself is not associated to increased mortality[447], the concomitant presence of FM was correlated with worst clinical outcomes (SJC, TJC enthesitis, and BASDAI), with poorer quality of life, higher fatigue and reduced efficacy of biologic drugs[441,440,448–452,439]. It is important to take into consideration that the disease activity scores in PsA often incorporate VAS pain and tenderness of joints which may overestimate final scores, when nociplastic pain is present.

1.2.3.2 *Inflammation in chronic pain*

The frequency of nociplastic pain syndromes is higher in individuals affected by a chronic inflammatory condition. When compared to the general population, the prevalence of FM is higher in patients affected by chronic inflammatory arthritis (detailed in section above), e.g. RA and PsA, or other system inflammatory/autoimmune syndromes, such as SLE[453]. Therefore, individuals with a chronic inflammatory condition appear susceptible to experiencing persistent pain, frequently involving mechanisms of nociplastic pain; collectively, the co-existance of inflammatory nociceptive and nociplastic pain characterises mixed pain states. The peripheral inflammation in arthritis represents a nociceptive input sustained over time which may lead to peripheral

and CNS neuroplastic changes, i.e. to sensitisation, in predisposed individuals[454]. This phenomenon might explain the higher prevalence of nociplastic pain in inflammatory arthritis populations. The immune system appears able to influence the somatosensory system at different levels from nociceptors to the brain[455].

Local inflammation, for example synovitis or enthesitis in PsA, is characterised by the release of pro-inflammatory mediators acting directly on nociceptors (e.g the transient receptor potential subfamily V member 1 [TRPV1]) or indirectly following tissue damage[456,457]. Nociceptors and neural bodies in the DRG express receptors for pro-inflammatory cytokines known to be involved in the pathogenesis of inflammatory arthritis, i.e., TNF, IL-17, IL-6 and IL-1β. Pro-inflammatory cytokines induce the expression of the NGF and, consequently, of neuropeptides, which act in synergy to increase nociceptor responsiveness[458,459]. Growing evidence supports the remarkable role of pro-inflammatory cytokines, including IL-17A and TNF, in peripheral and central sensitisation. For example, IL-17 appeared to be involved in the hyperalgesia associated with neuropathic and mechanical pain in animal models; this effect seems mediated by direct interactions with the peripheral nervous system which lead to enhanced neuroplasticity[460–464]. Moreover, IL-17 was able to sensitise the peripheral joint nociceptors in animal models of inflammatory arthritis, contributing to pain independently from the severity of the inflammatory arthritis[465,466]. Also TNF may contribute to neuroimmune interactions at the periphery. In fact, TNF can influence the synaptic transmission of the DRG and spinal cords neurons, sustaining peripheral and central sensitisation in animal models[467–471]. The persistence of TNF-mediated DRG neuroinflammation has been suggested to induce prolonged hyperalgesia after the resolution of joints inflammation in an animal model of inflammatory arthritis[472]. The neuro-immune interactions are bidirectional; neuropeptides can increase local blood flow, thereby facilitating the recruitment of immune cells, and activate them to express pro-inflammatory cytokines[473]. Innate and adaptive immune cells, mostly macrophages and T-cells can reach the DRG to release pro-inflammatory mediators nearby the neural bodies of nociceptors. At the intersection between the immune and nervous system there are the glial cells which have a putative central role in neuroinflammation. Glial cells are specialised macrophages resident within the

nervous system; the physiological role of glia is to sustain and protect the neuronal cells. Glial cells contribute to the pain sensitisation by releasing both pro-inflammatory mediators and neurotrophic factors, such as NGF[474]. The short-term effect of inflammation is increased nociceptor responsivity and activity resulting in local hypersensitivity, e.g. hyperalgesia[1475]. When inflammation is sustained over time, nociplastic changes can occur, resulting in peripheral sensitisation and increased pain sensitivity, potentially uncoupled from inflammation.

Systemic inflammation can also alter pain perception in individuals with chronic pain.The CNS is an immunologically privileged organ, with immune cells and cytokines are carefully “filtered” by the blood-brain barrier (BBB). However, some cytokines can pass the BBB and directly modulate the central sensory pathways. There are three ways circulating immune system cells and soluble mediators can reach the CNS: 1) selective permeability of the BBB; 2) choroid plexus and circumventricular organs that allow the passage to the CSF; or 3) retrograde migration throughout the vagus nerve[455]. Pro-inflammatory cytokines, including TNF, IL-1ß, IL-6 and IL-17, may have a direct excitatory effect on pain processing brain regions, as well as a stimulatory action on microglia and astrocytes within the brain[476]. For example, in animal models of migraine, IL-17 was a key mediator of neuroinflammation by passing the BBB and inducing hyperalgesia and migraine attack[477]. Chen and colleagues also demonstrated the direct neuro-modulatory properties of IL-17A on neurons and interneurons able to influence sensory and behavioural responses in a C. Elegans model[478]. TNF is a pivotal pro-inflammatory cytokine and has been directly linked to brain changes after peripheral nerve injury, moreover, the presence of TNF in the brain has been associated with increased neurotoxicity and central sensitisation in different animal models[479,480]. Intriguingly, TNF seems to also influence the psychological phenotype and affective components of pain (e.g., anxiety, depression)[481–483]. The glial cells within the CNS also contribute to the release of pro-inflammatory cytokines and chemokines centrally. Ultimately, the resulting increased firing activity of neurons can induce neuronal plasticity changes characteristic of central sensitisation[330]. Animal models have demonstrated that systemic inflammation can induce hyperalgesia and plethora of central symptoms, known as “sickness behaviours”, including fatigue, sleep impairment,

cognitive dysfunction and mood disturbances[484,485]. These symptoms are characteristic of nociplastic pain syndromes, such as FM. Moreover, a recent study demonstrated that treatment with serum immunoglobulin from individuals with FM, as opposed to healthy, can induce sensory hypersensitivity in mice [486]. Despite these results need to be further validated, suggest an additional potential mechanism of immune-mediated sensitisation of the somatosensory system.

Notably, the interactions and directionality of immune-nervous systems are yet to be fully understood. While somatosensory sensitisation by peripheral and systemic activation of the immune system may occur, the resolution of inflammation does not guarantee the resolution of chronic pain, supporting an uncoupling between nociplastic pain and inflammation. Nonetheless, both peripheral and systemic inflammation can still contribute to nervous system sensitisation and can act in association with genetic and psychosocial factors; altogether these factors collectively play a role in each individual susceptibility to develop a nociplastic pain state[487]. Indeed, chronic inflammatory conditions, including inflammatory arthritis, and their association with chronic pain states represents an intriguing and unique opportunity to investigate neuro-immune interactions and their contribute to the development of chronic pain as symptom.

1.2.3.3 *Inflammation in inflammatory arthritis*

Inflammation may indeed contribute to induce nociplastic pain in predisposed individuals or aggravate an already established nociplastic pain syndrome. Inflammation can interact with the nervous system via different mechanisms. At the periphery, pro-inflammatory mediators and neuropeptides are able to prompt nervous system sensitisation[488,489]. In the CNS, circulating pro-inflammatory mediators can sensitise the nervous system via various mechanisms leading to consistent behavioural responses, i.e. sickness behaviour[490]. In RA, has been demonstrated that levels of peripheral inflammation measured with ESR are associated with alteration in the connectivity of intrinsic connectivity networks and pain processing regions and a reduction in the grey matter volume of left inferior parietal lobule (IPL), observed in individuals with primary

FM[491,492]. From these observations stems the idea that tackling the inflammation in inflammatory arthritis might protect from CNS sensitisation and possibly improve nociplastic pain, once developed. In fact, advanced immunotherapies showed to have a benefit on pain, as well as features associated with CNS involvement, such as fatigue and depression. The cytokines keys in the pathogenesis are selectively targeted by bDMARDs and tsDMARDs currently available to treat PsA. The anti-TNF adalimumab showed approximately 5-times greater efficacy in reducing pain when compared to placebo[493]. Similarly, Secukinumab demonstrated to reduce long-term reported pain in a dose dependent fashion[278,494]. Also, the inhibition of the Il-23 pathway with ustekinumab and risankizumab demonstrated superiority on the placebo to reduce pain, fatigue and other PROs[495–497]. Inhibition of the intracellular pathways of multiple cytokines with JAKi demonstrated to be more effective in reducing pain. Tofacitinib and upadacitinib demonstrated to have sustained and quicker pain relief than placebo in patients with PsA[309,310,498].

1.2.3.4 *Clinical challenges in evaluating pain in PsA*

A clinical manifestation characteristic of PsA is the periarticular involvement, i.e. dactylitis and enthesitis. Symptoms associated with periarticular manifestations are typically characterised by difficult to localised pain. The clinical widespread pain can mimic FM, for example, resembling the tender points used in the early 1990 ACR FM classification criteria[499]. Consequently, individuals with PsA having a concomitant FM showed to have high enthesitis clinical scores[451]. A clinical distinction between the FM and enthesitis can be difficult in normal clinical settings. Ultrasound investigations have been suggested as a reliable objective investigation able to distinguish between enthesitis involvement and FM-associated widespread pain[500–505]. However, the examiner's experience and availability of ultrasound scanners represent limitations in the regular use of ultrasound in normal clinical settings. Not surprisingly, the clinical distinction and the decision towards the best management represent a challenge for health care professionals. PsA has a significant burden on the quality of life of the individuals affected[320]; the higher frequency of depression (up to 51%) and anxiety (61%) in PsA when compared with healthy subjects may contribute to this[506,507]. Anxiety and depression have

been deemed to be higher in PsA as a reflection of the link between inflammation and states of mental illness[508,509]. Surely, the detrimental effects on physical function and quality of life are likely to contribute to development of chronic pain and, in particular, of nociplastic pain syndromes. Further, anxiety and depression have an impact on disease activity scores and early discontinuation of anti-TNF drugs in PsA[506,510,511].

1.2.3.5 *Bridging Clinical Observations and Neurobiological Evidence*

PsA is characterised by a predominant nociceptive pain state as result of chronic inflammation. However, overlapping nociplastic pain appears to aggravate the pain and prolong pain duration beyond resolution of the inflammation. The frequencies of FM and pain persistence in PsA are similar. When FM is present, widespread pain, disease activity scores and quality of life are commonly worse. Higher pain and disease activity can lead to improper treatment escalation or investigations. Inflammation and mood disturbances are risk factors contributing to central sensitisation and development of nociplastic pain syndromes in PsA. While the clinical challenges in evaluating pain in PsA provide crucial insights into the complexity of pain manifestations, a significant gap persists between these valuable observations and the concrete neurobiological evidence needed to substantiate them. The existence of co-morbid nociplastic pain in PsA, suggested by the intricate interplay of inflammation, nociplastic pain, and mental health factors, requires objective validation to better understand the underlying mechanisms. To bridge this gap, it becomes imperative to employ objective measures that can shed light on the intricate neurobiological dynamics at play. Quantitative Sensory Testing (QST) and functional neuroimaging techniques, emerge as potent tools in the quest to uncover the objective neurobiological basis of pain in PsA. These advanced methodologies offer the potential to delve deeper into the underlying pain mechanisms and the interplay between peripheral inflammation and central sensitisation. The following sections delve into the QST and functional neuroimaging techniques to better understand how effectively use them to improve our understanding of nociplastic pain mechanisms in PsA.

1.2.4 Quantitative Sensory Testing

1.2.4.1 *Introduction*

QST is a comprehensive and validated set of sensory examinations able to assess the integrity and function of the central and peripheral somatosensory system[512]. Standardised protocols have been developed to evaluate and quantify the sensory responses to different stimulations, including mechanical (e.g., vibration, blunt or pinprick pressure), thermal (cold/heat), chemical (e.g., capsaicin), electrical, as well auditory, visual, odour, and gustative. The skin is the most assessed organ, however, deeper structures, such as tendons, muscles, and visceral organs can be investigated in QST protocols, including specific visual and acoustic stimulations[513,514]. QST is useful to explore central sensitisation manifestations more accurately than classical physical examination (e.g., tender points).

QST are divided in static and dynamic, based on the type and quality of the stimuli applied:

1. Static QST consists of stimuli with controlled intensity applied to selected body regions. With the increasing or decreasing of the intensity, it is possible to determine individuals' pain thresholds and/or tolerance to a specific modality. Overall, sensory hypersensitivity corresponds to reduced pain thresholds and tolerance. In contrast to localised hyperalgesia in areas injured or inflamed, a diffuse hypersensitivity in otherwise undamaged body areas, alludes to the presence of the nociplastic pain mechanisms[515]. Similarly, visual and acoustic hypersensitivities are associated to central sensitisation processes[516].

2. Dynamic QST is used to investigate somatosensory processes facilitating or inhibiting pain. Dynamic sensory testing can involve repeated stimulations over time or in progressively larger areas (i.e., temporal and spatial summation), or simultaneous stimuli in different body areas, such as in conditioned pain modulation (CPM). In dynamic testing, the variations in pain thresholds or tolerance detected reflect an altered sensitivity, either due to an increased excitatory activity (e.g., wind-up phenomenon) and/or a reduced inhibitory

control (CPM). Further, pain summation is considered the psychophysical correlate of the wind-up phenomenon (discussed in section 1.2.1.2) and it is commonly present in chronic pain conditions as an expression of nervous system sensitisation[517-519]. Conversely, CPM testing assesses the overall integrity of e descending pain inhibitory mechanisms by quantifying the variation of sensory perception following a noxious stimulus in a body area before and during a second stimulus in a remote area (with the same or different stimulus modality). In healthy subjects, the second stimulus prompts the descending inhibitory pathways resulting in reduced pain/discomfort. An impaired CPM (i.e., lack of pain reduction) is evident in different chronic pain conditions, including FM[520,521].

Overall, static QST manifests consistent results and acceptable reproducibility in healthy subjects, while dynamic testing, particularly CPM are more variable, justifying the more common use of the former in chronic pain research[522]. Of note, QST evaluates the contribution of the entire somatosensory system. Individual characteristics, including age, sex and gender, as well as psychological, cognitive-emotional factors[523], stress[524-526], and physical fitness[527] can influence QST results. For example, the examination environment (room setting), expectation (anticipation), and even the examiner's sex can have a differential impact[528]. Therefore, it is important to keep in mind confounders when interpretating the QST in both healthy subjects and patients. The QST application in normal clinical practice remains challenging due to limitations associated with assessment time, availability of instruments, and unclear predictive value, despite significant efforts to create standardised protocols in specific clinical conditions (mainly neuropathic pain[529]). However, QST represents an essential and unique research tool to help characterise pain phenotypes and to investigate pathophysiology of pain[530].

1.2.4.2 *Quantitative sensory testing in fibromyalgia*

Using QST protocols with noxious and non-noxious stimuli and different modalities, several studies have demonstrated a characteristic increased sensitivity and an impaired CPM in FM, when compared to healthy or other chronic pain conditions (table 1.2.3). Consistently, mechanical (including sharp,

blunt, and vibration) stimuli applied to painful and non-painful body areas showed widespread hypersensitivity and mechanical allodynia in individuals with FM, when compared to age-matched healthy subjects (and chronic low back pain)[531-534]. Several studies also have shown an increased sensitivity to thermal (cold and heat) and electrical stimuli in FM compared to heathy subjects[532-535]. Additional studies also support an impaired endogenous pain modulation in FM characterised by increased temporal summation (TS) and defective CPM[520,521,534,536-538]. Importantly, the hypersensitivity observed in FM is not restricted to painful stimuli but also involves other sensory inputs. These include acoustic[539], visual[540], odour[541,542], and taste[543]. Such characteristics can be considered a clinical epiphenomenon of central sensitisation. Taken together, QST is a valuable tool in assessing nociplastic pain conditions as demonstrated by the increasing pressure pain tolerance (PPTol) and thresholds (PPT), and improvement of CPM and TS following different pharmacological treatments, such as pregabalin and ketamine (a NMDA-antagonist)[544,545] . Moreover, PPT and TS were able to predict the response to non-pharmacological therapies, respectively, Emotional Awareness and Expression Therapy and acupuncture[546,547].

Table 1.2.3 Quantitative Sensory Testing evidence in fibromyalgia

QST	Modality	FM	Response to treatment	Change after treatment
Static	Pressure	↓PPT ↓PPTol (widespread)	↑PPTol ↑to EAET, no to CBT ↓PPTol ↑to CBT and EAET	↑PPT pregabalin ↑PTTol ketamine
	Temperature	↓ HPT ↓CPT	-	-
	Electrical	↓EPT	-	-
	Visual	↑VUR	-	-
	Acoustic	↑AA	-	-
	Odour	↑OA ↓TDI	-	-
	Taste	↓GF	-	-
Dynamic	TS*	↑	↑TS ↑ to acupuncture	↓ TS ketamine (electrical) ↓ TS acupuncture (pinprick)
	CPM*	↓	-	↑CPM pregabalin

AA: auditory augmentation; CBT: cognitive behavioural therapy; CPM: Conditioned Pain Modulation; CPT: Cold Pain Threshold; EAET: Emotional Awareness and Expression Therapy; EPT: Electrical Pain Threshold; FM: Fibromyalgia; GF: gustatory function; HPT: Heat Pain Threshold; PPT: Pressure Pain Threshold; PPTol: Pressure Pain Tolerance; OA: olfactory acuity; TDI: threshold, discrimination, and identification of odours; TS: Temporal Summation; VUR: visual unpleasantness ratings. * Different modalities can be used for CPM and TS.

1.2.4.3 *Quantitative sensory testing in psoriatic arthritis*

Pain is a key symptom in chronic musculoskeletal diseases and QST is a useful tool to investigate the underlying mechanisms responsible for the reported pain. In fact, in individuals with chronic musculoskeletal pain (neck and shoulder pain) the presence of mechanical and thermal hyperalgesia at the level of the hands, using a simple QST protocol, predicted the pain severity in more than 49% of participants; FM participants and healthy volunteers were used as controls[548]. In another study, subjects affected by OA or RA, but not FM, showed thermal and mechanical sensitivities similar to healthy subjects, as opposed to individuals with FM and complex regional pain syndrome. Interestingly, adults with RA, compared to pain-free subjects, showed reduced pressure pain threshold and tolerance only at articular areas, typically the target of chronic inflammation[549,550]; while wrist PPTs were lower in the relation to raised inflammation (CRP). Diffuse joints pain (i.e., TJC) and central symptoms (sleep) were associated with reduced threshold in non-joints areas (trapezius and thumbnail)[551]. Overall, the above data support the hypothesis that peripheral inflammation generates peripheral sensitisation (local hyperalgesia), and only individuals with overlapping nociplastic pain would demonstrate widespread hypersensitivity in extra-articular sites. A recent study including a large RA cohort with active disease, demonstrated a weak but significant correlation between QST and FM score, used as surrogate of central sensitisation[552].

According to a recent systematic literature review, QST studies in inflammatory arthritis mainly focused on RA, and axial SpA. Only a single study included individuals with PsA (a study evaluated as fair in quality according to NIH Quality Assessment tool) (table 1.2.4)[553]. Static QST using mechanical stimuli was the most commonly applied, as considered optimal for reproducing the pain associated with arthritis and because of easier execution compared to dynamic QST. As mentioned before, cross-sectional studies including RA participants demonstrated lower PPT and PPTol at the level of joints areas in comparison to healthy controls[549,554]. Interestingly, lower PPT were associated with pain levels, and with disease activity scores (DAS-28, Clinical Disease Activity Index [CDAI]),

TJC, PGA, and depression (Beck depression index score)[551,555-557]. To date, only one study showed an increased heat sensitivity in RA compared to healthy; however, due to the limited data available it is not possible to draw conclusive remarks regarding thermal sensitivity in this population[550]. TS seems to be increased in RA compared to healthy suggesting ongoing central sensitisation[558]. Despite some studies suggesting a direct association between TS and pain levels, VAS global and disease activity (CDAI and TJC), an increased pain summation alone was not able to predict response to DMARDs in different studies[555,557,559]. However, enhanced TS in presence of concomitant weaken CPM was demonstrated to have a negative predictive value for treatment response[560]. In the same study, RA individuals with impaired CPM were less likely to achieve a good response to treatment[560]. In another study, the CPM efficiency in RA subjects was lower compared to pain-free volunteers. The impaired CPM in RA has been linked to sleep disturbances[549], which are part of the key symptoms of nociplastic pain and a manifestation of nervous system sensitisation[561].

Only one cross-sectional study, which also included RA (n=50), AS (n=23), and healthy subjects (n=28), investigated the PPTs in PsA. Specifically, this study evaluated depression. A concomitant diagnosis of FM was an exclusion criterion. Depression was associated with lower PPTs evaluated with QST in PsA, as well as in RA and AS. Differently to AS, PPTs in RA and PsA individuals were lower than in controls. PPTs in all groups were not associated to pain intensity (VAS), however they were inversely correlated with depression levels (Hamilton Depression Rating scale). Compared to RA and AS, PsA subjects had higher depression scores and the correlation with the PPT was stronger compared to the other disease groups (PsA $r=-0.69$, $p<0.0002$; RA $r=-0.41$ $p=0.002$; AS $r=-0.45$ $p=0.02$) [554] . These data are interesting in respect to: 1) the reduced PPTs in PsA compared to controls, interestingly, not associated with pain VAS; 2) the strong association between depression and PPTs in PsA, probably due to the shared pathogenetic cytokines and the mutual influence on pain.

Additional data are available in axial SpA cohorts. Interestingly, reduced PPTs were more pronounced than healthy controls and were associated with higher disease activity and presence of enthesitis[562,563]. Moreover, in axial SpA subjects, lower PPTol and higher TS were also linked to disease activity, symptoms

duration, widespreadness of pain, fatigue, disability and anxiety[563]. Only one study explored CPM in axial SpA. It observed a significant reduction inversely associated with CSI[562].

To date, there is limited evidence available to support the presence of sensitisation of the nervous system in PsA. However, the current QST data available in SpA and RA point support a possible role of nociplastic pain in the PsA pain persistence, encouraging more studies to better characterise the pain phenotype in PsA. The inclusion of modalities characteristically linked to central sensitisation, i.e., visual, acoustic, and olfactory stimuli, would be useful to better distinguish the active pain mechanism in the cohort examined. Despite the limitations, QST represent a useful tool to characterise the pain phenotype in musculoskeletal chronic pain conditions, including inflammatory arthritis, relying on the ability to detect objective measures of peripheral and central sensitisation.

Table 1.2.4 Quantitative Sensory Testing evidence in inflammatory arthritis.

QST	Modality	RA	PsA	SpA (axSpA, AS)	Response to DMARDs	Association with clinical parameters
Static	Pressure	↓PPT (joints areas)	↓ PPT	↓/= PPT	-	RA: FMS, VAS pain and global, DAS-28, CDAI, TJC, depression; PsA: depression; SpA: depression, disease activity scores, enthesitis.
		↓ PPTol (joints areas)	-	↓ PPTol	-	SpA: symptom duration, higher disease activity, widespread pain, unacceptable pain, enthesitis, fatigue, disability, anxiety.
	Temperature	↓HPT ↓CPT	-	-	-	-
Dynamic	TS*	↑	-	↑	↑TS + ↓CPM	RA: VAS pain and global, CDAI, TJC,
	CPM*	↓	-	↓	↓CPM	RA: Sleep impairment; SpA: CSI

CDAI: Clinical Disease Activity Index; CPM: conditioned Pain Modulation; CPT: Cold Pain Threshold; CSI: central sensitisation inventory; DAS-28: Disease Activity Score-28; FMS: Fibromyalgia Score; HPT: Heat Pain Threshold; PPT: Pressure Pain Threshold; PPTol: Pressure Pain Tolerance; TJC: Tender Joints Count; TS: Temporal Summation; VAS: Visual Analogue Scale.
* Different modalities can be used for CPM and TS.

1.2.5 Multimodal MRI

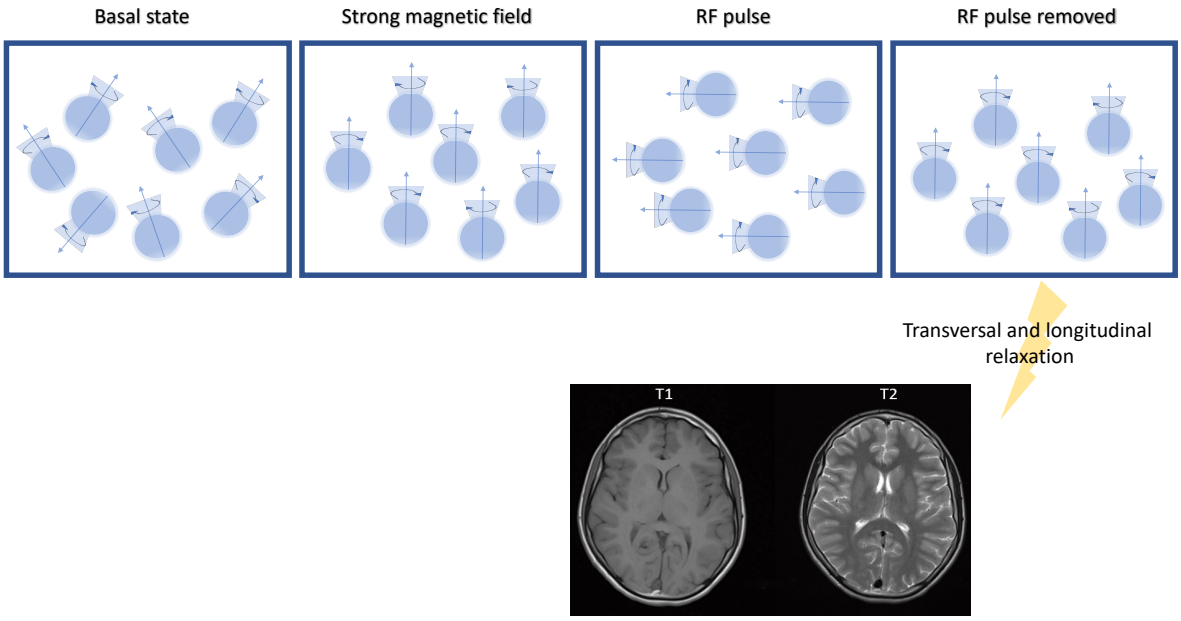
1.2.5.1 *Introduction*

Pain is subjectively perceived after the integration of sensory, affective, and cognitive information in the brain. Better understanding brain functioning is helpful to better comprehend pain mechanisms. In humans, investigating the CNS has been limited due to the practical safety constraints of tissue sampling, and findings from animal models are uncommonly translated to humans. In the last decades, advances in neuroimaging techniques have helped to overcome the experimental challenges in neuroscience and provided insight into the physiological and pathological pain processes within the CNS. Non-invasive neuroimaging methodologies provide multiple measures, including brain morphology, function (e.g., brain activity related to a specific task and/or functional connectivity), and chemical features, allowing researchers across different disciplines to have a better insight on brain functioning.

The basic principles of MRI are based on the alignment of the magnetic movements of the body's hydrogen molecules when exposed to a strong magnetic field. MRI scanners are equipped with an electromagnet that generates a significantly stronger magnetic field than the gravitational magnetic field of the Earth (0.00005 Tesla [T]), usually equal to 1.5, 3, and more recently 7 T. Hydrogens nuclei consist of a single proton that has a magnetic moment. The magnetic moment defines strength and direction of a magnetic field, and it is characterised by a vectorial direction (similar to a compass needle) and a spinning direction, or phase. In a strong magnetic field protons' magnetic vectors align generating the, so called, longitudinal magnetisation. Successively, a radio frequency pulse (RFP) is applied. The RFP has two effects: 1) alignment of the protons' phases (transversal magnetisation), and 2) "bending" the magnetic moment directions, reducing the previously established longitudinal magnetisation. When the RFP is removed its effects disappear, generating transversal and longitudinal relaxation. During this process a signal is released, and it can be detected by a receiving coil, usually applied around the body area of interest. Time constant 1 (T1), is a descriptor of the increasing longitudinal relaxation, while time constant 2 (T2) is a measure of the transversal

magnetisation reduction (figure 1.2.4). Body tissues have different relaxation times. Modulating the frequency of the RFP and the timing of signals detection, it is possible to emphasise the contrast for different tissues [564]. T1-weighted acquisitions have high spatial resolution and low temporal resolution, enhancing contrast for solid tissues; T2-weighted sequences, have low spatial resolution, but high temporal resolution and emphasise fluid-rich tissues. Therefore, structural and morphological information are of higher quality in T1-weighted sequences, while CSF and blood vessels are better investigated using T2-weighted MRI images (figure 1.2.4).

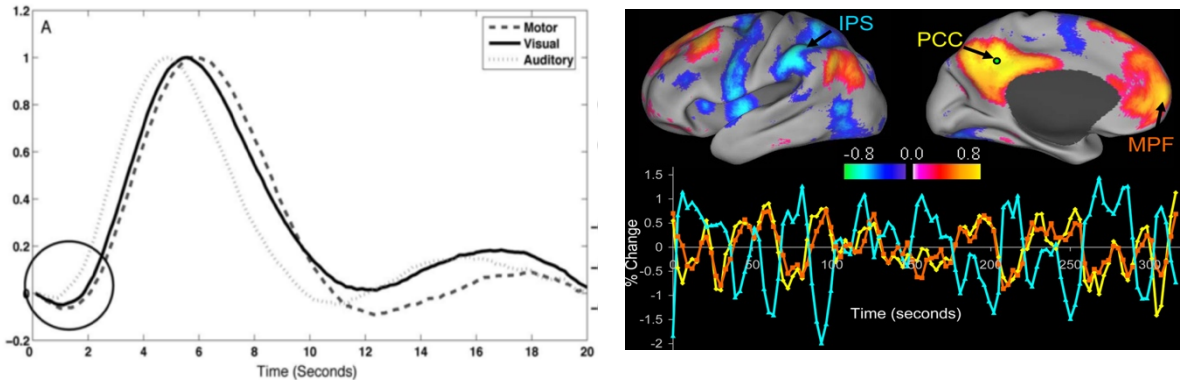
Figure 1.2.4 Fundamental physic principles of Magnetic Resonance Imaging



Legend: magnetic moments of hydrogen molecules (protons) are represented as a vector (arrows) pointing in the direction of the magnetic field generated by the spinning nuclei (phase). At basal conditions, protons have different vectorial directions and spinning phases. In a strong magnetic field proton magnetic moment aligns, but they preserve different phases (longitudinal magnetisation). A radiofrequency pulse (RFP) is applied at different frequencies and causing the magnetic moments to "flip over" and align the phases (transversal magnetisation). When the RFP is removed the nuclei return to the previous longitudinal magnetisation (longitudinal relaxation), and the phases realign (transversal relaxation). Transversal and longitudinal relaxation emit energy which detected by a coil, place around the body area of interest. T1-weighted and T2-weighted sequences put the emphasis on longitudinal relaxation and transversal relaxation, respectively, depending on the RFP frequency and timing of signal detection. Using on the signals detected it is possible to reconstruct the images.

A specific T2 acquisition technique, the T2 star (T2*), combines T2 properties and the influence of nearby local molecules on transversal relaxation (dephasing inhomogeneities). T2*-weighted sequences are sensitive to tissue oxygenation; In fact, haemoglobin has different magnetic properties depending on its oxygenation status: oxygenated haemoglobin (oxHb) increases, while deoxygenated haemoglobin (deoxHb) decreases the T2* MRI signal[565–567]. Therefore, using T2* sequences, it is possible to investigate oxygenation levels across the brain. Oxygenation levels reflects the metabolic demand of the group of neurons present in a certain brain area and it has been demonstrated to be an indirect, but reliable, measure of neuronal activity[568–572]. The most common approach used in functional MRI (fMRI) is the blood oxygenation level dependent (BOLD) contrast which is a measure of the ratio between oxHb and deoxHb. The use of the BOLD fMRI technique has increased significantly in the past 2 decades in neuroimaging research. This in part relates to the superior spatial and temporal resolution, when compared to other acquisition techniques, allowing metabolic activity to be measured in millimetric cubic units of the brain, also known as voxels, over time[383,573]. Approximately every 2 seconds an image is acquired for an experimentally determined period of time (usually minutes). The hemodynamic response function (HRF) is the changing over time of the BOLD signal corresponding to the neuronal firing activity that requires oxygen, in each voxel of the brain with each scan containing approximately 100.000 voxels. (figure 1.2.5)[574]. Based on the HRF, it is possible to compute the correlations between signals from different brain areas and to infer if 2 or more areas are activated at the same time, and therefore functionally connected, or viceversa uncoupled. Thus, functional connectivity is an indirect association between 2 or more brain areas, based on the correlation of BOLD signals across time, and it aims to describe how these interactions associates with the experimental conditions, tasks, or cognitive and behavioural measures.

Figure 1.2.5 Hemodynamic response function and correlations between a seed region and other brain voxels.



Legend: the image on the left from Zhang and colleagues[574] shows changes in BOLD signals triggered by neuronal activity, known as the hemodynamic response function (HRF). For short activatory events, it is possible to observe an initial signal reduction (*initial dip*), secondary to rapid oxygen consumption, followed by peak in the signal (4-6 secs after activation) that reflect a compensatory increasing of the local blood flow. Within approximately 12 secs, the BOLD signal decreases to the baseline level. The magnitude of changes is small, delayed, and slow compared to the neuronal firing activity (milliseconds); however, BOLD signals changes are reliable to detect the metabolic needs of neurons to perform the firing activity. The image on the right shows the work from Fox and colleagues[575]. A seed region in yellow BOLD changes (in yellow) is correlated with all brain voxels. Areas with similar activity patterns are similar to the red line and are positively correlated. Conversely, anticorrelation is shown in light blue.

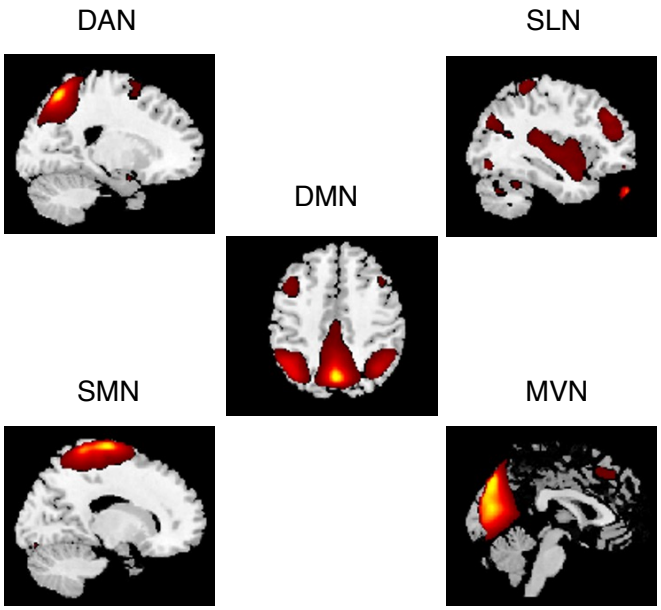
To quantify the brain functional connectivity, seed-based analysis is commonly used and often integrated with more advanced analysis such as, inverse covariance and independent component analysis (ICA). Seed-based connectivity is a bivariate analysis that informs the activity of a seed region, i.e., a predetermined region of interest (ROI), and another ROI (ROI-to-ROI) or all the other voxels of the brain (seed-to-voxel)[575–577] determining the regions which activity correlates with the seed (figure 1.2.5). Seed-analysis utilises correlations between time series from 2 separate brain regions obtaining a r value; for each subject it is possible to conduct a fisher transformation of the r values, obtaining normally distributed z-scores, and successively perform a group analysis[578–580]. Psychological, cognitive or pain parameters, for example, FM scores for nociplastic pain, can be associated with the z-scores for connectivity between 2 brain areas. The correlation between connectivity and the parameter of interest can be calculated[492]. It is also possible to correct for factors that confound BOLD signals, for example, sex, age, or heart rate and respiratory frequency. This is usually performed using regression methods[581]. More advanced analysis, such as ICA, can help to carry out data driven analysis investigating

multivariate brain connectivity, where the seed region(s) is tailored on the group of participants studied[582,583]. These methods allow a more exploratory and agnostic approach and can help in creating prediction models, for example, if the activity and connectivity of specific areas of the brain can predict the level of pain perceived.

FMRI data can be acquired not only when a subject is performing a certain task (task-based fMRI), but also at rest, investigating the brain spontaneous activity. Resting state fMRI (rs-fMRI) approaches aims to identify synchronous low frequency (0.01-0.08 Hz) BOLD signals in multiple brain regions independently from the anatomical distance, while the subject is not performing a specific task (either with eyes open or close)[584,585]. The rs-fMRI gained popularity in neuroimaging in the last decade. The advantages of this approach are that resting state scans can have a relatively short duration (5-10 mins) and there is no need for a specific experimental design or subject training (higher compliance), allowing comparisons across different studies and research centres[586-588]. Consistent results from rs-fMRI showed that spontaneous fluctuation of neuronal activity in certain specific areas evidenced a strong correlation between each other at rest, across different individuals and in different clinical conditions[589,590].The intrinsic functional connectivity between brain areas forms the so-called resting state networks or intrinsic connectivity networks (RSNs or ICNs). ICNs are believed to integrate and coordinate differently the specialised brain regions which support core somatosensory and cognitive functions[591,592]. ICNs seems to be hierarchically organised, with the default mode network (DMN) being the most prominent[593,594]. The DMN includes several interconnected brain areas including the mPFC, posterior cingulate cortex (PCC), precuneus, IPL, hippocampal formation and lateral temporal and parietal cortex[595,596]. The DMN is active by “default” at rest and showed to be involved in self-reflection, mind wandering, and other cognitive tasks[597]. Among others ICNs the Dorsal Attention Network (DAN) (classically anti-correlated with the DMN), Salience Network (SLN), Somatomotor Network (SMN), and the medial visual network (MVN) are enlisted (figure 1.2.6)[591]. ICNs connectivity is believed to be altered by previous co-activation history and therefore represents an expression of brain plasticity in response to environmental stimuli[598]. Interestingly, brain areas functionally connected at rest often show similar

activation patterns and ICNs can be upregulated or downregulated during specific tasks[587]. An average of the BOLD signals from regions within an ICN can be used as ROI (or seed) in functional connectivity analysis[575,580].

Figure 1.2.6 Intrinsic connectivity networks-DMN

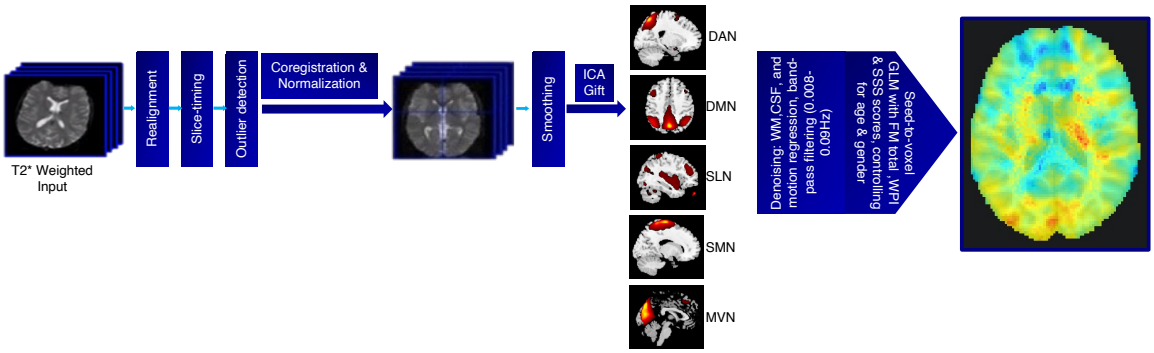


DAN - Dorsal Attention Network; DMN - Default Mode Network; SLN - Salience Network; SMN – Somatomotor Network; MVN - Medial Visual Network.

Overall, the data generated in fMRI scans is extensive and complex, demanding careful experimental planning and a predetermined data analysis pipeline to generate reliable results. Data processing pipelines in fMRI usually entail 4 main parts (figure 1.2.7): 1) data acquisition, 2) reconstruction, 3) pre-processing, and 4) data analysis. Data acquisition is influenced by the strength of the field, as well as other acquisition parameters, such as, the pulse sequence, tissue of interest, and outcomes variables. The reconstruction of the images is obtained via the transformation of the signals in brain images and other signals (for example, BOLD signals) essential for the data analysis. Following, the pre-processing has the aims of minimising artifacts. Usually, the location of brain structures across different subjects is standardised using localisations from open-source brain atlas. Pre-processing also encompasses the transformation and scaling of the data, and the frequency filtering for resting state. Lastly, the data analysis is based on different analytical approaches including seed-analysis, beta

analysis, or general linear model and ICA, depending on the research strategy and question.

Figure 1.2.7 Example of functional connectivity data pipeline.



Legend: The figure provides an example of pre-processing protocol of fMRI imaging data. A pre-processing pipeline is necessary before proceeding to fMRI data analysis.

1.2.5.2 *Brain functional MRI in fibromyalgia*

The new neurobiological insights provided by the advances of neuroimaging techniques have helped to shed a light on pain mechanisms, especially nociplastic pain[453]. A distinction between perceptive (sensory processing) and nociceptive (conscious perception) brain areas has been suggested[354]. Activation studies have showed the involvement of different brain areas in perception and nociception, those include the somatosensory and cingulate cortices (SI-II, ACC), IC, PFC, thalamus, and cerebellum. The ACC and IC appear to be activated in both FM and healthy controls when experiencing pain[342]; however, these areas showed a reduced grey matter volume only in individuals with chronic pain, the biologic meaning of these findings is still unclear[599]. Overall, results from fMRI studies in different chronic pain conditions point towards increased activity of limbic areas, including the IC and amygdala, which might influence the prefrontal cognitive evaluation of pain, e.g. learning and coping mechanisms (detailed in section 1.2.1). The altered functional interactions between different brain regions may reflect the maladaptive connectivity changes specific to chronic pain[354,600]. For example, in different chronic pain conditions altered activity connectivity has been demonstrated between DMN and the reward system (amygdala-nucleus accumbens), IC, and cingulate cortices[382,601-603].

Similar functional connectivity alterations, in association with grey matter volume changes, have been suggested as possible biomarkers of pain chronicity and pain intensity, independently from the underlying pain condition[604]. However, different chronic pain conditions seem to show distinct functional connectivity reorganizations. While this adds a degree of complexity in pain research it can also provide the opportunity to explore disease-specific neurobiological markers that can serve for diagnostic purposes and to predict treatment response[600]. In the past decades, FM neurobiological features have been extensively studied; despite the efforts, many of uncertainties still remain. In an “activation likelihood estimation" meta-analysis including 37 papers focussed on primary FM, the brain areas presenting consistent enhanced activation included the IC, ACC, amygdala, SI-II, superior temporal gyrus (involved in auditory perception), and the lingual gyrus (visual associations)[605]. Both perceptive and nociceptive brain areas appear more active in FM, and these include primarily somatosensory areas, limbic system, and areas with cognitive functions.

An altered resting state connectivity of key pain-processing brain areas and ICNs in FM has been demonstrated in different studies using BOLD signals and seed-based analysis. Insula is a key region in pain processing and an altered connectivity of the insula has been consistently shown in different studies including individuals with FM. Greater connectivity between IC to cingulate cortices was demonstrated in FM when compared to healthy controls and was also associated with reduced pressure pain thresholds and pain changes after acute pain stimulations[381,606–608]. Changes in IC-ACC connectivity in FM were also associated with the response to treatment with milnacipram, a SNRI drug[609]. In a rs-fMRI research study, brain functional connectivity was investigated before and after a deep tissues pressure pain applied to the lower of leg to participants with FM and healthy subjects; stimulation-associated pain intensity, as well as pain catastrophizing, deep-tissue pressure TS and cardiovagal response, were directly correlated with the connectivity between ROIs in the SI representing the stimulated body area (SI-stim) and the right antIC, while the connectivity of SI-stim to the left antIC was associated with attention ratings[610]. Moreover, an altered SI-IC connectivity was associated with reduction of pain levels and catastrophising scores, after electroacupuncture and CBT, respectively[611,612].

The studies above suggest, not only a different functional organisation of the insula between anterior and posterior (detailed in section 1.2.1.1), but also a laterality distinction between left and right, in terms of pain levels and salience. Additionally, an altered sensory processing of the perceptive areas extended to visual and auditory stimuli in FM and is confirmed by reduced somatosensory cortical connectivity to visual, and auditory cortices, and cingulate cortices correlating with levels of reported pain[606,613].

An altered connectivity of the DMN with different areas mentioned above has been demonstrated in FM subjects compared to healthy volunteers. Pain and depression scores were associated with DMN connectivity to cingulates cortices and SI-II, while symptoms duration inversely correlated with DMN-parahippocampal gyrus[614]. An altered DMN connectivity to IC seems to be characteristic of FM, because it showed to be directly correlated with spontaneous pain and its reduction after different treatments, including acupuncture, pregabalin and transcranial magnetic stimulation, and it was consistently correlated with clinical response and reported-pain reduction[382,384,615–617].

Resting state data in FM showed consistent results regarding DMN and IC altered functional connectivity. In fact, findings from rs-fMRI have been also successfully used to inform machine learning models to differentiate FM from pain-free subjects and to predict response to treatment[618,619]. However, longitudinal data, changes in response to treatment, and alteration specific to different conditions are still needed.

1.2.5.3 *Brain functional MRI in arthritis*

Advances in neuroimaging have enabled the exploration of the neurobiological features of pain in different chronic pain conditions, including inflammatory arthritis. To date, RA is the chronic inflammatory arthritis investigated the most with fMRI techniques, and findings in this condition might inform the mechanisms of pain persistence in other chronic immune-mediated inflammatory diseases.

Most of the available studies investigate task-based brain functional connectivity in RA. In two separate studies, brain activation and functional connectivity was inspected in subjects with RA (without FM) compared to individuals with FM (without RA) and healthy volunteers, while a painful pressure (individually calibrated) was applied at the most inflamed PIP joint (RA) and at the thumbnail (RA, FM, HC). RA participants displayed a higher activation of the SI-II, lingual gyrus, and left antIC during stimulation of the arthritic joint compared to stimulation at the non-involved area (in line with QST findings in section 1.2.4.3). In response to experimental pain at the inflamed joint, RA individuals presented a deactivation of the dlPFC compared to healthy individuals[620]. Moreover, the connectivity of the IPL to SMN and PFC after pain-evoking task was increased in RA compared to FM[621]. An altered activity of nociceptive and perceptive areas when inflamed areas are stimulated, but not for non-affected areas, may indicate the presence of peripheral sensitisation inducing aberrant central pain-processing. However, these studies excluded subjects with a diagnosis of FM, virtually eliminating the presence of concomitant central sensitisation, which is however frequent in rheumatic conditions. In fact, neuroimaging studies in RA considering the presence of central sensitisation, using the ACR FM criteria scale, demonstrated a significant correlation between DMN and pIC, consistent with findings in FM populations. To note, functional connectivity was analysed during fatigue-evoking task (paced auditory serial additional test, PASAT) and not during a resting state. Interestingly, despite the strong correlation with FM scores, an association with VAS for current pain was not found[492]; the latter observation suggests a higher specificity of the DMN-IC connectivity for central sensitisation (widespread pain and CNS symptoms) rather than a peripheral nociceptive pain. Therefore, DMN-IC connectivity can be a valid candidate as a neurobiological biomarker of central sensitisation across different chronic pain conditions[622]. Differently from primary FM, RA and other IA are characterised by chronic peripheral and systemic inflammation. In an interesting task-fMRI study using PASAT, higher levels of systemic inflammation in RA, measured with ESR, were associated with an increased connectivity of the IPL to the DMN and DAN, and between the mPFC and the DAN. Similar correlations between ESR and altered connectivity of the IPL and mPFC were replicated in the same participants (54 RA) at 6 months. The same

connectivity patterns were also associated with worse clinical symptoms including widespread pain and fatigue[623]. Similar correlations between inflammation and altered connectivity of IPL in RA was showed in a rs-fMRI study. Individuals with RA and concomitant FM showed a positive correlation between the functional connectivity of the left IPL to the IC, ACC, and mPFC, and the ESR levels when compared to RA subjects without FM[491]. Moreover, in a cohort of inflammatory arthritis, either RA or SpA, pIC-ACC resting state functional connectivity was able to predict the response to biologic treatments (in a separate validation group) and it correlated with disease activity scores, and the FM impact questionnaire[624].

These findings suggest that chronic inflammation induces changes in the brain circuitry involved in pain processing (cognitive function, attention and working memory) that can directly lead to a "bottom-up" central sensitisation in contrast to the conventional "top-down" nociplastic mechanism. This hypothesis can be also acceptable for chronic inflammatory conditions other than RA, i.e. SpA and PsA, however disease-specific studies are still lacking.

To date, only a single study has investigated evoked-pain brain activity in a single patient with PsA. Pain levels correlated with the activation of the left IC and right SII. The pain induced activation of these areas was significantly reduced at 1 and 3 hours after administration of an oral NSAID[625]. In a recent study focusing primarily on PsO and depression, using a large prospective cohort in UK[626], demonstrated that subjects with concomitant PsA (n=28) exhibited a functional decoupling between the frontal and occipital areas, when compared to the PsO only (n=234) and healthy subjects (n=393) groups which, instead, showed an anti-correlation between these brain areas[627]. From these studies is not possible to draw general conclusions due to the limitations of recruiting a single patient, or the absence of specific evaluation of PsA pain, respectively. Nonetheless, a hint towards pain-evoked activation of IC and somatosensory cortices, and fronto-occipital impaired connectivity (previously associated with chronic pain[628] and inflammation[629]) is given. No other studies using fMRI in PsA cohort are currently available to the best of my knowledge. However, fMRI studies on the associated AS might be of help to understand the neurobiological mechanism of pain in other SpA diseases, including PsA. For example, in a group

of 51 men affected by AS, clinical pain scores were positively correlated with DMN to SMN (SI-II) functional connectivity. A sub-analysis in AS men with high clinical pain evidenced an increased connectivity between DMN-SMN, as opposed to classical anticorrelation of these networks present in healthy subjects. However, AS individuals with low clinical pain showed no differences with healthy volunteers[630]. Importantly, differences between sexes requires to be carefully considered when evaluating functional connectivity in SpA, as demonstrated in a study focusing on ACC functional connectivity in AS[631]. An increased connectivity of the ACC to the DMN was present only in woman with AS, when compared to healthy women and men with AS. No difference in the ACC connectivity was found between men with AS and healthy. Additionally, women with AS showed lower ACC connectivity to hippocampus and frontal regions[631]. In another study including individuals with AS not receiving biologics, the IC to mPFC (part of the DMN) functional connectivity was positively associated to pain, fatigue, and disease activity (BASDAI). The ACC to PCC functional connectivity (both part of the DMN) negatively correlated with clinical pain in AS (sex differences are not mentioned in the study). Interestingly, the centrality (strength and numbers of connections within a network) of the mPFC within the DMN correlated with clinical scores of pain and fatigue, while centrality of the SLN (insula) and visual network nodes were associated with pain in AS[632]. The finding of changes in the visual network correlating to pain is interesting considering the findings in the recent study in PsO and depression (see above). In another well characterised AS cohort, including 20 subjects (naïve to biologics and with at least moderate current pain (>3/10), a greater connectivity between the DMN and SLN was demonstrated when compared to age-matched healthy participants. Moreover, in the same AS cohort, back pain and BASDAI scores positively correlated with DMN– SLN functional connectivity; in particular, PCC/precuneus (DMN) to antIC(SLN) increased connectivity and diminished DMN to PCC/precuneus connectivity correlated with BASDAI .

Overall, the evidence from the above 4 fMRI studies in AS suggest a central role of the DMN altered connectivity in pain; in particular, an increased DMN functional connectivity to SMN (SI-II) and SLN (IC), and a decreased connectivity within the DMN network (ACC-PCC, DMN-PCC) were present in AS, when compared to healthy individuals (with some sex differences), and this altered

DMN connectivity correlated with clinical pain and disease activity (BASDAI). Findings around dysfunctional visual network connectivity seems to be unique to this group of immune-mediated diseases (i.e. SpA), however, longitudinal disease-focused studies are needed to confirm this finding.

1.2.6 Confounder factors in pain: sex, age, and treatment

Due to the complexity of the underlying biology and the different mechanisms involved in pain perception, it is not surprising that different factors might have an impact in the perception, assessment, and treatment of pain. In fact, different individual characteristics influence pain, ranging from sex and age to the clinical condition(s) associated, especially inflammatory conditions, as well the medications taken and more complex psychosocial determinants. The following chapter will focus on the role of sex, pain modulating drugs, and inflammation on pain, as confounders of interest. Despite the relevance of the psychosocial factors[633], the in-depth analysis of these determinants was beyond the scope of this work.

1.2.6.1 *Role of sex and gender on pain perception*

Diseases associated with chronic pain burden are more common in women than men, which indirectly increases chronic pain occurrence in females[634]. For example, fibromyalgia (FM), the prototypical nociplastic disease, more frequently affects women compared to men worldwide[358]. Similarly, common forms of inflammatory rheumatic conditions, such as RA and SLE, are also more frequent in women compared to men[635]. Furthermore, while PsA has a more equal sex distribution, the condition seems to more frequently have peripheral involvement, higher TJC, worse reported pain and fatigue, and poorer treatment responses in women[636]. Moreover, differences in pain sensitivity, molecular pain processes, and hormones action, as well as, gender biased psychosocial traits and coping mechanisms, suggest that the impact of sex and gender on pain can involve a multitude of different mechanisms.

Pain sensitivity can be evaluated employing different standardised assessments, commonly defined as QST (detailed in section 1.2.4). These validated tests apply stimuli with different modalities (e.g. thermal, pressure, electrical, chemical, etc.) to assess different pain sensitivity determinants, i.e. pain thresholds and tolerance, pain summation, and CPM[637,638]. Studies in healthy adults shown an overall lower thermal/pressure pain thresholds and tolerance in female compared to male volunteers, as well as higher TS and reduced CPM. All these characteristics are associated with increased pain sensitivity accompanying nociplastic pain mechanisms. However, the increased sensitivity seems to be highly variable between the sexes, with and conflicting results are reported in different studies, depending on the modality and length of stimulations applied (tonic vs evoked). Despite the apparent increased pain sensitivity, women seem to show higher ability to adapt to repeated or tonic stimulations[639,640]. Furthermore, the psychosocial pain stereotypes associated with gender, i.e., femininity and masculinity, appears to influence the expectation of reported pain, as do some psychological factors including catastrophising, coping strategies, anxiety and depression[528]. In addition, sex-associated differences in genes coding for proteins involved in pain processing may account for different pain sensitivity in men and women[641]. Also, individual immune systems have been associated with sex-associated differences in pain processing[642]. For example, higher Th1:Th2 ratio and macrophages pro-inflammatory profile characterise females in comparison to males; the greater pro-inflammatory profile seems also to be present in astrocytes and microglia, mainly driven by sex hormones effects, and overall leads to greater pain sensitivity and hyperalgesia[642]. Sex hormones are also associated with altered pain sensitivity. Simplifying the complex endocrinological effects of sex hormones on pain processing, the available evidence suggests that testosterone, has an anti-nociceptive effect, while oestradiol has a pro-nociceptive role[643]. Interestingly, results from transgender individuals receiving hormonal therapy, seems to point towards an improvement of pain in transmasculine subjects receiving androgens and a worsening or new onset of chronic pain in transfeminine subjects on oestrogens hormonal therapy[644]. Presumably, difference in hormones quantity, quality, and fluctuations (e.g. menstrual cycle) may also contribute to difference in pain sensitivity between sexes[645] also with direct effects on the

excitability of brain cortical regions[646]. In fact, both "genetic" and psychosocial factors associated with sex and gender, discussed above, might also influence the pain processing within the brain. Neuroimaging studies showed that there are sex-specific brain signatures characterised by distinct brain connectivity between specific regions, including important pain processing areas, i.e. cingulate cortex, medial and lateral frontal cortex, temporoparietal regions, insula, and precuneus; machine learning approach was able to distinguish between males and females based on the connectivity of these brain areas, without a clear cut-off between the sexes, but more a continuum between them[647]. A recent "mosaic" hypothesis suggests that different degrees of "female-typical" and "male-typical" features coexists in the same individual and are influenced by a multitude of factors; therefore, the binary or continuum male-female brain features models are both limited to capture the complexity of the sex-related brain signature, while a "mosaic" of combinations of different features within each individual has been proposed as a more suitable model[648].

The clinical implications of sex and gender on pain processing are highly important and still not completely elucidated. Therefore, there is a need to expand the analyse based on sex and gender in research studies and consider more inclusive and detailed inclusion criteria in pain research[649]. In this study, differences between males and females in pain phenotypes were explored in a PsA cohort; however, no specific data were collected on gender features (masculinity and femininity independent from assigned sex), which could be considered in future studies.

1.2.6.2 *Age influence on pain sensitivity*

Age has also an impact on pain. The incidence of chronic pain seems to increase significantly from young adults (9%) to individuals over 65, reaching up to 60%; however, the prevalence in elderly adults can decline, suggesting a not linear relationship with pain[650–652]. Typically, the prevalence of clinical conditions associated with chronic pain, e.g., musculoskeletal degenerative or inflammatory diseases also rises with age[651]. Of note, several conditions associated with chronic pain, in particular inflammatory arthritis, are not exclusive to adults and can similarly affect paediatric population and

adolescents[650]. Different age-related changes can affect the somatosensory system in different ways and at different levels[653]. For example, heat-sensitive nociceptors are reduced with aging, while data on mechanoreceptors are controversial (altered density mainly at joints and muscles tissues); an overall increased sensitivity and spontaneous activations of C-fibers have been observed in elderly subjects[653,654]. Moreover, a progressive impairment of the descending pain modulation with age has been extensively demonstrated, and could certainly contribute to age-related altered pain sensitivity[655–657]. In fact, available data on pain sensitivity in elderly suggests reduced pain thresholds to heat stimuli, and increased sensitivity to mechanical stimuli (less consistent results), as well as increased TS[658–660]. Additionally, the cognitive decline observed in elderly can affect the assessment of pain due to patient difficulties in expressing location and intensity, and the influence of altered efficacy of pain treatments consequent to changes in drugs metabolism and interaction with concomitant medications[661,662]. Moreover, cognitive decline can contribute to increased pain sensitivity also with reduced physical function and cultural engagement, both predictive of chronic pain in elderly[663], suggesting higher vulnerability to pain compared to younger individuals.

Lastly, aging is characterised by increased basal inflammation with low-grade chronic inflammatory processes, known as “inflammaging”; chronic inflammation represents an important risk factor for the development of different disease and increased mortality[664]. Altered monocytes and microglia functions with aging, epiphenomenon of “inflammaging” have been associated with an increased expression of TNF and increased pain sensitivity in animal models[665,666](detailed in section 1.2.3.2).

1.2.6.3 *Effect of pain modulating drugs on pain*

The different pathogenetic mechanisms in pain syndromes are reflected in the variable efficacy of treatments with distinct mechanisms of action. Overall, anti-inflammatory drugs are usually effective in lessening nociceptive pain states, while centrally active drugs such as gabapentinoids and tricyclics are mainly effective for treating nociplastic and neuropathic pain. In inflammatory arthritis the nociceptive pain is prevalent, and driven by systemic and local

inflammation, thus anti-inflammatory (corticosteroids and NSAIDs) and immunotherapies (DMARDs) drugs are commonly used. The overlapping presence of nociplastic (or neuropathic) pain in some patients might partially explain the pain persistence. This observation points towards the need to stratify individuals by choosing drugs based on the dominant pain mechanism(s) present[667]. On the other hand, patients can present with multi-comorbidities and often take several drugs that potentially alter pain levels and interfere with pain assessment. As a result, in addition to the underlying pain mechanisms, considering the current treatment in each individual is particularly important when assessing pain levels in both research and clinical practice. Herein, a general overview of the most frequently used pain medication and the evidence of the efficacy in nociplastic pain is described.

Centrally acting drugs

Drugs acting on the nervous system have been demonstrated to be effective in treating FM[668];these include tricyclic antidepressants, dual reuptake inhibitors, gabapentinoids, and cannabinoids.

Amitriptyline is a tricyclic antidepressant approved to treat major depressive disorder, neuropathic pain, and to prevent migraine[669,670]. Furthermore, it has been shown to be effective in FM and other nociplastic pain conditions, e.g., irritable bowel syndrome and interstitial cystitis; however, it hasn't been officially approved, for lack of good quality evidence. Amitriptyline blocks the pre-synaptic transporter of NA and 5-HT, resulting in an increased activity of serotoninergic and noradrenergic transmission[671]. Despite being off-label in FM, amitriptyline is commonly used in clinical practice at a lower dose of its approved use in depression. A recent meta-analysis showed amitriptyline efficacy in ameliorating sleep, fatigue, and patients' reported quality of life[672].

Inhibitors of the 5-HT and NA reuptake (SNRIs) have been approved to treat depression, and anxiety, and also shown to be effective in chronic pain conditions, including FM and neuropathic pain[673]. SNRIs effects on pain involve the modulation of the descending pain pathways in both in the brain and the spinal cord; by increasing the descending inhibitory activity, SNRIs exert an anti-

nociceptive effect[674]. The effect seems mainly mediated by the effect on NA pathways, rather than 5-HT; in fact, selective inhibitors of 5-HT reuptake do not have the same benefit on pain[675]. However, the positive modulation of the 5-HT pathways is useful for mood disorders, such as anxiety and depression, which are often associated with nociplastic pain[676]. Duloxetine and milnacipram have been approved by FDA to treat FM[677,678]. Milnacipram is not available to use in Europe, while duloxetine is approved to treat neuropathic pain and is used off-label in FM. In a meta-analysis, duloxetine showed higher efficacy for pain levels and depression in FM compared to placebo, amitriptyline and pregabalin[672,679].

Gabapentinoids are inhibitors of the calcium channel 2δ and have a neuronal inhibitory activity analogous to the neurotransmitter GABA, originally approved to treat epilepsy, neuropathic pain, or anxiety disorders. The inhibition of calcium channels can inhibit central sensitisation, preventing the activation and activity of NMDA ion channels. Moreover, gabapentinoids can increase descending inhibitory pathways and reduce descending facilitation pathways, the pathophysiological mechanisms of maladaptive neuroplastic changes in nociplastic pain states[680]. Gabapentinoids also appear to improve patterns and quality of sleep, important factors in improving patients' outcomes and quality of life[681]. The most studied gabapentinoids to treat chronic pain disorders are pregabalin and gabapentin. Pregabalin was showed to be effective in reducing pain in FM in several meta-analyses and systematic reviews[682–684], which lead to approval from the FDA for treating FM[685]. Moreover, a neuroimaging study pre- and post-pregabalin showed restoration of altered brain connectivity in individuals with FM[384]. Pregabalin with duloxetine was reported to show effectiveness for pain and patients' reported outcomes with a good safety profile, in a Bayesian network meta-analysis[686], suggesting use of these as the first-line pharmacological treatment, potentially used in combination in selected cases[687]. The evidence for gabapentin is less robust with low quality of evidence suggesting less than 30% reduction in reported pain in almost 50% of subjects treated[688]. Gabapentin is not approved by FDA to treat FM; however, it is used commonly off-label due to the class effect on quality of sleep, anxiety and fatigue[689].

Cannabinoids have a modest level of evidence in FM and should only be used in selected cases. The most studied active cannabinoids are the cannabidiol (CBD), the tetrahydrocannabinol (THC), and its derivate nabilone. Cannabinoids have been used to treat different chronic pain conditions[690], therefore the interest in their use in nociplastic pain conditions, including FM, has been growing in the recent years[691]. Cannabinoids receptors are ubiquitous in the human body, the two known receptors are expressed predominantly in the nervous system (CB1) and on immune cells (CB2)[692]. The endogenous cannabinoids have a pain modulatory effect mainly via the descending inhibitory pathways. The current evidence for their efficacy remains unclear due lack of high quality randomised controlled trials and common patients' self-administration with high heterogeneity of dosage and route of administration. The effect of THC on pain, maximised when in combination with CBD, seems to be higher than CBD alone[693,694]. The safety profile appears good, albeit with non-severe adverse events that might, however, lead to discontinuation.

Opioids are a class of drugs acting on the endogenous μ-opioid receptor (MOR)[695]. Opioids are commonly prescribed to treat non-cancer chronic pain across the globe, including FM[696,697]. FM and rhematic conditions represent a risk factor for long-term opioids use[698]. However, the evidence supporting the efficacy of opioids in FM is modest and deleterious when used in long-term[699–701]. In fact, has been shown that individuals with FM have higher levels of endogenous opioids in the CSF[702] and that brain MOR availability is reduced in FM[386]. Moreover, reduced MOR binding potential was also linked to an increased brain activity in anti-nociceptive brain areas in response to pressure pain[387]. Therefore, taken together, this evidence suggests a high endogenous opioids dysregulation in FM, which might explain reduced efficacy of opioids in treating the disease, and potentially also other chronic pain conditions linked to nociplastic pain.

Naltrexone is an inhibitor of the MOR. When administered in low-doses, naltrexone has been shown to significantly reduce pain and improve fatigue and/or sleep in up to 32% subjects with FM[703]. These results further support hyperactivity of the endogenous opioids system in FM and may offer a promising therapeutic option. Tramadol is a low-potency opioids which showed some

benefit on pain in FM[704,705]. Tramadol binds MORs with low affinity and also inhibits the 5-HT and NA reuptake[706]; the latter mechanisms is more likely associated with its efficacy in FM.

Currently, the evidence for the efficacy of corticosteroids and analgesic drugs such as NSAIDs in nociplastic pain conditions (e.g. FM) is limited and they only appear effective in a limited number of patients, probably targeting concomitant pain mechanisms, i.e. in OA and inflammatory arthritis[707].

Chapter 2 Hypothesis and aims

Psoriatic arthritis is characterised by immune-mediated processes that leads to chronic inflammation targeting joints, periarticular and extra-articular structures. Chronic peripheral inflammation can lead to tissue damage. The peripheral nervous system is able to sense chemical stimulations induced by the release of pro-inflammatory mediators as well as the tissue damage. Consequently, peripheral sensory information is transmitted to the CNS. At this level, peripheral sensory information is integrated with and modulated by other sensory inputs, affective and cognitive dimensions to form the subjective pain perception. Pain is a hallmark feature of inflammatory arthritis, including PsA, and it represents a burden for the individual affected. In fact, pain can lead to reduced physical function and quality of life, disability, and even to increased mortality. Therefore, pain should be a high priority for rheumatologists treating and managing individuals with PsA. For years, the concept of pain in inflammatory arthritis has been mainly focussed on the level of peripheral inflammation, following the assumption that controlling the inflammation should sufficiently reduce, or even resolve the pain reported by patients. However, while modern immunotherapies effectively manage the clinical course and disease progression in PsA, they often fall short of adequately controlling the reported pain. In recent years growing literature support the evidence that persistent pain in PsA (and other inflammatory arthritis) is uncoupled with the peripheral inflammation. Pain persistence can lead to reduced patients' satisfaction and inappropriate treatment escalation, exposing individuals to unwanted risks, without significant incremental improvement in pain. Therefore, there is an unmet need to understand the pain phenomenon and its complexity in individuals with PsA. Recent advances in pain studies have helped define a new pain phenotype different from classical nociceptive and neuropathic pain, known as nociplastic pain. The dysfunctional processes in the somatosensory nervous system characteristic of nociplastic pain have been recently elucidated by cutting-edge non-invasive neuroimaging techniques, including fMRI brain studies in other clinical populations. Neuroimaging studies in FM, the prototypic disease of nociplastic pain, have shown that altered connectivity between specific pain processing areas or intrinsic networks of the brain, with increased

reported pain, reduced pain threshold and tolerance, and sensory hypersensitivity. Importantly, similar alterations have been shown to be present in RA. Currently, there is a lack of objective studies investigating the pain phenotype in PsA highlighting the need to fill this gap. PsA has heterogenic clinical characteristics, where articular and extra-articular inflammation often coexist in the same individual. The chronic inflammation causing enthesitis, psoriasis, uveitis, axial inflammation, and/or inflammatory bowel disease may also sensitise the nervous system, in predisposed subjects. For example, psoriasis can result in extended spatial skin noxious stimulations able to activate the somatosensory system. Moreover, enthesitis is a unique feature of PsA and clinically manifest with pain spreading across a larger area than the enthesis itself, emulating the classical nociplastic widespread pain. For this reason, differentiating between enthesitis and nociplastic pain can be clinically challenging for healthcare professionals, unless additional investigations are performed, i.e. ultrasound scans or MRI, with increased patients' burden and delays in starting the adequate treatment. In normal clinical settings, useful neurobiological biomarkers are not available to date. In this context, improving our understanding the neurobiology of pain in PsA would lead to a better disease management, improved patient care, and ultimately to a better quality of life for individuals affected by this disease.

- The first aim is to evaluate whether individuals with PsA and a high degree of nociplastic pain will present similar neurobiological features present in FM and RA. The hypothesis is that in PsA, similarly altered brain connectivity, namely an altered IC and DMN connectivity, and an increased pain sensitivity, measured with QST, would be associated with the degree of nociplastic pain. This hypothesis is in line with the idea that nociplastic pain can often overlap with other chronic pain condition in mixed pain states. In PsA, the overlapping of nociplastic pain can explain the persistence pain after control of inflammation. It has been demonstrated that the 2011 ACR FM criteria represent a useful clinical tool to stratify the degree of nociplastic pain in different chronic pain conditions. Therefore, the 2011 ACR FM criteria are employed to

determine the degree of nociplastic pain in the study participants. The presence or absence of FM allows the classification of subjects and comparison between the 2 groups, regarding clinical parameters and validated objective measures of pain assessed with QST techniques. The degree of FM (or FMness), determined with the total score derived from the 2011 ACR FM criteria (and subindexes, WPI and SSS), is used to describe the population and to compute correlations with the above-mentioned parameters, and with parameters of brain functional connectivity. These analyses contribute to the characterisation of the neurobiological features of pain in PsA, and to investigate whether the clinical presence of nociplastic pain reflects in similar altered connectivity of IC and DMN, as previously demonstrated in primary FM and in subjects with RA.

- The second aim is to investigate the role of different confounders in the pain phenotype of subjects with PsA. Different genetic and environmental factors can influence pain perception. In this thesis, I focused on exploring the effect of sex and current pain modulating drugs on the clinical and neurobiological features of pain in this well-characterised PsA cohort. Regarding the sex differences, the literature supports a higher pain sensitivity in females compared to males (detailed section 1.2.6.1). Therefore, the hypothesis is that FM would be more prevalent in females, and that the differences in the validated objective measures of pain sensitivity would be stronger in the female sex, compared to males. Pain modulating drugs may affect brain connectivity and reported pain levels (discussed in section 1.2.6.3). In this work, the differences in clinical, QST and neuroimaging variables are explored in participants taking pain modulating drugs and those who are not. A strengthening of the signal found in the primary analysis is hypothesised, particularly in those not taking the pain modulating drugs. A better understanding of the impact of different factors on pain perception in PsA could represent an important step toward patient stratification and improved management.

- The third aim is to explore the associations between inflammation and neurobiological features of nociplastic pain in PsA. Circulating levels of IL-

17A and TNF, and the response to immunosuppressive drugs prescribed are used as markers of inflammation in this study. Differently to primary FM, PsA has a predominant inflammatory component which cause nociceptive pain. However, in predisposed subjects the inflammation can induce the onset or aggravate an already established nociplastic pain condition. A direct influence of the inflammation on the nervous system might contribute to this phenomenon. Both IL-17A and TNF influence sensory processing at the periphery and centrally. In RA, levels of inflammation were associated with an altered brain connectivity of areas involved in pain processing. Therefore, in this agnostic exploratory analysis the circulating levels of IL-17A and TNF, as well as the response to treatments, assessed using the DAPSA score (section 1.1.3), are correlated with clinical and neurobiological parameters evaluated in the first aim. This unique data set offer the opportunity to explore the interaction between immune and nervous system in PsA and investigate its effect on pain phenotype.

Chapter 3 Methods

3.1 Introduction

This chapter describes the methods used to test the hypotheses addressed in the aims chapter. Herein the study design rationale and the experimental procedures performed are provided.

A single-centre observational study titled Characterising the Centralised Pain Phenotype in Chronic Rheumatic Disease - A Stride Towards Personalised Analgesia (CENTAUR)[708] was leveraged to address the aims of this thesis. The study received ethical approval from the West of Scotland Research Ethics Committee on the 14th of March 2019 (REC reference 19/WS/0033 – see Appendix 1). The primary objective of the CENTAUR study was to characterise neurobiological features of PsA patients in relation to FM scores. Additionally, it aimed to develop a concise self-report measure of centralised pain applicable to PsA and other rheumatic disorders. To achieve these objectives, the study employed various tools, including extensive questionnaires, QST, and cutting-edge neuroimaging methods. These methods investigated functional connectivity during rest and evoked-pain tasks, as well as spectroscopy of the right insular region. For the aims of this thesis, I specifically utilised clinical data, QST results, and resting-state functional MRI data.

In this clinical study, my role encompassed the selection of clinical parameters and disease-specific scores for PsA, particularly for assessing treatment response. As a clinical research fellow, I played a direct role in participant screening and recruitment. In my capacity as a sub-investigator for the study, I undertook responsibilities that included performing the study-related procedures for all participants, including supervision of baseline MRI protocols. These procedures involved the collection of data and samples during both baseline and follow-up visits, both in-person and remotely, the latter during the COVID-19 pandemic restrictions. I conducted independent analyses of clinical parameters and objective pain measures, i.e. QST. My colleague, Kristian Stefanov, handled the analysis of neuroimaging data, and I actively contributed to the interpretation of the findings.

3.2 Study design

3.2.1 Participants recruitment

Individuals with a diagnosis of PsA scheduled to start a new biologic or other DMARD for active disease as part of their usual care in NHS rheumatology out-patient clinics were identified as potential candidates by their health care professionals. The study participants' information sheet (PIS) was given to subjects expressing the interest in joining the study. The PIS included written information on the study procedures and the principal investigator contact details (Appendix 2). In accordance with the ethical committee indications, potentially eligible individuals were given a week to consider their participation. After a week, myself or a member of the research team contacted the interested subjects and gave them the opportunity to ask questions. At the same time inclusion and exclusion criteria (table 3.2.1) were used to confirm the participants' eligibility. Firstly, PsA individuals, fulfilling the CAPSAR criteria, had an active disease and were about to change or start a biologic or DMARDs at the time of recruitment. The inclusion of participants with similarly high disease activity was preferred to ensure a good homogeneity of the study population. Importantly, the degree of disease activity reflects the degree of inflammation, and both have an impact on the pain experienced. Moreover, the degree of active inflammation could be associated with sensitisation processes in the nervous system, both in the peripherally and centrally. Thus, including subjects with an active disease, supported by the clinical indication to start a biologic or a DMARD, served a crucial purpose in this study. This decision was based on the acknowledgement that patients experiencing an active disease were more likely to exhibit detectable inflammatory markers, including circulating cytokines. Therefore, targeting individuals with heightened inflammatory activity enabled a more robust exploration of the correlation between inflammatory markers and FM scores. Moreover, evaluating the response to treatment served as an indirect measure of inflammation. However, it is essential to acknowledge the inclusion of other variables, which could affect pain mechanisms differently. Examples include variations in disease duration and the initiation of various treatments, characteristic of cross-sectional studies. Among the inclusion criteria also the absence of contraindication for MRI and right-hand dominance were initially

assessed. The inclusion of only right-handed subjects is commonly preferred in fMRI studies; the intent is to reduce the data variability associated with the physiological laterality of brain functional and structural features[709]. In this study, individuals aged 75 and older were excluded. However, the exclusion of this age group from clinical studies is not a universal practice and is influenced by various factors. Firstly, health and safety concerns arise due to the potential complexity introduced by age-related changes, multiple health conditions, and various medications. These factors can impact the interpretation of study results, particularly in the context of neuroimaging investigations. Moreover, different challenges may stem from cognitive decline, posing ethical issues related to vulnerability and the ability to provide meaningful informed consent. These considerations contribute to decisions to exclude this age group.

Eligible and suitable subjects were finally invited to join the baseline research visit in the study clinical research facility within the NHS and affiliated with the University of Glasgow. All participants were asked to suspend current pain medications, including NSAIDs and paracetamol alone or in combination with short-lasting opioids, for 24-48 hours prior to the study visit, if possible. An exception was granted in case of severe levels pain due to ethical considerations related to increasing suffering in patients with active inflammation. This exception was left at the discretion of each patient. NSAIDs and paracetamol are commonly used as short-acting analgesics in inflammatory arthritis and chronic musculoskeletal conditions. The reason for suspending short-lasting pain medications, when possible, was that the pain reduction following consumptions of the above drugs can alter different study assessments, including the reported pain at the time of the visit and the QST[514,710]. The chosen timing of 24-48 hours allowed for a sufficient washout period for the drugs of interest. The suspension of longer-acting agents was not included, as it was unlikely to significantly alter the study's outcome, with potential implications for patient management. Notably, the option to not suspend the above medication may have generated an additional variability in the results of the study.

Table 3.2.1 Inclusion and exclusion criteria

The following inclusion criteria are used to identify eligible participants for the study:

Inclusion Criteria	Exclusion criteria
≥ 18 years at the time of consent	Inability to provide written informed consent
Fulfil the CASPAR criteria for PsA	Severe physical impairment (e.g., blindness, deafness, paraplegia)
Individuals who have active joint disease in the judgement of the rheumatologist and are being started on a new treatment with either a biologic or a DMARD(s)	Medical or psychiatric conditions that in the judgment of study personnel would preclude participation in this study (e.g., malignancy, psychosis, suicidal ideation)
Evidence of ongoing inflammation (synovitis or dactylitis on clinical examination; enthesitis on imaging)	Co-morbid medical conditions that may significantly impair physical functional status
Ability to read and speak English to allow for written informed consent	Pregnancy or breastfeeding
Right hand dominant	>75 years of age
	Contraindications to MRI (e.g. severe claustrophobia)
	BMI > 40 or unable to lie comfortably in MRI
	Diagnosed peripheral neuropathy
	A history of visual stimulus evoked migraine or epilepsy

3.2.2 Sample size and power of the study

The power of the study was calculated to be higher than 90%, using the error probability (or error type 1) at the conventional 5%, a sample size of 50 participants, and 0.50 correlation as effect size (Gpower v3.1 software). The power of the study is also in line with previous studies from the same research group, where a target of 50 participants provided the ability to detect correlations exceeding 0.45 between ACR FM score and objective pain outcomes with a power of at least 90%[623,711].

3.2.3 Study timeline

The study included 3 visits: a baseline and two follow-ups. During the baseline visit, the participants were asked to complete the consent form (Appendix 3), prior to performing any assessment. The consent form granted each patient permission to access NHS clinical records and to collect biological samples to be used for ethically authorized secondary studies, including this one. The baseline visit included collection of medical history, clinical evaluation, multi-modal subjective and objective pain assessment. The baseline visit was carried out before starting the new treatment prescribed. The baseline data alone provide robust data to address the question of whether nociplastic pain is present among individuals affected by PsA, by integrating questionnaire and neurobiological markers. The following 3 and 6 months (+/- 2 weeks) visits were performed to evaluate response to treatment. It is important to note that the inclusion criteria did not mention the new immunosuppressant started, resulting in different treatments across the recruited participants. During the follow-up visits, only the clinical and subjective pain assessments were performed, excluding multimodal-MRI and QST (table 3.2.2).

Table 3.2.2 Study matrix

Assessment	Baseline	3 months	6 months
CLINCAL EVALUATION			
Demographics	✓		
Family history	✓		
Smoking and alcohol history	✓		
PsA diagnosis date	✓		
Past medical history	✓	✓	✓
BMI	✓		
Complex Medical Symptom Inventory	✓		
Current drug treatment	✓	✓	✓
PsA CASPAR Criteria	✓		
66 swollen joint count	✓	✓	✓
68 tender joint count	✓	✓	✓
Dactylitis score (0-20)	✓	✓	✓
Leeds enthesitis index (0-6)	✓	✓	✓
Assessor global assessment VAS	✓	✓	✓
Patient global assessment VAS	✓	✓	✓
C reactive protein	✓	✓	✓
BASDAI	✓	✓	✓
Ultrasound scan of joints and enthesis	✓		✓
SUBJECTIVE MULTIMODAL ASSESSMENT			
Central Sensitization Inventory	✓	✓	✓
2011 FM survey criteria	✓	✓	✓
Brief Pain Inventory Overall	✓	✓	✓
PainDETECT	✓	✓	✓
10 day pain diary	✓	✓	✓
McGill pain questionnaire	✓	✓	✓
PROMIS physical function	✓	✓	✓
PROMIS pain interference	✓	✓	✓
PROMIS sleep-related impairment	✓	✓	✓

PROMIS fatigue	✓	✓	✓
MISCI	✓	✓	✓
PROMIS depression	✓	✓	✓
PROMIS anxiety	✓	✓	✓
PANAS	✓	✓	✓
Perceived Stress Scale	✓	✓	✓
PROMIS emotional support	✓	✓	✓
PROMIS social participation	✓	✓	✓
Life satisfaction Questionnaire	✓	✓	✓
Coping strategies questionnaire - catastrophizing	✓	✓	✓
Childhood traumatic events scale	✓		
Conner Davidson Resilience	✓	✓	✓
Patient Global Impression of Change		✓	✓
Hyperacusis questionnaire	✓		
OBJECTIVE MUTIMODAL ASSESSMENT			
Thumbnail Pressure pain sensitivity	✓		
Cuff algometry	✓		
Algometry on different body areas	✓		
Temporal summation	✓		
Aversion to visual stressors	✓		
Voxel based morphometry MRI	✓		
Resting state functional connectivity MRI	✓		
Evoked pain functional MRI	✓		
Proton magnetic resonance spectroscopy	✓		
Optional blood samples	✓	✓	

3.3 Collection of study outcomes and assessments

Herein, the methods used to collect clinical data, questionnaires on outcomes with a known impact on pain, objective and subjective pain assessment, as well as inflammatory and immunological status are provided. All baseline visits were conducted in person, adhering to government guidelines related to COVID-19. Personal protective equipment were consistently utilised by both myself and participants throughout these visits. When more restrictive measures were in place, baseline visits were temporarily suspended. However, follow-up visits were conducted remotely within the ethical approval granted for the study. The impact of COVID-19 led to delays in participants recruitment during the suspension periods. Furthermore, conducting follow-up visits remotely posed different challenges, particularly in assessing joint disease and collecting blood samples. However, the ethical approval granted the remote collection of questionnaires, mitigating the impact on questionnaire data collection to some extent.

3.3.1 Medical history and clinical status

Consent to access medical history from NHS records was given by participants. Medical records and subjects' interviews were used to document the medical history and comorbidities, previous exposure to DMARDs and current medications.

A set of clinical information was collected at the study entrance, including age, sex, smoking and alcohol consumption, height, weight and BMI (also part of the exclusion criteria), education, relationship and working status (via the questionnaire), as well as parameters of disease activity. Clinical variables were recorded on a clinical research form (CRF, in Appendix 4).

1.2.7 Comorbidities and presence of psoriasis

As part of the CASPAR criteria (table 1.1.1) the presence of a personal or family history of PsO is recorded. The presence of PsO, particularly when extensive can contribute to the reported pain via itching, which is a peculiar feature of PsD[712]

[713,714]. Moreover, the presence of several cardiometabolic comorbidities, the presence of other chronic pain conditions, or psychological disorders (e.g., anxiety and depression), was also documented for their known impact on chronic pain development and level of pain experienced. For example, the presence in FM of chronic painful conditions and/or psychological disorders, especially depression (up to 63%), is highly frequent, therefore, they need to be carefully evaluated[715].

3.3.2 Smoking and alcohol status

Participants were asked about their recreational use of tobacco smoke and alcohol. Smoking habits were recorded in 3 categories as current smokers, ex-smokers, and non-smokers. The effect of smoking on PsA and FM pain is not linear. The presence of psoriasis and depressive behaviours, respectively, seem to mediate the effect of smoking on arthritis and chronic pain[716,717]. In detail, smoking is a risk factor for PsO development and poor response to treatments; however, smoking has been negatively associated with the presence of PsO in patients with PsA ("smoking paradox")[716]. In FM, smoking has a negative impact on the reported pain and the association between the smoking and FM seems to be largely mediated by depressive symptoms[717].

Alcohol intake was evaluated with the participants-reported average of weekly units of alcohol intake using the UK's Alcohol unit online calculator[718]. Low alcohol intake was associated with reduced pain in FM, probably due to the GABAergic effect of low alcohol consumption[719,720]. However, excessive alcohol intake with (more than 53.6 units per week) showed to have an increased risk of developing chronic pain syndromes[721]. Moreover, the neurobiology of chronic pain syndromes and alcohol abuse seems to share similar brain areas and behavioural patterns; this evidence suggests a pathogenetic link between the two conditions[722].

3.3.3 Weight, height, and BMI

The presence of high BMI in PsA can reduce the achievement of remission or low disease activity[723]. Therefore, weight (Kg) and height (m) were measured during

the baseline visit for each participant. BMI was calculated with the following formula Kg/m^2. A BMI above 40 (Obesity class 3[724]) was also used as exclusion criteria.

3.3.4 Past therapeutic exposure and current medications

Previous and current medications were recorded. Previous exposure to biologics and DMARDs was recorded. The number of previous biologics or other class of DMARDs can reflect both disease duration (more time higher chances to change DMARDs) and severity of disease (lack of response to different DMARDs). Moreover, fibromyalgia in comorbidity can reduce the efficacy of DMARDs treatment in PsA[448]. Therefore, a higher number of previous treatments is expected to be associated with FM in this cohort. The newly prescribed immunosuppressant was also recorded.

The active ingredients and dosage of current DMARDs and concomitant medications were as well noted. A particular focus was dedicated to DMARDs and CNS-acting drugs, because of the potential impact on disease activity, severity of pain, and objective and subjective pain assessments. Importantly, pain medications can have an impact on pain perception at different levels, both at peripherally and centrally. The regular assumption of opioids (e.g. paracetamol/codeine, dihydrocodeine, tramadol, morphine), antidepressants or other psychoactive drugs (i.e. gabapentinoids, SNRIs and SSRIs, and benzodiazepines) was therefore recorded. These data were used for secondary sensitivity analysis in relation to pain.

3.4 Descriptors of disease-related activity and status

3.4.1 Diagnosis and disease duration

Clinical records and participants' survey were used to confirm the fulfilment of CASPAR criteria, including radiological findings and immunological status (RF). Chronic inflammation represents an opportunity to develop over time articular and peri-articular damage, especially when inadequately controlled. Moreover, it has been proposed that the greater cumulative exposure to inflammation, the

greater risk of nervous system sensitisation[725]. The participants' reported onset of symptoms was considered as the date of disease onset. The reported symptoms onset was preferred to the date of the first rheumatology visit mainly to overcome the underestimation associated with delays in referrals to a specialist; this delay varies across individuals and is associated with several factors, for example severity of clinical onset, rapidity of GP access and their knowledge of rheumatological conditions, presence of confounding comorbidities, i.e. OA or other diseases presenting with musculoskeletal symptoms. On the other hand, the patients' reported symptoms may overestimate the disease duration, as more generic musculoskeletal symptoms could be attributed to the arthritis unbeknown to the patient. To minimise this bias, the participants were asked to specify the onset of symptoms compatible with synovitis, enthesitis, or dactylitis. The disease duration is reported in years.

3.4.2 Blood samples collection and processing

CRP and ESR are commonly employed as markers of inflammation and often integrated in disease activity scores[726]. CRP is a test with greater sensitivity to acute phase inflammation when compared to ESR; in fact, ESR is known to be influenced by other haematological parameters[727]. Therefore, CRP was preferred over to ESR.

Blood investigations were conducted on the same day of the research visits. The samples were collected by trained team members following established procedures. In addition to CRP, any other additional blood tests requested by the standard care clinician (to avoid extra attendance to hospitals during the COVID-19 pandemic) were collected. In case of study visits conducted remotely, due to the pandemic, or if it was not possible to collect blood from the patient on the day of the visit, CRP values were found on the NHS system database, using a range of +/- 2 weeks from the visit. If CRP values were not available within this timeframe, CRP was recorded as a missing value. CRP was processed by local NHS laboratories, as per the routine testing methodologies. Additional optional blood samples were also collected at the baseline and 3 months' visits for future ethically approved research studies, including this thesis. Additional

blood samples were processed and stored within the University of Glasgow tissue biobank by trained personnel according to standardised protocol (Appendix 4).

3.4.3 Disease activity

In research oriented on PsA and other immune-mediated diseases, validated measures of disease activity are of highly importance for research outcomes[728]. Indeed, exploring the relationship between measures of disease activity and subjective and objective pain outcomes is crucial to better understand the bidirectional relationship between inflammation and pain phenotypes.

For the purpose of this study, different disease activity measures were collected. Keeping in mind the clinical heterogeneity of PsA, it is important to clarify that the primary focus was on the peripheral joints' involvement. The number of swollen and tender joints assessed by a trained researcher was documented respectively on 66 and 68 joints, as recommended for research studies on PsA[176,186]. Peri-articular manifestations were also considered as an important outcome, because are confounders for the presence of nociplastic pain, especially enthesitis. The presence of enthesitis was assessed using the LEI score ranging from 0 to 6 entheseal sites tender to touch[210]. The dactylitis score recorded the presence of dactylitis using a simple count of digits affected on both hands and feet (0-20). The questionnaire-based clinical score BASDAI was used to evaluate axial involvement[195]. The presence or absence of PsO was also noted, however severity or extension of skin inflammation were not evaluated. PhGA and PGA were also collected on VAS scales.

The DAPSA score was used to obtain a unique measure of disease activity at the peripheral joints to correlate with the primary outcome of nociplastic pain (2011 ACR FM score). DAPSA is computed by summing the reported pain on a VAS, the swollen (66) and tender (68) joint counts and CRP. All the above variables were collected in the study, without additional impact on participants. In addition to the simplicity of the score, DAPSA is a disease specific score which has been recommended to evaluate disease activity and clinical response by European guidelines (EULAR)[237].

3.5 Subjective pain assessment

3.5.1 Introduction

A comprehensive series of questionnaire were selected to characterise the pain phenotype and related bio-psychosocial determinants, including fatigue, quality of sleep, psychological status, cognitive and physical function. The full battery of questionnaire administered is in the study matrix (table 3.2.2). The primary aim of this study is around the presence of nociplastic pain in individuals with PsA and pain phenotype relationships with objective measures of pain, functional connectivity and QST, and inflammatory status, disease activity and inflammatory markers. Therefore, selecting a pain questionnaire able to detect presence of nociplastic pain was of primary importance for the aims of the study. The 2011 ACR FM criteria were used as primary outcome. The advantage of the questionnaire is that it has been validated for epidemiological studies, it is possible to self-administer it to participants, and it is a reliable measure of the degree of nociplastic pain when used in a continuum scale. Other validated scores to measure central sensitisation in primary FM, for example the CSI, represented valid alternative to the 2011 ACR FM criteria as primary outcome. However, the CSI has been validated as a screening tool only, without specific support in cohorts with different chronic musculoskeletal pain conditions. Therefore, the 2011 ACR FM criteria were preferred for this analysis.

Despite the importance of different psychosocial outcomes, including cognitive and emotional impact of pain, sleep impairment, anxiety and depression, the analysis of those parameters where beyond the aims of this thesis.

3.5.2 Data collection

Following the completion of the consent form, the baseline visit followed a precise order to avoid introducing variety between participants. After the initial collection of clinical history and BMI parameters, the set of questionnaires was administered. The participants had the opportunity to complete the questionnaire either on paper or on online (in an NHS GGC approved database: Qualtrics, Provo, UT), depending on personal preferences. During the baseline

visit a member of the research team was available if participants had any doubt or question. The assistance also allowed familiarisation with the questionnaires and increased the participants' confidence in self- administration before the follow-up visits. In fact, before the follow-up visits, participants had the opportunity to complete the questionnaires online or on paper up to a week before attending the research facility, according to individual preferences. At all visits, questionnaires were administered before physical examination and blood drawing to avoid eliciting pain or discomfort, which could negatively influence questionnaire responses. Following socio-demographic and self-reported family medical history on chronic pain and psychological disorders, the 2011 ACR FM criteria were the first to be administered to avoid mental tiredness when completing the primary outcome questionnaire. The order of the questionnaire administered remained unchanged from baseline to the 6 months' visit as showed in the study matrix (table 3.2.2).

3.5.3 Scoring the 2011 ACR Fibromyalgia survey

The 2011 ACR FM provided a measure of the degree of nociplastic pain by integrating two separated indexes, WPI and SSS, assessing two cardinal features, respectively the widespread body pain and the intensity of central-somatic symptoms.

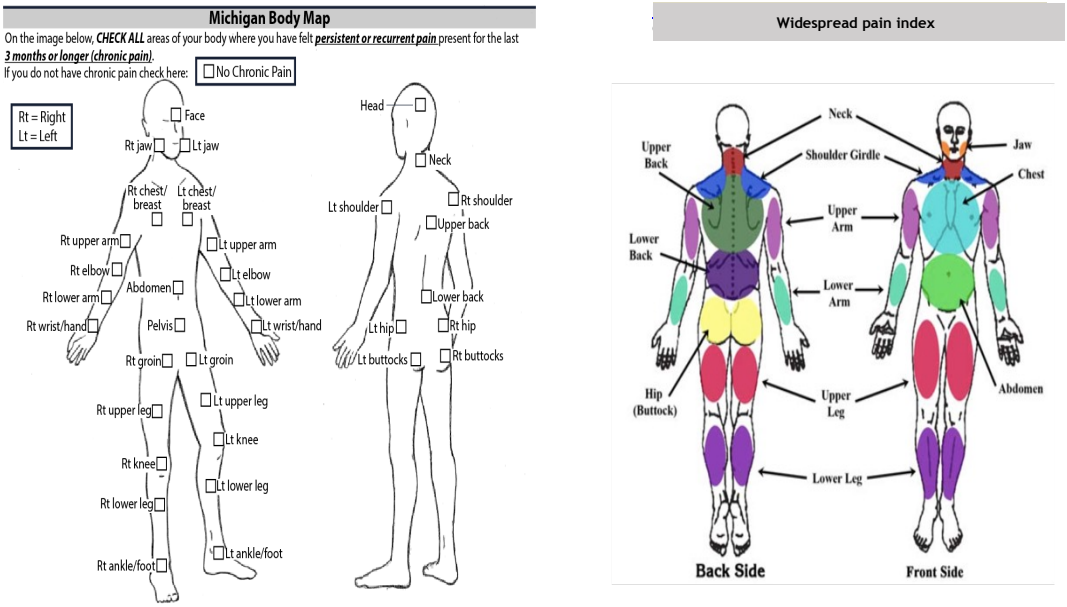
3.5.3.1 *Widespread pain index*

The Michigan body map (MBM) was used to calculate the WPI. The MBM is a self-assessment tool validated to measure widespread pain, a key component of the FM survey score[729]. The MBM was developed to accurately detect the distribution of pain in different bodily regions, including additional areas when compared to the traditional WPI (figure 3.5.1). Participants were asked to indicate the body areas presenting persistent or recurrent pain for at least 3 months, by ticking the appropriate box on the MBM. To calculate the WPI the three following steps were undertaken: 1) additional body areas, i.e., face, elbows, wrists/hands, pelvis, groins, knees, ankles/feet, and head were excluded; 2) hips and buttocks in each body side, and chest areas were counted a single area to reflect the

WPI; 3) summation of the remaining ticked boxes in the MBM and the areas in the point (2). The resulting WPI scores ranges from 0 to 19.

The use of WPI over MBM was preferred to evaluate the level of widespread pain. Despite the MBM is a useful tool to evaluate widespread pain in several chronic pain conditions, the additional areas included in the body map correspond to articular sites often painful in chronic inflammatory arthritis (Figure 3.5.1). Therefore, there is a risk of bias in evaluating the MBM in the present study, when considering that the overlapping nociceptive pain in a PsA population with active disease might overestimate the degree of nociplastic pain.

Figure 3.5.1 Michigan Body Map and widespread pain index in comparison



Legend: Michigan Body Map (MBM) and the widespread pain index (WPI) are body maps used to assess widespread pain in chronic pain conditions. MBM assess a higher number of body areas compared to the WPI. MBM demonstrated to be a valid, accurate, and reliable measure of pain widespreadness as component of the fibromyalgia score.

3.5.3.2 *Symptoms severity score*

The SSS questionnaire followed the MBM with the intent of assessing the presence and severity of three central symptoms and three somatic symptoms.

The three central symptoms include fatigue, waking unrefreshed, and trouble thinking or remembering. When symptoms are present, subjects are asked to rate the severity of the symptoms between mild (1), moderate (2), or severe (3). The intensity rating is referred to the past week and an additional question specify if the symptoms have been present for at least 3 months.

The three somatic symptoms include headaches, pain or cramps in the lower abdomen, and depression. The occurrence of those in the previous 6 months results in a score ranging from 0 to 3.

Finally, the severity of the central symptoms and the presence of somatic symptoms are summed to obtain the SSS (0-12).

3.5.3.3 *Fibromyalgianess*

A patient was classified as having FM when the three conditions of the 2011 ACR FM criteria were met: 1) WPI ≥ 7 and SSS ≥ 5, or WPI between 3–6 and SSS ≥ 9; 2) symptoms have been present at a similar level for at least 3 months; 3) the patient does not have a disorder that could otherwise sufficiently explain the pain [367] . The participants were divided into two groups meeting the criteria (FM+) or not (FM-) and the differences between the 2 groups were calculated. Some degree of nociplastic pain can be present also in individuals not meeting the FM criteria. Therefore, the total FM scores was used to express the degree of nociplastic pain, independently from the FM classification and from comorbidities associated. The FM scores obtained were used as continuum variable to calculate correlation with other neurobiological pain outcomes and clinical parameters.

3.5.4 Impression of global disease activity and body pain

Prior to the joint count a rating on the impression of global disease activity in the past week was asked to the participating subjects. A 10 cm long VAS was used. After Joint count the trained researcher blinded from participants' rating completed a similar VAS scale on the impression of global disease activity (Appendix 4). The ratings were transformed in numeric values using a centimeter's ruler, ranging from 0 to 10 (measured on a 10 cm line).

Different pain ratings were asked to participants on NRS scales, usually ranging from 0, “no pain” to 10 “worst pain you could possibly imagine”. During the questionnaire, participants were asked to rate the intensity of body pain at its worst over the past week and the average. The measure of overall body pain on average was preferred for the analysis conducted in this study. The overall body pain in the past week is a measure commonly used in clinical trials to assess the efficacy of treatments. Moreover, the average pain could be theoretically higher when hyperalgesia or allodynia are present with FM as comorbidity. Moreover, the currently experienced overall body pain and pain attributed to PsA were also completed before and after the fMRI scan using a 0-10 NRS scale.

Additionally, after each visit participants were asked to complete a pain diary for the following 10 days. Subjects were instructed to complete the diary with reporting the average pain experienced in the previous 24 hours, preferably at the same time of the day. According to participants preference, the 10 days pain diary was returned on paper (by a pre-paid envelope), telephone, online, text or at the follow-up visits. An average of the pain reported on the pain diaries was used as pain outcome.

3.6 Neurobiological correlates of pain

3.6.1 Introduction

Characterising pain phenotype is a challenging task mainly due to the complex interactions of many different contributors to the genesis of pain perception; among these neurobiological, immunological and psychosocial factors are involved. Recent advances in QST and fMRI methodologies allowed a better understanding of pain with a more objective characterisation of pain, as it does not rely solely on patients’ reported information. The integration of QST and fMRI is able to generate an extensive description of the neurobiological signature of pain in a group of individuals. To my knowledge, this is the first clinical study where a combination of these methodologies is used in a cohort of individuals affected by PsA.

3.6.2 Quantitative sensory testing

3.6.2.1 *Data collection*

The QST assessments were performed at the baseline visit only. The testing was carried out within the same clinical research facility, preferably in the same experimental room to avoid environmental biases between participants. The room was without windows to allow easier set-up of the aversion to visual stimulations test. The QST followed the collection of the medical history and clinical parameters, the questionnaire, followed by joints count and blood drawing. Prior to each testing a familiarisation procedure was carried out to reduce test-related anxiety and to train participants to better comply to each task.

The familiarization testing was carried out on the dominant right side, while the formal assessment was performed on the left side. The differences between left and right side within the same individual is negligible[529], however the evidence suggests that the dominant side is more sensitive, especially in right-handed individuals[730,731]. Therefore, carrying out the formal test on the non-dominant side (left) helped improve homogeneity of the data acquired and to reduce the potential bias linked to the different sensitivity.

The level of anxiety before and after the QST session were rated on a NRS from 0 (no anxiety) to 10 (the worst anxiety imaginable). The order of the testing remained consitent for each participant (table 3.6.1). For the QST session the same examiner read a script to ensure standardised instructions were given to each participant. Participants had the chance to ask questions or stop the test if too painful at any time. The trained researcher performing the QST was the same throughout the study, with the assistance of two other team members of the same gender (female). The advantage of having the same examiner and with the same gender is to reduce the gender-bias observed in a previous QST study[528]. The same gender examiner(s) granted consistency in the testing, despite it might have had an impact on participants with different sexes, for example males are more likely to have lower pain PPT is a female is the examiner (results discussion in section 5.4.1). Data were noted on paper CRF

format during the testing. At the end of each visit, the information was transferred on an approved electronic CRF, while performing a quality control on the collected data.

Table 3.6.1 Order of the QST protocol

	QST ASSESSMENT
1	MAST Familiarisation
2	Cuff Familiarisation
3	MAST Ascending Series
4	Cuff Evoked
5	Cuff Pseudorandom
6	MAST Random Series
7	Algometry
8	Temporal Summation Familiarisation
9	Temporal Summation Formal
10	Cuff Continuous
11	Visual Stimulation Task

The following QST protocol will describe the different modalities employed to deliver noxious and non-noxious stimuli to the study participants. The semi-objective method used required the examined subject to rate the pain sensation after each stimulus and/or the overall testing. The following QST battery assesses the somatosensory system at different levels obtaining different parameters associated to pain sensitivity, including pressure pain threshold, tolerance, indices of TS and sensitivity to visual stimulation. The QST methods used in this study have been validated and applied in other clinical studies on chronic pain associated with various conditions[732–734].

3.6.2.2 *Pressure pain sensitivity*

For both static and dynamic QST, the mechanical modality is commonly used in studies including cohorts of inflammatory arthritis (table 1.2.4). Mechanical stimulation can supposedly better emulate or trigger nociceptive pain secondary to joint inflammation. In fact, in different controlled studies, RA individuals showed a reduced pressure pain threshold (PPT) at the level of the inflamed joint, but also at non-affected body areas when FM was in comorbidity; similarly, the tolerance to pressure was reduced in RA[553]. To date, different stimulation modalities have been used rarely in RA, e.g., thermal stimuli, with

less robust results compared to pressure modality (detailed in section 1.2.4.1). Below will follow the different testing used the evaluate the pressure pain sensitivity which comprehend the majority of QST carried out in the study.

3.6.2.2.1 *Thumbnail algometry using the MAST device*

Algometers are electronic devices commonly used to manually apply and measure the pressure on a precise surface area. With a manual algometer it is possible to detect pressure pain threshold by applying an increasing pressure to a predetermined area and ask the subject to alert the investigator when pain is first experienced. The intensity of the pressure needed to reach the threshold can be highly variable between the different body areas tested. For example, the pressure to elicit pain on a small joint of the hand can be much less than the one needed for a larger articular structure, e.g., knee or hips. Additionally, it is important to mention the effect of the operator applying pressure, requiring dedicated training, and the selection of the area to be tested. The thumbnails have been commonly used to assess pressure pain sensitivity; mainly for the easy access to the body area and because thumbnail pressure sensitivity has demonstrated its validity as marker of overall body sensitivity and its ability to reflect the altered CNS sensory processing in FM[735].

Several studies consistently showed lower PPT at the thumbnail in individuals with FM when compared to healthy controls, either using manual[736,737] or automated algometers[515,738-740]. Measurements of sensitivity to pressure at the thumbnail were also used to assess or predict response to pharmacological and non-pharmacological treatments in FM[741-743]. Due to the easy accessibility of thumbnails during MRI scans, pressure stimulations in this area have been successfully used in pain-evoked fMRI studies showing an altered brain connectivity in FM in response to pressure pain[380,607,742,744-748]. To further confirm that pressure pain sensitivity at the thumbnail is a valid surrogate of FM central sensitisation, PPT at the thumbnails significantly correlated with excitatory neurotransmitters' levels detected in the insula with spectroscopy studies[749,750]. Moreover, pressure pain at thumbnails has been validated to be used as conditioning stimulus to assess CPM in FM[521].

In inflammatory arthritis the thumbnail pressure sensitivity was investigated as a non-articular area remote from sites primary involved by arthritis, i.e. joints. Therefore, an increased pressure sensitivity at the thumbnail may reflect the generalised hypersensitivity associated with central sensitisation also in individuals with arthritis.

Both digital and manual algometers have been used to deliver pressure at the thumbnail in studies enrolling subjects with RA, showing lower threshold compared to healthy (not statistically significant) and correlation with TJC and sleep impairment[549,551,557,561,751]. An automated computer-controlled piston machine was also used to deliver painful stimulations during fMRI studies in RA showing altered brain networks interactions and functional connectivity in RA following painful stimulations, distinct from both healthy and FM[620,621,752].

Despite the progresses to date, standardization processes are not straight-forward. To address these issues, automated algometers have been developed. Recently, the University of Michigan developed the multimodal automated sensory testing (MAST) device which is computer-based automated to deliver pressure on the thumbnail in a controlled and systematic manner[753]. Using a stimulation device also limits direct interaction between the examiner and the participant, limiting bias related to subjects' anxiety and operator physical strength. Additionally, automated devices allow the use of pressure pain stimulation during task-based fMRI. The MAST was successfully used to assess pressure pain sensitivity across different chronic pain conditions[546,732,754], including RA[621].

Study protocol

The MAST was used to apply pressure at the left thumbnail in a controlled and systematic manner. After a familiarization procedure on the right thumbnail, an ascending test was performed on the left thumbnail. The ascending test consists of delivering to the thumbnail increasing discrete pressures for 5 seconds. Between stimulation a recovery time of 20 seconds was given. The initial pressure was at 0.25 Kg/cm^2, followed by a 0.50 Kg/cm^2 stimulation. Rising in 0.50 Kg/cm^2 from the third pressure were applied. After each stimulus the pain

intensity was rated on a 0-100 NRS. The test stopped after reaching participant tolerance, defined as a rating ≥80/100 or highest pressure tolerated. The maximum pressure applied was equal to 10 Kg/cm^2. Pressure pain threshold (thumb-PPT), defined as the first thumb pressure to elicit a pain rating > 0, was also recorded. The recorded pain ratings of the ascending MAST test were used to interpolate the Thumb Pain50, defined as the pressure intensity that evokes a moderate level of pain (i.e., 50/100).

Depending on participant tolerance, a series of pressures were applied to the left thumbnail in a random order. Random tests are less sensitive to the "bias of expectancy" which can lead the examined subjects to provide higher ratings to end the test earlier[755]. On the other end, the awareness that the pressure will come in an ascending order might give a perception of increased control to the participants which can lower their pain ratings; in fact, in FM ratings in random tests with different modalities, including pressure, pain perception was higher compared to the ascending test[756]. Therefore, both ascending and random test can provide useful, but different information on individual pain sensitivity. In the random test delivered, to participants of this study, none of the pressures were higher than the tolerance reported in the ascending test.

3.6.2.2.2 *Deep tissues pressure pain sensitivity measured with cuff algometry*

In contrast to single-point algometry, which commonly applies pressure on a surface of around 1cm^2, cuff algometry has the advantage to deliver mechanical pressure to the deeper somatic tissues more effectively[757]. Skin desensitisation minimally affects pain responses elicited by cuff algometry, confirming that the primary target are deeper structures, including muscles and adipose tissue[758,759]. Computer-controlled cuff algometry is a safe, reliable, and validated technique to assess deep somatic tissues' pressure pain sensitivity, pain summation, and CPM[760–764]. Moreover, machine-delivered techniques have the advantage of delivering standardised pressures and being operator independent with high testing reliability[765]. In healthy subjects the cuff algometry pain thresholds and tolerances showed an excellent short- (2 weeks) and long-term reliability[761]. Cuff algometry has been demonstrated to be a useful assessment tool to evaluate

pressure pain sensitivity in musculoskeletal diseases, including inflammatory arthritis, where the involvement of deeper articular and peri-articular structures represents a key clinical feature[764,766–770].

In FM studies including evaluation of cuff pressure pain threshold and tolerance, a hypersensitivity in this population was demonstrated when compared to healthy or individuals with chronic low back pain[771,772]. Interestingly, muscle strength, but not depression or FM impact scores, showed a significant correlation with cuff algometry parameters. The authors justify this finding with the methodology used where the participants are able to stop the inflation directly and not via the examiner, minimising the bias due to expectation and control[771]. Moreover, neuropathic pain features (PDQ), known to be part of the central pain symptoms, were significantly associated with both cuff pressure pain threshold and tolerance in FM[773]. Cuff algometry can safely apply pressure for over 10 minutes[762], therefore, it has been assumed to emulate chronic pain when applied sustainedly. Following this principle, cuff algometry has been employed to deliver a sustained deep tissue stimulation in healthy, while performing fMRI scan for brain connectivity; in this study, during a 6-minutes cuff stimulation applied on the lower leg, it was demonstrated a shift of the somatosensory cortices (SI-II) connectivity from the SMN, coordinating motor reactions, to the SLN, responsible for attention and affective processing[774]. During task-fMRI scans, different cuff pressure intensities delivered to healthy subjects showed activation of different brain areas independently (SI-II) or dependently (DMN) to the pressure intensity applied[775]. Another study using tonic cuff stimulation during fMRI scans, compared brain activation between healthy to FM subjects, showing a strongly reduced activation of brain areas related to reward and punishment in FM[776].

Moreover, cuff algometry was also effectively used in RA cohorts. Pressure pain threshold and tolerance were reduced in RA subjects compared to healthy when measured with cuff algometry[777]. In the same study, TS was also assessed over the course of sustained cuff stimulations for 10-minutes; subjects with RA showed an increased cuff temporal summation. In another study, including participants with an active RA, the cuff TS was not able to predict response to newly started b- or cs-DMARDs at 4 months[559]. Cuff algometry was also assessed

in individuals with axial SpA in an uncontrolled study. The pressure pain tolerance was associated to different parameters of disease activity and duration, as well as more widespread and severe pain, and worst fatigue and anxiety, features of central sensitisation. Similarly, cuff TS in axial SpA was associated with pain severity, disease duration and spine mobility[563].

Collectively, these data suggest that cuff algometry is a reliable and objective assessment tool able to detect parameters of pain hypersensitivity characteristics of central sensitisation, seemingly independent from the subjective phenotype, and to elicit detectable brain activation and connectivity. Cuff algometry was therefore included in this study to obtain objective parameters of pain sensitivity related to deep somatic tissues, highly relevant for musculoskeletal diseases, e.g., PsA. Notably, this is the first study investigating cuff algometry in a PsA cohort.

Study protocol

Cuff algometry QST was used to assess deep muscle tonic pain. A velcro-cuff was applied at the largest circumference of the gastrocnemius muscle. A machine-controlled air compressor (Rapid Cuff Inflator, D.E. Hokanson Inc.) was used to deliver increasing pressures to the calf muscle. Familiarisation training was carried out on the right leg before proceeding to the formal test on the left. An ascending test was performed starting at a pressure intensity of 20 mmHg, with increasing 20 mmHg steps, until the maximum of 400 mmHg or the patient tolerance was reached. Each pressure was delivered for 10 seconds with a 20 seconds inter-stimulus interval. The participants were asked to rate the pain intensity of the previous stimulus on a 0-100 NRS. The Cuff-PPT and the Cuff-Tol were recorded. The ascending ratings were used to interpolate values from P10 to P70, i.e., pressure able to induce a rating equal to 10, 20, 30, and so on until 70 out of 100 on the NRS. The interpolated values were used to deliver a tailored pseudo-random test. With the obtained pain ratings a final Cuff Pain50 was calculated.

After a short calibration procedure to a find a pressure intensity that evokes moderate pain, starting from the interpolated Cuff Pain50, the final P50 was

applied for 6 minutes. Pain ratings were obtained every 60 seconds. At the end of the 6 minutes testing the overall pain and unpleasantness were obtained using the same 0-100 NRS. An elegant study demonstrated that pain unpleasantness can be affected by mood more than pain intensity ratings[778]. Therefore, unpleasantness rating during sustained cuff stimulations was included.

3.6.2.2.3 *Hand-held algometer to test PPT at different body sites*

Mechanical stimuli are the most employed in the studies investigating FM and chronic musculoskeletal conditions. Also the tender points, included in the 1990 ACR FM criteria, involve the manual application of mechanical pressure. The use of hand-held algometers to assess sensitivity to pressure pain is practical, versatile, and validated to assess pressure sensitivity; these features allowed its diffusion in pain research and in normal clinical settings, e.g., as a more sophisticated and quantifiable palpation of the tender points[755,779-781]. Not surprisingly the mechanical algometry probably constitutes the largest body of literature on pain QST. Algometers allow the measurement of the pressure applied on a predetermined body surface; the standardisation of the surface and shape of the probe is important to define accurate thresholds. In fact, the pressure is directly dependent on the surface and spatial summation is an effect to take into account with larger probes, especially in chronic nociplastic pain conditions[782]. Currently the most widely used probe has a 1 cm^2 flat disc surface. This has produced the most reliable results[783]. Importantly, the increasing of the pressure applied should be gradual and constant (usually at 1Kg/sec), as the pressure rate is known to influence the elicited pain levels[784]. The PPT, defined as the first pressure perceived as painful or discomforting, is the first quantifiable pain parameter obtained with algometry testing. Also tolerance, pain summation and CPM can be determined with algometry testing. The outcomes of hand-held algometry are indeed operator dependent because examiners' expectation, or the technique used, may influence the outcome[785]; thus, training of the examiner is crucial. Virtually all the areas of the body can be examined, however a careful selection of the target area is dictated by the disease of interest and the anatomical accessibility[786]. Independently from the body area chosen, anatomical differences between individuals would be physiologically present; therefore, it is important to identify precise anatomical

references to guarantee reliability and reproducibility of the test. In several FM studies using algometers to measure the pressure needed to elicit pain on the 18 tender points, a reduced PPT was repeatedly demonstrated compared to healthy individuals[739,787–789]. The PPTs at the tender points demonstrated sensitivity in distinguishing FM from healthy, however other body areas demonstrated similar accuracy (75 to 89%)[789]. Other studies assessing fewer body areas (4-7) showed similar findings[772,790]. Therefore, pressure hypersensitivity in FM is generalised involving the entire body, and not just the tender points[791]. Additionally, algometry was also integrated in clinical trials evaluating the efficacy of therapies in FM. These showed improved PTTs and tolerances after pharmacological and non-pharmacological treatments in FM, which further validates the relevance of pressure algometry in this condition[544,545,792–794].

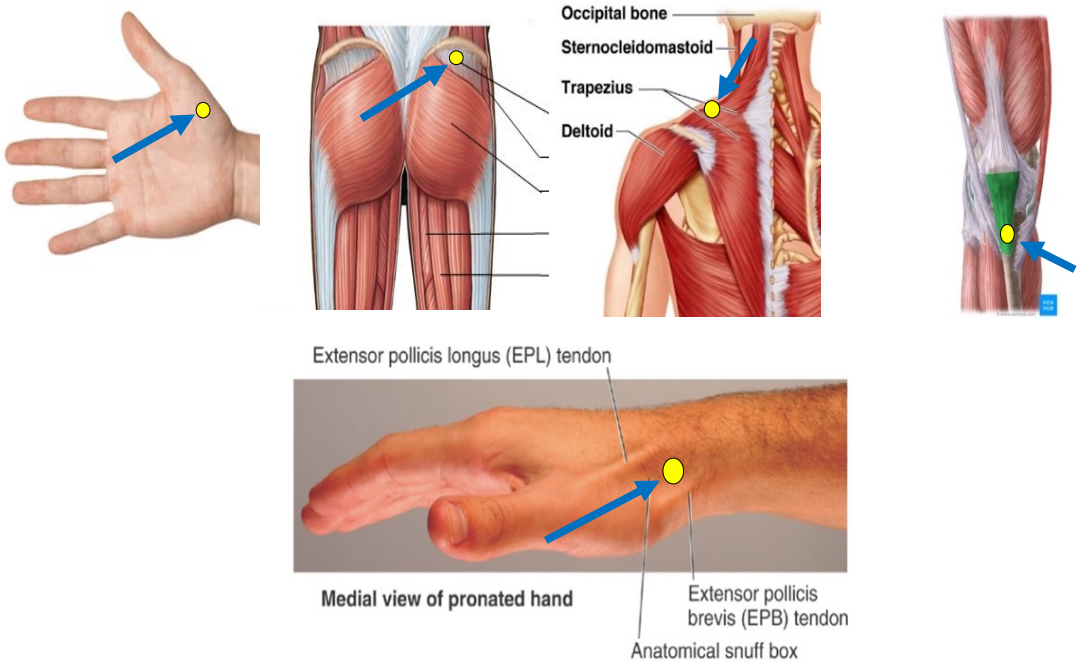
Algometry has been also employed to assess PTTs in RA were the areas mostly investigated were the hands (painful and non-painful sites) and the trapezium, the latter considered as a distant site from direct inflammatory involvement[553]. Other areas examined included lower leg structures, e.g., quadriceps, gluteal muscles, tibia, and knees. Overall, in RA reduced PPT were present mainly at articular inflamed joints compare to healthy, unless a degree of central sensitisation, measured with FM scores or sleep or depressive disorders, and high systemic inflammation were present. In these scenarios, reduced PTTs and increased TS (with pinprick pens – see section 3.6.2.2.4) was observed in non-affected areas[549–552,554–557]. In PsA and other phenotypes arthritis within the SpA group, the PTT or tolerance to pressure stimuli have not been directly correlated to central sensitisation, e.g., with FM scores. However, a connection between reduced PTT and depression, as well as reduced pressure tolerance, and TS with anxiety was demonstrated in these populations, suggesting a connection between central sensitisation mechanisms, phsychological disorders, and increased sensitivity to pressure [554,563,795].

These findings have been leveraged in this study to reduce testing time and to improve participants' compliance, by selecting nociplastic and nociceptive areas related to both PsA and FM.

Study protocol

The pressure pain threshold was measured at five different body sites using a handheld digital algometer with flat rubber probe tip with a surface of 1 cm^2 (Somedic, Hörby, Sweden). The body areas were assessed bilaterally, including the thenar eminence of the hand (hand), the gluteus medius insertion at the posterior superior iliac crest (hip), the point on the trapezius muscle (trap) that was most sensitive to touch (guideline of most painful area[779]), the anatomical snuffbox corresponding to the scaphoid bone (wrist), and the tibial insertion of the patellar tendon (knee) (figure 3.6.1). These areas were selected with the intent of representing body areas affected by inflammatory arthritis, or degenerative musculoskeletal diseases (e.g., OA), or those neutral to these conditions, and therefore more reliable in assessing the presence of central sensitisation. The trapezium was used as an area not involved by the inflammatory processes. The hand area is in close proximity with the carpo-metacarpal joint, often involved by degenerative OA processes. The wrist is often the target of synovitis processes, similarly the knee patellar tendon and the gluteus medius tendon insertion are common sites of enthesitis in PsA. Sites were tested in a random order[796] on the bare skin. The examiners applied the pressure manually, increasing it gradually at a rate of 50 kPA/sec (1000 kPa max). Participants were instructed to verbally express, or press a response button, as soon as the pressure was perceived as painful or discomforting. When the participant gave the signal, the testing was interrupted. The last pressure intensity was automatically recorded by the digital algometer, and immediately transcribed on the paper CRF as the PPT. Measurements were repeated 3 times on each site and the means were used for the analysis in this study.

Figure 3.6.1 Algometry – areas examined



Legend: The areas where the pressure pain threshold was assessed are highlighted with a yellow circle and a blue arrow. Hand – located on the Thenar eminence; Hip – located on posterior superior iliac crest insertion of the gluteus medius; Trapezius – located at the midpoint of the upper left and right trapezius; Wrist – located on the lateral side of the hand in correspondence of the scaphoid, at the so called anatomical snuffbox; Knee – located on the tibial insertion of the patellar tendon.
Image modified from: Pearson Education Inc., Moore & Dalley. Clinically Oriented Anatomy 5th Ed. 2006., and Ken Hub.com

3.6.2.2.4 *Temporal summation*

TS is a measure of the increased perception of pain when a stimulus with equal intensity is repeated over time, independently from the modality used (mechanical, thermal, electrical). An enhanced TS is a characteristic feature of central sensitisation and independent from peripheral sensitisation mechanisms[355,519,797-800]. In QST protocols, TS is usually quantified using the wind-up ratio (WUR) derived by the ratings after repeated stimuli versus single stimulus, representing the overall augmentation of painful sensation[780]. The frequency of the repeated stimulation applied is important to correctly elicit temporal summation, with the best induced responses at 1-2 Hertz (Hz)[801].

Lower stimulations frequencies can successfully evoke summation of second pain after the induction of summation, suggesting the possibility of sustaining the phenomenon overtime, reflecting central sensitisation as demonstrated in subjects with primary FM[802].There is no general consensus on the standard procedure to assess the magnitude of TS resulting in a great variety in the literature between modality, stimuli intensities and instruments used. Overall, it is important to consider that including different methodologies in the same protocol may result in excessive burden for the subjects tested. In fact, TS is influenced by several psychological factors, including anxiety and fear of pain, cognitive function, and stress[803–806]. Different modalities, including, electrical, mechanical, and thermal, have shown to be effective in demonstrating an increased TS in FM compared to healthy controls[534,772,806–810] and have also been linked to neurobiological alterations demonstrated with fMRI studies[811,812]. Electrical stimulations directly stimulate the spinal cord bypassing superficial and deep tissues[807]. Electrical stimuli are valid to assess previously establish TS and central sensitisation as demonstrated by the attenuation of electrical TS with ketamine in individuals with FM[545]. Mechanical and thermal modalities to elicit TS have been used more frequently in chronic musculoskeletal conditions. Some studies using thermal stimuli to induce TS have shown no significant differences between FM and healthy volunteers[813–815]. This evidence led to a preference for mechanical modalities, especially in our musculoskeletal clinical populations. Rolke and colleagues attempted to standardise different QST protocols for clinical trials settings, providing reference values computed from the testing of healthy subjects[529,816]. To assess temporal summation, the authors recommended the use of calibrated pinprick pens on different body areas, i.e., face, hand and feet. No differences between left-right side of the body were proved in their work. A single stimulus was followed by a train of 10 stimuli at a frequency of 1Hz in a $1cm^2$ area; the same procedure was repeated 5 times. The TS was measured using the WUR defined as the ratio between multiple and single stimuli (average of the pain ratings following 10 stimuli : average of the rating for the single stimulus)[529,816]. Importantly, TS can be effectively assessed in different body areas showing similar results, independently from the peripheral involvement of the area tested, in keeping with central pain mechanisms[547,817]. Of interest, a recent study showed that 3 repetitions of the

pinprick test had similar efficacy in eliciting TS to a 5 pinprick series in healthy individuals[818]. Moreover, in individuals with different chronic pain conditions, a 3 pinprick series successfully induced TS and it was possible to demonstrate its reduction following treatment[819]. The TS using the same protocol (3 pinprick series) was also able to predict response to acupuncture in FM[547]. Therefore, using 3 instead of 5 sequences of pinprick can reduce the testing time and increase participants' compliance, without compromising the testing accuracy.

TS has been also evaluated in individuals with inflammatory arthritis, using different methodologies. For example, repeated pulses of painful pressures applied at the lower leg using a cuff inflator induced higher TS in RA compared to healthy participants. Pain ratings collected during tonic cuff at the calf also confirmed higher TS in RA than in healthy controls[777], however a correlation with response to DMARDs was not proven[559]. Tonic cuff protocols also evidenced an association between TS and physical function in AS[563]. On the contrary, TS assessed with handheld algometers did not confirm these findings in both RA and AS[562,820]. Mechanical stimuli, applied with calibrated weighted pens at the forearm, appropriately induced TS which was significantly associated with pain ratings, disease activity parameters (CDAI, TJC, assessor and patient global VAS), and FM scores in RA cohorts[552,555,557]. Pinprick tests were also successfully employed to assess TS in RA[821,822]. To date TS and its relationship with central sensitisation has not been investigated in PsA.

The above evidence support that TS is a valid measure of central sensitisation and despite differences in testing protocols, mechanical stimulations are preferred for chronic musculoskeletal pain conditions. In this study, we employed the short-version of the standardized TS protocol by Rolke and collaborators, which uses 3 series of stimulations with pinprick-pointed pens.

Study protocol

To measure TS, a pinprick stimulus was applied to the dominant (right) forearm using 2 pinprick pointed pens (MRC Systems GmbH, Germany) calibrated at 256 and 512 millinewtons (mN). The forearm was chosen as neutral area distant from articular sites, minimising the possible impact of localized hyperalgesia due to

peripheral sensitisation; moreover, the forearm has been successfully used to assess TS in RA[552,555,557]. The 256mN pen, commonly used in various studies, was complemented by the 512mN pen to increase the chances of detecting the TS. Different protocols employed across various conditions might involve the use of multiple pinprick pens (up to 6-8), sometimes preceded by a pre-testing screening to select the pinprick pen able to induce ratings of at least 30-40/100 on the NRS. In this study, a heavier (512mN) pinprick pen was included in the protocol to reduce testing time needed for calibration testing, increasing participants' compliance, while collecting data from 2 intensities simultaneously. However, I acknowledge that this is the first study to investigate the presence of TS in PsA; therefore, it should be viewed as an exploratory undertaking that could be refined in future research.

The pinprick pens were sequentially applied to different areas on the same forearm, starting with the lighter pen (256mN) followed by the heavier one (512mN), unless participants indicated a preference to avoid it. Participants underwent practice on the left forearm to familiarise themselves with the test. An initial single pinprick stimulation was followed by a train of 10 identical stimuli at a frequency of 1 Hertz (Hz) (1 stimulation each second). Study participants were trained to rate the pain intensity of the pinprick sensation using a 0-100 NRS after both the single stimulus and the train of 10 stimuli. This procedure was repeated thrice for the 256mN and the 512mN pinprick pens, if tolerated. Two measures of TS were derived for each intesity: the WUR and the difference of the ratings on the NRS. The WUR is the mean pain rating of the three stimulus trains divided by the mean pain rating of the single stimuli; a WUR >1 indicates the presence TS[529]. To prevent data loss in cases where division was not possible due to ratings equal to 0 following the single stimulus, the presence of TS was also estimated by computing the difference between the mean of pain ratings of the 10 stimuli and the single one.

3.6.2.2.5 *Aversion to visual stressors*

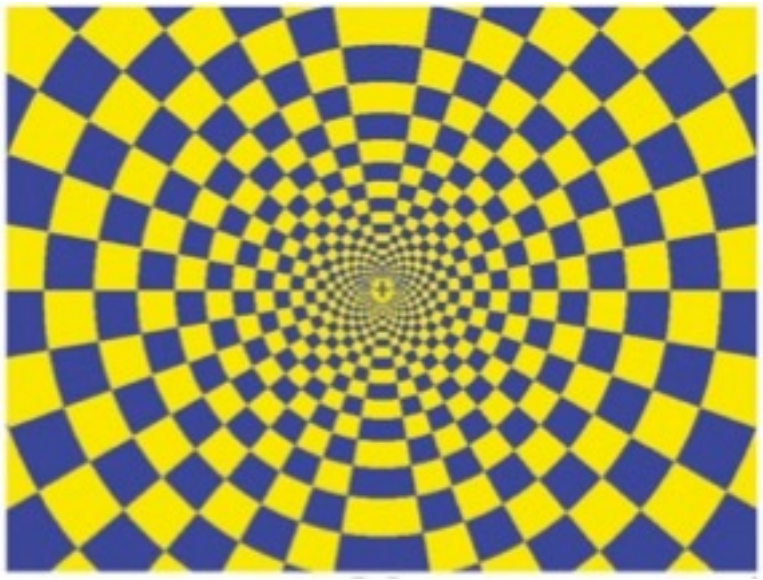
Beyond the somatic physical stimuli, e.g., mechanical and electrical, central sensitisation can characteristically involve a broader range of sensory sensitivity as demonstrated by the hypersensitivity to visual and acoustic stimuli of individuals with FM[740,823,824]. They enable the testing of general sensory sensitivity by directly stimulating the CNS without directly engaging the peripheral nociceptors or the spinal cord. Therefore, visual stimuli were included in this study to comprehensively assess central sensitisation and to better disentangle the different mechanisms contributing to pain in this PsA cohort. The M-VAST is a QST protocol recently developed to assess the sensitivity to visual stimuli [540]. This validation study showed a significantly increased level of unpleasantness ratings in the FM group compared to healthy. Interestingly, the unpleasantness ratings also significantly correlated with reported pain and pressure pain tolerance in the FM group. Moreover, the right antIC showed greater activation in the FM group during visual task fMRI. The greater insula activation was reduced after treatment with pregabalin. Support vector machine learning was able to distinguish FM versus controls, and pregabalin versus placebo based on fMRI data during visual stimulation. These data support the hypothesis that in FM an increased aversion to visual stimuli is present and linked to other features of central sensitisation, including altered brain activation and reduced tolerance to pressure pain. Therefore, evaluating visual sensitivity in PsA could help to understand whether central sensitisation is involved.

Study protocol

The aversion to visual stressors was tested by presenting a series of flashing blue-yellow and yellow-blue annular checkerboard patterns alternating at a frequency of 7.5 Hz (figure 3.6.2). The testing was developed using the PsychoPy2 software[825]. Participants were instructed on how to perform the test and allowed to sit in the examination room with the lights off for 5 minutes to provide a sufficient time for dark adaptation. The subjects of the study were seated with the eyes perpendicularly aligned at a 50.8 cm distance from a high-resolution LED monitor (EIZO, Japan). The visual stimuli varying in brightness

followed 10 seconds of dark screen and had a duration of 5 seconds. The illumination intensity included 6 different brightness levels, precisely 0.1, 0.2, 0.4, 0.6, 0.8, and 1 lumen. After each stimulus, participants were asked to rate the unpleasantness and brightness of the previous pattern on a 0-100 NRS. Initially, luminous stimuli were presented in an ascending order from 0.1 to 1. The first 6 visual stimulation were considered as part of the familiarisation to the procedure and relative ratings were excluded from the analysis. Subsequently, each luminous intensity was displayed for 3 times in a pseudo-random order. The mean of unpleasantness and brightness ratings were used for the analysis.

Figure 3.6.2 Dynamic visual stimulus presented to study participants to assess visual sensitivity



Adapted from Harte SE et al. Pain. 2016;157(9):1933-1945[540].

3.6.3 Functional neuroimaging

Advances in neuroimaging techniques have allowed a deeper understanding of brain function, morphology, and biochemical change. MRI methodologies compared to more traditional techniques, such as electroencephalography, have the advantage of exploring deeper areas within the brain. Other advantages of MRI methodologies are their non-invasiveness, availability, and lack of exposure to radiation[826]. Most functional neuroimaging studies use the BOLD contrast (section 1.2.5.1) which acts as a surrogate marker of brain activity. The BOLD contrasts are superior to other functional techniques like arterial spin labeling, in terms of accuracy in signal quantification and spatial resolution[827]. The BOLD signal stems from each voxel (three-dimensional pixel) of the brain, measured multiple times during the scan. The high spatial resolution of BOLD enables measuring activity from different-sized areas, from large-scale networks down to cortical columns at a sub-millimeter scale[828]. Beyond activation, BOLD imaging allows inference of the functional relationship between different brain areas through functional connectivity. Functional connectivity analysis correlates the recorded BOLD activity over time (timeseries) between multiple pairs of brain regions, or networks, allowing the mapping of functional relationships within the brain. When synchronised activation between brain areas is present, the involvement of those areas in the same neurobiological process is deduced; therefore they are inferred as being functionally connected. Functionally coupled brain regions will present correlated timeseries. Correlation between brain areas can be either positive, interpreted as brain regions active at the simultaneously, or negative (anticorrelated), when one region is active while the other is not and vice versa. Uncoupled regions will be active independently from each other resulting in a lack of correlation with values around zero. To determine connectivity, the raw fMRI data are pre-processed following several steps (figure 1.2.6).

The two most frequently used approaches to analyse resting-state fMRI data are: seed-to-voxel and ROI-to-ROI connectivity analyses. The former measures connectivity between a seed and every voxel in the brain, and thus is used to agnostically investigate functional connectivity within the whole-brain. In contrast, ROI-to-ROI measures connectivity between two selected brain regions.

Seeds are used to reduce the number of connections that need to be estimated; these can be over 60 billion for approximately 250,000 brain voxels, raising multiple comparisons problems[829]. The seed can be typically determined either by theory, previous study (e.g., task-dependent activation) or a meta-analysis of relevant studies. Importantly, variations in brain coordinates can significantly affect the results. While this approach relies on a previously known ROI in the brain, a seed can be also defined based on ICA, or hybrid strategies[576,580,830]. the ICA is a data-drive approach that robustly identifies brain networks with synchronised activity from the BOLD timeseries of the whole brain. The average activity within regions of the network would then be correlated the with every voxel in the brain or a single ROI. The seed-to-voxel approach may miss finer functional connectivity differences that are not present at the level of an entire network. Alternatively, ROI-to-ROI analysis addresses the multiple comparisons problem by using a parcellated brain (atlas) rather than any *a priori* hypothesis. However, conclusions from ROI-to-ROI analyses can suffer from reduced spatial specificity arising from selecting which brain atlas to use and its choice of ROIs.

Functional connectivity can be associated with selected clinical measures. Unless individual connections between two ROIs are tested, any results need to be corrected for multiple comparisons, either by family-wise error (FWE), Bonferroni, or false discovery rate (FDR)[583]. This study employed hybrid methods: seed-to-voxel analysis to quantify functional connectivity between ROIs (insula or networks like the DMN) and the whole brain; or ROI-to-ROI analysis using previously identified connections (e.g., DMN to insula). Ultimately, associations between brain functional connectivity and specific clinical outcomes (e.g., FM score or reported pain) can be effectively explored based on these analyses.

Study protocol

The participants undertook a 1-hour multi-modal MRI scan at the end of the baseline visit, following the QST session. Data were acquire using a 3 Tesla MRI scanner (Siemens Prisma, Erlangen, Germany) situated within the same building of the clinical research facility. Before and after the scan participants were asked to rate the intensity of their overall pain, pain secondary to psoriatic

arthritis, fatigue, and anxiety on a NRS 0-10. After the standard MRI screening procedures were carried out by the research radiographer team, subjects were instructed on the tasks involved during the fMRI scan. Individuals were asked to lie supine in the scanner. The motion was reduced by using foam pads placed around the head along with a forehead strap. A Nova 32 channel phased-array head coil was placed around the patient's head to acquire imaging during T2*-weighted gradient-echo echo-planar imaging pulse sequence, with coverage of the whole brain. During the resting state, the participants were instructed to remain awake without focusing on any particular task and keep their eyes open on a fixation cross[831,832]. Finally, the functional neuroimaging data were acquired in a 6-minutes of resting state. The MRI protocol also included a structural sequence for alignment with the functional data, two rs-fMRI sequences before and after an evoked pain fMRI sequence (deep muscle 141timulation using cuff algometry), and a spectroscopy sequence, which quantified brain metabolites from right insular regions. Data from the spectroscopy, evoked pain fMRI, and second resting-state were not analysed for this thesis. The analysis focused on the first rs-fMRI sequences of each participant.

3.7 Biological and clinical markers of inflammation

3.7.1 Circulating inflammatory biomarkers

3.7.1.1 *Introduction*

The individuals recruited in the study had the option to provide additional blood samples for research purposes. The aim for using the sera samples in this study was to explore the contribution of peripheral circulating mediators to the neurobiological features of pain in PsA. Despite the lack of diagnostic biomarkers available for nociplastic pain, recent evidence supports the associations between the circulating inflammatory biomarkers and pain phenotype. Recently, a distinct proteomic signature, including proteins involved in inflammatory and metabolic processes, was distinct in FM subjects compared to healthy subjects; intriguingly, those circulating proteins were also associated to reported pain,

PPT, and other psychological outcomes[833]. In a classic chronic inflammatory disease, i.e. RA, a relationship between peripheral inflammation (ESR) and an altered brain functional connectivity between key pain regions in the brain, IPL and PFC, and specific ICNs was demonstrated[623]. These results suggest a relationship between the inflammation and nociplastic pain features, potentially mediated by "inflammatory brain hubs" (IPL and PFC). The general mechanisms behind the inflammatory sensitisation of the CNS are still elusive. The relationship between pain phenotype, peripheral inflammation, and brain functional connectivity is yet to be investigated in PsA.

3.7.1.2 *Inflammatory biomarkers relevant in pain and psoriatic arthritis*

CRP was routinely tested in NHS laboratories at all study visits. Despite being a reliable inflammatory marker, CRP is uncommonly increased in individuals with PsA; differently from RA, low CRP levels do not always reflect low disease activity in PsA[834,835]. To better characterise the inflammatory profile in our population, other inflammatory markers of were investigated. The pro-inflammatory cytokines IL-17A and TNF have an established role in the pathogenesis of PsA[836] (section 1.1.2.3). In fact, immunotherapies targeting these cytokines are demonstrated to be effective in treating PsA[837]. The use of biologics and JAKi is effective to control pain independently from inflammation levels and disease progression, further supporting an interference of pro-inflammatory cytokines in the CNS-driven pain[316]. Indeed, chronic synovitis and enthesitis in PsA could lead to sensitisation and neuroplastic changes in the peripheral and CNS, with "bottom-up" mechanisms. Both IL-17A and TNF, key mediators of PsA pathogenesis, can also affect the somatosensory system inducing pain sensitisation at different levels of the nervous system (section 1.2.3.3)[838,839]. For their role in neuroinflammation, these cytokines were predilected, over CRP, as putative mediators for the inflammatory sensitisation of the CNS in our PsA cohort.

3.7.1.3 *Detection assays for pro-inflammatory cytokines*

From baseline and 3 months' visits, sera samples were processed and stored following a standardized protocol by trained researcher (Appendix 5). To

quantify the levels of circulating IL-17A and TNF stored sera samples form baseline visits were used. A multiplex ELISA (U-PLEX Metabolic Group 1 (hu), MSD Mesoscales Discovery, USA) was used to detect TNF levels, alongside other inflammatory and metabolic markers. The MSD protocol combines the classical sandwich immunoassay with electrochemiluminescence detection techniques. The MSD plates are designed to have multiple detection electrodes in each well for measuring the levels of various biomarkers in a single sample. I used 96-wells plates with 10 detection electrodes in each well. The cross-reactivity between proteins is possible in multiplex assays. Similarities in protein conformation or shared amino-acid sequences are responsible for this phenomenon. To overcome this issue, analytes combinations in each plate were carefully planned and optimised following producer's recommendations. The manufacturer protocol was followed. U-PLEX Linkers are proteins that selectively bind a single electrode in each well. U-PLEX Linkers were conjugated with captures monoclonal antibodies selective for a single analyte. At each stage, repeated washes to remove the reagents surplus were performed. Once the plate was coated with the conjugated capture antibodies, the samples and calibration standards were added. Samples were thawed on wet ice or at 2-8 °C before use. Calibration standards are recombinant cytokines at known concentrations. A total of 35 samples were analysed in duplicates. For technical reason, 10 samples were added to a single well, i.e., without duplicate measures (Appendix 6).

Successively, plates were incubated with monoclonal detection antibodies conjugated with electrochemiluminescent labels (MSD SULFO-TAG). A MSD reader instrument was used to apply a voltage to the electrodes. The excited labels emit light signals proportional to the amount of bound analyte. The MSD instrument can quantify the intensity of the light emitted by the SULFO-TAG, providing a quantitative measure of each analyte. TNF circulating values are expressed in pg/ul. Lower and upper limit of detection for TNF were respectively 0.51 - 3,650 pg/ml.

IL17A circulating levels are low in healthy, therefore, the detection is technically challenging. To obtain an accurate detection a plate with enhanced sensitivity from the same manufacturer (S-PLEX Human IL-17A Kit, MSD

Mesoscales, USA) was preferred. The S-PLEX immunoassay has the advantage of having an increased sensitivity and reproducibility, a reduced cross-reactivity, allowing the detection of lower abundance analytes. The detection antibody is conjugated with a different label (TURBO-TAG) able to produce larger light signals when activated by the electrodes. The average concentrations of IL-17A are expressed in fg/ul. The lower and upper limit of quantification for IL-17A were respectively 120 and 140,000 fg/ml.

For both assays nonspecific binding is estimated to be less than 0.5% with high detection accuracy. The signals generated by the standards are used to generate curve that fits a 4-parameter logistic model. The back-fitting of signals detected from samples to the calibrators curves was used to determine the pro-inflammatory cytokines concentrations in each well.

3.7.2 Response to anti-rheumatic treatment

Among the main inclusion criteria, active disease requiring a new immunosuppressant was present. There was not restriction on the prescribed drug which could have included any kind of DMARDs. All baseline visits were conducted prior to the start of the new medication. Treatment response was assessed at 3- and 6-months' visits. The simple computational DAPSA score was selected to define disease activity at baseline. DAPSA encompasses 5 clinical outcomes: TJC, SJC, PGA, patient-reported pain score, and CRP. By using these outcomes, PsA subjects can be classified into different categories of disease activity, including remission (REM, DAPSA < 4), low disease activity (LDA, DAPSA ≤ 14), moderate disease activity (MDA, 14 < DAPSA ≤ 28), and high disease activity (HAD, DAPSA > 28). DAPSA has emerged as a valuable tool, not only to classify disease activity, but also to assess response to treatment in PsA[840,841]. Moreover, DAPSA defined disease activity was associated with long-term radiographic progression and functional status[842]. Therefore, monitoring DAPSA scores over time can effectively evaluate disease progression and response to treatment. DAPSA is a valid tool to measure peripheral joints inflammation. However, other domains of the disease are not considered within the DAPSA score, i.e., enthesitis, dactylitis, spondylitis, and psoriasis[214]. The above disease's involvements have been recorded using manifestation specific scores,

respectively, LEI, dactylitis number, and BASDAI; the extension and severity of PsO was not noted. Despite the clinical value of these scores, the extended analysis of those domains from the follow-up visits was beyond the aims of this thesis. The focus on peripheral joints inflammation characterised by tender and swollen joints was predilected because it is supposed to be a stronger reflection of systemic inflammation. Conversely to axial, skin and entheseal inflammation, synovitis is the main clinical features shared between PsA and RA in which most of studies on inflammatory arthritis and pain phenotype have focused on. Extending the analysis to the ultrasound data collected at the 6 months' visit was beyond the scope of this work, however, in future analysis these data will help to deeply evaluate the response to treatment and the relationship between peripheral inflammation and central sensitisation.

Therefore, response to treatment at 3 and 6 months was described using changes in DAPSA scores at the follow-up visits compared to the baseline assessment. Participants were defined as responders to the new treatment when reaching REM, LDA, or at least 50% of improvement of their DAPSA score from baseline. The latter definition of clinical improvement is termed DAPSA50% and it was described to be in agreement with the standard ACR50% response, commonly used in clinical trials studying inflammatory arthritis[840]. More stringent cut-offs to define treatment response could have been used, such as DAPSA75/80%. However, due to the differences in drugs administered and the relatively short term of evaluation for treatment response, a less strict definition of responder to treatment was preferred.

3.8 Statistical analysis

Data collected during the visits were subjected to quality control checks just after the visits and before proceeding with the data analysis. Questionnaires completed online included an automatic reminder in case of missing answers, while more than one response per question was not allowed. Interactive questionnaire allowed to show only relevant questions and omit the non-required fields to make questionnaire completion user friendly. When possible, questions design with check box or drop-down menu was preferred to free text fields. During data cleaning suspect measure were traced back to the original

questionnaire, CRF, or NHS clinical records. Relevant outcomes for the study aims were calculated using pre-set worksheets and then verified manually.

3.8.1 Description of the recruited population

The first step of the analysis was to present the characteristics of the population included in the study. A simple descriptive statistic was used for this purpose. Variable were expressed as means and standard deviations (SD), or as ratio for dichotomic variable (e.g., sex). The frequency of specific variable, including kind of new medication, smoke and alcohol use, comorbidities and medications used at the time of the visits, was expressed in either percentages or with in absolute values. The normality of data distribution was determined by the Shapiro–Wilk test.

3.8.2 Stratification according with the 2011 ACR fibromyalgia criteria

To address the first aim, the population recruited was analysed based on the ACR FM scores. The participants were divided in 2 groups, either meeting the ACR FM criteria (FM+), or not (FM-). Significant differences between the 2 groups were determined using unpaired t-tests (for normally distributed variables, parametric), Mann Whitney tests (for non-normally distributed variables, or non-parametric) and Fisher's exact tests (for categorical variables). Correlations between the continuous ACR FM was computed using Pearson correlation coefficient for parametric variable, while the Spearman correlation was preferred for nonparametric variables. For all variable two-tailed P value was computed with confidence intervals at 95%. Comparison between repeated measures, for example, in ascending QST protocols, multiple t test was used to determine significant differences between groups. Multiple comparisons for clinical variables were adjusted using the FDR, Q was set equal to 1% (accepting 1% of false discovery rate). When outliers in the data set were suspected, the Tukey's fence method with a k = 3 was used to optimise identification of extreme outliers (values above the 75th quartile + k* interquartile range [IQR])[843]. The outliers identified were successively excluded from the analysis.

3.8.3 Functional neuroimaging analysis

Before proceeding to the analysis, the MRI data acquired was pre-processed to account for artefacts, and align to the structural data to templates for group analysis. Pre-processing included several steps (figure 1.2.7) following the default MNI (Montreal Neurological Institute average brain template) pipeline by functional connectivity toolbox CONN[844]: realignment, slice-timing correction, motion outlier detection, functional and structural segmentation, MNI normalization and smoothing (convolution with an 8 mm full-width at half maximum Gaussian Kernel). All scans were visually inspected for artifacts. The acquired data were corrected for motion: individual volumes were omitted from the analysis if they had over 2mm of motion and a global BOLD signal of over 9 SD; a patient would be considered for exclusion from analysis if they had over 20% of their functional volumes omitted[845]. No participants were excluded for these reasons. The brain networks were constructed using ICA on the functional smoothed data and identified using an established template in the literature; the Group ICA of fMRI Toolbox (GIFT) was used for these processes. These networks were then used as seeds in a seed-to-voxel analysis or as ROIs in a ROI-to-ROI analysis.

An initial *a priori* analysis was carried out using as predetermined Insula ROIs based on previous studies[492,608]. In more details, bilateral subdivisions of the IC, including anterior, middle and posterior cortices were selected as seeds[608]. Another ROI was positioned as 8mm sphere, centred around coordinates of the left middle insula, whose connectivity with the DMN was previously significantly associated with FM scores in RA[492]. The *a priori* analysis also included cross-correlations between DMN and left middle IC (8mm sphere seed), as well as the 6 insula ICs ROIs using general linear models, while correcting for age and gender. Subsequently, a more agnostic approach followed: the ROIs above were used as seeds in a seed-to-voxel analysis, to determine their connectivity with the whole brain. The analysis associated connectivity between the ROIs and voxels of the whole brain with the selected measure of nociplastic pain (2011 ACR FM scores). Age and sex were included in the model as covariates of no interest. Multiple comparisons correction was performed with significance set at

P < 0.05 FEW and significant discoveries were determined based on the FDR corrected P value < 0.05.

Data were pre-processed and later analysed to extrapolate variables of brain functional connectivity by a neuroscientist in the research group (Kristian Stefanov) using statistical parametric mapping software package version 12 (SPM12, Wellcome Department of Cognitive Neurology, London, United Kingdom), the CONN functional connectivity toolbox (Cognitive and affective neuroscience laboratory, MIT, Cambridge, USA), and GIFT toolbar, all running on MATLAB R2019b (Mathworks, Sherborn, MA).

3.8.4 Secondary sensitivity analyses

3.8.4.1 *Indexes of fibromyalgianess*

To better characterise the nociplastic pain features of the populations recruited multiple secondary sensitivity analyses were carried out. Firstly, the ACR FM score was deconstructed in the 2 sub-scores WPI and SSS. Those scores reflect different features of nociplastic pain, respectively, widespread somatic pain and central-related manifestations. For groups differences analysis, participants were divided in WPI-positive (WPI+) and WPI-negative (WPI-), or SSS-positive (SSS+) and SSS-negative (SSS-), using the median scores in the recruited population as cut-off.

3.8.4.2 *Effect of sex and pain modulating drugs*

To address the second aim, confounders factors known to have an impact of pain have been included in the sub-analysis. Specifically, differences between sexes and exclusion of individuals taking pain modulating drugs have been addressed to better assess the pain features of the participants recruited. Regarding sex differences, it is known that sex and gender can influence pain perception significantly; in fact, sex-based sensitivity analysis previously conducted in OA cohorts demonstrated meaningfully distinct associations with the FM score and various QST data between sexes. Despite the pain medication including opioids and NSAIDs were preferably stopped 42-24 hours before the baseline visit, other drugs known to act on nociplastic, and nociceptive pain were not listed in the

exclusion criteria. Therefore, a careful review of the CRF and, if necessary, of the NHS records, allowed to identify subjects who were taking such medications at the time of the baseline visit. The drugs considered to exclude participants fell in the following class: SNRIs, gabapentinoids, long-acting opioids. Subjects who have been taking one or more medications falling in the above classes were then removed from the FM scores analysis. The purpose was to eliminate the potential confounder factor associated with the above medications.

Crude groups differences and correlations between continuous variables were obtain with the same statistical methods used for the ACR FM scores.

3.8.4.3 *Inflammation and neurobiological features of nociplastic pain*

To address the third aim, pro-inflammatory cytokines and response to treatments were used as markers of inflammation. Herein, the statistical approaches used in these analyses.

The pro-inflammatory cytokines analysed were IL-17A and TNF. In the case of non-normally distributed values, a Log10 transformation was performed to improve the distributions and comparisons. Only IL-17A did not passed the normality test after transformation. Normalisation of the variables was additional performed to assess correlations with Z-scores reflecting the functional connectivity between brain areas of interest.

To assess the response to treatment, participants were classified according to DAPSA score standardised cut-offs into REM, LDA, MDA, and HAD across the 3 study visits. Subjects lost at follow-up, who did not start the treatment (independently from the reasons), or who stopped the new drug (for any reason at any follow-up visit) were excluded. Missing CRP values were recovered from NHS records including values obtained 1 week before the visit. If values were not available within the prefixed range of dates subjects were excluded from the analysis. When reaching remission, LDA, or at least 50% of improvement on their DAPSA scores (DAPSA50%), participants were classified as responders. Responders were further classified based on the response to treatment overtime. When the response was obtained at 3 months and sustained at 6 months, subjects were

classified as stable responders. Participants who did not responded at 3 months, but showed a significant improvement (REM, LDA, or DAPSA50%) at 6 months were classified as late responders. Conversely, who initially responded to treatment and successively worsened, was classified as relapsing. Groups differences were assessed using unpaired t-tests or Mann Whitney tests depending on distribution. Correlations between DAPSA scores and the 2011 ACR FM scores was computed using Pearson correlation coefficient (both parametric variables). Two-tailed P values with confidence intervals at 95% were preferred.

Chapter 4 Neurobiological features of pain in individuals with active psoriatic arthritis

4.1 Introduction

This chapter outlines the resulting neurobiological features of study participants with an active PsA. Initially, a clinical characterisation of the included population is described, with a specific focus on nociplastic pain features determined with the 2011 ACR FM score, as outlined in the first aim of this thesis. Both a dichotomic description of participants meeting the FM criteria (FM+) or not (FM-), as well as the "degree" of nociplastic pain, i.e. fMness, are reported. Then, the associations between nociplastic pain features and the different clinical features are examined to investigate the relationship between clinical inflammatory symptoms and central sensitisation. Next, the neurobiological signature of pain is delineated using the rs-fMRI and QST data and their relationship with fMness. Additionally, the associations between clinical and neurobiological features with the 2 components of the FM score, WPI and SSS, contribute to a deeper characterisation of nociplastic pain features in PsA.

4.2 Clinical features and prevalence of nociplastic pain

4.2.1 Study population

In total, 50 individuals met the inclusion and none of the exclusion criteria, and were enrolled in the study. Baseline visits and MRI scans were carried out in Glasgow between the 24.06.2019 and the 28.10.2021. The baseline data from 50 subjects was used to define the population characteristics. A total of 8 missing pain diaries reduced the number for this variable analysis to 42 for this variable only.

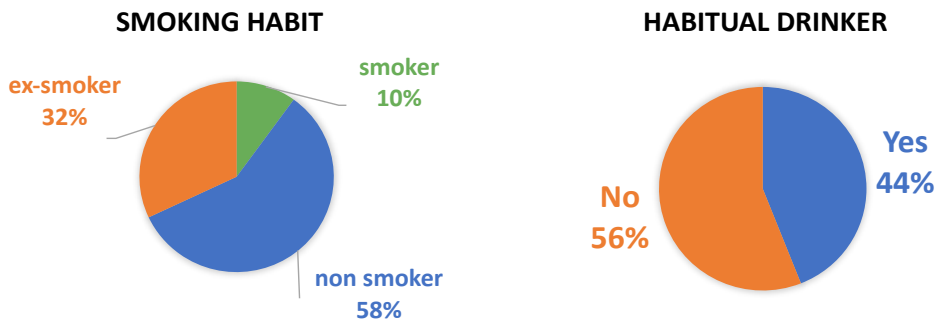
4.2.2 Baseline clinical characteristic

The clinical characteristics of the entire population recruited are illustrated in table 4.2.1. The majority of the individuals included were middle age (mean age

49 ± 11.4) with a slight prevalence of women (54%). On average the participants fell in the overweight-obese category (mean 30, SD ± 4.5). The preponderance of subjects were non-smokers (58%) and not habitual drinkers (56%) (figure 4.2.1). The presence of skin PsO was an overly common comorbidity (90%), followed by metabolic and endocrinological conditions (36%) and various gastrointestinal diseases (28%); cardiovascular diseases, chronic pain syndromes, and psychological/psychiatric conditions were equally represented in the population (16%) (figure 4.2.2). All subjects included presented a variable PsA disease duration, ranging from a recent diagnosis (a few months) up to 25 years of long-standing disease (mean 7 years, SD ± 5.8). The number of previous biologic or other DMARDs exposure was similarly variable (mean ±SD, respectively, 0.8 ±1.4 and 1.8 ±1.3). Only 13 participants were not taking any medication at the time of the baseline visits. Almost 50% were on a stable DMARDs and/or NSAIDs. Fewer individuals were taking opioids and/or antidepressants (figure 4.2.3).

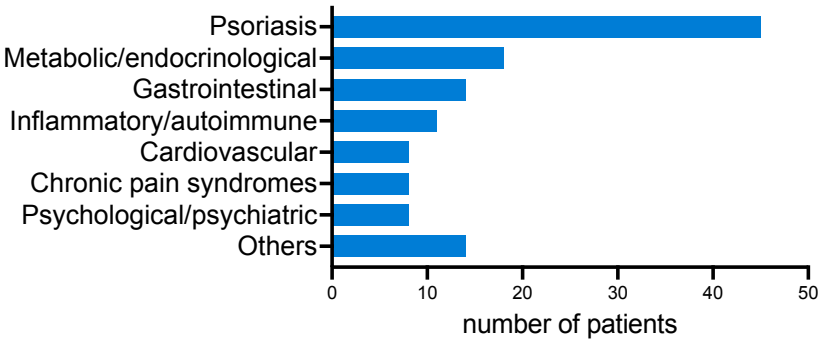
One of the main inclusion criteria was the presence of an active inflammatory disease indicating the commencing of a new biologic or other DMARD. More than half of the participants (64%) were prescribed with a biologic drug, a TNF or an IL-17A inhibitors. The remaining 36% of subjects were evenly divided in either a csDMARDs (methotrexate, leflunomide, or sulfasalazine) or a tsDMARDs (apremilast or tofacitinib) (figure 4.2.4).

Figure 4.2.1 Smoking and drinking use in the PsA population



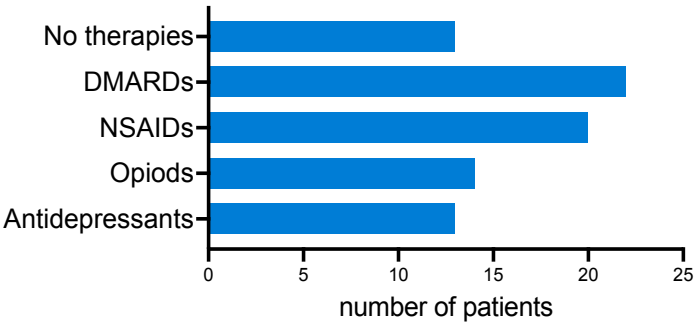
Legend: the figure illustrates the distribution of smoking and alcohol use in the total of subjects recruited. Most participants were non-smokers (58%), followed by ex-smokers (32%), while a minority was currently a smoker at the time of the baseline visit. A 44% of individuals enrolled were habitual drinkers.

Figure 4.2.2 Distribution of different comorbidities in the PsA cohort



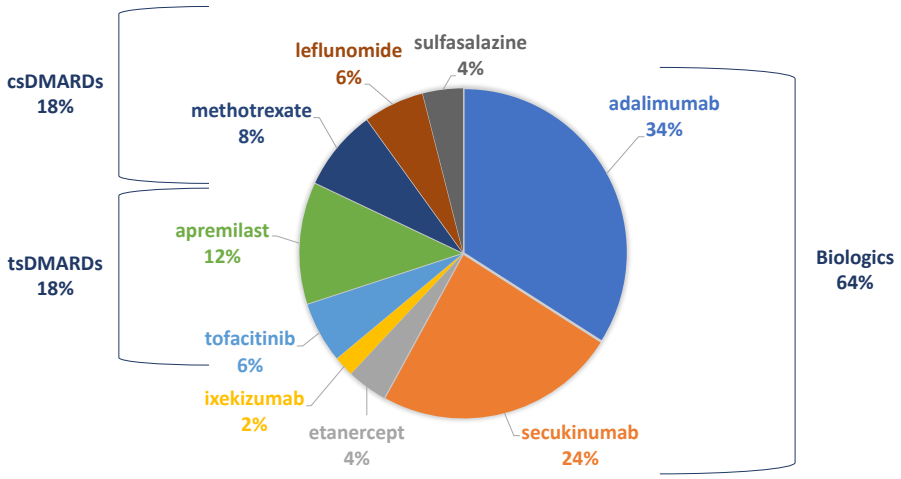
Legend: Number of participants affected by different comorbidities. Psoriasis is the most common with 45 subjects affected. Metabolic and endocrinological conditions follow with 18 individuals suffering from them. Gastrointestinal and other autoimmune comorbidities were present respectively, in 14 and 11 subjects. An equal number of 8 subjects presented cardiovascular, chronic pain syndromes or psychological disorders.

Figure 4.2.3 Distribution of DMARDs and pain modulating drugs at the baseline



Legend: The majority of participants (74%) was taking a different kind of medication at the time of the baseline visit. N=22 subjects were on an established DMARD treatment. Slightly less individuals (N=20) were assuming a regular NSAID to control the arthritis, whether alone on in combination with a DMARD. A similar number of participants had regular opioids or antidepressants with various mechanisms of action (e.g. SNRIs), respectively 14 and 13.

Figure 4.2.4 New anti-rheumatic drugs to commence after baseline visit



Legend: Biologics were the most prescribed treatment in the recruited population (64%). Anti-TNF mechanism of action is dominant, mainly in the form of adalimumab (34%) and to a lesser extent of etanercept (4%). IL-17A inhibition is the second most prescribed, with pre-eminence of secukinumab (24%) over ixekizumab (4%). In order of percentage of new prescriptions, the remaining DMARDs with different mechanisms of action are apremilast (12%) and methotrexate (8%), followed by tofacitinib and leflunomide (equal 6%), and lastly sulfasalazine (4%).

The mean ±SD of TJC and SJC of the individuals enrolled was respectively 21 ±14.4, and 7 ±4.5. In all participants at least one joint was swollen, despite the SJC was not in the inclusion or exclusion criteria. Up to 19 subjects had at least one finger (or toe) with evidence of dactylitis. Most participants (N=42) had a least 1 enthesis tender to touch, out of the 6 entheseal points included in the LEI score. The mean LEI score in all subjects is equal to 2.7 ±2 (mean ±SD). The BASDAI scores were overall high, 6.3±2 (mean ±SD); BASDAI scores above 4 indicate poor disease control [195] . The DAPSA scores were on average within the HDA classification (44.2 ±25, mean ±SD), reflecting an understandably high disease activity. The normal range defined in NHS laboratories for CRP is 10mg/L (or 1mg/dl). Most study participants showed CRP values within the normal range; the average CRP was slightly over 1 mg/dl (1.1 ± 2.5, mean ±SD). Both physician and patients' global VAS were above 50/100 (PhgVAS, 53.2 ±16.5; PtgVAS, 59.1 ±23.3). The pain reported on a VAS at the time of the visit was on average around 35/100 (35.1 ±25, mean ±SD). However, the 42 participants who completed the baseline 10 days pain diary, showed higher average pain (5.3 ±2.2, mean ±SD), closer to the values of global VAS. Additionally, fatigue and anxiety VAS collected during the visit were also above 50/100 (fatigue 6.7 ±2.6;

anxiety 7.7 ±13.5), suggesting a strong presence of both symptoms in our population (table 4.2.1).

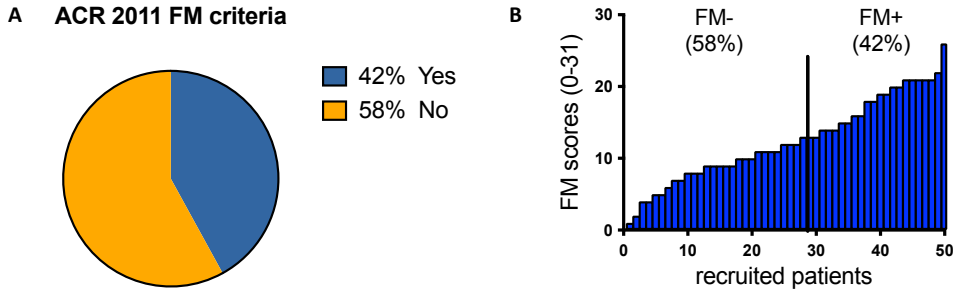
Table 4.2.1 Baseline clinical features of recruited participants

BASELINE CLINICAL CHARACTERISTICS	N = 50
Clinical characteristics	
Age (mean ± SD)	49 ± 11.4
Disease duration years (mean ± SD)	7 ± 5.8
Sex (male/female)	23/27
BMI (mean ± SD)	30 ± 4.5
Previous biologics (mean ± SD)	0.8 ± 1.4
N previous DMARDs (mean ± SD)	1.8 ± 1.3
N medications at baseline (mean ± SD)	3.3 ± 2.7
Disease activity	
TJC 66 (mean ± SD)	21 ± 14.4
SJC 68 (mean ± SD)	7 ± 4.5
Dactylitis (yes/no)	19/31
LEI score (mean ± SD)	2.7 ± 2
BASDAI (mean ± SD)	6.3 ± 2
DAPSA (mean ± SD)	44.2 ± 25
CRP mg/dl (mean ± SD)	1.1 ± 2.5
Physician gVAS (mean ± SD)	53.2 ± 16.5
Patient reported outcomes	
Patient gVAS (mean ± SD)	59.1 ± 23.3
Pain VAS (mean ± SD)	35.1 ± 25
10 days pain diary (n=42, mean ± SD)	5.3 ± 2.2
Fatigue VAS (mean ± SD)	6.7 ± 2.6
Pre-QST anxiety (mean ± SD)	7.7 ± 13.5

4.2.3 Nociplastic pain characteristics

Participants were classified according with the 2011 ACR FM criteria as having FM (FM-positive or FM+), or not (FM-negative or FM-). An equal of 21 individuals (42%) were classified as FM+, while 29 (58%) were FM- (figure 4.2.5 A). The distribution of the total FM scores in all subjects clearly illustrates the scores variability within the FM+ and FM- groups, ranging from 1 to 26 (figure 4.2.5 B). For example, it is clear that participants with FM scores from 1 to around 12 would be equally classified as FM-, despite presenting different degrees of nociplastic pain. Moreover, the vicinity of FMness scores between individuals belonging to different groups, evident at scores around 10 to 20, is also noticeable.

Figure 4.2.5 Distributions of 2011 ACR fibromyalgia scores in the population



Legend: Distribution of 2011 ACR FM criteria scores in the recruited participants. A. The 42% of subjects were classified as having FM (n = 21, FM+), while the remaining 58% (n = 29) did not satisfy the ACR FM criteria (FM-). B. the distribution of total scores for each patient from the lowest to the largest is illustrated in a histogram graph. The scores gradually increase from 1 to 26 with similar scores within the 2 groups. Higher degrees of nociplastic pain can be present in the FM- group, and vice versa in the FM+.

Age and BMI, as well as disease duration and number of csDMARDs were overall similar between the FM+ and FM- groups (table 4.2.2). Despite not reaching statistical significance, there was twice the number of females than males in the FM+ group. On the other hand, in the FM- the male/female ratio (16/13) slightly leant towards the male sex. The number of previous biologics and current medications taken (including any indication) were significantly higher in the FM+. The tender joints were significantly more in the FM+ group (P = 0.003), while the higher SJC did not reach the statical significance. The presence of dactylitis was modestly more frequent in absence of FM (41.4% vs 33.3%. for

FM+), however not significant. The LEI score calculate by the sum of reported tenderness at 6 entheseal points, was significantly higher in the FM+ group (P = 0.04). The CRP levels were similar in the 2 groups. Interestingly, both disease activity scores, BASDAI and DAPSA, resulted significantly higher (respectively, P = 0.006 and P = 0.0005) in participants classified as having FM. All the pain and fatigue VAS presented higher ratings in the FM+ group (table 4.2.2), with the expetion of the current VAS pain, which did not reach statistical significance. Anxiety ratings were also not significantly different; on average, the FM- showed slightly higher anxiety ratings.

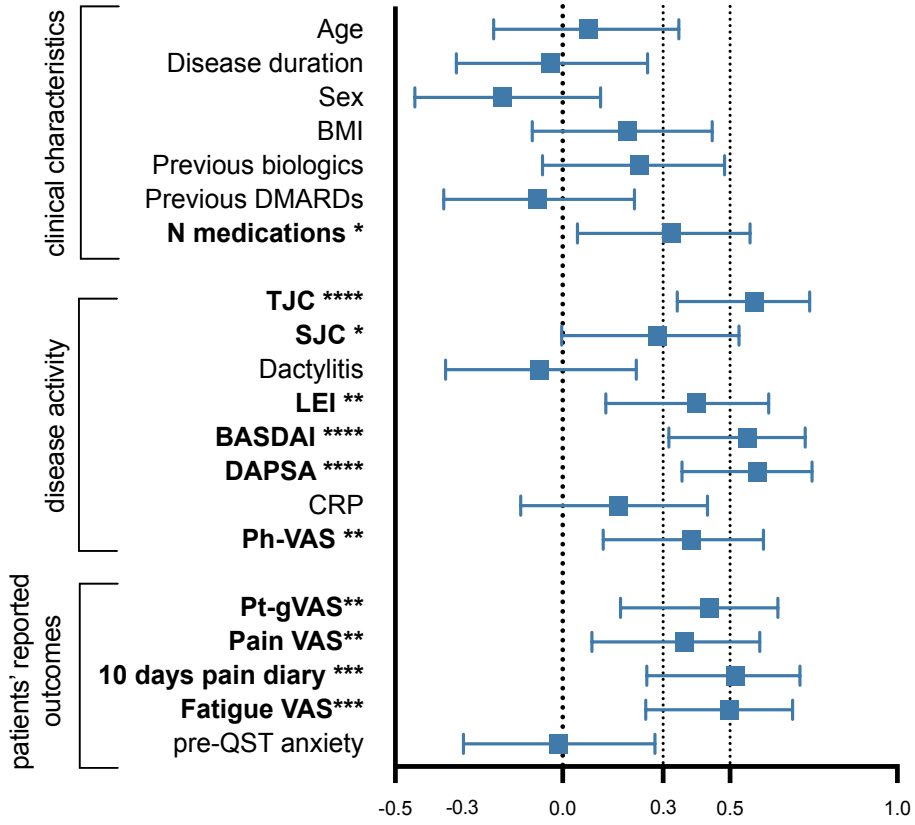
4.2.4 Relationship between clinical features and total FM scores

The simplistic dichotomic characterisation of FM risks overlooking nuances within widely distributed FMness features (figure 4.2.5 B). After confirmation of variables distributions, baseline clinical features were correlated with the FMness score, i.e., the total of 2011ACR FM score. In figure 4.2.6 are illustrated the correlation R and their confidence intervals (at 95%). Within the clinical characteristics, only the number of medications taken at the baseline, independently from indication and mechanism of action, significantly correlated with the FM scores (Spearman's R 0.327, P = 0.02). The number of previous biologics lost statical significance observed with FM groups. Regarding the disease activity variables, DAPSA, TJC, and BASDAI showed the strongest positive correlations with the FMness (Spearman's R respectively, 0.58, 0.57, and 0.55, all with P <0.0001). LEI scores and Physician gVAS followed with moderately strong positive correlations (respectively Spearman's R 0.40, P= 0.004, Pearson's R 0.39, P= 0.001). The SJC also showed a positive correlation, yet minor (Spearman's R 0.28, P<0.05). Interestingly, CRP and number of digits with dactylitis did not show associations with the FM scores. As showed in the group analysis, all the participants' reported outcomes, excluded the anxiety VAS, showed significant positive correlations with the FM scores. The average of 10 days diary pain ratings (n=42) and the fatigue VAS showed the strongest correlations (Pearson's R 0.52, P= 0.0005, and Spearman's R 0.5, P = 0.0002, respectively). Participants' gVAS scores were also moderately associated with FMness (Spearman's R 0.44, P= 0.0015). The correlation between FM scores and pain VAS at the time of the visit was less robust (Spearman's R 0.36, P= 0.009).

Table 4.2.2 Baseline clinical features in participants stratified according with the 2011 ACR fibromyalgia criteria.

BASELINE CLINICAL FEATURES	FM- (29)	FM+ (21)	P-values
Clinical characteristics			
Age (mean ± SD)	48 ± 12.5	49.4 ± 10	ns
Disease duration years (mean ± SD)	7.2 ± 6.5	5.6 ± 5	ns
Sex (male/female)	16/13	7/14	ns
BMI (mean ± SD)	29.1 ± 4.8	30.3 ± 4	ns
Previous biologics (mean ± SD)	0.4 ± 0.8	1.3 ± 1.9	0.03
N previous DMARDs (mean ± SD)	1.8 ± 1.2	1.7 ± 1.4	ns
N medications at baseline (mean ± SD)	2.5 ± 1.9	4.5 ± 3.2	0.02
Disease activity			
TJC 66 (mean ± SD)	16.7 ± 12.7	28 ± 14.3	0.003
SJC 68 (mean ± SD)	6.3 ± 3.5	8.6 ± 5.4	ns
Dactylitis (yes/no)	12/17	7/14	ns
LEI score (mean ± SD)	2.2 ± 2	3.4 ± 2	0.04
BASDAI (mean ± SD)	5.6 ± 2	7.1 ± 1.6	0.006
DAPSA (mean ± SD)	29.3 ± 15.4	49.3 ± 23	0.0005
CRP mg/dl (mean ± SD)	0.6 ± 0.6	1.8 ± 3.7	ns
Physician gVAS (mean ± SD)	48.6 ± 14.7	59.5 ± 17	0.02
Patient reported outcomes			
Patient gVAS (mean ± SD)	51.7 ± 23.8	69.3 ± 19	0.007
Pain VAS (mean ± SD)	29.8 ± 21.7	42.4 ± 27.8	ns
10 days pain diary (n=42,mean ± SD)	4.5 ± 2.2	6.6 ± 1.7	0.001
Fatigue VAS (mean ± SD)	6 ± 2.9	7.6 ± 1.9	0.04
Pre-QST anxiety (mean ± SD)	8.3 ± 14.9	6.9 ± 11.7	ns

Figure 4.2.6 Correlations between ACR fibromyalgia scores and clinical variables



Legend: Correlations between FM scores and clinical variables are illustrated. The dotted vertical lines at +0.3 and +0.5 on the x-axis represent the used cut-off to determine the strength of associations with the 2011 ACR FM scores. The number of medications taken at baseline was the only clinical characteristic significantly positively correlated with the FMness. Most of the disease activity-related variables were positively associated with the FM scores, with the exception of CRP and dactylitis. Also the ratings for current or 10 days pain on average, fatigue and global disease activity were positively associated with FM. Anxiety VAS was not. The clinical characteristics examined Spearman's and Pearson's correlations were used for non-parametric and normally distributed variables respectively. BMI - body mass index, DMARDs – disease modifying anti-rheumatic drugs, N - number, TJC – tender joint count, SJC – swollen joint count, BASDAI – Bath Ankylosing Spondylitis Disease Activity Index, DAPSA - Disease Activity in PSoriatic Arthritis, CRP – C-reactive protein, Ph – physician, Pt- Patient, VAS – visual analogue scale, QST – quantitative sensory testing.

4.2.5 Summary

This section investigated the clinical characteristics and prevalence of nociplastic pain in individuals with PsA. The data from the baseline visit were complete for all 50 participants enrolled, except for the 10 days pain diary which was available in 42 subjects. The study population consisted mainly of middle-aged individuals (mean age 49 years) with a slight female prevalence (54%), overall overweight-obese BMI (mean 30 ± 4.5) and with skin PsO (90%). Most individuals showed mildly raised or normal CRP, were taking medications (DMARDs, NSAIDs, opioids, and antidepressants), and they had a variable exposure other to biologics. At the time of the inclusion, and by study design, participants presented with high disease activity and were prescribed with a variety of new anti-rheumatic drugs, with biologics (64%) being the dominant choice. Reported fatigue and anxiety were also overall high.

Nociplastic pain characteristics were evaluated based on 2011 ACR FM criteria, classifying participants as FM+ (42%) or FM- (58%). Both groups exhibited similar age, BMI, disease duration, and previous DMARDs, while FM+ subjects had a significantly higher number of previous biologics and current medications taken. Additionally, TJC, LEI scores, disease activity scores (BASDAI and DAPSA), and patient-reported outcomes (VAS for global disease activity, 10 days pain diary, and fatigue) were significantly higher in the FM+ group. Classical features of inflammation, such as SJC, dactylitis, and CRP, were not different between the groups. Correlation analysis revealed that the number of medications at baseline, variables associated with disease activity (TJC, LEI DAPSA, and BASDAI), and most of the patient-reported outcomes (except for anxiety) were positively associated with FM scores. Interestingly, the pain VAS at baseline was not different between the groups, however it resulted positively associated with the FMness scores. Overall, this data suggests a slightly higher sensitivity of the 10-days pain diary and global disease activity ratings, compared to the pain VAS, in capturing nociplastic pain. Indeed, these results need to be confirmed due to several limitations, for example the lack of corrections for confounding factors in the correlation analysis, therefore, false associations may be present.

4.3 Neurobiological correlates of nociplastic pain

This chapter plans to describe the findings of the neurobiological signature of pain in PsA. Firstly, results on the association between Insula-DMN connectivity and FMness are illustrated. Secondly, the exploratory analysis of the connectivity of ICs or DMN with the whole brain are illustrated. Finally, the QST data analysis related to the FM categories, FM+ and FM-, as well as the FM scores are described.

4.3.1 Brain resting state functional connectivity in relationship with FM scores in PsA

4.3.1.1 *MRI data completeness and incidental findings*

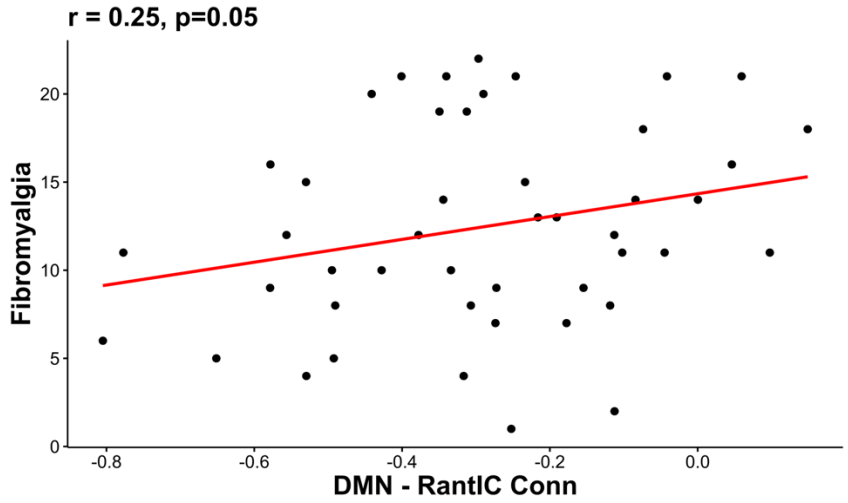
Two participants asked to stop the brain MRI at the initial T1 sequence, and a single additional patient failed to complete the first resting state sequence due to lack of compliance during the scanning. Therefore, a total of 48 T1 structural sequences were reviewed by NHS radiographers for structural abnormalities. An empty sella and an intrasellar cisternal herniation were reported in two subjects, however there were no suggestion of raised intracranial pressure, nor meaningful clinical relevance. Overall, no significant brain abnormalities were reported. Complete first rs-fMRI data from 47 participants were used for the neuroimaging analysis.

4.3.1.2 *DMN and right anterior insula functional connectivity correlates with FMness*

Guided by the *a priori* hypothesis of insula-DMN connectivity relevance in nociplastic pain, the ROI-to-ROI analysis between these brain areas showed a correlation with the FMness. In details, the connectivity of the DMN to right antIC resulted positively correlated with the total FM scores, although moderately ($r = 0.25$, $p = 0.05$) (figure 4.3.1). The DMN-RantIC connectivity values extend across the negative Z-scores, seemingly reflecting different degrees of anti-correlation. The greater anti-correlation is observed in participants with lower FM scores, while higher FMness tends to present a reduced anti-correlation up to uncoupling (i.e., connectivity around 0) between

DMN and insula. No other significant correlations were observed from the ROI-to-ROI analyses using the selected Insula cortices as seeds.

Figure 4.3.1 Functional connectivity between DMN and Insula correlates with fibromyalgia



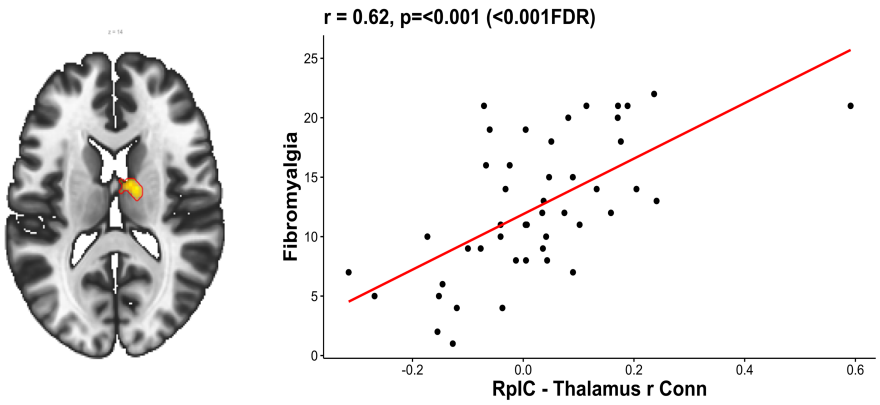
Legend: Correlation between FM scores and the functional connectivity between DMN to the right anterior Insula cortex (RantIC). DMN and the right anterior IC present a reduced anti-correlation at higher FM score.

4.3.1.3 *Nociplastic pain is associated with an altered mid-posterior insula connectivity in psoriatic arthritis*

The seed-to-voxel analysis explored the connectivity between the 6 bilateral ICs (seeds) and the whole brain in a more agnostic approach. An altered connectivity of mid-posterior insula cortices with areas involved in sensory processing and cognitive functions, namely the thalamus, parahippocampal gyri, and PFC, was observed.

The right pIC functional connectivity with the homolateral thalamus strongly correlated with the degrees of FM ($r = 0.62$, $p < 0.001$, FDR < 0.001) (figure 4.3.2). An increased right pIC to right thalamus connectivity is observed in individuals with high FMness, while anti-correlation is noticed within lower FM scores.

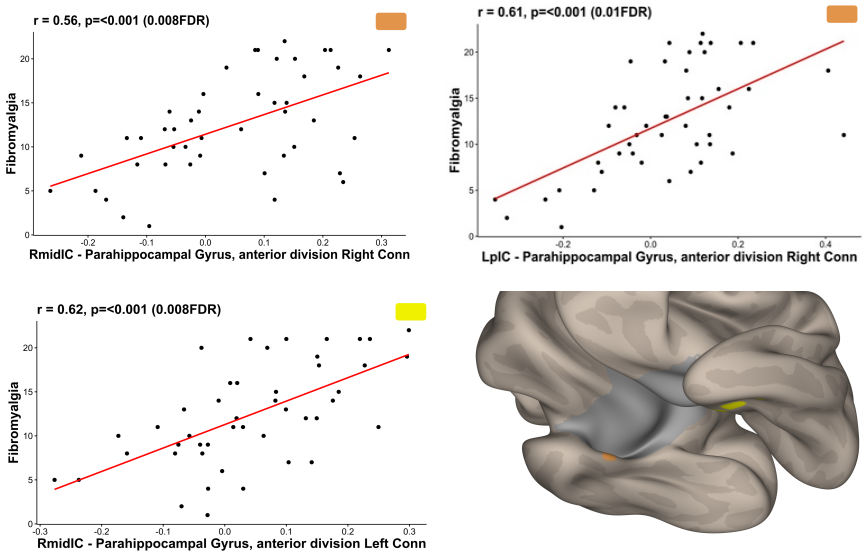
Figure 4.3.2 Increased Insula to Thalamus connectivity



Legend: functional connectivity right posterior Insula cortex (RpIC) to the right Thalamus is increased at the rising of the total FM scores. Lower FMness showed an anti-correlation between the 2 brain areas.

Whole brain analyses also revealed that bilateral interconnectivity between mid-posterior insula cortices and parahippocampal gyri is positively associated with our nociplastic pain outcome. The right mid insula connectivity to bilateral parahippocampal gyri correlations with FMness were fairly strong (right parahippocampal gyrus, r = 0.56, p <0.001, p = 0.008 FDR; left parahippocampal gyrus, r = 0.62, p <0.001, p = 0.008 FDR; figure 4.3.3). Similarly, the left posterior insula connectivity to the contralateral parahippocampal gyrus was positively associated with FM scores (r = 0.61, p <0.001, p = 0.01 FDR). In addition to the parahippocampal gyri, also the right mid insula connectivity to the dlPFC was positively associated with the total FM scores (frontal pole, r = 0.54, p <0.001, p = 0.001 FDR; figure 4.3.4 A).

Figure 4.3.3 Augmented insula connectivity to parahippocampal gyri in individuals with higher degrees of nociplastic pain



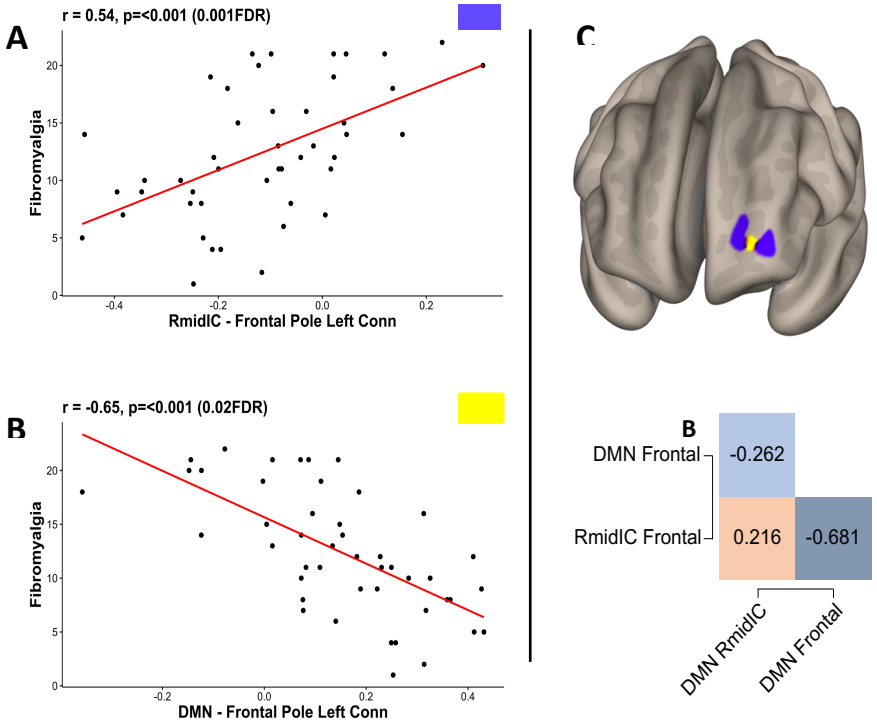
Legend: the right middle insula cortex (RmidIC) showed an increased functional connectivity to the right and left parahippocampal gyri to be associated with the FM scores (respectively, r = 0.56, p <0.001, p = 0.008 FDR, and r = 0.62, p <0.001, p = 0.008 FDR). Similarly, the left posterior insula cortex (LpIC) connectivity with the contralateral parahippocampal gyrus positively correlates with the total FM scores (r = 0.61, p <0.001, p = 0.01 FDR). In orange and yellow are respectively represented the right and left parahippocampal gyri and the colour reference in the corresponding correlation graphs.

4.3.1.4 *Diminished DMN connectivity to dlPFC is related to nociplastic pain and insula altered connectivity*

Seed-to-voxel analysis using the DMN as seed further unveiled a negative correlation between FMness and the DMN to left dlPFC functional connectivity (frontal pole left, r = 0.54, p <0.001, p = 0.001 FDR; figure 4.3.4 B). While in participants with low FM scores the DMN and left dlPFC showed a synchronised activity, at the increasing of the FMness the connectivity between these areas is lessen or anti-correlated (figure 4.3.4 B). Interestingly, the left dlPFC areas which functional connectivity with the DMN or the right mid insula is associated with FM (figure 4.3.4 A and B), were found to be across the same gyrus and sulcus in the frontal pole, within 0.3 mm distance at a 3T resolution (figure 4.3.4 C). The relationship between the functional connectivity of these 3 areas is illustrated in the correlation matrix in figure 4.3.4 D. The only significant correlation was a negative association between DMN-dlPFC and right midIC-dlPFC (r = -0.681, p <0.001). Additionally, despite being overall mildly and not reaching

the statistical significance, the connectivity between DMN and RmidIC increases in the same direction with the right midIC to left dlPFC, but inversely with the DMN to left dlPFC connectivity.

Figure 4.3.4 Functional connectivity between DMN, right insula and dlPFC in relationship to fibromyalgianess



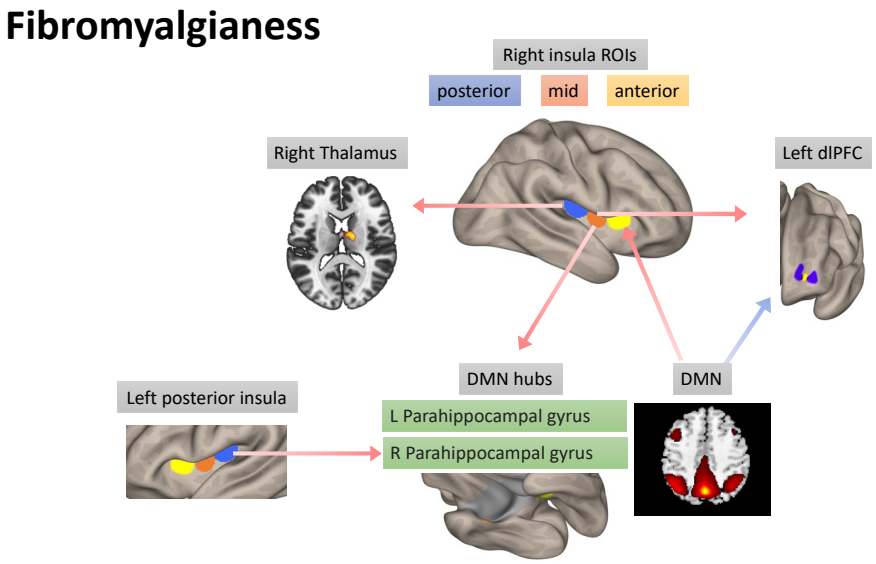
Legend: A. right middle insula cortex (RmidIC) functional connectivity to the left dlPFC (Frontal Pole Left) is positively correlated with degree of FM (r = 0.54, p <0.001, p = 0.001 FDR). B. DMN to left dlPFC (Frontal Pole Left) in negatively correlated with the FM scores (r = 0.54, p <0.001, p = 0.001 FDR). C. Graphical representation of the Frontal Pole. The purple areas are the dlPFC functionally connected with the RmidIC. The yellow area correspond to the voxel in the left dlPFC functionally connected with the DMN and correlating with the FM scores. D. Correlation matrix between functional connectivity of DMN-RmidIC-dlPFC. The DMN-dlPFC and the RmidIC-dlPFC are negatively correlated (r = -0.681, p <0.001).

4.3.1.5 *Summary*

The functional connectivity between the DMN and the right anterior insula was positively correlated with total FM scores, although only moderately (r=0.25, p = 0.05), using the robust ROI-to-ROI seed analysis. Additional exploratory findings using seed-to-voxel revealed an altered connectivity of the ICs and DMN to be related to the FMness. The functional connectivity of the right pIC to the

homolateral thalamus and left pIC to contralateral parahippocampal gyrus showed significant direct relationships with the FMness. Similarly, the right mid insula activity is more synchronous with the activity of bilateral parahippocampal gyri and dlPFC in participants presenting higher FM scores. Differently, the DMN connectivity to the dlPFC presented an inverse relationship with the FMness. The dlPFC areas were in close proximity to each other and their functional connectivity with DMN and the right mid insula are negatively correlated.

Figure 4.3.5 Altered functional connectivity associated with FMness in participants with active PsA



Legend: The figure summarises the findings in both ROI-to-ROI and seed-to-voxel analyses. Red arrows indicate a positive correlation between the connectivity between the two brain areas and the total FM score; the blue arrow highlights negative associations with the FM score. The arrows start from the ROIS used as seed and point towards the other ROI or the voxels resulted significant. The only ROI-to-ROI results is the positive correlation between the DMN to right anterior insula. From the seed-to-voxel analysis, the right mid insula connectivity with bilateral parahippocampal gyri and left dlPFC resulted positively correlated with the FMness. The right posterior insula connectivity with the homolateral thalamus, and the left posterior insula with the contralateral parahippocampal gyrus were also positively associated with the FM scores. Finally, the DMN to whole brain revealed a negative association between DMN to left dlPFC connectivity and FMness.

4.3.2 Quantitative Sensory Testing in PsA

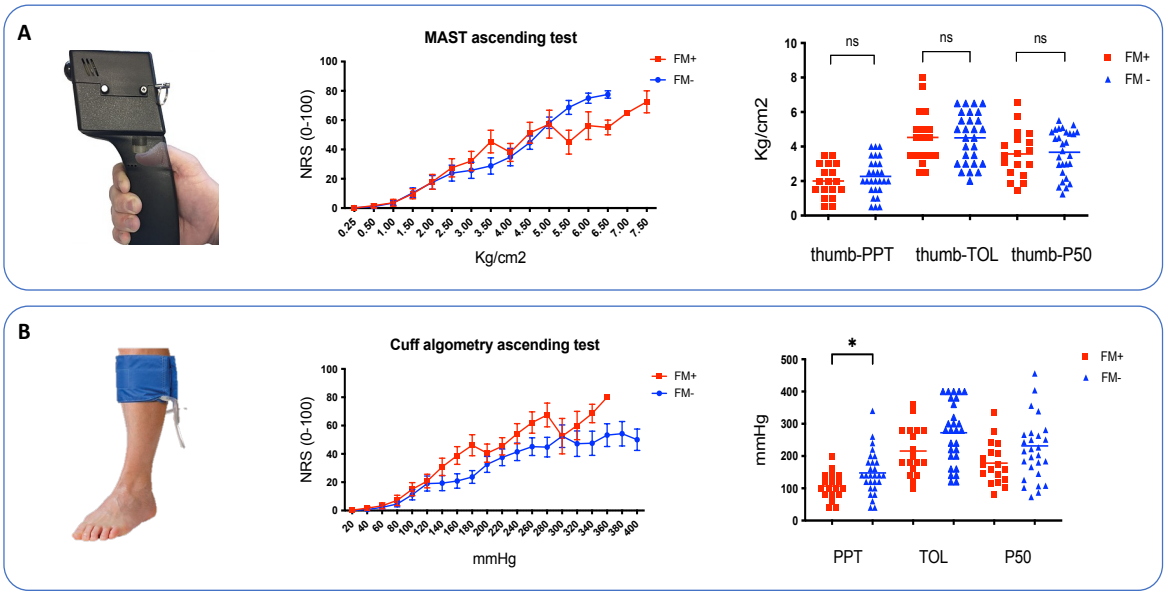
This chapter describes difference in the QST findings between individuals meeting the 2011 ACR FM criteria and those who are not. The most relevant findings are illustrated following the testing order (table 3.6.1), starting from MAST and cuff algometry, followed by handheld algometry and temporal summation, and finally the aversion to visual stimulation, M-VAST. Furthermore, correlations between the continuous FM scores and the QST parameters are also outlined.

4.3.2.1 *Concomitant FM in PsA increases sensitivity to pressure pain at deep tissues, joints and tendons areas*

A total of 29 participants were included in the FM- group. A patient was excluded from the FM+ group because their higher tolerance ratings were above the 75th quartile + 1.5IQR, and therefore, classified as outlier. This patient was taking different kind of medications with potential effect on pain perception, including opioids, anti-depressants and anti-psychotic drugs (i.e., opioid - paracetamol in combination with codeine, SNRIs– Venlafaxine, dopamine and serotonin agonist - Aripiprazole). Therefore, the number of FM+ subjects included in the MAST and cuff algometry both analyses was 20. The graphs for ascending MAST and cuff algometry sensory tests are illustrated in figure 4.3.6. The average ratings at increasing pressures applied at the thumbnail for the FM+ and FM- groups showed no significant differences (figure 4.3.6 A). The ratings between the groups are overall similar; only around a pressure equal to 3.5 Kg/cm^2 the FM+ group showed a slightly higher reported pain. However, the PPT, tolerance, and P50 observed with the MAST were not statistically significant between the groups (figure 4.3.6 A). Two subjects within the FM+ group had higher tolerance and P50 than the rest of the participants both in the FM+ or FM- groups, however they were not included in the outliers' classification. Pressure applied to the deep tissues of the calf with the cuff algometry revealed higher pain sensitivity in the FM+ group. At equal pressures, the FM+ subjects reported higher pain ratings than the FM- group, evident in the graphs in figure 4.3.6 B, from pressure equal to 140 mmHg. The average PPT was significantly lower in presence of FM ($p = 0.01$). However, tolerance and P50 did not differ

significantly, despite the pressures to reach tolerance or pain equal 50 were overall higher in the FM- group (figure 4.3.6 B), indicating overall lower pressure pain sensitivity compared to the FM+ group.

Figure 4.3.6 Differences in pressure pain sensitivity in PsA participants with and without FM

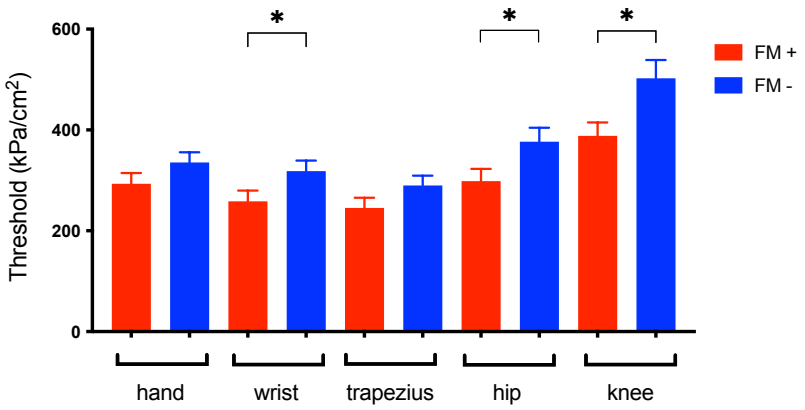


Legend: the figure illustrates the differences in pressure pain sensitivity elicited with the MAST device (A) or the cuff algometry (B) between participants with FM (FM+ in red), or not (FM- in blue). FM+ n = 20, FM- n = 29. No significant differences in pressure pain thresholds (PPT), tolerance (TOL), pressure to obtain pain rating of 50 (P50) were demonstrated for both MAST (A) and cuff algometry QST (B). On average pain ratings in the FM+ were higher than FM- when the same cuff pressure was applied. Cuff algometry PPT was significantly lower in the FM+ compared using Welch's t test ($p = 0.01$). Unpaired t-test and Mann-Whitney test showed no other significant differences between the groups.

The pressure pain thresholds were also recorded in different areas of the body using a digital handheld algometer. The average of three measures for each site bilaterally was computed. Comparisons between left and right side in all subjects, in FM+ and FM- groups showed no significant laterality differences in all groups for all body areas. The PPTs recorded in all participants included 2 values, both right and left averages, for each area. Some data were missing. Three individuals refused to perform the algometry test (on hands and knees) for acute pain, while 2 participants declined the test on knees for florid psoriasis at the site or previous total knee replacement surgery. The knee algometry was added in the protocol after the beginning of the study, therefore the knees PPTs of the first 6 participants were not present. The numbers for each dataset used

for the analysis are enlisted in figure 4.3.7. In all the 5 body areas tested, respectively hands, wrists, trapeziuses, hips, and knees, the subjects with FM showed on average lower PPTs. The differences were not significant on the hands and trapeziuses. The PPTs at the wrists were significantly lower in the FM+ compared to the FM- group (P= 0.04). Also the entheseal areas tested on the knees and hips resulted significantly more sensitive to pressure in the FM+ group (respectively, P = 0.02 and P = 0.04). The same patient identified as outlier in the FM analysis was excluded.

Figure 4.3.7 Algometry pressure pain threshold in different body areas



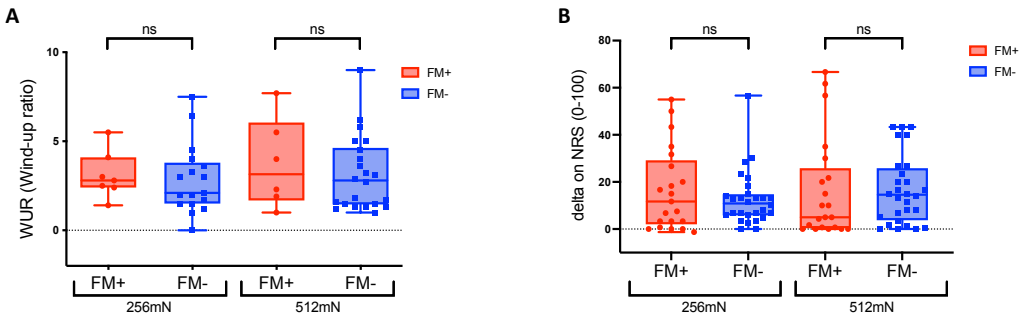
Legend: Differences between FM+ and FM- participants in pressure pain threshold at specific body areas. FM+ are represented in red, while the FM- group is represented in blue. Overall, FM+ individuals showed a reduced PPT elicited with a digital handheld algometer. Significant differences were observed at the joint area of the wrist (P = 0.04), and tendons areas of knees and hips (respectively, P = 0.02 and P = 0.04), using Mann-Whitney test. Number of values for each group: hand FM+ = 36; hand FM- = 55; hip, trapezius, and wrist FM+ = 38; hip, trapezius, and wrist FM- = 56; knee FM+ = 32; knee FM- = 47.

4.3.2.2 *Neither increased visual sensitivity nor temporal summation are distinctive for the presence of FM in PsA*

TS was evaluated using two weighted pinprick pens computing the WUR and delta TS using the average ratings of 3 singles and 3 trains of stimulations. Both pin prick intensities, 256mN and 512mN, did not show significant differences between the FM+ and FM- groups (figure 4.3.8 A and B). The WUR showed a slightly higher TS in the FM+, however differences were difficult to evaluate due to the loss of numbers in both groups for the WUR; because many participants

rated 0/100 at the single ratings, therefore was not possible to compute the WUR (figure 4.3.8 A). To avoid missing values, the deltaTS was calculated; the corresponding plots are illustrated in figure 4.3.8 B. In both groups, several subjects did not present an increased TS (individual values around zero). Two participants within the FM- present high TS measured as WUR and deltaTS.

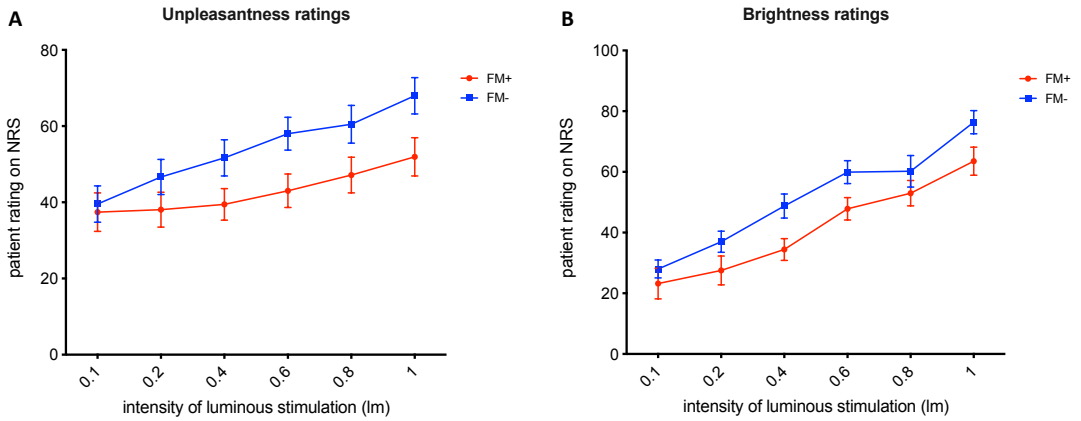
Figure 4.3.8 Temporal summation between PsA participants with and without FM



Legend: No significant differences in TS were demonstrated between FM+ (red) and FM- (blue). TS was evaluated with the Wind-up ratio (WUR), i.e., Single:Multiple ratings on the NRS (A). The difference between ratings of single and multiple stimuli (deltaTS) was also used to assess the degree of TS (B). The horizontal lines in the boxes represent the mean value for each group. Vertical bars range from the highest to the lowest values in each dataset. No significant differences between the groups were demonstrated with Mann-Whitney or unpaired t-test. Variables numbers are: WUR 256mN, FM+ n = 7 and FM- n = 17; WUR 512mN, FM+ n = 6 and FM- n = 22; deltaTS for both 256mN and 512mN, FM+ n = 21 and FM- n = 28.

The M-VAST tests was used to determine the aversion to visual stimuli using participants' ratings on unpleasantness and brightness associated to each luminous intensity. No significant differences were observed between the 2 groups. Surprisingly, the rating of unpleasantness and brightness were higher in the FM- negative group (figure 4.3.9 A and B). Of interest, the unpleasantness ratings were overall high (>40/100 on the NRS) even at low luminous intensity (0.1 lm) in both groups (figure 4.3.9 A).

Figure 4.3.9 Aversion to visual stressors



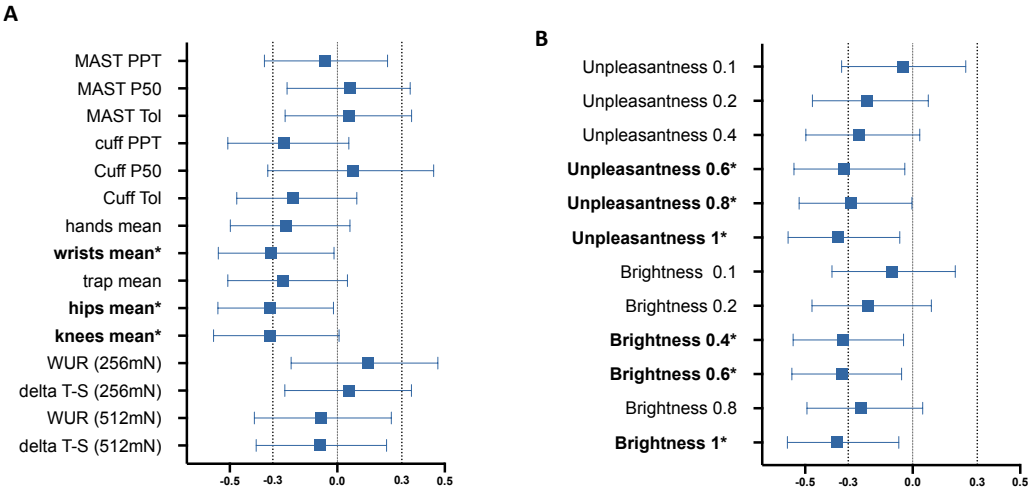
Legend: No significant differences were demonstrated between FM+ (red) and FM- (blues) in unpleasantness (A) and brightness ratings (B). FM+, n = 21; FM-, n = 29.

4.3.2.3 *Pressure pain thresholds and visual hypersensitivity are inversely associated with FMness*

Following the correlation between FM scores and QST was evaluated. Firstly, associations between QST and clinical parameters were assessed. No association with age was found, while the trapeziuses, hips, and knees algometry PPTs were associated with sex (Spearman's correlations respectively, R = 0.56, P <0.0001, R = 0.37, P = 0.01). However, female sex showed only trapeziuses PPT to be significantly lower than male (Mann-Whitney test P = 0.001, Appendix 7). Despite correlations between FM and QST variables were not adjusted for age or sex, correlations with the algometry PPT particularly at the trapeziuses might be influenced by sex. Correlations between FM scores and QST measuring pressure pain sensitivity, showed negative associations with the PPT measured at wrists, hips, and knees (respective Spearman's correlations, R = -0.31, P = 0.035; R = -0.31, P = 0.03; R = -0.31, P = 0.05). Correlation R and 95% confidence intervals are illustrated in figure 4.3.10 A. Despite not very strong associations were found and the results for the trapeziuses PPT might be affected by the sex, these correlations are in line with the findings in the FM groups, illustrated in the previous paragraph (figure 4.3.7). Regarding the visual unpleasantness and brightness ratings, negative associations with FM scores were demonstrated, especially at medium to high luminous intensities, as shown in figure 4.3.10 B

(brightness correlations for luminous intensities: 1, R = -0.35, P = 0.015; 0.6, R = -0.33, P = 0.02; 0.4, R = -0.32, P = 0.025; unpleasantness correlations for luminous intensities: 1, R = -0.35, P = 0.02; 0.8, R = -0.29, P = 0.05; 0.6 R = -0.32, P = 0.03). The correlations have the opposite direction than expected and suggest that the increased visual sensitivity is higher at lower FM scores.

Figure 4.3.10 Correlations between QST and FMness



Legend: Correlations between FM scores and QST variables are illustrated. Both graphs show the correlations R and the respective 95% confidence interval. A. The MAST, cuff algometry, algometry and temporal summation (TS) variables are included in the graph on the left. Only the PPT measured at wrists, hips, and knees resulted significantly associated with FM scores (respectively, R = -0.31, P = 0.035; R = -0.31, P = 0.03; R = -0.31, P = 0.05). B. In the graph on the right the unpleasantness and brightness rating from different luminous intensities are depicted. Stimulus of moderate or high intensities for both unpleasantness (0.6, 0.8, and 1 lm) and brightness (0.4, 0.6, and 1 lm) ratings were negatively correlated with the FMness (respectively, R and P, -0.32 and 0.02, -0.29 and 0.05, -0.35 and 0.02, and for brightness -0.32 and 0.025, -0.33 and 0.02, -0.35 and 0.02).

4.3.2.4 *Summary*

The sections above discuss the differences and associations between QST variables and FM in this PsA cohort. The analysis of pressure pain sensitivity measured with MAST and cuff algometry suggests an overall higher pain ratings in participants with FM than those without FM. However, the only significant difference between the two groups is observed for the cuff algometry PPT. Results from algometry measurements of PPTs in different body areas advocate that patient with FM are more sensitive in areas corresponding to joints (wrists) and enthesis (hips and knees). There were no significant differences between

the two groups in PPTs measured in other body areas, including trapeziuses and hands. These findings also reflect the negative associations between joint and enthesis PPTs and the total of FM scores, independently from the FM classification. Interestingly, no significant differences were found between the FM+ and FM- participants in TS and aversion to visual stimuli. The visual sensitivity was overall high in the entire cohort with unpleasantness rating above 40 out of 100 on the NRS, even for low luminous intensities. Brightness and unpleasantness ratings were negatively correlated with FM scores. Overall, the analysis suggests that concomitant FM in PsA increases sensitivity to pressure pain in deep tissues, joint and enthesis areas, and that the recruited population has an increased visual sensitivity which appears to be inversely associated with FMness.

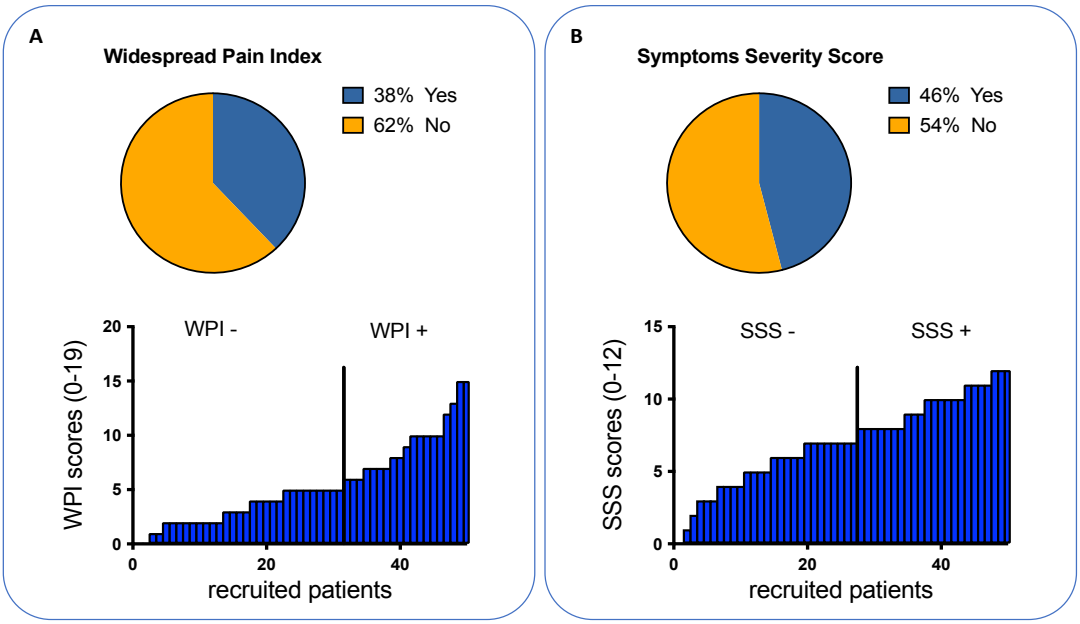
4.3.3 Relationship between the domains of the 2011 ACR fibromyalgia scores and neurobiological markers of nociplastic pain

WPI and SSS are two separate indices used to compute the ACR FM scores, validated to assess the degree of nociplastic pain. Both indices derive from questionnaire, however they measure different aspect of nociplastic pain; while WPI measures the intensity and distribution of widespread body pain, SSS assesses the severity of central-somatic symptoms, including fatigue, sleep quality, brain-fog, and depression, as well as headaches and abdomen discomfort. WPI scores range from 0 to 19 and higher scores indicate more widespread pain, likely to reflect a greater degree of nociplastic pain. In contrast, SSS scores range from 0 to 12, and a higher SSS score indicates a greater severity of somatic and central symptoms, epiphenomenon of central sensitisation. Because the aspects of nociplastic pain assessed by WPI and SSS are different, I progressed with a sensitivity analysis, based on the two indices, for the clinical, QST, and fMRI variables available to provide a more comprehensive understanding of the drivers of nociplastic pain in PsA, whether peripheral widespread pain or central-somatic symptoms.

4.3.3.1 *WPI and SSS distributions and associations with clinical variables are similar to FMness*

The medians values for the two indices were used to classify the participants in WPI and SSS high (WPI+/SSS+) or low (WPI-/SSS-). For the WPI, the median value was equal to 5, therefore subjects with WPI ≤5 were categorised in the WPI low (WPI-) group, while subjects with a WPI >5 were classified as WPI high (WPI+) (figure 4.3.11 A). Comparably, SSS high (SSS+) and SSS low (SSS-) were determined according with the median value of 7 (figure 4.3.11 B). The resulting distributions and frequencies of WPI and SSS were comparable with the FM classification, with largely overlapping classifications. A total of 12 individuals (6 to 7 individuals per index) with FM scores were differently classified using WPI or SSS, compared to FM scores which scores ranged from 10 to 15. Overall, the frequency of SSS+ subjects (46%, n=23) is higher than the corresponding WPI+ (38%, n=19) at apparently equal, but in opposite directions, distance from the percentage of FM+ participants (42%, n=21).

Figure 4.3.11 Distribution of WPI and SSS in the recruited population



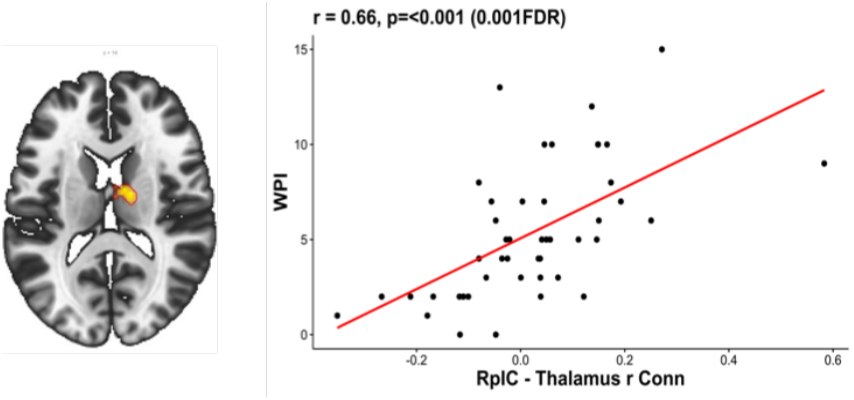
Legend: Distributions of the Widespread Pain Index (WPI) and Symptoms Severity Score (SSS) are illustrated. A. Based on the WPI median value of 5 calculated from the entire population recruited, the 38% of participants were classified as having a high WPI (WPI+) (n = 19), while the remaining 62% (n = 31) were classified as having a low WPI (WPI-). The histogram plot below shows the distribution of individual values across all the subjects. B. Similarly, SSS median value of 7 allowed the classification of subjects into 2 groups: high SSS (SSS+), representing the 46% of the individuals (n = 23), and low SSS (SSS-) resulting in most individuals (54%, n = 27). The distribution of individual SSS in an ascending order is shown in the bottom histogram graph.

Correlations between WPI and SSS with the clinical parameters collected at baseline are illustrated in the Appendix 8. Overall, similar correlations to the FMness were present also for WPI and SSS. Age and sex were not associated with both indices, while the number of all medications taken at baseline were positively correlated with both scores. Interestingly, FM and SSS showed similar associations with parameters of disease activity and participants' reported outcomes; conversely, WPI did not correlate with SJC, PhgVAS, and pain VAS at the moment of the visit.

4.3.3.2 *WPI-associated functional connectivity confirms an altered right mid-posterior insula connectivity with thalamus and dlPFC, and suggests the involvement of brain visual areas*

Similarly to FM, an initial ROI-to-ROI analysis to investigate the insula-DMN connectivity association with nociplastic pain was conducted. Differently from FMness, no significant association was found with the WPI and the functional connectivity between these brain areas. Successively, the seed-to voxel analysis using the DMN and the previously described 6 ROIs in the insula cortices as seeds was performed. Interestingly, similar connectivity between the mid-pIC and areas involved in sensory processing, i.e., the thalamus, and cognitive functions, i.e., the bilateral dlPFCs, was confirmed. The right pIC to right thalamus connectivity was directly associated with the WPI ($r = 0.66$, $p < 0.001$, FDR 0.001). In figure 4.3.12 is possible to observe the shift from anti-correlation between right pIC and right thalamus at low WPI, to the matched BOLD activities at higher WPIs.

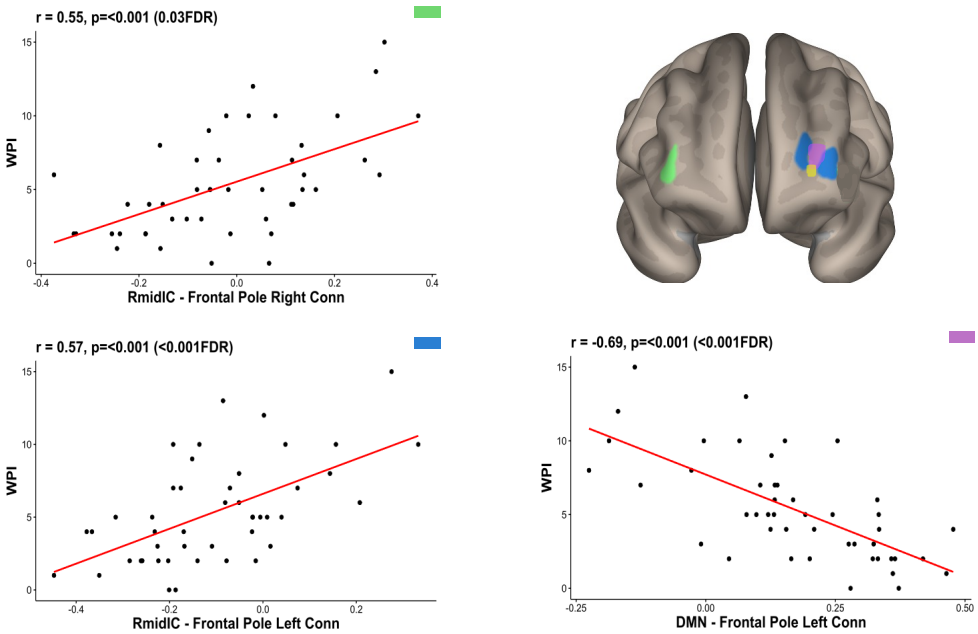
Figure 4.3.12 Right posterior Insula connectivity to homolateral thalamus association with the WPI



Legend: functional connectivity right posterior Insula cortex (RpIC) to the right Thalamus (Thalamus r) correlates with the WPI. Anti-correlation between the two areas characterises low WPI scores, while at the increasing of WPI synchronised BOLD signals (represented by values >0 on the X-axis) from the RpIC and the right thalamus are observed.

The whole brain analyses also confirmed the positive associations between the right midIC and DMN connectivity to dlPFC and nociplastic pain measured with WPI, previously showed with FMness. The left dlPFC brain areas functionally connected to the right midIC, and positively correlated with the WPI ($r = 0.57$, $p < 0.001$, $p = <0.001$ FDR), appear to overlay with the areas associated with FM (top right image in figure 4.3.13). In addition, the right midIC functional connectivity to the homolateral dlPFC is directly correlated with the WPI ($r = 0.55$, $p < 0.001$, $p = 0.03$ FDR, figure 4.3.13), which was not present with the FM scores. Interestingly, with the WPI was also observed a negative correlation between DMN to left dlPFC functional connectivity ($r = -0.69$, $p < 0.001$, $p < 0.001$ FDR, figure 4.3.13). Similarly to what observed in FM, low WPI scores showed a synchronised activity of DMN and left dlPFC, while at high WPI the connectivity between these areas is anti-correlated.

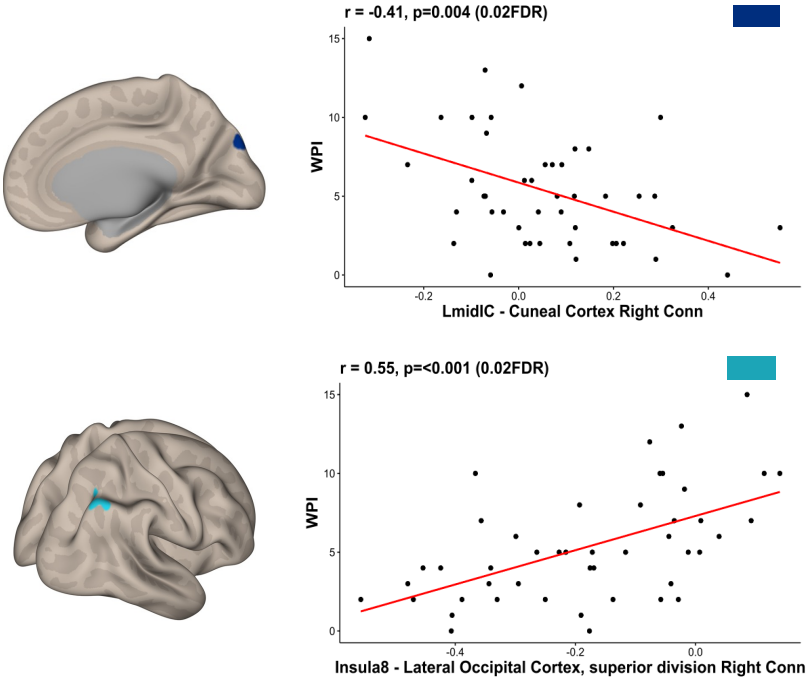
Figure 4.3.13 Right mid IC and DMN functional connectivity to dlPFC correlation to WPI



Legend: The two plots on the left illustrate the right middle insula cortex (RmidIC) functional connectivity to the right (top) and left (bottom) dlPFC (Frontal Pole) (respectively, r = 0.55, p <0.001, p = 0.03 FDR; r = 0.57, p <0.001, p <0.001FDR). The plot graph on the right shows the negative correlation between WPI and DMN to left dlPFC functional connectivity (r = -0.69, p <0.001, p <0.001FDR). On the top right image are represented the dlPFC areas correlated with RmidIC (green and blue), DMN (purple); in yellow is represented the left dlPFC area which connectivity with DMN correlates with FM.

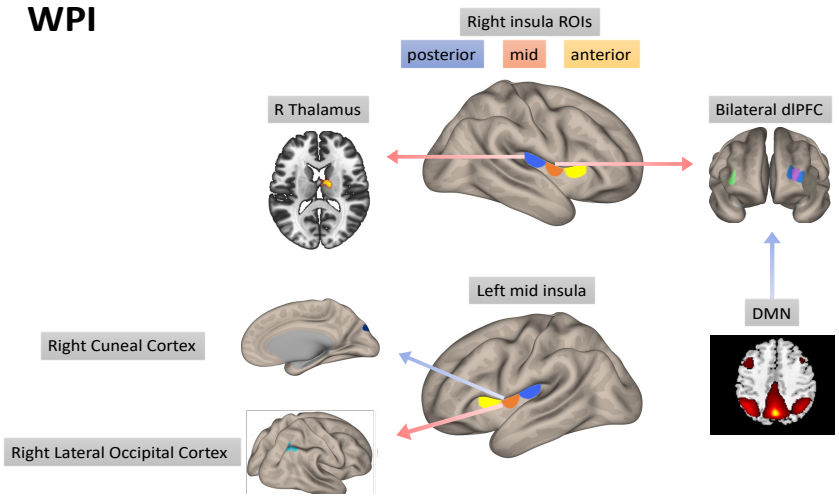
Additional findings from the seed-to-voxel analysis for WPI, different from FM scores, showed an altered connectivity of the left midIC to associative visual areas, involved in basic visual function, attentional and multimodal integrating functions, namely the cuneal (CC) and lateral occipital cortex (LOC). The left midIC functional connectivity to the primary visual area in the CC is negatively correlated to the WPI, showing anti-correlated activity at high WPI values, while their activities synchronise at lower WPI scores (r = -0.41, p = 0.004, p = 0.02 FDR, figure 4.3.14). Conversely, WPI is positively associated to the left midIC connectivity to visual associative area within the LOC (r = 0.55, p < 0.001, p = 0.02 FDR, figure 4.3.14). A similar transition, but in opposite directions, from anti-correlated to aligned BOLD signal in 2 brain areas is observed at the increasing of the WPI scores.

Figure 4.3.14 Altered left insula functional connectivity to visual areas



Legend: The top graph illustrates the relationship between WPI and the left middle insula cortex (LmidIC) to the right cuneal cortex functional connectivity (r = -0.41, p = 0.004, p = 0.02 FDR). The bottom graph represents the correlation between WPI and the left middle insula cortex (Insula 8 mm) to the right lateral occipital cortex functional connectivity (r = 0.55, p < 0.001, p = 0.02 FDR).

Figure 4.3.15 Summary of altered functional connectivity associated with the WPI

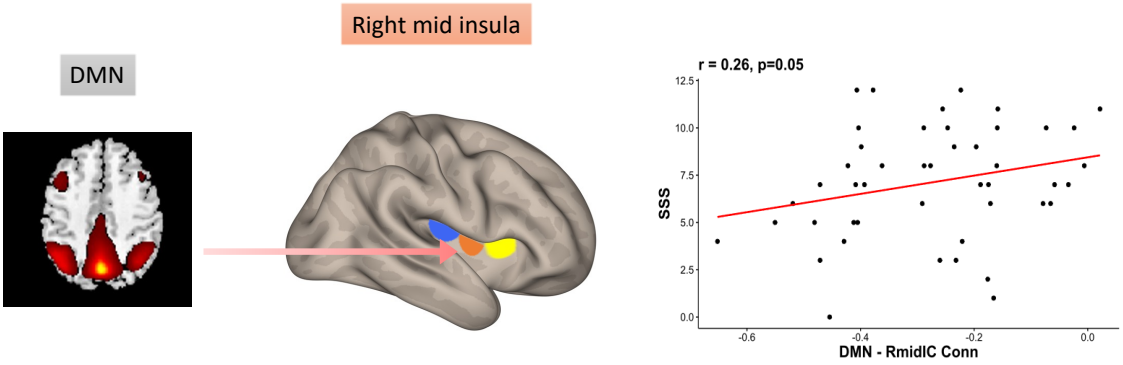


Legend: The figure summarises the seed-to-voxel analyses findings in relationship to WPI. Red arrows indicate a positive correlation with WPI, while the blue arrows show negative associations. The right posterior insula connectivity to thalamus, and the right mid insula connectivity to bilateral dlPFC resulted positively correlated with WPI. The DMN to whole brain revealed a negative association between DMN to left dlPFC connectivity and FMness. Moreover, the left mid insula connectivity to visual areas resulted associated with the WPI, revealing a negative association with the right cuneal cortex, and a positive correlation with the right lateral occipital cortex.

4.3.3.3 *Insula altered functional connectivity with DMN and occipital visual areas is associated with SSS*

Conversely to WPI, SSS was the only index with significant correlations with the DMN to right midIC functional connectivity, determined using ROI-to-ROI analysis. The positive correlation between DMN to right midIC functional connectivity and SSS (r = 0.26, p = 0.05) is illustrated in figure 4.3.16. In similarity with the FMness analysis, an anti-correlation between DMN and right midIC is observed in participants with low SSS, while the two brain areas appear uncoupled at higher SSS. Differently from FM, the DMN connectivity to the middle, and not anterior IC, was associated with the SSS.

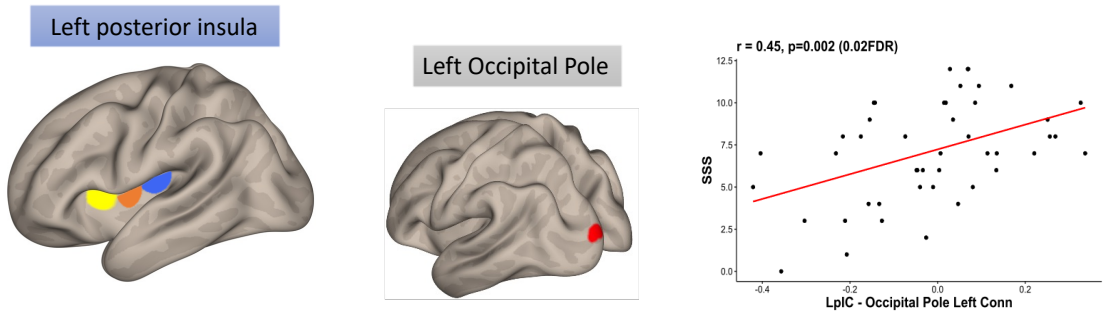
Figure 4.3.16 Functional connectivity of DMN to right mid-Insula positively correlates with SSS



Legend: DMN to the right middle Insula cortex (RmidIC) functional connectivity is positively correlated to the SSS. In the figure are illustrated the DMN (left) and the RmidIC (centre) and the positive correlation with the SSS (red arrow). The plot graph on the right illustrates the individual values for DMN-RmidIC connectivity and SSS and the positive correlation between the correlation (r = 0.26, p = 0.05)

The same seed-to-voxel analysis used for WPI and FM was carried out for the SSS. The SSS was positively associated with the connectivity of the left midIC to a primary visual area within the homolateral occipital pole (r = 0.45, p = 0.002, p = 0.02 FDR, figure 4.3.17). Thus, participants with higher SSS showed increased functional connectivity between the left pIC and the primary visual area in the occipital pole, as opposed to individuals with low SSS where an anti-correlation is observed between the same areas.

Figure 4.3.17 SSS is associated with left insula altered functional connectivity to the homolateral primary visual area



Legend: The graph shows the association between SSS and the left posterior insula cortex (LpIC) to the left occipital pole functional connectivity (r = 0.45, p = 0.002, p = 0.02 FDR). The LpIC is represented in blue in the image on the left, while the occipital pole functionally connected to it is in red (centre). The plot on the right displays the positive correlation showing increasing of LpIC-occipital pole connectivity at the rising of the SSS.

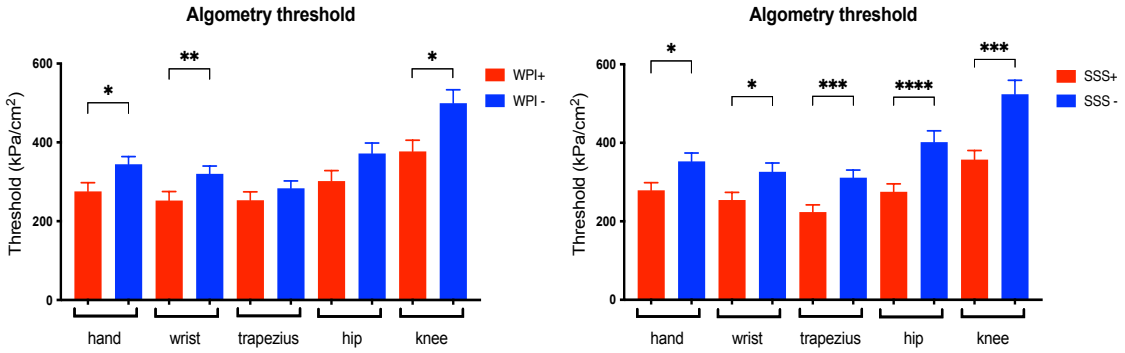
4.3.3.4 *SSS more than WPI drives the reduced PPTs in different body areas*

The differences in QST variables between participants with high or low indices (WPI and SSS) were investigated with the same analysis strategy used for the total FM scores. Firstly, differences in the MAST and cuff-algometry tests were explored. Similarly to FM, the MAST algometry analysis showed no significant differences in the PPT, Tolerance and P50, between the WPI+ versus WPI-, and SSS+ versus SSS+. The cuff algometry variables were not different in the 2 SSS groups, whereas the WPI+ group showed significantly higher PPTs than WPI-, in agreement with the results previously shown for FM groups (data shown in Appendix 9). Interestingly, TS and M-VAST did not show any significant difference between high versus low scores for both WPI and SSS. However, the unpleasantness and brightness ratings following high-moderate luminosity showed negative correlations with both WPI and SSS, as observed earlier in FM (Appendix 9).

The algometry PPTs tested in different body areas proved interesting results with the most significant differences seen in the QST analysis between WPI, SSS, and FM. In figure 4.3.18 A, the PPTs differences between WPI+ and WPI- for each body area is represented. With high WPI scores, the PPTs at wrists (Mann-Whitney, p = 0.005), followed by knees (Mann-Whitney, p = 0.0135) and hands

(unpaired t-test, p = 0.03), was significantly reduced compared to the WPI- subjects. Stronger differences in PPT between SSS+ and SSS- participants were observed in all the bodily areas tested (figure 4.3.1 B). The areas showing the most significant differences were the hips (Mann-Whitney, p <0.0001), knees (Mann-Whitney, p = 0.0005), and trapeziuses (Mann-Whitney, p = 0.0007). The remaining areas, hands (Mann-Whitney, p = 0.05) and wrists (Mann-Whitney, p = 0.01), also presented significantly lower PPT in the SSS- group.

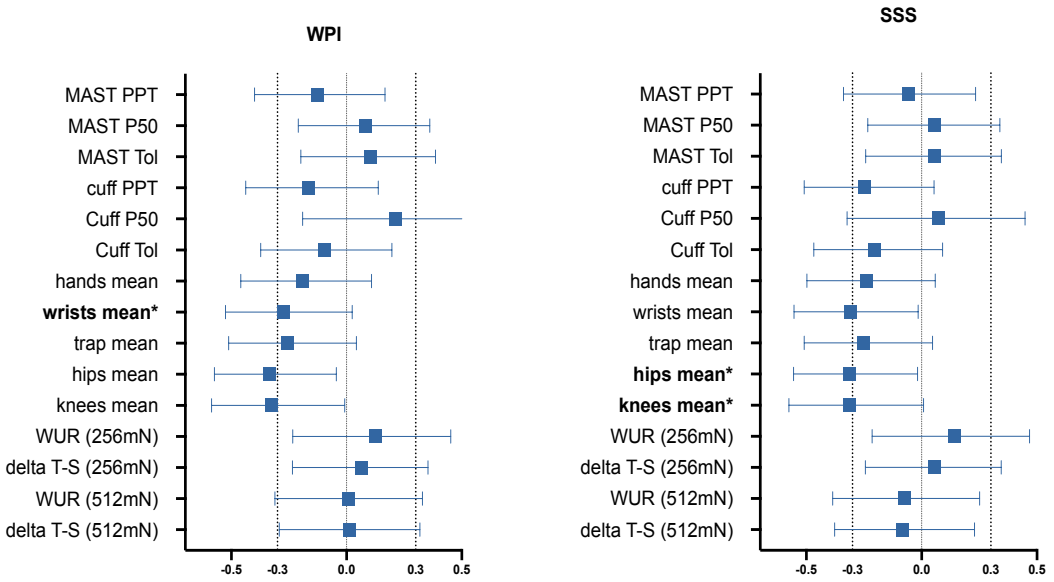
Figure 4.3.18 Algometry in different body areas: WPI and SSS



Legend: Distinctions between high and low FM indices, WPI (left) and SSS (right), in pressure pain thresholds (PPTs) measured in different body areas. A. Participants with high WPI showed reduced PPTs at the wrist (P = 0.04), knees and hands (respectively, P = 0.0135 and P = 0.04). B. SSS showed to have an influence on the PPTs of all body areas, in order of statistical significance observed, hips (p <0.0001), knees (p = 0.0005), trapeziuses (p = 0.0007), hands (p = 0.05) and wrists (p = 0.01). Number of values for each group: hand, WPI+ = 34 and WPI- = 57; hip, trapezius, and wrist, WPI+ = 36 and WPI- = 58; knee WPI+ = 28 and WPI- = 51; hand, SSS+ = 42 and SSS- = 49; hip, trapezius, and wrist, SSS+ = 42 and SSS- = 52; knee SSS+ = 32 and SSS- = 47.

The correlations between WPI and SSS indices and the pressure QST demonstrate significant negative correlation only with the algometry at the wrists, hips, and knees, supporting the findings in the FM groups. WPI correlated with wrists PPT (Spearman's R = -0.315, P = 0.03), while SSS was associated with entheseal areas, i.e., hips and knees (respectively Spearman's R and P, -0.335 and 0.02, -0.33 and 0.04).

Figure 4.3.19 Pressure QST variables associations with WPI and SSS



Legend: Correlations of QST variables with WPI and SSS. Pressure pain thresholds (PPTs) measured at joints and enthesis were significantly associated with WPI or SSS. Negative association between WPI and PPTs at the wrist (R = -0.315, P = 0.03). SSS significantly correlates with the PPTs at hips (R = -0.335 and P = 0.02) and knees (R = -0.33 and P = 0.04).

4.3.3.5 *Summary*

The findings above describe the relationships between WPI and SSS with neurobiological markers of nociplastic pain, i.e., fMRI functional connectivity and QST variables.

The functional connectivity between DMN and the right midIC was positively correlated with SSS, but not with WPI, as opposed to the previous findings in FMness (DMN- right antIC). An altered right mid-posterior insula connectivity with thalamus and dlPFC resulted associated with WPI, echoing the previous results in FM. With the WPI the connectivity of the right midIC extend also to the homolateral dlPFC; this association was not revealed with the FM scores only. However, similarly to FM, a negative association was found between the DMN and the left dlPFC, suggesting a potential important role of this area in nociplastic pain in PsA. Additionally, the WPI and SSS were associated with left pIC connectivity to primary and associative visual areas.

Indeed, these findings confirm the central role of insula its altered connectivity in nociplastic pain which might contribute to the manifestation of persistent pain in individuals with PsA.

Additionally, pressure pain sensitivity measured with MAST, and cuff algometry, as well as TS showed not significant differences between individuals with high or low WPI or SSS. Ratings following visual stimulations are not significantly different between high and low scores groups, however negative correlations were observed at high-moderate luminous intensities for both WPI and SSS, which had previously been observed in FM. The SSS showed the most significant results with PPTs algometry, presenting differences for all areas tested, mainly at knees, hips and trapeziuses. Correlations with knees and hips also presented significant negative associations with SSS. WPI was mainly associated with the joint areas at the wrists with significant differences between high versus low scores subjects, and significant correlations when analysing the WPI and PPTs continuously. The results overall support the findings in FM.

4.4 Discussion

4.4.1 Introduction

In this section I will discuss the results from the investigation on the neurobiological biomarkers of nociplastic pain in individuals with active PsA. The intent is to shed light on the neuroimaging correlates of pain perception and gain valuable insights into the complex neural mechanisms underlying pain in this population. The main focus was on nociplastic pain which was measured with the widely accepted 2011 ACR FM criteria score, encompassing the WPI and the SSS. Once defined the level of nociplastic pain in our PsA cohort, I tried to define the clinical characteristics and neurobiological signature using resting-state functional connectivity MRI and QST data.

4.4.2 Clinical pain phenotyping

FM is the prototype of chronic nociplastic pain disorders, and it is often observed in individuals with PsA. In line with the current evidence, a higher prevalence of FM among females participants was observed in the recruited population[358,846]. It is important to note that the recruited participants presented a higher prevalence of FM than expected from the current literature[439,440,448,450]. This might be explained by the higher probability to be referred and the personal interest to join the study in participants with chronic and severe pain. Otherwise, this result would suggest a higher prevalence of FM in the local Scottish community (no specific data available), however, it is inadequate to generalise due to the small population size and the single centre study.

The presence of FM is known to complicate the evaluation of disease activity due to overlapping symptoms and altered pain, posing challenges in distinguishing between inflammatory and non-inflammatory pain, and potentially leading to misinterpretation of disease activity[439,440,448,450]. Similarly, the presence of FM in this cohort was associated with different parameters used to assess disease activity, including TJC, enthesitis score, the PGA, and fatigue. In fact, a high widespread pain, previously measured with tender points, and currently with the WPI, can inflate the TJC and mimic enthesitis, as confirmed by these results. Interestingly, scores of disease activity, such as DAPSA and BASDAI, were also increased in presence of FM. However, more objective measure of active inflammation, i.e., SJC, dactylitis and CRP, were not different between participants with and without FM. Indeed, these clinical features might bias the clinical judgement and induce inappropriate treatment escalation; this phenomenon is also corroborated by the higher number of previous biologics demonstrated. Two alternative explanations which might further justify this finding are: 1) longer course of the disease (unlikely in this cohort because of lack of significant difference between FM+ and FM- participants); 2) a less controlled inflammatory disease, thus requiring more frequent treatment switch, could lead to nervous system sensitisation, increased FMness, and the development of a mixed pain state, at least in predisposed individuals. In our findings, the mild, but significant positive correlation between FMness and SSS with the SJC can support this interpretation. However, the cross-sectional study

and the limited number of participants for the power of a clinical study cannot strongly confirm this explanation.

Overall, FM is a challenging comorbidity in PsA, often mimicking high disease activity symptoms and potentially influencing treatment decisions; therefore, it should be carefully evaluated, particularly in patients with high levels of pain and not presenting classical inflammatory features, including swollen joints, dactylitis, or elevated CRP.

4.4.3 Neurobiology of FMness in PsA

To investigate the neurobiology of nociplastic pain in PsA, rs-fMRI and QST were employed. The analysis included a targeted approach (ROI-to-ROI) for the insula-DMN connectivity, as well as an exploratory analysis (seed-to-voxel) for the connectivity of the IC and DMN with the whole brain, both in relationship to FMness and the derived indices, WPI and SSS. The attention on the DMN and IC functional connectivity derived from their known relevance in pain processing and modulation, and more specifically in nociplastic pain[618,619]. In the context of different chronic pain conditions, the functional relationship between Insula, part of the salience network, and the DMN has been extensively investigated. In physiological conditions, the brain activity between the DMN and the insula within the SLN are usually anti-correlated, meaning that when one is active, the other one is inactive. Given their opposite role, one is active in self-reflection and rumination (DMN) and the other in attention to external input (IC) including acute pain[847], the functional uncoupling between these networks is understandable. Interestingly, a reduced anti-correlation or a synchronised activity has been proven in different conditions, including depression, dementia, FM and other chronic pain disorders[382,848-850]. Similar finding has been demonstrated in individuals with RA and a high degree of FMness showed similar DMN-IC relationship[492]. Our findings firstly demonstrated that a decreased anti-correlation between DMN and the right antIC is also present in PsA subjects with high FM scores. In support, a similar altered connectivity between DMN to right midIC was positively correlated to the SSS, incorporating the central symptoms characteristic of FM. This result is consistent with previous findings in FM where the right IC has a predominant role in pain processing compared to the left[608].

However, the correlation was not as strong as observed in RA, where the DMN to the left IC showed a strongly synchronised activity. Despite RA and PsA share similar immune-mediated pathogenesis, they are distinct diseases with their own peculiar clinical features and probably their unique pain signature. Overall, these findings suggest an increased integration of the right mid-antIC function in the painful perception, probably incorporating emotional/affective salience, and a neurobiological distinction between PsA and RA nociplastic pain.

To further investigate DMN and IC functional connectivity in PsA, the seed-to-voxel analysis was conducted, exploring the connectivity of these regions with the rest of the brain. Essentially 3 brain regions showed an increased connectivity, predominantly with the insula, in relationship with the FMness: the thalamus, parahippocampal gyrus (PHC), and dlPFC. These brain areas have established function in sensory processing or higher cognitive functions in keeping with their involvement in nociplastic pain. The thalamus has a well-established role in processing sensory information and both morphological and functional alterations have been associated with FM[851,852]. In our findings, a significantly increased functional connectivity is evident with the right pIC in relationship with both FMness and WPI. The posterior insula has the putative role to encode for intensity of sensory input from the periphery, as well as environmental monitoring[351,608]. Therefore, the increased connectivity right pIC to the homolateral thalamus is coherent with the current knowledge, and aligns well with the WPI driving this association in the PsA population. The PHC increased connectivity is a less expected finding, however, still consistent with its potential function in pain. First of all, the PHC gyri are part of the DMN, therefore the increased functional connectivity with both right midIC and left pIC further support the altered DMN-IC connectivity characteristic of nociplastic pain. Furthermore, the PHC itself recently emerged as a key brain area involved in primary FM. Growing evidence suggests that individuals with FM present reduced PHC grey matters volume, considered a sign of excitotoxicity or activation, often associated with cognitive deficits[853]. An increased PHC activation in FM after pressure or heat stimulation has been also demonstrated[811,854], and PHC activation is associated with poor prognostic implications[855]. These findings imply a direct role in pain perception, probably regulating unpleasantness and the affective component. In fact, PHC most

known function is in working memory and executive function[856] which might add a contextual memory to pain, contributing to an emotional evaluation of the sensory input, potentially leading to fear, stress, increased unpleasantness, and pain catastrophizing, all commonly present in primary FM[857,858]. Further investigation on the psychological phenotype in PsA, including stress, anxiety, cognitive function, and pain catastrophizing, would be important to integrate in future exploratory analysis around pain to validate our results. It is also important to note that all the studies in primary FM showing activation of the PHC have used different neuroimaging techniques, and a functional connectivity analysis was not available to the best of my knowledge. Finally, PHC and the antIC are part of the descending pain inhibitory pathways deficient in primary FM syndrome[859,860]. A dysregulation of the descending anti-nociceptive pathway might also have an important role in PsA with overlapping nociplastic pain. However, many limitations hinder this interpretation, including the seed-to-voxel methodology, which is explorative and lack of the accuracy of the ROI-to-ROI counterpart, and the unavailability of CPM data, which could corroborate this explanation. The hypothesis of an altered descending pain pathways should be further explored in PsA including CPM paradigm in future pain studies. Lastly, PHC connectivity was not confirmed with SSS and WPI separately. Overall, PsA participants who have higher FM scores look neurobiologically similar to people with primary FM, however the role of the PHC in the genesis of pain needs to be confirmed in future studies. The dlPFC is part of PFC where the most complex human cognitive functions are centred. Interestingly, the dlPFC showed an opposite connectivity to DMN (negative) and to IC (positive) in relationship with FM and WPI in this PsA cohort. Both DMN and IC showed connectivity within the same gyrus of the dlPFC, however the multiple complex function of this brain region in pain (generally considered an anti-nociceptive area) warrant caution in interpreting these findings. Nonetheless, high FMness seems associated to a synchronised activity between right midIC and the dlPFC which, in parallel, shows an uncoupled/anti-correlated activity with the DMN. These functional connectivities are negatively correlated between each other's, suggesting a meaningful relationship between the dlPFC and key pain areas in PsA. The dlPFC activity has been associated with affective pain, pain anticipation, and more complex phenomena, including placebo analgesia, anxiety, and pain

catastrophising[861]. Functional connectivity studies in FM, demonstrated a decreased coupling between right IC and dlPFC in a seed-to-voxel analysis, while the more precise ROI-to-ROI did not confirm the association[854]. Therefore, the DMN-dlPFC-IC relationship requires further validation, to ensure this result is not spurious, ideally using a ROI-to-ROI approach, or a targeted seed-to-voxel analysis using the dlPFC as the primary seed.

Another important piece to understand the neurobiology of pain in PsA is the subjects' sensitivity informed by the QST. In line with previous results in RA, the presence of nociplastic pain was associated with an increased sensitivity to pressure stimuli in disease specific body areas also in PsA. A reduced PPT at algometry testing on calf deep tissues, joints, and tendons areas was observed. In particular, the PPTs measured at the wrists, knees, hips, and calf were significantly reduced in PsA participants with high FMness. Those areas correspond, or are in proximity (calf muscle nearby knees and Achilles' tendons), with regions commonly affected in PsA. In neutral areas including carpometacarpal joints, typical of OA, trapezius, typical of FM, and the thumbnail a significant difference was not observed. Although the thumbnail is considered a neutral site in many chronic pain conditions, the reliability in PsA is affected by the potential psoriatic onychopathy. In our cohort most of individuals had an active or a personal history of PsO (around 90%), however, the nail involvement was not systematically recorded. Therefore, differentiating between inflammatory activity or nociplastic processes is problematic. Differently, the cuff PPT result is particularly relevant because the deep tissue stimulation has higher sensitivity in distinguish the presence of nociplastic pain in musculoskeletal diseases and it is less biased by psychological factors[765,771,862]. The increased pressure pain sensitivity aligns with previous studies in RA and knee OA, the active inflammation tends to reduce the PPTs in the areas directly affected, reflecting peripheral sensitisation[551]. However, in PsA the characteristic enthesitis represent an important confounding factor influencing the interpretation of this finding. In fact, while the peripheral inflammation represents a trigger for peripheral sensitisation in active PsA, the presence of high disease activity at baseline poses challenges in distinguish between nociceptive and nociplastic pain with QST. Similar difficulty in differentiating between the presence or not of FM with QST was recently showed in a clinical

study including subjects with an active RA[552]. Interestingly, the WPI confirmed the results observed in FM, with overall overlying results, in particular for the PPTs measured at the calf, knees, and wrists, with the addition of a reduced PPT at the hands. However, the pressure sensitivity in relationship with SSS, showed different results. The cuff algometry PPT lost its significance with SSS, while the differences in algometry PPTs strengthen significantly across all body areas. Despite both feeding the final FM score, WPI and SSS are intrinsically different, the first one measuring the widespread pain, while the second gauges central sensitisation features. This might explain the difference in our findings. Also, the modality of testing for the manual handheld algometer is substantially different from the computer-controlled cuff or MAST device and requires the direct application of the pressure by the examiner to the bare skin of the participant. The impression of a less standardised control of the pressure, especially when the testing is carried out after the MAST and the cuff algometry, and the closer interaction with the examiner will likely contribute to increase the testing anxiety (not recorded in between tests) and individuals with high SSS might be more susceptible to an emotional or psychological interference in pain ratings. Nevertheless, the WPI and SSS taken together as continuous scales confirmed the findings in FMness. Despite the increased sensitivity in specific body areas, a more generalised hypersensitivity was not demonstrated with the QST in our population, as suggested by the lack of significant differences in parameters classically linked to alteration of central processing, i.e., the TS, tolerance to pressure, or visual task. Regarding the TS, our data suggest that the presence of FM does not associate with an enhanced TS in PsA. To note, high ratings on the NRS scale following the initial single stimulation can impact the TS results. Moreover, TS can be evaluated using different modalities; considering the increased sensitivity at deep tissues in PsA, evaluating TS using deep tissues stimulation should be contemplated in future studies. Another interesting finding regards the visual sensitivity in PsA. All participants, independently from the FM classification, exhibited an overall high aversion to visual stimuli, suggested by high unpleasantness ratings when exposed to dim luminosity. Therefore, individuals with PsA may have heightened sensitivity to visual stimuli. However, there are a few caveats to consider. Firstly, the lack of control populations precludes the possibility to draw firm conclusions. Secondly, uveitis, a chronic

inflammatory eye disease, is often present in PsA (2-25%)[863,864], and light sensitivity is the most frequently reported symptom[865]. Despite only one patient had an episode of uveitis in the past, it is not possible to exclude the presence of subclinical eye inflammation in other participants, leading to increased visual sensitivity and, therefore, biasing the results. Thirdly, unpleasantness to visual stimulation has been shown to be affected by mood levels[778]; probably less meaningful, however, it should be considered when interpreting visual sensitivity. Overall, PsA subjects seem sensitive to visual stimuli, however, further studies including control populations, eye clinical evaluation, and psychological assessment are needed to confirm this interpretation. In our study however, other findings suggest involvement of the visual pathways. The seed-to-voxel sensitivity analysis in relationship with SSS and WPI demonstrated an altered connectivity of the left mid-posterior IC to visual areas. Participants with higher WPI and SSS showed an increased connectivity with primary and associative visual areas within the occipital cortex. Conversely, WPI scores were negatively associated the left midIC connectivity to a primary visual area. Therefore, independently from FM, both WPI and SSS associations point towards an activation of the visual areas which aligns with the QST findings. Whether these results are linked and neurobiologically meaningful cannot be elucidated with the data available in this study, however, advocate for further investigations. In fact, the cuneal and lateral occipital cortices, functionally connected with the left IC, are associative visual cortices, and have multiple and complex functions, including integration basic visual processing with working memory and reward/expectation, as well as a multimodal integrating function, such as feature-extracting and attention. The altered connectivity with the left insula in PsA might suggest an altered attentional and multimodal integrating function involving both areas (salience and visual-driven cognitive control), associated with central nociplastic pain features, WPI and SSS. Light sensitivity has been associated to development of central sensitisation in individuals with dry eyes, and it is a clinical feature of FM[866]. In a recent, large fMRI study focused on depression and PsO using the UK-biobank database demonstrated an impaired connectivity between the superior frontal and the temporo-occipital cortices, involved in visuospatial processing, in subjects with PsA, compared to PsO and controls[627]. Although the results are consistent with the available

literature and support the involvement of visual pathways in PsA, it is important to consider that seed-to-voxel analyses are susceptible for spurious results and visual areas are inclined to present BOLD signal artifacts due to vascular interactions[867]. Therefore, future targeted studies should include direct activation of the visual areas with evoked visual tasks fMRI with the intent to strengthen the signal from visual areas, as shown in FM[540], alongside a deeper clinical characterisation.

4.4.4 Study limitations

The study provides novel and valuable insight on nociplastic pain in PsA, however several limitations should be acknowledged.

Firstly, the use of functional connectivity analysis with fMRI has inherent limitations. BOLD fMRI signals represent an indirect measure of neuronal activity, based on the assumption that the increasing of neuronal activity reflects in a higher metabolic activity and oxygen demand. The temporal resolution is indeed reduced with delays of 4-6 seconds between neuronal activations and haemodynamic changes which limits the detection of event happening over a short course of time[574]. Additionally, the magnitude of the hemodynamic changes is quite small in single individuals, therefore, the assumptions derived from our fMRI analysis is established on population-based variations, hence the generalisation of the results is limited by inherent differences among participants. Moreover, the local blood is influenced by other factors, including anatomical variations, vascular comorbidities, and haemodynamic response independent from neuronal activation, for example anticipatory vasodilation and vascular interaction between brain areas[868,869]. Regarding interpretations of brain connectivity and its associations with nociplastic pain, I need to clarify that this analysis is based on correlations, which cannot establish causality. To unravel underlying mechanisms, more rigorous experimental designs like intervention studies and mediation analyses are necessary. Despite BOLD based fMRI has its limitations in capturing the full complexity of the brain, the technique has been validated and represent a reliable non-invasive approach to study brain function.

Ultimately, the study design and sample size limit the power of some findings. The cross-sectional design of the study limits the establishment of causal relationships. Longitudinal studies are needed to understand the temporal dynamics and potential causal links between nociplastic pain, brain connectivity, and clinical variables. The sample size of the study may impact the generalisability of the results, and a larger cohort, would enhance the findings' validity. Comparative analyses with control populations could also shed light on disease-specific neurobiological differences that may be specific to PsA compared to other chronic pain conditions. Moreover, the study relied on self-report measures, which can introduce biases and variations in reporting pain and symptom severity, however, pain is by definition a subjective experience, therefore, it is important to acknowledge the complexity of the phenomenon itself.

4.4.5 Conclusions

In this study, I aimed to explore the neurobiological biomarkers of nociplastic pain in individuals with active PsA using resting-state functional connectivity MRI and QST, in combination with clinical data. This study sheds light on the neuroimaging correlates of pain perception and provides valuable insights into the complex neural mechanisms underlying pain in this population. These results suggests that the presence of FM, or FMness, in PsA is challenging and requires careful evaluation, especially in individuals with high pain levels and atypical inflammatory features. Concomitant nociplastic pain can mimic high disease activity, inflating various clinical parameters including TJC, enthesitis, PGA, and fatigue. As a result, the distinction between inflammatory and non-inflammatory pain is arduous; our data also support a potential influence on treatment decisions. The unique finding of this study is that PsA subjects with high degree of FMness present similar neurobiological features of primary FM. The newly discovered altered functional coupling between two key networks in pain processing and modulation, the DMN and the Insula, is characteristic of nociplastic pain states and aligns with previous findings in FM and RA. In addition, our exploratory analysis also suggested that PsA has a unique pain signature with an increased connectivity with brain areas with complex functions all involved in somatosensory processing and affective pain perception. The

increase connectivity with areas involved in highly cognitive functions, such as the PHC and the dlPFC, drive towards a deeper characterisation of psychological and neurodegenerative states of individuals with PsA in relationship with pain. The relationship with the peripheral sensitivity is intriguing, in fact the PsA participants showed an increased pressure pain sensitivity in areas potentially involved by the inflammation, but not in “inflammation-neutral” areas. This finding advocate for a central key role of the peripheral sensitisation compared to the central, supporting a prevalent bottom-up versus top-down nociplastic pain. The combination of clinical (QST) and neurobiological (fMRI) findings also support different nervous system sensitisation routes, i.e., the visual pathways that have been rarely investigated in previous studies.

Despite the promising new advancements in understanding the PsA pain phenotype, I am aware that these findings require validation with targeted analyses and integration of different neuroimaging techniques and QST protocols to clarify the functional interactions within these networks and their impact on pain processing in PsA.

In conclusion, our study provides novel insights into the neurobiological basis of nociplastic pain in PsA. Peripheral sensitisation appears to play a prominent role in nociplastic pain mechanisms, and further investigations are needed to elucidate the complex interactions body and brain in subjects with PsA. Future studies with larger sample sizes, longitudinal designs, and more comprehensive clinical assessments are essential to validate the findings of this study.

Chapter 5 Effect of sex and pain modulating drugs on nociplastic pain phenotype in PsA

5.1 Introduction

Several factors can influence chronic pain in general, and nociplastic pain in particular; these include sex and medications able to modulate pain perception. This chapter addresses the second aim of this thesis by describing the effects of sex and pain-modulating drugs on pain sensitivity and neurobiological markers of nociplastic pain in PsA. Differences in clinical and QST variables between females and males, and participants taking pain-modulating drugs or not, are reported. The influence of pain modulating drugs on DMN and insula functional connectivity in relationship to our nociplastic pain measure, the 2011 ACR FM score, will be also described.

5.2 Effect of sex on pain sensitivity

The effect of sex on nociplastic pain was firstly investigated by describing the differences between female (n = 27) and male (n = 23) participants on the 2011 ACR FM scores and clinical features (table 5.2.1). Female participants showed higher frequency of FM, with 51.85% meeting the criteria compared to 30.43% of male participants; however, the difference in FM frequencies did not reach the statistical significance (Fisher's exact test). Similarly, the mean of the FM scores was slightly higher in females than males, despite not significant (13.8 ± 6.1 vs. 11.2 ± 5.5, respectively). The baseline clinical characteristics, including age, disease duration, BMI, current and previous medications, were overall similar between male and female subjects. However, there were several significant differences in disease activity and patient-reported outcomes between the sexes. Regarding disease activity indices, female participants showed overall higher mean values on most parameters, but only LEI scores, BASDAI, and DAPSA were significantly increased compared to males (respective P-values 0.04, 0.007, and 0.04). Nonetheless, pain intensity was significantly higher in female compared to male. In fact, females reported higher levels of pain on the VAS (P = 0.02) and the pain diaries (P = 0.004), as well as higher global disease activity on the VAS (P = 0.02). Furthermore, female participants also reported higher

levels of fatigue and anxiety, although the difference was not statistically significant. Overall, the findings suggest that there may be a distinction between male and female subjects, especially in terms of reported pain intensity which might influence disease activity scores. The higher prevalence of FM in the female participants, despite not significant, might contribute to these findings.

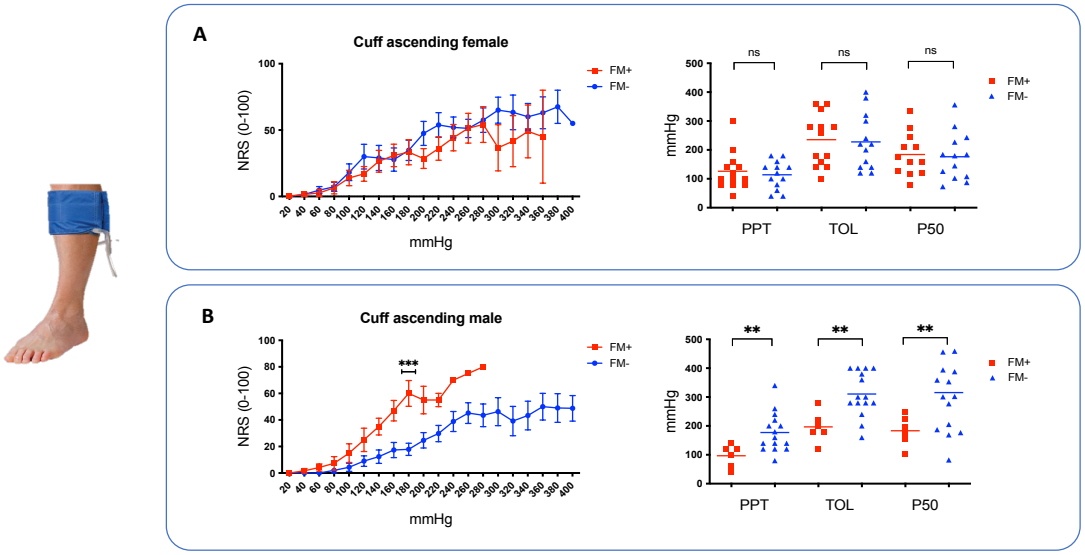
Table 5.2.1 Differences between male and female in clinical data and nociplastic pain, measured with 2011 ACR fibromyalgia criteria.

CLINICAL FEATURES	Female (27)	Male (23)	P-values
FM criteria (%)	51.85%	30.43%	ns
FM total score (mean ± SD)	13.8 ± 6.1	11.2 ± 5.5	ns
Clinical characteristics			
Age (mean ± SD)	48.3 ± 12	49 ± 11	ns
Disease duration years (mean ± SD)	5.4 ± 4.5	7.8 ± 7	ns
BMI (mean ± SD)	30.1 ± 4.9	29.2 ± 3.9	ns
Previous biologics (mean ± SD)	0.9 ± 1.45	0.65 ± 1.3	ns
N previous DMARDs (mean ± SD)	1.7 ± 1.2	1.8 ± 1.4	ns
N medications baseline (mean ± SD)	3.7 ± 2.5	2.8 ± 2.9	ns
Disease activity			
TJC 66 (mean ± SD)	23.96 ± 14.5	18.6 ± 14.1	ns
SJC 68 (mean ± SD)	7.9 ± 5.5	6.5 ± 2.9	ns
Dactylitis (yes/no)	1 ± 1.4	0.5 ± 1.2	ns
LEI score (mean ± SD)	3.2 ± 1.9	2.1 ± 1.9	0.04
BASDAI (mean ± SD)	6.9 ± 1.6	5.45 ± 2.1	0.007
DAPSA (mean ± SD)	50 ± 26.8	37.4 ± 21.2	0.04
CRP mg/dl (mean ± SD)	1.6 ± 3.3	0.45 ± 0.4	ns
Physician gVAS (mean ± SD)	56.85 ± 13.5	48.9 ± 18.8	ns
Patient reported outcomes			
Patient gVAS (mean ± SD)	66.3 ± 18.43	50.65 ± 25.9	0.02
Pain VAS (mean ± SD, n)	43 ± 25.1, 23	26 ± 22, 18	0.02
10 days pain diary (mean ± SD)	6.2 ± 1.8	4.25 ± 2.3	0.004
Fatigue VAS (mean ± SD)	7.3 ± 2.7	5.95 ± 2.4	ns
Pre-QST anxiety (mean ± SD)	9.3 ± 15.5	5.9 ± 10.8	ns

The sensitivity analysis of QST data was carried out to investigate differences in pressure and visual sensitivity between the sexes. Each sex was grouped in FM+ and FM-, weather individuals met or not the 2011 ACR criteria for FM. Cuff and manual algometry differences are described below, while additional QST data, including MAST, TS, and visual sensitivity, are available in Appendix 10.

Pressure pain sensitivity measured with cuff algometry showed significant differences between individuals with and without FM in male participants, but not in females (figure 5.2.1). Conversely, the ascending curves of females, with and without FM, were overall similar. In fact, derived pressure pain sensitivity variables, namely PPT, tolerance, and P50, were not statistically different between FM+ and FM- females (figure 5.2.1 A). Differently, male participants with FM showed higher pain ratings than the FM- group at each pressure applied; multiple comparison t-test showed significant differences only at a pressure equal to 180 mmHg ($P = 0.0004$, P-adjusted with Holm-Sidak method = 0.004). PPT, tolerance, and P50 were all significantly lower in the male subjects with FM (P-values respectively, 0.0035, 0.002, and 0.005), indicating increased sensitivity to pain in males with concomitant nociplastic pain.

Figure 5.2.1 Effect of sex on cuff algometry pain sensitivity in relationship with the 2011

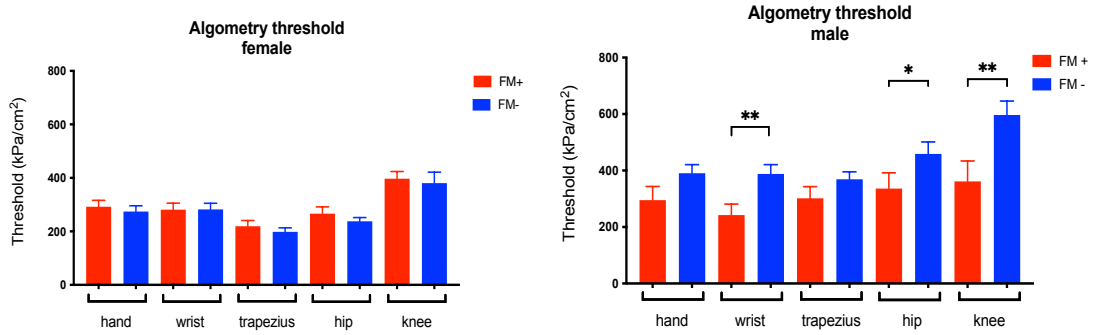


Legend: This figure displays the relationship between sex pain and nociplastic pain. Participants in each sex group are stratified by the 2011 ACR FM criteria in those with FM (FM+) and those without FM (FM-). Pressure pain sensitivity was measured using cuff algometry applied on the non-dominant calf. The data are presented separately for females (A; FM+ n = 13 and FM- n = 13) and males (B; FM+ n = 6 and FM- n = 15). Female showed no differences between FM+ and FM- across all algometry variables. Males with FM+ presented higher pain ratings than FM- at all pressure intensities, reaching statistical significance only at a pressure equal to 180 mmHg (P = 0.0004, P-adjusted with Holm-Sidak method = 0.005). PPT, tolerance and P50 were all significantly lower in the FM+ male participants using unpaired t-tests (P-values, respectively, 0.0035, 0.002, and 0.005).

Sex-specific pain sensitivity in relationship to nociplastic pain was also assessed measuring PPTs with a hand-held algometer at various anatomical sites, including hands, wrists, trapeziuses, hips, and knees (figure 5.2.2). It is interesting to note that, in male participants, PPTs measured in joints and tendons regions, i.e., wrists, knees and hips, differ significantly between FM+ and FM-. Specifically, these divergences yield a Mann-Whitney test p-value of 0.007, 0.0095, and 0.04, respectively for knees, wrists, and hips, suggesting an increased pressure pain sensitivity in males with FM. Conversely, the presence of FM seems to not influence the PPTs across different body areas in female participants. Actually, in the female group, no statistical differences in the pain response to algometry appears between FM+ and FM- individuals in any of the assessed anatomical sites. These findings suggest that the presence of nociplastic pain is associated with increased pressure pain sensitivity at joints

and tendons regions in males, but not in female participants. To note the FM presence is slightly more frequent in females than males (table 5.2.1), which might explain the tendency of females to present lower PPTs in different body sites reflecting higher pain sensitivity (Appendix 10). Overall, these observations suggest that the presence of FM influence pressure pain sensitivity in males, but not in females.

Figure 5.2.2 Differences of sex and nociplastic pain effect on algometry pressure pain sensitivity in different body areas.



Legend: the figure displays the relationship between sex pain and nociplastic pain and their influence on pressure pain sensitivity assessed with algometry at different anatomical sites, including the hand, hip, trapezius, wrist, and knee. Female participants are illustrated in the bar graph on the left, while males are depicted in the right graph. In each group enrolled individuals were stratified according with the 2011 ACR FM criteria as having FM (FM+, in red) or not (FM-, in blue). Only males showed significantly lower PPT in the FM+ group compared to FM-. Mann-Whitney test was used to test differences in PPT which resulted significant at the knees (P = 0.007), wrists (P = 0.0095), and hips (P = 0.04).The numbers of male and female participants in each body area are: females hands, wrists, trapeziuses, hips FM+ and FM- n = 26, and knees FM+ n = 24 and FM- n= 22; males wrists, trapeziuses, and hips FM+ n = 12, FM- n = 30; males hands FM+ n = 10 and FM- n = 29; males knees FM+ n = 8 and FM- n = 26.

5.3 Exploring the impact of pain modulating drugs on nociplastic pain in PsA

Pain modulating drugs, such as opioids, gabapentinoids, SNRIs, and certain antidepressants are commonly used in the management of various chronic pain conditions. Their impact on nociplastic pain in individuals with inflammatory arthritis and chronic nociplastic pain needs further investigation. The objective of this analysis is to investigate the impact of pain modulation drugs on nociplastic pain in our PsA cohort. The medication participants were taking at the baseline visit, including opioids, gabapentinoids, SNRIs, and specific

antidepressants such as tricyclic antidepressants (TCAs) and selective serotonin reuptake inhibitors (SSRIs), were reviewed knowing their multifaceted pharmacological actions that may impact pain perception and processing. This information is crucial in understanding their potential influence on the main results in this cohort (detailed in Chapter 4).

Firstly, participants were grouped into a pain modulating drugs (PM) group, when taking one or more of the drug classes mentioned above, or in a pain modulator free (PMF) group, if they were not. A total of 14 participants were included in the PM group, while the remaining 36 individuals were classified as PMF. The nociplastic pain classification, using the 2011 ACR FM criteria, alongside clinical features of the 2 groups are illustrated in the table 5.3.1. The analysis of the differences between the 2 groups indicates a significant difference in terms of presence of nociplastic pain. Of the PM subjects, 71.43% met the criteria for FM compared to 30.56% of PMF participants; this difference resulted statistically significant with the Fisher's exact test with a P-value of 0.01. There was also a significant difference in FM total scores (p=0.002) between participants who were treated with pain modulating drugs and those who were not, probably reflecting an already established treatment in individuals with chronic nociplastic pain and/or the concurrent presence of psychological disorders (not investigated in this study). Regarding the clinical characteristics, there were significant differences in age (p=0.0125), PM subjects were older than PMF, in the number of current medications (<0.0001), implying that PM individuals tend to take more medications, and in the number of previous biologics (0.0015), probably reflecting a more challenging control of the disease, potentially confounded by the concomitant FM. Despite proportionally more females were present in the PM group, there was no significant sex difference between the 2 groups, and neither in disease duration, BMI, or number of previous csDMARDs. In terms of disease activity, there was a significant difference in TJC 66 (p=0.02), BASDAI (p=0.002), and DAPSA (p=0.02) between PM and PMF subjects, entailing that higher level of pain on the joints areas and disease activity scores may have prompted the initiation of these drugs. Despite being on medications that should reduce pain levels, PM subjects still present more tender joints and higher disease activity indices than the PMF participants. Regarding the patient-reported outcomes, the reported pain on VAS and the pain diaries were overall

higher in the PM group compared to PMF, however these differences did not reach a statistical significance. The participants' impression of global disease activity (PtgVAS) and the levels of fatigue (fatigue VAS) were significantly higher in subjects treated with pain modulating drugs than those who were not (respectively, 0.001 and 0.03).

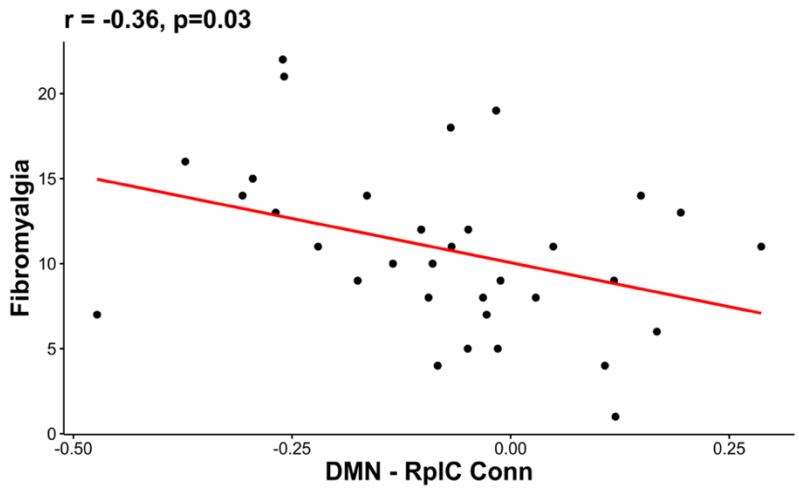
Overall, these findings suggest that PM subjects are more likely to meet the criteria for FM, and, despite reported pain is not significantly higher, the pain at joints, the participants' impression, and clinical indices of disease activity, as well as the reported fatigue remain high.

Table 5.3.1 Differences in 2011 ACR fibromyalgia criteria and clinical features between participants on pain modulating drugs and who are not.

CLINICAL FEATURES	Pain modulators (14)	Pain modulators-free (36)	P-values
FM criteria (%)	71.43%	30.56%	0.01
FM total score (mean ± SD)	16.36 ± 5.555	11.14 ± 5.378	0.002
Clinical characteristics			
Age (mean ± SD)	55 ± 11.95	46.17 ± 10.36	0.0125
Sex (male:female)	4:10	19:17	ns
Disease duration years (mean ± SD)	6.5 ± 5.814	6.5 ± 5.935	ns
BMI (mean ± SD)	30.64 ± 3.809	29.29 ± 4.727	ns
Previous biologics (mean ± SD)	1.571 ± 1.651	0.4722 ± 1.158	0.0015
N previous DMARDs (mean ± SD)	2.143 ± 1.748	1.639 ± 0.99	ns
N medications baseline (mean ± SD)	5.857 ± 2.248	2.333 ± 2.178	<0.0001
Disease activity			
TJC 66 (mean ± SD)	29.36 ± 14.2	18.42 ± 13.49	0.02
SJC 68 (mean ± SD)	8.714 ± 6.057	6.694 ± 3.686	ns
Dactylitis (yes/no)	0.3571 ± 0.6333	0.9722 ± 1.502	ns
LEI score (mean ± SD)	3.571 ± 2.102	2.361 ± 1.854	ns
BASDAI (mean ± SD)	7.354 ± 1.807	5.821 ± 1.893	0.002
DAPSA (mean ± SD)	53.49 ± 22.85	40.6 ± 25.14	0.02
CRP mg/dl (mean ± SD)	2.264 ± 4.352	0.6389 ± 0.8391	ns
Physician gVAS (mean ± SD)	56.07 ± 16.19	52.08 ± 16.71	ns
Patient reported outcomes			
Patient gVAS (mean ± SD)	71.43 ± 10.46	54.31 ± 25.22	0.001
Pain VAS (mean ± SD)	44.29 ± 23.93	31.53 ± 24.75	ns
10 days pain diary (mean ± SD, n)	6.2 ± 2.5, 14	4.9 ± 2, 28	ns
Fatigue VAS (mean ± SD)	8 ± 1.664	6.167 ± 2.783	0.03
Pre-QST anxiety (mean ± SD)	6.429 ± 14.99	8.194 ± 13.1	ns

The pain modulating medications investigated act on different components of the pain processing pathway, targeting neural receptors, neurotransmitter systems, and neuronal excitability. Therefore, a change of pain somatosensory processing would be expected with centrally acting drugs, as well as an alteration of the relationship between FMness and brain functional connectivity. To investigate the role of those medications in this population, the DMN and insula functional connectivity was assessed only in subjects not taking the pain modulating drugs. Of the 47 individuals for whom was available the resting state sequence, a total of 33 were not taking those medications. Firstly, the functional connectivity of DMN and different insula seeds with a ROI-to-ROI analysis was evaluated. In figure 5.3.1 is depicted the plot graph showing the negative association between FMness and DMN to the right pIC (r = -0.36, p = 0.03), as opposed to the results including all participants (figure 5.3.1). The DMN to right pIC functional connectivity is anti-correlated at high FM scores, while the 2 brain areas present synchronised activity when FMness is low.

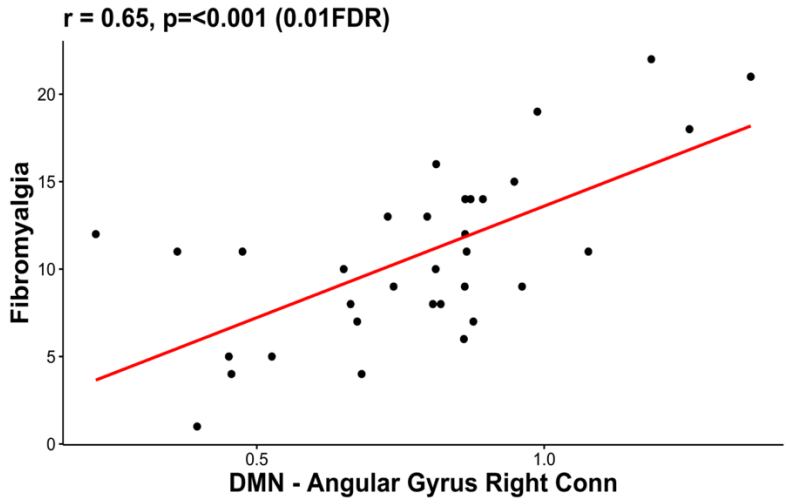
Figure 5.3.1 Relationship between FM scores and DMN functional connectivity to insula in participants without pain modulating drugs.



Legend: The figure shows the results of the ROI-to-ROI analysis for fMRI data in participants not taking pain modulating drugs. DMN to right posterior insula cortex (RpIC) functional connectivity is inversely associated with FM scores in the selected population (r = -0.36, p = 0.03).

Successively, a seed-to-voxel analysis was carried out using DMN and the 6 insula ROIs as seeds. The only significant correlation was observed between FM scores and the DMN to the angular gyrus (figure 5.3.2, r = 0.65, p<0.001, FDR p 0.01). The angular gyrus is located into the IPL, a key area of the DMN. Therefore, subjects free from the influence of pain modulators showed an increase DMN interconnectivity at growing FMness.

Figure 5.3.2 DMN interconnectivity is increased in individuals with high FM score not taking pain modulating drugs.

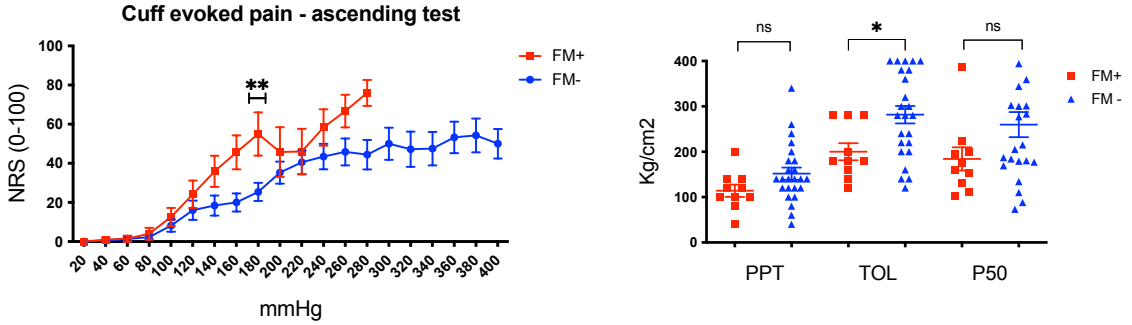


Legend: The figure displays the DMN-IPL functional connectivity in pain modulators-free subjects. The angular gyrus is a key region of the DMN. At high FM scores, participants present an increased interconnectivity of the DMN with the angular gyrus. The FM scores and DMN to angular gyrus functional connectivity resulted positively correlated at the seed-to-voxel analysis (r = 0.65, p<0.001, FDR p 0.01).

Finally, QST data were analysed to further investigate the neurobiological markers of nociplastic pain in individuals not taking pain modulating drugs. Below are described the significant algometry results, measured with the cuff at the non-dominant calf and with a hand-held algometer in different anatomical areas; the findings for the remaining QST data are illustrated in Appendix 11. The cuff algometry test on participants who were not using pain-modulating drugs are shown in figure 5.3.3. Subjects were stratified according with the 2011 ACR FM criteria as having FM (FM+, n = 11), or not (FM-, n = 25). Individuals with FM showed higher average pain ratings than the FM- group for most pressure intensity applied; multiple unpaired t-test yielded a significant difference

(0.0006) only at a pressure equal to 180 mmHg. The mean values of PPT and P50 were not significantly different between the FM groups, while the tolerance in the FM+ subjects was significantly lower than the FM- participants (0.02), reflecting an increased pressure pain sensitivity. Cuff algometry variables did not differ significantly in individuals taking the pain modulators stratified for the FM criteria (Appendix 11).

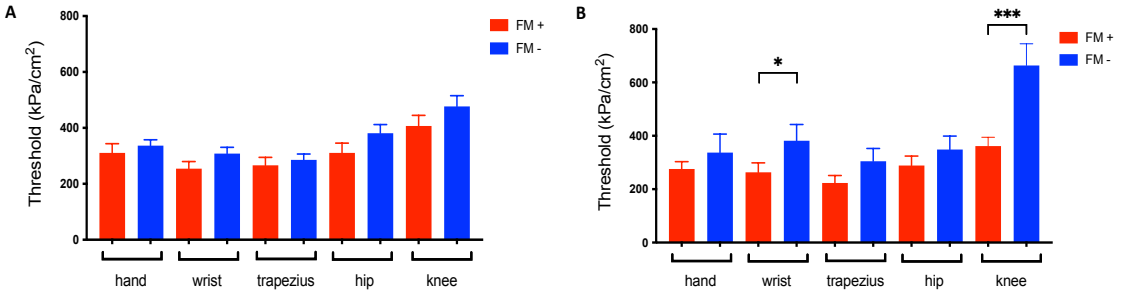
Figure 5.3.3 Cuff-algometry in individuals not using pain-modulating drugs



Legend: cuff algometry analysis in recruited subjects not taking pain modulators. Participants were divided in FM+ (n = 11, in red) when meeting the 2011 ACR FM criteria, and FM- (n = 25, in blue) when not. The ascending curves for both groups are illustrated on the left graph. The FM+ showed higher ratings than FM-, reaching statistical significance at 180 mmHg with multiple unpaired t-test (p = 0.0006). The tolerance (TOL) was significantly higher in the FM- group (p = 0.02). Pressure pain threshold (PPT) and pressure to elicit a pain rating of 50/100 (P50) were not different between the two groups.

Differently, pressure pain sensitivity in different body areas, measured with a hand-held algometer, did not differ between FM+ and FM- in pain modulators-free individuals (figure 5.3.4 A). However, in the participants taking pain modulating drugs, a significantly reduced PTT at the level of the wrists ($p < 0.05$) and tibial insertion of patellar tendons (knees, p = 0.0006) was observed in the FM+ compared to FM- (figure 5.3.4 B). Albeit different mechanisms of actions of the pain modulators, with variable efficacy on nociplastic pain, were included, the presence of FM seems to influence pressure pain sensitivity in the PM subjects, particularly on joints (wrists) and tendons (knees) body region.

Figure 5.3.4 Algometry pressure pain thresholds at different body areas in subjects grouped based on pain modulating medications taken and 2011 ACR fibromyalgia scores



Legend: The figure shows the effect of pain-modulating drugs on pressure pain sensitivity measured with algometry at different body areas and its relationship with nociplastic pain measured with the 2011 ACR FM scores. Differences in pressure-pain thresholds between FM+ (red) and FM- (blue) are illustrated for participants who are using pain-modulating drugs (B) and those who are not (A). No significant differences were demonstrated in pain modulating drugs-free subjects (A). PPTs measured at wrists and knees in individuals with FM on pain-modulating drugs (FM+, B) were significantly lower compared to the FM- participants (respectively, 0.05 and 0.0006).

5.4 Discussion

In this study, I aimed to investigate the effects of sex and pain-modulating drugs on nociplastic pain in individuals with PsA.

5.4.1 Sex-driven pain features

Our findings in the sex sub-analysis highlighted interesting gender differences in pain sensitivity and clinical manifestations, shedding light on potential sex-related influences on nociplastic pain in PsA. Firstly, a higher prevalence of FM in female subjects compared to males was observed. This result is in line with the current literature available, however, in our PsA cohort did not reach the statistical significance and the gender difference is less strong than in other populations worldwide[358]. Probably the presence of a chronic inflammatory disease may increase the chances of develop FM in males. The power of this study is limited to confirm this interpretation, and it should be taken as an overall indication to be verified in larger PsA and other inflammatory arthritis cohorts. Clinically, female participants reported higher levels of pain intensity, at the baseline visit and on the 10 days after, as well as impression of disease

activity. These might have also influenced the higher disease activity indices, namely BASDAI and DAPSA, observed in females compared to males. The LEI scores were also significantly higher in females; this result is in line with the previous literature showing higher enthesitis clinical scores in individuals with FM[451]. Overall, these results suggest a potential link between a higher degree nociplastic pain, and the clinical phenotype observed in female participants. Regarding the fMRI results, sex and age were accounted for in this analysis. Therefore, the neuroimaging results are not driven by sex or age differences in our population, emphasising the robustness of our neuroimaging findings. The QST analysis stratified by sex confirms a disparity in pain perception mechanisms between male and female participants. Similarly as observed in the whole population, the presence of FM did not showed a significant impact on QST variables linked with central sensitisation phenomena, such as TS, visual sensitivity, or PPTs in areas non-involved by PsA inflammatory processes. While these findings were analogous for other QST algometry variables in females, men presented different outcomes. In males with concomitant FM, the pressure pain sensitivity measured by cuff algometry was significantly increased compared to those without FM. Male participants with FM showed higher pain ratings at various cuff pressure intensities, with significantly lower PPT, tolerance, and P50, when compared to the FM- counterpart. Moreover, males with FM probably drove the algometry results found in the entire population, presenting an increased pressure pain sensitivity at joint and tendon regions, namely wrists, knees, and hips. Interestingly, female participants, irrespective of the presence of FM or not, presented similar PPTs to fibromyalgic males, indicating an overall higher pressure pain sensitivity. These observations are consistent with existing literature on sex and pain sensitivity, with previous studies reporting reduced pressure threshold and tolerance in females compared to males[639,640]. The impact of the gender of examiners, particularly the influence of female examiners in this study, is challenging to define with certainty. Overall, male subjects, when examined by females, tend to report lower pain ratings at the same pressure compared to examinations by examiners of the same sex[528]. Even if this effect was present in FM- male participants in this study, the FM+ group does not seem to exhibit the same pattern. However, this phenomenon may have contributed to the distinct separation between the examined populations,

resulting in significant differences. It is important to highlight that this is a speculative observation and challenging to confirm in the context of this study.

Overall, the impact of sex and gender on pain sensitivity can rely upon a multitude of different mechanisms, including immunological and genetic factors, as well as hormonal influences[643]. Beyond the genetically determined sex-differences, the psychosocial traits associated with gender constructsmay also influence these findings[528].

5.4.2 The exclusion of pain modulating agents suggests a PsA-specific neurobiological signature

The pain-modulating drugs investigated in this subanalysis, including opioids, gabapentinoids, SNRIs, and tricyclic antidepressants target various components of the pain processing pathway and may influence pain perception and processing. Taking one or more of above medications was not in the exclusion criteria of this study. To overcome possible biases due to the central effect of these medications, I divided the subjects enrolled in 2 groups (PM and PMF) with the aim of better understanding the impact of these drugs on pain sensitivity and functional connectivity associated with nociplastic pain in our PsA cohort. Firstly, a considerable portion of participants (71.43%) in the PM group met the criteria for FM with significantly higher FM scores compared to the PMF group. Despite taking medications aimed at controlling pain, PM subjects exhibited higher TJC, and disease activity indices compared to PMF individuals. These observations suggest that participants with a prevalent nociplastic pain component (top-down), driven primarily by central sensitisation, were also more likely to assume pain modulating-drugs, as well as other medications and have had more biologics prior to the baseline visit. Despite this, the pain levels remain high in the PM group, as shown in different participants' reported outcomes, in addition to TJC and disease activity scores. It is important to consider that the effect of those pain modulating drugs can indeed affect brain functional connectivity. In a primary FM cohort, Harris and colleagues demonstrated that pregabalin reverts the altered connectivity between anterior and posterior IC with IPL and PCC, key areas within the DMN[384]. Therefore, I moved to the analysis of functional connectivity in participants not taking the

above pain modulators with the hypothesis that the association between DMN-IC connectivity and FMness would be strengthen. Inversely to the initial hypothesis, the ROI-to-ROI analysis showed a negative correlation between the DMN to right pIC connectivity and the FM scores, in the PMF subjects. Possible explanations to this unexpected result might be different. First, the elegant study previously mentioned included only individuals with primary FM; in fact, the presence of rheumatic inflammatory diseases was an exclusion criterion in this study[384]. Furthermore, excluding participants taking pain-modulating drugs from the analysis meant to exclude those individuals with higher FM scores more likely to present a central "top-down" nociplastic pain phenotype. Conversely, individuals with a prevalent mixed pain phenotype, including a prospective "bottom-up" sensitisation driven by the peripheral inflammation, were probably predominant in the PMF group. Therefore, this result suggests a population-specific altered brain connectivity patterns associated to nociplastic pain. The result from the seed-to-voxel analysis further supports the interpretation of facing a mixed pain phenotype population. In fact, FMness positively correlated with an increased DMN interconnectivity, i.e. DMN to angular gyrus. Within the DMN, the angular gyrus is the posterior part of the IPL, and serves different important functions, including complex language tasks (e.g., reading by giving meaning to visual information), working memory (anatomically connected with the parahippocampal gyri, amongst other brain areas), and spatio-visual salience[870–872]. In RA subjects, a similar DMN connectivity to IPL resulted positively correlated with the peripheral inflammation (ESR), also on repeated fMRI scans after 6 months[623]. In this PsA cohort, an altered connectivity of brain regions believed to play a significant role in the central responses to peripheral inflammation are herein positively associated with the FM scores. These findings support a probable role of peripheral inflammatory sensitisation in the group of subjects not taking pain-modulating drugs, thus with a mixed pain phenotype relaying on bottom-up sensitisation mechanisms.

Further analysis of QST data also revealed interesting patterns. It is important to clarify that pain modulating drugs can indeed reduce the sensitivity to different stimuli, especially when pressure modalities are employed. Some medications, particularly SNRIs and gabapentinoids, can also modulate the descending pain-modulating pathways altering the finding in TS. Due to drugs mechanisms of

action and probably because of the reduced numbers of PM individuals (only 13), no significant results were found in the most of the QST analysis. The only exception was in the algometry measuring PPTs in different body areas. While PMF subjects showed no significant differences between FM+ and FM- in all body areas evaluated, the PM group with FM showed significantly lower PPTs at wrists and knees. While high disease activity can partially explain the lack of differences in both groups, it is difficult to express reliable interpretations for the PM group's results. Nonetheless, the increased pressure pain sensitivity in the FM+ participants might have been influenced by psychological (e.g., anxiety) factors associated with the presence of FM (SSS, not investigated in this small group, see results in section 4.3.3.4). Moreover, a reduced efficacy of pain modulating-drugs in individuals with higher FM compared to the FM- can also explain these findings. However, the interpretation of these finding is complicated by the heterogeneity of this group. For example, the kind of drug(s), dosage and duration of treatment are not uniform, and introduce multiple confounding factors which are not possible to disentangle with such a small samples size. Regarding the PMF group, significant differences were observed in the cuff algometry ratings and tolerance between subjects with and without FM. The pain modulating drugs probably increased participants' tolerance to pressure stimuli masking the results in the entire population. Excluding the subjects also better separated the ascending curves between FM+ and FM- participants, reaching the statistical significance at a moderate pressure of 180 mmHg. To note, similar results were also observed in the males' group, in fact the prevalence of male in the PMF group is higher, even if not significant, than the PM group. Overall, excluding the PM participants from the analysis confirmed an increased deep tissues pressure pain sensitivity in presence of FM in PsA. The increased sensitivity is reflected by a reduced tolerance, rather than a reduced PPT, despite the PPTs where overall lower in the FM+ compared to FM-.

5.4.3 Limitations

It is essential to acknowledge the limitations of this study with regards of the above results, including the relatively small sample size and potential confounding factors that were not fully accounted for. Indeed, those findings

need to be confirmed in other cohorts with prevalent peripheral sensitisation, e.g., RA and OA, ideally including control groups, primary FM and/or healthy. Despite the results being adjusted for the sex pf participants, a separate neuroimaging analysis might highlight differences between males and females that could elucidate the sex differences showed in the QST results. Moreover, a targeted approach using ROI-to-ROI from the IPL/angular gyrus, would be important to expand future analysis, especially factoring different peripheral markers of inflammation, including ESR. Additionally, future studies also should aim to distinguish between top-down and bottom-up sensitisation in nociplastic pain patients, however, no established clinical or biological marker of peripheral sensitisation is available to date. Furthermore, more stringent exclusion criteria for pain modulating agents or a more comprehensive assessments of potential confounders in larger cohorts should be considered.

Lastly, investigating the effects of central acting drugs in PsA individuals with concomitant FM would be beneficial to assess their impact on nociplastic pain and brain functional connectivity in inflammatory arthritis in general, and might assist to further disentangle the complex pain neurobiology in these inflammatory diseases.

5.4.4 Conclusions

Our study highlights the complex interactions between sex, pain-modulating drugs, pain phenotype, and brain functional connectivity in individuals with PsA.

The higher prevalence of FM and increased pain sensitivity in female participants, along with differences in pressure pain sensitivity, suggest that sex-related factors should be considered in the assessment and management of pain in PsA, as well as in every individual suffering from chronic pain. Similarly, excluding subjects taking pain-modulating drugs revealed distinct patterns of functional connectivity associated with FM scores, suggesting the involvement of peripheral inflammation in the observed brain connectivity changes. Overall, a unique neurobiological signature of individuals with PsA seems characterised by both classical DMN-IC altered connectivity, driven by participants with prevalent nociplastic features, and inflammatory sensitisation features, i.e., DMN-IPL

(angular gyrus), predominant in subjects with a mixed nociplastic-nociceptive pain phenotype.

This study provides a deeper understanding of the effects of different factors influencing nociplastic pain mechanisms in inflammatory arthritis. Despite the insightful observations from this exploratory analysis into the influence of sex and centrally acting drugs on nociplastic pain in PsA, more powered studies with larger sample sizes, including sex and gender factors, as well as more attention on drugs with influence on pain, are needed to confirm and expand on these findings. Moreover, finding a comprehensive biomarker able to distinguish between centrally- or peripherally- driven nervous system sensitisation in inflammatory arthritis may lead to more targeted treatments which would improve patients' quality of life.

Chapter 6 Relationship between inflammation and pain phenotype

6.1 Introduction

Inflammation plays a crucial role in the pathophysiology of pain, contributing to the development and maintenance of chronic pain. Emerging evidence suggests that dysregulated inflammatory processes may also contribute to the manifestation of nociplastic pain syndromes. Chronic inflammation contributes to create a pro-sensitising environment for the peripheral and CNS possibly linked to the frequent occurrence of FM in individuals with inflammatory arthritis. Despite recent advances, the mechanisms of crosstalk between the immune and nervous systems are not completely understood.

Leveraging the of presence chronic inflammation in this well-defined PsA cohort with active disease, I addressed the third aim of this thesis by exploring the relationship between inflammation and nociplastic pain states. Baseline levels of pathogenic pro-inflammatory cytokines and the response to immunosuppressants in the study participants were used as markers of inflammation. The associations between inflammatory markers and clinical features, and neurobiological determinants of nociplastic pain are illustrated below.

6.2 Peripheral blood pro-inflammatory cytokines

6.2.1 Introduction

Numerous pro-inflammatory cytokines have been implicated in somatosensory modulation and growing evidence support their involvement in pain pathways. Among these cytokines, IL-17A and TNF are key pathogenic mediators in PsA. IL-17A has been shown to contribute to the sensitisation of nociceptors and peripheral nerves by enhancing neuroplasticity and mediating neuroimmune crosstalk; IL-17A neurobiological effects lead to hyperalgesia and influence responses to sensory stimuli in different animal models [461,462,464–466,478]. Similarly, TNF has been associated with central and peripheral sensitisation through

facilitation of neuronal excitability and synaptic plasticity, augmenting pain perception even after resolution of the inflammation [469,470,472,479,481,873,874].

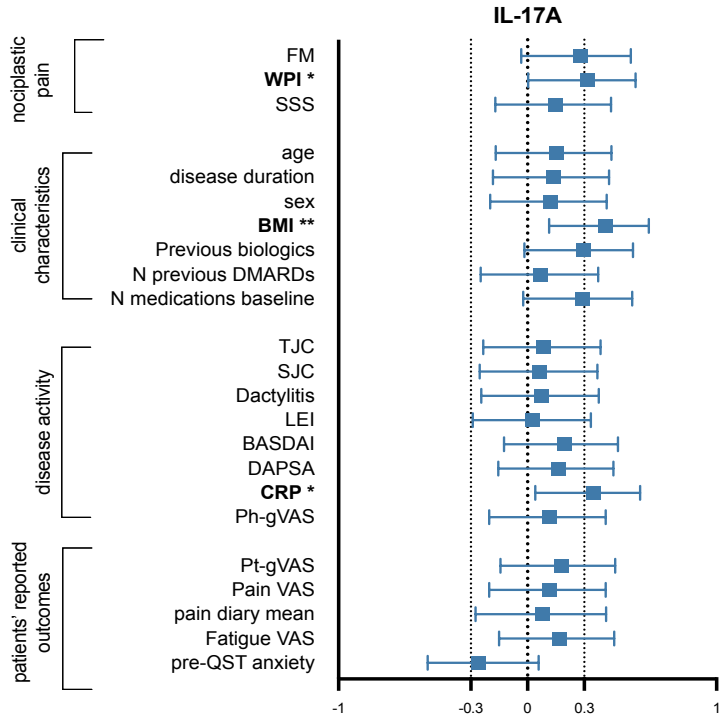
Notably, other markers of systemic inflammation, i.e., the CRP were within the normal range (10 mg/L) for most of participants, independently from their classification according with the 2011 ACR FM criteria. In light of the potential involvement of IL-17A and TNF in the nervous system sensitisation, my objective was to investigate their relationship with nociplastic pain in the context of chronic inflammation, in this PsA cohort. Circulating levels of IL-17A and TNF were measured in the biological samples available using MSD assays. Out of the 50 participants recruited a total of 45 samples were collected and used for this study. IL-17A was detectable in all 45 samples with a mean value of 4,377 fg/ml and a high SD of 13,950. IL-17A ranged from 217.1 to 85,007 fg/ml. Most of participants had levels of circulating IL-17A below 5,000 fg/ml, while 3 subjects had values above 20,000 fg/ml. Those 3 subjects (2 FM- and 1 FM+) were excluded from the analysis using the Tukey's fence method with k = 3 to optimise identification of extreme outliers (values above the 75th quartile + 3*IQR)[843]. Regarding TNF, the pro-inflammatory cytokine was detectable in 26 sera samples. Circulating TNF levels were on average 45.5 pg/ml ±SD of 79.4, and values were ranging from 0.16 to 300.6 pg/ml. In only one FM+ patient the TNF detected value was above the Tukey's outliers' threshold (TNF = 300.6); therefore, the single outlier was excluded from the analysis. Values distributions and assays detection curves are available in Appendix 11. Next, an analysis was performed to investigate the relationship between clinical parameters and circulating IL-17A and TNF was explored, focusing on the FM score and its sub-indices, WPI and SSS, as indicators of nociplastic pain. Additionally, the relationship between the pro-inflammatory cytokines detected and the objective neurobiological markers available, namely functional brain connectivity and QST, was explored.

6.2.2 IL-17A is associated with widespread pain and TNF with anxiety, while BMI correlates with both cytokines

To investigate the associations between circulating levels of IL-17A and TNF and nociplastic pain related outcomes and clinical variables I used Spearman's

correlations due to the distribution of the variables (Appendix 12). The levels of the 2 cytokines examined did not correlate significantly between each other (Appendix 12). After the exclusion of outliers, in the analysis were included 42 and 25 participants for IL-17A and TNF, respectively. The correlations between the nociplastic pain and clinical variables for both IL-17A and TNF are illustrated in figures 6.2.1 and 6.2.2. The analysis included parameters of nociplastic pain, as well as clinical characteristics, disease activity indices, and participants' reported outcomes. Regarding the parameters of nociplastic pain the total FM scores, as well as WPI and SSS were included. Only IL-17A resulted positively correlated with the WPI ($R = 0.315$, $p = 0.04$), however, the cytokine was not associated with the FM scores, nor the SSS. Positive correlations of IL-17A with BMI ($R = 0.41$, $p = 0.007$) and the circulating levels of CRP ($R = 0.35$, $p = 0.02$) were also observed. To evaluate the risk of biased results due to recent exposure to anti-IL-17 agents, 6 participants taking IL-17A inhibitors or interrupted before half-life of the drug were identified [875,876] . Amongst the 6 individuals only 3 had detectable levels of circulating IL-17A, but these corresponded to the 3 outliers; which had already been excluded from the analysis as potential confounders.

Figure 6.2.1 Correlations of circulating IL-17A with pain and clinical variables in individuals with psoriatic arthritis

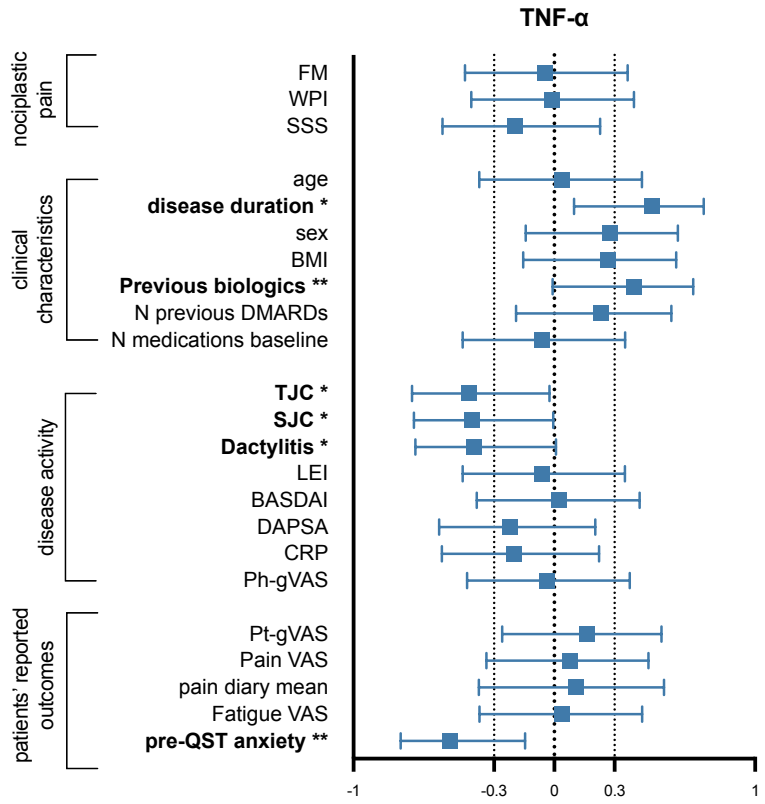


Legend: Correlations between circulating levels of IL-17A and various clinical variables are depicted. Correlation coefficients (Spearman's R-values) and corresponding 95% confidence interval are illustrated for each variable. Positive correlations of IL-17A levels with WPI (R = 0.315, p = 0.04), BMI (R = 0.41, p = 0.007), and CRP (R = 0.35, p = 0.02) are highlighted.

On the other hand, TNF resulted positively associated with the disease duration (R = 0.485, p = 0.01) and the number of previous biologic treatments received (R = 0.4, p = 0.05), surprisingly various measures of disease activity were negatively correlated with TNF, including TJC (R = -0.43, p = 0.03), SJC (R = -0.41, p = 0.04), and dactylitis scores (R = -0.4, p = 0.05). These results were not confirmed when participants with previous exposure to anti-TNF biologics were excluded[877,878](figure 6.2.3). Of the 11 subjects identified, 1 corresponded to an excluded outlier (FM+) and in 4 individuals TNF levels were not available; therefore, 6 subjects were excluded from the initial analysis, for a total of 19 participants. Interestingly, the secondary analysis showed a positive correlation between TNF levels and BMI (R = 0.46 p = 0.04). Despite none of the participants' reported pain or fatigue outcomes associating with the levels of TNF (or IL-17A), the self-reported anxiety prior QST session was negatively

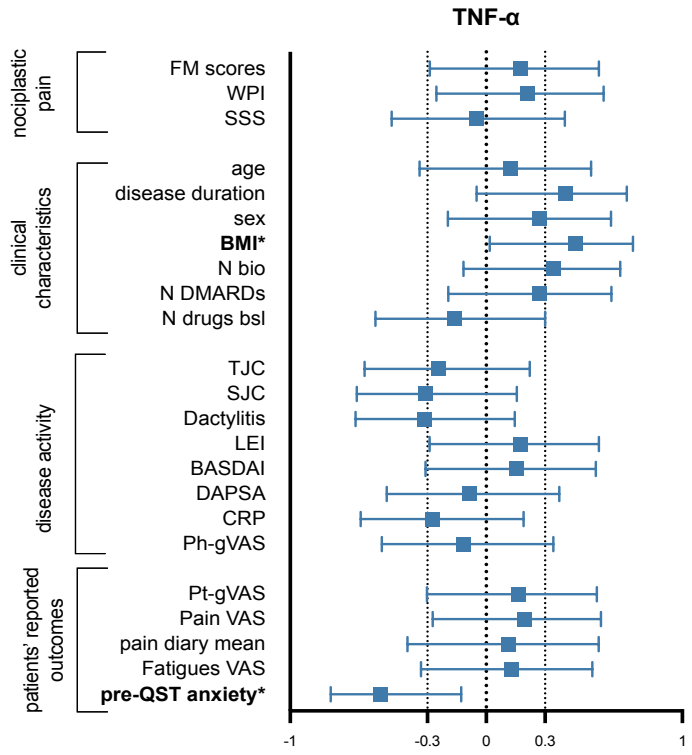
correlated with TNF; regardless of whether the subjects were exposed to biologic inhibitors were included or not (respectively, R = -0.52 p = 0.008, and R = -0.54 p = 0.01).

Figure 6.2.2 Correlations of circulating TNF with pain and clinical variables in subjects with psoriatic arthritis



Legend: The associations between circulating TNF-α levels and different clinical variables are presented. Spearman's correlation coefficients (R-values) along with their respective 95% confidence intervals are displayed for each variable. The graph shows the associations between TNF levels and disease duration (R = 0.485, p = 0.01), previous biologic treatments (R = 0.4, p = 0.05), and various clinical parameters, such as TJC (R = -0.43, p = 0.03), SJC (R = -0.41, p = 0.04), dactylitis (R = -0.4, p = 0.05), and anxiety reported before QST (R = -0.52, p = 0.008).

Figure 6.2.3 Correlations of circulating TNF with clinical variables after exclusion of subjects previously exposed to biologic inhibitors.

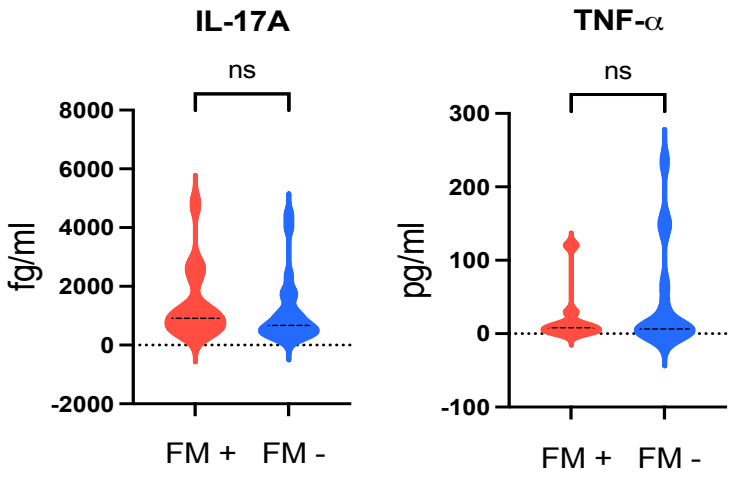


Legend: Correlations between circulating levels of TNF and various clinical variables in participants not recently exposed to TNF treatment. Correlation coefficients (Spearman's R-values) and corresponding 95% confidence interval are illustrated for each variable. BMI resulted positively correlated with the TNF (R = 0.46 p = 0.04), while the negative association with self-reported anxiety before QST were confirmed (R = -0.54 p = 0.01).

6.2.3 *Clinical and neurobiological markers of central sensitisation are not associated with circulating levels of IL-17A and TNF*

To investigate the relationship between IL-17A and TNF circulating levels with nociplastic pain, participants were stratified in 2 groups, with and without FM (FM+ and FM-), based on the 2011 ACR FM criteria. In line with the results showed above, the statistical analysis revealed no significant differences in the levels of both cytokines between individuals with higher (FM+) and lower (FM-) degrees of nociplastic pain (figure 6.2.4). The violin plots before the exclusion of the outliers for both IL-17A and TNF, as well as WPI sub-analysis are available in Appendix 12.

Figure 6.2.4 Differences in IL-17A and TNF levels of in PsA individuals with and without FM



Legend: Two violin plots show the circulating levels of IL-17A and TNF in study participants with fibromyalgia (FM+) and without fibromyalgia (FM-). On the left is represented IL-17A, while on the right TNF. FM+ participants are shown in red and FM- in blue. No significant differences were found for each pro-inflammatory cytokines in both groups. Participants numbers are: IL-17A, 18 FM+ and 25 FM-; TNF, 9 FM+ and 16 FM-.

Given the prior fMRI analysis (Chapter 4), it was important to investigate the potential relationships between circulating IL-17A and TNF, and functional brain connectivity. Initial analysis was focussed on DMN-IC connectivity, as previous findings demonstrated a positive correlation between ESR and the functional connectivity of specific brain areas in RA, i.e., the IPL and the mPFC connectivity with several ICNs[623]. To investigate whether similar relationship between inflammation and brain functional connectivity is present in PsA, the seed-to-voxel analysis using 5 ICA-determined ICNs (DMN, DAN, SLN, MVN, SMN) and 5 left IPL seeds was performed[623]. No significant associations were found in this PsA cohort between IL-17A or TNF with the functional connectivity of those brain regions (seeds representations and correlation graphs are available in Appendix 13). Similarly, no significant associations were found between pressure pain thresholds, temporal summation, and aversion to visual stimuli with the levels of IL-17A and TNF (Appendix 13).

6.3 Relationship between nociplastic pain and anti-inflammatory treatment response

6.3.1 Introduction

Pain is a complex and multifaceted phenomenon influenced by various neurobiological and psychosocial factors, including inflammation. Inflammatory processes, both systemic and peripheral, play a crucial role in the pathogenesis of nociceptive inflammatory pain and sickness behaviour, however, they can also represent a trigger, or a predisposing factor, to develop nociplastic pain. The interplay between inflammatory mediators, immune cells, and neural pathways contributes to the sensitisation of pain pathways and the amplification of pain signals. Immunotherapies, commonly used to treat inflammatory arthritis, have not only demonstrated efficacy in reducing inflammation, but have also shown beneficial effects on pain outcomes and central symptoms such as fatigue and psychological disorders. These observations highlight the potential of immunomodulatory therapies in alleviating both peripheral inflammatory and central symptoms in individuals with arthritis[309,310,493–498,879,880]. This study collected data before and after various immunosuppressive therapies in individuals with an active PsA and, therefore, represents an exciting opportunity to explore the relationship between the immune system and the nociplastic pain. In fact, the unique pain phenotyping in participants, including fMRI and QST, allowed to investigate the mutual effects of immunomodulatory treatments on nociplastic pain and vice versa.

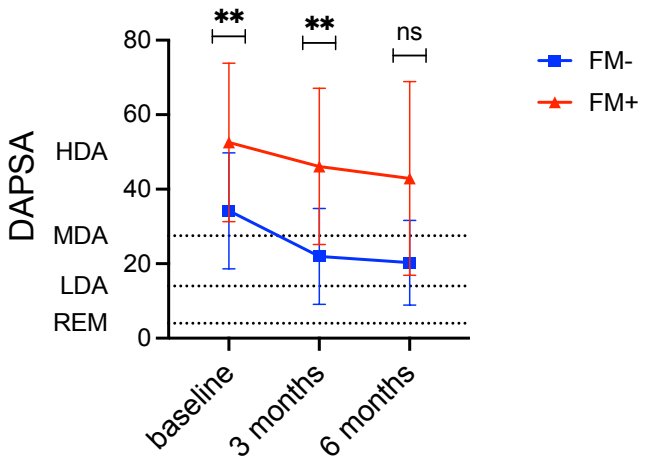
6.3.2 Clinical response after 3 and 6 months of immunosuppressive treatments

Disease activity was assessed at all study visits: baseline, 3 and 6 months. All participants at baseline fell into the HAD or MDA groups according to DAPSA classification (mean ±SD, 44.2 ±25). At 3 months, 37 subjects attended the follow-up visit in person with CRP data available to compute the DAPSA scores. However, 3 participants were excluded from the analysis for various reasons, including treatment not started (mainly for patient choice derived to fear of COVID-19 infection after consent), or drug intolerance; therefore, a total of 34

participants were included in the analysis (DAPSA mean ±SD, 30.4 ±28.6). At 6 months the completed data were available for a total of 30 subjects; for similar reasons, 3 participants were excluded from the analysis (DAPSA scores mean ±SD, 19.7 ±20.9). The clinical and demographic characteristic of participants included in the analysis were compared to those who were either lost at follow-up or excluded due to lack of data (predominately missing CRP for remote visits during the COVID-19 pandemic) at 3 and 6 months . These comparisons are detailed in Appendix 14. Reassuringly, both at the 3 and 6 months follow-ups, there were no significant differences in nociplastic pain determinants, overall clinical characteristics, and disease activity features. Interestingly, at 6 months is noticeable a signal suggesting an increased pain level (pain VAS) by the included participants (Appendix 14). Despite this, which might suggest that the included subjects feel more pain, the similarities between the other clinical features, especially those used to compute the DAPSA scores, are reassuring for the reliability of the findings. An additional significant difference was found in BMI and the 10-days pain diary at 3 months; however, these are not strongly significant and may represent spurious results, unlikely to bias the results in the included population.

In figure 6.3.1 are illustrated the means of DAPSA scores overtime of patients included in the longitudinal analysis, from baseline to 6 months, in participants with and without FM. Notably, mean DAPSA scores decrease in both groups from baseline to 6 months; it is also evident that individuals with FM (FM+, red) present overall higher DAPSA scores not only at baseline (29.3 ±15.4 versus 49.3 ±23 in table 4.2.2), but also at 3 months' (21.95 ±12.9 versus 46.1 ±21) and 6 months' visits (20.3 ±11.4 versus 42.9 ±26). Moreover, there was a significant difference in DAPSA scores between FM+ and FM- resulted significant at baseline and 3 months (adjusted P-values, respectively, 0.006 and 0.004), but not at 6 months.

Figure 6.3.1 Changes in DAPSA scores over time in individuals with Psoriatic Arthritis stratified by Fibromyalgia status



Legend: The graph illustrates the means and SD of Disease Activity in Psoriatic Arthritis (DAPSA) scores at baseline, 3 months, and 6 months for participants classified as Fibromyalgia positive (FM+) in red and Fibromyalgia negative (FM-) in blue. Statistically significant differences in DAPSA scores were observed between the two groups at baseline (P = 0.006) and 3 months (P = 0.004) using multiple t-test (Bonferroni corrected). The number of subjects in each group and time point: baseline, FM+ 29 and FM- 21 participants; 3 months, FM+ 24 and FM- 13 participants; 6 months, FM+ 19 and FM- 11 participants.

6.3.3 Relationship between response to treatment and baseline FM scores

The response to the different treatments was assessed using DAPSA scores at 3 and 6 months. Participants achieving LDA, REM, or at least 50% of reduction of DAPSA scores compared to baseline were classified as responders; while, those not meeting these criteria were considered non responders. At 3 months, 10 subjects met the definition of treatment response, while the remaining 24 participants did not achieve a satisfactory clinical response. The differences in clinical parameters between responders and not responders at 3 months are illustrated in the table 6.3.1. Non responders showed higher nociplastic pain variables than the responders (table 6.3.1). The percentage of non responder meeting the 2011 ACR FM criteria was significantly higher than that of responders (70.6% versus 49.4%). Additionally, total FM scores, as well as WPI and SSS sub-indexes, were significantly higher in non responders. Baseline clinical features associated with FM in the baseline cohort (table 4.2.2), including sex, TJC, LEI scores and PtgVAS, were greater in the non responders

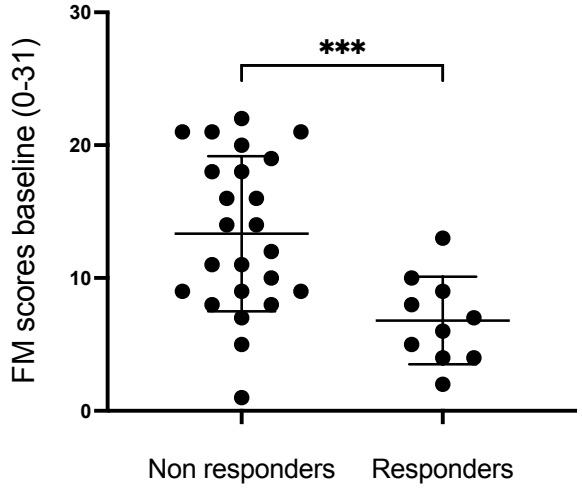
participants. Notably, both TJC and PtgVAS contribute to DAPSA scores, suggesting that higher baseline values in these parameters may inflate DAPSA scores, influencing the reduced treatments response observed.

Table 6.3.1 Baseline clinical features of responders and not responders at 3 months

CLINICAL FEATURES	non responders (24)	responders (10)	P-values
FM criteria (%)	70.6%	29.4%	0.05
FM total score (mean ± SD)	13.3 ±5.8	6.8 ±3.3	0.0003
WPI (mean ± SD)	6.2 ±3.9	2.5 ±1.8	0.004
SSS (mean ± SD)	7.1 ±2.8	4.3 ±2.2	0.005
Clinical characteristics			
Age (mean ± SD)	52.3 ±9.8	44.8 ±11.1	ns
Sex (male%)	20%	58.3%	0.04
Disease duration years (mean ± SD)	6.8 ±5.3	7.9 ±7.9	ns
BMI (mean ± SD)	30.3 ±4.4	28 ±5	ns
Previous biologics (mean ± SD)	0.7 ±1.2	0.6 ±1	ns
N previous DMARDs (mean ± SD)	2 ±1.4	1.6 ±0.8	ns
N medications baseline (mean ± SD)	3.6 ±2.8	2 ±2.5	ns
Disease activity			
TJC 66 (mean ± SD)	23.8 ±13.6	9.1 ±5.5	0.0025
SJC 68 (mean ± SD)	6.5 ±3.1	5.8 ±3.2	ns
Dactylitis (yes/no)	0.7 ±1.2	0.6 ±1.1	ns
LEI score (mean ± SD)	3.2 ±1.8	0.9 ±1.5	0.0005
BASDAI (mean ± SD)	6.6 ±1.4	4.3 ±2.	ns
CRP mg/dl (mean ± SD)	0.5 ±0.6	0.5 ±0.6	ns
Physician gVAS (mean ± SD)	50.8 ±13.4	43.5 ±18.3	ns
Patient reported outcomes			
Patient gVAS (mean ± SD)	61.5 ±16.6	38.5 ±32.9	0.01
Pain VAS (mean ± SD)	34.8 ±22	16.5 ±20.6	ns
10 days pain diary (mean ± SD)	6.1 ±3	1.8 ±2	ns
Fatigue VAS (mean ± SD)	7 ±2.3	4.5 ±2.4	ns
Pre-QST anxiety (mean ± SD)	4.2 ±8.9	6.5 ±9.7	ns

As mentioned earlier, FM scores at baseline were significantly higher in the non responders group compared to the responders to treatments at 3 months (13.3 ±6.8 versus 5.8 ±3.3, P = 0.0003, figure 6.3.2). The odds ratio (OR) for FM+ individuals not responding to treatment at 3 months, compared to the FM- group, was 7.615. These results suggest that the presence of nociplastic pain may influence the treatment response measured with DAPSA, possibly by inflating the TJC and PtgVAS scores. Therefore, nociplastic pain represents an important confounding factor to consider when managing individuals with PsA.

Figure 6.3.2 Baseline fibromyalgia scores in responders and non-responders after 3 months

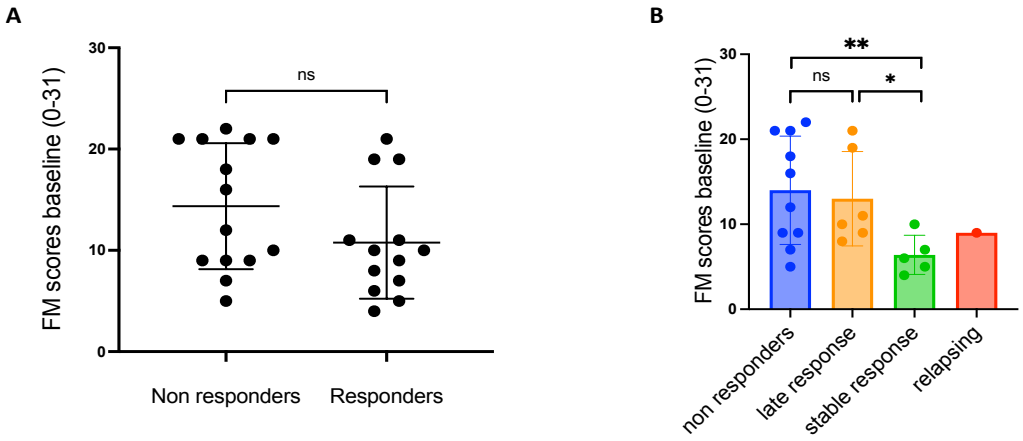


Legend: The graph illustrates the difference in baseline FM scores between participants responding or not to treatment at 3 months as defined by DAPSA criteria. A total of 24 subjects were non responders (70.6%), and 10 subjects responded to treatment (29.4%). FM scores at baseline were significantly higher in the non responders than the responders at 3 months (P = 0.0003).

In contrast, at 6 months the FM scores at baseline were not significantly associated with the response to treatment (figure 6.3.3 A). In fact, regarding the clinical variables the significant differences between responders and non responders at 3 months were not confirmed at the subsequent follow-up (Appendix 15). Among all clinical variables, only high CRP levels at baseline were significantly higher in the non responders (1.4 ±4.2 versus 0.8 ±0.7, P = 0.05). To better understand this finding, the variation across the visits of response to treatments was evaluated in a total of 22 participants with available DAPSA scores at both 3 and 6 months. Accordingly, individuals were classified as “late

responders" when the response was achieved at 6 months, but not at 3 months (n = 6), "stable responders" when the response was sustained over time (n = 5), and "relapsing" when the disease activity increased again at 6 months after an initial good response at 3 months (n = 1) (figure 6.3.3 B). Interestingly, participants with a sustained response from 3 to 6 months of treatment showed significantly lower FM scores at baseline compared to both non responders and subjects with late response at 6 months (P-values 0.0055 and 0.03). No significant difference was found between non responders and late responders in the baseline FM scores. To note, the few individuals responding to treatments at 6 months with high FM scores (study ID: C029, C030, and C046) were all females naïve to biologic medications, and all started the TNF inhibitor adalimumab. The other 3 participants with FM, biologic-naïve, and starting TNF inhibitors (no anti-IL-17A was prescribed) were 2 males (etanercept or adalimumab) who did not responded at 6 months. An additional female starting adalimumab with FM at baseline was loss at follow-up.

Figure 6.3.3 Association of 2011 ACR fibromyalgia criteria with response to treatment at 6 months



Legend: A. The difference in FM scores between responders (N = 13, 48%) and non responders (N = 14, 52%) after 6 months of immunosuppressive treatment. No significant difference was found. B. The bar graph shows the FM scores at baseline of participants classified in: "non responders" at both 3 and 6 months (N = 10, 45.5%); "late responders", presenting treatment response at 6 months only (N = 6, 27%); "stable responders", response at 3 months and sustained at 6 months (N = 5, 23%); "relapsing", responder at 3 months but relapsing at 6 months (N = 1, 4.5%). The baseline FM scores of "stable responders" were significantly lower than FM scores of "non responders" and "late responders" (0.0055 and 0.03, respectively).

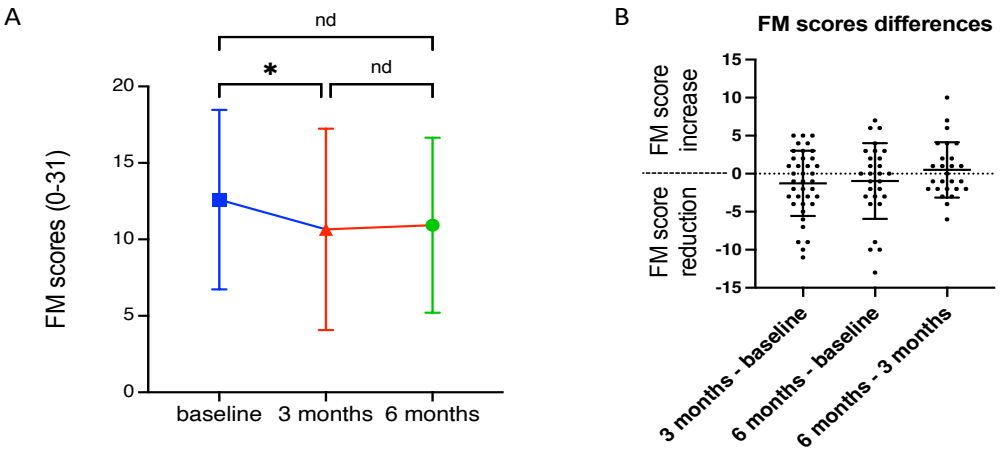
Despite FM scores not being different between responders and not responders at 6 months, the correlation between the FM scores at baseline and the DAPSA

score at 6 months showed a significant result (R = 0.6, P = 0.0005). Similar positive association was also observed between FM scores at baseline and DAPSA scores at 3 months (R = 0.68, P < 0.0001) (Appendix 15).

6.3.4 Variation of FM scores across the time points

To further investigate the relationship between treatment response and nociplastic pain, I extended the analysis to the variation of FM across the different time points. The completeness of FM scores data was greater compared to DAPSA, because the 2011 ACR FM questionnaires were completed remotely during the COVID pandemic emergency. All 50 participants completed the questionnaires at baseline, while at 3 and 6 months complete datasets were available for 38 and 28 subjects, respectively. The means of FM scores at each visit are illustrated in figure 6.3.4 A. On average FM scores tend to improve at 3 months and remain overall stable at 6 months. FDR correct multiple comparison showed a significant difference between the average of FM scores at baseline and 3 months (P = 0.006); however, no significant differences were observed between the other time points (baseline-6 months P = 0.06 and 3 to 6 months P = 0.7). Furthermore, the differences in FM scores for each patient between all visits was also investigated (figure 6.3.4 B). Fluctuation of FM scores in both directions, increasing and reduction, are observed between at all time points. At the end of the study (6 months) the reduction of FM scores was observed in the 50% of participants when compared to the baseline scores, with a range of variation equal to 16. Between baseline and 3 months the 54.1% of participants experienced a reduction of their FM scores (range 16), while between 3 and 6 months a fall of the FM scores was observed in the 52% of participants (range 20). The variation of FM scores between the 3 visits were not associated with the response to treatment at both 3 and 6 months (Appendix 15).

Figure 6.3.4 Changes across different time points of the 2011 ACR fibromyalgia scores



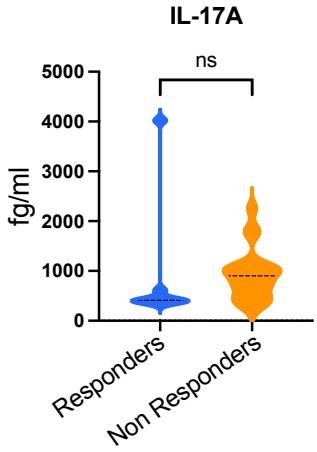
Legend: Variation of the FM scores across the study visits are illustrated in the graphs. A. The average of FM scores at baseline, 3 and 6 months are plotted. The initial reduction of FM scores between baseline and 3 months resulted significant at multiple comparison analysis (FDR adjusted P = 0.006). Differences amongst other time points were not significant (baseline to 6 months, P = 0.06; 3 to 6 months, P = 0.7). Number of participants: baseline, 50; 3 months, 38; 6 months, 28. B. Differences between FM scores across the study visits are illustrated in the scatter plot. Variation in both directions were observed at all time points. Differences between 3 months and baseline showed a 54.1% of FM reduction, 8.1% remained stable at the baseline levels, while the 37.8% of participants presented and increase. Similar percentages were present between baseline and 6 months (reduction 50%, stable 3.8%, increase 46.2%) and 3 and 6 months (reduction 52%, stable 4%, increase 44%).

6.3.5 Response to treatment do not correlate with neurobiological features of FM or circulating pro-inflammatory cytokines

Associations between treatment response and neurobiological features of nociplastic pain, including functional connectivity and QST parameters, were explored. The response to treatment at both 3 and 6 months did not show significant correlation with the functional connectivity (ROI to ROI) between DMN and ICs positively correlated with the baseline FM and SSS scores (Appendix 15). Similarly, no significant differences were found between responders and non responders at both 3 and 6 months in different QST variables, including pressure pain sensitivity, as well as TS and visual sensitivity (Appendix 15). Differences in baseline levels of IL-17A and TNF was also analysed. Levels of IL-17A and TNF did not differ significantly between responders and non responders at both 3 and 6 months. In the figure below are illustrated the difference in IL-17A between responders and non responders at 3 months (figure 6.3.5). Despite

the marked difference in mean values, and most individuals who responded to treatment at 3 months presenting with lower baseline levels of IL-17A compared to the non responders; however, there was no statistical significance. This difference was not confirmed when examining the 6 months treatment response (Appendix 15).

Figure 6.3.5 Baseline circulating levels of IL-17A in responders and non-responders to immunosuppressive treatments at 3 months



Legend: The plot graph illustrates the differences in baseline circulating levels of IL-17A between responders (n=7) and non-responders (n=21) to immunosuppressive treatment after 3 months. While no significant difference was demonstrated, the baseline levels of IL-17A were found to be lower in responders compared to non-responders.

6.4 Discussion

6.4.1 Introduction

The third objective of this thesis was to investigate the relationship between inflammation and nociplastic pain. Despite the mechanisms behind neuro-immune interactions are still unclear, the well neurobiologically characterised inflammatory population with active inflammation in this study represents a unique opportunity to better understand these relationships. Moreover, to my knowledge this is the first study investigating so in PsA. Hence, two measurements of inflammation were chosen to investigate their associations with clinical and neurobiological correlates of nociplastic pain: 1) levels of

circulating pathogenic pro-inflammatory cytokines, known to be directly involved in the disease's pathogenesis; 2) the response to treatment, to attempt to better understand the dynamic relationship between inflammation and pain.

6.4.2 IL-17 and TNF lack of associations with features of central sensitisation

The pro-inflammatory cytokines IL-17A and TNF have been implicated in the modulation of somatosensory and pain pathways, acting both on nociceptor and peripheral nerves activation, as well as in central sensitisation. More specific biomarkers of systemic immune activation were either not available, ESR, or most values were below the normal range according to NHS standard (10 mg/L) with a non-gaussian distribution, CRP. Therefore, I preferred to carry out the analysis on disease-specific cytokines supported by the current evidence on their effect on the nervous system. The findings revealed detectable levels of IL-17A and, to a lesser extent, of TNF in the peripheral blood of PsA participants. Both cytokines were not different in FM+ and FM- subjects, and neither were associated with neurobiological markers of nociplastic pain, i.e., brain functional connectivity and QST. These results pointing towards a lack of associations between circulating levels and clinical measure of nociplastic pain. However, IL-17A showed a positive correlation with WPI, suggesting a potential role in influencing peripheral pain perception, rather than central (FMness and fMRI) in PsA. In fact, IL-17A is a cytokine coordinating the peripheral immune responses and it is usually found in peripheral tissues affected by inflammation (e.g., lesional versus non lesional skin in PsO[881]). TNF levels showed no correlation with neither of the FMness, WPI, or SSS. Despite no interference between the 2 selected cytokines in our cohort (not correlating to each other) was presumed, participants with previous exposure to anti-TNF and anti-IL17A were identified to reduce interference with the correlation analysis. Regarding previous exposure to IL-17 inhibitors, the 3 subjects identified corresponded to the outliers excluded. Despite a lower level of IL-17A is expected after exposure to biologic inhibitors, there are no validate assays to measure the free IL-17A in the presence of secukinumab, however, previous data generated in a PsO cohort showed no significant reduction of the serum levels of IL-17F[882]. Weather the higher levels of IL-17A in individuals exposed to its inhibitors might reflect a lack

of response or a mechanism of tolerance is hard to define in this small cohort. Despite IL-17A levels correspond to skin disease activity in PsO, the relationship with joint inflammation has not been demonstrated. The exclusion of subjects recently exposed to TNF inhibitors did not confirm the initial associations with disease duration, number of previous biologic and measures of disease activity, including TJC, SJC, and dactylitis scores. Similar, lack of associations with clinical variables were observed for IL-17A. The proinflammatory cytokines rarely correlates with parameters of disease activity in inflammatory arthritis, with the exception of active skin PsO[883], therefore, the lack of associations found should not surprise. Interestingly, BMI was positively associated with both IL-17A and TNF. The immune-modulatory properties of the adipose tissue are known to have an impact on IL-17A and TNF pathways[884–888]. Moreover, increased body weight, particularly in visceral adipose tissue, has also been associated with different immune-mediated conditions, including PsA, PsA, and RA, and their risk to develop cardiovascular comorbidities[889–893]. IL-17A was also associated with CRP levels, even if less significantly than BMI. The combination of IL-17A association between WPI and BMI might suggest an interplay between increased body weight (mechanical stress), inflammation, and widespread pain. With the lack of central sensitisation correlates, one might contemplate whether these results imply a prevalent peripheral sensitisation linked to the peripheral immune imbalance. However, these correlations were not very strong, warranting further analysis to confirm these trends and establish the robustness of these conclusions and the involvement of IL-17A in peripheral sensitisation. Interestingly, TNF was also negatively associated with the reported anxiety prior to the QST session. This result is contradiction with the current evidence in primary depression and anxiety disorders, where TNF alongside other pro-inflammatory cytokines was associated with depressive and somatic symptoms[894,895]. In individuals with PsO, IL-17A and TNF resulted associated with both anxiety and depression[896]; however, the same cytokines did not differ between PsA subjects with and without depression, while IL-6 seems to have a predominant effect[897]. Probably a simple question before the QST session [1], could not capture the different phenotypes of depression and anxiety

[1] "What is your current anxiety on a scale of 0-100 where 0 is no anxiety and 100 is the worst anxiety imaginable?"

comprehensively, therefore it is difficult to draw certain conclusions. Interestingly, fatigue and participants' reported pain outcomes were not associated with IL-17A or TNF. These findings should be interpreted with caution due to the underpowered of this study to find significant findings; the several limitations are discussed in section 6.4.4.

6.4.3 Fibromyalgia is associated to early, but not late, response to treatment

Firstly, the effect of FM at baseline on the response to treatment was evaluated. Response to immunosuppressive treatment is expected to reduce inflammation and consequently parameter of disease activity. The DAPSA score, used to measure disease activity in our population, includes several variables, for example, TJC and PtgVAS, which resulted clearly associated with FM scores. This is in line with previous literature[373] and might explain the overall higher disease activity in participants with FM when compared to those without. This difference was evident across all study visits. Despite a reduction in the average of DAPSA in both groups can be observed, FM+ subjects did not reach below the MDA classification as opposed to the FM- participants. In fact, looking at the 3 months response is evident a clear distinction in FM classification between responders and non responders, with higher FM scores at baseline in individuals who did not respond to treatment. In fact, having FM at baseline greatly increases the chances to lack of response to treatments at 3 months (OR: 7.615). This result suggests that nociplastic pain may be related to treatment response in PsA. However, it is also possible that FMness might mask the initial beneficial response to treatment by inflating disease activity parameters and scores, leading to improper treatment changes, also suggested by higher number of previous biologics in the FM+ group in our cohort. Interestingly, at 6 months it was observed that having FM at baseline is not significantly associated with the response to immunosuppressive treatments. Certainly, the study is underpowered to confirm the loss of FM-effect on treatment response, due to data loss secondary to COVID-19 challenges, in addition to the common participants lost at follow-up. Aligned with the hypothesis that FMness can mask the initial response by inflating DAPSA (significantly correlating with FMness at 3 and 6 months), one could venture in the notion that participants with high FM

scores who responded at 6 months might present with a mixed pain phenotype. In fact, if the FM scores remain elevated while a response is observed at 6 months, one could suppose that the treatment response would be driven by components of the DAPSA score that are less associated with FMness. Unfortunately, I could not investigate this due the low numbers of subjects available in this study, which will not grant enough power to support reliable results. Additionally, a lack of association between FM variations across different time points and the response to treatment was also observed. Despite, this may suggest a lack of effect of inflammation on FMness, it is important to remember that DAPSA is influenced by FM and make this result of difficult interpretation. Indeed, it is still unclear how the nervous-immune systems interactions are working, and what directionality these communications have. Nonetheless, if the presence of chronic inflammation has been associated with sensitisation of the nervous system, it is not possible exclude that the inflammation reduction might lead to FMness reduction. Clinical trials in primary FM showed that altered brain functional connectivity can be restored in parallel with pain improvement using different treatments with direct action on the CNS, cognitive behavioral therapy, and acupuncture[373,384,609,611,898]. Interestingly, the changes in FM scores in individuals with OA following knee or hip replacement surgery were associated with pain persistence after procedure. This evidence supports the bottom-up mechanisms of nervous system sensitisation[899]. However, in PsA the inflammation is not always completely controlled, and rarely (less than 20%) a sustained remission is obtained in clinical practice[900]. This might suggest that in inflammatory arthritis the variation in FM scores might be observed on longer follow-up when the inflammation is steadily controlled over time; probably, mainly in subjects with bottom-up mixed pain states. This concept might explain the lack of association between FM scores variation and response to treatment observed. Surely larger cohort might help to address this hypothesis. Lastly, the lack of associations between neurobiological markers of central pain, pro-inflammatory cytokines and response to treatment is line with the results observed in the entire population. Data loss might have reduced the power to address this question. However, it is not possible to exclude that the connectivity between DMN, or IC, with other regions of the brain is associated with disease activity. Additionally, performing a whole brain approach with a

seed-to-voxel analysis including regions deemed more sensitivity to inflammation, IPL and PFC, might further help to understand the interchange between chronic inflammation and brain sensitisation. However, this analysis was not performed in this study. Probably, adding fMRI and QST assessment at follow-up after treatment in future studies would help to shed a light on the effect of immunosuppressive treatments on neurobiological correlates of nociplastic pain in PsA.

6.4.4 Limitations and future directions

There are several limitations that need to be carefully considered.

Firstly, the circulating levels of IL-17A and TNF, while informative, may not fully represent the complex processes occurring in peripheral areas such as the synovium and tendons. These peripheral sites play a significant role in the pathogenesis of PsA, and tissue inflammation not necessarily correlates with the systemic inflammation[883]. Furthermore, the sensitivity and specificity of cytokine assays in clinical practice are suboptimal for diagnosing and monitoring disease activity. The fluctuations of cytokine levels over time, coupled with the temporal gap between cytokine measurement QST (1 hour) and MRI scans (3-4 hours), may introduce potential biases. Indeed, this temporal misalignment could influence the correlation between cytokines and study variables. Collecting blood samples immediately before and/or after scans could mitigate these and increase the sensitivity of findings. The distribution of peripheral inflammation variables might have also impacted the results. For both evaluation of circulating markers of inflammation and assessment of treatment response, the heterogeneity of the patient recruited is important to consider. Outlier removal improved the distribution, although further analysis is warranted to explore the relationship between outliers probably with larger cohorts and with less heterogenic population. Subjects' prior exposure to different biologics could have influenced cytokine levels, potentially confounding the interpretation of results, despite the attempt of mitigation excluding participants with recent exposure to TNF and IL-17A inhibitors. The lack of associations between the cytokines and clinical features suggest that broader approaches should be considered in future studies. Probably the cytokines milieu, rather than a single

cytokine, would have a greater impact on disease manifestations and, consequently on pain phenotype. Integrating more advanced analytical approaches, for example cluster analysis or machine learning models, would enable to incorporate different variables, including multiple cytokines signature in combinations with clinical, fMRI, and QST determinants, facilitating a deeper investigations on this complex phenomenon. The integration of advanced immunological technologies would also allow to explore the immune phenotype at a deeper level than proteomics; for example, exploring transcriptomic and epigenetic profiles of key cellular types, e.g. IL-17 producing cells, could provide mechanistic insights into the role of these cells in pain phenotypes. Additionally, sampling peripheral synovial tissues could further address the question whether peripheral sensitisation is a key feature in PsA, linking peripheral tissue inflammation to altered brain processing.

Despite the efforts to address patient heterogeneity, limitations persist. Notably, the study lacked data on the time interval between stopping previous treatments and starting the study interventions, potentially affecting outcomes. The different mechanism of action of the treatments started at baseline can confound the interpretation of the treatment response and their impact on nociplastic pain determinants. Furthermore, the potential impact of concomitant DMARDs, aside from the newly initiated one, was not taken into consideration. This approach aligns with the common practice in clinical trials on inflammatory arthritis, wherein participants are commonly allowed to maintain any DMARD with a stable regimen throughout the study. Given the exploratory and cross-sectional nature of this study and recognizing that the primary objective did not involve assessing the efficacy of the initiated treatment, this aspect was not fully accounted for. It is essential to recognize that this may have introduced variability in the response to treatment and pro-inflammatory cytokines results, and as such, it should be acknowledged in future studies for a more comprehensive understanding of different mechanisms of pain in inflammatory arthritis. Additionally, the selected disease activity index to measure disease activity, DAPSA, might overlook at characteristic clinical features of PsA, for example enthesitis, dactylitis, and skin inflammation, while it can be influenced by clinical features associated to FM, e.g., TJC, pain and global VAS. Additionally, the low participant numbers, partly attributed to the

impact of the COVID-19 pandemic, limit the conclusiveness of the findings and the interpretations. With larger cohorts it would be possible to explore the impact of different disease domains on pain phenotype in PsA. Given the potential significance of FM scores as markers of pain and sensitisation in PsA, their integration into larger clinical trials could improve the comprehension of pain phenotype in this condition. Moreover, the impact of cytokine-targeted therapies on FM scores could provide insights into the effectiveness of these interventions in alleviating pain and nervous system sensitisation. Incorporating FM assessment into future clinical studies and trials will also allow comparisons across different studies and could enhance our understanding of the neurobiological mechanisms of CNS sensitisation in inflammatory arthritis. Lastly, the use of advanced neuroimaging techniques, expanding the understanding of brain connectivity, but also biochemical features with spectroscopy, or volumetric changes could reveal novel insights into the neural mechanisms underlying pain phenotype and treatment outcomes leading to the development of personalized treatment strategies.

Despite interesting interpretations can be inferred, the aforementioned limitations demand caution in the understanding of the results. These limitations also restricted further analysis in our population. The challenges in measuring peripheral inflammation in PsA, the patient heterogeneity, and potential biases associated emphasise the need for comprehensive and prospective investigations with larger sample sizes and robust methodologies. Addressing these limitations will be crucial to advancing our understanding the role of neuro-immune interactions in PsA pain phenotypes.

6.4.5 Conclusions

This study provides insight into the complex interplay of nociplastic pain and inflammation in PsA. Our findings suggest that a limited "systemic" inflammation in PsA may be insufficient to induce the central sensitisation in the brain, pointing towards a pre-eminence of peripheral over central mechanisms of sensitisation. The lack of associations between circulating pro-inflammatory cytokines selected and neurobiological correlates of nociplastic pain, as well the involvement of IL-17A and TNF in WPI, BMI, CRP and/or anxiety, support this

interpretation. However, the complexities of cytokine interactions and the current limitations of cytokine assays warrant further exploration to comprehensively elucidate their roles in PsA's nociplastic pain landscape. Nonetheless, these findings offer valuable insights into the potential role of nociplastic pain as a pivotal confounding element in evaluating treatment outcomes, which could potentially serve as a prognostic factor in PsA. In fact, baseline nociplastic pain relates to treatment response at 3 months. Indeed, the presence of FM in PsA is a key clinical challenge for rheumatologists nowadays. Elevated DAPSA scores in the FM+ group underscore heightened disease activity compared to the FM- group, accentuating the necessity of considering FM influence on disease activity. Despite the evident limitations, the data at 6 months propose to persist with immunosuppressive treatments in non responders with high FMness, because some individuals might responder at later stage. The influence on initial response assessment by concomitant FM and the presence of a mixed bottom-up sensitisation might contribute to this phenomenon. Indeed, robust research studies are needed to further understand nociplastic pain's role in PsA management and treatment.

In essence, this study highlights the intricate relationship between cytokines, pain phenotype, and disease characteristics in PsA, offering insight into potential avenues for future exploration. Despite acknowledged limitations and population heterogeneity, further investigations could utilise cutting-edge immune phenotyping methods and advanced neuroimaging techniques with integrative analytical approaches to unveil the dynamics between inflammation, central brain changes, and pain perception in PsA. Deciphering the interplay between chronic inflammation, nociplastic pain, and treatment outcomes would deepen our comprehension of the disease pathology and lead to optimisation of PsA therapeutic management and personalised interventions.

Chapter 7 Conclusions

This study delves into the intricate interplay between chronic inflammation, pain phenotype, and neurobiology in PsA. By investigating the relationships between nociplastic pain, immune markers, and treatment response, the study offers valuable insights into its pain phenotype.

Firstly, it's crucial to acknowledge the multifaceted nature of PsA, characterised by chronic inflammation and tissue damage. Despite significant advancements in understanding the immunopathogenesis and the development of targeted immunotherapies, persistent pain remains a substantial issue. Chronic pain is a symptom which can occur on its own, or in combination with different chronic conditions; with its adverse impact on quality of life and mortality, demands thorough investigations. While pain in PsA is classically rooted in nociceptive mechanisms, the clinical persistence of pain shifts the paradigm towards the emerging concept of nociplastic pain to explain this phenomenon. This hypothesis is supported by evidence suggesting the common coexistence of FM, the prototype of nociplastic pain, and PsA. While neurobiological features of nociplastic pain have been observed in individuals with RA, similar evidence is still lacking in PsA.

Focusing on nociplastic pain, this study explores the complexities of pain mechanisms in PsA. The investigation uncovers a high prevalence of FM among participants, compared to the available data. FM complicates disease assessment, mirroring high disease activity symptoms. The presence of FM correlates with various clinical parameters, including joint tenderness, enthesitis scores, global pain, and fatigue measures. Notably, disease activity scores like DAPSA and BASDAI are elevated in the presence of FM, despite no significant differences in objective inflammation markers. Altogether, the clinical data confirms that FM can influence clinical judgment and treatment decisions by mimicking high disease activity states.

Through the utilization of fMRI and QST, this study firstly revealed the presence of nociplastic neurobiological features in individuals with PsA. Subjects with a high degree of nociplastic pain, measured with the 2011 ACR FM criteria, exhibit heightened pressure sensitivity and altered functional connectivity patterns

within key pain processing areas, such as the DMN and IC. In details, QST analysis demonstrates that PsA individuals with high FMness have an increased sensitivity to pressure stimuli in areas directly affected by PsA, reflecting peripheral sensitisation mechanisms at play. However, QST does not reveal a generalised hypersensitivity in the population, therefore, it cannot confirm a prevalence of central sensitisation in the recruited population. Gender disparities are observed, suggesting that male participants primarily drive the main findings, while females present a generalised hypersensitivity independently from the degree of FMness. Moreover, functional connectivity changes between the insula and specific brain areas, including the thalamus, PHC, dlPFC, and associative visual areas, are linked to FMness, WPI, and SSS. These regions play roles in sensory processing and cognitive functions, often implicated in pain perception. This connectivity alteration aligns with FM and RA studies, albeit less strongly. These findings substantiate the presence of neurobiological features associated with nociplastic also in PsA, in addition to FM and RA; therefore, they support the existence of a generalised neurobiological marker that may be potentially applicable across various chronic pain cohorts. However, upon excluding participants taking pain modulating drugs, a significant proportion of individuals with FM were also excluded. Consequently, a higher degree of nociplastic pain was associated with an increased connectivity between the DMN and the Angular Gyrus. This results also imply the exhistence of a distinctive neurobiological signature for nociplastic pain in PsA, potentially overlapping with that in other inflammatory arthritis. Similar connectivity patterns associated with inflammation in RA suggest a sensitisation of this key “inflammatory hub” in the brains of individuals with an active inflammatory disease. The interplay between inflammation and nociplastic pain mechanisms is further investigated by correlating markers of inflammation in this PsA cohort, namely circulating pro-inflammatory cytokines and response to immunomodulatory treatments at follow-ups. IL-17A and TNF, implicated in central and peripheral sensitisation, do not show significant associations with 2011 FM scores, fMRI, and QST. This suggests a lack of effect of these cytokines in central sensitisation in PsA. However, IL-17A does show a correlation with widespread pain perception. BMI is found to be positively associated with both IL-17A and TNF levels, indicating a potential relationship between increased body weight, inflammation, and pain in

PsA. However, these data need to be confirmed in larger cohorts. The impact of FM on treatment response is also explored. The study participants with FM at baseline are less likely to respond to treatment at 3 months. However, this effect diminishes at the 6-month visit. This suggests that FM may initially mask the treatment response by inflating disease activity measures. Evaluating the response to treatment in longer periods when FM is present may prevent unnecessary treatment changes.

It is essential to acknowledge that this comprehensive analysis presents several limitations. The cross-sectional design, small sample size, and inherent biases of self-report measures necessitate cautious interpretation. Moreover, the impact of psychological factors on QST and technical challenges in functional neuroimaging results warrant caution in interpreting these results. Another significant limitation was that the study was conducted during the global COVID-19 pandemic. This had a negative impact not only on the recruitment and quality of data, especially for remote follow-up visits, but also on participants' day-to-day lives, potentially influencing their pain. The general uncertainties, anxiety related to immune suppression and infection risk, reduced mobility due to shielding restrictions, as well as the use of personal protective equipment during in-person visits, may have had a detrimental effect on the reported pain. Longitudinal studies in selected subjects would strengthen these findings. Furthermore, investigations involving homogeneous populations in terms of disease duration and treatments, with assessments of neurobiological changes post-treatment, will contribute to a better understanding the complex neuro-immune interactions. While this study did not explicitly investigate the influence of age within this cohort, it is a factor that merits careful consideration in future research, given its recognised impact on diverse physiological and neurobiological processes. Therefore, the study underscores the need for comparative analyses with control populations to better contextualise the findings within different chronic pain cohorts.

Additionally, the findings of this study support the evaluation of nociplastic pain in chronic conditions more generally. Despite each individual is unique, similar functional connectivity findings can be found across different conditions in presence of nociplastic pain features. In fact, pain as a symptom *per se* may

impact not only individuals with arthritis, but also the general population. Careful attention should be given especially to those individuals with multiple long-term conditions which may facilitate nervous system sensitisation through various mechanisms, not solely inflammation. Often, mixed pain states are more challenging to treat and may result in inappropriate management. Chronic pain should be carefully characterised in real-life settings to improve patients' management and quality of life. Incorporating specific pain assessments and questionnaires to detect the presence of nociplastic or neuropathic pain states might in research and clinical setting may aid in understanding the phenomenon across different conditions, in addition to inflammatory arthritis. Larger national and international cohorts will help to address and better understand the global pandemic of pain. Lastly, the identification of shared and disease-specific neurobiological features holds promise for establishing novel diagnostic tool for nociplastic pain in the future, while also supporting investigations into the effectiveness of interventions across various chronic pain conditions. Although this study was not interventional, I hope that it will contribute to future exploration on advanced interventions, including non-invasive brain modulation methodologies, and for monitoring the effectiveness of pharmacological and non-pharmacological approaches (such as exercise, psychological treatments, and holistic approaches) in both inflammatory arthritis and other chronic pain conditions.

In conclusion, this study's novel insights into the neurobiological phenotype of pain in PsA help to understand this debilitating symptom in a complex disease. The interplay between central and peripheral sensitisation mechanisms demands further exploration through larger-scale, longitudinal investigations. However, these findings hold the promise of enhancing our comprehension of PsA pain phenotype, and consequently pave the way for personalized interventions that can alleviate the burdensome impact of pain on PsA patients' lives.

Appendices

Appendix 1

WoSRES
West of Scotland Research Ethics Service

NHS
Greater Glasgow and Clyde

West of Scotland REC 3
West of Scotland Research Ethics Service
West Glasgow Ambulatory Care Hospital
Dalnair Street
Glasgow
G3 8SJ

Date 14 March 2019

Direct line 0141 232 1809
E-mail WoSREC3@ggc.scot.nhs.uk

Dr Neil Basu
Senior Clinical Lecturer of Rheumatology
University of Glasgow
Sir Graeme Davies Building
Glasgow
G12 8TA

Dear Dr Basu

Study title:	**Characterising the Centralised Pain Phenotype in Chronic Rheumatic Disease - A Stride Towards Personalised Analgesia**
REC reference:	**19/WS/0033**
Protocol number:	**1.0**
IRAS project ID:	**257990**

Thank you for your letter of 11 March 2019, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information was considered in correspondence by a Sub-Committee of the REC. A list of the Sub-Committee members is attached.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to make a request to postpone publication, please contact hra.studyregistration@nhs.net outlining the reasons for your request.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

The REC favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements. Each NHS organisation must confirm through the signing of agreements and/or other documents that it has given permission for the research to proceed (except where explicitly specified otherwise).

Guidance on applying for HRA and HCRW Approval (England and Wales)/ NHS permission for research is available in the Integrated Research Application System, at www.hra.nhs.uk or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of management permissions from host organisations

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database within 6 weeks of recruitment of the first participant (for medical device studies, within the timeline determined by the current registration and publication trees).

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to request a deferral for study registration within the required timeframe, they should contact hra.studyregistration@nhs.net. The expectation is that all clinical trials will be registered, however, in exceptional circumstances non registration may be permissible with prior agreement from the HRA. Guidance on where to register is provided on the HRA website.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Covering letter on headed paper [Cover letter for REC response]	1.0	11 March 2019
IRAS Application Form [IRAS_Form_01022019]		01 February 2019
Letter from funder [AWARD]		19 October 2018
Other [Telephone script]		11 March 2019
Participant consent form [Consent version2]	2.0	11 March 2019
Participant information sheet (PIS) [PIS_version2]	2.0	11 March 2019
Referee's report or other scientific critique report [PEER REVIEW]		27 June 2018
Research protocol or project proposal [PROTOCOL]	1.0	31 January 2019
Sample diary card/patient card [DIARY]	1.0	31 January 2019
Summary CV for Chief Investigator (CI) [CV]	1	31 January 2019
Validated questionnaire [Questionnaire version2]	2.0	11 March 2019

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website: http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

HRA Training

We are pleased to welcome researchers and R&D staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

19/WS/0033	**Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project.

Yours sincerely

for
Mrs Rosie Rutherford
Chair

Enclosures: List of names and professions of members who were present at the meeting and those who submitted written comments

"After ethical review – guicance for researchers"

Copy to: Dr Maureen Travers, NHS Greater Glasgow & Clyde

Lead Nation - Scotland: nhsg.NRSPCC@nhs.net

West of Scotland REC 3

Attendance at Sub-Committee of the REC meeting

Committee Members:

Name	*Profession*	*Present*	*Notes*
Mr Daniel Boyle	Scriptwriter	Yes	
Mrs Lorna Hammond	Senior Clinical Pharmacist	Yes	
Mrs Rosie Rutherford	Volunteer - Lay Plus Member and Chair	Yes	

Also in attendance:

Name	*Position (or reason for attending)*
Mrs Sharon Macgregor	REC Manager

Appendix 2

Characterising the Centralised Pain Phenotype in Chronic Rheumatic Disease - A Stride Towards Personalised Analgesia

You are being invited to take part in a research study. Before you decide whether or not to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish. Contact us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Introduction

Psoriatic arthritis is a disease of the immune system where patients experience significant pain. Unfortunately, it is common for pain to persist even after adequate treatment of the immune system. One possible explanation for this situation is that the pain in psoriatic arthritis originates from more than one source. In other disorders, the nervous system (brain and nerves) are known to generate pain and we propose that some of the pain experienced by psoriatic arthritis patients also comes from the nervous system.

What is the purpose of the study?

We are aiming to investigate the relationship between psoriatic arthritis pain and the nervous system by undertaking a comprehensive set of relevant measurements. We will then use this information to develop a simple questionnaire tool which may be used by doctors and patients in clinic to quantify this type of pain and inform treatment decisions.

Why have I been asked to take part?

You have been asked to take part as your doctors believe you have psoriatic arthritis and are currently experiencing pain.

Do I have to take part?

No, it is up to you to decide whether or not to take part. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part, you are still free to withdraw at any time and without giving a reason. Deciding not

to take part or withdrawing from the study will not affect the healthcare that you receive, or your legal rights.

In the unlikely event that you lose the capacity to consent to medical research during the course of the study, the research team would not collect any more of your details and you would be withdrawn from the study. Information that has already been collected will be retained and used in the study with your permission.

What will happen if I take part?

If you agree to take part, you will be invited to discuss the details of the study with a member of the research team who will make sure you understand everything. You will then be asked to sign a consent form. With your permission we will review your clinical records including medical history to confirm your eligibility for the study. We will also ask you some questions about your health.

If you are eligible to take part, the research study involves participants undertaking a a) clinical assessment, b) questionnaire, c) quantitative sensory testing, d) MRI scan e) optional blood samples f) optional ultrasound scan of your joints:

a) Clinical assessment – involves standard clinical questions and examination of your joints followed by collection of a blood sample which would be routinely taken as part of your standard care

b) Questionnaire – involves completing a number questions designed to evaluate your pain symptoms and also known risk factors of pain. This can be completed electronically using a hospital computer or at home. Paper versions will also be available.

c) Quantitative sensory testing – involves a number of procedures designed to measure your sensitivity to pain by presenting you with different types and intensities of stimuli (pressure and visual) and asking you to rate the stimuli on a variety of scales. They are designed to measure your individual sensitivity to these stimuli. Below is a brief description of the tests. You will receive detailed instructions prior to each and be given a chance to ask questions. You may also ask the study team member to provide more detail on these tests during the consenting process.

1. Pressure pain threshold – Using a pressure probe and a specialist semi-automatic

device, we will apply various pressures to your thumbnail beds, shoulders, hips, wrists and hands.

2. Cuff pressure – We will place a blood pressure cuff around your lower leg and inflate it at various pressures
3. Temporal summation – A series of stimuli will be applied to your forearm or hand in quick succession using a non-invasive pinprick stimulator
4. Visual sensitivity – You will sit in a dark room and view on a laptop a series of flashing checkerboard patterns of different levels of brightness and intensity.

All of these tests will be stopped as soon as you feel uncomfortable.

d) Magnetic Resonance Imaging (MRI) scan – involves monitoring areas of your brain that are involved in thinking about and processing pain. The scanner records information about your brain's physical structure and activity that we will analyse. The MRI procedure involves lying on a padded table that slides into a hollow machine. You will lie in the scanner with a coil around your head. We will ask you to keep your head still so the magnet can obtain clear images of your head and the blood flow in your brain. During the scan, we will take images of your brain function - some while you are resting and others while we are assessing your brain's responses to pain by replicating some of the pressure pain quantitative sensory testing procedures described above.

e) Optional blood tests – involves taking further tubes of blood which will be stored securely for future unspecified ethically approved research at the University of Glasgow, under the oversight of the NHS Greater Glasgow & Clyde Biorepository. These will include possible genetic studies.

f) Optional ultrasound scan of your joints – involves a scan of the joint of your hands and feet plus 1 or 2 joints that are particularly symptomatic at the time of the visit. Morevover, we will assess the inflammation at where the tendons attach to the bone (enthesitis). You will be asked to repeat the scan after 6 months to evaluate the clinical response to the treatment.

All of these procedures will take place over the course of half a day. You will then be asked to undertake the clinical assessments and questionnaires again at 3 and 6 months. Were possible, these visits will be coordinated to take place at the same time as your routine clinic appointments.

Finally, you will be asked to complete a 10 day daily diary which rates the severity of your pain over the previous 24hrs. This diary will be undertaken immediately following your first

visit and just prior to your subsequent visits (we will contact you to remind you by text/phone)

What are the possible benefits of taking part?

You may or may not get a direct benefit from taking part in this study, however by participating in clinical research you may provide benefit to the wider population who currently live with musculoskeletal diseases.

Rarely a new medical condition is found during these assessments. In the event that a new medical problem is identified following the assessments, you will be appropriately referred and treated. There is no payment for participation, although reimbursement for total travel costs will be offered.

What are the possible disadvantages and risks of taking part?

Magnetic resonance imaging (MRI) is a routine medical imaging test and is generally safe. It does not involve the use of any radiation. People with metal implants or devices including pacemakers cannot undergo MRI scanning and so will not be able to participate in the study. People who suffer from claustrophobia may find the process of an MRI scan uncomfortable or distressing and so it is worth considering whether you would wish to take part if you believe this may affect you. If you would like to take part but are concerned about claustrophobia, we may be able to provide a small dose of medication to help alleviate anxiety.

Quantitative sensory testing is a widely employed research tool. These tests may result in temporary discomfort, skin reddening and/or indentation marks, and, in rare cases, minor bruising, at the body areas tested. The discomfort usually resolves within minutes of test completion. In some cases, discomfort, skin/muscle tenderness, and skin marks may last from a couple hours to a day. Risks of cuff pain assessment include mild transient bruising associated with inflation of the cuff which is estimated to occur in less than 5% of cases. The possible discomfort of the visual stimulation task may be headache or nausea while or after

performing this task. The visual tasks may also result in transient discomfort that usually resolves in a few seconds to a couple minutes after the test stops. We've designed these experiments to follow strict safety standards and to be as brief as possible. You may stop these tests at any time by saying "Stop" to the operator or, if you are in the MRI, by squeezing a call ball that will notify us in the control room.

What if there is a problem?

If you have a concern about any aspect of this study, please contact Dr Basu.

In the unlikely event that something goes wrong and you are harmed during the research and this is due to someone's negligence then you may have grounds for a legal action for compensation against NHS Greater Glasgow & Clyde but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you (if appropriate).

What happens when the study is finished?

Your involvement with the study will be completed after your 6 month visit. Following this the data collected will be analysed. This will include transfer of anonymized data to our research collaborators in the USA where comparisons will be made with data from other research volunteers who do not have psoriatic arthritis.

If you agree, anonymised data and samples may be used for future studies in the UK and overseas, including commercial partners. You will not be identifiable in these data.

Will my taking part in the study be kept confidential?

All of the information collected during the course of the research will be kept confidential and there are strict laws that safeguard your privacy at every stage. The research team will require access to your medical records in order to carry out this research. Necessary data required for the study may be copied and stored in a secure password encrypted storage facility within the University of Glasgow computer network. This data will be anonymised. Storage in this network will allow confidential analysis. Any information held on computers will be securely stored and access granted to the research team only.

To ensure that the study is being run correctly, we will ask your consent for responsible representatives from the Sponsor, NHS Greater Glasgow and Clyde, to access your medical records and data collected during the study, where it is relevant to you taking part in this research. The Sponsor is responsible for overall management of the study and providing insurance and indemnity, the University of Glasgow will provide insurance in relation to the study design.

NHS Greater Glasgow and Clyde is the sponsor for this study based in the United Kingdom. We will be using information from you and your medical records in order to undertake this study and will act as the data controller for this study. This means that we are responsible

for looking after your information and using it properly. NHS Greater Glasgow and Clyde will keep identifiable information about you for 5 years after the study has finished.

Your rights to access, change or move your information are limited, as we need to manage your information in specific ways in order for the research to be reliable and accurate. If you withdraw from the study, we will keep the information about you that we have already obtained. To safeguard your rights, we will use the minimum personally-identifiable information possible.

You can find out more about how we use your information at https://www.hra.nhs.uk/information-about-patients of by contacting Dr Basu.

What will happen to the results of the study?

The results of this study will be written up as an academic research project and will be submitted for publication within a medical journal. It is possible that the results may also be presented at academic meetings or conferences. You will not be identifiable in any published results.

If you would like a summary of the results of the study, this may be provided via telephone or appointment. This can be arranged by contacting Dr Neil Basu (contact details are at the end of the information sheet).

Who is organising the research?

This study has been sponsored by NHS Greater Glasgow & Clyde.

Who has reviewed the study?

The study proposal has been reviewed by the NHS West of Scotland Research Ethics Committee. All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee. A favourable ethical opinion has been obtained from West of Scotland REC. NHS management approval has also been obtained.

If you have further questions about the study, please contact:

Dr Neil Basu, Rheumatology Consultant, NHS Greater Glasgow & Clyde on 0141 3301718 or email neil.basu@glasgow.ac.uk

If you would like to discuss this study with someone independent of the study, please contact: Prof Jonathan Cavanagh, Consultant, Queen Elizabeth University Hospital on 0141 330 7769 or email jonathan.cavanagh@glasgow.ac.uk.

If you wish to make a complaint about the study you can do this through your local complaints procedure

Phone: 0141 201 4500 (for complaints only)

E-mail: complaints@ggc.scot.nhs.uk

Thank you for taking the time to read this information sheet.

Appendix 3

Participant Consent Form

Study title: Characterising the Centralised Pain Phenotype in Chronic Rheumatic Disease - A Stride Towards Personalised Analgesia

Name of lead researcher: Dr Neil Basu

Participant ID:	**Please initial box**
1. I confirm that I have read and understood the information sheet dated _____ (version ____) for the above study. I have had the opportunity to consider the information, ask any questions and have these answered to my satisfaction	☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.	☐
3. I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from the Sponsor, research team, or regulatory authorities, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.	☐
4. I agree to the research team accessing my electronic and paper health records.	☐
5 I agree to my anonymised data being used for future ethically approved studies, including in countries other than the UK.	☐
6. I understand and agree that my optional blood samples (including genetics) will be stored securely at the University of Glasgow, under the oversight of the NHS Greater Glasgow & Clyde Biorepository, for use in this study or in future studies, and that some samples or data may be sent anonymously to other researchers, including ones outside the UK.	☐
7. I understand and agree that the optional ultrasound scan on selected areas will be performed during the study, at the baseline and at 6 months, and that all the scans are an optional procedure and I'm free to opt-out at any time.	☐ yes ☐ no
8. I agree to my health care providers being informed of my participation in this study and agree to their providing health information to the research team if necessary.	☐
9. I agree that my telephone number and email address will be collected and used by the authorized research team to contact me. The access to this information will be password controlled with personal access rights.	☐
10. I agree to take part in the above study.	☐

Name of Participant	Date	Signature
_____	_____	_____
Name of Person taking consent	Date	Signature
_____	_____	_____

Original (x1) to be retained in site file. Copy (x1) to be included in patient notes.
Copy (x1) to be retained by participant

Appendix 4

Participant ID | | | | | | |

Date | d | d | m | o | n | y | y | y | y |

Please specify the satisfied criteria in the list below

a) History of Psoriasis (unless a present) Yes ☐ No ☐

b) Family history of Psoriasis (unless a and b) Yes ☐ No ☐

c) Dactylitis Yes ☐ No ☐

d) Juxta-articular new bone formation Yes ☐ No ☐

e) Rheumatoid factor negativity Yes ☐ No ☐

f) Typical nail dystrophy Yes ☐ No ☐

B6. Has the patient ever had psoriatic skin involvement? Yes ☐ No ☐

*If **yes**, please indicate the date of symptom onset (dd-mm-yy)* ☐☐ ☐☐ ☐☐

B7. Change or indication to modify in immunomodulatory therapy since the last medical review, because of active disease

*If **no**, please exclude patient to the study* Yes ☐ No ☐

*If **yes**, please name the new drug the patient is about to start* _____

B8. Severe physical impairment Yes ☐ No ☐

e.g. blindness, deafness, paraplegia are exclusion criteria.

*If **yes**, please specify* _____

B9. Medical or psychiatric conditions that in the judgment of study personnel would preclude participation in this study Yes ☐ No ☐

e.g. malignancy, psychosis, suicidal ideation may be exclusion criteria.

*If **yes**, please exclude patient to the study* _____

B10. Contraindications to MRI Yes ☐ No ☐

e.g. severe claustrophobia

*If **yes**, eventually exclude patient to the study* _____

Participant ID ☐☐☐☐☐☐

Date ☐☐☐☐☐☐☐☐

B11. Height ☐☐☐

Body weight ☐☐☐

BMI ☐☐☐

*If **>40**, participant ineligible*

B12. Diagnosis of peripheral neuropathy Yes ☐ No ☐

*If **yes**, participant ineligible* _____

B13. History of visual stimulus evoked migraine or epilepsy Yes ☐ No ☐

*If **yes**, participant ineligible* _____

B14. Current, recent, or habitual use of artificial nails Yes ☐ No ☐

*If **yes**, please specify* _____

Participant confirmed eligible Yes ☐ No ☐

Researcher signature _____

Section C – Clinical Data

C1. Sex Female ☐ Male ☐

*If **female, is the** participant pregnant or breastfeeding?* Yes ☐ No ☐

*If **yes**, please exclude patient to the study*

C2. Smoking habit Yes ☐ No ☐ Ex ☐

e.g. blindness, deafness, paraplegia are exclusion criteria.

*If **yes** or **ex**, please specify start and/or cessation dates (yyyy)* ***start*** ☐☐☐☐ ***quit*** ☐☐☐☐

C3. Alcohol Intake (unit/week) ☐☐

*An **alcohol unit** is equivalent to half pint of beer, half glass of wine, or 25ml of whiskey.*

Participant ID | | | | | | |

Date | d | d | m | o | n | y | y | y | y |

C4. Significant co-morbidities Yes ☐ No ☐

*If **yes**, please specify in the table below*

Disease	***Date of onset***

C5. Previous DMARDs, Biologics and Steroids Yes ☐ No ☐

Medications that participant are now NO LONGER TAKING.

*If, **yes** please specify the name of the drug(s)*

Generic name of drug and dose (if possible)	***Date started***	***Date stopped***

Participant ID | | | | | | |

Date | d | d | m | o | n | y | y | y | y |

C6. Current pharmacological therapies (at date of review) Yes ☐ No ☐

*If **yes**, please specify in the table below*

Generic name of drug	***Dose***	***Date started***

Researcher signature _____

Participant ID | | | | | | |

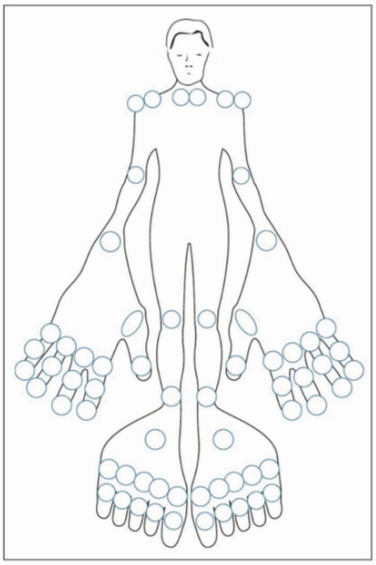
Date | d | d | m | o | n | y | y | y | y |

CENTAUR CRF - BASELINE

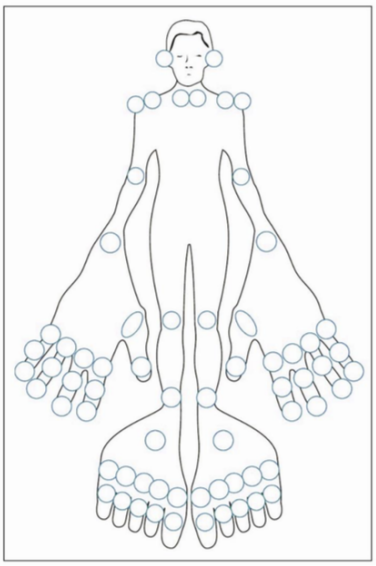
Section D - Clinical assessment

D1. Joint count

Tender Joints Count (0-68)



Swollen Joints Count (0-66)



Participant ID | | | | | | |

Date | d | d | m | o | n | y | y | y | y |

D2. Dactylitis Yes ☐ No ☐

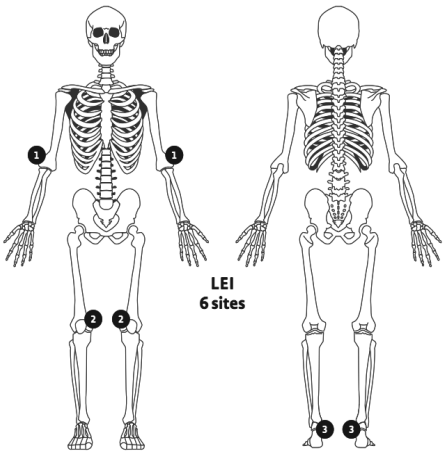
Dactylitis is defined as a uniform swelling of the digits where the joints cannot be defined

*If **yes**, please identify which DIGIT(S) is affected and* **score (0-20)**

Sites	Digit(s) and Count
Right Hand	
Left Hand	
Right Foot	
Left Foot	
Total score (0-20)	

D3. Enthesitis (Leeds Enthesitis Index) Yes ☐ No ☐

The Leeds Enthesitis Index (LEI) examines 6 sites: Achilles tendon insertions, medial femoral condyles, and lateral epicondyles of the humerus, as shown in the figure below. Tenderness at every site is scored 1.



LEI
6 sites

LEI score	Right	Left
Lateral epicondyle humerus		
Medial condyle femur		
Achilles tendon insertion		
Total score (0-6)		

Participant ID						
Date	d d	m o	n	y y	y y	

D4. PATIENT global assessment VAS

"Considering all the **ways your disease affects you**, how well are you doing in the **past week**?"

Please rate how the patient is doing on a scale of 0-100 with 0 being very well and 100 being very poorly

0mm _____ ***100mm***

D5. ASSESSOR global assessment VAS

0mm _____ ***100mm***

Participant ID | | | | | | |

Date | d | d | m | o | n | y | y | y | y |

D6. Bath Ankylosing Spondylitis Disease Activity Index (BASDAI)

Please place a mark on each line below to indicate your answer to each question relating to **the past week**

1. How would you describe the overall level of **fatigue/tiredness** you have experienced?

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

2. How would you describe the overall level of AS **neck, back or hip pain** you have had?

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

3. How would you describe the overall level of pain/swelling in **joints other** than neck, back, hips you have had?

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

4. How would you describe the overall level of **discomfort** you have had from any areas tender to touch or pressure?

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

5. How would you describe the overall level of **morning stiffness** you have had **from the time you wake up?**

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

6. How long does your morning stiffness last from the time you wake up?

hours **0** **½** **1** **1½** **2 or more**

Assessor use only - BASDAI score calculator

A. Calculate the mean of questions 5 and 6 [(5+6)/2]

B. Sum the score from questions 1-4 plus A and divide by 5

NB: question 6 is from 0 to 10. Value Q6 = hours/2*10

Total Score

Participant ID | | | | | | |

Date | d | d | m | o | n | y | y | y | y |

D7. Blood Test for CRP and additional blood sample

Blood test for CRP Yes ☐ No ☐

CRP results in mg/dl ☐☐☐☐

Additional blood samples Yes ☐ No ☐

Time of blood collection (hh:mm) ☐☐☐☐

Additional Note:

Appendix 5

Centaur	

Centaur
Sample Handling
And Processing Manual

Main Study

	Name	Signature	Date
Compiled by:	Louise Bennett		20/06/2019
Approved by clinical research fellow:	Flavia Sunzini		22/06/2019
CI acceptance:	Neil Basu		
Review date:	20th June 2019		

1. Purpose

To describe the procedures to collect and process blood samples for the Centaur study.

2. Summary Sample Collection Schedule

Biological sample	Purpose	Baseline	Month 3	Month 6
Routine biochemistry and haematology (SST + EDTA)	Screening & monitoring	10 ml	10ml	-
STUDY BLOODS				
Plasma/PBMC (EDTA)	Protein Arrays/ELISA	10ml	10ml	I
Serum (SST)	Cytokines	5ml	5ml	
RNA (Pax gene))	Transcriptional studies	3ml	3ml	
Total volume of blood draw		**18 ml**	**18 ml**	

3. Sample Collection Packs.

Centaur sample collection packs will contain all the relevant vacutainers for *university* samples, pre-printed barcode labels and sample collection record log sheet.

Please note, vacutainers for routine blood tests (CRP) are not included, so please remember to take a separate EDTA & SST tube at each visit, label as per TrakCare, send to the NHS laboratory, and record results in the eCRF.

3.1 Main Study Bloods-baseline and month 3:

5ml SST vacutainers x1
10ml EDTA vacutainers x1
Paxgene RNA tube x1

Sundry Items (supplied)

Sample collection record log sheet
Barcode labels

Sundry Items (required but not supplied)

Alcohol swab x1
Cotton swab x1
Sharps bin x1

12 inch blood collection set x1
e.g. BD Vacutainer® Push Button

or BD Vacutainer® Safety-Lok™ Blood Collection Set

4. Patient IDs, Time Point Identifiers and Sample Tube Labels

4.1 Patient IDs

Each participant will be assigned a study ID recorded in the eCRF, sample collection record log and laboratory information management system (LIMS).

4.2 Study site & visit number

The study site (Glasgow only) and visit number e.g. baseline, Month 3 and Month 6, should be recorded in the eCRF, sample collection record log and LIMS.

4.3 Sample tube labelling

- Samples taken as part of routine clinical care (CRP) will be labelled as per standard NHS protocol (e.g. by Trakcare labelling in NHS GGC).

- All other samples will be labelled with the following unique study codes:

Blood samples:

Purpose	Prefix	Number	LABEL
SST 5ml for serum	A	1000	A_1001
SST 5ml for serum	A	1000	A _1001
EDTA 6ml for plasma	B	1000	B _1001
EDTA 10ml for FACs and ex vivo studies	C	1000	C _1001
EDTA 10ml for FACs and ex vivo studies	C	1000	C _1001
Paxgene RNA 3ml	E	1000	E _1001

6. Laboratory Sample Processing

6.1 Routine blood samples

Blood sample for CRP (x1 SST 5ml vacutainer) should be sent to the local NHS laboratory as per routine care.

6.2 Study specific blood samples and urine sample

Study specific blood samples will be sent to Clinical Trial Laboratory (tel: 0141 330 2025/ tel: 0141 330 2282), Sir Graeme Davies Building, 126 University Place, BHF for aliquoting, freezing, and storage.

NOTE: please ship all blood at room temperature and ship **as soon as possible** to the GBRC labs to allow processing of the bloods.

A) SST 5ml vacutainer for serum (x1)

Local CRF:

1. Note collection time of each SST tube in the sample collection record log & eCRF, and register the tube barcodes.

GBRC:

2. Note arrival time of samples in the sample collection record log
3. The vacutainer should be allowed to stand for at least 30 minutes from collection before being centrifuged.
4. Centrifuge blood samples at 1200g at room temperature for 10 minutes
5. Aliquot serum into 5 x 0.5 ml aliquots in pre-labelled FluidX tubes with orange lids (maximum capacity of 750ul).
6. Seal each FluidX tube and scan into the LIMS system
7. Place FluidX tubes into 96 well box, then into -80^{o}C freezer.

B) 10ml EDTA vacutainer for plasma and PBMCs isolation (x1)

Local CRF:

1. Note collection time of the 6ml EDTA tube in sample collection record log & eCRF and register the tube barcodes

GBRC:

2. Note arrival time of samples in the sample collection record log
3. Centrifuge blood sample at 1200g at room temperature for 10 minutes
4. Pipette plasma into plastic tube, leaving 0.5 ml of plasma above buffy layer **taking care not to disturb it.**
5. **DO NOT BIN THE REMAINING BLOOD IN THE EDTA TUBE PLACE TO THE SIDE FOR FURTHER PROCESSING.**
6. Aliquot plasma from plastic tube into 5 x 0.5 ml aliquots in pre-labelled FluidX tubes (maximum capacity of 750ul).
7. Seal each FluidX tube and scan into the LIMS system
8. Place FluidX tubes into 96 well box, then into -80^{o}C freezer.

C) 10ml EDTA vacutainer (continued) for PBMCs isolation

Reagents

- Ficoll - Paque^{TM}PLUS (GE-Healthcare #17-1440-03)

- Dulbeccos's PBS (dPBS, Ca++ and Mg++ free) (#14190-094)
- Fetal Bovine Serum (FBS)(Invitrogen, #10270-106)
- Dimethyl Sulfoxide (DMSO) (Sigma #D2650-100ML)
- Freezing buffer 10% DMSO in FCS
    - Add 200ul DMSO into 1800ul FCS, mix thoroughly and store on ice.

GBRC:

1. Transfer the remaining blood in the EDTA tube into a new 15ml conical tube and top up to 10ml with sterile dPBS and invert to mix.
2. Place 3ml RT Ficoll into a new 15 ml conical tube.
3. Using a Pasteur pipette VERY SLOWLY layer the blood on top of the Ficoll ensure the 2 liquids do not mix.
4. Centrifuge the blood sample at 400g at room temperature for 30 minutes, with NO break.
5. Carefully remove the PBMCs and place them into a fresh 50ml falcon tube, be careful not to disrupt the pelleted granulocytes or erythrocytes at the bottom of the tube.
6. Top up the PBMCs to 50ml with sterile dPBS
7. Spin at 300g for 10 mins at RT, then remove the supernatant and resuspend the cell pellet.
8. Top up to 50ml with sterile dPBS
9. Spin at 200g for 10 mins at RT, then remove the supernatant and resuspend the cell pellet.
10. Carefully top up to 25ml with sterile dPBS and count the cells (see section E) recording the cell number in the quality control work sheet.
11. Spin at 600g for 10 mins at RT, then completely remove the supernatant leaving a dry cell pellet
12. Resuspend the cell pellet **slowly** in 2ml freezing buffer.
13. Aliquot the cell suspension into 2 pre-labelled FluidX tubes (maximum capacity of 1250ul) placing 1ml of the cell suspension per tube.
14. Seal each FluidX tube and scan into the LIMS system for temporary storage.
15. Place FluidX tubes into a MR Frosty for overnight storage in the -80^{o}C freezer.
16. The next day identify the Liquid nitrogen storage location for the samples using the LIMS system and transfer the samples on dry ice to the Liquid Nitrogen tank.

D) Paxgene RNA tube

Local CRF:

1. Note collection time of tube in sample collection record log & eCRF and register tube barcode

GBRC:

2. Note arrival time of sample in the sample collection record log
3. Paxgene RNA vacutainers should be kept upright, at room temperature for 3 hours after blood collection and then transferred to -20^{o}C freezer overnight.
4. The following day place in the LIMS defined location in the -80 Freezer
5. Record any deviations from this time point in the meta data.

E) Counting cells using haemocytometer

- Prepare the glass haemocytometer and coverslip by cleaning both with 70% alcohol. Moisten the coverslip with water and affix to the haemocytometer. The presence of Newton's refraction rings under the coverslip indicates proper adhesion
- Gently swirl the centrifuge tube containing your cells resuspended in 25ml of dPBS, to ensure that the cells are evenly distributed.
- Add 60µl of 0.4% trypan blue trypan blue to a well of a 96-well plate (round bottom)
- Add 20µl of cell suspension to the trypan blue in the 96-well plate and MIX THOROUGHLY
- Gently pipette 10µl of the cell/trypan solution into the chamber underneath the coverslip, allowing the cell suspension to be drawn out by capillary action
- Using a microscope, focus on the grid lines of the haemocytometer with a 10X objective
- Using a hand tally counter, count the live, unstained cells (live cells do not take up trypan blue) in one set of 16 squares (labelled A in figure 1). Move the haemocytometer to the next set of 16 squares (labelled B in figure 1), and then repeat for the other 2 remaining sets of 16 squares (C and D in figure 1)

- Take the average cell count from each set of 16 squares: (A+B+C+D)/4
- Multiply by 10,000 (10^4)
- Multiply by 4 to correct for the 1:4 dilution from the trypan blue addition
- The final value is the number of viable cells/ml in the original cell suspension
- Multiply this value by 25 to calculate the total number of cells in a 25ml cell suspension
- Record this in the Centaur record sheet

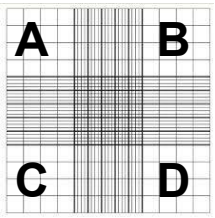


Figure 1: Haemocytometer grid

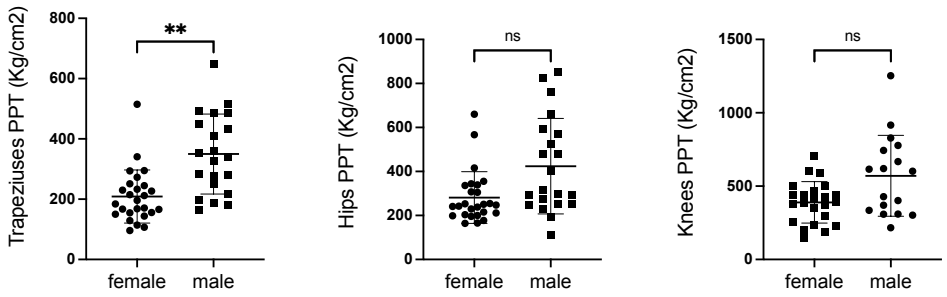
Appendix 6

MSD plate samples template

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL	DUP	C001	C001	C019	C019	C031	C031	C040	C040	C003	C025
B	CAL	DUP	C002	C002	C021	C021	C032	C032	C043	C043	C004	C028
C	CAL	DUP	C006	C006	C022	C022	C035	C035	C044	C044	C012	C029
D	CAL	DUP	C013	C013	C023	C023	C036	C036	C045	C045	C009	C042
E	CAL	DUP	C015	C015	C024	C024	C037	C037	C047	C047	C014	C011
F	CAL	DUP	C016	C016	C026	C026	C038	C038	C048	C048	C005	C005
G	CAL	DUP	C017	C017	C027	C027	C039	C039	C049	C049	C010	C010
H	CAL	DUP	C018	C018	C030	C030	C041	C041	C050	C050	C020	C020

Appendix 7

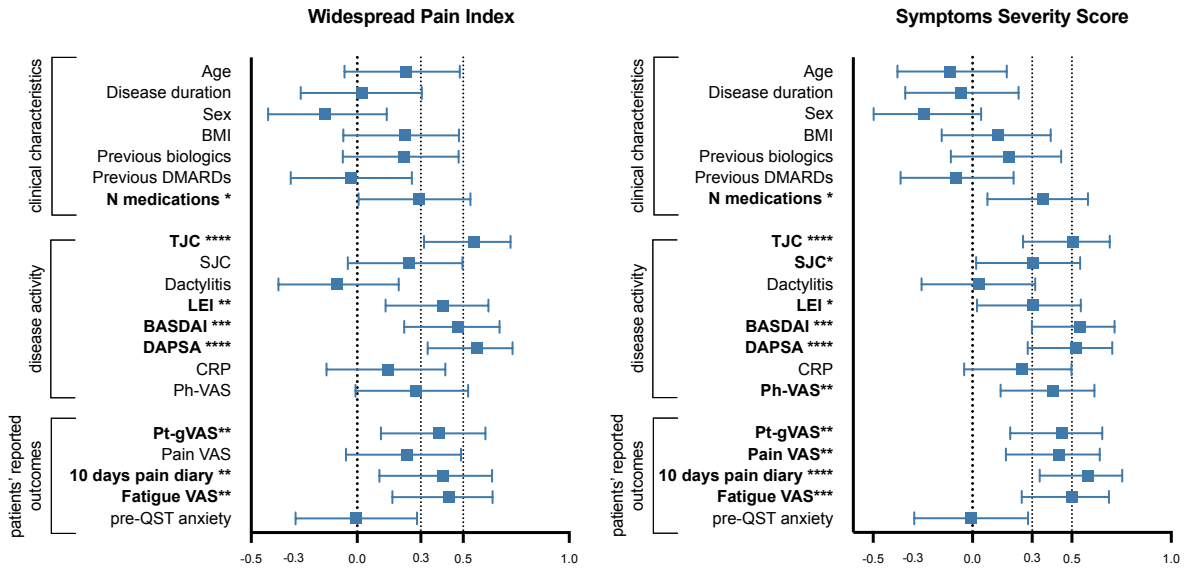
Difference between male and female in trapeziuses, hips, and knees PPT



Legend: trapeziuses PPTs in females are significantly lower than males (P = 0.001 with Mann-Whitney test).

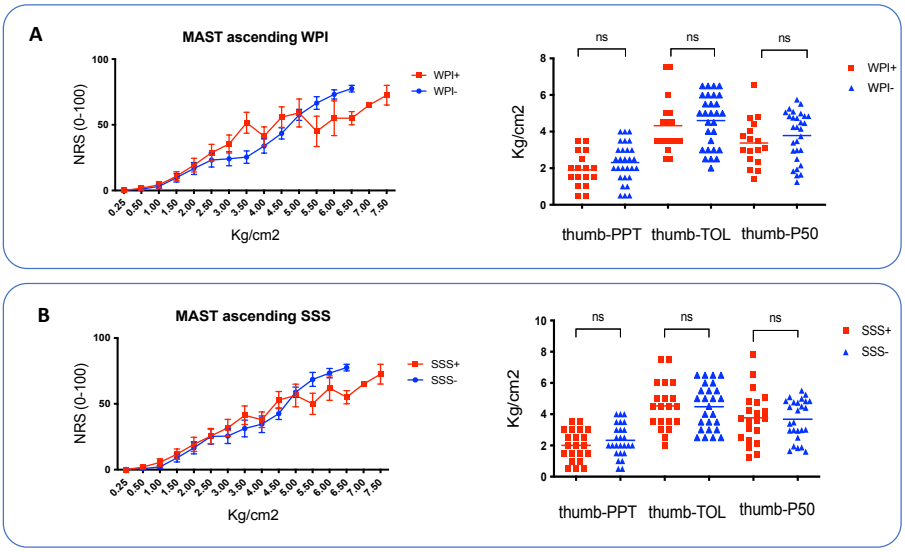
Appendix 8

WPI and SSS correlations with clinical parameters



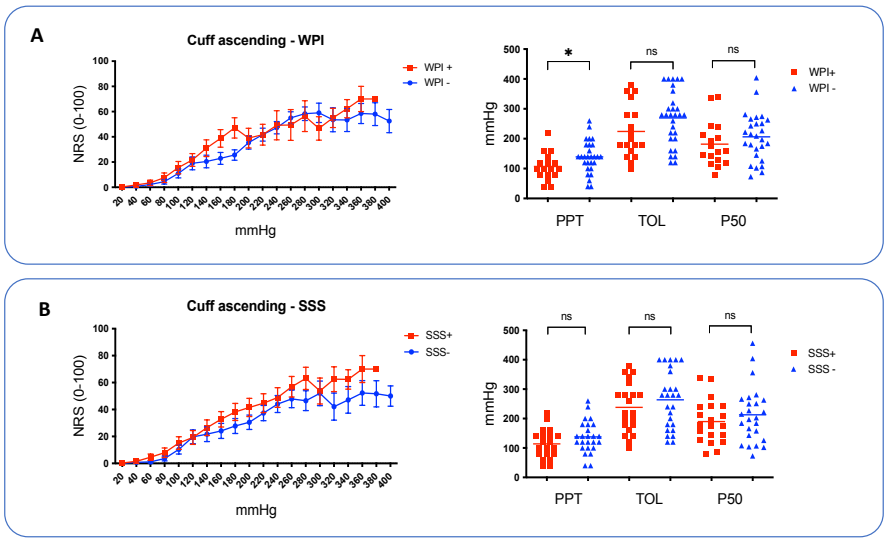
Appendix 9

MAST sensitivity analysis for WPI and SSS



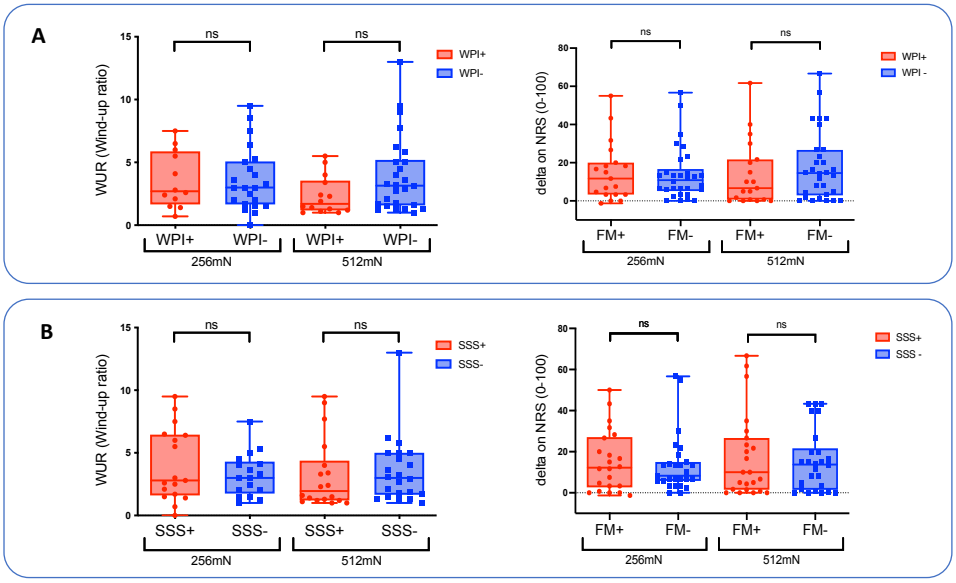
Legend: the figure depicts the QST variables measured with the MAST, including pressure pain thresholds (thumb-PPT), tolerance (thumb-TOL), and pressure to obtain pain rating of 50 (thumb-P50). Ascending curves (left) and plot graphs (right) illustrate the difference in participants grouped according with WPI (A) or SSS (B) scores. Number of subjects in each group correspond to the following: WPI+ n = 18, WPI- n = 31, SSS+ n = 22, SSS- n = 27. No significant differences in pressure pain thresholds (PPT), tolerance (TOL), pressure to obtain pain rating of 50 (P50) were demonstrated for both WPI and SSS groups using unpaired t-test and Mann-Whitney.

Cuff algometry sensitivity analysis for WPI and SSS



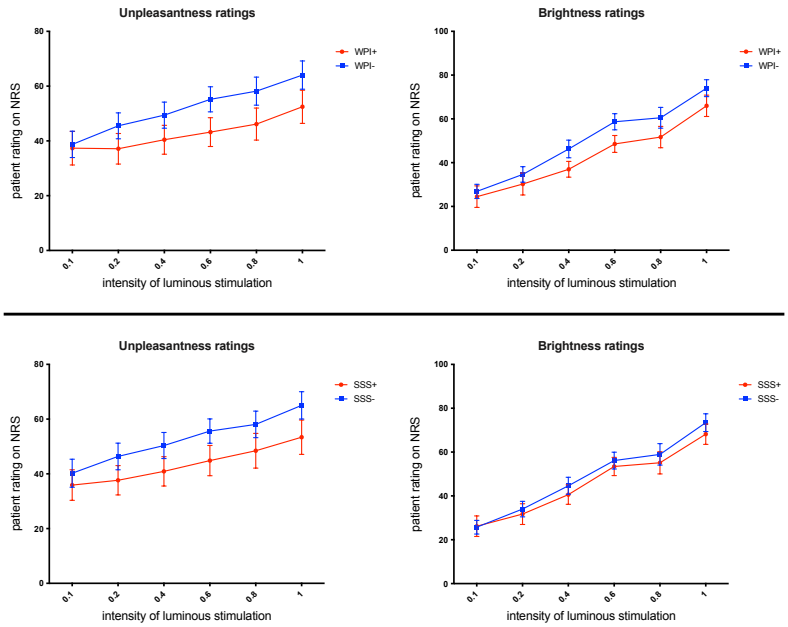
Legend: Differences in pressure pain sensitivity elicited with cuff algometry in participants grouped according to WPI (A) and SSS (B). FM+ n = 20, FM- n = 29. The pressure pain thresholds (PPT) the WPI+ was significantly lower compared to the WPI- group (unpaired t-test, p = 0.0391) (A). No other significant differences PPT, tolerance (TOL), nor pressure to obtain pain rating of 50 (P50) were demonstrated for SSS (B). Number of subjects in each group: WPI+ n = 18, WPI- n = 31, SSS+ n = 22, SSS- n = 27.

Temporal summation is distinct in individuals stratified for WPI and SSS



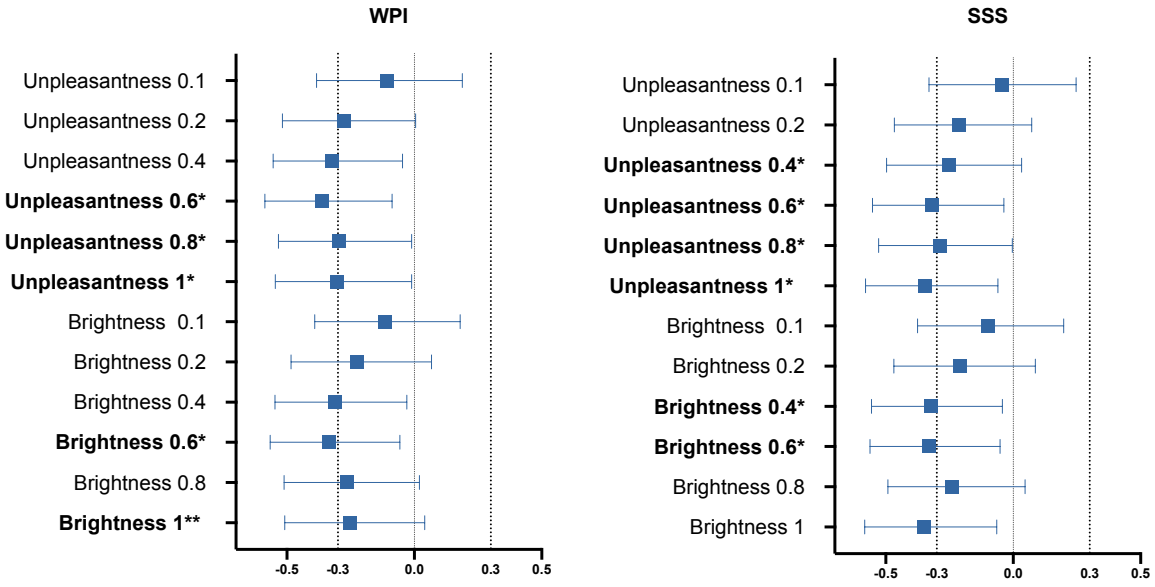
Legend: TS do not differ between WPI+ and WPI- (A), nor between SSS+ and SSS- (B). TS was evaluated with the Wind-up ratio (WUR, left) and difference between ratings of single and multiple stimuli (deltaTS, right). The horizontal lines in the boxes represent the mean value for each group. Vertical bars range from the highest to the lowest values in each dataset. Participants numbers are: WUR 256mN, WPI+ n = 12, WPI- n = 22, SSS+ n = 17, and SSS- n = 17; WUR 512mN, WPI+ n = 14, WPI- n = 26, SSS+ n = 18, and SSS- n = 22; deltaTS 256mN WPI+ n = 19, WPI- n = 30, SSS+ n = 22, and SSS- n = 27; deltaTS 512mN, WPI+ n = 19, WPI- n = 30, SSS+ n = 23, and SSS- n = 26.

Aversion to visual stressors for WPI and SSS



Legend: No significant differences were demonstrated for participants stratified for WPI (top) and SSS (bottom). No significant differences between the groups were demonstrated with Mann-Whitney test. WPI+ n = 19; WPI- n = 30. SSS+ n = 23; SSS- n = 26.

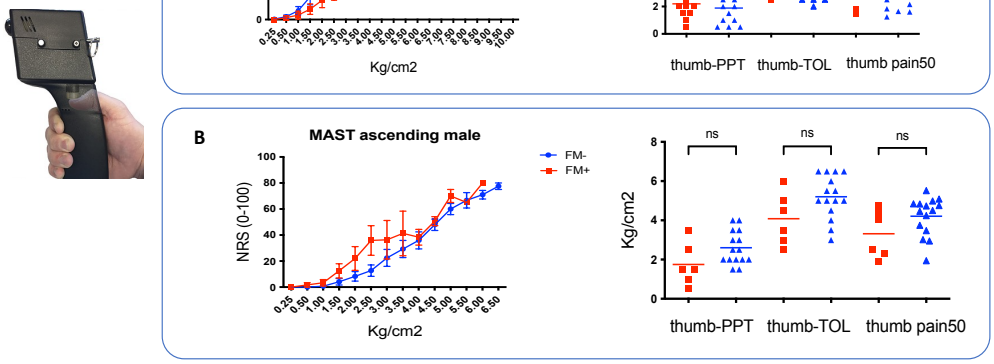
Correlations for visual stimulations ratings versus WPI and SSS



Legend: the figure illustrates the correlation r and the corresponding 95% confidence interval.

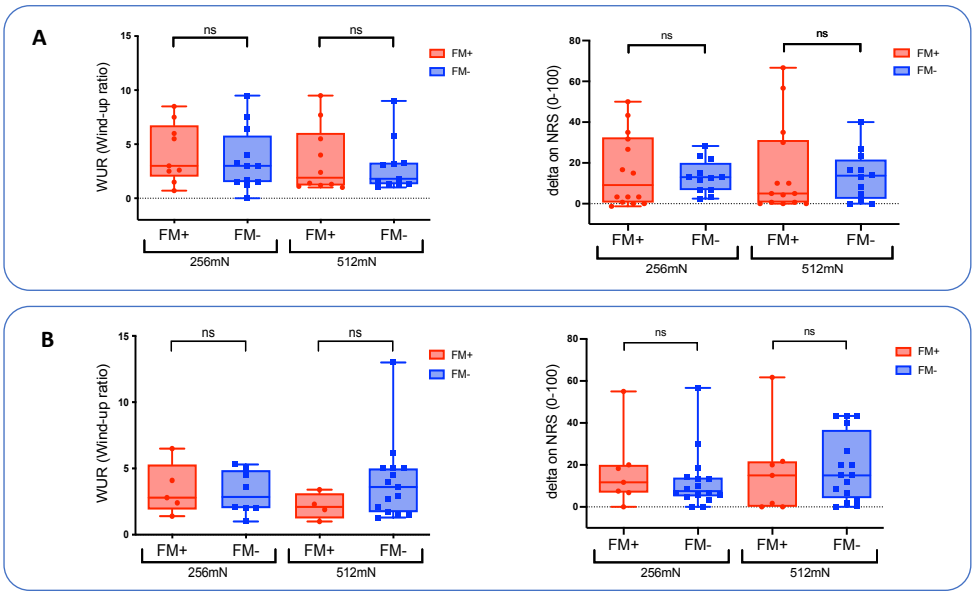
Appendix 10

Effect of sex on pressure pain sensitivity, measured with the MAST device, in relationship with the 2011 ACR fibromyalgia scores.



Legend: This figure displays the relationship between sex pain and nociplastic pain in regards of thumbnail pressure pain sensitivity. Results for females (A) and males (B) are illustrated separately. Participants in each sex group are divided in FM+ and FM- whether they meet the 2011 ACR fibromyalgia criteria or not. Number of subjects in each group are: females FM+ n = 13 and FM- n = 13 (A); males FM+ n = 6 and FM- n = 15 (B). No significant differences were found between FM+ and FM- in both sexes.

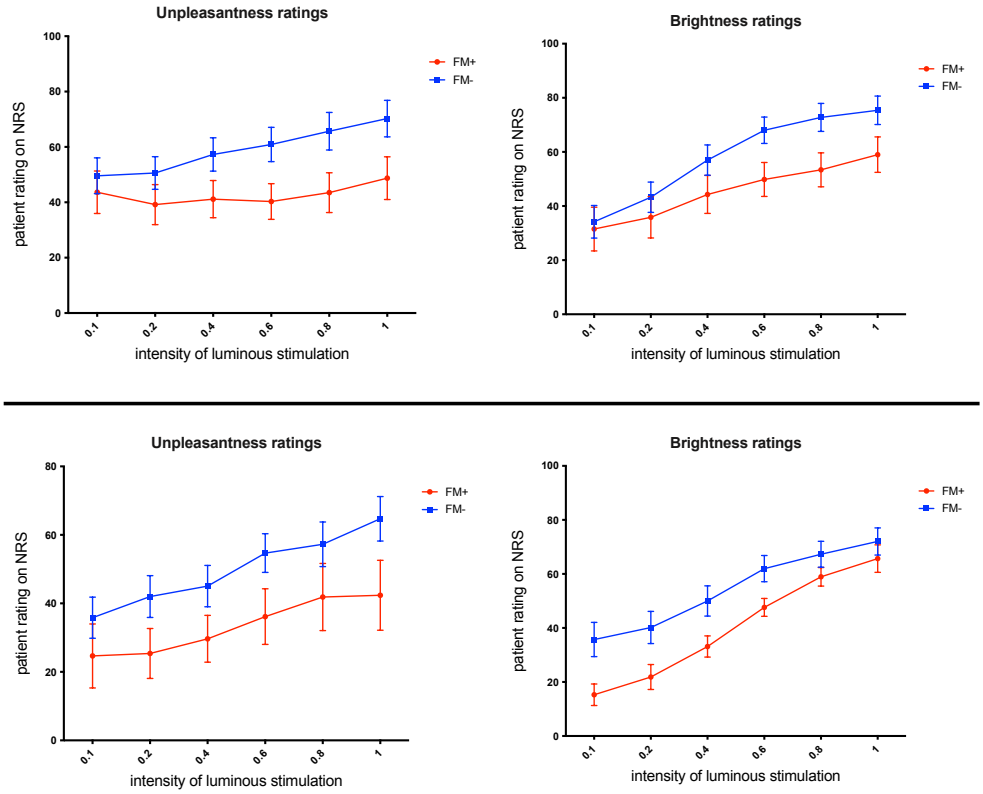
Effect of sex on temporal summation.



Legend: The temporal summation in females (A) and males (B) participants. Temporal summation (TS) was evaluated with 2 pinprick pens calibrated to deliver a force of 256mN or 512 mN. The wind-up ratio (WUR, 10 repeated stimulations ratings divided single stimulation rating) and the delta TS ratings (deltaTS, 10 repeated stimulations - single stimulation ratings) are represented for each sex, respectively on the left and the right bars graphs. Participants are grouped in FM+ or FM-, if satisfying the 2011 ACR fibromyalgia

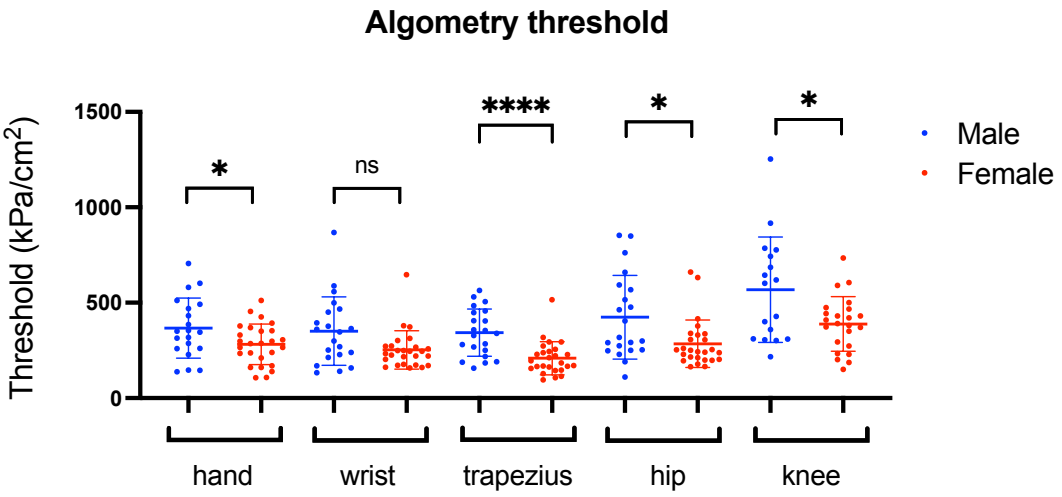
criteria, or not. Number of female subjects in figure A: WUR 256 mN, FM+ n = 9 and FM- n = 12; 512 mN, FM+ n = 10 and FM- n = 11; deltaTS, FM+ n = 14 and FM- n = 12. Numbers for male participants in figure B: WUR 256 mN, FM+ n = 5 and FM- n = 8; 512 mN, FM+ n = 4 and FM- n = 15; deltaTS, FM+ n = 7 and FM- n = 15. No significant differences were found in both sexes.

Unpleasantness and brightness in response to visual stressors in female and male PsA individuals stratified according with the 2011 ACR FM criteria.



Legend: The sensitivity to visual stimuli is represented in in females (A) and males (B) participants grouped according with the 2011 ACR FM criteria in FM+ and FM-. Ratings of unpleasantness (left) and brightness (right) are illustrated above. Number of female participants in figure A: FM+ n = 14 and FM- n = 13. Numbers for male participants in figure B: FM+ n = 7 and FM- n = 15. No significant differences were observed, despite higher ratings were overall present in the FM- group, compared to the FM+.

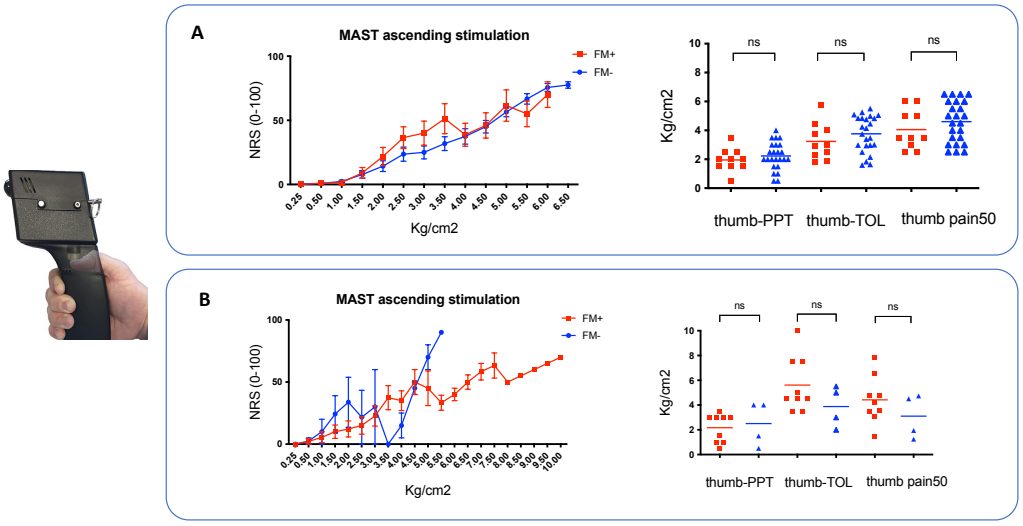
Differences between males and females in pressure pain thresholds measured at different body areas.



Legend: differences in algometry pressure pain thresholds (PPT) tested at different anatomical sites. Female (F) participants are represented in red dots, while males (M) are illustrated in blue dots. Differences in PPT at hands (M = 20, F = 26), hips (M = 21, F = 26), trapeziuses (M = 21, F = 26), wrists (M = 21, F = 26), and knees (M = 17, F = 23) are described. The PPTs at trapeziuses in females resulted strongly lower than males (P <0.0001). No significant difference in wrists PPTs was observed between males and females. Females presented lower PPTs at the hands, hips, and knees than males (respective P-values 0.048, 0.016, and 0.0227).

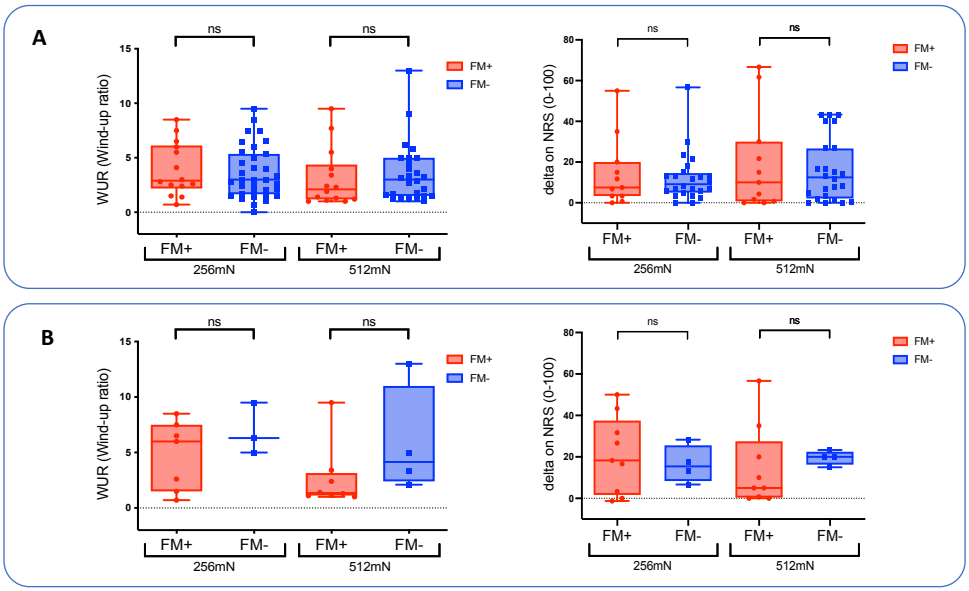
Appendix 11

Thumbnail pressure pain sensitivity in participants on pain modulating drugs or not.



Legend: participants were divided in individuals taking pain modulating drugs (B), including opioids, gabapentinoids, SNRI and other antidepressants, or not (A). In each group subjects were divided according with the 2011 ACR FM criteria in FM+ (red) and FM- (blue). A. A total of 36 participants were included in the pain modulators free group (FM+ = 11, FM- = 25). B. Thirteen subjects were taking at least one pain modulator (FM+ = 9, FM- = 4). No significant differences were found between FM+ and FM- in both groups. A participants with FM in figure B exhibits higher pain tolerance compared to rest of the participants in the same group.

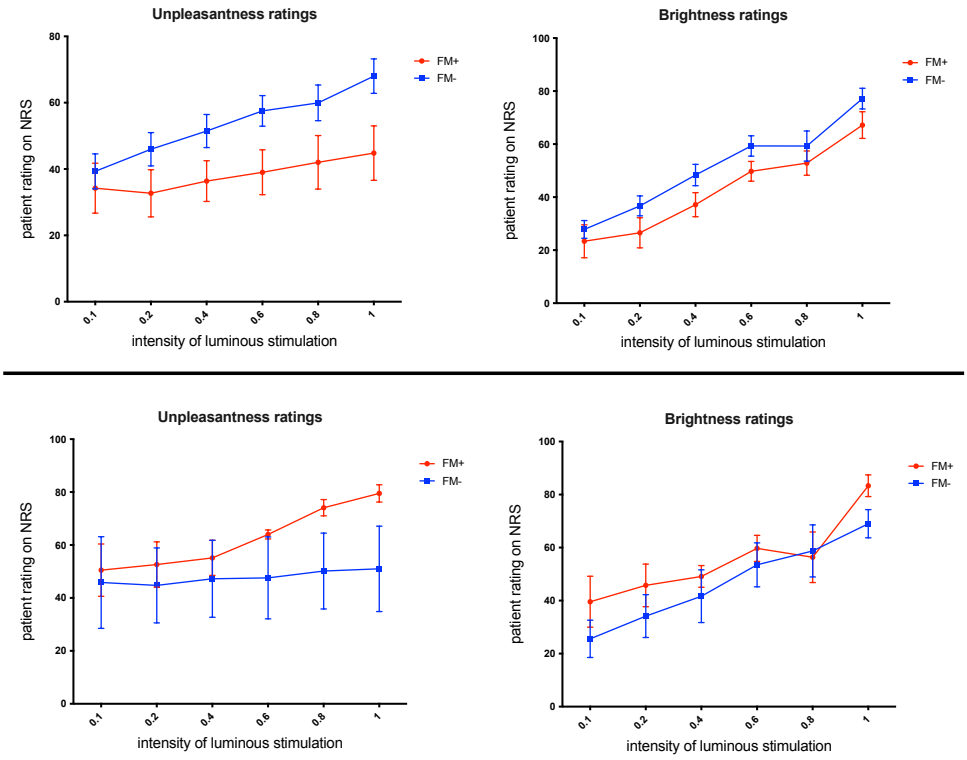
Temporal summation is not altered by pain modulators



Legend: Temporal summation in participants taking pain modulating drugs (B) or not (A) participants. Two pinprick pens calibrated to deliver a force of 256mN or 512 mN were used

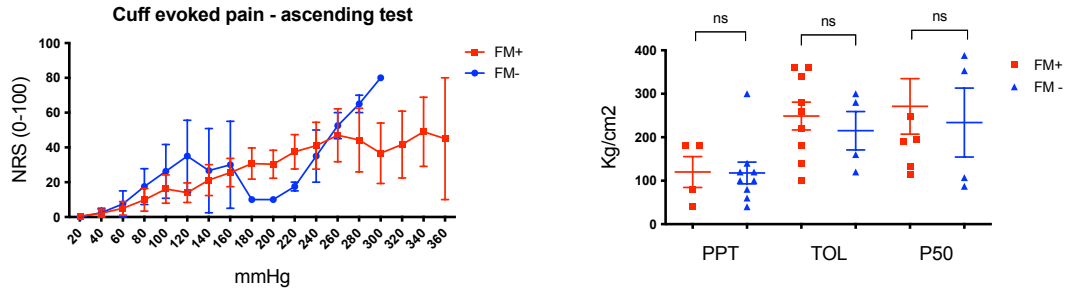
to determine temporal summation. The wind-up ratio (WUR) and the delta TS ratings (deltaTS) are depicted in separate bar graphs for each group. The WUR is calculated by dividing the ratings obtained from 10 repeated stimulations by the rating from a single stimulation. The delta TS ratings are determined by subtracting the ratings from a single stimulation from those obtained from 10 repeated stimulations. The bar graphs on the left represent the WUR, while the bar graphs on the right represent the delta TS ratings.
Participants were categorized into 2 groups, FM+ (red) or FM- (blue), based on whether they met the 2011 ACR fibromyalgia criteria or not. Figure A presents the number of subjects free from pain modulators for different parameters: WUR at 256 mN, FM+ n = 14 and FM- n = 34; WUR at 512 mN, where FM+ n = 14 and FM- n = 26; deltaTS at 256 mN, FM+ n = 11 and FM- n = 24; deltaTS at 512 mN, FM+ n = 12 and FM- n = 23. In Figure B, the numbers for participants taking pain modulating drugs are presented: WUR at 256 mN, FM+ n = 7 and FM- n = 3; WUR at 512 mN, FM+ n = 8 and FM- n = 4; and deltaTS, FM+ n = 9 and FM- n = 4. No significant differences were observed between the two groups.

Evaluating Visual sensitivity in individuals PsA stratified based on pain modulators and the 2011 ACR FM criteria.



Legend: The sensitivity to visual stimuli is depicted for participants not taking pain modulators (A), and for individuals who were (B). Participants were categorized based on the 2011 ACR Fibromyalgia (FM) criteria as FM+ (red) and FM- (blue). Ratings of unpleasantness (left) and brightness (right) are displayed. In Figure A, the number of pain modulators-free subjects is indicated as FM+ n = 11 and FM- n = 24. Despite higher ratings being generally observed in the FM- group compared to FM+, no statistically significant differences were found between the groups. For Figure B, the numbers of participants taking pain modulators at the time of the visit are FM+ n = 9 and FM- n = 4. In participants fulfilling the FM criteria the unpleasantness and brightness were overall higher than the FM- group, however, the differences did not resulted significant.

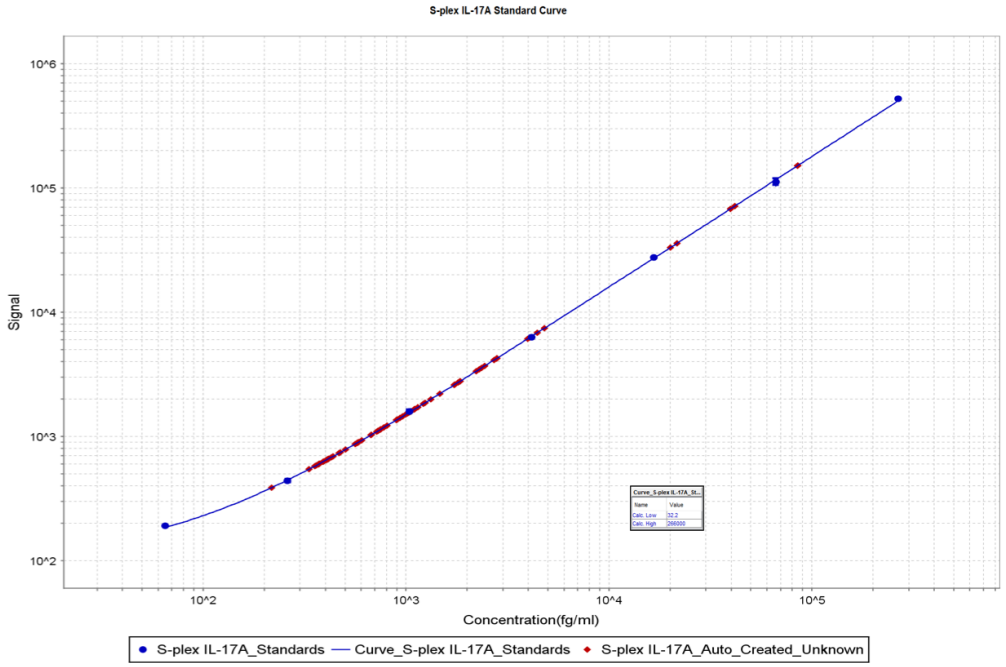
Cuff-algometry in participants using pain-modulating drugs

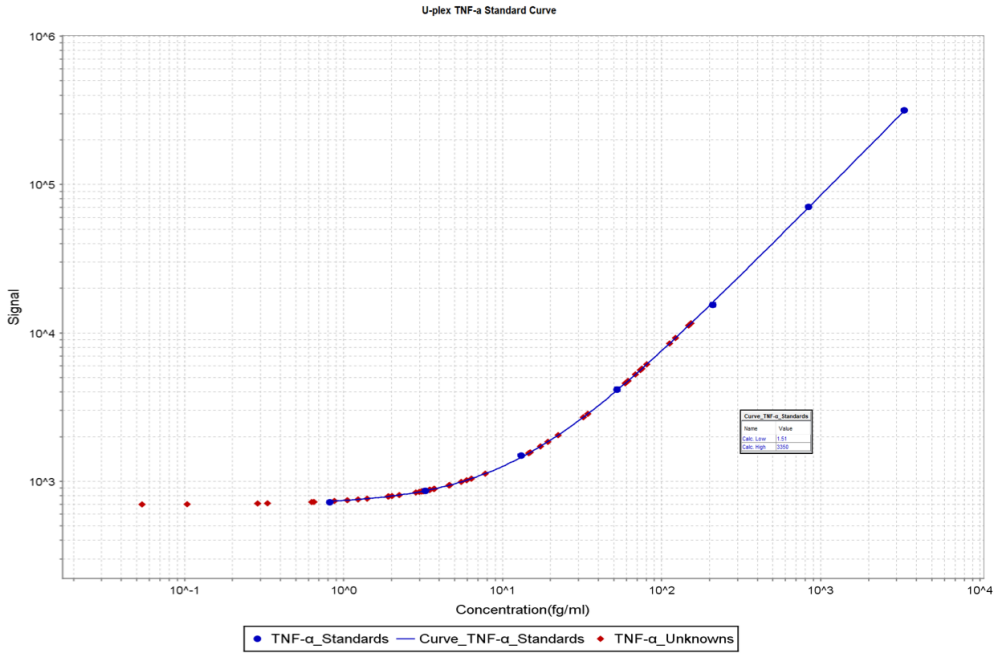


Legend: The figure presents cuff-algometry analysis in individuals who were using pain-modulating drugs. The participants were categorized as FM+ (n = 9, indicated in red) if they met the 2011 ACR Fibromyalgia (FM) criteria and as FM- (n = 4, indicated in blue) if they did not. The ascending curves for both groups were overall similar, as illustrated in the left graph. The FM+ group exhibited higher tolerance (TOL) ratings compared to the FM- group, however the significance was not reached. Similarly, there were no significant differences between the two groups in terms of pressure pain threshold (PPT) and pressure to elicit a pain rating of 50/100 (P50).

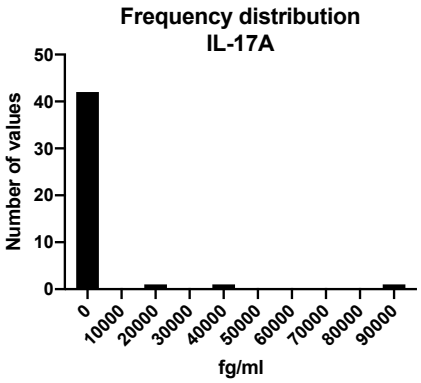
Appendix 12

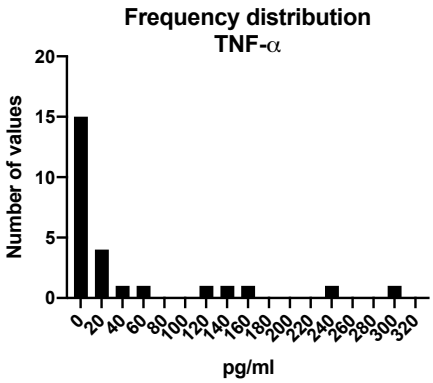
Detection curves for IL-17A and TNF-α are illustrated below.



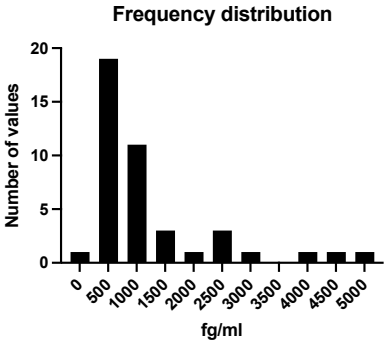


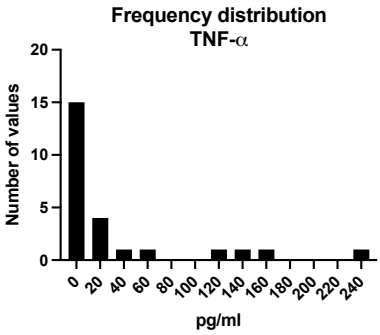
Distributions IL-17A and TNF-α including all values detected.



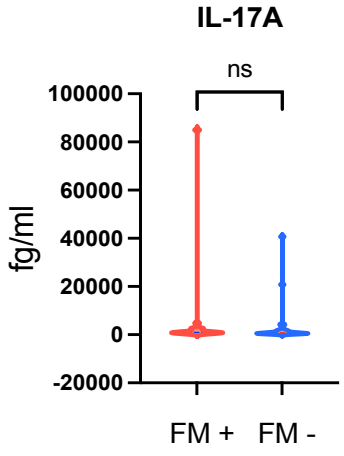


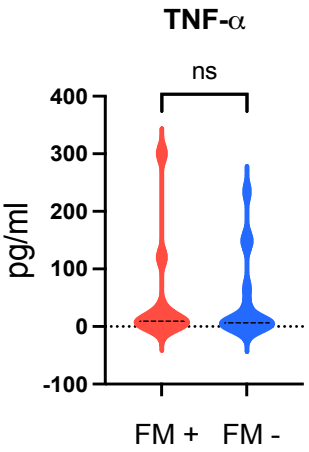
Distributions IL-17A and TNF-α after exclusion of outliers.



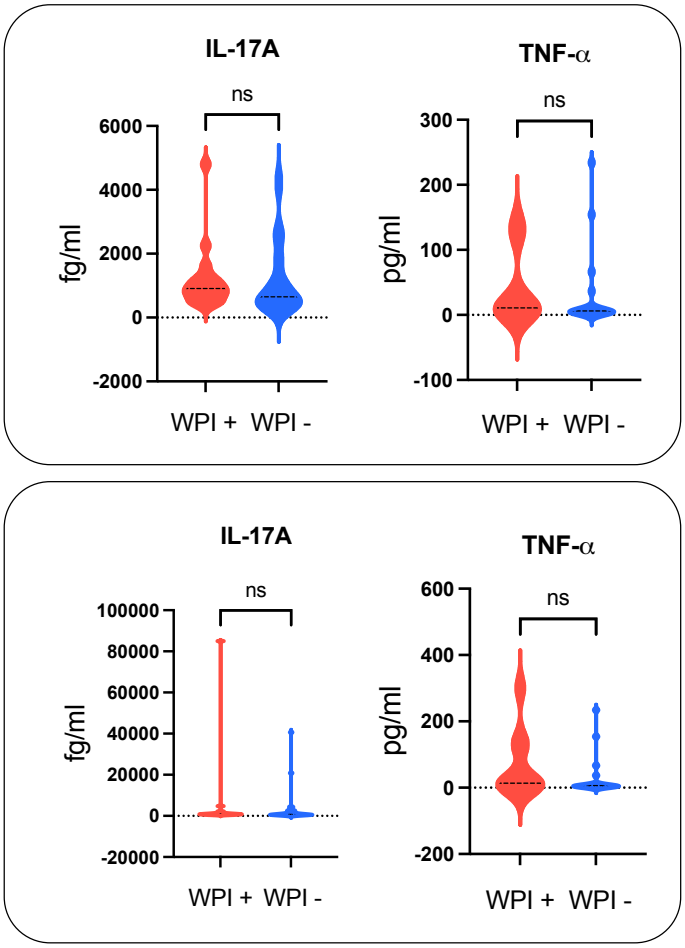


Differences in IL-17A and TNF-α in patient with and without FM.



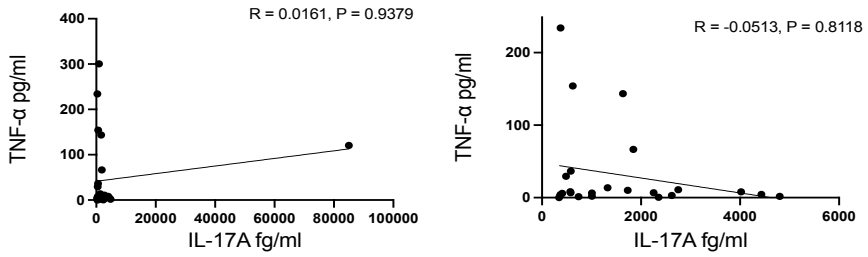


Differences in pro-inflammatory cytokines in participants stratified according with WPI.



Legend: difference in IL17A and TNF-α circulating levels between subjects with high (WPI+) and low (WPI-) WPI. The WPI was determined using the wpi from the 2011 ACR FM criteria, and the median of all the indexes collected was used as cut-off. The top graph depicts the differences in cytokines values excluding the outliers determined with the Tukey's fence method. The bottom graph illustrates the difference when all the values detected were included in the analysis.

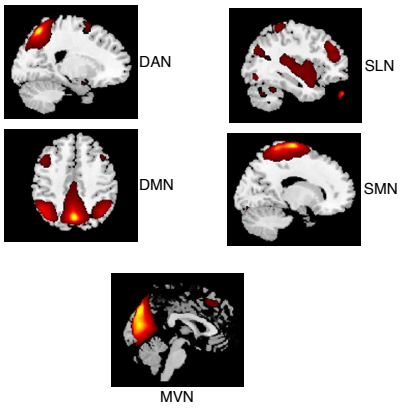
Correlation plot bteween circulating levels of TNF-α and IL-17A at baseline.

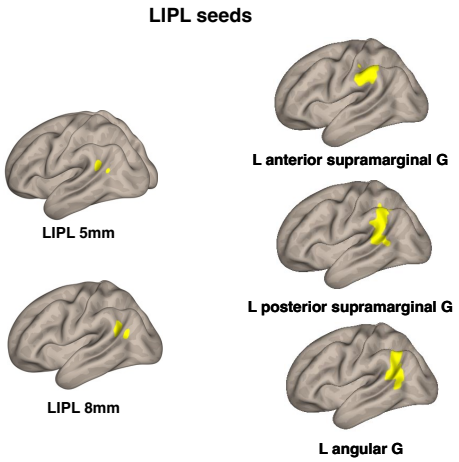


Legend: Circulating levels of the 2 pro-inflammatory cytokines analysed, TNF-α and IL-17A are not significanlty associated. The graph on the left illustrates the relation ship between all levels of cytokines detected, while the graph on the right shows the correlation plot after outliers exclusion

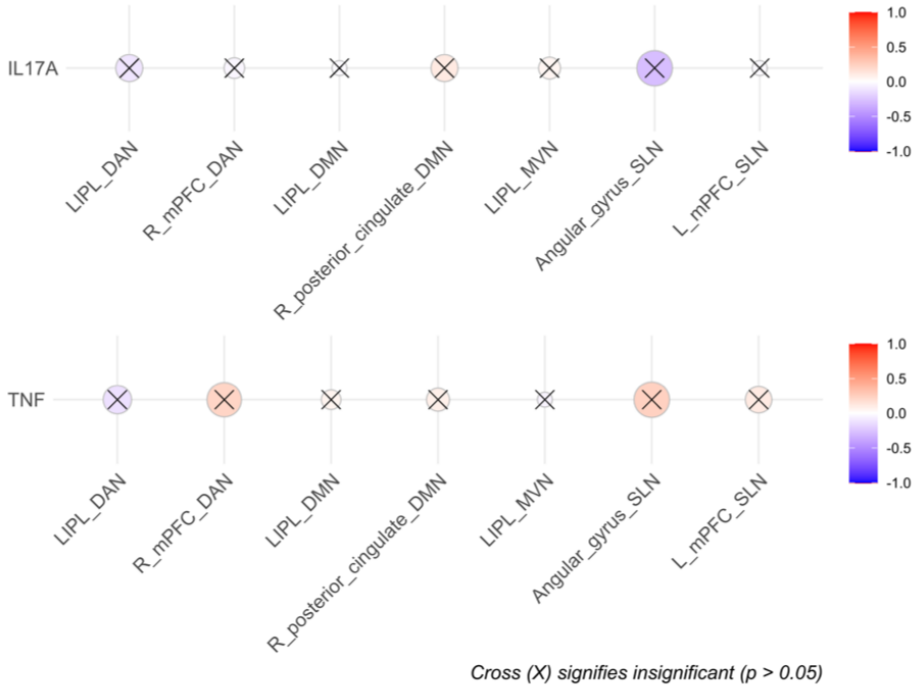
Appendix 13

ROIs used for seed-to-voxel analysis in relationship with circulating pro-inflammatory cytokines.



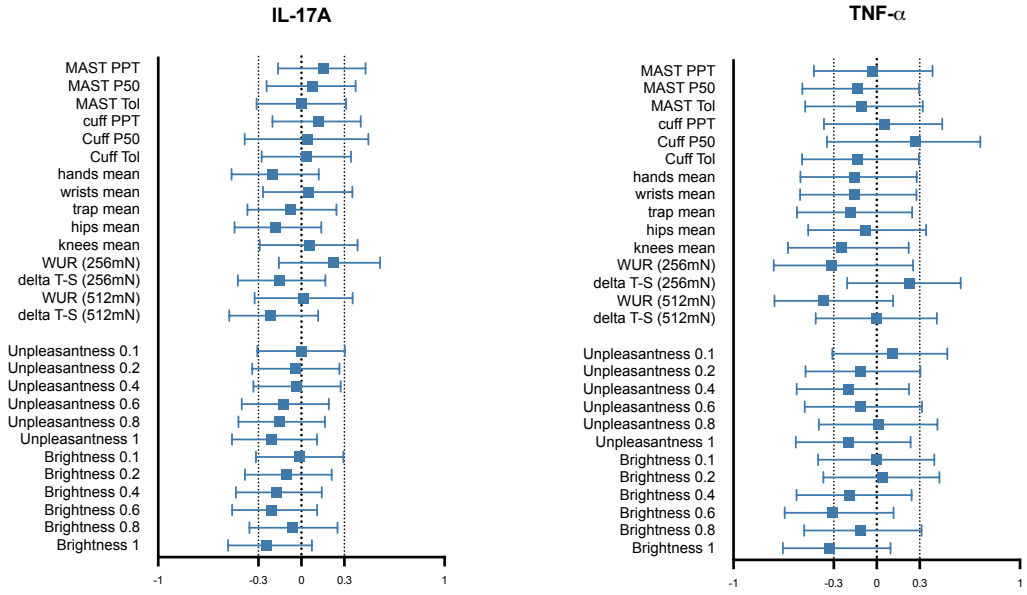


Results from seed-to-voxel analysis for both IL-17A and TNFα



Cross (X) signifies insignificant (p > 0.05)

Correlation plots of associations between QST and pro-inflammatory cytokines.



Appendix 14

Differences in baseline clinical features between participants included and excluded from the 3 months response to treatment analysis

CLINICAL FEATURES	3 months included (34)	3 months excluded (16)	P-values
FM criteria (%)	47.1%	35.7%	ns
FM total score (mean ± SD)	13 ±5.9	11 ±5.5	ns
WPI (mean ± SD)	5.9 ±3.9	4.6 ±3.2	ns
SSS (mean ± SD)	7.4 ±3	6.6 ±2.9	ns
Clinical characteristics			
Age (mean ± SD)	49 ±12	47 ±9.1	ns
Sex (male%)	50%	80%	ns
Disease duration years (mean ± SD)	7 ±6.2	5.4 ±4.9	ns
BMI (mean ± SD)	29 ±3.7	31 ±5.7	0.05
Previous biologics (mean ± SD)	0.76 ±1.5	0.81 ±1.2	ns
N previous DMARDs (mean ± SD)	1.6 ±1.2	2.1 ±1.4	ns
N medications baseline (mean ± SD)	3.3 ±2.7	3.4 ±2.9	ns
Disease activity			
TJC 66 (mean ± SD)	22 ±14	21 ±16	ns
SJC 68 (mean ± SD)	7.3 ±4.8	7.3 ±4.1	ns
Dactylitis (yes/no)	2.1 ±1.4	2 ±1.7	ns
LEI score (mean ± SD)	3 ±2	2.1 ±1.7	ns
BASDAI (mean ± SD)	6.3 ±1.7	6.1 ±2.5	ns
CRP mg/dl (mean ± SD)	1.3 ±3	0.74 ±0.6	ns
Physician gVAS (mean ± SD)	53 ±16	53 ±17	ns
Patient reported outcomes			
Patient gVAS (mean ± SD)	61 ±19	56 ±32	ns
Pain VAS (mean ± SD)	31 ±22	43 ±29	ns
10 days pain diary (mean ± SD)	5.3 ±1.8	5.5 ±2.9	0.04
Fatigue VAS (mean ± SD)	6.5 ±2.3	7 ±3.3	ns
Pre-QST anxiety (mean ± SD)	7.2 ±12	8.8 ±17	ns

Differences in baseline clinical features between participants included and excluded from the 6 months response to treatment analysis

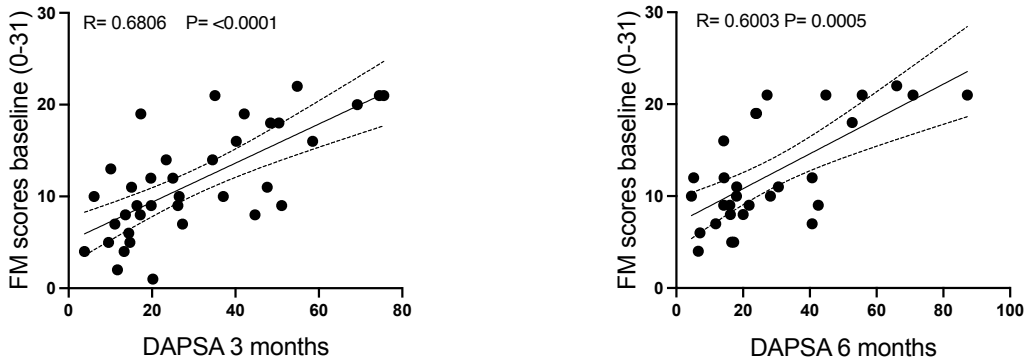
CLINICAL FEATURES	6 months included (27)	6 months excluded (23)	P-values
FM criteria (%)	37%	47.8%	ns
FM total score (mean ± SD)	13 ±5.8	12 ±6.1	ns
WPI (mean ± SD)	5.8 ±3.9	5 ±3.5	ns
SSS (mean ± SD)	7.3 ±2.6	7 ±3.3	ns
Clinical characteristics			
Age (mean ± SD)	48 ±12	49 ±11	ns
Sex (male%)	44.4%	47.8%	ns
Disease duration years (mean ± SD)	7.1 ±6.7	5.8 ±4.8	ns
BMI (mean ± SD)	29 ±4.2	31 ±4.7	ns
Previous biologics (mean ± SD)	0.85 ±1.4	0.7 ±1.5	ns
N previous DMARDs (mean ± SD)	1.7 ±1.3	1.9 ±1.2	ns
N medications baseline (mean ± SD)	3.2 ±2.6	3.5 ±2.8	ns
Disease activity			
TJC 66 (mean ± SD)	25 ±16	18 ±12	ns
SJC 68 (mean ± SD)	7.4 ±5	7.1 ±4	ns
Dactylitis (yes/no)	2.3 ±1.6	1.9 ±1.1	ns
LEI score (mean ± SD)	2.9 ±2.3	2.5 ±1.5	ns
BASDAI (mean ± SD)	6.5 ±1.7	6 ±2.3	ns
CRP mg/dl (mean ± SD)	1.3 ±3.2	0.83 ±0.96	ns
Physician gVAS (mean ± SD)	53 ±16	53 ±18	ns
Patient reported outcomes			
Patient gVAS (mean ± SD)	64 ±16	54 ±29	0.006
Pain VAS (mean ± SD)	35 ±21	35 ±29	ns
10 days pain diary (mean ± SD)	5.4 ±2	5.3 ±2.6	ns
Fatigue VAS (mean ± SD)	6.9 ±2.3	6.5 ±3	ns
Pre-QST anxiety (mean ± SD)	10 ±15	4.8 ±12	ns

Appendix 15

Baseline clinical features of responders and not responders at 6 months

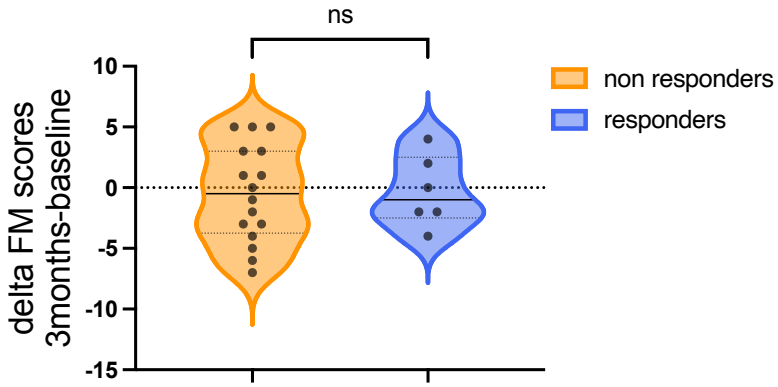
CLINICAL FEATURES	non responders (14)	responders (13)	P-values
FM criteria (%)	23.08%	50.00%	ns
FM total score (mean ± SD)	14.36±6.222	10.77±5.54	ns
WPI (mean ± SD)	6.5±4.816	4.308±2.594	ns
SSS (mean ± SD)	7.857±2.143	6.462±3.307	ns
Clinical characteristics			
Age (mean ± SD)	50±13.15	47.15±11.19	ns
Sex (male %)	61.54%	57.14%	ns
Disease duration years (mean ± SD)	5.5±5.08	9.385±7.355	ns
BMI (mean ± SD)	28.41±3.594	30.09±5.076	ns
Previous biologics (mean ± SD)	0.6429±1.336	0.5385±0.9674	ns
N previous DMARDs (mean ± SD)	1.286±0.9139	2±1.414	ns
N medications baseline (mean ± SD)	3.071±2.947	2.615±2.022	ns
Disease activity			
TJC 66 (mean ± SD)	22.79±15.87	21.38±15.56	ns
SJC 68 (mean ± SD)	8.857±5.934	6.538±3.431	ns
Dactylitis (yes/no)	0.9286±1.639	1.154±1.519	ns
LEI score (mean ± SD)	3.214±2.259	1.846±1.908	ns
BASDAI (mean ± SD)	6.186±1.784	6.4±2.032	ns
CRP mg/dl (mean ± SD)	1.4±4.2	0.8±0.7	0.05
Physician gVAS (mean ± SD)	50.71±17.19	51.15±14.16	ns
Patient reported outcomes			
Patient gVAS (mean ± SD)	54.64±18.55	58.08±22.32	ns
Pain VAS (mean ± SD)	32.5±21.1	31.92±23.32	ns
10 days pain diary (mean ± SD)	5.533±1.986	5.019±2.428	ns
Fatigue VAS (mean ± SD)	6.071±2.702	6.923±2.629	ns
Pre-QST anxiety (mean ± SD)	10.71±12.84	1.923±6.934	0.01

FM scores at baseline correlate positively with the disease activity at follow-up visits.



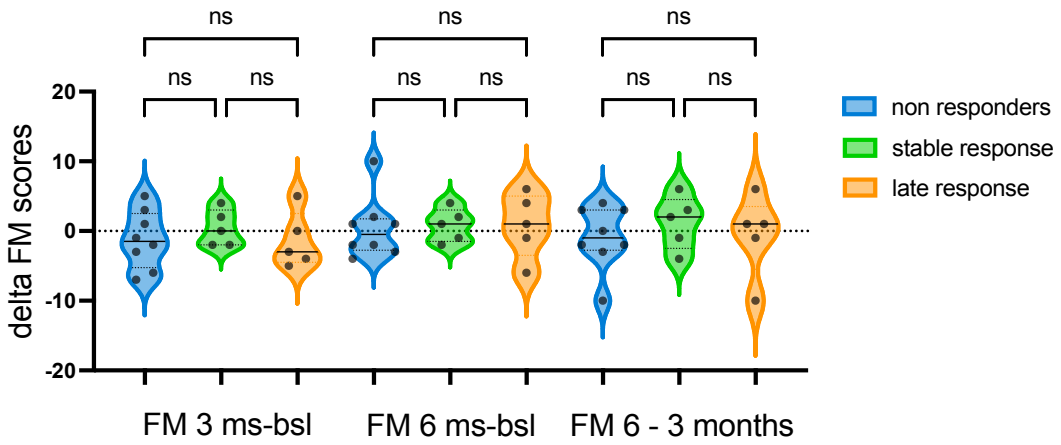
Legend: Correlations between ACR FM scores and disease activity at 3 and 6 months. The plot graphs show positive correlations between the FM scores at baseline and the disease activity at 3 months (left) and 6 months (right) measured with the DAPSA score (respectively, R = 0.6806, P < 0.0001, and R = 0.6003, P = 0.0005).

Variation of ACR FM 2011 Scores in non-responders and responders to Immunosuppressive treatment at 3 months



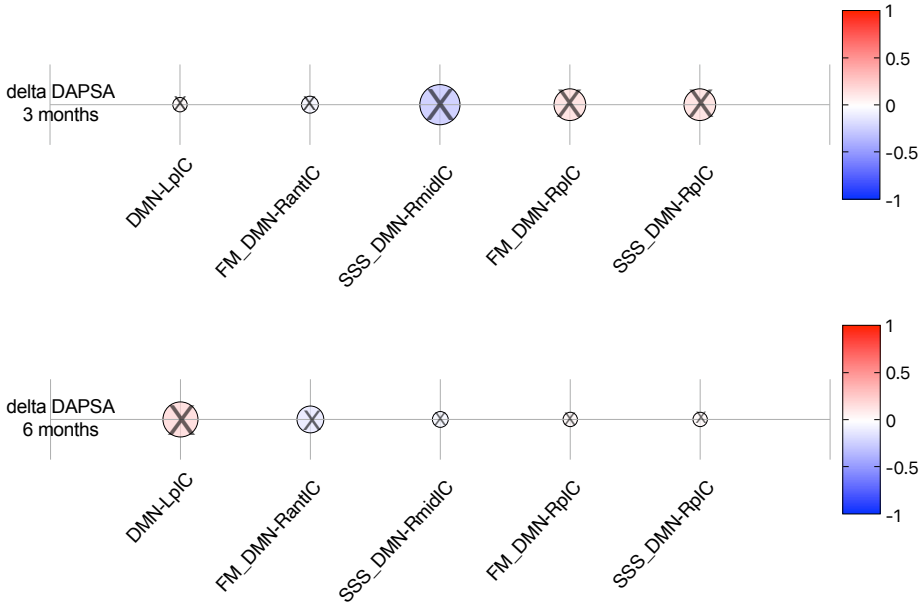
Legend: The figure shows two violin plots representing the variation in ACR FM 2011 scores between baseline and 3 months after immunosuppressive treatment for responders (on the right) and non-responders (on the left). On the left violin represents the non-responders, while the right violin represents the responders. Despite a slightly higher range of variation in the non-responders (12) compared to the responders (8), the difference between the two variations did not reach statistical significance.

Variations in 2011 ACR FM scores at different visits after 6 months of treatment: exploring non-responders, stable responders, and late responders



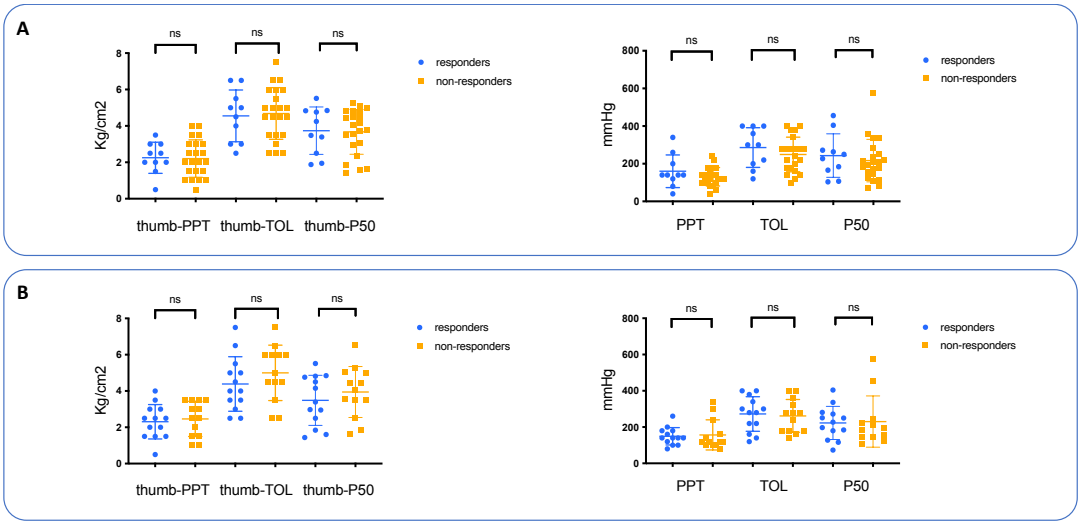
Legend: The figure presents violin plots illustrating the differences in 2011 ACR FM scores between baseline and 3 months, baseline and 6 months, and 3 months and 6 months, for subjects categorized into three groups: non-responders, stable responders (responding at both 3 and 6 months), and late responders (responding at 6 months, but not at 3 months). Stable responders showed the narrowest range of variation across all three differences (6-10), while non-responders and late responders exhibited wider ranges of variations (12-14 and 10-16, respectively). However, none of the variations were found to be statistically significant between the groups.

Correlations between DMN to Insula functional connectivity and changes in DAPSA after 3 and 6 months of treatment.



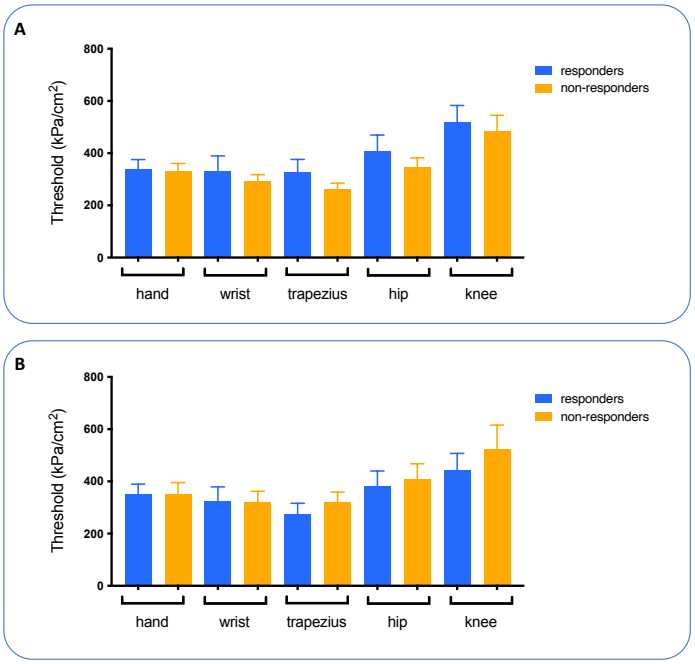
Legend: ROI to ROI functional connectivity between DMN and Insula resulted significantly associated with baseline determinants of nociplastic pain, namely FM and SSS, were correlated with variation of disease activity after 3 months (top, delta DAPSA 3 months) and 6 months of treatment (bottom, delta DAPSA 6 months). The size of the spheres corresponds to the correlation R. The only R higher than |0.30| was observed between SSS_DMN-RmidIC and delta DAPSA at 3 months (R= -0.3021, P = 0.0777), however no correlation resulted statistically significant.

Pressure pain sensitivity in participants treated with immunosuppressive treatment: comparison between responders and non-responders at 3 and 6 months



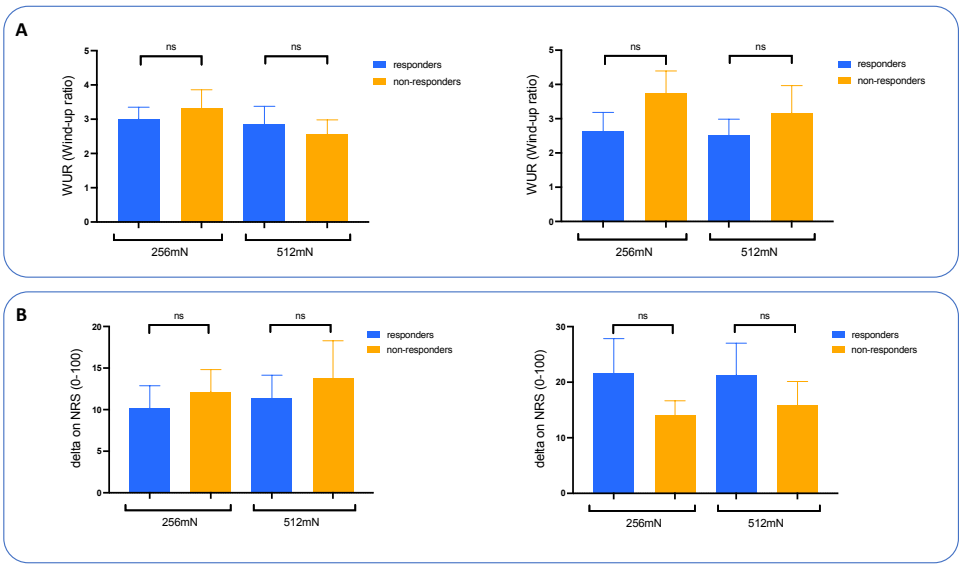
Legend: the figure displays pressure pain sensitivity assessed at the thumbnail using the MAST device and calf with cuff algometry. Participants with an active PsA were treated with immunosuppressive treatment and response to treatment was evaluated at 3 (A)and 6 months (B). Both at 3 and 6 months, the groups of responders (R) and non-responders (NR) were compared in terms of pressure pain threshold (PPT), tolerance (TOL), and pressure needed to elicit a pain rating equal to 50/100 (P50). The number of subjects in each group for both time points is indicated within the parentheses. At 3 months the non responders were 22, while the responders 10. At 6 months, noth responders and non responders were equal to 13. At both time points (3 and 6 months), there were no significant differences in pressure pain sensitivity measures (PPT, TOL, and P50) between the responder and non-responder groups.

Comparison of pressure pain thresholds in different body areas between responders and non-responders to immunosuppressive treatment at 3 and 6 months



Legend: The figure displays the variations in pressure pain threshold (PPT) measured in various body areas using a digital handheld algometer. The top graph (A) represents PPT differences between responders (R) and non-responders (NR) to immunosuppressive treatment after 3 months, while the bottom graph (B) illustrates the variations at 6 months. At 3 months, 22 participants were included in the non responders group for each body area, except for the knees with 18 subjects; while, the responders were 10, except the hand area which included a total of 8 subjects. At 6 months, both responders and non responders were 13 in both groups, apart from the knee having 12 responders and 10 non responders. No significant differences were observed in pressure pain threshold between responders and non-responders at both 3 months and 6 months.

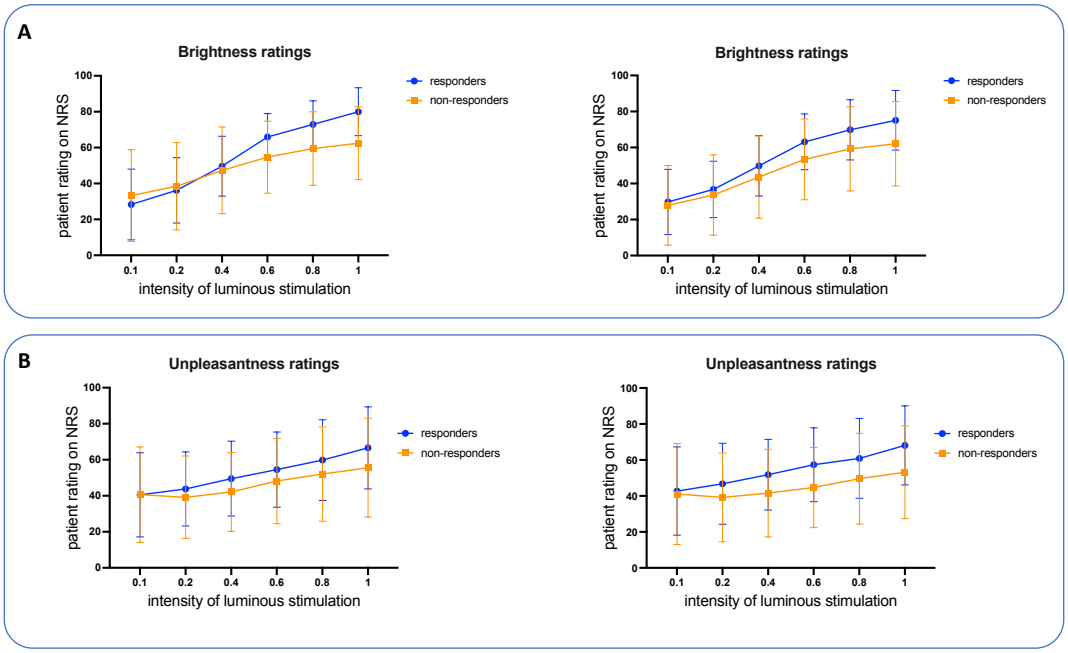
Temporal Summation differences in responders and non-responders to immunosuppressive treatments at 3 and 6 months



Legend: The figure presents plot graphs illustrating the differences in temporal summation (TS) between responders (R) and non-responders (NR) to immunosuppressive

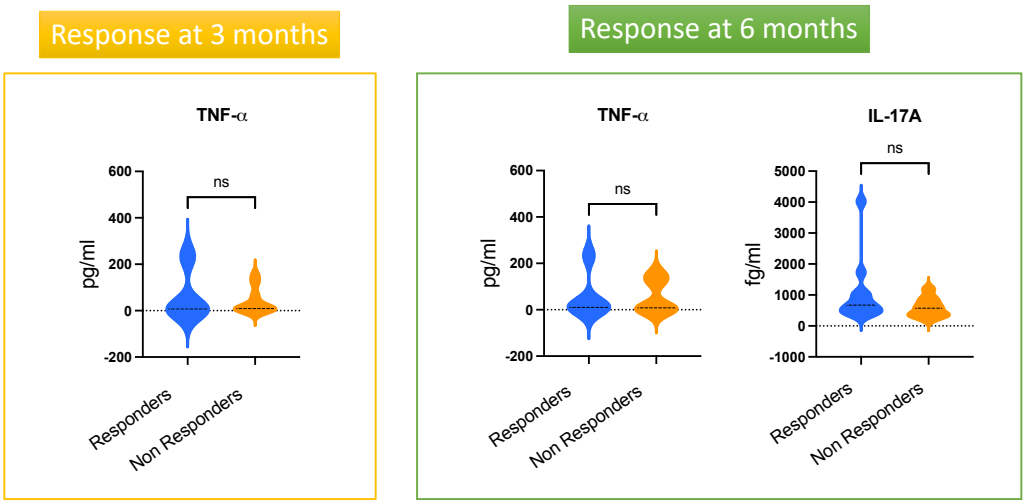
treatments at 3 months (left) and 6 months (right). Temporal summation was assessed using pointed pin prick pens calibrated to apply a force of 256mN or 512mN. The temporal summation was measured using the wind-up ratio (WUR), representing the ratio between the pain ratings following 1 and 10 repeated stimulations (A), or using the differences between the same pain ratings (dTS) (B). Participants numbers: 256 WUR, responders n=6, and non responders n=18; 512 WUR, responders n=9, and non responders n=20; 256 dTS, responders n=10, and non responders n=21; 512 dTS, responders, n=9, and non responders n=21. No significant differences in temporal summation were found between responders and non responders at both 3 and 6 months.

Differences in visual sensitivity between responders and non-responders to immunosuppressive treatments at 3 and 6 months



Legend: The figure illustrates the visual sensitivity differences between responders (R) and non-responders (NR) to immunosuppressive treatments at 3 months (left graphs) and 6 months (right graphs). Visual sensitivity was assessed by collecting the average of three brightness (A) and unpleasantness (B) ratings following visual stimulations with different luminous intensities (X-axis). At both 3 and 6 months, data from 13 responders and 14 non-responders were analysed. No significant differences were demonstrated in visual sensitivity between responders and non-responders at both time points.

Baseline levels of il-17A and TNF-α in responders and non responders to immunosuppressive agents at 3 and 6 months



Legend: The graphs illustrate the differences in baseline levels of the pro-inflammatory cytokines IL-17A and TNF-α between responders and non-responders to immunosuppressive agents. Response to treatment was evaluated after 3 (left) and 6 months (right). No statistically significant differences were found in the circulating levels of both cytokines at both time points (3 months and 6 months). Numbers at 3 months: TNF-α, responders n=4, and non-responders n=10. Numbers at 6 months: IL-17A, responders n=11, and non-responders n=9; TNF-α, responders n=5, and non-responders n=5.

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