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Most of all, I am indebted to my beloved wife Rashmi, my darling daughter Anushka, and my Mum for encouraging me, supporting me and always being there for me. I dedicate this thesis to them.
DECLARATION

In accordance with University of Bath regulation 16.5 (l) (PhD), this thesis is the result of work done by the author, who is registered as a candidate for the Degree of Doctor of Philosophy at the University of Bath. Where others have contributed data or assisted with data analyses they are acknowledged accordingly in each thesis chapter.
Psoriatic arthritis (PsA) is a chronic heterogenous inflammatory arthritis with five phenotypes. The two least studied phenotypes are investigated in this thesis, including: psoriatic spondyloarthropathy (PsSpA) and psoriatic arthritis mutilans (PAM). The aims of this thesis were to determine the prevalence, clinical characteristics and radiographic characteristics of PsSpA and PAM in a cohort of PsA patients, and serum-soluble bone-turnover biomarkers of these phenotypes. Comparisons were made with PsA patients without axial disease (pPsA), and ankylosing spondylitis (AS) patients.

A prospective single-centre cross-sectional study was conducted of PsA and AS patients. Serum on psoriasis-only patients (PsC) and healthy controls (HC) were also obtained. Multivariate clinical, radiographic, genetic and serum biomarker comparisons were made between these five groups of subjects.

The study enrolled 201 PsA and 201 AS patients, who were then reclassified as 118 PsSpA, 127 pPsA and 157 AS cases, alongside 200 PsC and 50 HC subjects. Several clinical biomarkers, imaging biomarkers, serum-soluble biomarkers and genetic biomarkers were identified that differentiate PsSpA from pPsA and AS. PsSpA affected a significant proportion of PsA patients, and was not a milder version of AS. PsSpA involvement was as disabling and clinically impactful as AS. PAM was found to be associated with PsSpA, and clinical biomarkers of PAM occurrence and radiographic progression were identified.

In conclusion, this thesis indicates that PsSpA is on a spectrum of musculoskeletal disease, in between pPsA and AS; with PsSpA comprising a continuum itself, and with a phenotype expression related to disease duration. These findings may prompt the inception of an international-consensus classification system for PsSpA, for which there is a great clinical need. Given that PsSpA has its own discrete clinical and biomarker signature, its clinical management and research should be tailored from that of pPsA and AS. Ultimately this may further the effort for stratified and personalised medicine.
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ADIPSA</td>
<td>Axial Disease In Psoriatic Arthritis Study</td>
</tr>
<tr>
<td>IRR&lt;sub&gt;adj&lt;/sub&gt;</td>
<td>adjusted incidence risk ratio</td>
</tr>
<tr>
<td>OR&lt;sub&gt;adj&lt;/sub&gt;</td>
<td>adjusted odds ratio</td>
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<tr>
<td>ALP</td>
<td>bone alkaline phosphatase</td>
</tr>
<tr>
<td>AN</td>
<td>Alison Nightingale</td>
</tr>
<tr>
<td>AP</td>
<td>antero-posterior</td>
</tr>
<tr>
<td>AS</td>
<td>ankylosing spondylitis</td>
</tr>
<tr>
<td>ASDAS</td>
<td>Ankylosing Spondylitis Disease Activity Score</td>
</tr>
<tr>
<td>BASDAI</td>
<td>Bath Ankylosing Spondylitis Disease Activity Index</td>
</tr>
<tr>
<td>BASFI</td>
<td>Bath Ankylosing Spondylitis Functional Index</td>
</tr>
<tr>
<td>BASMI</td>
<td>Bath Ankylosing Spondylitis Metrology Index</td>
</tr>
<tr>
<td>BASRI</td>
<td>Bath Ankylosing Spondylitis Radiology Index</td>
</tr>
<tr>
<td>BIRD</td>
<td>Bath Institute for Rheumatic Diseases</td>
</tr>
<tr>
<td>BMP</td>
<td>bone morphogenetic protein</td>
</tr>
<tr>
<td>CASPAR</td>
<td>Classification for Psoriatic Arthritis criteria</td>
</tr>
<tr>
<td>COMP</td>
<td>cartilage oligomeric matrix protein</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>CTX-1</td>
<td>cross-linked telopeptide of collagen-1</td>
</tr>
<tr>
<td>CV%</td>
<td>co-efficient of variation percentage</td>
</tr>
<tr>
<td>DJ</td>
<td>Deepak Jadon</td>
</tr>
<tr>
<td>Dkk-1</td>
<td>dickkopf 1</td>
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<tr>
<td>DMARD</td>
<td>disease modifying anti-rheumatic drug</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<tr>
<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>ERAP1</td>
<td>endoplasmic reticulum aminopeptidase 1</td>
</tr>
<tr>
<td>eSpA</td>
<td>enteropathic spondyloarthritis</td>
</tr>
<tr>
<td>ESR</td>
<td>erythrocyte sedimentation rate</td>
</tr>
<tr>
<td>ESSG</td>
<td>European Spondyloarthropathy Study Group</td>
</tr>
<tr>
<td>GR</td>
<td>Graham Robinson</td>
</tr>
<tr>
<td>GRAPPA</td>
<td>Group for Research and Assessment of Psoriatic Arthritis</td>
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<tr>
<td>HAQ</td>
<td>Health Assessment Questionnaire</td>
</tr>
<tr>
<td>HC</td>
<td>healthy control</td>
</tr>
<tr>
<td>HCEBC</td>
<td>Illumina Infinium HumanCoreExome BeadChip</td>
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<tr>
<td>HLA</td>
<td>human leucocyte antigen</td>
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<tr>
<td>ICC</td>
<td>intra-class correlation coefficients</td>
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<td>IQR</td>
<td>inter-quartile range</td>
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<tr>
<td>Abbreviation</td>
<td>Term</td>
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<tr>
<td>IRR</td>
<td>incidence risk ratio</td>
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<td>LEI</td>
<td>Leeds Enthesitis Index</td>
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<tr>
<td>LOPAS</td>
<td>Long-term Outcomes in Psoriatic Arthritis Study</td>
</tr>
<tr>
<td>M-CSF</td>
<td>macrophage colony- stimulating factor</td>
</tr>
<tr>
<td>MASES</td>
<td>Maastricht Ankylosing Spondylitis Enthesitis Score</td>
</tr>
<tr>
<td>MICD</td>
<td>minimum important clinical difference</td>
</tr>
<tr>
<td>MMP-3</td>
<td>matrix metalloproteinase</td>
</tr>
<tr>
<td>mNAPSI</td>
<td>modified Nail Psoriasis Severity Index</td>
</tr>
<tr>
<td>mNY</td>
<td>modified New York criteria for the diagnosis of ankylosing spondylitis</td>
</tr>
<tr>
<td>mSASSS</td>
<td>modified Stoke Ankylosing Spondylitis Scoring tool</td>
</tr>
<tr>
<td>NSAIDs</td>
<td>non-steroidal anti-inflammatory drugs</td>
</tr>
<tr>
<td>OC</td>
<td>osteocalcin</td>
</tr>
<tr>
<td>OD</td>
<td>optical density</td>
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<tr>
<td>OPG</td>
<td>osteoprotegerin</td>
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<tr>
<td>OR</td>
<td>odds ratio</td>
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<tr>
<td>PAM</td>
<td>Psoriatic arthritis mutilans</td>
</tr>
<tr>
<td>PASI</td>
<td>Psoriasis Area and Severity Index</td>
</tr>
<tr>
<td>PASRI</td>
<td>Psoriatic Arthritis Spondylitis Radiology Index</td>
</tr>
<tr>
<td>PIIINP</td>
<td>amino-terminal propeptide of procollagen type III</td>
</tr>
<tr>
<td>PROM</td>
<td>patient-reported outcome measure</td>
</tr>
<tr>
<td>PsA</td>
<td>psoriatic arthritis</td>
</tr>
<tr>
<td>PsC</td>
<td>psoriasis cutaneous (without arthritis)</td>
</tr>
<tr>
<td>PsSpA</td>
<td>psoriatic spondyloarthritis</td>
</tr>
<tr>
<td>RA</td>
<td>rheumatoid arthritis</td>
</tr>
<tr>
<td>RAD</td>
<td>radiographic axial disease</td>
</tr>
<tr>
<td>RANKL</td>
<td>receptor activator of nuclear factor-kappa B ligand</td>
</tr>
<tr>
<td>reSpA</td>
<td>reactive spondyloarthritis</td>
</tr>
<tr>
<td>RNHRD</td>
<td>Royal National Hospital for Rheumatic Diseases</td>
</tr>
<tr>
<td>RR</td>
<td>relative risk</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SIJ</td>
<td>sacroiliac joint</td>
</tr>
<tr>
<td>SpA</td>
<td>spondyloarthritis</td>
</tr>
<tr>
<td>TRAIL</td>
<td>tumor necrosis factor-related apoptosis-inducing ligand</td>
</tr>
<tr>
<td>VAS</td>
<td>visual analogue scale</td>
</tr>
<tr>
<td>95% CI</td>
<td>95% confidence interval</td>
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</table>
PUBLICATIONS AND CONFERENCE CONTRIBUTIONS
 ARISING FROM THE WORK OF THIS THESIS

PEER-REVIEWED PAPERS


BOOK CHAPTERS


CONFERENCE ABSTRACTS (oral presentation)


CONFERENCE ABSTRACTS (poster presentation)


PATIENT LEAFLET

Jadon D, McHugh N. 2013 Psoriatic Arthritis: Did you know? *Psoriasis and Psoriatic Arthritis Alliance (PAPAA)*. London: PAPAA.

PRIZES

June 2015 Royal Society of Medicine, Eric Bywaters Rheumatology Research Prize (for the Axial Disease in Psoriatic Arthritis, ADIPSA study)

Jan 2015 Royal National Hospital for Rheumatic Diseases, Researcher of the Year Award

Sept 2013 Paediatric Rheumatology Europe (PReS), Young Investigator Award (2nd place)

May 2012 Bath Institute for Rheumatic Diseases, Davies-Maitland Scholarship (2nd place)

May 2012 Royal National Hospital for Rheumatic Diseases, Researcher of the Year Award (nomination)

OTHER PUBLICATIONS ACHIEVED DURING THIS PERIOD OF RESEARCH RELATED TO THE THEME OF SPONDYLOARTHRITIS

PEER-REVIEWED PAPERS


**CONFERENCE ABSTRACTS (oral presentation)**


**CONFERENCE ABSTRACTS (poster presentation)**


1 CHAPTER I: INTRODUCTION

This thesis aims to determine biomarkers of psoriatic arthritis (PsA) phenotypes. In particular, I will investigate serum-soluble bone-turnover biomarkers of both occurrence and severity of two subsets of PsA: psoriatic spondyloarthritis (PsSpA) and psoriatic arthritis mutilans (PAM).

The aims will be achieved through a review of the current literature, followed by the enrolment and clinical assessment of a cohort of patients as part of a prospective cross-sectional single-centre observational study.

PsA, PsA subsets, related conditions including ankylosing spondylitis (AS), and the systems that have been devised and employed to classify these clinically overlapping conditions will be described briefly in this chapter. Biomarker research is currently very topical in rheumatology research globally; so the importance and utility of biomarkers in PsA will also be outlined here. More detailed descriptions of each sub-study are provided in related chapters, that combine the thesis.

1.1 PSORIATIC ARTHRITIS

PsA is a chronic inflammatory musculoskeletal disease usually accompanied by cutaneous psoriasis. PsA has clinical, radiological and immuno-pathogenic features distinct to other forms of arthritis.

Psoriasis is a chronic inflammatory skin condition characterised by red scaly patches commonly on the elbows, knees, trunk and scalp. There are several potential reports of cutaneous psoriasis in the historical literature, but it is difficult to know for certain since psoriasis can be confused with leprosy. For example, the Ebers papyrus found in Luxor in 1873, and written in the 15th century B.C., cites numerous skin diseases, and uses the term Šuf.t (translated as ‘scale’) [1]. The Charaka Samitha, a book from Indian ayurvedic medicine, describes a disease named ‘khusta’, which has been proposed as being either psoriasis or leprosy [2]. Hippocrates in the 5th century B.C., used the word ‘psora’ for itchy lesions of the eyelids and genitals, and for which he recommended tars and climate as treatment [3]. The most convincing earliest report of psoriasis came in the 1st century A.D. series of books by A. Cornelius Celsus, De re Medica Libri Octo [4]. He described psoriasis as impetigo appearing on the skin of the extremities and nails, and managed with medications containing pitch and sulphur.
The association of inflammatory arthritis and cutaneous psoriasis is accredited to a report in 1822 by a French dermatologist, Jean-Louis Alibert, working at the Hospital of St. Louis in Paris dedicated to skin diseases [5] (Figure 1.1). The condition was later termed ‘Psoriasis Arthritique’ in 1860 by another French dermatologist in Paris, Pierre-Antoine-Ernest Bazin [6] (Figure 1.1). Only in 1964, with the advent of rheumatoid factor (RF) testing, and the observation that RF was rarely present in PsA patients, was the condition first recognised by the Arthritis and Rheumatism Association of North America [7], enabling it to progressively gain global recognition.

Figure 1.1. Founding fathers of the study of psoriatic arthritis
Jean-Louis Alibert (1768-1837) [left], and Pierre-Antoine-Ernest Bazin (1807-1878) [right]

1.1.1 Epidemiology of PsA

PsA affects women and men equally [8-11], and tends to present between the ages of 30 and 50 (median 48) years [11]. In the U.K. population, the prevalence of psoriasis is estimated to be 2 per 100 of the population [12], and the prevalence of PsA to be 1 - 2.5 per 1000 of the population [9, 13]. The prevalence of PsA among psoriasis cases in two large primary care cohorts in the U.K. was 13.8% [10]. The incidence of PsA in the general population is estimated to be 6 cases per 100,000 per year [9, 13-15]. A prospective study of 313 psoriasis patients without arthritis (PsC) followed over four years, found an annual incidence of newly diagnosed arthritis of 1.87 (95% CI 0.71, 3.03) cases per 100 psoriasis cases per year [16]. Predictors of incident arthritis in PsC patients included the severity of psoriasis, the presence of nail lesions, and the presence of scalp and inter-gluteal lesions [16].
1.1.2 Presentation of PsA

PsA patients characteristically present with inflammatory early-morning musculoskeletal stiffness lasting at least thirty minutes, pain affecting peripheral joints (commonly hands, wrists, knees, ankles and feet), enthesitis (inflammation of tendon, ligament or joint capsule attachment to bone), tenosynovitis (inflammation of the sheath around tendons), inflammatory spinal pain (termed spondylitis), and dactylitis (inflammation of the joint, tendons, enthesis and subcutaneous tissues) [8]. Peripheral joints are often affected in an asymmetric manner [17].

Five distinct patterns of PsA presentation were originally described by Moll and Wright, based upon clinical and radiographic characteristics [18]:

- symmetric polyarthritis (five or more affected joints; 15% prevalence)
- asymmetric oligoarthritis (four or fewer affected joints; 70% prevalence)
- distal interphalangeal joint (DIPJ) predominant (affecting the joint at the end of fingers; 5% prevalence)
- psoriatic spondyloarthropathy (PsSpA; 5% prevalence)
- psoriatic arthritis mutilans (PAM; 5% prevalence)

Patients can migrate between subsets, or have two subsets concomitantly during the course of their disease [19]. Oligoarthritis and polyarthritis are the most common subsets of PsA [20], whilst PAM is the rarest albeit most destructive form of PsA. Peripheral PsA is characterised radiologically by articular (pertaining to the joint) bone destruction (erosion), articular cartilage destruction (joint space narrowing), and articular new bone formation (osteo proliferation).

Cutaneous psoriasis usually precedes the onset of arthritis in PsA (70%), but in 15% of individuals the arthritis precedes psoriasis, and in a further 15% of individuals psoriasis is present at the time of arthritis onset, but not detected by the clinician [8, 21]. PsA patients are prone to psoriatic nail disease (dystrophy) that is indistinguishable from that seen in those with psoriasis-only [22]. Of note, the prevalence of psoriatic nail dystrophy is more common in PsA (especially in those with DIPJ arthritis) than in psoriasis alone [23].

Other extra-articular manifestations can include uveitis and inflammatory bowel disease (IBD). Uveitis presents as a painful red eye, and is likely related to inflammation of the entheseseal structure within the eye. IBD is a chronic inflammatory condition of the gastrointestinal system manifesting as bloody diarrhoea and weight loss, with ulcerative colitis and Crohn’s disease being the two most common forms.
1.1.3 Investigation and diagnosis of PsA

There are no diagnostic criteria or laboratory tests specific to PsA. A diagnosis of PsA is usually made on the basis of an inflammatory arthritis accompanying psoriatic skin and / or nail disease, in a characteristic distribution, accompanied by enthesitis and / or tenosynovitis usually in the absence of rheumatoid factor.

The Classification for Psoriatic Arthritis (CASPAR) criteria were devised in 2006 to aid the classification of participants enrolled in research studies (Table 1.1) [24]. The CASPAR criteria comprise a stem mandating inflammatory peripheral / axial arthritis or enthesitis, accompanied by at least three of the following features: psoriasis (current psoriasis scoring two points, previous psoriasis scoring one point, or family history of psoriasis scoring one point); dactylitis; psoriatic nail dystrophy; negative rheumatoid factor serology; and / or osteoproliferation on peripheral radiographs. The sensitivity and specificity of the CASPAR criteria are 91.4% and 98.7%, respectively [24].

Table 1.1. CIAsification criteria for Psoriatic Arthritis (CASPAR) criteria
(from Taylor et al. 2005 [24])

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>i)</td>
<td>Evidence of current psoriasis, a personal history of psoriasis, or a family history of psoriasis. Current psoriasis is defined as psoriatic skin or scalp disease present today as judged by a rheumatologist or dermatologist.*</td>
</tr>
<tr>
<td></td>
<td>(A personal history of psoriasis is defined as a history of psoriasis that may be obtained from a patient, family physician, dermatologist, rheumatologist, or other qualified health care provider. A family history of psoriasis is defined as a history of psoriasis in a first- or second-degree relative according to patient report).</td>
</tr>
<tr>
<td>ii)</td>
<td>Typical psoriatic nail dystrophy including onycholysis, pitting, and hyperkeratosis observed on current physical examination.</td>
</tr>
<tr>
<td>iii)</td>
<td>A negative test result for the presence of rheumatoid factor by any method except latex but preferably by enzyme-linked immunosorbent assay or nephelometry, according to the local laboratory reference range.</td>
</tr>
<tr>
<td>iv)</td>
<td>Either current dactylitis, defined as swelling of an entire digit, or a history of dactylitis recorded by a rheumatologist.</td>
</tr>
<tr>
<td>v)</td>
<td>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.</td>
</tr>
</tbody>
</table>

* Current psoriasis is assigned a score of 2; all other features are assigned a score of 1.
1.1.4 Management of PsA

Patients with mild PsA are managed with non-steroidal anti-inflammatory drugs (NSAIDs), simple analgesia, physiotherapy, occupational therapy, education and support. Moderate to severe PsA cases require more intensive therapy with synthetic (e.g. methotrexate, sulfasalazine, and leflunomide) and/or biologic (anti-tumour necrosis factor-alpha drugs, anti-TNF; and interleukin 23 / 12 inhibitors) disease modifying anti-rheumatic drugs (DMARDs) [25, 26].

1.1.5 Prognosis of PsA

As there is no cure for PsA, long-term pharmacologic and physical therapies are required to slow and/or halt the progression of disease [25]. Two years after the diagnosis of PsA, 47% of cases are reported to have peripheral radiographic erosions, and 56% require the use of DMARDs [8]. In another study, over a period five year from presentation, the majority of cases showed progression in the number of joints affected and 68% had peripheral radiographic erosions [19]. In a study from Toronto (Canada) of 391 PsA cases, 18% achieved remission with use of DMARDs; remission lasted a mean of 2.9 (standard deviation, SD 1.7) years, but the majority (52%) of patients had a relapse within a mean of 1.8 (SD 1.3) years [27]. Predictors of severe peripheral disease in PsA include: female sex [28, 29]; high acute phase response at diagnosis or during disease course [19, 30]; high number of actively inflamed joints at presentation [29, 31, 32]; and a delay in receiving specialist care of greater than two years [33].

There is emerging evidence that patients with PsA are at increased risk for cardiovascular disease and/or death. One study reported a significant increased risk of myocardial infarction (standardised prevalence ratios, SPR 2.57; 95% CI 1.73, 3.80), angina (SPR 1.97; 95% CI 1.24, 3.12), and hypertension (SPR 1.90; 95% CI 1.59, 2.27), in patients with PsA compared with the general population, particularly in those with more severe cutaneous psoriasis, diabetes, and dyslipidaemia [34]. The risk of cardiovascular-related death in PsA compared with the general population is less certain, with conflicting reports. Conflicting data exist concerning the association between PsA and increased mortality [9, 35, 36]. There was no significant difference in mortality risk in a cohort of 453 patients with PsA followed in a single-centre in Bath (UK) compared with the general population of the UK (standardised mortality ratio 81.82; 95% CI 57.61, 112.78) [35].

Work disability is high in patients with PsA. A recent systematic literature review found that levels of unemployment range between 20 to 50% and work disability ranges from 16
to 39%, and can be in the form of absenteeism (unable to attend work due to symptoms related to PsA) and presenteeism (present at work but not working to full capacity due to symptoms related to PsA) [37]. Several factors have been shown to be associated with work disability in PsA, including longer disease duration, worse physical function, high joint count, low educational level, female gender, erosive disease and manual work [37]. The same systematic review identified some evidence that work disability is worse in those with PsA than those with psoriasis alone [37]. A large prospective multi-centre UK study of work disability reported that reduced effectiveness at work in PsA patients was associated with measures of disease activity, whilst unemployment was associated with employer factors, age and disease duration [38].

1.2 SPONDYLOARTHRITIS

Spondyloarthritis (SpA) is the term encompassing a family of conditions with shared clinical manifestations, and is characterised by inflammatory axial disease, extra-articular manifestations, and often peripheral arthritis. Axial structures include the spine, sacroiliac joints (SIJs) and pelvis. Inflammatory axial disease manifests as inflammatory back pain and stiffness that is worse in the morning, exacerbated by rest, and improved by both activity and NSAIDs. SpA is characterised by inflammation of the SIJ(s) that bridge the sacrum (tail bone) and ilium of the pelvis (resulting in sacroiliitis), and / or the vertebral bodies (resulting in spondylitis). Sacroiliitis is inflammation of the lower third of the SIJ, where it is primarily a synovial joint. Spondylitis is inflammation of the interface between vertebral bodies and adjacent structures, such as intervertebral discs, ligaments and joint capsules. Inflammation of the SIJ(s) can result in osteoproliferation, with bridging of bone across the joint (ankylosis) leading, in severe cases, to fusion of the joints. Inflammation of the entheses can result in osteoproliferation at the corner of vertebral bodies, resulting in fine bone growths termed syndesmophytes (Figure 1.2). Both SIJ ankylosis and vertebral syndesmophytes result in pain, stiffness and restricted range of movement.
Conditions within the continuum of SpA include: PsA, ankylosing spondylitis (AS), reactive arthritis, spondyloarthritis (SpA) associated with inflammatory bowel disease (IBD), juvenile SpA and undifferentiated SpA (uSpA) (Figure 1.3).

**Figure 1.3. The spectrum of spondyloarthritis**

(including the overlapping clinical phenotype of psoriatic arthritis)
1.3 ANKYLOSING SPONDYLITIS

Ankylosing spondylitis (AS) is the prototypical SpA. AS usually presents in the second to fourth decade of life, and the prevalence of AS is approximately three times higher in males [40]. It has a strong genetic component, with up to 95% of patients carrying variants of the human leucocyte antigen (HLA)-B27 [41].

1.3.1 Presentation of AS

AS presents with inflammatory axial pain, early morning stiffness that can wake from sleep, restricted range of movement, sacroiliitis manifesting as alternating buttock pain, usually spondylitis manifesting as spinal pain, occasional peripheral oligoarthritis, and extra-articular manifestations such as enthesitis, uveitis (painful inflammation of the eye) and IBD [41, 42].

1.3.2 Investigation and diagnosis of AS

The modified New York (mNY) criteria for diagnosing AS were devised in 1984 [43]. These criteria comprise a stem requiring the exclusion of spinal fracture, intervertebral disc disease and fibromyalgia as the cause of axial symptoms. A patient must then have plain radiographic sacroiliitis (grade $\geq 3$ unilaterally or $\geq 2$ bilaterally) and $\geq 1$ of the following three clinical criterion: insidious onset inflammatory low back pain and stiffness of at least three months duration that improves with exercise and is not relieved by rest; limitation of motion of the lumbar spine in both the sagittal and frontal planes; and / or limitation of chest expansion relative to normal values correlated for age and sex [43].

1.3.3 Management of AS

AS is a chronic progressive condition, without cure, and managed primarily with physiotherapy, occupational therapy, hydrotherapy, NSAIDs, and anti-TNF therapy [40, 42].

Predictors of severe disease in AS include: axial syndesmophytes at presentation; high acute phase response at presentation; reduced range of movement of the lumbar spine; poor NSAID response; dactylitis or oligoarthritis; tobacco smoking; onset before the age of 16 years; and early hip involvement [44-47].
1.4 PSORIATIC SPONDYLOARTHRITIS

Psoriatic spondyloarthritis (PsSpA) is a more precise term for SpA accompanying psoriasis. PsSpA shares clinical features of both PsA and AS. PsSpA patients have predominantly axial symptoms, but a significant proportion have concomitant peripheral arthritis and enthesitis.

Some propose that isolated radiographic features alone may be sufficient to classify a patient as PsSpA [48], arguing that PsSpA patients often do not have a history of inflammatory axial symptoms [49]; in essence a symptomatically-silent form of radiographic-PsSpA.

The association between psoriasis and AS was first reported in 1928 by Zellner [50]. After a number of case series supporting this association, Fletcher and Rose coined the term 'psoriasis spondylitica' in the Lancet journal in 1955 [51]. In 1961 Wright reported that radiographic sacroiliitis was more common in PsA patients compared with RA patients [52]. Later the same year, Dixon et al. published a paper demonstrating a higher prevalence of radiographic sacroiliitis in PsA patients compared with a group of controls [53]. Three years later, Kaplan et al. added to the canon of knowledge with the observation that facet joint narrowing / sclerosis and anterior ligamentous calcification in the cervical spine were common in patients with psoriasis, even in those without peripheral arthritis [54]. They observed that changes were indistinguishable from AS, and that the lumbar spine and SIJs were not always affected. In 1965, Bywaters et al. proposed that PsSpA patients demonstrate para-marginal syndesmophytes (PMS; thorn-like and angulated), which are different in shape and location to the marginal syndesmophytes (MS; slim and vertical) seen in AS, and may therefore help distinguish PsSpA and AS radiographically [55].

Example plain radiographs from healthy individuals are shown in Figure 1.4, and pathological plain radiographs from patients with PsA and AS are shown in Figures 1.5, 1.6 and 1.7.
Figure 1.4. Normal radiographs of the axial skeleton
Cervical spine (lateral view) [top-left image], lumbar spine (antero-posterior and lateral views) [top-middle and top-right images] and sacroiliac joints (antero-posterior view) [bottom image]
Figure 1.5. Plain radiographs of the lateral cervical spine
Showing syndesmophytes in PsSpA (left & middle images; broad-based thorn-like & diagonal orientation) and AS (right image; narrow slim vertical orientation). The right image also shows multi-level flowing facet joint fusion in AS (diagonal arrow).

Figure 1.6. Plain radiographs of the lateral lumbar spine
Showing syndesmophytes in PsSpA (left & middle images; broad-based thorn-like & diagonal orientation) and AS (right image; narrow slim vertical orientation).
Figure 1.7. Plain radiographs of the sacroiliac joints (SIJs)
Showing: bilateral SIJ sclerosis and erosions in PsSpA [upper image]; and bilateral complete fusion of the SIJs in AS [lower image].
1.4.1 Classification of PsSpA and AS

The classification of PsSpA has been somewhat neglected, and there is no internationally-recognised consensus on the best classification to use either in clinical practice or for research studies. As is discussed below, this is not due to a lack of effort or recognition of the importance to do so, but is in large part due to a lack of research investigating the clinical, radiographic and biomarker characteristics of PsSpA, and how they compare with AS. Only through further research will it be possible to devise a robust classification system, for the benefit of patients clinically and for research.

The CASPAR classification criteria are used to classify PsA patients for research studies [24], and the 1984 modified New York (mNY) diagnostic criteria are used to diagnose AS [43]. However, no classification or diagnostic criteria exist for PsSpA, who are left to be captured by one, but sometimes neither of these two classification systems. The CASPAR classification system was devised using PsA cohorts with a predominantly peripheral phenotype (pPsA) [24]. Relatively little clinical and radiographic data was available in terms of the presence or absence of axial disease. As a result the CASPAR criteria perform well classifying pPsA patients (sensitivity 98.2%, specificity 99.1%) but did not have the remit to capture PsSpA patients [24]. It was assumed that such PsSpA patients would be captured by the mNY diagnostic criteria for AS. However, clinical experience and research studies show that PsSpA cases have a different clinico-radiographic expression to AS and may therefore not fulfil mNY criteria for AS [56-59]. Some PsSpA cases experiencing significant clinical impact from their axial disease therefore remain unclassified. This may have an impact on a patient’s management, especially once they have more active disease refractory to first and second-line treatment. For example, some PsA patients are denied escalation of therapy to anti-TNF therapy because they have predominantly axial symptoms, and do not fulfil the peripheral-weighted PsA criteria for anti-TNF, that requires them to have at least three tender or at least three swollen joints. Conversely, some AS patients are denied escalation of therapy to anti-TNF therapy because they have predominantly peripheral symptoms and do not fulfil the axial-weighted AS criteria for anti-TNF that require them to have a BASDAI of at least 4/10 and pain score of at least 4/10. In such circumstances exceptional funding for anti-TNF can be sought on named individual basis, but this may result in variability in the provision of anti-TNF between hospital and geographic regions.

Taylor et al. reviewed the differences between PsA, AS, enteropathic SpA (eSpA), and SpA related to reactive arthritis (reSpA) [57]. They proposed that both symptoms and radiographics (spine and SIJs) must be considered together to capture the totality of axial
manifestations in PsA. Unfortunately, studies of axial disease in PsA have classified PsSpA using a variety of systems. Some have classified patients as PsSpA based upon isolated unilateral grade $\geq 2$ sacroiliitis, whilst other studies have used a more stringent classification in keeping with that used to classify AS patients: radiographic sacroiliitis of grade $\geq 3$ unilaterally, or grade $\geq 2$ bilaterally. Some studies have classified patients as PsSpA based upon symptomatic back pain with restricted range of movement, one isolated syndesmophyte, or a combination of clinical and radiographic features [48]. Some studies have used the European Spondyloarthropathy Study Group (ESSG) criteria [60]. The ESSG criteria require: inflammatory spinal pain or synovitis (asymmetric or predominantly in the lower limbs), together with at least one of the following: positive family history, psoriasis, inflammatory bowel disease, urethritis, or acute diarrhea, alternating buttock pain, enthesopathy, or sacroiliitis as determined from radiography of the pelvic region. Whilst the ESSG criteria have a sensitivity of 87% and a specificity of 87% for SpA [60], they have not shown good specificity or sensitivity for PsSpA [24, 57].

If the classification employed for PsSpA is that of the 1984 modified New York criteria, which requires radiographic sacroiliitis, then PsSpA cases with vertebral spondylitis but no sacroiliac changes, would be overlooked. Taylor et al. (2004) have proposed vertebral spondylitis without sacroiliitis to be a feature of PsSpA distinguishing it from AS [56].

One cannot robustly compare the severity of PsSpA and AS if the classification systems for each are not the same and restricted in certain ways. A study classifying PsSpA as unilateral sacroiliitis of grade $\geq 2$ and / or $\geq 1$ syndesmophyte of the spine, whilst classifying AS as bilateral sacroiliitis grade $\geq 2$, or unilateral sacroiliitis grade $\geq 3$, will by the entry criteria show PsSpA cases to be less radiographically severe than AS, and possibly clinically less severe as well. The most sensitive, specific and / or accurate classification system for PsSpA may be determined once we know: the proportion of psoriatics with unilateral grade 2 sacroiliitis demonstrating symptomatic and / or metrological evidence of axial disease; the proportion of such patients progressing to develop more extensive sacroiliitis or spondylitis; and the prevalence of grade 2 sacroiliitis in an otherwise healthy population [48]. This information may help determine whether unilateral grade 2 sacroiliitis is a ‘pre-PsSpA’, or simply an acceptable normal variant within the general population [48].

The different systems that have been used in studies to classify PsA patients as PsSpA, with decreasing order of stringency, are summarised in Table 1.2.
### Table 1.2. Classification systems for psoriatic spondyloarthritis

<table>
<thead>
<tr>
<th>Study</th>
<th>Classification criteria for psoriatic spondyloarthritis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Helliwell et al. 1998 [58]</td>
<td>Cutaneous psoriasis and fulfilling 1984 modified New York diagnostic criteria for AS [43] (sacroiliitis of grade ≥2 bilaterally or grade ≥3 unilaterally; with decreased range of movement in two planes and inflammatory back pain)</td>
</tr>
<tr>
<td>Chandran et al. 2010 [61]</td>
<td>Radiographic sacroiliitis (grade ≥2 bilaterally, or grade ≥3 unilaterally), with inflammatory spinal pain and / or limited spinal mobility.</td>
</tr>
<tr>
<td>Gladman et al. 1993 [49]</td>
<td>Inflammatory back pain / stiffness; and / or sacroiliitis on clinical examination (Gaenslen test, Patrick-FABER test, or direct compression); and / or grade ≥2 sacroiliitis; and / or ≥1 paramarginal syndesmophyte.</td>
</tr>
<tr>
<td>Hanly et al. 1988 [63]</td>
<td>Either inflammatory pain / stiffness of the spine, and / or clinical sacroiliitis disease (Gaenslen test, Patrick-FABER test, or direct compression), and / or sacroiliitis of grade ≥2 unilaterally.</td>
</tr>
<tr>
<td>Fernandez-Sueiro et al. 2009 [64]</td>
<td>Spinal symptoms (defined as a combination of inflammatory back pain plus back stiffness), and radiographic sacroiliitis grade ≥2 unilaterally.</td>
</tr>
<tr>
<td>Battistone et al. 1999 [65]</td>
<td>Radiographic sacroiliitis grade ≥2 unilaterally.</td>
</tr>
<tr>
<td>Lubrano et al. 2009 [12]</td>
<td>Meet CASPAR criteria for PsA [24], and the presence of clinical (axial inflammatory pain according to the Calin criteria [13]) and / or radiographic axial disease.</td>
</tr>
<tr>
<td>Queiro et al. 2002 [66]</td>
<td>Spondyloarthritis according to the European Spondyloarthropathy Steering Group (ESSG) criteria, plus radiographic sacroiliitis. ESSG criteria require: inflammatory spinal pain or synovitis, together with at ≥1 of the following: positive family history, psoriasis, inflammatory bowel disease, urethritis, or acute diarrhea, alternating buttock pain, enthesopathy, or sacroiliitis as determined from radiography of the pelvic region [60].</td>
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</tbody>
</table>

#### 1.4.2 Prevalence of PsSpA in PsA cohorts

The prevalence of PsSpA in PsA cohorts varies markedly from 25 to 70%, and is likely related to a number of factors. As described above, a number of different classification systems for PsSpA have been employed; some less stringent than others. Most of the studies have recruited participants of Caucasian ancestry, and only a few studies have recruited non-Caucasian patients, resulting in confounding; *HLA-B27* variants are known to vary in populations of different ancestry, and *HLA-B27* is important in the aetiology of PsSpA [62, 67]. Study design and sampling methods might also explain the wide estimate of prevalence. PsSpA is known to manifest several years after PsA onset, and studies have reported cohorts with quite different disease duration at assessment [49, 68]. Another reason is selection bias, e.g. specialist cohorts in tertiary referral centres having more severe or complex disease, compared with secondary-care centres.

A study of 75 PsA patients from Bath (U.K.) published in 1994 found a high prevalence of cervical spine involvement: 45% of those examined and 36% of patients with radiographs [68]. Eighty five percent had a pattern of disease comprising of ankylosis, syndesmophytes and / or ligamentous ossification. ‘Silent’ radiographic cervical
spondylitis without clinical (symptomatic or examination) evidence of disease was present in 12% of cases.

1.4.2.1 Prevalence of PsSpA in PsA cohorts using the 1984 mNY criteria for AS

Studies classifying PsA cases as PsSpA using the mNY criteria for AS, have relatively consistent estimates of PsSpA prevalence. In a prospective cohort study by Chandran et al. (2010) of 206 PsA cases with a mean disease duration of 6.6 years at their first clinic visit, 50/206 (24%) were deemed to have PsSpA [61]. PsA patients were classified as PsSpA if they had radiographic sacroiliitis (grade ≥2 bilaterally, or grade ≥3 unilaterally), with inflammatory spinal pain and/or limited spinal mobility. In a cross-sectional study of 125 PsA cases attending a tertiary referral hospital in Hong Kong (2011), a similar proportion (29/125; 24%) were classified as PsSpA using the same radiographic criteria, although did not include clinical criteria [62].

An earlier study by Hanly et al. of 220 PsA cases in Toronto (Canada), demonstrated a prevalence of PsSpA of 52/220 (24%) at entry to the cohort [63]. In addition, they found that the prevalence increased to 194/377 (52%) as the cohort was followed over a mean of 57 months [49].

1.4.2.2 Prevalence of PsSpA in PsA cohorts using less stringent criteria

When less stringent criteria are used, especially those based on more subjective patient-reported or clinical-examination rather than more robust imaging criteria, the estimated prevalence of PsSpA in PsA cohort varies more markedly. In a study of 130 PsA cases the prevalence of PsSpA was 40%, and the prevalence of sacroiliitis was 21% [69]. The definition of PsSpA was not detailed in the manuscript, and the paper was published in 1977, prior to the advent of the 1984 modified New York criteria for AS. In addition, fewer than half the cohort (51/130) had a complete set of axial radiographs for review. Similarly, in a study of 498 PsA cases from Naples (Italy) published in 1988 [70], the prevalence of PsSpA was only 43/498 (11%) cases, defined clinically on the basis of Bennett and Wood’s suggestion [71]. The disease duration of these PsA patients was not stated, which is important, as others have shown that the prevalence of axial disease in PsA increases with increasing disease duration [49, 68].

A multi-centre study recruiting patients from North America as part of a randomised placebo-controlled trial comparing the efficacy of sulfasalazine for SpA, published a post-
hoc analysis in 1999 of 221 PsA cases [65]. Pelvic radiographs, performed in all patients but interpretable in 202/221, were assessed by a blinded musculoskeletal radiologist for sacroiliitis using a less stringent definition (grade ≥2 unilaterally). Sacroiliitis was identified in 78% (157/202) of PsA cases. The prevalence of sacroiliitis decreased to 55% (112/202) when a more stringent definition was used (grade ≥3 unilaterally, or grade ≥2 bilaterally). Comparisons were made with other clinical parameters, using the less stringent definition of sacroiliitis, and likely explain the relatively low frequency of HLA-B27 positivity (17.2% of cases). However, a relatively high frequency of current inflammatory axial night pain (85%) was reported. The estimated prevalence of sacroiliitis of 55% is likely higher than reported by other studies because these PsA patients had more established disease (mean disease duration of 12 years) than those in other studies.

In a cross-sectional Spanish study of 100 PsA and 103 AS cases, patients were classified as having PsSpA based on grade ≥2 unilateral sacroiliitis in the presence of spinal symptoms (defined as a combination of inflammatory back pain plus back stiffness) [64]. Using this less stringent criteria for PsSpA, 46/100 (46%) were classified as PsSpA, and 54/100 as pPsA. Four PsA cases (4/100; 4%) were classified as having ‘pure-PsSpA’, without current peripheral arthritis. However, since a history of peripheral arthritis was not assessed by the investigators, the concept of ‘pure-PsSpA’ in this study was not robust and is therefore not a reliable estimate.

1.5 PSORIATIC ARTHRITIS MUTILANS

Psoriatic arthritis mutilans (PAM) is the rarest, albeit most destructive subtype of PsA. PAM is characterised by severe destruction of the joint and adjacent bone through erosion (small-scale destruction of bone) and subsequent osteolysis (large-scale destruction of bone along its length). As a result of lost bone stock and soft-tissue laxity, fingers and toes can shorten, giving rise to thick transverse folds of skin, and digits that can be elongated with traction [72] (Figures 1.8, 1.9 and 1.10). The prevalence of PAM is estimated to be 5 per 100 PsA cases [14, 18, 73, 74]. The clinical and radiographic characteristics of PAM are discussed in more detail in Chapter 7 of this thesis.

Given the significant bone destruction (resorption) and paradoxical osteoproliferation seen in PAM, I hypothesise that serum-soluble biomarkers of bone resorption and / or formation may associate with this extreme subphenotype of PsA. Since PAM is a rare subphenotype within PsA, and from clinical experience appears to be active for a relatively short period of time, followed by a protracted inactive ‘burnt-out’ state, it can be difficult to capture and
investigate. Most research cohorts of PsA around the world are relatively small, perhaps <200 cases, so few PAM cases are available for research. As a result, studies of PAM to date have been cross-sectional, with no longitudinal data [73-82]. Additionally, the natural clinical and / or radiographic history of PAM have never been described.

Figure 1.8. Psoriatic arthritis mutilans - clinical images
(Ventral [upper image] and dorsal [lower image] views of the hands of a patient with psoriatic arthritis mutilans demonstrating classical digit deformity and shortening, with secondary transverse fold of skin and soft-tissue)
Figure 1.9. Psoriatic arthritis mutilans of the hands - radiographic images
(Characterised by osteolysis, erosion and joint space narrowing resulting in loss of bone stock, shortened and deformed digits, and the characteristic pencil-in-cup deformity affecting both thumbs)

Figure 1.10. Psoriatic arthritis mutilans of the feet - radiographic images
(Characterised by osteolysis, erosion and joint space narrowing resulting in loss of bone stock, shortened and deformed digits. Classical pencil-in-cup deformity affecting several toes are seen, and several phalanges have been entirely resorbed)
1.6 SERUM-SOLUBLE BIOMARKERS IN PSORIATIC ARTHRITIS

Relatively little is known about the utility of serum-soluble bone and cartilage-turnover biomarkers in PsA, PsSpA or PAM. Given that PsA is characterised by osteoproliferation and bone / cartilage destruction, it seems plausible that biomarkers of their turnover may have value in monitoring disease activity, contributing to a diagnosis, and / or indicating prognosis. As in other diseases, serum-soluble biomarkers may potentially inform stratified medicine approaches. However, identifying and monitoring biomarkers in PsA is difficult due to the heterogeneity of PsA disease. In PsA, bone loss can occur in the form of bone erosion, osteolysis, and bone mineral density loss [83]. Bone formation can occur in the form of osteoproliferation, ankylosis and / or syndesmophytes.

Several bone and cartilage turnover biomarkers might be of interest in PsA. Some directly cause bone resorption by virtue of their enzymatic or cytokine properties, e.g. matrix metalloproteinase-3 (MMP-3) enzymatically degrades the extra-cellular matrix of bone and cartilage [84]. Osteoprotegerin (OPG) is a glycoprotein secreted by osteoblasts and stromal cells, acting as a decoy receptor to receptor activator of nuclear factor-kappa B ligand (RANKL), thereby inhibiting osteoclastogenesis, resulting in reduced bone resorption. Others are by-products of bone resorption, thereby acting as markers of the process, e.g. cross-linked telopeptide of collagen-1 (CTX-1) is the product of excess metalloproteinase degradation of type 1 collagen. There are several by-products of cartilage turnover: C2C and C1-2C are neoepitopes that are released when type 2 cartilage is degraded by collagenases; C-propeptide of type II collagen (CPII) is released during pro-collagen 2 synthesis; and CPII:C2C is the ratio of cartilage degradation versus by-product formation [85].

Serum-soluble bone-turnover biomarkers have shown some utility in AS. Low levels of sclerostin have been shown to be associated with syndesmophyte formation in AS [86], and MMP-3 appears to correlate with radiographic progression [87]. MMP-3 and OPG have been shown to be elevated in PsA patients [88]. Dickkopf 1 (Dkk-1) suppresses bone formation by interfering with the Wnt pathway, and blockade of Dkk-1 has been shown to lead to sacroiliac joint fusion in mouse models [89].

A full systematic literature review of serum-soluble bone and cartilage-turnover biomarkers in PsA, PsSpA and PAM is presented in Chapter 2 of this thesis, and will therefore not be repeated here.
1.7 GENETICS OF PSORIATIC ARTHRITIS

PsA has a strong genetic component to its aetiology, and is likely a polygenic disease. It is known to aggregate in families, being 55-fold more frequent if a first degree relative is affected by PsA [90]. Human leucocyte antigen (HLA) -B27 [66], HLA-Cw*06:02 [91], interleukin 12B (IL12B) [92, 93], interleukin 23 receptor (IL23R) [92, 94], interleukin 23A (IL23A) [95], late cornified envelope gene cluster [96], TRAF3IP2 [97], and TNIP1 [95] genes among others, have shown association with PsA.

Unlike other gene polymorphisms, HLA-B27 [98-100] and interleukin 13 (IL13) [101] polymorphisms have shown association with PsA, independently of psoriasis. Three haplotypes containing HLA-B*27:05 or HLA-B*39:01 were significantly increased in frequency in PsA but not psoriasis cases [100]. A similarly designed study in an independent cohort confirmed several HLA alleles to be significantly associated with PsA compared with psoriasis: HLA-B*08 (OR 1.61; 95% CI 1.12, 2.30; p=0.009), HLA-B*27 (OR 5.17; 95% CI 2.95, 9.04; p<0.0001), and HLA-B*38 (OR 1.65; 95% CI 1.06, 2.57; p=0.03) [102]. Conversely, HLA-C*06:02 is more associated with psoriasis than PsA (frequency 57.5 vs. 28.7%; p=9.9x10^-12) [100]. The same study showed that HLA-C*06 is associated with more penetrant psoriasis and delayed musculoskeletal phenotype (interval ten years between skin and articular manifestations) [100]. In contrast HLA-B alleles (especially HLA-B*27) are associated with coincident skin and musculoskeletal manifestations (interval one year), and almost equivalent penetrance of musculoskeletal and psoriasis [100].

AS is strongly associated with HLA-B27, but its role in PsA has not been precisely defined [79, 103]. As in AS, the exact mechanism of the association between HLA antigens and PsA has not been established. HLA polymorphisms may result in altered antigen presentation to T-cells, misfolding of the HLA-molecule resulting in altered morphology, altered intracellular peptide handling by aminopeptidases, or simply be in linkage disequilibrium with a pathogenic susceptibility gene at another locus [104]. Allotypes of HLA-B*38:01 and HLA-B*08 encoding P2 pockets that bind side chains opposite in charge from those encoded by the HLA-B*27 and HLA-B*39 molecules may exert a ‘protective’ role [100].

1.7.1 Genetic markers of PsA phenotypes

In 1974 Brewerton et al. first reported an association between HL-A 27(W27) and PsA [105]. In a cross-sectional study of 70 PsA cases, 56% of patients with isolated axial PsA
were HLA-B27 positive, compared with 24% of polyarthritic-axial PsA and 31% with oligoarthritic-axial PsA [66]. HLA-B27 positive cases tended to be younger at psoriasis onset and arthritis onset, and were more likely to be male, have bilateral sacroiliitis, and have uveitis. A separate study published recently of 282 PsA patients compared HLA-B and HLA-C sequencing data with clinical phenotype in PsA [106]. Enthesitis (OR 3.7; 95% CI 1.9, 7.1; p<0.001), dactylitis (OR 2.5; 95% CI 1.2, 5.0; p=0.009) and symmetric sacroiliitis (atypical for PsA) (OR 10.6; 95% CI 3.8, 29.0; p<0.0001) were more common in patients carrying variants of HLA-B*27:05:02. Joint fusion (OR 2.0; 95% CI 1.2, 3.4; p=0.006), joint deformities (OR 2.0; 95% CI 1.2, 3.7; p=0.004), and dactylitis (OR 1.8; 95% CI 1.1, 2.9; p=0.03) were more common in patients carrying the HLA-B*08:01:01-C*07:01:01 haplotype and its component alleles. Asymmetrical sacroiliitis (OR 0.45; 95% CI 0.21, 0.98; p=0.04) was less frequent in patients carrying HLA-C*06:02:01 variants. HLA-B*44:02:01 was associated with a lower frequency of joint fusion (OR 0.21; 95% CI 0.07, 0.60; p=0.002), and dactylitis (OR 0.37; 95% CI 0.20, 0.70; p=0.003). Of note for the aims of this thesis, signals were present for osteolysis (a marker of PAM) to be more frequent in patients carrying HLA-C*02:02:02 variants (OR 3.1; 95% CI 1.2, 8.1; p=0.02).

1.7.2 Prevalence of HLA-variants in PsSpA

Several studies report that HLA-B27 is associated with PsSpA, especially with radiographic sacroiliitis [66, 107-110], as shown in Table 1.3.

**Table 1.3. Association between HLA-B27 and sacroiliitis in spondyloarthritis**

(table from Queiro et al. 2002 [66])

<table>
<thead>
<tr>
<th>Disease</th>
<th>HLA-B27 Positivity (%)</th>
<th>Bilateral SI (%)</th>
<th>Bilateral SI/HLA-B27 Association (%)</th>
</tr>
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<tbody>
<tr>
<td>AS</td>
<td>95</td>
<td>99</td>
<td>95</td>
</tr>
<tr>
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<td>80</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>PsA</td>
<td>25</td>
<td>10</td>
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<tr>
<td>AIIBD</td>
<td>25</td>
<td>10</td>
<td>70</td>
</tr>
<tr>
<td>uSpA</td>
<td>70</td>
<td>70</td>
<td>75</td>
</tr>
<tr>
<td>PsSpA*</td>
<td>34</td>
<td>44</td>
<td>75</td>
</tr>
</tbody>
</table>

Abbreviations: AIIBD, arthritis associated with inflammatory bowel disease; AS, ankylosing spondylitis; PsPA, psoriatic spondyloarthropathy; PsA, psoriatic arthritis; ReA, reactive arthritis; SI, sacroiliitis; uSpA, undifferentiated spondyloarthropathy.
Lambert et al. performed HLA-typing on 82 PsA patients in Yorkshire [110]. The prevalence of HLA-B27 was 28% in the whole PsA cohort, 60% in PsA cases with some form of spinal disease, 71% in PsA cases fulfilling the New York criteria for AS, and 5% in a control group (details not specified, but likely a general population control group). The prevalence of HLA-B27 positivity in patients with sacroiliitis alone was 78%, 36% in those with syndesmophytes alone, and 75% in those with both sacroiliitis and syndesmophytes. HLA-B27 positivity prevalence was not increased in those with peripheral arthritis only (11%). The prevalence of HLA-B13 was lower in those with PsSpA (though not statistically significant). The overall prevalence of HLA-BW17 was increased, especially in those with syndesmophytes (though not statistically significant). Gladman et al. found a similar prevalence of HLA-B27 positivity in males and females with PsSpA [111]. However, this finding was not corroborated by a later study by Queiro et al. [66], who found males PsSpA patients to be more likely HLA-B27 positive. HLA-B27 variants were prevalent in 8% of patients with PsA in a Korean study, but sub-analyses in PsSpA cases were not performed [67].

Mazzanti et al. argue that HLA-B27 is not a true marker of PsSpA [103]. They sub-categorised 66 PsA patients into nine groups on the basis of the presence of: peripheral arthritis; axial disease fulfilling the modified New York criteria for AS or; axial disease with or without peripheral involvement; and bilateral or unilateral sacroiliitis. Only the group with sacroiliitis, spondylitis, no peripheral arthritis and not fulfilling the modified New York criteria showed an increased prevalence of HLA-B27 positivity. However, in this atypical group, only two patients had a true ankylosing pattern of spondylitis. Surprisingly, only 1/9 cases in the group with axial disease fulfilling the modified New York criteria was HLA-B27 positive. The authors therefore concluded that HLA-B27 is not a true marker of PsSpA [103].

1.7.3 Contribution of HLA-variants to disease expression in PsSpA

In a prospective cohort study by Hanly et al. of 52 PsSpA cases, over a mean follow-up of 57 months, HLA-B27 status did not appear to influence disease progression [63]. In the same PsA cohort, results published seven years later showed that of 194 PsSpA (from a cohort of 498 PsA) cases carrying HLA-B27 in the presence of HLA-DR7, had more severe disease [98]. In an Italian study published in 1992, predictors of radiographic cervical spine disease patterns were considered in a cohort of 57 PsA cases; 40/57 with PsSpA [112]. Multivariate analysis showed that the best predictors of radiographic cervical spondylitis were the presence of HLA-B39 and HLA-DR4 variants, and the absence of the HLA-DR5 variants [112].
Queiro et al. studied the influence of HLA-B27 variants on PsSpA susceptibility and disease expression, using a retrospective cross-sectional single-centre study in northern Spain [66]. Axial disease was defined as grade >2 sacroiliitis, or inflammatory back pain plus syndesmophyte, facet fusion, or aseptic discitis. Of 70 consecutive PsSpA patients, identified from a previous study of the entire PsA cohort (mean age 48 years, mean arthritis duration 12 years), 16/70 cases were classified as pure axial, 29/70 mixed axial and oligoarthritis, and 25/70 mixed axial and polyarthritis. HLA-B27 positivity was more likely in the PsA cases (24/70; 34%), compared with 7% in a ‘control’ group (details not given, but likely a general population comparator) (OR 6.4; p<0.0004). HLA-B27 positivity was significantly more likely in pure-axial (9/16; 56%), compared with mixed axial-oligoarthritis (9/29; 31%), or mixed axial-polyarthritis (6/25; 24%) (p=0.02 across groups). Univariate analyses showed that HLA-B27 positive cases were significantly more likely than HLA-B27 negative cases to have bilateral sacroiliitis (75% vs. 28%; p=0.002), an earlier age of onset of psoriasis (26 vs. 33 years; p=0.03), an earlier age of onset of arthritis (32 vs. 40 years; p=0.01), male:female preponderance (7:1 vs. 1:1; p=0.002), and uveitis (33 vs. 11%; p=0.03). HLA-B27 negative patients developed significantly more peripheral erosions than HLA-B27 positive patients (p=0.05). No statistically significant differences were found between HLA-B27 positive and negative cases in terms of inflammatory spinal (neck or back) pain, radiographic cervical involvement, presence of marginal syndesmophytes, presence of paramarginal syndesmophytes, forward lumbar flexion (as measured by the Schober test), distal interphalangeal joint disease, nail dystrophy, psoriasis onset before arthritis, family history, or disability (as measured by the spinal health assessment questionnaire; HAQ-S). Syndesmophytes were present in 44% of cases, peripheral erosions in 44%, enthesitis in 43%, and psoriatic nail disease in 55%. Univariate analyses showed that the mixed axial-polyarthritis group fared significantly worse than the other two groups in terms of disability (p-value not stated), ESR (p=0.0001), and more peripheral erosions (p=0.0001).

1.7.4 HLA-variants in PsSpA compared with AS

The association of HLA-B27 with PsSpA has not been consistent, and is usually weaker than with AS. This might be because some studies have misclassified classical AS cases with coincidental psoriasis as PsSpA, and not considered the radiographic and clinical features thought to differentiate the two conditions; spondylitis without sacroiliitis in PsSpA, younger age at axial disease onset in AS, and more positive family history in AS. In a study of 66 PsA and 40 AS cases from Toronto (Canada) in 1993, AS cases compared with PsA cases had a significantly higher frequency of both HLA-B27 (83 vs. 27%; p=0.0001) and HLA-Cw02 (47 vs. 16%; p=0.01), and a significantly lower frequency
The presence of peripheral radiographic erosions have been associated with a polymorphism of the *IL23R* gene [93], *HLA-C*01:02:01 [106], the *HLA-B*27:05:02-C*01:02:01 haplotype [106], interleukin-4 receptor (*IL4R*), *IL50V* gene [119], *TNF-alpha gene* [120], and *TNF-beta* gene [120]. A pilot study by our group, published in *Rheumatology (Oxford)* 2013, confirmed that patients carrying a variant of the *IL12B* gene are significantly more likely to have PsA (OR 1.70; 95% CI 1.3, 2.2; p<0.001), and a trend
for peripheral erosive disease to be less frequent in cases carrying a variant of the interleukin-23 receptor (IL23R) gene (OR 0.47; 95% CI 0.18, 1.20; p=0.16) [93]. However, a variant of the endoplasmic reticulum aminopeptidase 1 (ERAP1) gene was not associated with axial radiographic disease in PsA even after stratifying for HLA-B27 and HLA-C*0602 status, suggesting that spinal involvement in PsA may be genetically different from that in AS, which is in keeping with previous observations that the clinical and radiographic pattern of axial disease also differ. Variants of the TNF-alpha and TNF-beta genes have also been investigated, but similarly did not show association with either a history of inflammatory back pain or radiographic sacroiliitis / spondylitis [120].

1.8 SUMMARY

In summary, relatively little is known about the clinical, radiographic, serum and genetic biomarker characteristics of two subphenotypes of PsA; PsSpA and PAM. Estimates of the prevalence of PsSpA and PAM in PsA cohort have markedly varied, in part due to a lack of an international consensus classification for PsSpA and PAM, and due to the scarcity of large well-characterised PsA cohorts. The demographic, articular and extra-articular manifestations, functional and metrology impairments that PsSpA experience has yet to be established, in particular how they compare to pPsA and AS. Only a few moderate-sized studies have described sites, patterns, severity and morphological features of axial radiographic disease in PsSpA, and even fewer have made comparisons with AS. Furthermore, the reliability of some of these studies has been reduced by the stringency of the classification systems for PsSpA used, case selection and sample size. The association of HLA-B27 variants with PsSpA has yet to be fully established. Other genetic variants have not been thoroughly tested for association with PsSpA or PAM. Studies investigating PAM have been cross-sectional, likely underpowered, and had limited imaging data. No longitudinal study has been published describing the distribution of joints affected by PAM, or the natural radiographic history of PAM. Serum-soluble bone-turnover biomarkers have been investigated in AS, and shown some utility for clinical and research purposes. However, only a few small studies with variable study designs have investigated serum biomarkers in PsA, and no study has investigated in PsSpA or PAM.

It is hoped that through a better understanding of the clinical, radiographic and serum-biomarker features of these two phenotypes of PsA, patients can be better classified and managed using the principles of stratified medicine, and a better understanding of their pathogenesis can be attained, thereby elucidating therapeutic targets.
2 CHAPTER II: SERUM-SOLUBLE BONE AND CARTILAGE TURNOVER BIOMARKERS IN PSORIATIC ARTHRITIS AND PSORIATIC SPONDYLOARTHROPATHY: A SYSTEMATIC REVIEW

2.1 ABSTRACT

Background: Psoriatic arthritis (PsA) is an inflammatory disease of joints. Therefore, serum soluble biomarkers specific for chronic joint and bone inflammation may predict future disease severity and response to therapy, thereby informing stratified medicine approaches. The objectives of this systematic review were to determine if serum-soluble bone and cartilage turnover biomarkers are: (i) associated with PsA or psoriatic spondyloarthropathy (PsSpA); (ii) associated with disease activity, disease severity, or clinical phenotype.

Methods: A search was conducted of several databases (Medline, EMBASE, and Cochrane Controlled Trials Register) and conference proceeding sites using the following MeSH, EMTree or key term stems: Arthritis, Psoriatic; Psoriatic spondylarthritis; Biological markers; Bone turnover markers; osteoprotegerin (OPG), matrix metalloproteinase (MMP-3), sclerostin, dickkopf 1 (Dkk-1), bone alkaline phosphatase (ALP), osteocalcin (OC), macrophage colony-stimulating factor (M-CSF), receptor activator of nuclear factor-kappa B ligand (RANKL), collagen type II, extracellular matrix proteins, glycoproteins, procollagen, amino-terminal propeptide of procollagen type III (PIIINP), cross-linked telopeptide of collagen-1 (CTX-1), tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), bone morphogenetic protein (BMP), and cartilage oligomeric matrix protein (COMP). Two reviewers independently assessed abstracts for inclusion in the review, and extracted data from papers meeting study eligibility criteria. One reviewer (Deepak Jadon) critiqued the papers and results.

Results: The search identified 155 unique studies, and ten of these met the eligibility criteria of the systematic review. MMP-3, Dkk-1, M-CSF, CTX-1, and TRAIL were associated with PsA, with equivocal results for OPG and ALP. MMP-3, Dkk-1, M-CSF, CPII:C2C, and possibly OPG associated with PsA independently of psoriasis. C1-2C was associated with both tender and swollen joint counts, and BMP-4 with patient global assessment of disease, pain score and BASDAI. Bone ALP was associated with disease activity. M-CSF and RANKL were associated with several plain radiographic features.
Conclusions: Whilst several studies have investigated serum-soluble bone and cartilage biomarker associations with PsA, no studies have investigated association specifically with PsSpA. Studies to date have had small samples sizes and may have been underpowered to detect associations. Studies have used inconsistent clinical end-points and laboratory methods, making comparison between studies difficult. We have identified several candidate biomarkers that warrant further investigation for association with PsA and PsSpA in the first instance, and subsequently for association with hard end-points such as radiographic outcome.
2.2 INTRODUCTION

The following systematic review was published as a paper in The Journal of Rheumatology in January 2014 (J Rheumatol. 2015;42:21-30) [121]. There is some repetition in this chapter of the content of that paper. The manuscript was written by Deepak Jadon (DJ). Raj Sengupta (RS) and Alison Nightingale (AN) helped in the screening of eligible papers and data extraction, respectively. All co-authors contributed to the review inception, design, and internal peer review of the final draft written by DJ.

Psoriatic arthritis (PsA) is a chronic inflammatory musculoskeletal disorder with characteristic patterns of peripheral and axial joint inflammation, and extra-articular manifestations that can include skin psoriasis, psoriatic nail disease, enthesitis, dactylitis or uveitis. As such, candidate serum soluble biomarkers specific for chronic joint and bone inflammation may predict future disease severity and response to therapy, thereby informing stratified medicine approaches. However, identifying and monitoring biomarkers in PsA is difficult due to the heterogeneity of PsA disease. In PsA, bone loss can occur in the form of bone erosion, osteolysis, and bone mineral density loss [83]. Bone formation can occur in the form of osteoproliferation, ankylosis and syndesmophytes.

Several bone and cartilage turnover biomarkers might be of interest in PsA. Some directly cause bone resorption by virtue of their enzymatic or cytokine properties, e.g. matrix metalloproteinase-3 (MMP-3) enzymatically degrades the extra-cellular matrix of bone and cartilage [84]. Osteoprotegerin (OPG) is a glycoprotein secreted by osteoblasts and stromal cells, acting as a decoy receptor to receptor activator of nuclear factor-kappa B ligand (RANKL), thereby inhibiting osteoclastogenesis, resulting in reduced bone resorption. Others are by-products of bone resorption, thereby acting as markers of the process, e.g. cross-linked telopeptide of collagen-1 (CTX-1) is the product of excess metalloproteinase degradation of type 1 collagen. There are several by-products of cartilage turnover: C2C and C1-2C are neoepitopes that are released when type 2 cartilage is degraded by collagenases; CPII is released during pro-collagen 2 synthesis; and CPII:C2C ratio of cartilage degradation versus by-product formation [85].

Although there have been several editorial review articles, there have been no systematic reviews published on the clinical and prognostic value of serum soluble bone-turnover biomarkers in PsA. The objectives of this systematic review were to determine if serum-soluble bone and cartilage turnover biomarkers are: (1) associated with PsA or psoriatic spondyloarthropathy (PsSpA); (2) associated with disease activity, disease severity, or clinical phenotype in PsA cases vs. healthy controls, and PsA vs. PsC cases.
2.3 METHODS

Methods of analysis and eligibility criteria were specified in advance and documented in an *a priori* protocol. This study aligns with the ‘meta-analysis of observational studies in epidemiology (MOOSE) guidelines [122].

2.3.1 Eligibility criteria

Inclusion Criteria

(i) Cohort, case-control, cross-sectional studies and randomised controlled trials published in the form of a journal paper, journal abstract or conference abstract that compared the serum concentration of bone and cartilage-turnover biomarkers in PsA cases to that in healthy controls, with or without an additional psoriasis (PsC) comparator group.

(ii) Study participants with PsA must have fulfilled Classification for Psoriatic Arthritis (CASPAR) criteria [24] for PsA, or Moll and Wright criteria for PsA [18], or PsSpA [123].

(iii) The following bone and cartilage-turnover biomarkers were included (as defined in key indexing terms below; MeSH, EMTree or key terms): osteoprotegerin (OPG), matrix metalloproteinase (MMP-3), sclerostin, dickkopf 1 (Dkk-1), bone alkaline phosphatase (ALP), osteocalcin (OC), macrophage colony-stimulating factor (M-CSF), receptor activator of nuclear factor-kappa B ligand (RANKL), collagen type II, extracellular matrix proteins, glycoproteins, procollagen, amino-terminal propeptide of procollagen type III (PIIINP), cross-linked telopeptide of collagen-1 (CTX-1), tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), bone morphogenetic protein (BMP), and cartilage oligomeric matrix protein (COMP).

(iv) Outcome variables were: (i) peripheral and / or spinal involvement: clinical symptoms, radiographic disease (ii) disease severity, as measured by axial and / or peripheral radiographic disease (iii) disease activity: tender joint counts, swollen joint counts, Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), Bath Ankylosing Spondylitis Functional Index (BASFI), Bath Ankylosing Spondylitis Metrology Index (BASMI), enthesitis, C-reactive protein (CRP), composite scores, and other outcome measures.
Exclusion criteria

We excluded studies without a healthy control (HC) group. We excluded studies where participants were using biological agents, since tumour necrosis factor inhibitors [88, 124, 125], but not conventional DMARDs [88, 126], have been reported to directly influence serum bone and cartilage turnover biomarkers [127], thereby confounding results when comparing HC with PsA cases.

2.3.2 Searches

The search date was 1 February 2014. The search was repeated on 1 July 2015 prior to submission of this thesis, and no additional papers were identified. The following databases were searched using key indexing terms: Medline (1950 - present); EMBASE (1974 - present); Cochrane Controlled Trials Register (1993 - present). The following MeSH, EMTree or key term stems were used: Arthritis, Psoriatic; Psoriatic spondylarthritis; Biological markers; Bone turnover markers. No language restrictions were applied to publications.

The reference lists of all papers fulfilling inclusion criteria and all review articles were scrutinised for any references not identified in the original database search, but still meeting the inclusion criteria. Two key authors (Dr. Vinod Chandran, VC; Professor Oliver Fitzgerald, OF) were contacted to determine if any important unpublished papers or not indexed (e.g. conference proceedings) should be screened.

2.3.3 Study selection

Two reviewers (DJ, RS) independently assessed abstracts for inclusion in the review. Where there was disparity in opinion, the full paper was obtained and consensus for inclusion or exclusion reached (DJ, RS). An assessment was made at this point for potential publication bias, or selective reporting within studies. Two reviewers (DJ, AN) independently extracted data from the papers onto a standardised data-extraction Excel spreadsheet, that was initially pilot-tested. The papers were critically appraised using the Critical Appraisal Skills Programme toolkit [128] for cohort and cross-sectional studies, including sources of bias, both at study and outcome level. DJ and AN reached consensus on data for use in the subsequent analyses.
2.3.4 Synthesis of results

The primary summary measure was odds ratios (OR) for serum biomarker levels in PsA vs. healthy controls, or PsA vs. PsC, including p-values for the analyses. The secondary summary measures were odds ratios or Spearman Rho correlation co-efficient for clinical outcomes in PsA vs. healthy controls, or PsA vs. PsC, including 95% confidence intervals (95% CI) and p-values for the analyses.

We initially intended to combine the results of different studies mathematically as a meta-analysis, including tests for heterogeneity. However, due to the differing methods used in the included studies (cohorts, laboratory techniques using enzyme-linked immunosorbent assay or immunoassays with different reference ranges, statistical analyses) and a lack of homogeneity in the reporting of results it was not possible to combine the results of different studies statistically. Therefore we have reported the results of the studies quantitatively, without meta-analysis.

2.4 RESULTS

155 unique studies were identified; ten of these met the eligibility criteria and were included in the systematic review. Two papers [129, 130] that met inclusion criteria were unobtainable from several libraries (including the British Library) or on contacting the first authors, and insufficient detail of results were provided in the abstract to allow inclusion in the systematic review. Four papers were excluded because they did not have a healthy control group for comparison with the PsA group [131-134]. Eight papers were excluded because the PsA cases were using biological agents, and either didn’t have a healthy control comparator group or did not provide pre-biological initiation biomarker data [88, 124, 125, 127, 134-137]. The remaining 131 articles were excluded because they did not fulfil several eligibility criteria. No further articles were identified on scrutinising the reference list of included articles, or by recommendation of two key authors (VC, OF).

Figure 2.1 details the flow of study selection in the systematic review, and Table 2.1 summarises the characteristics of the ten studies meeting the eligibility criteria of the systematic review.
Figure 2.1. Study selection in the systematic review

(Figure reproduced from Jadon et al. J Rheumatol 2015) [121]
### Table 2.1. Characteristics of the ten studies meeting eligibility criteria of the systematic review

<table>
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<tr>
<th>Authors</th>
<th>Journal</th>
<th>Country</th>
<th>Design</th>
<th>Setting</th>
<th>Case selection</th>
<th>Classification criteria used</th>
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<th>PsA</th>
<th>PsC</th>
<th>Other cohorts</th>
<th>Mean age (y)</th>
<th>Mean disease duration (y)</th>
<th>Matching</th>
<th>Male : female ratio</th>
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<td>Prospective single centre</td>
<td>OPD</td>
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<td>n/s</td>
<td>ESSG</td>
<td>41</td>
<td>23</td>
<td>-</td>
<td>30 AS 10 ReA</td>
<td>45.2</td>
<td>-</td>
<td>n/s</td>
<td>17:6</td>
</tr>
<tr>
<td>Franck et al. 2000 (23)</td>
<td>Rheumatology International</td>
<td>Germany</td>
<td>Prospective single centre</td>
<td>OPD</td>
<td>Consecutive OPD attendees</td>
<td>Moll &amp; Wright</td>
<td>50</td>
<td>32</td>
<td>17</td>
<td>-</td>
<td>45</td>
<td>-</td>
<td>Age &amp; sex</td>
<td>21:11</td>
</tr>
<tr>
<td>Magaro et al. 1989 (29)</td>
<td>Clinical Rheumatology</td>
<td>Italy</td>
<td>Prospective single centre</td>
<td>IPD</td>
<td>n/s</td>
<td>n/s</td>
<td>25</td>
<td>25</td>
<td>-</td>
<td>25 RA</td>
<td>50.3</td>
<td>11.3</td>
<td>Age &amp; sex</td>
<td>Females only</td>
</tr>
</tbody>
</table>

HC: healthy control  PsA: psoriatic arthritis  PsC: cutaneous psoriasis without arthritis  AS: ankylosing spondylitis  RA: rheumatoid arthritis  OA: osteoarthritis  ReA: reactive arthritis  Steroids: corticosteroids  n/s: not stated  CASPAR: Classification criteria for psoriatic arthritis  ESSG: European spondyloarthropathy study group

(Table reproduced from Jadon et al. J Rheumatol 2015) [121]
2.4.1 Comparison of biomarkers levels in PsA cases vs. healthy controls

The results of comparisons between biomarkers levels in PsA cases vs. healthy controls are shown in Table 2.2. The serum concentration of several biomarkers (MMP-3, Dkk-1, M-CSF, CTX-1, and TRAIL) was significantly higher in PsA vs. healthy controls. whereas the serum concentration was not significantly different in PsA vs. healthy controls for RANKL, BMP, OC, PIIIINP, COMP, C1-2C and CPII:C2C. The results for OPG and ALP were equivocal.
### Table 2.2. Comparison of biomarkers levels in PsA cases and healthy controls

<table>
<thead>
<tr>
<th>Domain</th>
<th>Biomarker</th>
<th>Author</th>
<th>PsA vs. HC</th>
<th>Sample size (HC, PsA, PsC)</th>
<th>OR</th>
<th>95% CI</th>
<th>p-value</th>
<th>Mean serum conc.</th>
<th>Serum conc. units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone resorption marker</td>
<td>MMP-3</td>
<td>Chandran 2010</td>
<td>Higher</td>
<td>28,26,26</td>
<td>1.40</td>
<td>1.12, 1.18</td>
<td>p&lt;0.003</td>
<td>25.8 vs. 8.8</td>
<td>ng/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ribbens 2001</td>
<td>Higher (in females)</td>
<td>96,18,0</td>
<td>p&lt;0.05</td>
<td>29.5 vs. 9.1</td>
<td>ng/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ribbens 2001</td>
<td>Higher (in males)</td>
<td>96,18,0</td>
<td>p&lt;0.05</td>
<td>39.5 vs. 19.2</td>
<td>ng/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Shibata 2009</td>
<td>Higher</td>
<td>11,16,15</td>
<td>14.9</td>
<td>1.90, 397.40</td>
<td>p&lt;0.01</td>
<td>OR of levels above cut-off</td>
<td>ng/ml</td>
</tr>
<tr>
<td></td>
<td>Dkk-1</td>
<td>Dalbeth 2010</td>
<td>Higher</td>
<td>12,38,10</td>
<td>p&lt;0.01</td>
<td>charted</td>
<td>pg/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>RANKL</td>
<td>Chandran 2010</td>
<td>Higher</td>
<td>26,26,26</td>
<td>1.01</td>
<td>1.00, 1.01</td>
<td>p=0.04</td>
<td>319.8 vs. 266.4</td>
<td>pg/l</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dalbeth 2010</td>
<td>Equal</td>
<td>12,38,10</td>
<td>p&gt;0.05</td>
<td>charted</td>
<td>pmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M-CSF</td>
<td>Dalbeth 2010</td>
<td>Higher</td>
<td>12,38,10</td>
<td>p&lt;0.01</td>
<td>charted</td>
<td>pg/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CTX-1</td>
<td>Grisar 2002</td>
<td>Higher</td>
<td>41,23,0</td>
<td></td>
<td></td>
<td></td>
<td>4.8 vs. 3.3</td>
<td>ng/ml</td>
</tr>
<tr>
<td>Bone formation markers</td>
<td>OPG</td>
<td>Chandran 2010</td>
<td>Higher</td>
<td>26,26,26</td>
<td>1.01</td>
<td>1.00, 1.02</td>
<td>p&lt;0.01</td>
<td>683.8 vs. 609.2</td>
<td>pg/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gisar 2002</td>
<td>Higher</td>
<td>41,23,0</td>
<td></td>
<td></td>
<td></td>
<td>47.5 vs. 35.2</td>
<td>ng/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hoffbauer 2006</td>
<td>Higher (in females)</td>
<td>90,116,0</td>
<td>p&lt;0.05</td>
<td>6.7 vs. 5.4</td>
<td>pmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hoffbauer 2006</td>
<td>Equal (males)</td>
<td>90,116,0</td>
<td>p=0.38</td>
<td>5.09 vs. 5.4</td>
<td>pmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dalbeth 2010</td>
<td>Equal</td>
<td>12,38,10</td>
<td>p&lt;0.05</td>
<td>charted</td>
<td>pg/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BMP-2</td>
<td>Groevic 2010</td>
<td>Equal</td>
<td>25,23,0</td>
<td>p&gt;0.05</td>
<td>n/s</td>
<td>n/s</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BMP-4</td>
<td>Groevic 2010</td>
<td>Equal</td>
<td>25,23,0</td>
<td>p&gt;0.05</td>
<td>n/s</td>
<td>n/s</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BMP-6</td>
<td>Groevic 2010</td>
<td>Equal</td>
<td>25,23,0</td>
<td>p&lt;0.05</td>
<td>n/s</td>
<td>n/s</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ALP</td>
<td>Gisar 2002</td>
<td>Higher</td>
<td>41,23,0</td>
<td>p&lt;0.05</td>
<td>12.5 vs. 10.1</td>
<td>ng/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Franck 2000</td>
<td>Lower (in females)</td>
<td>50,32,17</td>
<td>p&lt;0.05</td>
<td>91 vs. 125</td>
<td>U/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Franck 2000</td>
<td>Equal (males)</td>
<td>50,32,17</td>
<td>p&lt;0.05</td>
<td>136 vs. 125</td>
<td>U/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>OC</td>
<td>Gisar 2002</td>
<td>Equal</td>
<td>41,23,0</td>
<td>p&lt;0.05</td>
<td>20.7 vs. 17.8</td>
<td>ng/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Magaro 1989</td>
<td>Equal</td>
<td>25,25,0</td>
<td>p&lt;0.05</td>
<td>4.83 vs. 7.25</td>
<td>ng/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Franck 2000</td>
<td>Equal</td>
<td>50,32,17</td>
<td>not stated</td>
<td>3.0 vs. 3.6</td>
<td>ng/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Franck 2000</td>
<td>Lower (in females)</td>
<td>50,32,17</td>
<td>p&lt;0.05</td>
<td>2.28 vs. 4.11</td>
<td>ng/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PIINP</td>
<td>Sharif 1996</td>
<td>Equal</td>
<td>16,12,0</td>
<td>p=0.079</td>
<td>0.39 vs. 0.30</td>
<td>U/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cartilage turnover markers</td>
<td>COMP</td>
<td>Chandran 2010</td>
<td>Equal</td>
<td>26,26,26</td>
<td>1.00</td>
<td>1.00, 1.00</td>
<td>p=0.47</td>
<td>2325.1 vs. 1669.7</td>
<td>ng/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Shibata 2009</td>
<td>Higher</td>
<td>11,16,15</td>
<td>p&lt;0.01</td>
<td>12.7 vs. 8.9</td>
<td>U/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C1-2C</td>
<td>Chandran 2010</td>
<td>Equal</td>
<td>28,26,26</td>
<td>0.46</td>
<td>0.001, 217.74</td>
<td>p&lt;0.01</td>
<td>0.6 vs. 0.5</td>
<td>mcg/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CPR2/C2C</td>
<td>Equal</td>
<td>26,26,26</td>
<td>2.17</td>
<td>0.59, 8.04</td>
<td>p&lt;0.03</td>
<td>4.76 vs. 3.70</td>
<td>no units (ratio)</td>
</tr>
<tr>
<td>Synovial integrity marker</td>
<td>TRAIL</td>
<td>Hoffbauer 2006</td>
<td>Higher</td>
<td>90,116,0</td>
<td></td>
<td></td>
<td></td>
<td>66.1 vs. 50</td>
<td>pg/ml</td>
</tr>
</tbody>
</table>

OR: odds ratio
95% CI: 95% confidence interval
HC: healthy control
PsA: psoriatic arthritis
PsC: cutaneous psoriasis without arthritis
Dkk-1: Dickkopf 1
OPG: osteoprotegerin
BMP: bone morphogenetic protein
M-CSF: macrophage colony-stimulating factor
MMP-3: matrix metalloproteinase-3
PIINP: amino-terminal propeptide of procollagen type III
TRAIL: tumor necrosis factor-related apoptosis-inducing ligand
C1-2C: collagen 1 telopeptide
C2/C3: ratio of procollagen 2 to collagen 2 neoepitope

(Table reproduced from Jadon et al. *J Rheumatol* 2015) [121]
2.4.2 Comparison of biomarker levels in PsA cases vs. PsC cases

The results of comparisons between biomarkers levels in PsA cases vs. PsC cases are given in Table 2.3. The serum concentration of MMP-3, Dkk-1, M-CSF, and CPII:C2C was significantly higher in PsA vs. PsC. The results for OPG were equivocal.

**Table 2.3. Comparison of biomarkers levels in PsA and PsC cases**

<table>
<thead>
<tr>
<th>Domain</th>
<th>Marker</th>
<th>Reference</th>
<th>PsA vs. PsC</th>
<th>Sample size (HC, PsA, PsC)</th>
<th>OR</th>
<th>95% CI</th>
<th>p-value</th>
<th>Mean serum concentration</th>
<th>Serum conc. units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone resorption marker</td>
<td>MMP-3</td>
<td>Chandran 2010</td>
<td>Higher</td>
<td>26,26,26</td>
<td>1.28</td>
<td>1.02, 1.60</td>
<td>p=0.03</td>
<td>5.80 vs. 11.30</td>
<td>ng/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Shibata 2009</td>
<td>Higher</td>
<td>11,16,15</td>
<td>6.30</td>
<td>1.30, 37.80</td>
<td>p=0.01</td>
<td>OR of levels above cut-off</td>
<td>ng/ml</td>
</tr>
<tr>
<td></td>
<td>Dkk-1</td>
<td>Dalbeth 2010</td>
<td>Higher</td>
<td>12,38,10</td>
<td>p=0.001</td>
<td>charted</td>
<td></td>
<td></td>
<td>pg/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dalbeth 2010</td>
<td>Higher (if erosive)</td>
<td>12,38,10</td>
<td>p=0.001</td>
<td>charted</td>
<td></td>
<td></td>
<td>pg/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dalbeth 2010</td>
<td>Higher (if non-erosive)</td>
<td>12,38,10</td>
<td>p=0.05</td>
<td>charted</td>
<td></td>
<td></td>
<td>pg/ml</td>
</tr>
<tr>
<td></td>
<td>RANKL</td>
<td>Chandran 2010</td>
<td>Equal</td>
<td>26,26,26</td>
<td>1.00</td>
<td>0.99, 1.00</td>
<td>p=0.77</td>
<td>319.80 vs. 319.80</td>
<td>pg/l</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dalbeth 2010</td>
<td>Equal</td>
<td>12,38,10</td>
<td>p=0.05</td>
<td>charted</td>
<td></td>
<td></td>
<td>pmol/L</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dalbeth 2010</td>
<td>Equal (if erosive)</td>
<td>12,38,10</td>
<td>p=0.05</td>
<td>charted</td>
<td></td>
<td></td>
<td>pmol/L</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dalbeth 2010</td>
<td>Equal (if non-erosive)</td>
<td>12,38,10</td>
<td>p=0.05</td>
<td>charted</td>
<td></td>
<td></td>
<td>pmol/L</td>
</tr>
<tr>
<td></td>
<td>M-CSF</td>
<td>Dalbeth 2010</td>
<td>Higher</td>
<td>12,38,10</td>
<td>p=0.01</td>
<td>charted</td>
<td></td>
<td></td>
<td>pg/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dalbeth 2010</td>
<td>Higher (erosive)</td>
<td>12,38,10</td>
<td>p=0.001</td>
<td>charted</td>
<td></td>
<td></td>
<td>pg/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dalbeth 2010</td>
<td>Equal (if non-erosive)</td>
<td>12,38,10</td>
<td>p=0.05</td>
<td>charted</td>
<td></td>
<td></td>
<td>pg/ml</td>
</tr>
<tr>
<td>Bone formation marker</td>
<td>OPG</td>
<td>Chandran 2010</td>
<td>Higher</td>
<td>26,26,26</td>
<td>1.01</td>
<td>1.00, 1.02</td>
<td>p=0.02</td>
<td>863.80 vs. 595.50</td>
<td>pg/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dalbeth 2010</td>
<td>Equal</td>
<td>12,38,10</td>
<td>p=0.05</td>
<td>charted</td>
<td></td>
<td></td>
<td>pg/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dalbeth 2010</td>
<td>Equal (if erosive)</td>
<td>12,38,10</td>
<td>p=0.05</td>
<td>charted</td>
<td></td>
<td></td>
<td>pg/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dalbeth 2010</td>
<td>Equal (if non-erosive)</td>
<td>12,38,10</td>
<td>p=0.05</td>
<td>charted</td>
<td></td>
<td></td>
<td>pg/ml</td>
</tr>
<tr>
<td></td>
<td>OC</td>
<td>Franck 2000</td>
<td>Lower (in females)</td>
<td>50,32,17</td>
<td>p=0.05</td>
<td></td>
<td></td>
<td>2.28 vs. 3.00</td>
<td>ng/ml</td>
</tr>
<tr>
<td>Cartilage turnover marker</td>
<td>COMP</td>
<td>Chandran 2010</td>
<td>Equal</td>
<td>26,26,26</td>
<td>1.00</td>
<td>01.00, 1.00</td>
<td>p=0.35</td>
<td>2329.10 vs. 2516.50</td>
<td>ng/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chandran 2010</td>
<td>Equal</td>
<td>26,26,26</td>
<td>0.02</td>
<td>&lt;0.001, 16.44</td>
<td>p=0.26</td>
<td>0.60 vs. 0.50</td>
<td>mcg/ml</td>
</tr>
<tr>
<td></td>
<td>CPII:C2C</td>
<td>Chandran 2010</td>
<td>Higher</td>
<td>26,26,26</td>
<td>4.76</td>
<td>1.35, 16.77</td>
<td>p=0.02</td>
<td>4.76 vs. 3.28</td>
<td>ratio</td>
</tr>
</tbody>
</table>


(Table reproduced from Jadon et al. J Rheumatol 2015) [121]
2.4.3 Association of biomarker levels with demographic parameters

Franck et al. [138] demonstrated two biomarkers to be higher in males vs. female PsA patients: ALP (mean serum concentration 137 U/l in males vs. 91 U/l in females; p<0.05) and OC (mean serum concentration 3.62 ng/ml in males vs. 2.28 ng/ml in females; p<0.05). However, Hofbauer et al. [139] did not corroborate the findings for OC, demonstrating OC levels to be no different in male and female PsA patients (23.7 ng/ml in males vs. 23.1 ng/ml in females; p=0.82). However, the study populations of the two studies differed with Hofbauer et al. excluding patients using DMARDs or corticosteroids and took fasting blood samples whereas Franck et al. took un-fasted samples and included patients using DMARDs and corticosteroids. Similarly, the association between OPG levels and sex were conflicting, with higher levels in females vs. males in the study by Hofbauer et al. (6.7 in females vs. 2.09 pmol/L in males; p=0.001) [139] but no difference by sex in the smaller study by Dalbeth et al. (mean serum concentrations or p-values not stated) [126]. No correlation has been reported between sex and Dkk-1 [126], M-CSF [126], RANKL [126], PIIINP [140], [139], or cross-laps [139].

No association has been reported for PIIINP and age of PsA patient at the time of sampling (p=0.925) [126, 140], Dkk-1, RANKL, M-CSF or OPG (p-values or correlation coefficients not stated) and body weight in kilograms [126].

2.4.4 Association of biomarker levels with clinical parameters

Three studies investigated for association between PsA disease duration and serum biomarkers [138, 140, 141]. However, none of the studies defined whether duration was analysed as a continuous or categorical variable. Disease duration was positively associated with serum CTX-1 concentrations (r=0.670; p=0.009) [141], but not with OPG [141], ALP [141], PIIINP [140], or OC [138, 141].

Chandran et al. demonstrated a positive correlation between C1-2C and both tender joint counts and swollen joint counts [88]. However, p-values, Spearman Rho correlation coefficients, and tender or swollen joint counts per unit increase in C1-2C were not stated. Grcevic et al. reported a positive association between BMP-4 and both patient global assessment of disease (r=0.54; p=0.02), and pain score on a visual analogue scale (r=0.49; p=0.04) [142]. No such associations were found between the same parameters and either BMP-2 or BMP-6 [142].
2.4.5 Association of biomarkers levels with laboratory parameters

An association was demonstrated between C-reactive protein (CRP) levels and both CTX-1 [141] and TRAIL [139] in PsA patients, but not with either MMP-3 [143] or OPG [139]. Erythrocyte sedimentation rate (ESR) was positively associated with both CTX-1 [141] and OPG [139]. TRAIL was not associated with ESR levels [139]. Two studies consistently showed ALP to be positively associated with OC [138, 144]. Serum creatinine levels were not associated with Dkk-1, RANKL, M-CSF or OPG levels in the single study that tested for this correlation [126].

2.4.6 Association of biomarkers levels with composite indices

Franck et al. reported an association between disease activity and both ALP (mean serum concentration in patients with ‘no disease activity’ 69 U/l vs 148 U/l in patient with ‘high’ disease activity; p<0.005) and OC (mean serum concentration in patients with ‘no disease activity’ 2.2 ng/ml vs. 3.92 ng/ml in patient with ‘high’ disease activity; p<0.05) although the number of patients in these groups were very small (4 vs. 98, respectively) and no definition of ‘no’ vs. ‘high’ diseases activity was stated. [138]. Disease Activity Score 28-joints using CRP (DAS28-CRP) was not associated with Dkk-1, M-CSF, RANK-L, OPG [126], BMP-2, BMP-4 or BMP-6 [142]. BMP-4 associated with BASDAI in the one study that tested this correlation (r=0.46; p=0.04) [142]. No association was demonstrated between BASDAI and either BMP-2 or BMP-6 [142]. COMP positively correlated with the Psoriasis Area and Severity Index (PASI) in one study (analyses not stated in paper) [88]. No association was demonstrated between DAS28-CRP and Dkk-1, M-CSF, RANKL, OPG, BMP-2, BMP-4, or BMP-6 [126]. No association was demonstrated between BASFI and BMP-2, BMP-4, or BMP-6 [142].

2.4.7 Association of biomarkers levels with radiographic parameters

Four studies [88, 126, 139, 142] investigated the association between biomarker levels and radiographic parameters with two studies providing the majority of the data [88, 126] (Table 4). Joint space narrowing was associated with RANKL (p<0.05) and M-CSF (p<0.01), but not with Dkk-1 or OPG in the one study testing these associations [126]. Similarly, osteolysis (defined as pencil-in-cup deformity) was associated with both RANKL (p<0.05) and M-CSF (p<0.05), but not with Dkk-1 or OPG in the one study testing these associations [126]. Osteoproliferation was not associated with RANKL, M-CSF, Dkk-1 or OPG in the one study testing these associations [126]. Two studies tested for association
between peripheral radiographic erosions and serum biomarkers. M-CSF was positively associated with peripheral erosions (p<0.001) [126], but the remaining biomarkers were not (MMP-3, Dkk-1, RANKL, OPG, COMP, C2C, C1-2C, CPII) [88, 126]. The modified van der Heijde score for PsA (VDH) is a composite score encompassing joint space narrowing and erosions in peripheral radiographs [145]. Dalbeth et al. demonstrated an association between the VDH and both M-CSF (p<0.01) and RANKL (p<0.05), but not with Dkk-1 or OPG.

Three studies [88, 126, 142] tested for association between radiographic sacroiliitis and several biomarkers: no associations were found with MMP-3, RANKL, OPG, BMP-2, BMP-4, BMP-6, COMP, C2C, C1-2C, CPII; and results were equivocal for Dkk-1 and M-CSF [88, 126, 142].

No association was found between bone mineral density (BMD) at the hip, lumbar spine or femur and several biomarkers, including Dkk-1, RANKL, M-CSF, OPG, or TRAIL (Table 4) [139].
2.5 DISCUSSION

A summary of biomarkers that are associated with PsA and its clinical parameters are shown in Figure 2.2. The following biomarkers were associated with PsA: MMP-3, Dkk-1, M-CSF, CTX-1, TRAIL, and the results were equivocal for OPG and ALP. MMP-3, Dkk-1, M-CSF, CPII:C2C, and possibly OPG, were associated with PsA independently of PsC. ALP was associated with male sex in PsA. CTX-1 was associated with disease duration, C1-2C with both tender and swollen joint counts, and BMP-4 with both patient global assessment of disease and pain score. CRP was associated with both CTX-1 and TRAIL, ESR associated with both CTX-1 and OPG, and ALP associated with OC. Disease activity was associated with ALP and possibly OC; BASDAI associated with BMP-4, and skin score (PASI) correlated with COMP. The following biomarkers were associated with radiographic features: M-CSF with the PsA-modified van der Heijde composite score, joint space narrowing, peripheral radiographic erosions, and osteolysis; RANKL with the van der Heijde composite score, joint space narrowing, and osteolysis.

Figure 2.2. Summary of serum bone and cartilage-turnover biomarkers associated with PsA

(Figure reproduced from Jadon et al. J Rheumatol 2015) [121]
2.5.1 Disparity in study findings

There are several potential reasons for disparity in study results. Firstly, most of the studies have been cross-sectional, rather than prospectively conducted cohort studies. A study by Young-Min et al. of early RA demonstrated biomarkers associations with swollen / tender joint counts and disease activity score only when longitudinal data were analysed, and not when cross-sectional baseline data was analysed [146]. Studies have investigated differing clinical parameters, used differing collection protocols (overnight fasted in two studies [139, 144]) and laboratory techniques, making comparison difficult. Several studies had small samples [140-142, 144, 147, 148] and were likely to be underpowered.

We acknowledge that there may be publication bias towards studies with positive results. However, we suspect that due to several biomarkers being reported in each study, with a mixture of both positive and negative findings, that selective reporting bias may be less of a problem.

Whilst it is common practice in studies of metabolic bone disease to test bone markers in the morning and in a fasted state, few PsA studies have undertaken testing in this manner. Clowes et al. investigated the effect of feeding vs. fasting on several markers in twenty women, and demonstrated little effect on bone biomarkers, except in the case of serum CTX [149]. Other factors influencing serum levels: circadian rhythm [150], sex, oral contraceptive pill use, menstrual cycle, growth, diet, meal composition and timing of sample post-ingestion [149]. Standardisation of diet prior to sampling may improve measurement variability, but at the expense of feasibility.

Conflicting reports of associations between serum biomarker concentrations and demographic parameters may be, in part, attributable to uncontrolled confounding due to a lack of matching or adequate adjustment for age and sex within the study design and analysis. Dalbeth et al. did not adjust for the higher proportion of women in their HC vs. PsA group [126]. Hofbauer et al. had entirely men in their HC group, since this was a ‘convenience sample’ derived from participants in a coronary artery study [139]. They also reported higher OPG serum concentration in females compared with males, due to a lack of adjustment for sex; oestrogen is known to stimulate OPG production. Ribbens et al. sex-matched their participants, and since corticosteroid use alters MMP-3 levels, analyses were made in non-corticosteroid using patients only [147].
Sharif et al. did not state the source of their HC group, which appears much younger than the PsA cohort [140]. Significant differences in mean age, sex, and disease duration of the patients in all three disease groups were noted. Whilst Shibata et al. [148] matched for age in their study, further inspection demonstrates that the HC group was much younger than both the PsA and PsC groups [148].

2.5.2 Priorities for future research

Biomarker identification in PsA may help identify PsC patients with subclinical arthritis, and aid both prognostication and stratified medicine approaches. Biomarkers may facilitate monitoring of disease activity and treatment response, so that non-efficacious treatment is switched, rather than waiting several years for radiographic progression. Our knowledge of the pathogenesis of PsA, and how it overlaps with AS and RA, may be improved through such research. Biomarkers may guide the development of new drugs, both to obtain proof of principle in an early stage of drug development, and avoid reliance on slow structural damage outcomes requiring lengthy clinical trials [137]. Serum biomarkers may offer a more economic and readily available alternative to imaging. All such knowledge is important for the individual patient, public health and health policy.

Despite the theoretical advantages, “novel” biochemical markers have not translated to the bedside. This may be in part due to a lack of longitudinal prospective studies and robust evidence of superiority over existing biomarkers e.g. CRP. PsA is a heterogeneous disease with several subphenotypes, varied clinical course, and often comorbidities that can confound the interpretation of results [151].

There is a need for longitudinal studies to identify biomarkers that correlate with or predict long-term clinical, radiographic, functional outcomes and treatment response. Research will be most valuable if it identifies biomarkers that fulfil the Outcome Measures in Rheumatology Clinical Trials (OMERACT) filter: truth; discrimination and feasibility [152]. It is likely than a panel of biomarkers, rather than a single biomarker, will achieve this [127, 153].
2.6 PUBLICATIONS ARISING FROM THE WORK OF THIS THESIS

CHAPTER

Peer-reviewed papers


Book Chapter


Conference abstract (poster presentation)

3 CHAPTER III: AIMS AND OBJECTIVES

3.1 AIMS

The aims of this thesis are to determine the prevalence and characteristics of psoriatic spondyloarthritis (PsSpA) and psoriatic arthritis mutilans (PAM) in a cohort of psoriatic arthritis (PsA) patients, and serum-soluble bone-turnover biomarkers of these PsA phenotypes.

3.2 OBJECTIVES

3.2.1 OBJECTIVE 1: Prevalence and characteristics of PsSpA in PsA

1a) To determine the prevalence and characteristics of axial involvement in a PsA cohort, using patient reported outcome measures (PROMs), clinical examination indices and radiographic indices.

1b) To compare the pattern of axial involvement in a PsA cohort with that of an AS cohort, using PROMs, clinical examination indices and radiographic indices.

3.2.2 OBJECTIVE 2: Prevalence, characteristics and natural radiographic history of PAM

2a) To determine the prevalence of PAM in a cohort of PsA cases.

2b) To compare the clinical characteristics of PAM and non-PAM cases in a cohort of PsA cases using PROMs, clinical history and radiographic indices.

2c) To determine the natural radiographic history of PAM.
3.2.3 **OBJECTIVE 3: Serum-soluble bone-turnover biomarkers of PsSpA**

3a) To investigate four potential serum-soluble bone-turnover biomarkers (osteoprotegerin, OPG; Dickkopf 1, Dkk-1; matrix metalloproteinase 3, MMP-3; and macrophage colony stimulating factor, M-CSF) as predictors of PsSpA occurrence, by comparison with PsA, AS, psoriasis-only (PsC) cases and healthy controls (HC).

3b) To investigate four potential serum-soluble bone-turnover biomarkers (OPG, Dkk-1, MMP-3 and M-CSF) as predictors of disease severity in patients with PsSpA.

3c) To investigate four potential serum-soluble bone-turnover biomarkers (OPG, Dkk-1, MMP-3 and M-CSF) as predictors of PAM occurrence, by comparison with non-PAM PsA cases.
CHAPTER IV: STUDY DESIGN AND GENERAL METHODS

1.1 STUDY DESIGN

A single-centre prospective observational study was conducted at the Royal National Hospital for Rheumatic Diseases (RNHRD) in Bath, U.K.. Consecutive PsA patients were recruited from those attending a dedicated PsA clinic (seeing 600 unique PsA cases per year), and consecutive AS patients were recruited from those attending a dedicated AS clinic (seeing 500 unique AS cases per year). This study was named the Axial Disease in Psoriatic Arthritis (ADIPSA) study. The study data collection proforma is included in Appendix 1. The patient information sheet (PIS) and consent form are included in Appendix 2.

4.1.1 Study eligibility criteria

Study inclusion criteria

Either:
(i) psoriatic arthritis (PsA) patients fulfilling the Classification of Psoriatic Arthritis (CASPAR) criteria for PsA [24], or
(ii) ankylosing spondylitis (AS) patients fulfilling the 1984 modified New York diagnostic criteria for AS [43].

Exclusion criteria

(i) Patients younger than 18 years of age.
(ii) Patients with severe mechanical spinal disease (e.g. spinal canal stenosis, disc prolapse) that would significantly complicate the assessment of inflammatory spinal disease.
(iii) Patients with other concomitant inflammatory arthritis (e.g. rheumatoid arthritis, crystal arthritis, polymyalgia rheumatica, diffuse idiopathic skeletal hyperostosis, etc.).
4.2 STUDY RECRUITMENT

Recruitment of study participants commenced on 1st August 2012, and completed on 10th October 2013. Axial radiographic scoring and laboratory testing were performed after all participants were recruited.

All patients had previously given consent to be screened and approached for research studies. Four weeks in advance of scheduled PsA and AS outpatient clinics, patients meeting the stated eligibility criteria were identified by DJ for potential study enrolment, using the existing PsA and AS clinical research databases.

Three weeks prior to their routine outpatient clinic attendance, 250 consecutive PsA patients and 250 consecutive AS patients were posted a ‘Study Invitation Letter’ and ‘Patient Information Sheet’ (PIS) that included detailed information about the study rationale, design and personal implications of the study. Patients interested in taking part returned the enclosed ‘Study Interest Reply Slip’. Patients identified by DJ were also approached about study participation by the rheumatologist consulting them in clinic.

4.2.1 Consent

On the day of routine outpatient clinic appointment, invited patients were approached by their routine clinician for further face-to-face counselling with DJ. Confirmation of eligibility and completion of informed written consent for study enrolment were performed.

Completion of patient-reported outcome measure (PROM) questionnaires, clinical measurements and blood sampling took take place immediately.

Patients were given further opportunity to discuss the study either by telephone or in person with DJ if they wished. The right of a patient to refuse consent without giving reasons was respected. The patient was free to withdraw from the study at any time without giving reasons and without prejudicing further management.

4.2.2 Blinding

As only one researcher (DJ) was available to identify eligible patients, recruit patients and perform the clinical assessment, it was not possible to blind the assessor as to the
patient's underlying diagnosis of PsA or AS. However, during axial radiographic scoring, the patients’ rheumatological diagnosis was undisclosed.

4.3 PATIENT REPORTED OUTCOME MEASURES

All participants independently completed the following patient reported outcome measures (PROMs): Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) [154], Bath Ankylosing Spondylitis Functional Index (BASFI) [155], Stanford Health Assessment Questionnaire (HAQ) [156]. The Ankylosing Spondylitis Disease Activity Score (ASDAS) [157] was derived from the completed BASDAI, BASFI and C-reactive protein blood test. All PROMs were checked after completion by DJ to ensure no missing data.

Patients were questioned by DJ for the presence or absence of inflammatory axial symptoms (current or ever), using the Assessment of Spondyloarthritis International Society (ASAS) definition [123]. Current or previous dactylitis, peripheral enthesitis (including plantar fasciitis and greater trochanteric pain), and extra-articular manifestations (EAMs) were also queried, including: chest wall pain, uveitis and IBD. A standardised description was made as follows:

- **Inflammatory pain**: Insidious onset pain with stiffness that is worse first thing after waking or after sitting down for an hour; it improves later in the day with some activity / exercise; it lasts for >30 min each time; improved by NSAIDs.
- **Chest wall pain**: Pain and / or swelling either side of the breast bone.
- **Enthesitis**: Pain and / or swelling where ligaments join bone e.g. tennis elbow, golfer's elbow, Achilles tendon, front of knee cap.
- **Plantar fasciitis**: Pain on the sole of the foot near the heel base.
- **Dactylitis**: When a finger or toe swells up along its entire length "like a sausage", with a shiny stretched skin appearance.
- **Uveitis**: Red painful eye that affects vision. Lasts for a week or more. Often needs steroid eyedrops to settle.
- **IBD**: A formal diagnosis of Crohn's disease or ulcerative colitis, presenting as bloody diarrhoea, even at night, weight loss, and lasting for several months.

An enquiry was made of a family history in first-degree relatives of SpA (PsA, AS or undifferentiated SpA), psoriasis, uveitis and / or IBD.

The data collection proforma is included in Appendix 1.
4.3.1 Bath Ankylosing Spondylitis Disease Activity Index (BASDAI)

The BASDAI is a PROM of musculoskeletal disease activity (mainly axial, but some peripheral), that was originally devised in 1994 for use in AS [154]. It consists of 10 cm visual analogue scores (VAS) to answer six questions, pertaining to the five major symptoms of AS over the preceding week: fatigue; spinal pain; joint pain or swelling; areas of localised tenderness; and morning stiffness (Appendix 1). To give each symptom equal weighting, the mean of the two scores relating to morning stiffness is taken. The resulting 0 to 50 score is divided by five, to give a final score range for the BASDAI of 0 to 10. A BASDAI score of greater than or equal to four, is considered to indicate active disease [158, 159]. A score of less than four is considered to indicate inactive disease [158, 159]. Clinical response is regarded as a reduction in BASDAI of 2 units or more [158, 159]. The minimum clinically important difference (MCID) for the BASDAI is considered to be one unit [160]. Absolute values for BASDAI in AS compared with age and sex-matched healthy controls have been determined as reference centile charts [161]. In AS the BASDAI has shown to be feasible, reliable, have good score distribution, and good sensitivity to change [154, 162]. The BASDAI has subsequently been validated for use in PsSpA [163, 164], and this is discussed in more detail in Chapter 5.

4.3.2 Bath Ankylosing Spondylitis Functional Index (BASFI)

The BASFI is a PROM designed to determine the degree of functional limitation in patients with AS, and was conceived in 1994 [155]. It consists of 10 cm visual analogue scores (VAS) to answer ten questions relating to functional ability during the preceding week. Eight questions consider activities related to functional anatomy, and two questions assess the patient’s ability to cope with everyday life (Appendix 1). The resulting 0 to 100 score is divided by ten, to give a final score range for the BASFI of 0 to 10. A BASFI score of greater than or equal to five, is considered to indicate poor functional ability [155, 165]. A score of less than five is considered to indicate good functional ability [155, 165]. Clinical response is regarded as a reduction in BASFI of 2 units or more [155, 160]. The MCID for the BASDAI is considered to be 0.7 unit [160]. Absolute values for BASFI in AS compared with age and sex-matched healthy controls have been determined as reference centile charts [161]. Further details for the BASFI are given in Chapter 5. In AS the BASFI has shown to be feasible, reliable, have good score distribution, and good sensitivity to change [155, 162]. The BASFI has subsequently been validated for use in PsSpA [64], and this is discussed in more detail in Chapter 5.
Ankylosing Spondylitis Disease Activity Score (ASDAS)

The ASDAS is a composite score comprising PROMs and one objective laboratory measure of inflammation, the C-reactive protein (CRP). The ASDAS comprises three questions from the BASDAI and one question from the BASFI. It was originally devised for AS as an indicator of musculoskeletal (axial mainly, but also including peripheral) disease activity. Its main strength over the BASDAI is thought to be its inclusion of an objective biochemical marker of inflammation, the CRP [157]. The ASDAS comprises four grades of disease activity; inactive (score $\leq 1.2$), moderate (score 1.3 to 2.0), high (score 2.1 to 3.4) and very high (score $\geq 3.5$) [166]. A clinically important improvement is regarded as a 1.1 unit change, and a major improvement as a $\geq 2$ unit change [157]. The ASDAS has subsequently been validated for use in PsSpA [163, 164], and this is discussed in more detail in Chapter 5.

Health Assessment Questionnaire (HAQ)

The HAQ was originally devised for use in RA to measure physical function. It was subsequently validated for use in PsA [167-170]. Twenty questions relating to a patient's ability to perform activities related to daily living over the last week are asked in eight domains: dressing and grooming; rising; eating; walking; hygiene; reach; grip; and activities (Appendix 1). Each question is graded by the patients on a 0-3 Likert scale from “no difficulty” to “unable to do”. The score range of the final HAQ is continuous from 0 to 3, and is graded as follows: 0 to 1, mildly disabled; >1 to 2, moderately disabled; >2 to 3, severely disabled [162, 167]. The MCID for the HAQ in PsA is considered to be 0.35 [171]. The HAQ was modified for use in SpA by including two spinal domains (HAQ-S) [172], and for use in PsA by the addition of a skin domain (HAQ-SK) [167, 173]. However, the HAQ, HAQ-S and HAQ-SK have been shown to perform similarly [174]. Additionally, use of the same version of the HAQ across all rheumatological conditions may allow for more reliable comparison across diseases. Therefore in this study, the original HAQ was used.

CLINICAL EXAMINATION

All patients were examined and scored by DJ using the following clinical examination indices: Bath Ankylosing Spondylitis Metrology Index (BASMI) [175], chest expansion, Leeds Enthesitis Index (LEI) [176], 68 tender joint count (TJC) [177], 66 swollen joint

67
count (SJC) [177], Psoriasis Area and Severity Index (PASI) [178], and modified Nail Psoriasis Severity Index (mNAPSI) [179].

DJ was trained to perform the BASMI and chest expansion from AS specialist physiotherapists in the RNHRD who regularly perform it, alongside a review of the papers reporting the inception and application of the tools. DJ was trained to perform the PASI, TJC, SJC and mNAPSI from the PsA specialist nurses who regularly perform it as part of clinical and observational trials, alongside a review of the papers reporting the inception and application of the tools.

4.4.1 **Bath Ankylosing Spondylitis Metrology Index (BASMI)**

The BASMI is a clinical examination index designed to determine the degree of axial range of movement (metrology) limitation in patients with AS, and was conceived in 1994 [175]. It is performed by a trained health professional and measures five key metrology domains: cervical rotation (with a value ≤70 degrees considered to be limited); ear tragus to wall distance (a marker of abnormally forward neck position); lumbar side-flexion (with a value ≤10 cm considered to be limited); modified Schober’s (a measure of forward lumbar flexion; with a value ≤5 cm considered to be limited); and inter-malleolar distance (a marker of hip disease) (Appendix 1). For cervical rotation, lumbar side flexion and tragus to wall, a mean of the left and right measurements are taken. The absolute values of each of the five domains are graded for metrology limitation, to give a final score range for the BASMI of 0 to 10. The MCID for the BASMI is considered to be one unit [160]. Absolute values for BASMI in AS compared with age and sex-matched healthy controls have been determined as reference centile charts [161]. In AS the BASMI has shown to be feasible, accurate, reliable, and have good sensitivity to change [162, 175]. The BASMI has subsequently been validated for use in PsSpA [62, 64, 180], and this is discussed in more detail in Chapter 5.

4.4.2 **Chest expansion**

Chest expansion is measured by the health professional at the level of the nipple using two hands clasped against the chest wall during full exhalation. As the patient inhales, the expanded distance between the examiner’s thumbs is recorded in centimeters. Normal chest expansion is ≥5 cm, but can be adjusted for the height and sex of the patient. Chest expansion tends to be reduced in patients with AS due to arthritis of the thoracic joints, but
the inter-rater reliability of the method has been variable in clinical studies [175]. The validity of chest expansion measurement in PsSpA has not been established.

4.4.3 Leeds Enthesitis Index (LEI)

The LEI measures six peripheral sites for the presence or absence of enthesitis as a binary outcome, and was both devised and validated for use in PsA [176]. The sites (three on each side of the body) are the: lateral epicondyle of the humerus (outer edge of the elbow); medial femoral condyle (inner edge of the knee); and Achilles tendon insertion at the calcaneum. The examiner applies moderate pressure (enough to blanche the finger nail) to these six sites to potentially elicit tenderness. The total score range is 0 to 6. Higher count represents greater enthesitis burden, but the score range has not been further sub-categorised in PsA [181].

4.4.4 Psoriasis Area and Severity Index (PASI)

The PASI was devised and validated for use in psoriasis [178]. The examiner grades the head, arms, trunk and legs according to four domains: surface area affected; erythaema (redness); desquamation (scaling); and infiltration (elevation of the plaque above the adjacent skin surface). The calculation of the final PASI is somewhat complex and is therefore detailed in Figure 4.1. The PASI score range is 0 to 72, but most patients are clustered between 0 and 15, and few patients have PASIs at the upper end of the scale; patients with very severe psoriasis usually have a PASIs of ≤40 [182]. A reduction in PASI score of ≥50% is regarded as a clinically significant end-point in the assessment of psoriasis, but an improvement of ≥75% is often the primary end-point in clinical trials [183]. However, there is evidence that PASI-75 calculation (delta-PASI) may under-represent clinical improvement, such that a PASI-75 actually represents a 95% clinical improvement [182].
The mNAPSI was devised and validated for use in psoriasis [179]. It scores all ten fingernails for: onycholysis (lifting of the distal nail; graded 0-3); pitting (small depressions in the nail; graded 0-3); crumbling (graded 0-3); leuconychia (excess whitening of the nail; graded 0-1 as present / absent); splinter haemorrhage (fine black lines of the nail; graded 0-1 as present / absent); hyperkeratosis (thickening of the nail; graded 0-1 as present / absent); and red spot lunula (red discolouration of the proximal nail bed; graded 0-1 as present / absent), as detailed in Figure 4.2. These individuals scores are added to give the final mNAPSI score range of 0 to 140. Higher scores represent worse nail disease, but have not been further sub-categorised for severity as yet. The toe nails are not scored, as they are frequently affected by fungal nail disease, that can mimic psoriatic nail disease. The patient and clinician also grade their overall impression of the nail disease using a 0 to 10 cm VAS; although this is not included in the final mNAPSI score.
Figure 4.2. Modified Nail Psoriasis Severity Index (mNAPSI)

4.4.6 Tender and swollen joint counts

A selection of 68 peripheral and root (hip, shoulder and costal) joints commonly affected in PsA are usually evaluated in cross-sectional and longitudinal clinical studies of PsA [177]. Sixty eight of these joints are assessed for tenderness (tender joint count; TJC) and 66 for swelling (swollen joint count; SJC) by the examiner (Appendix 1). Tenderness is elicited by the application of enough pressure to blanche the examiner’s finger nail. The hip joint can not reliably be examined for swelling, and both hips are therefore excluded from the SJC. Whilst this method has shown good intra-rater reliability [177], the inter-reliability is poorer [184].

4.5 AXIAL RADIOGRAPHIC ASSESSMENT

The most recent axial radiographs of the cervical (lateral), lumbar (lateral and anteroposterior) and pelvis (including SIJs) making a complete set were scored using the two most validated axial radiographic scoring tools for PsA; the Psoriatic Arthritis Spondylitis Radiology Index (PASRI) [185] and the modified Stoke Ankylosing Spondylitis Spinal Scoring tool (mSASSS) [186]. These scoring tools and their validation for use in
this study, along with the full methods for scoring are described in more detail in Chapter 6.

Where possible, radiographs taken at the same time-point were scored as a set. Where radiographs were taken at different time-points, the most recent time-point was taken as the date of radiographic assessment, on the condition that the interval between the earliest and latest radiograph was ≤5 years. All but six cases had radiographs taken within five years of study recruitment. The remaining six patients had one region of the spine radiographed between 5-7 years prior to study enrolment.

4.5.1 Definition of inflammatory radiographic axial disease

In keeping with previous publications, inflammatory radiographic axial disease (RAD) was defined as: the presence of modified New York criteria unilateral grade ≥3, or bilateral grade ≥2 sacroiliitis on AP pelvic radiograph; and / or ≥1 marginal / paramarginal syndesmophyte(s) of the cervical or lumbar spine [93, 187].

4.6 BLOOD SAMPLING

Each patient had a blood sample taken for serum-soluble biomarker testing and high-sensitivity CRP testing using commercially available enzyme-linked immunosorbent assay (ELISA) kits. Each patient also had a blood sample taken for deoxyribonucleic acid (DNA) extraction at the Bath Institute for Rheumatic Diseases (BIRD) laboratory, followed by HLA-B and HLA-C -sequencing by Professor Robert Winchester’s team at Columbia University, New York, USA. Full details of methods used to test for serum-soluble biomarkers are given in Chapter 7.

4.7 DATA-HANDLING

All data were collected and stored confidentially in Case Report Files (CRFs) at the RNHRD. All information collected during the course of the study will be kept strictly confidential. Information will be held securely on paper and electronically at the RNHRD, under the auspices of the RNHRD Data Protection Officer, in accordance with the Data Protection Act 1998. Operationally this included:
(i) Consent from patients to record personal details including name, date of birth, address and telephone number, National Health Service (NHS) identification number, hospital identification number, General Practitioner name and address.

(ii) Appropriate storage, restricted access and disposal arrangements for patient personal and clinical details.

(iii) Consent from patients for access to their medical records by responsible individuals from the research staff, the sponsor or from regulatory authorities, where it is relevant to study participation.

(iv) Consent from patients for the data collected for the study to be used to evaluate safety and develop new research.

In accordance with the principles of Good Clinical Practice and U.K. Clinical Trial Regulations guidelines, at the end of the study, data will be securely archived for a minimum of ten years. Arrangements for confidential destruction will then be made. Any patient withdrawing consent for their data to be used, will have their records confidentially destroyed.

4.8 STATISTICAL ANALYSIS & POWER CALCULATION

4.8.1 Power calculation

The target for study recruitment was 200 PsA and 200 AS cases. The power calculations for each element of the study (clinical, radiographic, serum biomarker and genetic biomarker) are described separately in the corresponding chapter of this thesis.

4.8.2 Statistical analysis

The statistical analyses specific to each study within this thesis are described in the relevant chapters.

Data were analysed using STATA 12.1 (2011 Texas, USA). Some data were analysed using ‘R’ (GNU Project, The R Foundation, Vienna, Austria) when help was sought from academic colleagues at the University of Bath (Dr. Gavin Shaddick, Reader of Mathematics, Department of Mathematical Sciences; and Ms Amelia Jobling, PhD Student, Department of Pharmacy and Pharmacology).
Univariate analyses were performed using the continuity-corrected Chi-squared test for categorical variables, independent Student’s t-test for normally distributed continuous data, the Mann-Whitney U test for non-normally distributed continuous data, Poisson regression for non-normally distributed continuous data comprised of integers, and zero-inflated Poisson regression for non-normally distributed continuous data with an excess of zero-values. Negative binomial regression and zero-inflated negative binomial regression were considered as an alternative to Poisson regression where the mean did not approximate the variance of the data.

Multivariate analyses were performed using logistic regression for categorical variables, linear regression for normally distributed continuous data, Poisson regression for non-normally distributed continuous data, and zero-inflated Poisson regression for non-normally distributed continuous data with an excess of zero-values. Negative binomial regression and zero-inflated negative binomial regression were considered as an alternative to Poisson regression where the mean did not approximate the variance of the data. A reverse step-wise regression approach was used, setting the alpha-level at 0.1 to determine inclusion or exclusion of covariates in the model. This allowed adjustment for potentially significant covariates including: sex, age at clinical assessment, age at spinal radiographic assessment, disease duration at clinical assessment, disease duration at radiographic assessment, Biologic agent use, synthetic DMARD use, smoking and body mass index; whilst minimising noise and maximising the statistical power of the analyses.

Data are presented as mean, median, inter-quartile range (IQR), standard deviation (SD), odds ratio (OR), adjusted OR (OR_{adj}), incidence risk ratio (IRR), adjusted IRR (IRR_{adj}), 95% confidence interval (95% CI), and p-value as appropriate to the data. The alpha-level for statistical significance was set at 0.05.

4.8.3 Missing data handling

Patients uncertain how to complete the PROMs correctly were given support by DJ to ensure correct and full completion. No missing data were therefore expected for the PROMs or clinical examination.

If an entire region of radiographic data was missing, e.g. no cervical spine images available, those patients were omitted from that component of the analyses. However, available radiographs were still scored as data remained valuable for specific sub-analyses e.g. associations with osteoporosiation as a binary outcome measure. As per methods used by Wanders et al. [188] and later by Lubrano et al. [185]: if >3 scoring sites
(e.g. lower border of T12 vertebrae) were missing, the radiographs were excluded; if ≤3 scoring sites were missing, the mean of the other scoring sites were used as a substitute for the missing sites.

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4.10 Ethical Approval

The study was awarded national ethical approval in May 2012 by Frenchay Research Ethics Committee, and local ethical approval by the RNHRD Research and Development committee in June 2012 (Appendix 3). The Axial Disease in PsA study (ADIPSA) study is a National Portfolio Study (Study I.D. 12579).
CHAPTER V: CLINICAL CHARACTERISTICS OF AXIAL DISEASE IN PSORIATIC ARTHRITIS, AND A COMPARISON WITH ANKYLOSING SPONDYLITIS

5.1 ABSTRACT

**Background:** Few studies have characterised psoriatic spondyloarthritis (PsSpA) using a robust classification system, and only four studies have compared the clinical characteristics of PsSpA and ankylosing spondylitis (AS). The objectives of this chapter of the thesis were to: (i) determine the prevalence of PsSpA in a cohort of SpA cases (psoriatic arthritis, PsA; and AS) with psoriasis; (ii) compare the sociodemographic and treatment characteristics of PsSpA, peripheral-only PsA (pPsA) and AS cases; (iii) compare patient-reported outcome measures (PROMs) and clinical examination indices in PsSpA, pPsA and AS cases.

**Methods:** A prospective single-centre cross-sectional observational study was conducted, recruiting consecutive PsA and AS cases. Participants completed PROMs, were examined, and their axial radiographs were scored. Univariate and multivariate analyses were performed.

**Results:** The 402 enrolled cases (201 PsA and 201 AS) were reclassified based on radiographic axial disease and psoriasis into three more precise groups: 118 PsSpA, 127 pPsA, and 157 AS cases. The prevalence of PsSpA in this SpA cohort was 118/245 (48.16%). The modified New York diagnostic criteria for AS were fulfilled by 48/201 (23.88%) of PsA cases, and the Classification of Psoriatic Arthritis (CASPAR) criteria were fulfilled by 49/201 (24.38%) of AS cases.

Symptomatically-silent but radiographically-evident PsSpA was present in 30/118 (25.42%). pPsA compared with PsSpA cases: were less likely to be HLA-B27 positive (odds ratio, OR 0.12; 95% CI 0.05, 0.25; p<0.001), and have inflammatory bowel disease (adjusted OR, OR_{adj} 0.12; 95% CI 0.03, 0.55; p=0.01); had older age at arthritis symptom onset (median 37.98 vs. 31.27 years; p=0.01); had lower psoriatic nail disease severity (mNAPSI; adjusted incidence risk ratio, IRR_{adj} 0.90 per unit increase; 95% CI 0.83, 0.97; p=0.01), in particular nail onycholysis severity (IRR_{adj} 0.84 per unit increase; 95% CI 0.74, 0.95; p=0.01). Male PsA cases were no more likely to be classified as PsSpA than female patients.

AS and PsSpA cases had similarly high disease activity (as measured by ASDAS, BASDAI, and PaGA). Axial metrology as measured by the BASMI was no different in AS
compared with PsSpA cases, although tragus to wall distance (adjusted mean difference 2.00 cm; 95% CI 0.58, 3.42; p=0.006), and modified Schober test (adjusted mean difference -0.65 cm; 95% CI -1.14, -0.15; p=0.01) were poorer in AS compared with PsSpA cases. Disability (HAQ) was no different across the three disease groups. Function was poorer in AS compared with PsSpA cases (BASFI, IRR adj 1.17 for every 0.7 unit increase in score; 95% CI 1.06, 1.26; p=0.002).

Conclusions: A significant proportion of PsA cases have PsSpA, and it is often asymptomatic. Up to one quarter of PsA and AS cases may be classified as the other disease using the currently available classification systems. Several predictors of PsSpA occurrence in PsA were identified. Measures of disease activity, metrology and disability were equally poor in PsSpA and AS cases. All three groups were equally likely to proceed to anti-TNF therapy, at a similar interval from diagnosis. PsSpA should no longer be regarded as a clinically-milder form of AS.
5.2 INTRODUCTION

5.2.1 Clinical characteristics of psoriatic spondyloarthritis

Ten studies have compared the sociodemographic and clinical characteristics of psoriatic spondyloarthritis (PsSpA) and peripheral-only PsA (pPsA; without axial disease) [61-64, 67-69, 111, 189, 190]. From the literature, the prevalence of PsSpA in PsA cohorts is estimated to be 24 - 40 per 100 PsA cases [63, 69, 111]. There are no estimates of asymptomatic (no clinical symptoms or signs) radiographic PsSpA, but one study found asymptomatic radiographic cervical spondylitis to be present in 41% of PsSpA cases [190]. Clinical predictors for PsSpA occurrence in PsA include: male sex [58, 63, 69], HLA-B27 positivity [61], psoriatic nail dystrophy [61], higher number of radiographically damaged joints [61], high erythrocyte sedimentation rate (ESR) [61], and longer disease duration, such that axial disease in PsA is a late-onset feature [68, 111]. The latter clinical predictor may explain much of the variation in estimated prevalence of PsSpA in PsA cohorts; many studies have investigated PsA cases with relatively recent onset disease, allowing insufficient time for the PsSpA phenotype to have fully expressed. There are conflicting reports for both early-age at psoriasis onset [67], and later-age [69] at psoriasis onset being a clinical predictor of PsSpA in PsA. The following are reported as being more common in PsSpA compared with pPsA patients: chronic inflammatory back pain [69], uveitis [69], inflammatory bowel disease (IBD) [68], and peripheral joint ankylosis [68]. The following have been reported to be higher (i.e. poorer) in PsSpA compared with pPsA patients: peripheral joint counts [68], ESR [61, 62], C-reactive protein (CRP) [62], and axial metrology as measured by the Bath AS Metrology Index (BASMI) [62, 64] or by axial region [62, 69, 189]. Reports of composite indices pertaining to function (Bath AS Functional Index; BASFI) [62, 64], disease activity (Bath AS Disease Activity Index; BASDAI) [62], and disability (Health Assessment Questionnaire; HAQ) [62, 64, 68] have had conflicting results; with some reporting either no difference or poorer scores in PsSpA compared with pPsA patients. No differences between PsSpA and pPsA patients have been reported in terms of the occurrence of enthesitis [62], or skin severity (Psoriasis Area and Severity Index; PASI) [62]. In terms of prognosis, in one study male PsSpA cases had more advanced disease as measured by the proportion with grade 4 sacroiliitis and lumbar forward-flexion [111].

Certain themes for similarities and differences between PsSpA and pPsA emerge, but many features are investigated by only one study, and are therefore never corroborated nor challenged. Some studies have used convenience samples as part of a retrospective study, with incomplete clinical and radiographic data [65]. Several studies have not or only
partially adjusted for confounders. Some of the earliest studies from the 1960s and 1970s have not statistically compared the features of PsSpA and AS, providing descriptive analyses only [59, 191]. One of the major reasons for disparity in study results, is that there is no international consensus on how to classify cohorts of PsA and ankylosing spondylitis (AS) cases as PsSpA, pPsA and classical AS with coincidental psoriasis. Studies have therefore markedly varied in their case definition, and classification systems employed have often not been sufficiently stringent. Case definition is the fundamental first stage in studies comparing clinical characteristics and outcome across groups. Without a robust and consistent case definition, comparisons are simply unreliable and as described in the literature review above, are often not reproducible in independent studies. Knowledge on how to best classify PsSpA has improved over time through progressively more clinical experience, awareness of its occurrence as an entity, inception and validation of clinical indices, growth of radiographic repositories to allow better powered studies to be conducted, newer more sensitive and specific imaging techniques, and the expansion of genetic testing available for these cohorts. The studies reporting these results are summarised in turn below, along with a critique of their study methods, reliability of results, and where possible an explanation for any disparity between study results. This will allow the methods and results of this chapter of the thesis to be put into context of the existing literature comparing the clinical characteristics of PsSpA and pPsA.

5.2.2 Clinical features of PsSpA compared with pPsA

In 1977 Lambert and Wright reported the largest detailed description of axial disease in PsA [69]. They described spinal disease in 130 PsA patients, and noted a prevalence of PsSpA of 40%, although only 51/130 (39%) had complete axial radiographs for assessment. Whilst the criteria to classify cases as PsSpA were not stated, they are likely to have been strict given that 86% of PsSpA patients, but only 27% of PsA cases with peripheral-only and without radiographic axial disease (pPsA) fulfilled the New York criteria for AS. Compared with the pPsA group, the PsSpA group had proportionally more males (male:female ratio, 0.6:1.0 vs. 1.2:1.0, respectively; p<0.05), less prevalent nail dystrophy (mean 43 vs. 86%, respectively; p<0.05), and patients had developed psoriasis at a later age (mean 35 vs. 24 years, respectively; p<0.05). Chronic back pain (100 vs. 37%, respectively; p<0.05), restriction of neck movement (83 vs. 32%, respectively; p<0.05), lumbar movement (71 vs. 5%, respectively; p<0.05), chest expansion (71 vs. 11%, respectively; p<0.05), uveitis (14 vs. 5%, respectively), higher values of erythrocyte sedimentation rate (ESR, a measure of biochemical inflammation), and HLA-B27 positivity
(71 vs. 18%, respectively; p<0.05) were more common in the PsSpA than in the pPsA groups. Of note, pPsA patients rarely had axial symptoms or signs.

In a cohort study from Toronto (Canada) by Hanly et al. published in 1988, of 220 PsA cases followed-up for at least 30 months. 52/220 (24%) were identified as PsSpA clinically or radiographically at study enrollment [63]. Clinical criteria for PsSpA classification were moderately stringent: inflammatory pain / stiffness of the cervical / lumbar spine, with evidence of clinical sacroiliac disease (Gaenslen test, Patrick-FABER test, or direct compression). Radiographic criteria included the presence of sacroiliitis (unilateral grade ≥2). A slight male predominance in the PsSpA group was noted (male:female ratio 30:22). A subsequent report in 1992, from the now larger Toronto cohort of 498 PsA cases, showed a higher prevalence of PsSpA of 194/498 (39%) cases [111]. This might indicate that the prevalence of PsSpA rises with increasing disease duration, and that axial disease in PsA is late-onset feature. The primary objective of the study was to compare males and females with PsSpA. No differences in the degree of damage, type of peripheral arthritis, or medication use were noted between male and female PsSpA cases. However, male PsSpA cases appeared to have more advanced disease as measured by the proportion with grade 4 sacroiliitis (16 vs. 5%; p=0.04) and finger to floor distance (11.2 vs. 6.7 cm; p=0.03). These differences were not explained by HLA- differences in males and females.

In a study of 75 PsA cases from Bath, cases with radiographic cervical spondylitis, compared to those without, had more frequent sacroiliitis (50 vs. 7%; p<0.001), higher peripheral joint counts (1.88 vs. 1.31; p<0.001), peripheral joint ankylosis (49 vs. 0; p<0.001), and more disability as measured by the HAQ (p<0.001) [68]. IBD was found to be particularly frequent in those with PsSpA, emphasising the overlap of PsSpA and AS. IBD was present in 7/23 (30%) PsSpA, 3/15 (20%) oligoarthritis, and 0/26 polyarthritic PsA cases [192]. However, the prevalence of IBD in PsSpA cases appears to be significantly lower than that reported in non-psoriatic SpA [192]. In another study, this time from Finland in 2002, investigating cervical spondylitis in 160 PsA cases, 65/160 had cervical radiographs, and 12/65 were noted to have radiographic cervical disease. Cervical rotation was measured in 40/65 of these PsSpA cases, and was limited to less than 45 degrees on either side in 20/40 (50%) of cases [189].

A study from Spain by Queiro et al. identified 14 symptomatically-silent PsSpA cases (radiographic axial disease in the absence of inflammatory lumbar or buttock pain) from a cohort of 70 PsSpA cases (20%) [190]. However, the criteria for radiographic axial disease was not detailed. An association between symptomatic disease and longer
duration of arthritis (p=0.04) was demonstrated, but no adjustment was made for sex or age at assessment. Sacroilitis was present in 11/14, facet joint disease (erosion / fusion) in 2/14, and aseptic discitis in 1/14. This study also indicated that radiographic cervical disease in particular may be asymptomatic; of 29/70 (41%) cases with radiographic cervical disease, only 17/29 (59%) had pain and rigidity at that level, whilst 12/29 (41%) were asymptomatic (without clinical symptoms or signs).

The same group published a study six years later proposing that the phenotype of PsSpA varies according to the age at PsSpA onset [193]. Seventy patients from a SpA clinic were diagnosed with PsSpA according to the European Spondyloarthropathy Steering Group (ESSG) for SpA criteria plus radiographic sacroilitis, and prospectively followed. Patients were divided into two groups: young-onset PsSpA (disease onset <40 years of age; n=39), and late-onset PsSpA (disease onset ≥40 years of age; n=31). Young-onset cases tended to have a higher frequency of bilateral sacroilitis (62 vs. 29%; p=0.01) and be HLA-B27 positive (51% vs. 13%; p=0.001). Late-onset PsSpA cases had a higher frequency of unilateral sacroilitis (71 vs. 38%; p=0.01), silent axial disease (32% vs. 10%; p=0.02), and polyarthritis (45 vs. 23%; p=0.02). No differences were found between the two groups in terms of composite outcome measures: BASDAI, BASFI, BASMI, Maastricht Ankylosing Spondylitis Enthesitis Score (MASES), or BASRI. Importantly, no multivariate modeling was performed in this study to adjust for the differing disease duration, age at assessment, or sex in the two groups. The difference in sacroilitis frequency in the two groups may simply be a cohort effect; with young-onset PsA cases having had disease for longer, therefore more time to develop to sacroilitis. Given that the ESSG criteria [60] have not shown good specificity or sensitivity for axial disease in PsA [24, 57], one might argue that: the young-onset PsSpA cases were in fact patients with classical AS with coincidental psoriasis, with the classical bilateral sacroilitis and higher prevalence of HLA-B27 variants; and that the late-onset PsSpA cases were the ‘true’ PsSpA cases, with polyarthritic often symptomatically-silent unilateral sacroilitis.

Lubrano et al. conducted a multi-centre study in Italy of 77 ‘PsSpA’ cases recruited consecutively from the outpatient department (mean age 49 years, and mean disease duration 14 years) [186]. PsSpA was defined on the basis of meeting CASPAR criteria, and the presence of clinical (axial inflammatory pain according to the Calin criteria [194]) and / or radiographic axial disease). The classification criteria used in this study were less stringent, and resulted in half the cohort (37/75) being classified as PsSpA on the basis of axial symptoms but no radiographic axial disease. If aligned with the classification criteria used by most other studies, only 40/77 cases were in fact PsSpA. As a result of the classification criteria employed, only 19/60 (32%) were HLA-B27 positive, and little further
can be gleaned from the study’s secondary objectives of describing the clinical and radiographic features of PsSpA.

A cross-sectional study of 125 PsA cases attending a tertiary-referral hospital in Hong Kong, 29/125 (23%) classified PsA cases as PsSpA on the basis of radiographic sacroiliitis (grade ≥2 bilaterally, or grade ≥3 unilaterally) and 96/125 (77%) as pPsA [62]. Multivariate analyses were not conducted, and no adjustments were made for the longer disease duration in the PsSpA cases compared with pPsA cases. Univariate analyses showed no difference between the two groups in terms of proportion of males, tender joint counts, swollen joint counts, current dactylitis, enthesitis burden as measured by the MASES, BMI, or psoriasis severity as measured by the PASI. Compared with pPsA cases, the PsSpA cases had higher CRP (9.6 vs. 17.4 mg/L; p=0.02) and ESR (28.4 vs. 43.6 mm/h; p=0.01) levels. However, no adjustment was made for BMI which is known to influence CRP levels.

5.2.3 Risk factors for axial disease in PsA

Risk factors for axial involvement in 206 PsA cases were investigated in a prospective cohort study by Chandran et al., published in 2010 [61]. PsA patients were classified as PsSpA using stringent criteria: sacroiliitis (grade ≥2 bilaterally or grade ≥3 unilaterally), and inflammatory spinal pain and / or limited spinal mobility. Multivariate analyses showed that the risk of having PsSpA, rather than pPsA, was significantly increased by higher radiographically damaged peripheral joint count (OR 1.12; 95% CI 1.07, 1.17; p<0.0001), HLA-B27 positivity (OR 5.75; 95% CI 2.22, 14.90; p=0.0003), and an elevated ESR (OR 1.02; 95% CI 1.01, 1.04; p=0.003).

In the same study, of the 156 PsA patients without evidence of PsSpA at their first clinic visit, 28/156 developed PsSpA during a median follow-up period of 6.4 (range 0.5 to 31) years [61]. The risk of developing PsSpA during follow-up was increased by the presence of nail dystrophy (relative risk, RR 7.07; 95% CI 1.56, 43.04; p=0.01), the presence of periostitis (RR 5.67; 95% CI 2.11, 15.27; p<0.0001), higher radiographically damaged peripheral joint count (RR 1.11; 95% CI 1.05, 1.17; p<0.0001), elevated ESR (RR 1.03; 95% CI 1.01, 1.05; p=0.001). After ten years of follow-up, it was estimated that 15% would develop PsSpA. The findings were similar when the criteria for PsSpA were relaxed to radiographic criteria alone. Male sex, number of radiographically damaged joints, and elevated ESR increased the odds of having PsSpA at the first clinic visit, and developing it during follow-up. These risk factors align with the currently accepted theory for the pathogenesis of SpA: psoriatic nail dystrophy is thought to indicate inflammation of the
synovial enthesal complex; and periostitis is thought to be a precursor to osteoproliferation [195].

Jenkinson et al. reported disease duration to be longer in PsA cases with cervical spondylitis, than in those without (18.7 vs. 6.6 years; p<0.001) (n=75), implying that the prevalence of cervical spondylitis rises with increasing disease duration [68]. Radiographic cervical spondylitis was not related to sex, age at arthritis onset, arthritis severity, skin psoriasis severity as measured by the PASI, psoriatic nail dystrophy severity as measured by the Bath Nail Score, or any of the other four subgroups of PsA. It is difficult to generalise these risk factors for cervical spondylitis to PsSpA affecting the whole spine, nonetheless, this study identifies potential risk factors to explore further in PsSpA.

Most studies of PsSpA have been performed in Caucasian populations. In a Korean population, Baek et al. demonstrated an earlier age of psoriasis onset (median age 22 vs. 31 years; p=0.03), and an earlier age of arthritis onset (median age 29 vs. 44 years; p=0.01) in PsSpA cases compared with pPsA cases [67]. The authors reported the prevalence of HLA-B27 positivity in PsA cases to be 8%, which is comparable to other general PsA cohorts, but unfortunately did not report the prevalence of HLA-B27 in their PsSpA cases. This would have been particularly interesting given the unique non-Caucasian ancestral origin of this PsA cohort. In addition, comparisons between PsSpA and pPsA cases were not adjusted for HLA-B27 status, which would certainly have influenced the results.

5.2.4 Prognosis of PsSpA

In two studies, both using the Toronto cohort, PsSpA cases did not have a poorer prognosis than pPsA cases [30, 196]. However, males PsSpA cases were found to have more advanced disease than females, as measured by grade 4 sacroiliitis and finger to floor distance, even after adjustment for HLA-B27 status, age and disease duration [111].

The prospective Toronto cohort was used to evaluate disease course in PsSpA patients at study entry, five-year and ten-year follow-up [187]. Of 297 cases at study entry (mean age 43 years, arthritis duration 8 years), 56% had axial symptoms, 43% had radiographic sacroiliitis, and 13% had syndesmophytes. The frequency of patient-reported spinal pain, spinal stiffness, and clinical-sacroiliitis decreased significantly at both five and ten-year follow-up. However, there was a significant increase in the number of patients with clinician-measured restricted cervical mobility at both five and ten-year visits. This highlights the disparity between patients-reported and clinician-measured clinical indices,
and their potential reliability for use in longitudinal studies of PsSpA. Of those cases without sacroiliitis at baseline, 37% developed sacroiliitis (grade ≥2) at five-years, and 52% developed sacroiliitis at ten-years follow-up. Of those cases who presented with grade two sacroiliitis at baseline, 47% progressed to a higher grade at five-years, and 52% at ten-years follow-up. 25% of cases progressed from grade three to four sacroiliitis at ten-years follow-up. Of the patients without cervical, thoracic, or lumbar syndesmophytes at baseline: 11, 16, and 14% developed syndesmophytes in these regions at five-year follow-up, respectively; and 14, 21, and 20% at ten-years follow-up, respectively [187].

There have been no clinical trials specifically recruiting PsSpA cases. A very limited amount of data is available from a handful of biological studies of general PsA cohorts that have performed sub-analyses in those with PsSpA [197]. The impact of anti-TNF and NSAIDs therapy on structural progression in the related condition AS, also remains inconclusive [198].

5.2.5 Patient reported outcome measures in PsSpA: Utility and validation

Some patient reported outcome measures (PROMs) have been used and at least partially validated in axial PsSpA. These were briefly discussed in Chapter 4, and are discussed in more detail below.

5.2.5.1 Health Assessment Questionnaire Disability Index (HAQ)

In a Spanish study of 46 PsSpA, 54 pPsA, and 103 AS patients, the Health Assessment Questionnaire Disability Index (HAQ) was a good measure for disability related to peripheral arthritis, but less so for disability related to axial arthritis [64]. However, it did not differentiate between the PsSpA and pPsA groups. Although used widely in clinical practice for PsA, the results of this single study indicate that the HAQ may not be an effective tool for evaluating both peripheral and spinal disability in PsSpA. The HAQ was modified for use in SpA by including two spinal domains (HAQ-S) [172], and for use in PsA by the addition of a skin domain (HAQ-SK) [167, 173]. However, the HAQ, HAQ-S and HAQ-SK have been shown to perform similarly [174]. Additionally, use of the same version of the HAQ across all rheumatological conditions may allow for more reliable comparison across diseases.
5.2.5.2 Bath Ankylosing Spondylitis Functional Index (BASFI) and Bath Ankylosing Spondylitis Disease Activity Index (BASDAI)

In the same Spanish study, PsSpA cases reported higher BASFI scores than pPsA cases [64]. The BASFI showed good correlation with radiographic structural damage, as measured by the Bath AS Radiology Index (BASRI). Therefore, the authors proposed that the BASFI may be a useful tool for measuring function in PsSpA.

BASDAI performed similarly in evaluating disease activity in PsSpA, pPsA, and in AS (Cronbach’s alpha 0.65, 0.78, and 0.79, respectively) [199]. The analysis of convergent validity showed that in pPsA and PsSpA patients, the BASDAI was significantly correlated with other subjective disease activity parameters. For responsiveness, no association was found between changes in the BASDAI and changes in disease activity either in pPsA or in PsSpA. A study from Wellington (New Zealand) by Taylor et al., showed BASDAI performs similarly for PsSpA and pPsA, but does not correlate well with external indicators of disease activity, such as treatment decisions [200]. 133 PsA patients identified from a disease-register, completed a postal questionnaire, and 86/133 consented to be examined. BASDAI correlated highly with patient perception of disease activity (Spearman r=0.739), but PsSpA and pPsA performed no differently. BASDAI was not associated with high or low disease activity as measured by other means: physician PGA, HAQ, Short Form-36 (SF-36), joint counts or acute phase reactants.

A prospective cross-sectional study comparing 1072 AS, 147 PsSpA, and 45 enteropathic SpA (eSpA) patients (with similar disease duration, but younger age in the AS group) found that BASDAI, BASFI, and quality of life (as measured by the AS Quality of Life score; ASQoL) were no different across the three groups [201]. In another study, twenty nine PsSpA cases were compared with 96 pPsA cases. The two groups were no different in terms of HAQ, BASDAI, BASFI, global pain (patient reported on a 100mm VAS), global health (patient reported on a 100mm VAS), or quality of life (SF-36) [62].

5.2.5.3 Ankylosing Spondylitis Disease Activity Score (ASDAS)

The discriminative ability and correlation of the Ankylosing Spondylitis Disease Activity Score using CRP (ASDASCPRP) and BASDAI with disease activity in PsSpA was tested in a study by Eder et al. in a large prospective cohort of PsA cases, and published in 2010 [163]. Patients were categorised into high and low disease activity states based on the patient’s global assessment score (PaGA), physician’s (PhGA) global assessment score, and physician’s decision to change treatment. In 201 PsSpA patients enrolled, ASDAS
and BASDAI showed good correlation with disease activity as reflected by the PaGA (correlation coefficient for BASDAI r=0.84, ASDAS r=0.81) and the PhGA (BASDAI r=0.53, ASDAS r=0.55). Both scores showed good discriminative ability between high and low disease activity states. However, there were no significant differences between areas under the curve for the models that compared ASDAS with BASDAI for each definition of disease activity state. BASDAI and ASDAS were therefore deemed to have similar good-moderate discriminative ability and correlation with different constructs of disease activity in PsSpA.

Subsequently in 2014, Kilic et al. compared the discriminative ability of ASDAS and BASDAI in 54 PsSpA cases from the Erciyes Spondyloarthritis Cohort and Anatolian Group for the Assessment in Rheumatic Disease cohorts [164]. ASDAS (both CRP and ESR versions) and BASDAI showed similar good discriminative ability between high and low disease activity, as reflected by the area under the curve analysis. The cut-off values for inactive disease (1.59) and high disease activity (2.03) were relatively similar to the cut-off values used in AS.

5.2.6 Clinical examination in PsSpA: Utility and validation

Some clinical examination indices have been used and at least partially validated in PsSpA, and will be discussed now. The International Spondyloarthritis Inter-observer Reliability Exercise (INSPIRE) study first demonstrated that the BASMI and its components (occiput to wall distance, tragus to wall distance, cervical rotation, Schober test) and chest expansion, all originally devised for use in AS, are also reliable in PsSpA [180]. These findings were corroborated in a more recent study of 46 PsSpA, 54 pPsA and 103 AS patients in Spain [64]. PsSpA patients demonstrated poorer metrology compared with pPsA cases, but of note, only minor differences were demonstrated between PsSpA and AS cases. The modified Schober test, lumbar side flexion, and cervical rotation were the more suitable measurements for assessing axial PsA.

The usefulness of various spinal mobility measurements in predicting PsSpA were evaluated in a cross-sectional study of 125 PsA cases in Hong Kong [62]. 29/125 (23.2%) were classified as PsSpA (based on sacroiliitis of grade ≥2 bilaterally, or grade ≥3 unilaterally) and 96/125 as pPsA. The BASMI differentiated PsSpA and pPsA. Components of the BASMI; tragus-to-wall distance, modified Schober test, and lumbar side-flexion showed good sensitivity and specificity in predicting PsSpA. In the logistic regression model, lumbar side flexion (independently) and modified Schober test best differentiated PsSpA and pPsA.
5.2.7 Comparison of PsSpA and AS

Only four studies have compared the sociodemographic and clinical characteristics of PsSpA and AS [49, 58, 64, 201]. The following are reported to be more common in PsSpA compared with AS: enthesitis [201], peripheral arthritis [201], and dactylitis [201]. Spinal pain and / or stiffness has been shown to be more frequent in AS compared with PsSpA patients [49, 201]. The following are reported to be poorer or have higher scores in AS compared with PsSpA: metrology [201] as measured by the BASMI [64], occiput to wall distance [64], Schober test of lumbar forward-flexion [49, 58, 64], and chest expansion [64] although not consistently [58]. Radiographic severity as measured by the Bath AS Radiology Index (BASRI) in one study was higher in AS compared with PsSpA [201], but no different as measured by five radiographic indices in another study [202]. Several composite indices are reported to be no different in AS and PsSpA cases: disease activity (BASDAI) [201], function (BASFI) [201], and quality of life [201].

In these studies certain themes for similarities and differences between PsSpA and AS emerge. However, on closer inspection it is noted that these studies have used various classification systems for their cases, for some clinical characteristics no other studies have attempted to replicate results in an independent cohort, different sampling methods and study design have been used, and the disease duration in some PsA cohort may simply be to short to have allowed full expression of the PsSpA phenotype. The studies reporting these results are summarised in turn below, along with a critique of their study methods, reliability of results, and where possible an explanation for any disparity between study results. This will allow the methods and results of this chapter of the thesis, investigating and comparing the clinical characteristics of PsSpA and AS, to be put into context of the existing literature.

5.2.7.1 Clinical features in PsSpA and AS cases

In 1974, an expert in the field of PsA, Professor John Moll drew on the literature and his clinical experience, and wrote that PsSpA is probably a specific entity, as an alternative expression of PsA, but that in most cases there are no clinical or radiological differences from classical AS [203]. This has been supported, but also contested by researchers and commentators. Only four studies have directly compared PsSpA with AS cases in terms of clinical characteristics [49, 58, 64, 201], however, all but one have had a small sample size.
In 1993, Gladman et al. compared 66 PsSpA and 40 AS in terms of clinical, radiographic and HLA-status [49]. The criteria for classifying the PsA patients as PsSpA were relatively weak: the presence of inflammatory back pain / stiffness; and / or sacroiliitis on clinical examination; and / or grade ≥2 sacroiliitis; and / or ≥1 paramarginal syndesmophyte. The proportion of PsSpA patients earning their classification by each of the four routes was not reported, and a patient could have been classified as PsSpA on the basis of symptoms alone or clinical-examination sacroiliitis alone, without any radiographic evidence of disease. Even if radiographic disease was present, grade 2 sacroiliitis is relatively easy to attain, might not be pathological, and tends to have only moderate inter-rater reliability. In this study, clinical examination was performed by several different assessors, and intra- and inter-rater reliability testing not reported. Nonetheless, the authors reported that after adjusting for the pronounced differences in sex, age at assessment, and disease duration between the two groups, the AS patients were noted to have a higher frequency of inflammatory spinal (neck and / or lumbar) pain, inflammatory spinal stiffness (p<0.0002), and limitation of lumbar spinal movements (p<0.0006).

In a study by Helliwell et al. comparing 34 PsSpA, 91 AS, 31 eSpA, and 7 reSpA patients, all fulfilling modified New York criteria for AS, the PsSpA and AS cases were no different in terms of male:female ratio (approximately 3:1) [58]. Given that the ratio of males to females in general PsA cohorts is usually 1:1, and although the findings in this paper were not adjusted for age or disease duration, it does infer that males are more likely to experience PsSpA. A significant difference in lumbar forward flexion (using the modified Schober’s method) was found across the groups, and poorest in AS. Chest expansion was no different across groups. The results of this thesis chapter will be less directly comparable with the study by Helliwell et al. because our cases were not required to fulfill mNY criteria for AS; which require inflammatory spinal pain, radiographic sacroiliitis, and reduced range of movement in two planes. Simply through study eligibility criteria, our PsSpA cases may therefore be less severe than those reported by Helliwell et al.

Perez-Alamino et al. compared 2,044 consecutive SpA patients meeting ESSG criteria for SpA, to determine differential characteristics of axial involvement in PsSpA, AS and enteropathic SpA (eSpA) in a cohort of Ibero-American cases [201]. A total of 1,264 patients met the 1984 modified New York criteria for AS, and were further subclassified as 1072 AS, 147 PsSpA, and 45 eSpA. Median disease duration was comparable across the groups, but AS patients were significantly younger (p=0.01), and had a higher proportion of males (p=0.01). Analyses were therefore adjusted for sex, age and disease duration. AS patients had more prevalent inflammatory back and / or sacroiliac pain (p=0.05) than PsSpA patients. The frequency of dactylitis, enthesitis, and peripheral arthritis was higher.
in PsSpA patients than in AS patients (p=0.05). Compared with PsSpA patients, patient with AS have more severe axial involvement in terms of metrology, radiographic severity as measured using the BASRI total, and prevalence of IBP. Other clinical indices were no different between the two groups: disease activity, function, and quality of life. In summary, PsSpA appears to be as disabling as primary AS for two reasons: the disease progresses for a long time period, and brings with it the burden of peripheral arthritis.

5.2.7.2 Examination indices in PsSpA and AS

In a prospective cross-sectional study comparing 1072 AS, 147 PsSpA, and 45 eSpA patients (with similar disease duration, but younger age in the AS group), AS patients had more limited spinal mobility than the other two groups (p=0.0001) [201]. Another study comparing AS and PsSpA patients showed significantly worse occiput to wall distance, chest expansion and a lower modified Schober score in the AS patients [64].

5.2.7.3 Prognosis in PsSpA and AS

In a study with the primary objective to compare the reliability of four radiographic scoring methods for PsSpA (n=40) and AS (n=18), the two groups were found to differ in terms of severity score [202]. The PsSpA and AS cases were no different in terms of sex, age at radiograph or disease duration. Acknowledging the small sample size, and this being a secondary outcome analysis, axial disease severity was no different in PsSpA and AS cases as measured by the PASRI (p=0.12), mSASSS (p=0.49), BASRI-spine (p=0.26) or RASSS (p=0.70).

The clinical course of PsSpA was observed to be less disabling than that of AS in one study [70]. Whether clinical and radiographic severity differences between AS and PsSpA exist, as reported by some studies [58, 111, 204], is determined by the PsSpA classification criteria employed. Thresholds for classification of PsSpA have been very low in some studies.

5.2.8 Summary

Through a robust clinical and radiographic case classification system, this thesis chapter aims to investigate areas of limited knowledge and features of PsSpA important to patients. Comparisons will be made with the existing literature, to either corroborate or challenge the results of previous studies, and where possible explain any disparity.
5.3 STUDY OBJECTIVES

The objectives of this study chapter were to:

(i) determine the prevalence of PsSpA in a cohort of SpA (PsA and AS) cases with psoriasis.

(ii) compare the sociodemographic and treatment characteristics of PsSpA, pPsA and AS cases.

(iii) compare patient reported outcome measures in PsSpA, pPsA and AS cases.

(iv) compare clinical examination indices in PsSpA, pPsA and AS cases.
5.4 METHODS

A comprehensive description of general methods for this component of the Axial Disease in PsA (ADIPSA) study are detailed in Chapter 4 (Study Design and General Methods). Specific methods for this component of the study are described below. To avoid repetition, some items pertaining to radiographic methods, are provided in more detail in Chapter 6.

5.4.1 Patient reported outcome measures

All participants completed the following patient reported outcome measures (PROMs): Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) [154], Bath Ankylosing Spondylitis Functional Index (BASFI) [155], Stanford Health Assessment Questionnaire Disability Index (HAQ) [156]. The Ankylosing Spondylitis Disease Activity Score (ASDAS) [157] was derived from the completed BASDAI, BASFI and C-reactive protein blood test. To ensure no missing data all PROMs were checked after completion by DJ.

Patients were also questioned by DJ for the presence or absence of inflammatory axial symptoms (current or ever), using the ASAS definition [123]. Current or previous extra-articular manifestations (EAMs) were also queried, including: dactylitis, peripheral enthesitis (including plantar fasciitis and greater trochanteric pain), chest wall pain, uveitis and IBD. An enquiry was made of a family history in first-degree relatives of SpA, psoriasis, uveitis and / or IBD.

5.4.2 Clinical examination

All patients were examined and scored by DJ using the following clinical examination indices: Bath Ankylosing Spondylitis Metrology Index (BASMI) [175], chest expansion, Leeds Enthesitis Index (LEI) [176], 68 tender joint count (TJC) [177], 66 swollen joint count (SJC) [177], Psoriasis Area and Severity Index (PASI) [178], and modified Nail Psoriasis Severity Index (mNAPSI) [179].

DJ was trained to perform the BASMI and chest expansion from AS specialist physiotherapists in the RNHRD who regularly perform them, alongside a review of the papers devising these tools. DJ was trained to perform the PASI, TJC, SJC and nail score from the PsA specialist nurses who regularly perform it as part of clinical and observational trials, alongside a review of the papers devising these tools.
5.4.3 Blinding

As only one researcher (DJ) was available to perform the clinical assessment, it was not possible to blind the assessor as to the patient’s underlying diagnosis of PsA or AS.

5.4.4 Radiographic assessment and re-classification of the cohort

Detailed methods for radiographic assessment and scoring are provided in Chapter 6 of this thesis, and will therefore not be repeated here. In brief, the most recent axial radiographs of the cervical (lateral), lumbar (lateral and anteroposterior) and pelvis (including SIJs) making a complete set were scored using the two most validated axial radiographic scoring tools for PsA; the Psoriatic Arthritis Spondylitis Radiology Index (PASRI) [185] and the modified Stoke Ankylosing Spondylitis Spinal Scoring tool (mSASSS) [186]. Where possible, radiographs taken at the same time-point were scored as a set. Where radiographs were taken at different time-points, the most recent time-point was taken as the date of radiographic assessment, on the condition that the interval between the earliest and latest radiograph was ≤5 years.

PsA patients (all fulfilling CASPAR criteria) were reclassified as either PsSpA or PsA using a radiographic definition. In keeping with previous publications, inflammatory radiographic axial disease (RAD) in psoriatic patients was defined as: the presence of New York criteria sacroiliitis (unilateral grade ≥3, or bilateral grade ≥2) on AP pelvic radiograph; and / or ≥1 syndesmophyte (marginal or paramarginal) of the cervical or lumbar spine. The entire study cohort were then reclassified into three more precise groups: psoriatic cases with RAD were reclassified as PsSpA; psoriatic cases without RAD were reclassified as peripheral-only PsA without axial disease (pPsA); and the remaining patients were all classified as AS, since by study eligibility criteria, all fulfilled modified New York diagnostic criteria for AS (radiographic and clinical). This reclassification is described in more detail in Figure 5.1 in the Results section of this chapter.

5.4.5 DNA extraction and testing methods

Each patient had a blood sample taken for deoxyribonucleic acid (DNA) extraction at the BIRD laboratory using a standard salting technique. Extracted DNA was aliquoted and diluted in 100 mcl of deionised water and stored at -20 degrees Celsius for analysis later. Extracted DNA samples were forwarded as one batch to Professor Robert Winchester
(Department of Medicine, Columbia University Medical School, New York, USA) for HLA-B and HLA-C sequencing. HLA-B27 data are presented in this thesis, to allow HLA-B27 positivity prevalence to be compared across the three disease groups, and to allow HLA-B27 to be used a covariate in the regression models of clinical and radiographic outcome.

5.4.6 Statistical methods

5.4.6.1 Sample size calculation

Using an estimated prevalence of axial inflammatory pain in AS cases of 99% and 75% in PsSpA cases, the sample required to detect a difference between the two groups, with a power of 0.90 (setting the alpha level at 0.05) would be 48 PsSpA and 48 AS cases.

Based upon the mean HAQ in SpA cited by previous studies, and the minimum clinically important difference (MCID) for the HAQ in PsA estimated to be 0.35 [171], the sample size required to detect a difference in HAQ of 0.35 between PsSpA and AS cases (assuming a standard deviation of 0.3, an alpha level of 0.05, and a study Power of 0.90) would be 15 PsSpA and 15 AS cases.

5.4.6.2 Statistical analysis

Data were analysed using STATA 12.1 (2011 Texas, USA).

Univariate analyses were performed using the continuity-corrected Chi-squared test for categorical variables, independent Student’s t-test for normally distributed continuous data (e.g. age at clinical assessment, age at recent axial radiographs, and BMI), the Mann-Whitney U test for non-normally distributed continuous data (disease duration at clinical assessment, recent axial radiographs, and to first anti-TNF agent use), Poisson regression for non-normally distributed continuous data comprised of integers (e.g. BASDAI, Patient Global Assessment of axial disease activity, BASFI, and BASMI), and zero-inflated Poisson regression for non-normally distributed continuous data with an excess of zero-values (e.g. HAQ, tender joints counts, swollen joint counts, mNAPSI, nail onycholysis score, PASI and LEI). Negative binomial regression and zero-inflated negative binomial regression were considered as an alternative to Poisson regression where the mean did not approximate the variance of the data.

Multivariate analyses were performed using logistic regression for categorical variables, linear regression for normally distributed continuous data (e.g. tragus to wall distance,
Schober test, lumbar side flexion, inter-malleolar distance, chest expansion), Poisson regression for non-normally distributed continuous data (e.g. BASDAI, Patient Global Assessment of axial disease activity, BASFI, and BASMI), and zero-inflated Poisson regression for non-normally distributed continuous data with an excess of zero-values (e.g. HAQ, tender joints counts, swollen joint counts, mNAPSI, nail onycholysis score, PASI and LEI). Negative binomial regression and zero-inflated negative binomial regression were considered as an alternative to Poisson regression where the mean did not approximate the variance of the data. A reverse step-wise regression approach was used, setting the alpha-level at 0.1 to determine inclusion or exclusion of covariates in the model. This allowed adjustment for potentially significant confounders including: sex, age at clinical assessment, age at spinal radiographic assessment, disease duration at clinical assessment, disease duration at radiographic assessment, anti-TNF use at any time point during disease course (although it was found that all patients who had ever used an anti-TNF agent, were still using at the time of assessment), synthetic DMARD use (at any time point during disease course), smoking (current and ex-smokers were grouped together, and compared with never smokers) and body mass index (most recently recorded). It is acknowledged that indices such as BMI change over time, and that both smoking and medication use as a binary outcome does not capture the various durations and doses used by the patients. Univariate and multivariate reduced-models are presented in the tables as indicated.

Data are presented as mean, median, inter-quartile range (IQR), standard deviation (SD), odds ratio (OR), adjusted OR (OR_{adj}), incidence risk ratio (IRR), adjusted IRR (IRR_{adj}), 95% confidence interval (95% CI), p-value, and adjusted p-value as appropriate to the data. The alpha-level for statistical significance was set at 0.05.

5.4.6.3 Missing data handling

Patients uncertain how to correctly complete the PROMs were given support by DJ to ensure correct and full completion. No missing data were therefore expected for PROMs.
5.5 RESULTS

5.5.1 Demographic, lifestyle and treatment characteristics of the cohort

The study enrolled 402 SpA patients (201 PsA and 201 AS), and all completed clinical assessment. A full set of axial radiographs (cervical, lumbar and pelvis) were available on 392/402 cases, with an incomplete series of axial radiographs in 10/402 cases. Of these: 118/402 (29.35%) cases were classified as PsSpA based upon psoriasis (current or previous) and radiographic axial disease (RAD); 127/402 (31.60%) cases were classified as peripheral PsA (pPsA) based upon psoriasis (current or previous), peripheral arthritis and without RAD; and 157/402 (39.05%) cases were classified as AS based upon mNY criteria but no psoriasis (current or history of) (Figure 5.1).

Only ten PsA patients had an incomplete series of axial films to determine RAD, and were assumed to have peripheral arthritis only, and therefore classified as pPsA.

\[\text{pPsA: peripheral PsA  PsSpA: psoriatic spondyloarthropathy  AS: ankylosing spondylitis  mNY: modified New York criteria for AS}\]

**Figure 5.1. Classification of the study cohort**

5.5.1.1 Prevalence of PsSpA

The prevalence of PsSpA amongst the PsA cases was 74/201 (36.82%; 95% CI 30.14, 43.89 using Fisher Exact Clopper-Pearson). The prevalence of PsSpA in all SpA (PsA and AS) cases with psoriasis was 118/245 (48.16%; 95% CI 41.76, 64.61 using Fisher Exact Clopper-Pearson).
5.5.1.2 Fulfilment of CASPAR and modified New York classification criteria

Of 201 cases clinically coded as PsA according to hospital case-notes, as many as 48/201 (23.88%) also fulfilled modified New York criteria for AS, whilst 153/201 (76.12%) did not.

Of 201 cases clinically coded as AS according to hospital case-notes: 49/201 (24.38%) fulfilled CASPAR criteria for PsA; 46/201 indeterminate, though likely to fulfil CASPAR (score +1; RF is usually negative in AS, and the absence of peripheral radiographs suggests that the patient did not have peripheral arthritis to indicate having imaging and therefore likely would not have radiographic osteoproliferation); 3/201 were unlikely to fulfil CASPAR (score +2; no peripheral radiographs available); and 103/201 did not fulfil CASPAR. Thus, at least 49/201 (24.38%) AS patients fulfil CASPAR criteria for PsA.

5.5.1.3 Demographic characteristics of the pPsA, PsSpA and AS cases

The proportion of male cases in the PsSpA group was 74/118 (62.71%), 66/127 (51.97%) in the pPsA group, and 118/157 (75.16%) in the AS group (Table 5.1). There was a significantly higher proportion of males in the AS group compared with the PsSpA group (OR 1.80; 95% CI 1.07, 3.03; p=0.03), but not in the pPsA group compared with the PsSpA group. Male PsA cases were no more likely to be classified as PsSpA than female patients (OR_{adj} 0.65; 95% CI 0.39, 1.08; p=0.09) (adjusted for age at radiographic assessment, disease duration at radiographic assessment, HLA-B27 status, synthetic DMARD use ever, anti-TNF use ever, and smoking ever).
### Table 5.1. Sociodemographic and treatment characteristics of the pPsA, PsSpA and AS cases (categorical variables)

<table>
<thead>
<tr>
<th></th>
<th>pPsA (n=127)</th>
<th>PsSpA (n=118)</th>
<th>AS (n=157)</th>
<th>Comparison</th>
<th>Odds ratio (mean difference)</th>
<th>95% CI</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
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<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td>Ref (PsSpA)</td>
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<td></td>
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<tr>
<td></td>
<td>66/127 (51.97)</td>
<td>74/118 (62.71)</td>
<td>118/157 (75.16)</td>
<td>AS</td>
<td>1.80 (0.65)</td>
<td>1.07, 3.03</td>
<td>0.03*</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>pPsA</td>
<td></td>
<td>0.39, 1.08</td>
<td>0.09*</td>
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<td>HLA-B27 positive</td>
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<tr>
<td></td>
<td>9/127 (7.09)</td>
<td>47/118 (39.83)</td>
<td>140/157 (89.17)</td>
<td>Ref (PsSpA)</td>
<td>12.44 (0.12)</td>
<td>6.67, 23.22</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>AS</td>
<td></td>
<td>0.05, 0.25</td>
<td>&lt;0.001*</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>pPsA</td>
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<td></td>
<td></td>
<td>Smoking ever</td>
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<td></td>
<td>67/109 (61.47)</td>
<td>57/110 (51.82)</td>
<td>72/157 (45.86)</td>
<td>Ref (PsSpA)</td>
<td>0.79 (1.49)</td>
<td>0.48, 1.28</td>
<td>0.34*</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>AS</td>
<td></td>
<td>0.87, 2.56</td>
<td>0.15*</td>
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<td></td>
<td></td>
<td></td>
<td>pPsA</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Body mass index, Mean (SD)</td>
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<tr>
<td></td>
<td>29.69 (5.36)</td>
<td>29.52 (5.16)</td>
<td>29.92 (4.96)</td>
<td>Ref (PsSpA)</td>
<td>(-1.60) (-0.18)</td>
<td>-2.21, -0.84</td>
<td>0.01*</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td>AS</td>
<td></td>
<td>-0.62, 0.51</td>
<td>0.81*</td>
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<td></td>
<td>Synthetic DMARD use during disease course</td>
<td></td>
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<tr>
<td></td>
<td>83/127 (65.35)</td>
<td>53/118 (44.92)</td>
<td>26/157 (16.56)</td>
<td>Ref (PsSpA)</td>
<td>0.24 (2.33)</td>
<td>0.14, 0.42</td>
<td>&lt;0.001*</td>
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<tr>
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<td></td>
<td>AS</td>
<td></td>
<td>1.39, 3.85</td>
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<td></td>
<td>pPsA</td>
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<td></td>
<td></td>
<td>Anti-TNF use during disease course</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>47/127 (37.01)</td>
<td>53/118 (44.92)</td>
<td>59/157 (37.58)</td>
<td>Ref (PsSpA)</td>
<td>0.74 (0.72)</td>
<td>0.45, 1.20</td>
<td>0.22*</td>
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<td></td>
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<td></td>
<td>AS</td>
<td></td>
<td>0.43, 1.22</td>
<td>0.21*</td>
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<td></td>
<td></td>
<td></td>
<td>pPsA</td>
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<td></td>
<td></td>
<td></td>
<td>&gt;1 anti-TNF used during disease</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12/127 (9.45)</td>
<td>12/118 (10.17)</td>
<td>6/157 (3.82)</td>
<td>Ref (PsSpA)</td>
<td>0.40 (1.17)</td>
<td>0.14, 1.14</td>
<td>0.09*</td>
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<td>AS</td>
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<td>0.47, 2.94</td>
<td>0.74*</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>pPsA</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

*continuity adjusted Chi² test
pPsA: peripheral PsA
PsSpA: psoriatic spondyloarthropathy
AS: ankylosing spondylitis
DMARD: disease modifying anti-rheumatic drug
anti-TNF: anti-tumour necrosis factor drug
n: number / proportion
95% CI: 95% confidence interval
mean diff: mean difference

Peripheral psoriatic arthritis mutilans (PAM) was evident clinically and radiographically in 19 cases; 8/127 in the pPsA group, and 11/118 in the PsSpA group.

### 5.5.1.4 Age and disease duration characteristics of the PsA, PsSpA and AS cases

The mean age at clinical assessment was 58.13 (SD 11.71) years for pPsA, 58.09 (SD 11.42) years for PsSpA, and 53.44 (SD 13.61) years for AS cases (Table 5.2). At clinical assessment, AS cases were a mean 4.63 years younger than PsSpA cases (95% CI -7.68, -1.58; p=0.003) (Table 5.2), but there was no significant difference between the mean ages of PsSpA and pPsA cases.
Table 5.2. Clinical characteristics of the pPsA, PsSpA and AS cases (continuous variables)

<table>
<thead>
<tr>
<th></th>
<th>pPsA (n=127)</th>
<th>PsSpA (n=118)</th>
<th>AS (n=157)</th>
<th>PsSpA vs. pPsA</th>
<th>AS vs. PsSpA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (Mean)</td>
<td>IQR (SD)</td>
<td>Median (Mean)</td>
<td>IQR (SD)</td>
<td>Difference in means</td>
</tr>
<tr>
<td>Age at (years)</td>
<td>Clinical assessment</td>
<td>58.13 (11.71)</td>
<td>58.09 (11.42)</td>
<td>53.44 (13.61)</td>
<td>-0.03</td>
</tr>
<tr>
<td></td>
<td>Arthritis onset</td>
<td>37.98</td>
<td>28.37, 46.32</td>
<td>31.27</td>
<td>21.06, 46.24</td>
</tr>
<tr>
<td></td>
<td>Arthritis diagnosis</td>
<td>39.99</td>
<td>29.70, 48.73</td>
<td>35.99</td>
<td>27.27, 52.00</td>
</tr>
<tr>
<td></td>
<td>Psoriasis onset</td>
<td>27.00</td>
<td>16.00, 41.00</td>
<td>31.50</td>
<td>17.50, 41.50</td>
</tr>
<tr>
<td></td>
<td>Psoriasis diagnosis</td>
<td>30.00</td>
<td>19.00, 46.00</td>
<td>33.00</td>
<td>19.00, 43.00</td>
</tr>
<tr>
<td></td>
<td>Recent axial radiograph</td>
<td>56.80 (12.21)</td>
<td>56.36 (11.56)</td>
<td>51.48 (13.67)</td>
<td>-0.44</td>
</tr>
<tr>
<td>Disease duration At (years)</td>
<td>Clinical assessment</td>
<td>15.00</td>
<td>7.00, 26.00</td>
<td>18.00</td>
<td>9.00, 27.00</td>
</tr>
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<td></td>
<td>Recent axial radiograph</td>
<td>13.00</td>
<td>6.00, 23.00</td>
<td>15.50</td>
<td>7.00, 26.00</td>
</tr>
<tr>
<td></td>
<td>To first anti-TNF</td>
<td>13.00</td>
<td>4.00, 23.00</td>
<td>16.00</td>
<td>9.00, 27.00</td>
</tr>
</tbody>
</table>

* two-tailed Student's t-test
^ Mann-Whitney-U test
pPsA: peripheral PsA
PsSpA: psoriatic spondyloarthropathy
AS: ankylosing spondylitis
anti-TNF: anti-tumour necrosis factor drug
IQR: interquartile range
SD: standard deviation
95% CI: 95% confidence interval
Compared with pPsA cases, PsSpA cases tended to have a younger age at arthritis symptom onset (median 31.27 vs. 37.98 years; p=0.01), but were no different in terms of age at arthritis diagnosis (p=0.33), psoriasis onset (p=0.68) or psoriasis diagnosis (p=0.20) (Table 5.2).

The mean age at the most recent axial radiograph (most often the pelvis film) was 56.80 (SD 12.21) years in pPsA, 56.36 (SD 11.56) years in PsSpA, and 51.48 (SD 13.67) years in AS cases. AS cases were a mean 4.88 years younger than PsSpA cases (95% CI - 7.95, -1.81; p=0.002) at the most recent axial radiograph (Table 5.2).

The year of arthritis diagnosis was known for all 402 SpA cases. It was extracted from the case notes and corroborated by the patient. The disease duration, as measured from year of diagnosis to the year of clinical assessment, was a median 15.00 (IQR 7.00, 26.00) years in pPsA, 18.00 (IQR 9.00, 27.00) years in PsSpA, and 22.00 (IQR 10.00, 31.00) years in AS cases, with no statistically significant differences between the PsSpA and AS groups, or pPsA and PsSpA groups (Table 5.2). Similarly, the disease duration to the most recent axial radiograph was no different across the three groups: median 13.00 (6.00, 23.00) years in pPsA, 15.50 (IQR 7.00, 26.00) years in PsSpA, and 19.00 (IQR 10.00, 29.00) years in AS (Table 5.2).

5.5.1.5 HLA-B27 characteristics of the pPsA, PsSpA and AS cases

All but two of the 402 SpA cases were of self-reported (patient questioned by DJ) Caucasian ethnicity. One case was Indian and the other Persian.

HLA-B27 variants (primarily HLA-B2705) were present in 9/127 (7.09%) pPsA, 47/118 (39.83%) PsSpA and 140/157 (89.17%) AS cases (Table 5.1). AS cases were more likely to be HLA-B27 positive than PsSpA cases (OR 12.44; 95% CI 6.67, 23.22; p<0.001). pPsA cases were less likely to be HLA-B27 positive than PsSpA cases (OR 0.12; 95% CI 0.05, 0.25; p<0.001).

Quantification and assessment of strand length was performed on four DNA samples at the Department of Pharmacy and Pharmacology (University of Bath) under the supervision of a post-doctoral student, Dr. Benoit Roux. DNA quantification was performed using the ‘Qubit 2.0 Fluorometer (Invitrogen, by Life technologies)’ using the manufacturers instructions. Concentrations were very high (Table 5.3).
**Table 5.3. DNA concentrations and total quantity in ADIPSA samples**

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Concentration (ng/µl)</th>
<th>Quantity per 100mcl stored sample (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASINP004</td>
<td>439</td>
<td>43.9</td>
</tr>
<tr>
<td>ASINP005</td>
<td>710</td>
<td>71.0</td>
</tr>
<tr>
<td>ASINP006</td>
<td>610</td>
<td>61.0</td>
</tr>
<tr>
<td>ASINP010</td>
<td>428</td>
<td>42.8</td>
</tr>
</tbody>
</table>

Gel electrophoresis was performed on the same four random samples of DNA, using a standard operating procedure. Very little DNA fragmentation was detected, with the majority of DNA remaining as long strands (Figure 5.2). The first and last lanes are the DNA ladder, with the strand lengths detailed in the standards on the right; lanes 2 - 5 are the four samples (ASINP004, 5, 6, 10, respectively); lane 6 is a negative control comprising of fragmented DNA.

![Gel electrophoresis](image)

*Figure 5.2. Gel electrophoresis to determine DNA fragmentation in the ADIPSA samples*

(The first and last lanes are the DNA ladder, with the strand lengths detailed in the standards on the right; lanes 2 - 5 are the four patient samples; lane 6 is a negative control comprising of fragmented DNA).

### 5.5.1.6 Lifestyle characteristics of the pPsA, PsSpA and AS cases

A history of tobacco smoking (current or ex-smoker) was present in 61.47% of pPsA, 51.82% of PsSpA and 45.86% of AS patients; with no difference between PsSpA and pPsA cases, or PsSpA and AS cases (*Table 5.1*).
The mean body mass index (BMI) in the pPsA group was 29.69 (SD 5.36), 29.52 (SD 5.16) in the PsSpA group, and 27.92 (SD 4.96) kg/m² in the AS group; with a lower BMI in AS compared with PsSpA group (mean difference -1.60; 95% CI -2.21, -0.84; p=0.01), but no significant difference between PsSpA and pPsA cases (Table 5.1).

5.5.1.7 Acute-phase response in the pPsA, PsSpA and AS cases

A multivariate reverse-stepwise Poisson regression was used to compare high sensitivity CRP concentrations in pPsA, PsSpA and AS groups, adjusted for: sex, age at sampling, disease duration as sampling, anti-TNF use ever, DMARD use ever and BMI. CRP concentration was lower in PsSpA compared with pPsA cases (IRR_adj 0.79 for every 1 unit increase in concentration; 95% CI 0.74, 0.84; p<0.001). CRP concentration was no different in PsSpA compared with AS cases (IRR_adj 1.06 for every 1 unit increase in concentration; 95% CI 0.99, 1.12; p=0.07).

5.5.1.8 Treatment characteristics of the pPsA, PsSpA and AS cases

Anti-TNF therapy had been used during disease course by 47/127 (37.01%) of pPsA, 53/118 (44.92%) of PsSpA, and 59/157 (37.58%) of AS cases (Table 5.1), with no differences demonstrated between the groups. Similarly, no difference was found between the groups in the likelihood of using more than one anti-TNF agent during their disease course (Table 5.1). The interval between diagnosis and commencement of the first anti-TNF agent was a median 13.00 (IQR 4.0, 23.00) years in the pPsA, 16.0 (IQR 9.0, 27.0) years in PsSpA, and 16.0 (IQR 8.0, 26.0) in the AS group; with no statistically significant difference between groups (Table 5.2).

pPsA cases had commonly used methotrexate (65/127; 51.18%), sulfasalazine (48/127; 37.80%) and less commonly leflunomide (12/127; 9.45%) (Table 5.1). PsSpA cases had commonly used methotrexate (38/118; 32.20%), sulfasalazine (36/118; 30.51%) and more rarely leflunomide (10/118; 8.47%). pPsA cases were more likely to have used a synthetic DMARD during the course of their disease than PsSpA cases (OR 2.33; 95% CI 1.39, 3.85; p=0.001). AS cases were less likely to have used a synthetic DMARD (methotrexate 8/157; sulfasalazine 17/157; leflunomide 1/157) than PsSpA cases (OR 0.24; 95% CI 0.14, 0.42; p<0.001).
5.5.1.9 Summary

The PsSpA and pPsA groups were different to each other in terms of HLA-B27 status, and synthetic DMARD use. The PsSpA and pPsA groups were no different to each other in terms of age at clinical assessment, age at axial radiographic assessment, disease duration at clinical assessment, disease duration at axial radiographic assessment, disease duration at first anti-TNF use, anti-TNF agent use, sex ratio, smoking history, or body mass index. The PsSpA and AS groups were different to each other in terms of proportion of males, HLA-B27 status, synthetic DMARD use, age at clinical assessment and age at axial radiographic assessment. The PsSpA and AS groups were no different to each other in terms of disease duration at clinical assessment, disease duration at axial radiographic assessment, disease duration at first anti-TNF use, anti-TNF agent use, or smoking history. However, synthetic DMARD use has not been shown in the literature to have axial disease-modifying effects in either PsA or AS. These univariate analyses guided the choice of covariates to include in the reverse-stepwise multivariate models.

5.5.2 Patient reported outcome measures in pPsA, PsSpA and AS cases

5.5.2.1 Inflammatory axial symptoms

A history (current or ever) of inflammatory axial symptoms was present in 77/127 (60.63%) pPsA, 88/118 (74.58%) PsSpA, and 156/157 (99.36%) AS cases (Table 5.4). Of particular note, 30/118 (25.42%) PsSpA cases, with radiographic axial disease by classification, did not report a history of inflammatory axial symptoms. Whilst some of this may be explained by patient recall error, this estimate of ‘symptomatically-silent’ axial disease in PsSpA is significant, and far exceeds the estimate in AS of 1/157. This finding may also in part be explained by PsA patients being more likely than AS patients to be attending clinic for peripheral arthritis, giving the opportunity for imaging of the axial skeleton and subsequent detection of symptomatically-silent radiographic axial disease.

The sites most commonly affected by a history of inflammatory pain: in PsSpA cases were the cervical (71/118; 60.17%) and lumbar (70/118; 59.32%) spine; and in AS cases were the lumbar (149/157; 94.90%), cervical (132/157; 84.08%) spine and buttocks (132/157; 84.08%).

Multivariate analyses showed that AS cases were far more likely than PsSpA cases to have a history of inflammatory axial symptoms (OR_{adj} 28.45; 95% CI 3.65, 221.74; p=0.001), even when analysed by axial site: thoracic spine (OR_{adj} 2.20; 95% CI 1.23, 3.92;
Multivariate analyses showed that whilst pPsA cases were not less likely than PsSpA cases to report a history of inflammatory axial symptoms (adjusted OR 0.69; 95% CI 0.38, 1.25; p=0.22), a difference was noted when analysed by axial site: thoracic spine (adjusted OR, OR(adj) 0.48; 95% CI 0.26, 0.88; p=0.02), and buttocks (OR(adj) 0.38; 95% CI 0.20, 0.71; p=0.003) (Table 5.4). This indicates that a history of inflammatory axial symptoms of the thoracic spine and buttocks has a role in identifying PsSpA cases from a PsA cohort, but also detects some false-positive cases (pPsA cases) based on a radiographic definition of RAD. Whether these cases are false-positives based on a magnetic resonance imaging (MRI) definition of axial disease remains to be determined.

**Table 5.4. Axial inflammatory symptoms in pPsA, PsSpA and AS cases**

<table>
<thead>
<tr>
<th>Site</th>
<th>Condition</th>
<th>n</th>
<th>%</th>
<th>Comparison</th>
<th>Adjusted OR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>History of inflammatory axial symptoms</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spine / buttock(s)</td>
<td>pPsA</td>
<td>77/127</td>
<td>60.63</td>
<td>Ref (PsSpA)</td>
<td>28.45</td>
<td>3.65, 221.74</td>
<td>0.001 *</td>
</tr>
<tr>
<td></td>
<td>PsSpA</td>
<td>88/118</td>
<td>74.58</td>
<td>AS</td>
<td>0.69</td>
<td>0.38, 1.25</td>
<td>0.22 *</td>
</tr>
<tr>
<td></td>
<td>AS</td>
<td>156/157</td>
<td>99.36</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cervical</td>
<td>pPsA</td>
<td>54/127</td>
<td>42.52</td>
<td>Ref (PsSpA)</td>
<td>1.82</td>
<td>0.94, 3.55</td>
<td>0.08 **</td>
</tr>
<tr>
<td></td>
<td>PsSpA</td>
<td>71/118</td>
<td>60.17</td>
<td>AS</td>
<td>0.73</td>
<td>0.42, 1.27</td>
<td>0.26 **</td>
</tr>
<tr>
<td></td>
<td>AS</td>
<td>132/157</td>
<td>84.08</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thoracic</td>
<td>pPsA</td>
<td>27/127</td>
<td>21.26</td>
<td>Ref (PsSpA)</td>
<td>2.20</td>
<td>1.23, 3.92</td>
<td>0.01 *</td>
</tr>
<tr>
<td></td>
<td>PsSpA</td>
<td>50/118</td>
<td>42.37</td>
<td>AS</td>
<td>0.48</td>
<td>0.26, 0.88</td>
<td>0.02 *</td>
</tr>
<tr>
<td></td>
<td>AS</td>
<td>114/157</td>
<td>72.61</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumbar</td>
<td>pPsA</td>
<td>58/127</td>
<td>45.67</td>
<td>Ref (PsSpA)</td>
<td>6.75</td>
<td>2.82, 16.16</td>
<td>&lt;0.001 **</td>
</tr>
<tr>
<td></td>
<td>PsSpA</td>
<td>70/118</td>
<td>59.32</td>
<td>AS</td>
<td>0.82</td>
<td>0.47, 1.43</td>
<td>0.48 **</td>
</tr>
<tr>
<td></td>
<td>AS</td>
<td>149/157</td>
<td>94.90</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buttock(s)</td>
<td>pPsA</td>
<td>24/127</td>
<td>18.90</td>
<td>Ref (PsSpA)</td>
<td>3.19</td>
<td>1.67, 6.07</td>
<td>&lt;0.001 ***</td>
</tr>
<tr>
<td></td>
<td>PsSpA</td>
<td>55/118</td>
<td>46.61</td>
<td>AS</td>
<td>0.38</td>
<td>0.20, 0.71</td>
<td>0.003 ***</td>
</tr>
<tr>
<td></td>
<td>AS</td>
<td>132/157</td>
<td>84.08</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Current inflammatory axial symptoms</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spine / buttock(s)</td>
<td>pPsA</td>
<td>54/127</td>
<td>42.52</td>
<td>Ref (PsSpA)</td>
<td>2.76</td>
<td>1.62, 4.71</td>
<td>&lt;0.001 ****</td>
</tr>
<tr>
<td></td>
<td>PsSpA</td>
<td>65/118</td>
<td>55.08</td>
<td>AS</td>
<td>0.57</td>
<td>0.34, 0.96</td>
<td>0.03 ****</td>
</tr>
<tr>
<td></td>
<td>AS</td>
<td>53/157</td>
<td>33.76</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

pPsA: peripheral PsA  PsSpA: psoriatic spondyloarthropathy  AS: ankylosing spondylitis  n: proportion  OR: odds ratio  95% CI: 95% confidence interval

* Multivariate reverse-stepwise logistic regression model (reduced model adjusted for: disease duration at assessment, and HLA-B27 status)
** Multivariate reverse-stepwise logistic regression model (reduced model adjusted for: disease duration at assessment, HLA-B27 status, and synthetic DMARD use ever)
*** Multivariate reverse-stepwise logistic regression model (reduced model adjusted for: sex, age at assessment, HLA-B27 status, and synthetic DMARD use ever)
**** Multivariate reverse-stepwise logistic regression model (reduced model adjusted for: sex, and age at assessment).

Current inflammatory axial symptoms were present in 54/127 (42.52%) of pPsA, 65/118 (55.08%) of PsSpA, and 53/157 (33.76%) of AS cases (Table 5.4). Multivariate analyses demonstrated that AS cases were significantly more likely than PsSpA cases to report
current inflammatory axial symptoms (OR_{adj} 2.76; 95% CI 1.62, 4.71; p<0.001), and pPsA cases to be less likely than PsSpA cases (OR_{adj} 0.57; 95% CI 0.34, 0.96; p=0.03).

### 5.5.2.2 Personal history of extra-articular manifestations

Multivariate analyses showed that pPsA cases were less likely to have a clinical history of IBD than PsSpA cases (OR_{adj} 0.12; 95% CI 0.03, 0.55; p=0.01), but no different in terms of a clinical history of dactylitis, enthesitis, chest wall pain, or uveitis (*Table 5.5*).

AS cases were less likely to have a clinical history of dactylitis compared with PsSpA cases (OR_{adj} 0.18; 95% CI 0.07, 0.42; p<0.001), but were no different in terms of a clinical history of enthesitis, chest wall pain, uveitis and IBD (*Table 5.5*).

**Table 5.5. Extra-articular manifestations in pPsA, PsSpA and AS cases**

<table>
<thead>
<tr>
<th>Clinical history of</th>
<th>n</th>
<th>%</th>
<th>Comparison</th>
<th>Adjusted OR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dactylitis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pPsA</td>
<td>50/127</td>
<td>39.37</td>
<td>Ref (PsSpA)</td>
<td>0.18</td>
<td>0.07, 0.42</td>
<td>&lt;0.001 *</td>
</tr>
<tr>
<td>PsSpA</td>
<td>37/118</td>
<td>31.36</td>
<td>AS</td>
<td>1.09</td>
<td>0.62, 1.93</td>
<td>0.76 *</td>
</tr>
<tr>
<td>AS</td>
<td>8/157</td>
<td>5.10</td>
<td>pPsA</td>
<td>1.09</td>
<td>0.62, 1.93</td>
<td>0.76 *</td>
</tr>
<tr>
<td>Enthesitis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pPsA</td>
<td>59/127</td>
<td>46.46</td>
<td>Ref (PsSpA)</td>
<td>0.70</td>
<td>0.43, 1.14</td>
<td>0.15 **</td>
</tr>
<tr>
<td>PsSpA</td>
<td>56/118</td>
<td>47.46</td>
<td>AS</td>
<td>0.93</td>
<td>0.56, 1.55</td>
<td>0.79 **</td>
</tr>
<tr>
<td>AS</td>
<td>58/157</td>
<td>36.94</td>
<td>pPsA</td>
<td>0.93</td>
<td>0.56, 1.55</td>
<td>0.79 **</td>
</tr>
<tr>
<td>Chest wall pain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pPsA</td>
<td>34/127</td>
<td>26.77</td>
<td>Ref (PsSpA)</td>
<td>1.21</td>
<td>0.70, 2.19</td>
<td>0.52 ***</td>
</tr>
<tr>
<td>PsSpA</td>
<td>52/118</td>
<td>44.07</td>
<td>AS</td>
<td>0.63</td>
<td>0.35, 1.15</td>
<td>0.13 ***</td>
</tr>
<tr>
<td>AS</td>
<td>102/157</td>
<td>64.97</td>
<td>pPsA</td>
<td>1.21</td>
<td>0.70, 2.19</td>
<td>0.52 ***</td>
</tr>
<tr>
<td>Uveitis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pPsA</td>
<td>11/127</td>
<td>8.66</td>
<td>Ref (PsSpA)</td>
<td>1.44</td>
<td>0.79, 2.62</td>
<td>0.24 ****</td>
</tr>
<tr>
<td>PsSpA</td>
<td>31/118</td>
<td>26.27</td>
<td>AS</td>
<td>0.52</td>
<td>0.22, 1.22</td>
<td>0.13 ****</td>
</tr>
<tr>
<td>AS</td>
<td>84/157</td>
<td>53.50</td>
<td>pPsA</td>
<td>1.44</td>
<td>0.79, 2.62</td>
<td>0.24 ****</td>
</tr>
<tr>
<td>Inflammatory bowel</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pPsA</td>
<td>2/127</td>
<td>1.57</td>
<td>Ref (PsSpA)</td>
<td>0.85</td>
<td>0.36, 1.98</td>
<td>0.70 *****</td>
</tr>
<tr>
<td>PsSpA</td>
<td>13/118</td>
<td>11.02</td>
<td>AS</td>
<td>0.85</td>
<td>0.36, 1.98</td>
<td>0.70 *****</td>
</tr>
<tr>
<td>AS</td>
<td>15/157</td>
<td>9.55</td>
<td>pPsA</td>
<td>0.85</td>
<td>0.36, 1.98</td>
<td>0.70 *****</td>
</tr>
</tbody>
</table>

pPsA: peripheral PsA  PsSpA: psoriatic spondyloarthropathy  AS: ankylosing spondylitis
n: proportion  OR: odds ratio  95% CI: 95% confidence interval

* Multivariate reverse-stepwise logistic regression model (reduced model adjusted for: sex, and HLA-B27 status)

** Multivariate reverse-stepwise logistic regression model (reduced model adjusted for: sex, and anti-TNF use ever)

*** Multivariate reverse-stepwise logistic regression model (reduced model adjusted for: age at assessment, disease duration at assessment, and HLA-B27 status)

**** Multivariate reverse-stepwise logistic regression model (reduced model adjusted for: age at assessment, disease duration at assessment, and synthetic DMARD use ever)

5.5.2.3 Family history

AS cases were more likely than PsSpA cases to have a family history of uveitis (OR_{adj} 2.27; 95% CI 0.94, 5.51; p=0.07), although this did not reach statistical significance (*Table
5.6. The two groups were no different in terms of a family history of SpA and IBD. PsA and PsSpA cases were no different in terms of a family history of SpA, psoriasis, uveitis and IBD (Table 5.6).

Table 5.6. Family history of disease in pPsA, PsSpA and AS cases

<table>
<thead>
<tr>
<th>Family History of</th>
<th>n</th>
<th>%</th>
<th>Comparison</th>
<th>OR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spondyloarthritis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pPsA</td>
<td>16/127</td>
<td>12.60</td>
<td>Ref (PsSpA)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PsSpA</td>
<td>25/118</td>
<td>21.19</td>
<td>pPsA</td>
<td>1.26 *</td>
<td>0.67, 2.34</td>
<td>0.47 *</td>
</tr>
<tr>
<td>AS</td>
<td>52/157</td>
<td>33.12</td>
<td>AS</td>
<td>0.73 *</td>
<td>0.34, 1.53</td>
<td>0.40 *</td>
</tr>
<tr>
<td>Psoriasis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pPsA</td>
<td>56/127</td>
<td>44.09</td>
<td>Ref (PsSpA)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PsSpA</td>
<td>58/118</td>
<td>49.15</td>
<td>AS</td>
<td>0.16</td>
<td>0.08, 0.30</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AS</td>
<td>20/157</td>
<td>12.74</td>
<td>pPsA</td>
<td>0.79</td>
<td>0.46, 1.36</td>
<td>0.40</td>
</tr>
<tr>
<td>Uveitis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pPsA</td>
<td>4/127</td>
<td>3.15</td>
<td>Ref (PsSpA)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PsSpA</td>
<td>7/118</td>
<td>5.93</td>
<td>AS</td>
<td>2.27 *</td>
<td>0.94, 5.51</td>
<td>0.07 *</td>
</tr>
<tr>
<td>AS</td>
<td>37/157</td>
<td>23.57</td>
<td>pPsA</td>
<td>1.84 *</td>
<td>0.42, 7.99</td>
<td>0.42 *</td>
</tr>
<tr>
<td>Inflammatory bowel disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pPsA</td>
<td>11/127</td>
<td>8.66</td>
<td>Ref (PsSpA)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PsSpA</td>
<td>9/118</td>
<td>7.63</td>
<td>AS</td>
<td>1.57</td>
<td>0.59, 4.17</td>
<td>0.37</td>
</tr>
<tr>
<td>AS</td>
<td>17/157</td>
<td>10.83</td>
<td>pPsA</td>
<td>1.10</td>
<td>0.42, 2.90</td>
<td>0.84</td>
</tr>
</tbody>
</table>

pPsA: peripheral PsA  PsSpA: psoriatic spondyloarthropathy  AS: ankylosing spondylitis
n: proportion  OR: odds ratio  95% CI: 95% confidence interval  
* Reverse-stepwise logistic regression model adjusted for HLA-B27 status

5.5.2.4 Composite measures

Similar proportions of PsSpA and AS cases were found to be in each of the four ASDAS activity categories, with no statistically significant differences between the diseases (Table 5.7). Of note, disease activity was generally high or very high in both PsSpA (64/118; 54.24%) and AS (80/157; 50.96%) cases, with no statistically significant difference between the two diseases.

Table 5.7. ASDAS in PsSpA and AS cases

<table>
<thead>
<tr>
<th>ASDAS category</th>
<th>Disease</th>
<th>n</th>
<th>%</th>
<th>Comparison</th>
<th>Adjusted OR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inactive</td>
<td>PsSpA</td>
<td>20/118</td>
<td>16.95</td>
<td>Ref (PsSpA)</td>
<td>0.80</td>
<td>0.40, 1.63</td>
<td>0.59 *</td>
</tr>
<tr>
<td></td>
<td>AS</td>
<td>25/157</td>
<td>15.92</td>
<td>AS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>PsSpA</td>
<td>34/118</td>
<td>28.81</td>
<td>Ref (PsSpA)</td>
<td>1.19</td>
<td>0.71, 2.53</td>
<td>0.51 *</td>
</tr>
<tr>
<td></td>
<td>AS</td>
<td>52/157</td>
<td>33.12</td>
<td>AS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>PsSpA</td>
<td>50/118</td>
<td>42.37</td>
<td>Ref (PsSpA)</td>
<td>0.72</td>
<td>0.39, 1.40</td>
<td>0.21 *</td>
</tr>
<tr>
<td></td>
<td>AS</td>
<td>54/157</td>
<td>34.39</td>
<td>AS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very high</td>
<td>PsSpA</td>
<td>14/118</td>
<td>11.86</td>
<td>Ref (PsSpA)</td>
<td>1.89</td>
<td>0.80, 3.69</td>
<td>0.17 *</td>
</tr>
<tr>
<td></td>
<td>AS</td>
<td>26/157</td>
<td>16.56</td>
<td>AS</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PsSpA: psoriatic spondyloarthropathy  AS: ankylosing spondylitis  ASDAS: AS Disease Activity Score
n: proportion  OR: odds ratio  95% CI: 95% confidence interval
These data were corroborated when using other composite measures of disease activity, BASDAI and patient global assessment of axial disease activity (PaGA). A BASDAI of ≥3.5 [205] and ≥4 [159] have been cited in the literature as indicating high disease activity in AS. The MCID cited for the BASDAI in AS is 1 unit [160]. The median BASDAI in PsSpA (3.60; IQR 2.2, 5.60) and AS (3.20; IQR 2.10, 5.40) cases suggests near active disease in both conditions, bearing in mind that data for each disease were negatively skewed (Figure 5.3). The BASDAI was statistically no different in PsSpA and AS cases; both had similarly active disease (adjusted IRR, IRR\textsubscript{adj} 1.03 for every 1 unit increase in score; 95% CI 0.92, 1.17; p=0.54).

**Figure 5.3. BASDAI in PsSpA and AS cases**

A PaGA of ≥4 cm is considered to indicate active disease in AS. The median PaGA in PsSpA (3.00 cm; IQR 2.00, 5.00 cm) and AS (3.00 cm; IQR 2.00, 6.00 cm) cases suggested active disease, with no differences between the two disease groups (IRR\textsubscript{adj} 1.13 for every 1 cm increase in score; 95% CI 1.00, 1.28; p=0.15) (Figure 5.4).
Multivariate reverse-stepwise Poisson regression model (reduced model adjusted for: sex, age at clinical assessment, disease duration at clinical assessment, and anti-TNF use)

**Figure 5.4. Patient global assessment of axial disease activity in PsSpA and AS cases**

The MCID for the BASFI in AS is cited as 0.7 units [160]. Function as measured by the BASFI was poorer in AS (median 3.90 units; IQR 2.20, 5.60) compared with PsSpA (median 3.10 units; IQR 1.40, 5.20) cases, with a clinically and statistically significant difference in BASFI score between the two groups (IRR_{adj} 1.17 for every 0.7 unit increase in score; 95% CI 1.06, 1.26; p=0.002) (**Figure 5.5**). Expressed in another way, for every 0.7 unit increase in BASFI score, the probability of a test patient being categorised in the AS rather than PsSpA group, increases by 17%.
Disability was compared between the three diseases using the HAQ. The median HAQ score was 0.75 (IQR 0.25, 1.50) in pPsA, 0.63 (IQR 0.13, 1.25) in PsSpA, and 0.50 (IQR 0.13, 1.13) in AS cases. The MCID for the HAQ in PsA has been cited as 0.35 units [171]. Using this value in a multivariate zero-inflated Poisson regression, no statistically significant differences were demonstrated between AS and PsSpA (IRR_{adj} 1.04 for every 0.35 unit increase in score; 95% CI 0.90, 1.19; p=0.61), or pPsA and PsSpA (IRR_{adj} 1.09 for every 0.35 unit increase in score; 95% CI 0.95, 1.26; p=0.21) (Figure 5.6).
Multivariate reverse-stepwise zero-inflated Poisson regression model (reduced model adjusted for: sex, age at clinical assessment, disease duration at clinical assessment, anti-TNF use, and smoking)

**Figure 5.6. HAQ scores in pPsA, PsSpA and AS cases**

5.5.3  Clinical examination indices in pPsA, PsSpA and AS cases

5.5.3.1  Axial metrology in PsSpA and AS cases

Axial metrology as measured by the BASMI was no different in AS (median score 3.0; IQR 1.00, 5.00) compared with PsSpA (median score 2.0; IQR 1.00, 5.00) cases (IRR$_{adj}$ 1.07 for every 1 unit increase in score; 95% CI 0.95, 1.21; p=0.26) (**Figure 5.7**).
As has been performed in previous studies [64, 189, 201], the components of the BASMI using their absolute values, rather than their BASMI grade, were also compared in PsSpA and AS cases (Table 5.8). Tragus to wall distance (adjusted mean difference 2.00 cm; 95% CI 0.58, 3.42; p=0.006), and modified Schober test (adjusted mean difference -0.65 cm; 95% CI -1.14, -0.15; p=0.01) were poorer in AS compared with PsSpA cases. However, lumbar side-flexion, inter-malleolar distance, cervical rotation (Table 5.8), and chest expansion (Figure 5.8) were no different in PsSpA and AS cases.

BASMI scores were no different in pPsA compared with PsSpA cases. When analysed by the BASMI subdomains, the Schober test was paradoxically poorer in pPsA compared with PsSpA cases (adjusted mean difference –0.59 cm; 95% CI -1.08, -0.09; p=0.02), but the other domains were no different in the two groups (Table 5.8). These exploratory analyses imply that the BASMI may not be useful to differentiate PsA cases with and without axial disease.
Table 5.8. BASMI subdomains in PsSpA and AS cases

<table>
<thead>
<tr>
<th>BASMI domain</th>
<th>Disease</th>
<th>Mean</th>
<th>SD</th>
<th>Comparison</th>
<th>Adjusted Mean difference</th>
<th>Adjusted 95% CI</th>
<th>Adjusted p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tragus to wall distance (cm)</td>
<td>pPsA</td>
<td>12.04</td>
<td>3.18</td>
<td>Ref (PsSpA)</td>
<td>2.00</td>
<td>0.58, 3.42</td>
<td>0.006 *</td>
</tr>
<tr>
<td></td>
<td>PsSpA</td>
<td>13.56</td>
<td>4.59</td>
<td>AS pPsA</td>
<td>-0.43</td>
<td>-1.79, 0.93</td>
<td>0.54 *</td>
</tr>
<tr>
<td></td>
<td>AS</td>
<td>16.77</td>
<td>7.29</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schober (cm)</td>
<td>pPsA</td>
<td>5.87</td>
<td>1.52</td>
<td>Ref (PsSpA)</td>
<td>-0.65</td>
<td>-1.14, -0.15</td>
<td>0.01 **</td>
</tr>
<tr>
<td></td>
<td>PsSpA</td>
<td>5.13</td>
<td>1.92</td>
<td>AS pPsA</td>
<td>-0.59</td>
<td>-1.08, -0.09</td>
<td>0.02 **</td>
</tr>
<tr>
<td></td>
<td>AS</td>
<td>4.30</td>
<td>2.10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumbar side flexion (cm)</td>
<td>pPsA</td>
<td>12.07</td>
<td>4.50</td>
<td>Ref (PsSpA)</td>
<td>-0.42</td>
<td>-1.89, 1.05</td>
<td>0.58 ***</td>
</tr>
<tr>
<td></td>
<td>PsSpA</td>
<td>11.45</td>
<td>4.77</td>
<td>AS pPsA</td>
<td>0.09</td>
<td>-1.46, 1.63</td>
<td>0.91 ***</td>
</tr>
<tr>
<td></td>
<td>AS</td>
<td>11.05</td>
<td>7.49</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inter-malleolar distance (cm)</td>
<td>pPsA</td>
<td>94.93</td>
<td>15.75</td>
<td>Ref (PsSpA)</td>
<td>0.74</td>
<td>-3.87, 5.15</td>
<td>0.74 ****</td>
</tr>
<tr>
<td></td>
<td>PsSpA</td>
<td>91.00</td>
<td>19.10</td>
<td>AS pPsA</td>
<td>4.54</td>
<td>-0.18, 9.10</td>
<td>0.05 ****</td>
</tr>
<tr>
<td></td>
<td>AS</td>
<td>94.21</td>
<td>20.69</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poor cervical rotation (≤70 degrees)</td>
<td>pPsA</td>
<td>89/127</td>
<td>70.06%</td>
<td>Ref (PsSpA)</td>
<td>OR 1.13</td>
<td>OR 0.63, 2.06</td>
<td>0.68 ^</td>
</tr>
<tr>
<td></td>
<td>PsSpA</td>
<td>85/118</td>
<td>72.03%</td>
<td>AS pPsA</td>
<td>OR 1.05</td>
<td>OR 0.58, 1.92</td>
<td>0.87 ^</td>
</tr>
<tr>
<td></td>
<td>AS</td>
<td>110/157</td>
<td>70.06%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PsSpA: psoriatic spondyloarthritis  
AS: ankylosing spondylitis  
BASMI: Bath AS Metrology Index  
95% CI: 95% confidence interval  
SD: standard deviation  
OR: odds ratio  
cm: centimetres

* multivariate reverse-stepwise linear regression model (reduced model adjusted for: sex, HLA-B27 status, and synthetic DMARD use)  
** multivariate reverse-stepwise linear regression model (reduced model adjusted for: sex, HLA-B27 status, age at assessment, disease duration at assessment, and synthetic DMARD use)  
*** multivariate reverse-stepwise linear regression model (reduced model adjusted for: age at assessment, disease duration at assessment, anti-TNF use, and synthetic DMARD use)  
**** multivariate reverse-stepwise linear regression model (reduced model adjusted for: age at assessment, disease duration at assessment, smoking, synthetic DMARD use, and age at assessment)  
^ multivariate reverse-stepwise logistic regression model (reduced model adjusted for: sex, HLA-B27 status, age at assessment, and disease duration at assessment)

Figure 5.8. Chest expansion in PsSpA and AS cases

Multivariate reverse-stepwise linear regression model (reduced model adjusted for: HLA-B27 status, and age at assessment)
5.5.3.2 Peripheral examination indices in pPsA and PsSpA cases

Nail disease severity, as measured by the mNAPSI was lower in pPsA than PsSpA cases (median 2.0 vs. 2.0, respectively; IRR\textsubscript{adj} 0.90 per unit increase in score; 95% CI 0.83, 0.97; p=0.01) (Table 5.9). Similarly, a signal was detected for nail onycholysis to be less severe in pPsA cases than PsSpA cases (IRR\textsubscript{adj} 0.84 per unit increase in score; 95% CI 0.74, 0.95; p=0.01) (Table 5.9). Nail onycholysis was tested as it is a more specific marker of synovial-enthesal complex disease, a process considered to be important in the pathogenesis of SpA [195]. Nail pitting scores and nail crumbling scores were no different in PsSpA compared with pPsA cases.

Table 5.9. Peripheral examination measures in pPsA, PsSpA and AS cases

<table>
<thead>
<tr>
<th>Clinical Parameter</th>
<th>Condition</th>
<th>Median</th>
<th>IQR</th>
<th>Comparison</th>
<th>Adjusted IRR</th>
<th>Adjusted 95% CI</th>
<th>Adjusted p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tender joint count</td>
<td>pPsA</td>
<td>3.0</td>
<td>0.0, 10.0</td>
<td>Ref (PsSpA)</td>
<td>0.81</td>
<td>0.76, 0.88</td>
<td>&lt;0.001 *</td>
</tr>
<tr>
<td></td>
<td>PsSpA</td>
<td>2.0</td>
<td>0.0, 7.0</td>
<td>pPsA</td>
<td>1.17</td>
<td>1.11, 1.24</td>
<td>&lt;0.001 *</td>
</tr>
<tr>
<td></td>
<td>AS</td>
<td>0.0</td>
<td>0.0, 2.0</td>
<td>Ref (PsSpA)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Swollen joint count</td>
<td>pPsA</td>
<td>0.0</td>
<td>0.0, 1.0</td>
<td>Ref (PsSpA)</td>
<td>1.13</td>
<td>0.89, 1.45</td>
<td>0.31 **</td>
</tr>
<tr>
<td></td>
<td>PsSpA</td>
<td>0.0</td>
<td>0.0, 1.0</td>
<td>pPsA</td>
<td>1.08</td>
<td>0.92, 1.27</td>
<td>0.33 **</td>
</tr>
<tr>
<td></td>
<td>AS</td>
<td>0.0</td>
<td>0.0, 0.0</td>
<td>Ref (PsSpA)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enthesitis score (LEI)</td>
<td>pPsA</td>
<td>0.0</td>
<td>0.0, 2.0</td>
<td>Ref (PsSpA)</td>
<td>1.33</td>
<td>1.02, 1.74</td>
<td>0.04 **</td>
</tr>
<tr>
<td></td>
<td>PsSpA</td>
<td>0.0</td>
<td>0.0, 1.0</td>
<td>pPsA</td>
<td>1.20</td>
<td>0.92, 1.57</td>
<td>0.18 **</td>
</tr>
<tr>
<td></td>
<td>AS</td>
<td>0.0</td>
<td>0.0, 1.0</td>
<td>Ref (PsSpA)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nail severity (mNAPSI)</td>
<td>pPsA</td>
<td>2.0</td>
<td>0.0, 9.0</td>
<td>Ref (PsSpA)</td>
<td>0.90</td>
<td>0.83, 0.97</td>
<td>0.01 ***</td>
</tr>
<tr>
<td></td>
<td>PsSpA</td>
<td>2.0</td>
<td>0.0, 10.0</td>
<td>pPsA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AS</td>
<td>0.0</td>
<td>0.0, 1.0</td>
<td>Ref (PsSpA)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nail onycholysis score</td>
<td>pPsA</td>
<td>0.0</td>
<td>0.0, 4.0</td>
<td>Ref (PsSpA)</td>
<td>0.84</td>
<td>0.74, 0.95</td>
<td>0.01 ***</td>
</tr>
<tr>
<td></td>
<td>PsSpA</td>
<td>1.0</td>
<td>0.0, 4.0</td>
<td>pPsA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AS</td>
<td>0.0</td>
<td>0.0, 1.0</td>
<td>Ref (PsSpA)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin severity (PASI)</td>
<td>pPsA</td>
<td>0.8</td>
<td>0.0, 2.6</td>
<td>Ref (PsSpA)</td>
<td>0.91</td>
<td>0.82, 1.01</td>
<td>0.08 ***</td>
</tr>
<tr>
<td></td>
<td>PsSpA</td>
<td>0.9</td>
<td>0.0, 2.9</td>
<td>pPsA</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

pPsA: peripheral PsA
mNAPSI: modified Nail Psoriasis Severity Index
IRR: incidence risk ratio
95% CI: 95% confidence interval
IQR: interquartile range

* Multivariate reverse-stepwise zero-inflated Poisson regression model (reduced model adjusted for: sex, age at assessment, disease duration at assessment, and anti-TNF use ever)
** Multivariate reverse-stepwise zero-inflated Poisson regression model (reduced model adjusted for: sex, and anti-TNF use ever)
*** Multivariate reverse-stepwise zero-inflated Poisson regression model (reduced model adjusted for: sex, HLA-B27 status, and smoking ever)
**** Multivariate reverse-stepwise zero-inflated Poisson regression model (reduced model adjusted for: sex, HLA-B27 status, disease duration at assessment, and synthetic DMARD use ever)

The median tender joint count was 3.0 (IQR 0.0, 10.0) in pPsA, and 2.0 (IQR 0.0, 7.0) in PsSpA cases, with a clinically and statistically significant difference between the two diseases on multivariate analyses (IRR\textsubscript{adj} 1.17 for every 1 joint increase; 95% CI 1.11, 1.24; p<0.001) (Table 5.9). No differences were found between pPsA and PsSpA cases in terms of swollen joint count, peripheral enthesitis as measured by the Leeds Enthesitis Index, or skin psoriasis severity as measured by the PASI (Table 5.9). Similarly, the LEI was no different in AS compared with PsSpA cases (Table 5.9). All of these PROM and clinical examination results are summarised in Figure 5.9 and Figure 5.10.
Figure 5.9. A comparison of clinical characteristics in pPsA and PsSpA cases

More likely or higher in pPsA
- Tender joint count (IRR = 1.17)
- Synthetic DMARD use during disease course (OR = 2.33)

 Less likely in pPsA
- HLA-B27 positivity (OR = 0.12)
- History of:
  - IBD (OR = 0.12)

Factors associated with developing pPsA rather than PsSpA:
- Younger age at onset of arthritis (p = 0.01)
- Nail psoriasis severity; mNAPSI (IRR = 0.90)
- Nail onycholysis severity (IRR = 0.84)

No different in pPsA & PsSpA cases
- Socio-demographics:
  - Osteoarthritis diagnosis
  - Age at psoriasis symptom onset and diagnosis
  - Body mass index

History of:
- Axial inflammatory symptoms
- Chest wall pain

Swollen joint count

Extra-articular manifestations:
- Personal history of dactylitis, enthesitis, uveitis
- Enthesitis burden (LEI)

Family history of SpA, psoriasis, uveitis, IBD

Disability (HAQ)

Treatment:
- Proceeding to anti-TNF use
- Interval from diagnosis to anti-TNF use

Figure 5.10. A comparison of clinical characteristics in AS and PsSpA cases

More likely or higher in AS
- Male preponderance (OR = 1.80)
- HLA-B27 positivity (OR = 12.44)

- History of axial inflammatory symptoms:
  - All regions (OR = 28.45)
  - Thoracic spine (OR = 2.20)
  - Lumbar spine (OR = 6.75)
  - Buttocks (OR = 3.19)

- Current axial inflammatory symptoms
  - OR = 2.76

Function:
- BASFI (IRR = 1.17 per 0.7 unit increase in score)

Metrology:
- Tragus to wall distance (adjusted mean difference 2.00 cm)
- Schober test (adjusted mean difference -0.65 cm)

Less likely in AS
- Synthetic DMARD use (OR = 4.17)
- Personal history of dactylitis (OR = 0.18)
- Family history of psoriasis (OR = 0.16)

No different in AS & PsSpA cases
- History of inflammatory cervical spine symptoms
- Disability (HAQ)
- Disease activity (ASDAS, BASDAI, PaGA)

Metrology (BASMI, cervical rotation, lumbar side-flexion, inter-malleolar distance, chest expansion)

Extra-articular manifestations:
- Personal history of enthesitis, chest wall pain, uveitis, IBD
- Enthesitis burden (LEI)

Family history of SpA, uveitis, and IBD

Treatment:
- Proceeding to anti-TNF use
- Interval from diagnosis to anti-TNF use
5.6 DISCUSSION & CONCLUSIONS

This study used a large cohort of SpA patients attending a single centre to compare the sociodemographic, lifestyle, treatment, patient-reported and clinical examination characteristics of pPsA, PsSpA and AS.

To my knowledge, this is the first study to directly compare PsSpA with AS patients in terms of inflammatory axial symptoms by region, disability as measured by the HAQ, disease activity as measured by the ASDAS and PaGA, treatment in terms of anti-TNF and synthetic DMARD use, enthesitis burden as measured by the LEI, and personal history of chest wall pain and IBD. This is also the first study to compare PsSpA and pPsA patients in terms of psoriatic nail disease severity using the mNAPSI, occurrence of global and regional inflammatory spinal pain, and personal history of dactylitis. Importantly, for the purpose of future research and devising a classification system for PsSpA, we are the first to report the proportion of PsSpA patients with radiographic sacroiliitis, spondylitis or both, and how these correlate with clinical parameters and HLA-B27 status.

5.6.1 Clinical characteristics in PsSpA compared with pPsA cases

The prevalence of PsSpA in this PsA cohort (36.82%) is very similar to that reported by Lambert et al. of 40% [69] and Gladman et al. of 39% [111]. A study by Leung et al. reported a lower prevalence of 23%, but the disparity is likely explained by the shorter disease duration in their cohort of 9.2 (SD 6.7) years [62], compared with 18 (IQR 9, 27) years in this study. A previous study from Bath has shown that the prevalence of PsSpA rises with increasing disease duration [68].

The prevalence of PsSpA in all SpA (PsA and AS) cases with psoriasis was 118/245 (48.16%), which is higher than the prevalence stated in the ‘PsA cohort’. The difference is explained by the set-up of clinical services in our hospital, which is similar to that in many other large secondary-care hospitals in the UK. Historically, patients initially presenting with psoriasis and predominantly peripheral symptoms have been cared for in the dedicated PsA clinic; whilst patients initially presenting with psoriasis and axial symptoms have been cared for in the dedicated AS clinic. The clinical diagnosis is therefore influenced by their initial predominant presentation. As described in the Results section: of 201 cases clinically coded as PsA in hospital case-notes, 24% also fulfil modified New York criteria for AS; and of 201 cases clinically coded as AS in hospital case-notes, at least 24% also fulfilled CASPAR criteria for PsA. This finding is important for both clinicians and patients, and for both clinical and academic purposes. For the clinician,
these data would support the establishment of a specialist ‘SpA service’, whereby patients with pPsA, PsSpA, AS and other forms of SpA attending the same service, have access to the same medical and nursing expertise, specialist physiotherapy and occupational therapy services, and medications. For the patient, this would allow them to utilise services based on their need, rather than their ‘NHS diagnosis’, which as is demonstrated above, has its pitfalls. Such a ‘SpA clinic’ may also encourage and improve quality of research. SpA patients scattered in several general rheumatology clinics seeing clinicians without an academic interest in SpA may be disadvantaged by not having the same access to SpA-related research. In many hospitals in the UK, PsA patients are seen by one group of clinicians with a research interest in PsA, and AS patients are seen by another group of clinicians with an interest in AS. This has potentially impeded research of PsSpA, which bridges the two services. Given the overlap between PsA and AS, a ‘SpA clinic’ would serve to integrate and improve research of these overlapping diseases.

Symptomatically-silent radiographic axial disease was present in a significant proportion (30/118; 25.42%) of PsSpA cases, which is similar to the prevalence of 20% reported by Queiro et al. [190]. Ten PsA patients had an incomplete series of axial films to determine RAD, and were therefore classified as pPsA. It was assumed that the absence of a complete set of radiographs implied no widespread axial symptoms. However, given that almost a quarter of PsSpA cases were shown in this study to have symptomatically-silent radiographic axial disease, it is possible that three or more of these cases may have had PsSpA. This is a significant proportion of patients with potentially sub-optimised treatment. Although one might argue that awareness of these asymptomatic patients is not necessary given that medical intervention is not required, one might repost that given the natural clinical history of such patients has yet to be determined, preventative early intervention through physiotherapy and medications may halt or slow disease progression to the symptomatic state. This would benefit patients and the health service.

We did not find that male PsA patients are more likely to develop PsSpA than female patients. These results are in keeping with a study from Hong Kong of 125 PsA cases using stringent classification criteria [62], a study from Bath of 75 PsA cases with cervical spondylitis [68], and a large prospective cohort study from Toronto (Canada) of 206 PsA cases [61]. The latter study did however, suggest that PsSpA develops at an earlier stage (often at first clinic attendance) in male compared with female patients [61]. Two studies reported male PsA cases being more prone to PsSpA [63, 69], although the study by Hanly et al. classified PsSpA cases using less stringent criteria (sacroiliitis unilaterally of grade ≥2, or clinical sacroiliitis) [63].
HLA-B27 was far less likely to be positive in pPsA than in PsSpA cases. This is discussed further in Chapter 6, including how HLA-B27 appears to relate to radiographic subsets of PsSpA.

PsSpA cases in our cohort had a younger age at arthritis onset than pPsA cases. This was also shown in a study of PsA cases in Korea [67]. We did not find a younger age at psoriasis onset in the PsSpA compared with pPsA groups, as has been reported elsewhere [67, 69]. The disparity may be explained by recall bias in our PsA cases, who had a mean arthritis disease duration of 18 (IQR 7, 27) years, and with skin psoriasis onset approximately ten years prior to that.

We found that psoriatic nail severity, especially nail onycholysis severity, was significantly higher in PsSpA compared with the pPsA cases, and may therefore be a clinical indicator of PsSpA occurrence; this might be especially useful in those with symptomatically-silent PsSpA. A similar association was noted by Chandran et al. who in a prospective longitudinal cohort also demonstrated a higher risk of developing PsSpA in PsA cases with nail dystrophy (RR 7.07; p=0.01) [61]. Nail onycholysis is a more specific marker of synovial-enthesal complex disease, a process considered to be important in the pathogenesis of SpA [195]. The nailbed is attached by ligaments and entheses to the distal interphalangeal joint (DIPJ), and it is therefore hypothesised that PsA-related DIPJ inflammation encompasses and is transmitted to the nailbed, manifesting as psoriatic nail disease [195]. The pathogenesis of PsSpA is considered to involve both entheses and synovial joints [195]. Therefore the same axial enthesitis may be manifesting as nail onycholysis in this study. The association between PsSpA and nail onycholysis demonstrated in this study aligns appropriately along a causal pathway and is biologically plausible, further strengthening the validity of the result.

A significant proportion of our pPsA cases reported a past (60.63%) or current (42.52%) history of inflammatory axial symptoms. These findings differ to those of Lambert et al. who reported that their pPsA cases rarely had axial symptoms [69]. We used the ASAS criteria for ‘inflammatory spinal symptoms’ [123], but Lambert et al. did not state the criteria they used [69]. Taken together, this questions the sensitivity and specificity of the ASAS screening questions for use in PsSpA cases ultimately classified radiographically. However, further exploration is beyond the remit of this thesis.

Our finding of higher peripheral joint counts in PsSpA compared with pPsA cases corroborates a previous study by Jenkinson et al. [68], but not a more recent smaller study of 125 PsA cases which found no difference between the groups [62]. However, the latter
study did not perform multivariate analyses, and no adjustments were made for the longer disease duration in the PsSpA cases compared with pPsA cases.

A personal history of dactylitis, enthesitis, chest wall pain and uveitis, and a family history of SpA or psoriasis were not associated with developing PsSpA rather than pPsA in this study; in keeping with the results of another study [61]. Our results of PsSpA and pPsA cases showing no difference in terms of personal history of dactylitis, skin severity or enthesitis burden corroborate those of a recent study from Hong Kong [62], although enthesitis burden in that study was measured using a more detailed and sensitive clinical index, the MASES. These findings are somewhat surprising. Whilst both pPsA and PsSpA are both entheseal-related diseases, PsSpA is considered by some to be more so [206, 207], and one might therefore expect PsSpA to present with a more entheseal-related disease e.g. peripheral enthesitis, chest wall pain and uveitis once adjusted for HLA-B27 status. The cross-sectional design of this study, use of dichotomous outcome measures (e.g. chest wall pain and uveitis), and possible insensitivity of the LEI may explain these results.

The higher frequency of IBD in PsSpA compared with pPsA cases is corroborated by a previous study [192], although the latter had a higher frequency (30%) compared to ours (11%). Our results align well with the research framework of ‘association, causality, and biological plausibility’. The pathogenesis of both SpA [208-210] and IBD [211-213] have been shown to be related to the interleukin-23 pathway. The higher prevalence of IBD in PsSpA compared with pPsA cases therefore potentially ties-in with the interleukin-23 pathway and is biologically plausible.

Disability, as measured by the HAQ, was no different in PsSpA and pPsA cases, which corroborates a smaller Spanish study of 46 PsSpA and 54 pPsA cases [64], and a small study from Hong Kong of 29 PsSpA and 96 pPsA cases [62]. A previous smaller study from Bath of 75 PsA cases published in 1994, demonstrated that PsA cases with radiographic cervical spondylitis have a higher HAQ than those without radiographic cervical spondylitis [68]. The difference may be due to that study only comparing PsA patients with cervical spondylitis or not, whereas this study assessed the entire axial skeleton. Very few of the questions in the HAQ relate to specifically to neck function, and it is therefore more appropriate to compare HAQ with axial disease of the entire skeleton, as we performed here.

BASFI was higher in PsSpA than in the pPsA cases in this study, which corroborate the results of a smaller Spanish study [64], but not those of a small study from Hong Kong
which showed no difference in BASFI between the groups [62]. The Hong Kong study was probably underpowered to detect a difference as it compared only 29 PsSpA with 96 pPsA cases. Our findings are important since BASFI, unlike BASMI, BASDAI, ASDAS, and PaGA appears to distinguish PsA patients with and without axial disease.

We found evidence of high disease activity (ASDAS, BASDAI and PaGA) in both PsSpA and AS cases, although with no difference between groups. These findings are corroborated by previous studies investigating BASDAI [62] and PaGA [62]. A cross-sectional observational study comparing the utility of ASDAS and BASDAI in unselected established 201 PsSpA cases, with classification criteria similar to ours, also demonstrated moderate-high disease activity in their PsSpA cohort: mean ASDAS 2.4 (SD 1.1), and mean BASDAI 3.5 (SD 2.4) [163]. Similarly, another validation study of ASDAS in PsSpA demonstrated moderate-high disease activity in their 54 PsSpA cases: mean BASDAI 3.96 (SD 2.65), mean PaGA 4.25 (SD 2.79), and mean ASDAS 2.68 (SD 1.13) [164].

Metrology as measured by the BASMI, and its individual domains (apart from the Schober test, although in the opposite direction to expected) were no different in PsSpA and pPsA cases. Although it is beyond the remit of this thesis chapter to compare in more detail, this exploratory analysis does suggest that the BASMI and it domains may have little clinical utility to differentiate PsA cases with and without axial disease.

5.6.2 Clinical characteristics in PsSpA compared with AS cases

The proportion of males in our AS (75%) group was higher than in the PsSpA (63%) group. These results are different to a study by Helliwell et al., which showed approximately 75% males in both groups [58]. The disparity might be explained by the classification criteria used. All of their PsSpA cases fulfilled modified New York criteria for AS, and were subsequently sub-classified on the basis of their psoriasis. Only 65 of our 118 PsSpA cases (55%) fulfilled modified New York criteria for AS, and may therefore more truly represent PsSpA rather than a mixture of PsSpA and AS cases with coincidental psoriasis.

HLA-B27 was significantly more likely to be positive in AS than in PsSpA cases. This is discussed further in Chapter 6, in the context of the radiographic similarities and difference between PsSpA and AS.
Our findings of a higher prevalence of all-region axial inflammatory symptoms in AS compared with PsSpA cases, is corroborated by two previous studies [49, 201]. This is in large part explained by the 25% of radiographic-PsSpA cases who have never been symptomatic.

AS cases in this study were less likely to have a personal history of dactylitis than PsSpA cases. This has been noted previously, in a study that also reported a higher frequency of enthesitis in PsSpA compared with AS cases [201]; a finding we did not corroborate. Our AS cases were no more likely than our PsSpA cases to have a personal history of uveitis, once adjusted for HLA-B27 status. This challenges the results of another study, which did not adjust for HLA-B27 status [49].

AS cases compared with PsSpA cases in this study were more likely to have a family history of SpA. No such difference was reported in the only other study investigating this [201].

We found that function as measured by the BASFI was poorer in AS compared with PsSpA cases. A large prospective cross-sectional study comparing 1072 AS and 147 PsSpA cases, found no difference in BASFI between the groups on multivariate analysis [201]. This disparity might be explained by differing classification systems being used. Perez-Alamino et al. classified PsSpA cases on the basis of SpA through ESSG criteria and concomitant psoriasis. The ESSG criteria have not shown good specificity or sensitivity for axial disease in PsA [24, 57]. In addition, both their AS (median 6 vs. 18 years, respectively) and PsSpA (median 6 vs. 15 years, respectively) patients had much shorter disease duration than those in our cohort, permitting less time for differential functional impairment to develop between the two diseases.

Disease activity as measured by the BASDAI were no different in AS and PsSpA cases, a finding corroborated by another study [201]. Metrology as measured by the BASMI was no different in AS compared with PsSpA cases in this study. This is supported by one other study comparing BASMI and its component domains [64], but challenges the poorer BASMI demonstrated in AS compared with PsSpA cases in another study [201]. Lumbar forward flexion was poorer in AS compared with PsSpA cases in our study, which corroborates the results of three studies [49, 58, 201], but challenges one study that reported no difference [64]. Tragus to wall distance was higher in the AS cases compared with the PsSpA cases. A comparable measure, the ‘occiput to wall distance’ was similarly shown to be poorer (higher) in AS compared with PsSpA cases in two studies [64, 201].
Cervical rotation was no different in our AS and PsSpA cases. This corroborates the result of one study [64], but refutes the results of another study where cervical rotation was more limited in AS compared with PsSpA [201]. Although the cohort in the Fernandez-Sueiro et al. [64] study was older than in the study by Perez-Alamino et al. [201], adjustments were made for age in the multivariate model, and both studies used similar definitions of restricted cervical rotation (less than or greater than seventy degrees of rotation). Intermalleolar distance was no different in AS compared with PsSpA cases our study or in one other study [64]. Chest expansion was no different in AS compared with PsSpA cases in our study, and in another study [58]. Such a difference was not found in another study [64], which may have been underpowered to detect what can be quite small differences in expansion.

In summary, it appears that PsSpA and AS are no different in terms of disease activity and several domains of metrology, but AS patients have poorer function. Patients adapt to their disease over time and find novel ways to circumvent a physical limitation through the use of aids, using their body in a different way, pacing and carer-support. Given that the management of axial disease in PsA is sometimes considered after peripheral disease in PsA, it is important that PsSpA patients have as much access as AS patients to axial-specific education, physiotherapy and occupational therapy, in order for them to optimise their remaining physical ability. Clinicians need to be pro-active to enable patients to achieve this.

5.6.3 Classification of the study cohort using a stricter definition

It might be argued that PsSpA should have been classified as psoriasis plus sacroiliitis (grade ≥2 bilaterally, or grade ≥3 unilaterally), and not permitted cases with spondylitis but no sacroiliitis to qualify as PsSpA. Using this classification system, this study would have 79 PsSpA (instead of 118), 166 pPsA (instead of 127), and 157 AS cases. The prevalence of PsSpA in the psoriatic cohort would have been 79/245 (32.24%). I chose not use this classification system as there is evidence and expert clinical opinion that the radiographic expression of PsSpA and AS is different [57, 214]. Exclusion of spondylitis-only patients from the PsSpA group would have ignored PsA patients with genuine inflammatory axial disease.
5.6.4 Study critique

This study had several strengths, especially the large sample size, the robust case classification system employed, and no missing PROM or clinical examination data. The latter is a rarity in clinical studies, and has both improved the study's power and the reliability of the results. Covariate data were collected including anti-TNF use, synthetic DMARD use, smoking and BMI to include in the multivariate regression models; thereby allowing for some adjustment for potential confounders. The findings of this study are generalisable, because the RNHRD is a secondary referral centre, rather than a centre receiving high volumes of specialist tertiary-centre referrals.

All PsA and AS clinic attendees were invited to participate in the study, thereby minimising selection bias. However, as the study was titled the ‘Axial Disease in PsA’ study and despite the patient information leaflet explicitly stating that all PsA patients were invited, it is possible that the study attracted PsA patients with axial symptoms more so than those without axial symptoms, thereby causing some selection bias. Some clinical indices such as BASDAI are known to vary somewhat over time, despite other indicators of disease activity being stable [215, 216]. BASFI and BASMI have been shown to have better test-retest reliability [215, 216]. The study may have been improved by averaged clinical assessment at two time-points. However, this would have significantly impacted on study recruitment, inconvenience to the participants, and clinical assessor's time. Whilst recall bias is likely to have been present, particularly in patients without longer disease duration, its impact is likely to have been curtailed by a similar magnitude and direction of effect in each of the three disease groups.

For clinical examination indices, the study might have been improved by having more than one clinical assessor, thereby reducing assessor bias and / or performance bias. At study inception, I intended to have three clinical assessors, but this later became impossible due to insufficient research staff. Having other clinical examination assessors may have enabled the diagnosis of participants to have been partially blinded, as their might be a bias for an unblinded assessor of AS patients to expect, for example, their axial metrology to be poorer than that in PsSpA patients. Such a bias would have made it more likely for BASMI to be higher in AS compared with PsSpA cases; a result not seen in this study. However, one might also argue that having one assessor for clinical examination is a strength of this study, given that inter-rater reliability for clinical assessment in PsA has been shown to be notoriously poor [184].
In this study, smoking was defined as a history (past or present) of tobacco consumption, and was therefore present in 61.47% of pPsA, 51.82% of PsSpA and 45.86% of AS patients, with no significant difference between groups. These ‘lifetime prevalence’ values are much higher than estimates of ‘point prevalence’ of smoking in the general UK population, which as published by the Office of National Statistics is 19.16% (95% CI 18.27, 20.06%) for all persons aged over 16 years in year 2013 [217]. The disparity may in part be related to: ‘point’ compared with ‘lifetime’ prevalence, the latter also comprising ex-smokers. Age, sex and socio-demographics of the two study populations may also contribute to the disparity. However, it may also implicate smoking in the aetio-pathogenesis of PsA and AS.

One must also consider the presence of uncontrolled confounding or unmeasured confounders. Despite our best attempts with the data collected, time-varying variables such as smoking, BMI and medications have not been fully adjusted for in these analyses. For example, a ‘history of smoking’ encompasses patients who are both ex-smokers and current smokers, and smokers of varying duration, dose (cigarettes per day or ounces of tobacco) and form (filtered cigarettes, self-rolled cigarettes, pipes). BMI changes over time, often rising with increasing age, and this study has used the most recently recorded BMI within the last one year. For these two uncontrolled confounders, the direction and magnitude of their confounding is unlikely to be significantly different across the three disease groups, and therefore their effect on the results of this study may be minimal. Medication use is another time-varying variable that may differ across the three groups in terms of agent, dose and duration. We only included synthetic DMARD and anti-TNF use as dichotomous covariates in the analyses. Whilst we have some data on anti-TNF start and end date, and exact anti-TNF and synthetic DMARDs used, there is some missing data, medications have differential efficacy, patients don’t necessarily take their medications compliantly, and such longitudinal data analyses would require complex mathematical modelling considered to be somewhat beyond the remit of this thesis.

We also acknowledge that there are unmeasured confounders, of which some we may not even be aware. For example, functional ability (as measured by the BASFI) is somewhat influenced by the use of aids and patients’ personal and environmental adaptations. If axial disease in AS, is more likely than axial disease in PsSpA to have drawn the clinician’s attention and prompted a referral for education and occupational therapy services, it is possible that functional impairment in AS may be more compensated for in AS than in PsSpA. The higher BASFI this study has demonstrated in AS compared with PsSpA cases may therefore be even higher if this unmeasured confounder had been adjusted for. For similar reasons, AS compared with PsSpA patients may have been more
likely to have been referred for specialist axial physiotherapy. This unmeasured confounder may have differentially improved AS compared with PsSpA patients’ metrology, resulting in no apparent difference in BASMI between the two disease groups. After adjustment for this confounder, AS patients may have demonstrated poorer metrology than PsSpA patients. Other potential unmeasured confounders include NSAID use, analgesia use, delay from symptom-onset to diagnosis. The latter may be different both within and between groups. For example, if the index of suspicion for axial disease in PsA is not high, then there may be greater delay in diagnosis in PsSpA than in AS, resulting in longer untreated axial disease in PsSpA and resultant poorer clinical outcomes. Similarly, given that AS is more common in males than females, the index of suspicion for AS in a female presenting with axial symptoms may be low, resulting in greater delay to diagnosis in female compared with male AS patients. This may translate into poorer clinical outcomes in female than in male AS patients. Furthermore, juvenile-onset AS patients have poorer clinical outcomes than adult-onset AS patients [218, 219], although in this study we have grouped both sub-subsets of AS into one group for comparison with PsSpA.

5.6.5 Conclusions

Through the robust classification of a large cohort of SpA cases, we have demonstrated that a significant proportion of PsA cases have axial disease, and that it is often asymptomatic. Occurrence of axial disease in PsA appears to be associated with HLA-B27 positivity, younger age at arthritis onset, and with psoriatic nail disease severity, in particular nail onycholysis severity. Other indicators include axial inflammatory symptoms, and the occurrence of IBD. PsSpA appears not to be the clinically-milder ‘sister’ of AS. Measures of disease activity were high in both PsSpA and AS cases, with no difference between groups. All three disease groups have significant disability, with no difference between groups. Metrology was just as impaired in PsSpA as in AS. Up to one quarter of PsA and AS cases may be classified as the other disease using the currently available classification systems.
5.7 PUBLICATIONS ARISING FROM THE WORK OF THIS THESIS

CHAPTER

CONFERENCE ABSTRACT (oral presentation)


CONFERENCE ABSTRACT (poster presentation)

6  CHAPTER VI: RADIOGRAPHIC CHARACTERISTICS OF AXIAL DISEASE IN PSORIATIC ARTHRITIS AND ANKYLOSING SPONDYLITIS

6.1  ABSTRACT

Background: Only four studies have compared the radiographic characteristics of psoriatic spondyloarthritis (PsSpA) and ankylosing spondylitis (AS), and findings have not been entirely consistent. The objectives of this chapter of the thesis were to: (i) compare the pattern of radiographic axial disease in PsSpA and AS; (ii) compare the morphological (osteoproliferation and erosion) features of radiographic axial disease in PsSpA and AS; (iii) compare axial radiographic severity in PsSpA and AS.

Methods: A prospective single-centre cross-sectional observational study was conducted, recruiting consecutive PsA and AS cases. Axial radiographs of the cervical spine, lumbar spine and pelvis taken for clinical reasons in the five-years preceding study enrolment were read by two raters and scored using the modified Stoke Ankylosing Spondylitis Spinal Score (mSASSS) and the Psoriatic Arthritis Spondylitis Radiology Index (PASRI). Univariate and multivariate analyses were performed.

Results: The 402 enrolled cases (201 PsA and 201 AS) were reclassified based on radiographic axial disease and psoriasis into three more precise groups: 118 PsSpA, 127 pPsA, and 157 AS cases.

Of the PsSpA cases: 45/118 (38.14%) had sacroiliitis with spondylitis; 34/118 (28.81%) had sacroiliitis without spondylitis; and 39/118 (33.05%) had spondylitis without sacroiliitis. HLA-B27 positivity was significantly more likely in PsSpA cases with both sacroiliitis and spondylitis (adjusted odds ratio, OR_{adj} 9.10; 95% CI 3.01, 27.47; p<0.001) compared with PsSpA cases with spondylitis alone. The majority of PsSpA cases with spondylitis alone were symptomatic (56.41%); although were less likely to be symptomatic than PsSpA cases with sacroiliitis with / without spondylitis (83.54%) (OR 0.26; 95% CI 0.11, 0.61; p=0.002).

Radiographic sacroiliitis was more likely in AS (147/157; 93.63%) compared with PsSpA (79/118; 66.95%) cases (adjusted OR, OR_{adj} 3.78; 95% CI 1.63, 8.77; p=0.002). AS cases were more likely to have complete ankylosis of the SIJs (OR_{adj} 2.96; 95% CI 1.42, 6.15; p=0.004), but not SIJ sclerosis, erosion or partial ankylosis. Both PsSpA and AS cases tended to have bilateral (82.28 and 96.60%, respectively) symmetrical-grade (75.95 and 80.95%, respectively) sacroiliitis.
Bridging syndesmophytes were significantly more common in AS compared with PsSpA (OR\textsubscript{adj} 2.78; 95% CI 1.49, 5.18; p=0.001), and may therefore be more useful to distinguish PsSpA from AS than paramarginal syndesmophytes. There was no significant difference between AS and PsSpA cases in terms of the occurrence of cervical vertebrae spondylitis (OR\textsubscript{adj} 1.20; 95% CI 0.65, 2.25; p=0.56), lumbar vertebrae spondylitis (OR\textsubscript{adj} 1.37; 95% CI 0.75, 2.50; p=0.30), or cervical facet joint fusion (OR\textsubscript{adj} 1.48; 95% CI 0.81, 2.72; p=0.20).

Radiographic axial disease was more severe in AS compared with PsSpA as measured quantitatively using the PASRI (adjusted incidence risk ratio, IRR\textsubscript{adj} 1.13 for every 1 unit increase in score; 95% CI 1.09, 1.19; p<0.001), mSASSS (IRR\textsubscript{adj} 1.09 for every 1 unit increase in score; 95% CI 1.04, 1.14; p<0.001) and osteoproliferative burden (IRR\textsubscript{adj} 1.31 for every 1 unit increase in score; 95% CI 1.09, 1.57; p=0.004), but not erosion burden.

**Conclusions:** Spondylitis without sacroiliitis was both common and usually symptomatic in PsSpA, so must be considered in classification systems for PsSpA. *HLA-B27* positivity is more specific to sacroiliitis than to spondylitis, implying different pathogenesis. Complete ankylosis of the SIJs and bridging syndesmophytes appear to distinguish AS from PsSpA radiographically. AS is radiographically more severe than PsSpA as measured by the mSASSS and PASRI scores, both globally and regionally.
6.2 INTRODUCTION

Assessment of structural radiographic damage in psoriatic spondyloarthritis (PsSpA) is important since it can help classify cohorts, quantify disease burden, monitor disease progression, and has been shown to be a prognostic marker in several rheumatological conditions including ankylosing spondylitis (AS) [44].

Eleven studies have reported on the radiographic characteristics of PsSpA [49, 55, 58, 59, 63, 68-70, 112, 189, 201]. From these studies, it seems that radiographic sacroiliitis in PsSpA is usually of symmetrical grade [69], but whether it is unilateral [70] or bilateral [59, 69] remains to be determined in a cohort of PsSpA cases with long enough disease duration to ascertain this likely time-dependent variable. Vertebral syndesmophytes are reported in up to a quarter of PsSpA cases [69], with an unilateral asymmetrical random distribution along the spine [70]. Para-marginal shaped syndesmophytes may be more specific to PsSpA than AS [58, 59, 70], although in the cervical spine of PsSpA cases, marginal syndesmophytes are reported to be more common than para-marginal syndesmophytes [68]. According to four studies, vertebral spondylitis of the cervical and / or lumbar spine without concurrent sacroiliitis is common in PsSpA, with an estimated prevalence of 16 – 62%, albeit different definitions of sacroiliitis have been used [63, 69, 70, 112]. Radiographic axial disease can manifest in PsSpA in other forms, such as (in order of decreasing frequency): facet joint space narrowing or fusion [68, 189], posterior ligament ossification [68], para-vertebral ossification [55], narrowing of the lower cervical disc space with subsequent fusion of the vertebral bodies [68], atlanto-axial subluxation [189], and atlanto-axial impaction [189]. Very little has been reported on the natural radiographic history of axial disease in PsSpA. Only one study has reported that syndesmophytes and sacroiliitis progress over time, although not necessarily accompanied by increasing spinal pain, SIJ tenderness or deterioration of thoracolumbar spinal metrology [63].

Some findings have been consistent in studies using independent cohorts, whilst others have not been replicated. This may be explained by the variety of classification systems for PsSpA employed, sampling methods, and differing disease durations between cohorts; a factor believed to influence the expression of PsSpA. Some studies have used convenience samples as part of a retrospective study design, with incomplete radiographic data and selective reporting of available data. Several studies have either not or only partially adjusted for confounders. Some of the earliest studies from the 1960s and 1970s did not statistically compare radiographic features in PsSpA and AS, providing descriptive analyses only. The studies reporting these results are summarised in turn
below, along with a critique of their study methods, reliability of results, and where possible an explanation for any disparity between study findings. This will allow the methods and results of this thesis chapter to be put into context of the existing literature.

6.2.1 Radiographic features of PsSpA

Para-vertebral ossification is a phenomena typically seen in PsA, and was first reported in 1965 by Bywaters and Dixon as a case series of four patients [55]. Sacroiliitis or syndesmophytes were not always present, but peripheral joint erosions and periosteal bone reaction often accompanied.

In a study of 130 PsA patients by Lambert et al. (1977), 40% were classified as PsSpA and syndesmophytes were noted in 25% of cases [69]. However, the paper did not state the classification criteria used for PsSpA, and only 51/130 had complete sets of axial radiographs for review. Isolated anterior syndesmophytes of the cervical spine, without sacroiliitis, were noted in 60% of PsSpA cases. This group had a slight male preponderance, and more prevalent nail dystrophy, but were no more likely to have symptoms or signs of spinal disease than those with normal spine radiographs; suggesting symptomatically-silent radiographic PsSpA. Lateral syndesmophytes were most commonly found in the lumbar and lower thoracic spine; although that might be explained by the often poor visualisation of syndesmophytes on anteroposterior views of the cervical spine. Radiographic sacroiliitis was observed in 17% (22/130) of cases, with the majority having bilateral sacroiliitis (17/22; 77%). Bilateral sacroiliitis was of symmetrical-grade in the majority (12/22), and asymmetrical-grade in 5/22.

In a cohort study by Hanly et al. (1988) of 220 PsA, 52/220 (24%) were classified as PsSpA using relatively weak criteria: either inflammatory pain / stiffness of the spine, and / or clinical sacroiliitis disease (Gaenslen test, Patrick-FABER test, or direct compression), and / or sacroiliitis of grade ≥2 unilaterally [63]. Sacroiliitis of grade ≥2 was noted in 24/220 (11%) of PsA cases. Of 22 patients with syndesmophytes, only 13/22 had sacroiliitis (even using the less strict definition of grade ≥2 unilaterally) and 9/22 (41%) had normal SIJs. Although the classification criteria were less stringent, these data support the observation of Lambert et al. [69] that syndesmophytes without sacroiliitis are a common occurrence in PsSpA, thereby distinguishing PsSpA from AS. However, since AS patients are only classified as such by virtue of their sacroiliitis, perhaps a better comparison in this setting might be PsSpA with non-radiographic axial SpA. Unfortunately, no studies have investigated this.
In the same study, serial radiographs over a mean follow-up of 57 months showed a significant increase in the number of patients with syndesmophyte formation and sacroiliitis. There was no significant increase in the number of patients with spinal pain, SIJ tenderness, or change in thoracolumbar spine metrology [63]. The authors concluded that although PsSpA patients experience radiographic progression, it remains clinically silent and does not compromise spinal mobility.

Of 57 consecutive PsA patients enrolled in a cross-sectional study in Italy by Salvarani et al., 40/57 (70%) demonstrated radiographic cervical spondylitis [112]. Two patterns of cervical spondylitis were noted: a pattern similar to that seen in AS (n=25/40); and a pattern similar to that seen in RA with erosion and / or subluxation (n=15/40). Isolated cervical spondylitis without lumbar spondylitis or sacroiliitis was present in 9/57 cases; all of whom had peripheral disease and all were HLA-B27 negative.

In a study of 75 PsA patients from Bath (UK) in 1994, 43/75 (57%) cases had radiographic cervical spondylitis. Cervical spondylitis changes included: facet joint space narrowing or fusion in 21/43 cases; syndesmophytes in 7/43 cases; vertebral body fusion in 6/43 cases; and posterior ligament ossification in 4/43 cases [68]. When present, marginal syndesmophytes were noted in the majority of cases (8/13), compared with paramarginal syndesmophytes in only 5/13 cases. This indicates that marginal syndesmophytes are not exclusive to AS, and can be more frequent in PsSpA than paramarginal syndesmophytes. Narrowing of the lower cervical disc space with subsequent fusion of the vertebral bodies was relatively frequent (6/43). Atlanto-axial subluxation (n=3, but asymptomatic in 2/3) and sub-axial subluxation (n=1) were rare. Unlike in RA, odontoid peg erosion was not demonstrated in any PsA cases.

In a study of 498 PsA cases, 43/498 were classified as PsSpA [70] on the basis of Bennett and Wood’s clinical suggestion [71]. All patients were later confirmed as having radiographic spondylitis and / or sacroiliitis (further details not provided in the paper). Three further subgroups were derived by the researchers: 22/43 with axial arthritis only; 8/43 with mixed axial and DIPJ arthritis; and 13/43 with mixed axial and symmetrical polyarthritis. Neither, sacroiliitis nor HLA-B27 positivity were present in 8/13 cases with mixed axial-DIPJ disease. Psoriatic nail dystrophy was present in 13/43 (30%) of PsSpA cases, and particularly so in the group with mixed axial-DIPJ disease (at 40%). Only one case had a history of acute anterior uveitis. Of 17/43 patients with sacroiliitis, sacroiliitis was unilateral in 11/17, and bilateral in 6/17. Spinal disease was characterised by unilateral asymmetrical syndesmophytes, often para-marginal, and randomly distributed along spine. This was especially so in the mixed axial-polyarthritis group. One might
conjecture: the axial-only group were AS cases with coincidental psoriasis; the mixed axial-DIPJ group had diffuse idiopathic hyperostosis (DISH) rather than inflammatory axial disease; and the mixed axial-polyarthritis group were the ‘true’ PsSpA cases.

A paper published in 2002, reports 160 consecutive PsA patients enrolled and assessed in a secondary-care teaching hospital in Finland [189]. Cervical radiographs were only available in 65/160 cases. In 12/65 (18%) cases, inflammatory radiographic cervical changes were observed. Facet joint ankylosis was the most frequent pathology (7/65; 11%), followed by anterior atlanto-axial subluxation (5/65; 8%) and atlanto-axial impaction (3/65; 5%). The reliability of this study is however, severely diminished by the large amount of missing cervical radiographic data.

No studies have explored whether the pathogenesis of osteoproliferation and / or erosion is a systemic process, nor whether it occurs in axial and peripheral sites concomitantly.

6.2.2 Radiographic features of PsSpA compared with AS

Only four studies have compared the axial radiographic characteristics of PsSpA and AS [49, 58, 59, 201]. Three of these studies had small (n=34, 39 and 66, respectively) sample sizes, and one was large with 147 PsSpA cases, although relied upon the European Spondyloarthropathy Study Group (ESSG) criteria for SpA [60] which have not shown good specificity or sensitivity for PsSpA [24, 57]. Several similarities and differences were found between AS and PsSpA in these studies. Sacroiliitis appears to be commonly bilateral in AS (even in early disease), but either unilateral or bilateral in PsSpA [59]. Sacroiliitis tends to be of symmetrical-grade in AS of recent onset, but of asymmetrical-grade in recent-onset PsSpA [58, 59]. In terms of severity of sacroiliitis, whilst Gladman et al. (1993) demonstrated a higher frequency of grade 4 sacroiliitis in AS compared with PsSpA [49], Helliwell et al. (1998) did not corroborate this [58]. In AS syndesmophytes have been shown to be symmetrically distributed [58, 59], progressing cranially from the lumbar to the cervical spine [58, 59], with a primarily marginal shape and rarely paramarginal [59]. Whereas in PsSpA, the same two studies found that syndesmophytes progress in a more random fashion along the spine [58, 59], with both marginal and paramarginal syndesmophytes being equally common [58, 59]. Gladman et al. found a higher frequency of syndesmophytes in AS compared with PsSpA, although did not subclassify by shape [49]. The following axial radiographic features appear to be more common in PsSpA than AS, albeit data being from one study only [59]: pubic symphesisitis, osteoporosis, lumbar hyperextension, thoracic kyphosis, frequency and severity of facet joint involvement. Acknowledging the frequently poor radiographic projection, Helliwell et
al. propose that lumbar facet joint fusion is also more common in AS than in PsSpA [58]. Data in terms of ligamentous ossification and vertebral squaring is somewhat conflicting, with McEwen et al. [59] reporting both to be more common in AS than PsSpA, but Helliwell et al. [58] reporting no difference. One study found that vertebral corner lesions, paravertebral ossification, and discitis are no more common in AS than in PsSpA [58]. According to two studies, radiographic severity is worse in AS than in PsSpA as measured by the Bath AS Radiology Index (BASRI) total [201] and BASRI whole spine [58], particularly when comparing the lumbar spine [58]. However, another study with the primary objective to validate four different axial radiographic scoring indices in PsSpA, found that BASRI-spine, PASRI, mSASSS and RASSS scores to be no different in AS compared with PsSpA [202]. Finally, Helliwell et al. observed that in AS, BASRI scores were similar in the three spinal regions (cervical, thoracic and lumbar), whereas in PsSpA, BASRI scores were highest in the cervical followed by the thoracic then lumbar spine [58].

The small sample sizes of these studies, variety of radiographic endpoints used, selective reporting of incomplete datasets, lack of confirmed or attempted replication of results in other studies, and different classification systems for PsSpA utilised, limits the reliability of the comparisons between PsSpA and AS listed above. The four studies are summarised in turn below, along with a critique of their study methods, reliability of results, and where possible an explanation for any disparity between study.

Paramarginal syndesmophytes (also termed non-marginal syndesmophytes) were first reported in a study comparing 18 PsSpA, 19 AS, 19 reSpA, and 18 eSpA cases, and presented as an abstract at the 1964 American College of Rheumatology Annual Meeting [191]. It was noted that the radiographic features of AS and eSpA were similar, but different to the radiographic features of PsSpA and reSpA. The cohort was added to, and in 1971 McEwen et al. reported a North American multicentre study comparing the radiographic features of 39 PsSpA, 29 AS, 38 eSpA, and 34 reSpA [59]. None of the AS cases has clinical evidence of psoriasis. The method for classifying cases as PsSpA were not described in detail; other than they were SpA cases with psoriasis confirmed on clinical examination by a physician, and occasionally with biopsy. This was an entirely descriptive paper, with proportions of radiographic features cited for each condition, and no statistical comparisons made between groups. The majority (50%) of PsSpA cases had onset of SpA after the onset of psoriasis, and 18% synchronously. Axial radiographs comprising a set, were taken at different time-points during disease course, with significant variation. This paper preceded the advent of the 1984 modified New York criteria for AS; although the SIJs were scored using the New York grading system. Axial disease without peripheral radiographic involvement was present in 10% of PsSpA
compared with 34% of AS cases. All PsSpA cases had DIPJ disease, but in none of the AS cases. Both PsSpA and AS cases were prone to hip and shoulder joint radiographic damage.

In AS cases, sacroiliitis was usually bilateral and of symmetrical-grade, even in early disease [59]. In PsSpA cases, sacroiliitis could be unilateral or bilateral, and asymmetrical-grade in early disease. The following were more common in AS than in PsSpA cases: pubic symphisisitis; osteoporosis; lumbar hyperextension; thoracic kyphosis; more limited chest expansion; frequency and severity of facet joint involvement; vertebral squaring; greater frequency of syndesmophytes; bilateral symmetrical-grades of syndesmophytes progressing cranially from the lumbar to the cervical spine in AS, whilst PsSpA cases having syndesmophytes progressing in a more random fashion along the spine; AS cases mainly having marginal syndesmophytes and rarely having paramarginal syndesmophytes, whilst PsSpA cases commonly having both marginal and paramarginal syndesmophytes; greater ligamentous ossification.

In 1993, Gladman et al. compared 66 PsA and 40 AS cases in terms of clinical, radiographic and genetics [49]. The criteria for classifying the PsA patients as PsSpA was relatively weak: the presence of inflammatory back pain / stiffness; and / or sacroiliitis on clinical examination; and / or grade ≥2 sacroiliitis; and / or ≥1 paramarginal syndesmophyte. The proportion of PsSpA patients earning their classification by each of the four routes was not reported, and a patient could have been classified as PsSpA on the basis of symptoms alone or clinical-examination sacroiliitis alone, without any evidence of disease on radiographs. After adjusting for the pronounced differences in sex, age at assessment and arthritis duration between the two groups, the AS patients were noted to have a higher frequency of grade four sacroiliitis (p=0.007), and syndesmophytes (p=0.005).

A medium-sized hospital-based study in Leeds (UK) of 34 PsSpA, 91 AS, 31 eSpA, and 7 reSpA patients by Helliwell et al. in 1998 corroborated the findings of McEwen et al., including: symmetry of sacroiliitis in AS, symmetry of lumbar spinal involvement in AS, frequency and size of syndesmophytes [58]. However, this study also demonstrated less severe radiographic axial disease in PsSpA compared with AS cases, as measured by the BASRI-total, and more frequent lumbar facet joint fusion in AS than in PsSpA. All 163 patients fulfilled the modified New York criteria for AS, and further subdivision was based on concomitant disorders e.g. psoriasis diagnosed by a dermatologist, IBD diagnosed by a gastroenterologist, and reactive arthritis diagnosed using the criteria of Calin et al. [220]. Radiographs had been taken in the previous twelve months, and although read in tandem
by two blinded readers, no intra- or inter-rater reliability testing was performed. The PsSpA and AS cases were no different in term of sex ratio, median age (46 vs. 46 years), or median disease duration (16 vs. 18 years).

Severity of SIJ involvement (using the higher grade of the two SIJs) did not differ significantly between the groups [58]. However, AS, PsSpA and eSpA differed when compared for sacroiliitis grade-symmetry (p=0.02). Groups were no different in terms of pubic symphysitis, iliac enthesitis, ischial enthesitis, or hip involvement.

Syndesmophyte morphology, as recorded by the percentage of paramarginal syndesmophytes, was not significantly different between PsSpA, AS and eSpA cases. However, the degree of syndesmophyte symmetry differed, especially when comparing the lumbar spines of PsSpA, AS, and eSpA groups (p=0.04) [58]. The asymmetry of syndesmophytes in PsSpA may simply be a function of the lower frequency of syndesmophytes in PsSpA compared with AS. In addition, the cervical spine was only assessed on the lateral view, therefore not allowing for assessment of left-right symmetry. The groups were no different in terms of vertebral squaring, vertebral corner erosions, paravertebral ossification, ligamentous calcification, discitis, or spinal pseudarthrosis.

6.2.3 Radiographic severity in PsSpA and AS cases

Whilst two studies have used the BASRI to quantitatively compare radiographic severity in PsSpA and AS cases [58, 201], to date no study has with a key study objective compared radiographic severity, as measured by the mSASSS or PASRI scores, in PsSpA and AS cases.

Regional variation in radiographic severity occurs between diseases, according to a study by Helliwell et al. [58]. Compared with PsSpA cases the AS cases showed: more severe changes (as measured by the BASRI), particularly of the lumbar spine (p=0.02), with a higher percentage of grade three and four involvement; more lumbar facet joint involvement (p=0.03); and more complete ankylosis. The grading system was as follows: 0 (normal); 1 (erosion of the superior anterior corner of a vertebra); 2 (≤3 syndesmophytes); 3 (>3 syndesmophytes); 4 (complete ankylosis or facet joint fusion throughout the region). On this basis, and despite having similar disease duration to the AS group, the PsSpA group were considered to have less severe disease overall.

Whilst the inter-regional pattern of severity differed across AS, PsSpA, eSpA and reSpA, it was not consistently so between groups. In AS the worst affected area was the lumbar
spine, but there was little difference between the spinal areas (grade 3-4 disease: lumbar 55%; cervical 50%; thoracic 45%). In the PsSpA group the worst affected area was the cervical spine (grades 3 - 4 disease: cervical 47%; thoracic 36%; lumbar 25%).

A recent large prospective cross-sectional study by Perez-Alamino et al. comparing 1072 AS, 147 PsSpA, and 45 eSpA patients found that radiographic severity, as measured by the BASRI-total score, to be different across the three groups [201].

A study with the primary objective to compare the reliability of four radiographic scoring methods for PsSpA (n=40) and AS (n=18), found that the two groups did not differ in terms of radiographic severity [202]. The PsSpA and AS cases were no different in terms of sex, age at radiograph or disease duration. Acknowledging the small sample size, and this being a secondary outcome analysis, axial disease severity was no different in PsSpA and AS cases as measured by the PASRI (p=0.12), mSASSS (p=0.49), BASRI-spine (p=0.26) or RASSS (p=0.70).

6.2.4 Syndesmophyte morphology in PsSpA and AS

Marginal syndesmophytes represent calcification in the outer fibres of the annulus fibrosis of the intervertebral disc, and are the predominant form in all of the SpAs [58]. Paramarginal syndesmophytes that are broader and bulkier have been observed to occur commonly but not exclusively, in patients with PsSpA. Axial syndesmophyte pathogenesis may parallel the periosteal osteoblastogenesis seen in pPsA, in the form of an enthesopathy at the attachment of the annulus fibrosis to the vertebrae. De Vlam et al. proposed that reduced spinal mobility secondary to facet joint fusion, more common in AS, is associated with marginal syndesmophyte formation. Whereas in PsSpA, preservation of facet joint mobility results in greater anterior tensile forces, and increased damage coupled with reparative osteoproliferation leading to the bulkier para-marginal syndesmophytes of PsSpA [221]. More detailed longitudinal pathological data on the origin and progression of syndesmophytes are needed.

6.2.5 Radiographic scoring tool inception and design

Three key scoring methods have been devised to assess radiographic axial disease in AS: the modified Stoke Ankylosing Spondylitis Spinal Score (mSASSS) [222], the Bath Ankylosing Spondylitis Radiology Index (BASRI) [223], and the Radiographic Ankylosing Spondylitis Spinal Score (RASSS) [224]. The mSASSS and BASRI were later validated.
for use in PsSpA [186]. The Psoriatic Arthritis Spondylitis Radiology Index (PASRI) is a modification of the mSASSS, designed to capture cervical facet joint fusion, which is thought to be more specific to PsSpA than AS [225].

The mSASSS scores the superior and inferior corner of each vertebrae as follows: 0 = normal; 1 = erosion, sclerosis or squaring; 2 = non-bridging syndesmophyte; 3 = bridging syndesmophyte (Figure 6.1) [226]. This is done from the lower border of the second cervical vertebrae to the upper border of the first thoracic vertebrae (using a lateral cervical radiograph), then from the lower border of the twelfth thoracic vertebrae to the upper border of the first sacral vertebrae (using lateral and anteroposterior lumbosacral radiographs). The total score range is 0 to 72 (cervical 0 to 36; lumbar 0 to 36). Of note, the mSASSS does not score the facet joints or SIJs.

Figure 6.1. Radiographic scoring of the vertebral corner using the mSASSS (Figure from Maksymowych et al. 2010 [227])

The PASRI scores the superior and inferior corner of each vertebrae using the same grading system as the mSASSS (Figure 6.1) [225]. This is done from the lower border of the second cervical vertebrae to the upper border of the sixth cervical vertebrae (using a lateral cervical radiograph), then from the lower border of the twelfth thoracic vertebrae to the upper border of the first sacral vertebrae (using lateral and anteroposterior lumbosacral radiographs). The facet joints between C2 and C3, C3 and C4, C4 and C5, and C5 and C6 are also scored as follows; 0 = non-fused; 1 = fused. In addition, the PASRI scores each SIJ using the New York grading system: 0 = normal; 1 = suspicion of sacroiliitis; 2 = sclerosis either side of the joint; 3 = erosions, joint space widening or partial ankylosis of the joint; 4 = complete ankylosis of the joint. The total score range is 0 to 72 (cervical 0 to 24; lumbar 0 to 36; cervical facet joints 0 to 4; SIJs 0 to 8).
6.2.5.1 Radiographic scoring tool validation

All four tools have now been validated for use in PsSpA [186, 202, 225].

The PASRI was developed for use in PsSpA and validated in 73 PsSpA cases with established disease (54 males, mean age 49.4 years, mean disease duration 14.0 years) [225]. During development, the PASRI scored facet joints in both the cervical and lumbar spine. The lumbar facets were then removed because their visualisation and interpretation was not reliable. The construct validity of the PASRI was tested by examining the correlation with PROMs (Revised Leeds Disability Questionnaire, RLDQ; and BASFI), anthropometric measures, and comparison of these domains with the other two validated tool in PsSpA, the mSASSS and BASRI. The PASRI encompassed a greater range of the radiographic axial features of PsSpA. Correlation with anthropometric and patient reported outcomes was good for both the PASRI and BASRI, with both these measures outscoring the mSASSS. The PASRI had a score range that exceeded that of the mSASSS and BASRI. The PASRI had less complete data than the BASRI, as it scored more vertebral levels, so had more potential for poorly-visible levels.

In 2009, the BASRI and mSASSS scores were first validated for use in PsSpA by Lubrano et al. in terms of construct validity, reliability, and feasibility [186]. Seventy seven PsA cases (58 males, mean age 49 years, mean disease duration 14 years) fulfilling CASPAR criteria and with clinical, functional and / or radiological axial involvement were studied. When compared with metrology (as measured by the BASMI), both the BASRI (Spearman rho=0.47; p<0.001), and mSASSS (Spearman rho=0.39; p<0.001) showed modest correlation. No correlation was found between mSASSS or BASRI and BASFI, BASDAI or HAQ. Test-retest analysis demonstrated good reliability of the scores. Both were feasible, although the BASRI was quicker to perform.

All four tools appear to have face validity, as required by the Outcome Measures in Rheumatology (OMERACT) filter [152], given that they measure osteoproliferation and erosion at both SIJs and vertebrae; key features of PsSpA. The four scores have since been compared in terms of intra- and inter-rater reliability in PsSpA (n=40) and AS (n=18), as read in random order by each of four rheumatologists [202]. In PsSpA, the intra- and inter-rater intraclass correlation coefficients (ICC) were: 0.81 (95% CI 0.73, 0.88) and 0.67 (95% CI 0.54, 0.79) for sacroiliitis grade; 0.92 (95% CI 0.87, 0.95) and 0.88 (95% CI 0.82, 0.93) for PASRI; 0.91 (95% CI 0.86, 0.95) and 0.65 (95% CI 0.49, 0.80) for mSASSS; 0.77 and 0.52 for BASRI-spine; and 0.90 and 0.68 for RASSS, respectively. In AS, the intra- and inter-rater ICC were: 0.91 and 0.80 for sacroiliitis grade; 0.99 and 0.93 for
PASRI; 0.98 and 0.86 for mSASSS; 0.96 and 0.86 for BASRI-spine; and 0.96 and 0.75 for RASSS, respectively [202]. All radiographic scoring tools had moderate to excellent intra- and inter-rater reliability when used in PsSpA. In particular, the PASRI had excellent intra- and inter-rater-reliability in PsSpA, and the inter-rater reliability of the PASRI was significantly better than the other three measures tested. The PASRI may therefore be the scoring method of choice for assessing structural damage in PsSpA. In AS, the PASRI had excellent intra- and inter-rater-reliability, whilst the mSASSS and RASSS had very good reliability. Of note, the reliability of sacroiliitis scoring was lower in PsSpA compared to AS. The authors noted that all four methods were less reliable in PsSpA compared with AS, especially in terms of sacroiliitis, and reasoned that this was because the PsSpA population were older than their AS population (53 vs. 45 years; p=0.05) and therefore had a higher prevalence of degenerative arthritis (87 vs. 50%; p=0.01) complicating radiographic interpretation [202].

These four radiographic scoring tools appear to have moderate sensitivity, but high specificity to detect 'true' change in PsSpA, with little difference between them to detect change, according to one study by Chandran et al. 2011 [228]. Axial radiographs of 105 PsSpA patients (71 males, mean age 52 years, mean PsA duration 16 years; defined as sacroiliitis of grade ≥2 and inflammatory back pain and / or restricted spinal mobility) were retrieved for two time points at least two years apart. All radiographs were scored by three rheumatologists using the PASRI, mSASSS, BASRI-spine, and RASSS. The proportion of patients with worsening scores between the two time intervals (mean 3.5 years) was: 32% with the PASRI, 5% with the mSASSS, 29% with the BASRI-spine, and 3% with the RASSS. During this interval the mSASSS (p=0.02), PASRI (p=0.01) and BASRI-spine (p=0.02) showed statistically significant difference in mean scores. The sensitivity for any increase in score to detect true change was as follows: PASRI 0.52 (95%CI 0.32, 0.72); mSASSS 0.52 (95%CI 0.32, 0.72); BASRI-spine 0.48 (95%CI 0.28, 0.68); and RASSS 0.44 (95%CI 0.25, 0.65). The specificity for any increase in score to detect true change was as follows: PASRI 0.74 (95%CI 0.63, 0.83); mSASSS 0.84 (95%CI 0.73, 0.91); BASRI-spine 0.78 (95%CI 0.67, 0.86); and RASSS 0.84 (95%CI 0.73, 0.91) [228].

6.2.6 Summary

Only four studies have compared the axial radiographic characteristics of PsSpA and AS [49, 58, 59, 201]. Radiographic findings across these studies are somewhat more consistent than the clinical findings (PROM and clinical examination) reported Chapter 5. This is likely to be a reflection of the more consistent classification methods used, and the more robust nature of radiographic outcome measures compared with several clinical
outcome measures. However, most of these studies had a small sample size (PsSpA, n=34 to 39; AS n=29 to 91) and may have been underpowered to detect a difference between the two diseases. Perez-Alamino et al. had a larger cohort, but the only radiographic outcome reported was the BASRI [201]. There is an unmet need to compare PsA and AS cases in terms of syndesmophyte morphology, radiographic severity, osteoproliferative and erosive burden. This will allow for the phenotypes of both diseases to be better compared in terms of serum and genetic biomarkers.
6.3 STUDY OBJECTIVES

Our study objectives were to:

(i) compare the pattern of radiographic axial disease in PsSpA and AS.

(ii) compare the morphological (osteoproliferation and erosion) features of radiographic axial disease in PsSpA and AS.

(iii) compare axial radiographic severity in PsSpA and AS.
6.4 METHODS

PsSpA and AS patients were identified, recruited and clinically assessed for the Axial Disease in PsA (ADIPSA) study using the methods described in Chapter 4.

6.4.1 Axial radiographic assessment

The most recent axial radiographs of the cervical (lateral), lumbar (lateral and anteroposterior) and pelvis (including SIJs) making a complete set were scored using the two most validated axial radiographic scoring tools for PsA; the Psoriatic Arthritis Spondylitis Radiology Index (PASRI) [185] and the modified Stoke Ankylosing Spondylitis Spinal Scoring tool (mSASSS) [186].

Where possible, radiographs taken at the same time-point were scored as a set. Where radiographs were taken at different time-points, the most recent time-point was taken as the date of radiographic assessment, on the condition that the interval between the earliest and latest radiograph was ≤5 years.

In keeping with previous publications, inflammatory radiographic axial disease (RAD) was defined as: the presence of New York criteria sacroiliitis (unilateral grade ≥3, or bilateral grade ≥2) on AP pelvic radiograph; and / or ≥1 syndesmophyte (marginal or paramarginal) of the cervical or lumbar spine.

6.4.1.1 Axial radiographic scoring: Rate of progression pilot study

Since the axial radiographs were not taken for research purposes, they were not taken at fixed time intervals. Studies of AS report a very slow rate of axial radiographic progression, of only 1.3 (SD 2.5) units/year using the mSASSS [229]. However, little has been published regarding progression in PsSpA; one study reported that 20% of patients without syndesmophytes at baseline, developed them after ten years [187]. No studies have reported PASRI progression in PsSpA.

Therefore, a pilot study was undertaken to estimate the rate of axial radiographic progression in PsSpA, and guide the decision whether it is reasonable to compare current clinical data and historic radiographs. Sequential axial radiographs of a case-series of 22 PsSpA patients attending the RNHRD over the last three decades with at least two sequential radiographs were scored.
6.4.1.2 Axial radiographic scoring: Reliability exercise

Radiographs were scored by two raters (DJ and AN), but due to time constraints, not in duplicate. Therefore, a reliability exercise was performed to test whether DJ and AN score consistently with each other, and according the ‘gold-standard’ rater Dr. Graham Robinson (GR, Consultant Musculoskeletal Radiologist).

Ten random cases were selected from the ADIPSA cohort (five PsA and five AS cases) for scoring independently by DJ, AN and GR using the PASRI and mSASSS scoring tools.

6.4.1.3 Axial radiographic scoring

Axial radiographs were anonymised for the patient’s rheumatological diagnosis prior to the scoring. Radiographs were scored by two raters (DJ and AN) using the PASRI and mSASSS. The absolute score served as a marker of disease severity, for comparison with clinical characteristics and serum-soluble biomarkers. Subdomains of these composite scores allowed for comparisons between different pathologies and serum-soluble biomarkers.

All but a few radiographs were available for scoring on the RNHRD Picture Archiving Computer System (PACS), and both raters used the same monitor, viewing settings and office environment. Scoring data was entered into an electronic database designed by DJ.

6.4.2 Statistical methods

6.4.2.1 Power calculation

200 PsA patients were compared with 200 AS patients. To date, no studies have defined the relationship between axial radiographic change and minimum clinically important difference (MCID) using the PASRI or mSASSS. Therefore it was not possible to perform a power calculation for this component of the study. However, it should be noted that the proposed sample size was more than double other published studies comparing PsSpA with AS radiographically [49, 58, 59].

Perez-Alamino et al. found a difference in BASRI-total in AS and PsSpA cases [mean 8.7 (SD 4.0) in AS; mean 6.1 (SD 4.0) in PsSpA] [201]. A sample of size of 77 PsSpA and 77 AS would be needed to detect a difference in BASRI-total between the two groups (with an alpha level of 0.05 and Power of 0.90).
6.4.2.2 Statistical analysis

Data was analysed using STATA 12.1 (2011 Texas, USA).

Intra-rater and inter-rater reliability testing was performed for axial radiographic assessments using intra-class correlation coefficients (ICC).

Univariate analyses was performed using the continuity-corrected Chi-squared test for categorical variables, Poisson regression for non-normally distributed continuous data comprised of integers (e.g. PASRI, mSASSS, and vertebral osteoproliferation burden scores in AS compared with PsSpA cases), and zero-inflated Poisson regression for non-normally distributed continuous data with an excess of zero-values (e.g. cervical vertebrae PASRI, lumbar vertebrae PASRI, cervical facet joint PASRI, and vertebral erosion burden scores in AS compared with PsSpA cases). Negative binomial regression and zero-inflated negative binomial regression was considered as an alternative to Poisson regression when the mean did not approximate the variance of the data.

Multivariate analyses was performed using logistic regression for categorical variables, Poisson regression for non-normally distributed continuous data (e.g. PASRI, mSASSS, and vertebral osteoproliferation burden scores in AS compared with PsSpA cases), and zero-inflated Poisson regression for non-normally distributed continuous data with an excess of zero-values (e.g. cervical vertebrae PASRI, lumbar vertebrae PASRI, cervical facet joint PASRI, and vertebral erosion burden scores in AS compared with PsSpA cases). Negative binomial regression and zero-inflated negative binomial regression was considered as an alternative to Poisson regression when the mean did not approximate the variance of the data. A reverse step-wise regression approach was used, setting the alpha-level at 0.1 to determine inclusion or exclusion of covariates in the model. This allowed adjustment for potentially significant covariates (derived from Chapter 5) including: sex, HLA-B27 status, age at axial radiographic assessment, disease duration at axial radiographic assessment, anti-TNF use, synthetic DMARD use, smoking and body mass index, to minimising noise and maximise statistical power. It is acknowledged that indices such as BMI change over time, and that both smoking and medication use as a binary outcome does not capture the various durations and doses used by the patients. Univariate and multivariate reduced-models are presented in the tables as indicated.

Data are presented as mean, median, inter-quartile range (IQR), standard deviation (SD),
odds ratio (OR), adjusted OR (OR_adj), incidence risk ratio (IRR), adjusted IRR (IRR_adj), 95% confidence interval (95% CI), and p-value as appropriate to the data. The alpha-level for statistical significance was set at 0.05.

6.4.2.3 Missing data handling

If an entire region of radiographs were missing, e.g. no cervical spine images available, then those patients were omitted from that component of the analyses. However, available radiographs were still scored as data remained valuable for specific sub-analyses e.g. associations with osteoproliferation as a binary outcome measure. As per methods used by Wanders et al. [188] and later by Lubrano et al. [185]: if >3 scoring sites (e.g. lower border of T12 vertebrae) were missing, the radiographs were excluded; if ≤3 scoring sites were missing, the mean of the other scoring sites were used as a substitute for the missing sites.
6.5 RESULTS

6.5.1 Rate of axial radiographic progression in PsSpA: An exploratory study

Twenty two patients (14 males; mean age at baseline 42.24 years, defined as the first radiograph available) were selected: 12/22 had a diagnosis of PsA with / without axial symptoms; and 10/22 had a diagnosis of PsSpA (7/10 with peripheral symptoms, and 3/10 without peripheral symptoms). Follow-up of the patients ranged from 2 to 31 years, and the interval between radiographs ranged from 2 to 33 years.

The median mSASSS at baseline was 8.57 (range 0, 24) and 11.29 (range 1, 30) at the second time-point. During a median of 5.98 years (range 1.83, 11.00) the annual mSASSS progression was a median 0.61 units/year (range -0.67, 2.18).

The median PASRI at baseline was 12.57 units (range 4, 30) and 15.00 units (range 2, 34) at the second time-point. Over a median of 5.98 years (range 1.83, 11.00) the annual PASRI progression was a median 0.40 units/year (range -0.67, 1.64).

In summary, the rate of mSASSS progression in PsSpA is approximately half-that reported in AS (0.6 vs. 1.3 units/year). This data suggests that it is reasonable to use films in this study that are up to ten years old. This explorative study has limitations, including: an assumption of linear progression; small sample size; differing time intervals between films, non-random patient selection (with a potential bias towards symptomatic patients, who might be the progressors); and time between symptom onset and baseline film were unknown. A large prospective longitudinal study using pre-defined imaging intervals is needed to fully understand radiographic progression of axial disease in PsSpA.

6.5.2 Reliability of axial radiographic scoring

There was excellent (>0.75) inter-rater reliability between DJ, AN and GR for both of the composite radiographic scoring tools (PASRI and mSASSS) and their regional subdomains (cervical, lumbar and SIJs), as measured by the intra-class correlation coefficient (ICC) [230]. Compared to the gold standard rater (GR), ICCs ranged between: 0.98 (95% CI 0.90, 1.00) to 0.98 (95% CI 0.92, 1.00 ) for the PASRI; 0.98 (95% CI 0.90, 0.99) to 0.99 (95% CI 0.96, 1.00) for the mSASSS; and 0.85 (95% CI 0.33, 0.96) to 0.90 (95% CI 0.56, 0.98) for the SIJs (Table 6.1).
Table 6.1 Inter-rater reliability of axial radiographic scoring in PsSpA and AS across the three raters

<table>
<thead>
<tr>
<th>Site</th>
<th>Rater</th>
<th>Intraclass correlation coefficient (ICC)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>PASRI</td>
<td>DJ vs. AN</td>
<td>0.99</td>
<td>0.97, 1.00</td>
</tr>
<tr>
<td>PASRI</td>
<td>DJ vs. GR</td>
<td>0.98</td>
<td>0.90, 1.00</td>
</tr>
<tr>
<td>PASRI</td>
<td>AN vs. GR</td>
<td>0.98</td>
<td>0.92, 1.00</td>
</tr>
<tr>
<td>mSASSS</td>
<td>DJ vs. AN</td>
<td>0.99</td>
<td>0.97, 1.00</td>
</tr>
<tr>
<td>mSASSS</td>
<td>DJ vs. GR</td>
<td>0.98</td>
<td>0.90, 0.99</td>
</tr>
<tr>
<td>mSASSS</td>
<td>AN vs. GR</td>
<td>0.99</td>
<td>0.96, 1.00</td>
</tr>
<tr>
<td>SIJs</td>
<td>DJ vs. AN</td>
<td>0.97</td>
<td>0.90, 0.99</td>
</tr>
<tr>
<td>SIJs</td>
<td>DJ vs. GR</td>
<td>0.90</td>
<td>0.56, 0.98</td>
</tr>
<tr>
<td>SIJs</td>
<td>AN vs. GR</td>
<td>0.85</td>
<td>0.33, 0.96</td>
</tr>
<tr>
<td>C-spine vertebrae</td>
<td>DJ vs. AN</td>
<td>0.98</td>
<td>0.91, 0.99</td>
</tr>
<tr>
<td>C-spine vertebrae</td>
<td>DJ vs. GR</td>
<td>0.99</td>
<td>0.94, 1.00</td>
</tr>
<tr>
<td>C-spine vertebrae</td>
<td>AN vs. GR</td>
<td>0.97</td>
<td>0.84, 0.99</td>
</tr>
<tr>
<td>C-spine facet joints</td>
<td>DJ vs. AN</td>
<td>0.99</td>
<td>0.94, 1.00</td>
</tr>
<tr>
<td>C-spine facet joints</td>
<td>DJ vs. GR</td>
<td>0.89</td>
<td>0.58, 0.97</td>
</tr>
<tr>
<td>C-spine facet joints</td>
<td>AN vs. GR</td>
<td>0.96</td>
<td>0.83, 0.99</td>
</tr>
<tr>
<td>L-spine vertebrae</td>
<td>DJ vs. AN</td>
<td>1.00</td>
<td>0.99, 1.00</td>
</tr>
<tr>
<td>L-spine vertebrae</td>
<td>DJ vs. GR</td>
<td>0.94</td>
<td>0.78, 0.99</td>
</tr>
<tr>
<td>L-spine vertebrae</td>
<td>AN vs. GR</td>
<td>0.95</td>
<td>0.82, 0.99</td>
</tr>
</tbody>
</table>

DJ: Deepak Jadon
AN: Alison Nightingale
GR: Graham Robinson
SIJs: sacroiliac joints
C-spine: cervical spine
L-spine: lumbar spine
95% CI: 95% confidence interval
PASRI: Psoriatic Arthritis Spondylitis Radiology Index
mSASSS: modified Stoke Ankylosing Spondylitis Spinal Score

There was excellent (>0.75) intra-rater reliability by both DJ and AN with the PASRI, mSASSS and, and their regional subdomains (cervical, lumbar and SIJs), when the same ten cases were re-scored a month later by each rater. ICCs for DJ ranged between 0.99 (95% CI 0.95, 1.00) for the cervical spine, to 1.00 (1.00, 1.00) for the SIJs and cervical facet joints; and for AN ranged between 0.88 (95% CI 0.53, 0.97) for cervical spine, to 1.00 (0.98, 1.00) for the lumbar spine (Table 6.2).
Table 6.2. Intra-rater reliability of axial radiographic scoring in PsSpA and AS across the two raters

<table>
<thead>
<tr>
<th>Site</th>
<th>Rater</th>
<th>Intraclass correlation coefficient (ICC)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>PASRI</td>
<td>DJ vs. DJ</td>
<td>1.00</td>
<td>0.99, 1.00</td>
</tr>
<tr>
<td>mSASSS</td>
<td>DJ vs. DJ</td>
<td>1.00</td>
<td>0.98, 1.00</td>
</tr>
<tr>
<td>SIJs</td>
<td>DJ vs. DJ</td>
<td>1.00</td>
<td>1.00, 1.00</td>
</tr>
<tr>
<td>C-spine vertebrae</td>
<td>DJ vs. DJ</td>
<td>0.99</td>
<td>0.95, 1.00</td>
</tr>
<tr>
<td>C-spine facet joints</td>
<td>DJ vs. DJ</td>
<td>1.00</td>
<td>1.00, 1.00</td>
</tr>
<tr>
<td>L-spine vertebrae</td>
<td>DJ vs. DJ</td>
<td>0.99</td>
<td>0.97, 1.00</td>
</tr>
<tr>
<td>PASRI</td>
<td>AN vs. AN</td>
<td>0.99</td>
<td>0.96, 1.00</td>
</tr>
<tr>
<td>mSASSS</td>
<td>AN vs. AN</td>
<td>0.98</td>
<td>0.93, 1.00</td>
</tr>
<tr>
<td>SIJs</td>
<td>AN vs. AN</td>
<td>0.99</td>
<td>0.94, 1.00</td>
</tr>
<tr>
<td>C-spine vertebrae</td>
<td>AN vs. AN</td>
<td>0.88</td>
<td>0.53, 0.97</td>
</tr>
<tr>
<td>C-spine facet joints</td>
<td>AN vs. AN</td>
<td>0.99</td>
<td>0.95, 1.00</td>
</tr>
<tr>
<td>L-spine vertebrae</td>
<td>AN vs. AN</td>
<td>1.00</td>
<td>0.98, 1.00</td>
</tr>
</tbody>
</table>

DJ: Deepak Jadon
AN: Alison Nightingale
SIJ: sacroiliac joint
PASRI: Psoriatic Arthritis Spondylitis Radiology Index
mSASSS: modified Stoke Ankylosing Spondylitis Spinal Score

In summary, the mSASSS and PASRI were reliable methods to score axial radiographic disease in PsSpA and AS.

6.5.3 Axial radiographic characteristics of PsSpA and AS cases

The study enrolled 402 SpA patients (201 PsA and 201 AS), and all completed clinical assessment. A full set of axial radiographs (cervical, lumbar and pelvis) were available on 392/402 cases, with an incomplete series of axial radiographs in 10/402 cases. Only ten PsA patients had an incomplete series of axial films to determine RAD, and were assumed to have peripheral arthritis only, and therefore classified as pPsA. Of these: 118/402 (29.35%) cases were classified as PsSpA on based upon psoriasis (current or previous) and radiographic axial disease (RAD); 157/402 (39.05%) cases were classified as AS based upon RAD but no psoriasis (current or history of); 127/402 (31.60%) cases were classified as peripheral PsA (pPsA) based upon psoriasis (current or previous), peripheral arthritis and without RAD.

All the vertebral levels were visible for scoring in the PsSpA and AS cases; so there were no missing data. A comparison of the axial radiographic characteristics of the 118 PsSpA and 157 AS cases are presented below.
6.5.3.1 Socio-demographic and treatment characteristics of the PsSpA and AS cases

A more detailed description is given in Chapter 5, but a summary for the purpose of this chapter is given here. There was a preponderance of males in the AS group compared with the PsSpA group (OR 1.80; 95% CI 1.07, 3.03; p=0.03). AS cases were a mean 4.88 years younger than PsSpA cases at the most recent axial radiograph (51.48 vs. 56.36 years, respectively; p=0.002). Disease duration from arthritis diagnosis to the most recent axial radiograph was no different in AS compared with PsSpA cases (19.00 vs. 15.50 years, respectively; p=0.13). AS cases were more likely than PsSpA cases to be HLA-B27 positive (89.17 vs. 39.83%; OR 12.44; 95% CI 6.67, 23.22; p<0.001). AS and PsSpA cases were no different in terms of a history of tobacco smoking (45.85 vs. 51.82%, respectively; p=0.34). BMI was lower in AS compared with PsSpA cases (mean difference -1.60 kg/m^2; p=0.01). AS cases were far less likely to have used a synthetic DMARD than PsSpA cases (OR 0.24; 95% CI 0.14, 0.42; p<0.001). Anti-TNF therapy use during disease course was no different in AS compared with PsSpA cases (37.58 vs. 44.92%, respectively; p=0.22); nor was the interval between diagnosis and commencement of the first anti-TNF agent (16.0 years in PsSpA vs. 16.0 years in AS; p=0.62%). Multivariate regression models were used to adjust for differences between the AS and PsSpA groups.

6.5.3.2 Axial radiographic pattern

Radiographic sacroiliitis was more likely in AS (147/157; 93.63%) compared with PsSpA (79/118; 66.95%) cases (OR_{adj} 3.78; 95% CI 1.63, 8.77; p=0.002) (Table 6.3). The ten cases in the AS group deemed not to have sacroiliitis were re-reviewed after the formal scoring exercise. Their classification as AS was deemed appropriate as the sacroiliitis was equivocal, and might justifiably have been scored as New York criteria sacroiliitis in a non-research clinical setting. They remained in the AS cohort (n=157), and their PASI and SIs scores, as scored in the formal scoring exercise, were not altered for subsequent analyses.
Table 6.3. Axial radiographic characteristics in PsSpA and AS

<table>
<thead>
<tr>
<th>Domain</th>
<th>Clinical Parameter</th>
<th>Disease</th>
<th>Yes (%)</th>
<th>Adjusted OR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiographic</td>
<td>Sacroiliitis</td>
<td>PsSpA</td>
<td>79/118 (66.95)</td>
<td>3.78</td>
<td>1.63, 8.77</td>
<td>0.002 *</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AS</td>
<td>147/157 (93.63)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spondylitis</td>
<td>PsSpA</td>
<td>84/118 (71.19)</td>
<td>0.94</td>
<td>0.52, 1.69</td>
<td>0.83 **</td>
</tr>
<tr>
<td></td>
<td>(cervical and / or</td>
<td>AS</td>
<td>109/157 (69.43)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>lumbar)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sacroiliitis pattern</td>
<td>Bilateral</td>
<td>PsSpA</td>
<td>65/79 (82.28)</td>
<td>6.14</td>
<td>2.08, 18.15</td>
<td>0.001 ***</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AS</td>
<td>142/147 (96.60)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Symmetrical grade</td>
<td>PsSpA</td>
<td>60/79 (75.95)</td>
<td>1.17</td>
<td>0.59, 2.32</td>
<td>0.65 ****</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AS</td>
<td>119/147 (80.95)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spondylitis pattern</td>
<td>Cervical vertebrae</td>
<td>PsSpA</td>
<td>52/116 (44.83)</td>
<td>1.20</td>
<td>0.65, 2.25</td>
<td>0.56 *****</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AS</td>
<td>85/157 (54.14)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cervical facet joint</td>
<td>PsSpA</td>
<td>29/118 (24.58)</td>
<td>1.48</td>
<td>0.81, 2.72</td>
<td>0.20 *****</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AS</td>
<td>60/157 (38.22)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lumbar vertebrae</td>
<td>PsSpA</td>
<td>50/117 (42.74)</td>
<td>1.37</td>
<td>0.75, 2.50</td>
<td>0.30 ******</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AS</td>
<td>85/157 (54.14)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Multivariate reverse-stepwise logistic regression model (reduced model adjusted for: HLA-B27 status)
** Multivariate reverse-stepwise logistic regression model (reduced model adjusted for: sex, age at radiographic assessment, disease duration at radiographic assessment)
*** Multivariate reverse-stepwise logistic regression model (reduced model adjusted for: disease duration at radiographic assessment)
**** Multivariate reverse-stepwise logistic regression model (reduced model adjusted for: age at radiographic assessment, and disease duration at radiographic assessment)
***** Multivariate reverse-stepwise logistic regression model (reduced model adjusted for: sex, HLA-B27 status, age at radiographic assessment, anti-TNF use ever, and synthetic DMARD use ever)
****** Multivariate reverse-stepwise logistic regression model (reduced model adjusted for: HLA-B27 status, and age at radiographic assessment)

Bilateral sacroiliitis was present in the majority of PsSpA cases (65/79; 82.28%), with unilateral sacroiliitis present in only 14/79 (17.72%) (Table 6.3). Similarly, bilateral sacroiliitis was present in the majority of AS cases (142/147; 96.60%), with unilateral sacroiliitis present in only 5/147 (3.40%). Despite this, AS cases were statistically more likely to have bilateral sacroiliitis than PsSpA cases (OR_{adj} 6.14; 95% CI 2.08, 18.15; p=0.001).

Symmetrical-grade sacroiliitis was present in the majority of PsSpA cases (60/79; 75.95%), with asymmetrical-grade sacroiliitis present in only 19/79 (24.05%) (Table 6.3). Similarly, symmetrical-grade sacroiliitis was present in the majority of AS cases (119/147; 80.95%), with asymmetrical-grade sacroiliitis present in only 28/147 (19.05%). Of note, AS cases were no more likely to have symmetrical-grade sacroiliitis than PsSpA cases (OR_{adj} 1.17; 95% CI 0.59, 2.32; p=0.65).

There was no significant difference between AS (109/157; 69.43%) and PsSpA (84/118; 71.19%) in terms of the occurrence of spondylitis (cervical and / or lumbar) (OR_{adj} 0.94; 95% CI 0.52, 1.69; p=0.83) (Table 6.3). Regional analysis showed that cervical vertebrae spondylitis was present in 52/116 (44.83%) PsSpA and 85/157 (54.14%) AS cases, with no difference between diseases (OR_{adj} 1.20; 95% CI 0.65, 2.25; p=0.56) (Table 6.3).
Similarly, cervical facet joint fusion was present in 29/118 (24.58%) PsSpA and 60/157 (38.22%) AS cases, with no difference between diseases (OR_{adj} 1.48; 95% CI 0.81, 2.72; p=0.20) (Table 6.3). Lumbar vertebrae spondylitis was present in 50/117 (42.74%) PsSpA and 85/157 (54.14%) AS cases, with no difference between diseases (OR_{adj} 1.37; 95% CI 0.75, 2.50; p=0.30) (Table 6.3).

Of 118 cases classified as PsSpA: 45/118 (38.14%) had sacroiliitis with spondylitis; 34/118 (28.81%) had sacroiliitis without spondylitis; and 39/118 (33.05%) had spondylitis without sacroiliitis (Figure 6.2). The majority of PsSpA cases with spondylitis but no sacroiliitis were symptomatic (22/39; 56.41%); although were less likely to be symptomatic than PsSpA cases with sacroiliitis with / without spondylitis (66/79; 83.54%) (OR 0.26; 95% CI 0.11, 0.61; p=0.002).

![Figure 6.2. Pattern of radiographic axial disease in PsSpA cases](image)

All 39 cases with spondylitis but not sacroiliitis were enrolled from the PsA clinic. In that group, 4/39 had borderline unilateral grade 2 sacroiliitis. Spondylitis had been scored in this group on the basis of cervical non-bridging syndesmophyte(s) (n=18/39; 46.15%), or cervical bridging syndesmophyte (n=3/39; 7.69%), or lumbar non-bridging syndesmophyte(s) (n=23/39; 74.36%).
HLA-B27 was positive in 6/39 (15.38%) of PsSpA cases with spondylitis-alone, 11/34 (32.35%) of cases with sacroiliitis-alone, and 30/45 (66.67%) of cases with both sacroiliitis and spondylitis. Logistic regression (adjusted for sex, age at radiographic assessment and disease duration at radiographic assessment) demonstrated that compared with PsSpA cases with spondylitis-alone, there is a significantly increased probability of HLA-B27 positivity in PsSpA cases with both sacroiliitis and spondylitis (unadjusted OR 11.00; 95% CI 3.78, 32.00; p<0.001. Adjusted OR_{adj} 9.10; 95% CI 3.01, 27.47; p<0.001), and a trend for the same when comparing spondylitis-alone with sacroiliitis-alone (unadjusted OR 2.63; 95% CI 0.85, 8.13; p=0.09. Adjusted OR 2.60; 95% CI 0.80, 8.42; p=0.11).

Peripheral hand and feet radiographs of all of the 201 PsA cohort had been scored by DJ during a previous study using the modified Van der Heijde scoring tool [231]. Peripheral joint ankylosis (≥1 joint) was observed in 12/127 (9.45%) pPsA cases and 11/74 (14.86%) PsSpA cases; 44 PsSpA cases derived from the AS cohort didn’t have peripheral radiographs for scoring. A logistic regression model included sex, HLA-B27 status, age at axial radiograph, disease duration at axial radiograph, anti-TNF use ever and synthetic DMARD use ever demonstrated that the odds of having peripheral joint ankylosis was no higher in the PsSpA compared with the pPsA group (OR_{adj} 1.61; 95% CI 0.66, 4.20; p=0.29), although may be underpowered to detect a difference.

6.5.3.3 Axial radiographic morphology

AS cases were significantly more likely to have SIJ complete ankylosis (grade 4 sacroiliitis) than PsSpA cases (43.31 vs. 15.25%; OR_{adj} 2.96; 95% CI 1.42, 6.15; p=0.004) (Table 6.4). However, there was no statistically significant difference between AS and PsSpA cases in terms of the probability of SIJ joint partial ankylosis (29.30 vs. 21.19%; OR_{adj} 1.08; 95% CI 0.56, 2.10; p=0.81), SIJ erosion (33.76 vs. 35.59%; OR_{adj} 1.07; 95% CI 0.63, 1.81; p=0.80), and SIJ bilateral simple sclerosis (5.73 vs. 5.08%; OR_{adj} 1.75; 95% CI 0.56, 5.48; p=0.34).
### Table 6.4. Axial radiographic morphology in PsSpA and AS

<table>
<thead>
<tr>
<th>Site</th>
<th>Morphological feature</th>
<th>Disease</th>
<th>Yes (%)</th>
<th>Adjusted OR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sacroiliac joint</td>
<td>Simple sclerosis bilaterally (Grade 2)</td>
<td>PsSpA</td>
<td>6/118 (5.08)</td>
<td>1.75</td>
<td>0.56, 5.48</td>
<td>0.34 *</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AS</td>
<td>9/157 (5.73)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Erosion (Grade 3)</td>
<td>PsSpA</td>
<td>42/118 (35.59)</td>
<td>1.07</td>
<td>0.63, 1.81</td>
<td>0.80 **</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AS</td>
<td>53/157 (33.76)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Partial ankylosis (Grade 3)</td>
<td>PsSpA</td>
<td>25/118 (21.19)</td>
<td>1.08</td>
<td>0.56, 2.10</td>
<td>0.81 ***</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AS</td>
<td>46/157 (29.30)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Complete ankylosis (Grade 4)</td>
<td>PsSpA</td>
<td>18/118 (15.25)</td>
<td>2.96</td>
<td>1.42, 6.15</td>
<td>0.004 ****</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AS</td>
<td>68/157 (43.31)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vertebral</td>
<td>Erosion</td>
<td>PsSpA</td>
<td>3/118 (2.54)</td>
<td>1.58</td>
<td>0.38, 6.57</td>
<td>0.53 **</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AS</td>
<td>6/157 (3.82)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Non-bridging syndesmophyte</td>
<td>PsSpA</td>
<td>47/118 (39.83)</td>
<td>0.93</td>
<td>0.57, 1.56</td>
<td>0.79 ****</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AS</td>
<td>58/157 (36.94)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bridging syndesmophytes</td>
<td>PsSpA</td>
<td>12/118 (10.17)</td>
<td>2.78</td>
<td>1.49, 5.18</td>
<td>0.001 ****</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AS</td>
<td>36/157 (22.93)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Multivariate reverse-stepwise logistic regression model (reduced model adjusted for: synthetic DMARD use ever)
** Multivariate reverse-stepwise logistic regression model (reduced model adjusted for: sex, and disease duration at radiographic assessment)
*** Multivariate reverse-stepwise logistic regression model (reduced model adjusted for: HLA-B27 status, age at radiographic assessment, and disease duration at radiographic assessment)
**** Multivariate reverse-stepwise logistic regression model (reduced model adjusted for: sex, HLA-B27 status, and disease duration at radiographic assessment)
***** Multivariate reverse-stepwise logistic regression model (reduced model adjusted for: sex, and age at radiographic assessment)
****** Multivariate reverse-stepwise logistic regression model (reduced model adjusted for: sex, age at radiographic assessment, anti-TNF use ever, and synthetic DMARD use ever)

Bridging syndesmophytes were relatively common in both AS (22.93%) and PsSpA (10.17%) cases, but significantly more common in AS cases (OR_{adj} 2.78; 95% CI 1.49, 5.18; p=0.001) (Table 6.4). Non-bridging syndesmophytes were common in both AS (36.94%) and PsSpA (39.83%) cases, with no difference in frequency between groups (OR_{adj} 0.93; 95% CI 0.57, 1.56; p=0.79). Vertebral erosion (also known as the Romanus lesion) was remarkably rare in both AS (3.82%) and PsSpA (2.54%) cases, with no significant difference in frequency between groups (OR_{adj} 1.58; 95% CI 0.38, 6.57; p=0.53).

#### 6.5.3.4 Axial radiographic severity

Axial radiographic severity was higher in AS than in PsSpA cases, as measured by the PASRI (median score 15 vs. 6, respectively; IRR_{adj} 1.13 for every 1 unit increase in score; 95% CI 1.09, 1.19; p<0.001) (Figure 6.3), and the mSASSS (median score 10 vs. 3, respectively; IRR_{adj} 1.09 for every 1 unit increase in score; 95% CI 1.04, 1.14; p<0.001) (Figure 6.4).
Multivariate reverse-stepwise Poisson regression model adjusted (reduced model adjusted for: sex, HLA-B27 status, age at radiographic assessment, disease duration at radiographic assessment, anti-TNF use ever, synthetic DMARD use ever, and smoking ever)

**Figure 6.3.** Axial radiographic severity as measured by the PASRI in PsSpA and AS cases

Multivariate reverse-stepwise Poisson regression model (reduced model adjusted for: sex, HLA-B27 status, age at radiographic assessment, disease duration at radiographic assessment, anti-TNF use ever, synthetic DMARD use ever, and smoking ever)

**Figure 6.4.** Axial radiographic severity as measured by the mSASSS in PsSpA and AS cases

The same held true when analysed by axial region: cervical vertebral PASRI in AS and PsSpA (median score 2 vs. 0, respectively; IRR$_{adj}$ 1.57 for every 1 unit increase in score;
95% CI 1.35, 1.83; p<0.001) (Figure 6.5); and lumbar vertebral PASRI in AS and PsSpA (median score 4 vs. 0, respectively; IRR_{adj} 1.42 for every 1 unit increase in score; 95% CI 1.29, 1.58; p<0.001) (Figure 6.6).
Cervical facet joint PASRI was also higher in AS (median score 0; IQR 0, 3) compared with PsSpA (median score; IQR 0, 0) cases (IRRadj 1.47; 95% CI 1.08, 1.99; p=0.01) (multivariate reverse-stepwise zero-inflated Poisson regression model adjusted for: sex, age at radiographic assessment, disease duration at radiographic assessment, anti-TNF use ever, and synthetic DMARD use ever).

The mSASSS scores the same number of levels in the cervical and lumbar spine, thereby allowing score comparisons to be made; whereas the PASRI scores a different number of levels in the cervical and lumbar spine. Using the mSASSS, the cervical spine was more severely affected than the lumbar spine in PsSpA (IRRadj 1.54; 95% CI 1.20, 1.74; p<0.001), but no different in AS.

The burden of vertebral osteoproliferative disease as measured by the PASRI (bridging and / or non-bridging syndesmophytes) was higher in AS compared with PsSpA cases (median score 6 vs. 2, respectively; IRRadj 1.31 for every 1 unit increase in score; 95% CI 1.09, 1.57; p=0.004) (Table 6.5). The burden of vertebral erosive disease was not statistically different in AS and PsSpA cases (IRRadj 0.99; 95% CI 0.43, 2.31; p=0.99).

**Table 6.5. Burden of osteoproliferation and erosion in PsSpA and AS cases**

<table>
<thead>
<tr>
<th>Vertebral morphology using the PASRI</th>
<th>Disease</th>
<th>Median score</th>
<th>IQR</th>
<th>Adjusted IRR</th>
<th>Adjusted 95% CI</th>
<th>Adjusted p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erosion burden</td>
<td>PsSpA</td>
<td>0</td>
<td>0, 0</td>
<td>0.99</td>
<td>0.43, 2.31</td>
<td>0.99 ^</td>
</tr>
<tr>
<td>AS</td>
<td>0</td>
<td>0, 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osteoproliferation burden (syndesmophytes)</td>
<td>PsSpA</td>
<td>2</td>
<td>0, 6</td>
<td>1.31</td>
<td>1.09, 1.57</td>
<td>0.004 *</td>
</tr>
<tr>
<td>AS</td>
<td>6</td>
<td>0, 30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

IQR: inter-quartile range  
IRR: Incidence risk ratio  
PsSpA: psoriatic spondyloarthritis  
AS: ankylosing spondylitis  
PASRI: Psoriatic Arthritis Spondylitis Radiology Index

^ Multivariate reverse-stepwise zero-inflated Poisson regression model, adjusted for: age at radiographic assessment, and smoking ever.

* Multivariate reverse-stepwise Poisson regression model, adjusted for: sex, HLA-B27 status, age at radiographic assessment, disease duration at radiographic assessment, anti-TNF use ever, synthetic DMARD use ever, and smoking ever.

There was a moderate-to-good [232] positive correlation that was statistically significant between the PASRI and mSASSS in: PsSpA cases (Spearman rank correlation coefficient, r_s 0.62; p<0.0001), and in AS cases (r_s 0.97; p<0.0001); although the strength of the correlation was greater in AS than in PsSpA cases.
6.5.4 An exploratory analysis of axial radiographic severity in male and female cases

The objective of this chapter of the thesis was to compare PsSpA and AS cases, rather than subgroups within each cohort. However, due to the findings of a few previous studies, exploratory analyses was performed to determine if axial radiographic severity was related to sex, adjusted for HLA-B27 status and other covariates.

In the PsSpA cohort: PASRI was lower in female (median 5.5; IQR 4, 6) compared with male (median 7; IQR 4, 23) cases (IRR\textsubscript{adj} 0.62 per 1 unit increase in score; 95% CI 0.57, 0.67; p<0.001); and mSASSS was lower in female (median 2.5; IQR 0, 6) compared with male (median 4; IQR 0, 18) PsSpA cases (IRR\textsubscript{adj} 0.60 per 1 unit increase in score; 95% CI 0.54, 0.66; p<0.001). A zero-inflated Poisson regression adjusting for HLA-B27 status, age at axial radiograph, disease duration at axial radiograph, anti-TNF use ever, synthetic DMARD use ever and smoking was used for each analysis.

The same was found in the AS cohort: PASRI was lower in female (median 8; IQR 6, 23) compared with male (median 18; IQR 7, 40) AS cases (IRR\textsubscript{adj} 0.67 per 1 unit increase in score; 95% CI 0.61, 0.73; p<0.001); and mSASSS was lower in female (median 0; IQR 0, 20) compared with male (median 12; IQR 0, 36) AS cases (IRR\textsubscript{adj} 0.58 per 1 unit increase in score; 95% CI 0.53, 0.64; p<0.001). A Poisson regression adjusting for HLA-B27 status, age at axial radiograph, disease duration at axial radiograph, anti-TNF use ever, synthetic DMARD use ever and smoking was used for each analysis.

6.5.5 An exploratory analysis of correlation between axial radiographics and metrology in both PsSpA and AS

Given that two radiographic indices (mSASSS and BASRI)-spine have shown correlation with metrology in PsSpA in a previous study [204], we performed an exploratory analysis comparing metrology (BASMI and its subdomains) with radiographic indices (PASRI and mSASSS) in our cohort. These exploratory analysis showed moderate-to-good positive correlation that was statistically significant in: PsSpA between PASRI and BASMI (Spearman rank correlation coefficient, $r_s = 0.57$; p<0.0001) (Figure 6.7), and between mSASSS and BASMI ($r_s = 0.56$; p<0.0001); and in AS between PASRI and BASMI ($r_s = 0.57$; p<0.0001) (Figure 6.8), and between mSASSS and BASMI ($r_s = 0.57$; p<0.0001).
Further exploratory analyses for association between regional PASRI and corresponding regional BASMI showed a moderate-to-good positive/negative correlation that was statistically significant in PsSpA cases between: cervical-PASRI (vertebral and facet scores combined) and cervical rotation ($r_s = -0.54; p<0.0001$), cervical-PASRI and tragusto-wall distance ($r_s = 0.64; p<0.0001$), lumbar-PASRI and lumbar forward flexion as measured by the Schober test ($r_s = -0.56; p=<0.0001$), and lumbar-PASRI and lumbar side-flexion ($r_s = -0.38; p<0.0001$). Similarly, there was moderate-to-good positive/
negative correlation that was statistically significant in AS cases between: cervical-PASRI (vertebral and facet scores combined) and cervical rotation \( (r_s = -0.56; p<0.0001) \), cervical-PASRI and tragus-to-wall distance \( (r_s = 0.44; p<0.0001) \) lumbar-PASRI and lumbar forward flexion as measured by the Schober test \( (r_s = -0.56; p<0.001) \), and lumbar-PASRI and lumbar side-flexion \( (r_s = -0.39; p<0.0001) \).

6.5.6 Summary of the results

The typical radiographic axial phenotype of AS and PsSpA are summarised in Figure 6.9. The axial radiographic similarities and difference between AS and PsSpA are summarised in Figure 6.10.

![Figure 6.9. The typical axial radiographic phenotype of AS and PsSpA](image)
More likely or higher in AS
Bilateral sacroiliitis (OR$_{adj}$ 6.14), although usually bilateral in both AS and PsSpA cases.
Complete ankylosis of the SIJs (OR$_{adj}$ 2.96).
Bridging syndesmophytes (OR$_{adj}$ 2.78).
Axial radiographic severity, as measured by:
- PASRI (IRR$_{adj}$ 1.13 per unit increase)
- mSASSS (IRR$_{adj}$ 1.09 per unit increase)
- cervical PASRI (IRR$_{adj}$ 1.57 per unit increase)
- cervical facet joint PASRI (IRR$_{adj}$ 1.47 per unit increase)
- lumbar PASRI (IRR$_{adj}$ 1.42 per unit increase)
Vertebral osteoproliferative burden (IRR$_{adj}$ 1.31 per unit increase)

Less likely in AS
No features

AS
(n=157)

PsSpA
(n=118)

No different in AS & PsSpA cases
Prevalence of:
- Cervical / lumbar vertebrae spondylitis.
- Cervical facet joint fusion.
- Symmetrical grade sacroiliitis (common in both groups).
- SLI partial ankylosis, erosion or sclerosis.
- Non-bridging syndesmophytes (common in both AS & PsSpA).
- Vertebral erosion (rare in both AS & PsSpA).
Vertebral erosion burden (PASRI)

Other features of note
Of 118 PsSpA cases:
- 38% sacroiliitis with spondylitis
- 29% sacroiliitis without spondylitis
- 53% spondylitis without sacroiliitis
(21/39 with cervical spondylitis; 23/39 with lumbar spondylitis)
Of 157 AS cases
- 69% sacroiliitis with spondylitis
- 31% sacroiliitis without spondylitis

Figure 6.10. A comparison of the axial radiographic characteristics of AS and PsSpA
6.6 DISCUSSION & CONCLUSIONS

To our knowledge, this is the largest sample of PsSpA and AS cases with a detailed description of plain radiographic characteristics, and the first study to compare PsSpA and AS cases in terms of: inflammatory symptoms in PsSpA cases with and without sacroiliitis; symptoms in unilateral grade 2 sacroiliitis; prevalence of bridging and non-bridging syndesmophytes; radiographic severity as measured by the PASRI and mSASSS; cervical facet joint severity; vertebral osteoproliferative burden; and vertebral erosion burden. This is also the first study to investigate the influence of HLA-B27 variants on the radiographic expression of PsSpA in terms of sacroiliitis, spondylitis, or both.

6.6.1 Radiographic axial disease in PsSpA

Spondylitis in the absence of sacroiliitis was present in 33% (39/118) of our cohort of PsSpA cases. This is lower than the prevalence of 60% reported by Lambert at al., although that study did not state the classification system used for cases, and only 51 of their 130 PsA cases had complete sets of axial radiographs for review [69]. The missing radiographs are most likely to have been of the cervical or lumbar spine, given that those cases were often asymptomatic. Hanly et al., using a less stringent definition of sacroiliitis (of grade ≥2 unilaterally) demonstrated a 17% (9/52) prevalence of spondylitis without sacroiliitis [63]. This figure would have risen had they used a definition of sacroiliitis similar to ours. Salvarani et al. demonstrated a prevalence of cervical spondylitis without sacroiliitis and / or lumbar spondylitis of 16% (9/57) [112]. Unfortunately, the prevalence spondylitis without sacroiliitis was not reported.

Taylor et al. (2004) proposed that vertebral spondylitis without radiographic sacroiliitis is a feature that may distinguish PsSpA from AS [56]. This has important implications when classifying PsSpA, when a requirement of sacroiliitis (as per AS classification), may incorrectly exclude genuine PsSpA cases with vertebral spondylitis but no sacroiliitis. We support this position given that a significant proportion (56%) of our PsSpA cases with spondylitis-alone had inflammatory axial symptoms.

This is the first study to investigate the influence of HLA-B27 variants on the radiographic expression of PsSpA in terms of sacroiliitis, spondylitis, or both. When compared with PsSpA cases with spondylitis-alone, HLA-B27 variants were significantly more common in PsSpA cases with both sacroiliitis and spondylitis and with sacroiliitis-alone (trend for significance). This implies that sacroiliitis may be the results of abnormal HLA-B27-related
pathways, whereas spondylitis may involve pathways unrelated to HLA-B27. Whilst osteoporosis is a shared feature of both sacroiliitis and spondylitis, their aetio-pathogenesis may be different.

Taken together, our data showing that a significant proportion PsSpA suffer with spondylitis alone, the majority are symptomatic, and that it might not be appropriate to group sacroiliitis and spondylitis together given that they may have different pathogenesis, would argue against having the same the treatment criteria for PsSpA and AS. Requiring PsSpA patients to fulfill AS criteria for anti-TNF therapy, which mandates AS criteria sacroiliitis, seems strict. Similarly, requiring PsSpA patients with primarily axial disease to fulfill PsA criteria for anti-TNF therapy, which mandates significant peripheral arthritis, also seems strict. One might therefore advocate PsSpA-specific criteria for access to specialist therapies such as anti-TNF, whereby symptomatic PsSpA patients with spondylitis but no sacroiliitis can access anti-TNF therapy with equal opportunity. Clinical trials testing the efficacy of specialist therapies such as anti-TNF, specifically in PsSpA patients would be required to justify this. In addition, a new international-consensus classification system for PsSpA, perhaps recognizing that spondylitis alone is sufficient to classify a patient as having PsSpA, would improve the classification of cohorts in research studies and clinical trials, improve the quality of research, and raise the profile of PsSpA.

6.6.2 Radiographic axial disease pattern in PsSpA compared with AS

Bilateral sacroiliitis was the most common pattern in both PsSpA and AS cases in this study. These findings replicate the results of two other studies [59, 69], but challenges the predominantly unilateral sacroiliitis in PsSpA cases reported by Scarpa et al. [70]. The disparity may be related to Scarpa et al.'s cases being younger than ours (mean age 42 vs. 56 years, respectively), so they may not have expressed their complete sacroiliitis phenotype. Another explanation is the classification system used by Scarpa et al. may have misclassified patients with either DISH or AS with coincidental psoriasis, as PsSpA.

Bilateral sacroiliitis was usually symmetrical-grade in our PsSpA and the AS cases, which corroborates the findings of Lambert et al. [69]. McEwen et al. noted asymmetrical grade sacroiliitis in PsSpA cases with early disease, but did not comment on grade-symmetry in established PsSpA cases such as ours [59]. Helliwell et al. reported symmetrical sacroiliitis to be more common in their AS cases [58]. The PsSpA cases in that study were younger than ours (46 vs. 56 years, respectively), and so may not have expressed their complete sacroiliitis phenotype. Complete ankylosis of the SIJs (grade 4) was more
common in our AS compared with PsSpA cases; a finding corroborated by one previous study [49].

Cervical vertebrae spondylitis was noted in 21% (52/245) of our PsA cases, which is similar to the prevalence reported by Laiho et al. of 18% [189]. However, this prevalence is far lower than the 70% reported by Salvarani et al. [112], and the 57% reported by Jenkinson et al. [68]. Such a difference may have arisen if both those studies classified patients as cervical spondylitis on the basis of facet joint disease alone.

AS and PsSpA cases in our cohort had a similar frequency of cervical facet joint disease. McEwen et al. noted a greater frequency of facet joint involvement in AS, but did not compare the groups statistically [59]. However, just as found in our cohort, McEwen et al. also reported a greater severity of facet joint involvement in AS compared with PsSpA.

Just like Helliwell et al. [58], we found no difference between the groups in terms of the frequency of vertebral corner erosion, or vertebral squaring. Neither our study nor that by Jenkinson et al. [68] demonstrated RA-like odontoid peg erosion in the PsSpA cases. We found a greater frequency of bridging syndesmophytes in AS cases compared with PsSpA cases; but no difference in non-bridging syndesmophytes frequency. This is the first study to separately analyse bridging and non-bridging syndesmophytes in the two disease. Three other studies noted a greater frequency of syndesmophytes in AS, but did not sub-analyse by bridging [49, 58, 59].

According to Helliwell et al., the percentage of paramarginal syndesmophytes is not significantly different between PsSpA and AS [58]. However, McEwen et al. noted AS cases rarely having paramarginal syndesmophytes, mostly marginal syndesmophytes; whilst PsSpA cases commonly having both types of syndesmophytes [59]. Jenkinson et al. [68] noted that in PsSpA cases, cervical marginal syndesmophytes were more common than paramarginal syndesmophytes.

6.6.3 Radiographic axial disease severity in PsSpA and AS

Axial radiographic severity as measured by the PASRI and / or mSASSS, was more severe in our AS cases compared with PsSpA cases; even when analysed by spinal region. As measured by the mSASSS, we also found that the cervical spine was more severely affected than the lumbar spine in PsSpA, but was no different in AS. Helliwell et al. measured axial radiographic severity using the BASRI-total, and support our findings both for the whole spine, and for the cervical versus lumbar spine [58]. A large prospective
cross-sectional study by Perez-Alamino et al. using the BASRI-total also supports our findings [201]. Only one study challenges our results, and found no difference in radiographic severity as measured by the PASRI, mSASSS or BASRI-spine [202]. However, that study had much fewer cases (PsSpA n=40, and AS n=18), their cases had shorter disease duration than ours, and the comparison was a secondary objective of the study, so the study most likely was underpowered to detect a difference in severity. Nonetheless, the burden of axial radiographic damage in PsSpA cases was still high and widespread, and is therefore still clinically impactful. In summary, axial disease in PsSpA is not mild, it is clinically important and should be actively treated.

6.6.4 The significance of unilateral grade 2 sacroiliitis

A study classifying PsSpA as unilateral sacroiliitis of grade ≥2, whilst classifying AS as bilateral sacroiliitis grade ≥2 or unilateral sacroiliitis grade ≥3, simply by the entry criteria will show PsSpA cases to be less radiographically severe than AS, and possibly clinically less severe as well. One author has proposed that three key pieces of information will help determine the best definition of PsSpA [48]. Firstly, the proportion of psoriatics with unilateral grade 2 sacroiliitis demonstrating symptomatic and / or metrological evidence of axial disease needs to be determined. Secondly, the proportion of such patients progressing to develop more extensive sacroiliitis or spondylitis needs to be ascertained. Thirdly, the prevalence of grade 2 sacroiliitis in an otherwise healthy population needs to be determined for comparison with the suspected PsSpA patients. This information may help determine whether unilateral grade 2 sacroiliitis is a ‘pre-PsSpA’, or simply an acceptable normal variant within the general population. In our cohort, of eight cases with unilateral grade two sacroiliitis, 5/8 reported a history of inflammatory lumbosacral pain. Unfortunately, data on progression of grade 2 sacroiliitis, or the prevalence in the general healthy population are not available in our study.

6.6.5 Correlation between axial radiographics and metrology in PsSpA and AS

This study is the first to compare mSASSS with BASMI, and PASRI with BASMI and BASMI-subdomains. We found moderate-to-good positive correlation that was statistically significant between PASRI and BASMI, and between mSASSS and BASMI, in both PsSpA and AS cases. Further analyses of BASMI subdomains showed moderate-to-good correlation that was statistically significant between cervical-PASRI and cervical rotation, cervical-PASRI and tragus-to-wall distance, lumbar-PASRI and Schober test, in both PsSpA and AS cases. Our results indicate that the PASRI has construct validity, and
supports our choice to use it in this study as a radiographic outcome measure in both PsSpA and AS cases. Our data are supported by the results of a study from Toronto (Canada) published in 2007, of ten PsSpA and nine AS cases with similar disease duration to our cohort, as part of the International Spondyloarthritis Inter-observer Reliability Exercise (INSPIRE) study [204]. Spinal metrology were correlated with the mSASSS and BASRI-spine. Moderate-to-good correlation was demonstrated between mSASSS and cervical rotation, tragus-to-wall distance, modified Schober test, and lumbar side-flexion in the entire group ($r_s > ±0.54$ for each measure; $p<0.05$ for each measure). The clinical-radiographic correlations were comparable in the PsSpA and AS groups, although, cervical rotation correlated better with mSASSS in PsSpA, than in AS.

### 6.6.6 Other imaging modalities in PsSpA

In a study from Oxford (U.K.) of 68 PsA patients, moderate-to-severe sacroiliitis on clinical examination (clinical-sacroiliitis) was demonstrated in 24/68 (35%) cases [233], and MRI-sacroiliitis was present in 26/68 (38%) cases. However, there was no association between clinical-sacroiliitis and MRI-sacroiliitis, or between HLA-B27 status and MRI-sacroiliitis, MRI-sacroiliitis was associated with restricted spinal movements, and the duration of PsA. These data question the reliability of ‘clinically-demonstrated sacroiliitis’ using physical maneuvers. The correlation between radiographic-sacroiliitis and MRI-sacroiliitis in PsSpA has not been reported.

A retrospective cross-sectional study of MRI scans of the lumbar spine and SIJs from 33 PsSpA, 24 non-radiographic axial SpA (nr-axSpA), and 19 AS patients (of comparable age across groups) found that total MRI scores for bone marrow oedema were higher in AS patients than in PsSpA and nraxSpA patients [234]. HLA-B27 positivity was no different in PsSpA and nraxSpA cases, but higher in AS patients. HLA-B27 negative PsSpA patients had lower MRI scores than both HLA-B27 positive PsSpA patients and AS patients, whereas MRI scores were similar in HLA-B27 positive PsSpA patients and AS patients. Based on this single study, it appears that HLA-B27 might be a predictor of disease severity (burden) in PsSpA, as measured by axial bone marrow oedema on MRI.

It is not known whether the pathogenesis of osteoproliferation and / or erosion is systemic, nor whether it occurs in axial and peripheral sites concomitantly. To determine this, a prospective inception cohort study with concurrent spinal and peripheral imaging would be required.
6.6.7 Study critique

This study had several strengths, in particular the large sample size, and the robust case classification system employed. Secondly, the quantitative radiographic scoring with detailed sensitive scores allowing the groups to be compared using continuous rather than binary outcome measures, improves the study’s power to detect a difference. The collection of morphology data embedded within the scoring tools, allowed comparison of morphologies and patterns of disease. The collection of covariate data including anti-TNF use, synthetic DMARD use, smoking and BMI to include in the multivariate regression models allowed for some adjustment for potential confounders, although we did not measure exposure to these covariates at the exact time of the radiographs; therefore treatment and smoking may not be entirely controlled for. As is discussed in detail in Chapter 5, based on the data we collected, it was not possible in analyses to fully adjust for time-varying variables such as smoking, BMI and medication. The differential magnitude and direction of effect of potential confounders such as anti-TNF use have yet to be determined in AS and PsSpA. There are conflicting reports of anti-TNF therapy both inhibiting [235, 236] and having no effect [237, 238] on the formation of vertebral syndesmophytes in AS. No data exist in PsSpA. Lastly, through education and practice we achieved very high inter- and intra-rater reliability for radiographic scoring.

This study would have been improved by having the entire axial skeleton imaged on the day of the clinical assessment rather than using historical radiographs, a fixed interval between diagnosis and imaging, and sequential radiographs in each case, ideally from diagnosis. This could only have been achieved through a prospective inception cohort, with protocolised imaging at predefined intervals. Although the PsSpA patients were 4.88 years older at imaging, we were fortunate that the disease duration to imaging was no different across the disease groups on univariate analyses. In addition, we made adjustments for age and disease duration at imaging in the multivariate regression models. We do acknowledge that such modelling can only in part, adjust for the differences between the groups. A more sensitive imaging method such as MRI may have detected subtle early imaging difference between the groups. Such a study would not have been affordable or time-feasible in this number of cases, and likely would have reduced study recruitment. As is described above, the utility of MRI in PsSpA has yet to be established.

There are potentially a few unmeasured and therefore uncontrolled confounders in this study. For example, data on participation in regular physiotherapy was not collected in the patients groups. For reasons discussed in Chapter 5, AS patients may be more likely to
be referred for and perform regular physiotherapy. Higher participation may inhibit radiographic disease development and progression. Although the direction of confounding may be the same in PsSpA and AS, the magnitude of effect may be different. Another unmeasured potential confounder is NSAID use, which has been shown to inhibit vertebral syndesmophyte for in AS [239]. Whilst the direction of confounding might be the same in PsSpA and AS, the magnitude of effect and use of NSAIDs may be different in PsSpA and AS cases.

6.6.8 Conclusions

In this study of established PsSpA and AS cases, both groups tended to have bilateral symmetrical-grade sacroiliitis. Unilateral or asymmetrical-grade sacroiliitis reported in previous studies to be characteristic of PsSpA, may simply reflect an earlier stage in its radiographic phenotype expression. AS cases were more likely than PsSpA cases to have complete ankylosis of the SIJs, and / or bridging syndesmophytes. These two features may therefore be more useful than paramarginal syndesmophytes to distinguish PsSpA from AS. The frequency of cervical vertebral spondylitis, lumbar vertebral spondylitis, and cervical facet joint spondylitis was no different in AS and PsSpA cases. Radiographic axial disease appears to be more severe in AS compared with PsSpA as measured quantitatively using the PASRI, mSASSS and osteoproliferative burden, and morphologically as measured by complete ankylosis of the SIJs. PASRI and mSASSS showed good-to-moderate correlation with metrology in both disease. Spondylitis without sacroiliitis was both common and usually symptomatic in PsSpA, so must be considered in classification systems for PsSpA. Aetio-pathogenic differences may exist between sacroiliitis and spondylitis in PsSpA, given that the prevalence of \textit{HLA-B27} variants differed in subsets of PsSpA with and without these radiographic features. Taken together, these data indicate that PsSpA is on a spectrum of musculoskeletal disease, starting at pPsA, then PsSpA, then AS; with PsSpA being a continuum itself, and with a phenotype expression related to disease duration. Current qualification criteria for anti-TNF use may be unnecessarily disadvantaging PsSpA patients. Our findings could guide an internationally agreed classification system for PsSpA with the purpose of improving clinical care and research.
6.7 PUBLICATIONS ARISING FROM THE WORK OF THIS THESIS
CHAPTER

CONFERENCE ABSTRACT (oral presentation)


CONFERENCE ABSTRACT (poster presentation)

CHAPTER VII: PSORIATIC ARTHRITIS MUTILANS: PREVALENCE, CLINICAL CHARACTERISTICS, AND NATURAL RADIOGRAPHIC HISTORY

7.1 ABSTRACT

Objectives: The objectives of this thesis chapter were to: (i) determine the prevalence of psoriatic arthritis mutilans (PAM) in a cohort of psoriatic arthritis (PsA) cases; (ii) compare the clinical characteristics of PAM and non-PAM cases in a cohort of PsA cases using patient reported outcome measures (PROMs), clinical history and radiographic indices; (iii) determine the natural radiographic history of PAM.

Methods: A retrospective cohort study was conducted of all PsA patients attending a teaching hospital. The most recent hand and feet radiographs were screened for PAM. Serial radiographs (earliest to most recent) were quantitatively scored for osteolysis, erosion, joint space narrowing, and osteoproliferation by two raters using validated scores.

Results: Of 610 PsA cases, 36/610 (5.9%) had PAM. PAM cases were younger at diagnosis of PsA than non-PAM cases (p=0.04), had more prevalent psoriatic nail dystrophy (odds ratio, OR 5.43; 95% CI 2.21, 13.30; p<0.001), and more disability (adjusted incidence risk ratio, IRR_adj 1.26 per 0.35 unit increase in HAQ score; 95% CI 1.07, 1.49; p=0.005). Radiographic axial disease (adjusted odds ratio, OR_adj 2.31; 95% CI 1.07, 4.97; p=0.03), and especially radiographic sacroiliitis (OR_adj 2.99; 95% CI 1.33, 6.73; p=0.01) were more prevalent in PAM cases compared with non-PAM cases. PAM cases were more likely than non-PAM cases to have used a synthetic DMARD (OR 16.36; 95% CI 3.88, 68.96; p<0.001), but not an anti-TNF agent (OR 1.25; 95% CI 0.62, 2.54; p=0.54). 29/33 PAM cases had initiated a synthetic DMARD, and 4/13 had initiated anti-TNF, prior to first demonstration of PAM.

A median five radiographs were scored for each PAM case (IQR 3-7). PAM progressed from monoarticular (60%) to polyarticular (80%) involvement, with certain joints being more frequently affected (big toe IPJ, MTPJ2-5, and hand MCPJ1, DIPJ2, and PIPJ5). Osteolysis was initially rapid and progressive in the hands and feet, tapering later during disease course. Nail dystrophy predicted more severe osteolysis (p=0.03).

Conclusion: Compared to non-PAM cases, PAM cases have earlier age at PsA diagnosis, more disability (HAQ), more prevalent nail dystrophy and radiographic axial disease / sacroiliitis. The rate of osteolysis is higher in earlier disease, and more severe in those with nail dystrophy. DMARDs and anti-TNF appear not to prevent PAM occurrence.
7.2 INTRODUCTION

The content of this chapter of the thesis was published as a peer-reviewed paper in The Journal of Rheumatology in July 2015 (J Rheumatol 2015;42:1169-76) [240]. Therefore there is some repetition in this chapter of the content of that paper. The paper was written by Deepak Jadon (DJ). Dr. William Tillett (WT; Consultant Rheumatologist, Royal National Hospital for Rheumatic Diseases, Bath) contributed to radiographic data collection, and complex longitudinal statistical modeling was performed by Dr. Gavin Shaddick (GS; Reader of Mathematics, Department of Mathematical Sciences, University of Bath, Bath). All co-authors contributed to the study inception, design, and internal peer review of the final draft of the paper written entirely by DJ.

Five patterns of psoriatic arthritis (PsA) were described in 1973 by Moll et al.: asymmetric oligoarthritis, symmetric polyarthritis, distal interphalangeal joint-predominant arthritis, psoriatic spondyloarthritis (PsSpA), and arthritis mutilans [18]. The most extreme, albeit rarest form of PsA is psoriatic arthritis mutilans (PAM).

PAM is a condition characterised by severe destruction of the joint and adjacent bone through erosion and subsequent osteolysis. As a result of soft-tissue laxity and loss of bone stock, fingers and toes can shorten, giving rise to thick transverse folds of skin, and digits that can be elongated with traction. Clinical and radiographic images of PAM were provided in Figures 1.8, 1.9 and 1.10 in Chapter 1 of this thesis. Two such cases were first described in 1913 by Marie et al., who compared the phenomena with the telescopic action of an opera glass (la main en lorgnette) [72]. Earlier in 1888, Bourdillon first reported a resorptive arthropathy associated with psoriasis [241]. Arthritis mutilans can occur in association with several diseases, including psoriatic arthritis (PAM) [18], rheumatoid arthritis [242, 243], chronic reactive arthritis, juvenile chronic arthritis and mixed connective tissue disease [244]. Its pathogenesis is unknown.

Research of PAM has been impeded by the rarity of the subphenotype, and the lack of an agreed clinical or radiographic definition. Estimates of the prevalence of PAM in PsA cohorts range from 0.6 to 21 per 100 PsA patients [14, 18, 73, 74, 245, 246], but are likely to be closer to the 5 per 100 PsA patients as was originally described by Moll and Wright [18] and subsequently by others [14, 73, 74]. A recent population-based study estimated the prevalence of PAM in the adult Nordic population to be 3.69 per million inhabitants, and with similar sex distribution [82]. Studies to date have been cross-sectional, with no longitudinal data. Recent reports are mostly case series [75-77], with a few studies of
between 6 to 24 cases each [73, 74, 78-81], and only one large study of 59 cases [82]. The majority of studies simply note the occurrence of PAM in a general PsA cohort. To our knowledge, the natural clinical and / or radiographic history of PAM has never been described.
7.3 STUDY OBJECTIVES

The objectives of this thesis chapter were to:

(i) determine the prevalence of PAM in a cohort of PsA cases.

(ii) compare the clinical characteristics of PAM and non-PAM cases in a cohort of PsA cases using patient reported outcome measures (PROMs), clinical history and radiographic indices.

(iii) determine the natural radiographic history of PAM.
7.4 METHODS

A retrospective cohort study was conducted of all PsA patients attending the dedicated PsA outpatient clinic at the Royal National Hospital for Rheumatic Diseases (RNHRD) in Bath, and enrolled in the Long-term Outcomes in Psoriatic Arthritis Study 1 (LOPAS1). Patients attending clinic had plain radiographs of hands and feet performed at intervals determined by usual clinical care; and dated from 1974 to 1 December 2013 (census). The case notes and clinical data collection proforma (from the LOPAS1 and Axial Disease in PsA study, ADIPSA) of PsA cases provided clinical data including: sex, date of birth, age at psoriasis symptom onset, age at psoriasis diagnosis, age at PsA arthritis symptom onset, age at PsA diagnosis, smoking history (never, ever, current), alcohol consumption (never, ever, current), nail dystrophy at any time during disease course, most recent Stanford Health Assessment Questionnaire (HAQ) [167], anti-cyclical citrullinated peptide antibody (ACPA) serology, synthetic disease modifying anti-rheumatic drug (DMARD) use (agent, date of initiation, date of cessation), biological therapy use in particular anti-tumour necrosis factor-alpha (anti-TNF) (agent, number of biologicals used, date of initiation, date of cessation), and corticosteroid use (in oral, intramuscular or intravenous form).

7.4.1 Peripheral radiographic scoring methods

As no existing plain radiographic score for PsA captures the various radiographic features of PAM, components of existing radiographic tools that have been validated for use in PsA, were used. Plain radiographs of hands and feet were quantitatively scored for osteolysis (see below), erosion (using the PsA-modified Sharp score; mSS [247, 248]), joint space narrowing (mSS), osteoproliferation (PsA Ratingen Score; PARS [249]), periostitis of the shaft and juxta-articular area (mSS), tuft resorption (mSS), and osteopaenia using the PsA-modified Steinbrocker score [250]. A new domain to score osteolysis was devised to aid the detection of PAM and capture progression; with a range of 0 to 12, and based upon lysis of bone from the articular surface of the epiphysis, through the metaphysis, to the diaphysis (Figure 7.1). With some variability according to the radiographic domain, the following joints were scored in the hands (all small joints of the fingers and thumb, the majority of carpal articulation, radio-carpal joints, and ulnar-carpal joints) and feet (big toe interphalangeal joint, IPJ1; first to fifth metatarsophalangeal joints, MTPJ 1-5). The other toes’ interphalangeal joints were not scored due their poor visibility on radiographs likely to cause unreliable and difficult scoring (see radiographic data collection proforma, Appendix 4).
Figure 7.1. Osteolysis score

(Osteolysis is scored as blocks of bone. Either side of the joint, there are two blocks in the epiphysis, two blocks in the metaphysis, and two blocks in the diaphysis. Score range 0 to 12)

7.4.2 Reading strategy and reliability exercise

Standard digital antero-posterior radiographs of the hands and feet were viewed and scored using the hospital’s Picture Archiving and Communication System (PACS). Historical hard-film plain radiographs, preceding the advent of PACS in our institution (February 2007), were digitised to HiPAX Image Viewer for scoring. All images were read on the same viewing monitor, preserved at the original 1:1 ratio. All radiographs included a ‘phantom phalanx’ as a reference for normal bone mineral density.

The two readers (DJ and WT) underwent pre-study training in the precise definitions of radiographic findings of PsA [251], practice, supervision and discussion with an experienced Consultant musculoskeletal radiologist (Dr Graham Roinson, GR). To determine inter-rater reliability, ten random pairs of hand and feet radiographs were scored by both the readers, in random order.

7.4.3 Identification of PAM cases

In keeping with previous publications on PAM [78, 79, 246], plain radiographic PAM was defined as osteolysis affecting ≥50% of the visualised articular surface on both sides of the joint. A large erosion qualified as osteolysis once the epiphyseal plate was
encroached. PAM was therefore only called once the osteolysis score in an individual joint was $\geq 4$ units.

The most recent plain radiographs of hands and feet were evaluated for PAM (DJ). Group consensus (DJ, WT, GR and Professor Neil McHugh) was sought for uncertain cases. All available radiographs of PAM cases (earliest to most recent) were then randomised and scored by the two readers (DJ, WT). Radiographs were scored in chronological order. An assumption of non-regression of the score was made in advance to allow for variability in radiographic exposure and projection, and facilitate longitudinal statistical modelling.

7.4.4 Axial radiographic scoring methods

All cases had their most recent axial plain radiographs (cervical spine, lumbar spine, and sacroiliac joints, as available) scored for evidence of radiographic axial disease (RAD) by one reader (DJ) following education, practice and reliability testing as described in Chapter 6 of this thesis. In keeping with Chapter 6 of this thesis, radiographic axial disease was defined as: the presence of New York criteria unilateral grade $\geq 3$, or bilateral grade $\geq 2$ sacroiliitis on AP pelvic radiograph; or $\geq 1$ marginal / paramarginal syndesmophyte(s) of the cervical, and / or lumbar spine.

7.4.5 Statistical analysis

Data were analysed using STATA 12.2 (2011 Texas, USA) by DJ, and more complex longitudinal modelling was performed by Dr. Gavin Shaddick (GS) using ‘R’ [252]. Interrater reliability testing for peripheral radiographic scoring was determined using the intra-class correlation coefficient (ICC).

For univariate analyses continuity-corrected Chi-squared tests were used to test for differences between categorical variables, and independent t-tests or Mann-Whitney U-tests used for normally and non-normally distributed continuous variables, respectively.

Logistic regression models were used to compare the prevalence of radiographic axial disease in PAM and non-PAM, adjusted for sex and age at radiographic assessment. As the HAQ data had an excess of zeros and is not a truly continuous variable, univariate analyses were performed using a zero-inflated Poisson regression model, and multivariate analyses were performed using a revere-stepwise (alpha level set at 0.1 for significance) zero-inflated Poisson regression adjusted for sex and age at HAQ assessment. Univariate
and reduced-multivariate models are presented. Median, inter-quartile range (IQR), mean, standard deviation (SD), odds ratios (OR), adjusted OR (OR_{adj}), incidence risk ratio (IRR), adjusted IRR (IRR_{adj}), 95% confidence interval (95% CI), and p-value were calculated as appropriate to the data. The alpha-level for statistical significance was set at 0.05.

Patterns and rates of deterioration of individual joints over time were examined for all patients by Dr. Gavin Shaddick (GS) using the following method (written by GS): Initial analysis consisted of plotting the data together with locally-weighted polynomial regression smoothers (lowess) [253]. Formal modelling of the changes in scores over time was performed using generalised additive mixed models (gamm) [254], using penalised splines to assess the complexity of the model required for each joint and allowing a random effect for each patient. For each joint, this complexity was expressed as the effective degrees of freedom (EDF) required to model the changes over time. For this part of the study GS undertook the analyses and DJ was involved in providing a clinical interpretation of the results.

7.4.6 Ethical considerations

Ethical approval for the study was given by the Local Regional Ethics Committee and informed written consent obtained from participants as part of the on-going prospective LOPAS1 study (see Appendix 5).
7.5 RESULTS

All 610 PsA cases fulfilling Classification Criteria for Psoriatic Arthritis (CASPAR) [24] in the LOPAS1 study [255] were screened for the presence or absence of plain radiographic PAM. PAM was present in 36/610 (5.9%) PsA cases, with 35/36 having serial radiographs. 483/610 had no evidence of PAM. 91/610 either had no radiographs of hands and feet, or only one of the two sites imaged, and therefore although unlikely, PAM could not be excluded.

7.5.1 Inter-rater reliability of plain radiographic scoring

The inter-rater reliability of peripheral plain radiographic scoring was very high: intra-class correlation coefficient, ICC 0.99 (95% CI 0.98, 1.00) for osteolysis; ICC 0.95 (95% CI 0.91, 0.99) for erosion; ICC 0.97 (95% CI 0.95, 0.99) for osteoproliferation; and ICC 0.90 (95% CI 0.84, 0.93) for osteopaenia. The reliability of radiographic scoring between the two raters (DJ and WT) was therefore very high.

7.5.2 Clinical characteristics of PAM and non-PAM cases

The proportion of females in the PAM (19/36; 52.78%) and non-PAM (228/483; 47.20%) groups was statistically no different (odds ratio, OR 1.25; 95% CI 0.63, 2.46; p=0.52). PAM cases were significantly younger at PsA diagnosis than non-PAM cases (median age 33.00 vs. 40.00 years; p=0.04), but no different in terms of age at PsA arthritis symptom onset, psoriasis symptom onset, psoriasis diagnosis, or age at census (Table 7.1).

Psoriatic nail dystrophy at anytime during disease course was significantly more common in PAM cases than non-PAM cases (OR 5.43; 95% CI 2.21, 13.30; p<0.001) (Table 7.2). For the patients in whom ACPA serology was available, no difference was demonstrated in ACPA-positivity in PAM (0/16) compared with non-PAM (8/226) cases (p=0.44) (Table 7.2).
Table 7.1. Clinical characteristics of PAM and non-PAM cases (numerical variables)

<table>
<thead>
<tr>
<th>Clinical parameter</th>
<th>PAM (n=36)</th>
<th>Non-PAM (n=483)</th>
<th>Unadjusted</th>
<th>Adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>IQR</td>
<td>Median</td>
<td>IQR</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at Psoriasis symptom onset</td>
<td>29.00</td>
<td>16.00, 45.00</td>
<td>28.00</td>
<td>18.00, 42.00</td>
</tr>
<tr>
<td>Psoriasis diagnosis</td>
<td>30.00</td>
<td>16.00, 52.00</td>
<td>33.00</td>
<td>21.00, 47.00</td>
</tr>
<tr>
<td>PsA symptom onset</td>
<td>32.00</td>
<td>23.00, 45.00</td>
<td>38.00</td>
<td>28.00, 49.00</td>
</tr>
<tr>
<td>PsA diagnosis</td>
<td>33.00</td>
<td>24.00, 45.00</td>
<td>40.00</td>
<td>30.00, 51.00</td>
</tr>
<tr>
<td>Census</td>
<td>67.37</td>
<td>51.56, 73.53</td>
<td>59.05</td>
<td>48.54, 67.73</td>
</tr>
<tr>
<td>HAQ Total score</td>
<td>1.25</td>
<td>0.38, 2.13</td>
<td>0.63</td>
<td>0.13, 1.38</td>
</tr>
<tr>
<td>HAQ-dressing</td>
<td>1</td>
<td>0.2</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>HAQ-rising</td>
<td>1</td>
<td>0.2</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td>HAQ-eating</td>
<td>1</td>
<td>0.2</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td>HAQ-walking</td>
<td>1</td>
<td>0.2</td>
<td>0</td>
<td>0.2</td>
</tr>
<tr>
<td>HAQ-hygiene</td>
<td>2</td>
<td>0.3</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>HAQ-reach</td>
<td>2</td>
<td>1.3</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>HAQ-grip</td>
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<td>0.2</td>
<td>0</td>
<td>0.2</td>
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<tr>
<td>HAQ-activity</td>
<td>1</td>
<td>0.2</td>
<td>1</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Radiographic events

| Number of films scored per case     | 5.00       | 3.00, 7.00    | -          | -        | -      | -      | -       | -      | -      | -       |
| Interval: earliest to most recent   | 10.87      | 5.48, 16.51   | -          | -        | -      | -      | -       | -      | -      | -       |
| film (years)                        |            |               |            |          |        |        |         |        |        |         |

Age at (years)

| Earliest film                       | 47.37      | 37.46, 61.71  | -          | -        | -      | -      | -       | -      | -      | -       |
| Recent film                         | 64.54      | 48.71, 70.37  | -          | -        | -      | -      | -       | -      | -      | -       |
| Census                              | 67.37      | 51.56, 73.53  | -          | -        | -      | -      | -       | -      | -      | -       |

PAM first demonstrated (restricted to cases with PAM-onset during follow-up)

| Age (years)                         | 49.70      | 42.92, 58.21  | -          | -        | -      | -      | -       | -      | -      | -       |
| Years after diagnosis of PsA        | 12.50      | 6.00, 18.00   | -          | -        | -      | -      | -       | -      | -      | -       |

PAM: psoriatic arthritis mutilans
PsA: psoriatic arthritis
HAQ: Health assessment questionnaire
IQR: inter-quartile range
IRR: incidence risk ratio per 1 unit increase in HAQ-subscore
* Zero-inflated Poisson regression model
** Zero-inflated Poisson regression model (adjusted for: sex and age at HAQ)
<table>
<thead>
<tr>
<th>Clinical Parameter</th>
<th>PAM (n=36)</th>
<th>Non-PAM (n=483)</th>
<th>Unadjusted</th>
<th>Adjusted *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>OR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Female sex</td>
<td>19/36</td>
<td>52.78</td>
<td>228/483</td>
<td>47.20</td>
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<tr>
<td>Nail dystrophy</td>
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<td>83.33</td>
<td>211/440</td>
<td>47.95</td>
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<td>0.00</td>
<td>8/226</td>
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<td></td>
</tr>
<tr>
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<td>16/29</td>
<td>55.17</td>
<td>129/366</td>
<td>35.25</td>
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<td>Cervical-spondylitis</td>
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<td>33.33</td>
<td>93/360</td>
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<td>22.22</td>
<td>57/352</td>
<td>16.19</td>
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<td>9/11</td>
<td>81.82</td>
<td>56/69</td>
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</tr>
<tr>
<td>Grade</td>
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</tr>
<tr>
<td>2</td>
<td>0/11</td>
<td>0.00</td>
<td>12/69</td>
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</tr>
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<td>36.37</td>
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<td>Yes</td>
<td>33/36</td>
<td>91.67</td>
<td>236/470</td>
<td>50.21</td>
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<tr>
<td>Methotrexate</td>
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<td>189/470</td>
<td>40.21</td>
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<td>Sulfasalazine</td>
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<td>5.11</td>
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<td>DMARD initiated prior to PAM onset</td>
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<tr>
<td>Yes</td>
<td>29/33</td>
<td>87.88</td>
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<td>-</td>
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<tr>
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<td>4/33</td>
<td>12.12</td>
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<td>Never used DMARD</td>
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<td>Yes</td>
<td>4/13</td>
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<td>23.08</td>
<td>-</td>
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* logistic regression model adjusted for sex & age at most recent pelvic radiograph
PAM: psoriatic arthritis mutilans
ACPA: anti-cyclic citrullinated peptide antibody
DMARD: synthetic disease modifying anti-rheumatic drug
Anti-TNF: anti-tumour necrosis factor
OR: odds ratio
95% CI: 95% confidence interval

Table 7.2. Clinical characteristics of PAM and non-PAM cases (categorical variables)
Physical function, as measured by the HAQ total score was significantly more impaired in PAM (median HAQ 1.25; IQR 0.38, 2.13) cases compared with non-PAM (median HAQ 0.63; IQR 0.13, 1.38) cases using a zero-inflated Poisson regression model adjusted for sex and age at HAQ assessment (adjusted incidence risk ratio, $\text{IRR}_{\text{adj}}$ 1.26 per 0.35 unit increase in HAQ score; 95% CI 1.07, 1.49; p=0.005) (Table 7.1). Differences in sub-domains of the HAQ between the PAM and non-PAM cases were clinically significant, and approached statistical significance for the HAQ-rising domain ($\text{IRR}_{\text{adj}}$ 1.62 per 1 unit increase in subscore; 95% CI 0.98, 2.66; p=0.06), HAQ-reach domain ($\text{IRR}_{\text{adj}}$ 1.34 per 1 unit increase in subscore; 95% CI 1.00, 1.79; p=0.05), and the HAQ-grip domain ($\text{IRR}_{\text{adj}}$ 1.44 per 1 unit increase in subscore; 95% CI 0.99, 2.08; p=0.06) (Table 7.1). Other subdomains of the HAQ were statistically no different in PAM compared with non-PAM cases.

PAM cases were significantly more likely to have radiographic axial disease than non-PAM cases ($\text{OR}_{\text{adj}}$ 2.31; 95% CI 1.07, 4.97; p=0.03) (Table 7.2). PAM cases were significantly more likely to have radiographic sacroiliitis than non-PAM cases ($\text{OR}_{\text{adj}}$ 2.99; 95% CI 1.33, 6.73; p=0.01). When sacroiliitis was present in PAM cases it tended to be bilateral (9/11; 81.82%) and of grade ≥3 (11/11; 100%) (Table 7.2). PAM cases were no more likely to have radiographic spondylitis than non-PAM cases ($\text{OR}_{\text{adj}}$ 1.46; 95% CI 0.65, 3.28; p=0.36). When spondylitis was present in PAM cases it affected both the cervical (6/27; 22.22%) and lumbar spine (5/24; 20.83%), no differently in frequency to that seen in non-PAM cases (Table 7.2).

During the course of their disease, PAM cases were significantly more likely than non-PAM cases to have used a synthetic DMARD (OR 16.36; 95% CI 3.88, 68.96; p<0.001); including methotrexate (OR 5.20; 95% CI 2.32, 11.66; p<0.001), sulfasalazine (OR 4.71; 95% CI 2.28, 9.73; p<0.001) and leflunomide (OR 6.94; 95% CI 2.98, 16.18; p<0.001) (Table 7.2). Of particular note, 29/33 (87.88%) of PAM cases had initiated a DMARD before PAM was first observed radiographically (a median of six years before), implying that DMARDs do not prevent the onset of PAM. Corticosteroids (in oral, intramuscular or intravenous form) had been used during disease course in 15/36 (41.67%) PAM cases.

PAM cases were no more likely than non-PAM cases to have used an anti-TNF agent (OR 1.25; 95% CI 0.62, 2.54; p=0.53) (Table 7.2). In 4/13 PAM cases, anti-TNF had been initiated before the onset of radiographic PAM (5.67, 3.89, 3.72, and 1.08 years beforehand). In 6/13 PAM cases anti-TNF had been initiated after radiographic PAM onset, and 3/13 cases already had PAM at their earliest radiograph.
7.5.3 Radiographic characteristics of PAM cases

The median number of films (pairs of hands and feet) scored per patient was 5 (IQR 3, 7) (Table 7.1). The median interval from baseline to most recent film (i.e. duration of radiographic follow-up) was 10.87 (IQR 5.48, 16.51) years. The median age of PAM cases at the earliest film was 47.37 (IQR 37.45, 61.71) years, and at the most recent film was 64.54 (IQR 49.71, 70.37) years (Table 7.1).

Whilst radiographic PAM was evident in the earliest film in 13/35 (37.14%) cases, the majority of cases (22/35; 62.86%) developed it during the course of their follow-up (Table 7.3). In the 22 cases where PAM developed during the course of follow-up, the time from diagnosis of PsA to onset of radiographic PAM was a median of 12.50 (IQR 6.00, 18.00) years, at a median age of 49.70 (IQR 42.92, 68.21) years (Table 7.1).

PAM was most commonly monoarticular (21/35; 60%) when first demonstrated, but progressed to being polyarticular at the most recent film (28/35; 80%) (Table 7.3). At the most recent film, the most frequently affected joint was the big toe interphalangeal joint (IPJ1) (8.81%), followed in equal frequency (6.92%) by the thumb metacarpophalangeal joint (MCPJ1), index finger distal interphalangeal joint (DIPJ2), little finger proximal interphalangeal joint (PIPJ5), and feet metatarsophalangeal joints 2 to 5 (MTPJ2-5) (Figure 7.2).
Figure 7.2. Eight joints most commonly affected by PAM at the most recent radiography (frequency in white box)

Of note, 12/35 (34.29%) PAM cases had concurrent evidence of joint osteolysis and ankylosis within the same hand or foot (Table 7.3). Two of 35 PAM cases had proceeded to having surgery on the joint affected by PAM; due to impaired hand function in one case, and pain in the other case (Table 7.3).

Table 7.3. Radiographic characteristics of PAM cases with serial radiographs

<table>
<thead>
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<th>%</th>
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<tbody>
<tr>
<td>PAM present on earliest radiograph</td>
<td>13/35</td>
<td>37.14</td>
</tr>
<tr>
<td>PAM pattern on earliest radiograph</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mono-articular</td>
<td>21/35</td>
<td>60.00</td>
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<tr>
<td>Polyarticular</td>
<td>14/35</td>
<td>40.00</td>
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<td>PAM pattern on most recent radiograph</td>
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<tr>
<td>Mono-articular</td>
<td>7/35</td>
<td>20.00</td>
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<tr>
<td>Polyarticular</td>
<td>28/35</td>
<td>80.00</td>
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<tr>
<td>Concurrent joint ankylosis in hand / foot</td>
<td>12/35</td>
<td>34.29</td>
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<tr>
<td>Surgery to joint affected by PAM</td>
<td>2/35</td>
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PAM: Psoriatic arthritis mutilans

7.5.4 Radiographic progression of PAM

Serial radiographs were available for scoring in 35/36 PAM cases. For hands and feet combined, at the most recent film the median osteolysis score was 2.00 (IQR 0.00, 15.00) units, erosion score 9.00 (IQR 0.00, 26.00) units, joint space narrowing score 14.00 (IQR
0.00, 31.00) units, osteoproliferation score 3.00 (IQR 0.00, 6.00) units, and osteopaenia score 0.00 (IQR 0.00, 2.00) units.

A significant change in osteolysis score over time was observed for all joints (p<0.008). After allowing for patient differences in a random effects model, there was a strong indication that different joints had different patterns of deterioration over time (p<0.001).

Figure 7.3. shows curves from the generalised additive mixed model, including data from all 159 joints affected by PAM. The best fit regression curve, 95% confidence interval bands, and the effective degrees of freedom (EDF) for each curve are shown for the hands and feet joints separately. In the feet, there is an initial high rate of osteolysis, followed by a tapering rate, and eventually little further progression of osteolysis (EDF=3.1). However, in the hands the curve is more complex (EDF=4.3) with an initially high increase in osteolysis, followed by a tapering in the rate. For some patients there was indication of a second surge of osteolysis in some joints later during disease course, adjusted for the time since diagnosis.

**Figure 7.3. Rate of osteolysis progression over time**

[additive mixed model of all joints: smoothed regression line (black), 95% confidence interval (grey bands) and effective degrees of freedom (EDF)]

PAM cases with a history of psoriatic nail dystrophy (30/36) had significantly higher overall osteolysis scores in topographically related joints (DIPJ / PIPJ / IPJ) of the hands, than PAM cases without nail dystrophy (6/36) (mean scores: 3.0 with nail dystrophy, 1.3 without nail dystrophy; p=0.03)
7.6 DISCUSSION & CONCLUSIONS

This study takes advantage of a large well-characterised cohort of PsA patients followed longitudinally in a single centre to estimate the prevalence of PAM in a PsA cohort, compare the clinical characteristics of PAM and non-PAM cases, and to investigate the natural radiographic history of PAM. To our knowledge, this is the largest sample of PAM cases with a detailed description of plain radiographic characteristics, and the first study to report on radiographic progression.

A previous study by our group showed that nail dystrophy is more common in PsA patients with DIP joint disease, and is significantly associated with adjacent DIP joint disease (33). Here we demonstrate that psoriatic nail dystrophy is a clinical biomarker of both PAM occurrence, and of having more severe osteolysis in topographically adjacent joints (hand DIP, PIP and IP joints). This has not previously been reported. Furthermore, PAM was most commonly seen in the weight-bearing joints of the feet (MTPJ 2-5, IPJ1) and hand joints involved in power / precision grip (DIPJ2, MCPJ1). Little is known about the pathogenesis or trigger for PAM. The concept of altered biomechanical loads or stress on the synovial-entheseal complex being involved in the pathogenesis of SpA, as proposed by McGonagle et al. [195, 206], is supported by our current findings in PAM.

There has been debate as to whether involvement of a single joint is sufficient for a case to be defined as PAM [256]. Our data indicates that 7/35 (20%) have monoarticular PAM, even after several years of follow-up. Review of these seven cases in our outpatient clinic demonstrated clinical evidence of PAM, with shortened telescopic digits. We therefore propose that PAM can be monoarticular, although it is more frequently polyarticular and the notion is supported by data from other studies [18, 75, 80, 82].

We found that disability as measured by the HAQ, is worse in PAM compared with non-PAM cases. This seems reasonable given the damage and deformity that PAM inflicts on the hands and feet. Closer inspection of the data shows that disability was often in HAQ subdomains pertaining to hand and / or foot function, such as HAQ-grip, HAQ-reach and HAQ-rising. No other studies have investigated disability in PAM compared with non-PAM patients.

Several of our results corroborate those of the Classification of Psoriatic Arthritis (CASPAR) dataset [80], which showed that PAM cases are usually polyarticular, of long disease duration, and are more prone to spinal involvement compared to their non-PAM counterparts. In our cohort there was a higher prevalence of sacroiliitis, that tended to be
more severe than in non-PAM cases, and some increase in other features of spondylitis. However, we did not replicate the CASPAR findings of more frequent ACPA-positivity (albeit of low titre) in PAM compared with non-PAM cases. Joint surgery rates in our cohort of PAM cases were far lower than the 48% reported in that study.

A further intriguing issue is the nature of the relationship between osteolysis and ankylosis. In our dataset, 12/35 (34.29%) cases had concurrent osteolysis and ankylosis in the same hand or foot. Occasionally, osteolysis progressed to joint ankylosis in the same joint, but more commonly we saw joint space narrowing progressing to ankylosis, suggesting that there are two separate pathological processes, although both may occur in the same patient. Axial osteoproliferation causing cervical myelopathy in PAM despite peripheral bone erosion has been described [257].

None of our PAM cases had improvement of osteolysis, erosion or osteoproliferation scores. Joint space narrowing, osteopaenia and periostitis scores fluctuated, with both regression and progression. There are emerging reports in the literature of improvement in erosion [258, 259] and joint-space narrowing [259] scores following anti-TNF use, implying filling-in or ‘healing’ of previous structural damage. However, a paper reporting the follow-up of three PAM cases treated with etanercept, demonstrated no improvement in deformities resulting from several years of progressive disease [76]. Furthermore, our data suggests that neither synthetic DMARDs nor anti-TNF prevent the onset of radiographic PAM. Our findings need to be interpreted with caution in a retrospective study where the radiographs were not taken at fixed time intervals, hence sensitivity to detect PAM onset is reduced, and the doses of some agents such as methotrexate were lower than those used nowadays.

The sequence of pathological events leading to the frank osteolysis of PAM is of much interest, particularly as it could prompt the clinician to instigate more intensive disease modifying therapy. Magnetic resonance imaging has demonstrated higher bone proliferation and oedema scores in PAM compared with non-PAM cases, and has therefore been proposed as a potential radiological biomarker of progressing to PAM [78]. In our study we did not see osteopaenia or periostitis preceding the onset of erosions or osteolysis. Nor did we overtly see progressive erosions prior to the onset of osteolysis. However, we acknowledge that the varied time interval between radiographs may have reduced our sensitivity to track such changes. In our general observations we noted progressive joint space narrowing prior to the onset of osteolysis.
7.6.1 Study critique

This study has particular strengths. This is the largest sample of PAM cases with a detailed description of plain radiographic characteristics, and is unique in being the first study to report on radiographic progression. Our large sample size for PsA cases, and relatively large sample size for PAM given its rarity, has increased the statistical power of our analyses to detect a difference between the PAM and non-PAM cases. Several of our analyses were adjusted for potential confounders, including sex and age at clinical / radiographic assessment. The findings of our study are generalisable to PsA cohorts in other centres in the UK, since the RNHRD takes unselected secondary-care referrals from the local community, spanning the spectrum of mild to severe PsA.

We acknowledge that our study has limitations. Whilst it appears that 36/519 (6.94%; 95% CI 4.91, 9.47 using Fisher-exact Clopper-Pearson) of our cohort have PAM, we are aware that 91/610 PsA cases had insufficient or no radiographs to assess the presence or absence of PAM. The lack of radiographs may imply minimal disease activity at these sites, or may be due to non-clinical factors. Assuming that all 91 were non-PAM cases, the prevalence of PAM in our cohort falls to 36/610 (5.90%; 95% CI 4.17, 8.08 using Fisher-exact Clopper-Pearson). However, both estimates and their 95% confidence interval values are within the range of prevalence described by other authors [14, 18, 73, 74, 245, 246], especially those using a radiographic rather than clinical definition of PAM.

Since the interval between films was determined by clinical need, rather than predefined intervals, we were unable to determine the incidence of PAM onset. Since PAM was evident in the earliest available radiograph in 13/35 (37.14%) of cases, the initial rate of radiographic progression could not be determined in this subgroup of patients. To overcome this issue one would need to conduct a prospective inception cohort study with radiographs taken on all patients at protocolised regular time intervals. This would be difficult given the rarity of PAM, late-age at onset of PAM (median in this cohort of 49.70; IQR 42.92, 68.21 years), and natural radiographic history as demonstrated in this study characterised by much osteolytic damage happening in the first few years after onset. A very large inception cohort, followed over 20-40 years, and with longitudinal radiographs taken every 1-2 years would be required. This would be an enormous logistical, financial and ethical challenge.

We did not adjust for anti-TNF use and synthetic DMARD use as the proportion of patients using these agents was too small to include in the regression models. In addition, we did not have detailed longitudinal data on medication use, and there is no evidence that these
agents either prevent PAM or alter its radiographic progression. We report in Table 7.2 the proportion of PsA cases who have ever used corticosteroids. These data have their limitations, in particular the varied routes of administration with differing efficacy, the variety of doses commonly used in clinical practice, and the significant potential for incomplete data give that some corticosteroids are administered in primary care and never documented in the hospital case-notes. We had dichotomous smoking data on all of the PAM cases, but data was incomplete on a third of non-PAM cases. Furthermore, as discussed previously in Chapters 5 and 7 of this thesis, as smoking is a time-varying variable, we elected not to report the smoking data or include it in the multivariate models.

This study has potential unmeasured confounders, such as disease duration. As there was a significant proportion of incomplete data on disease duration (from diagnosis to census) in this cohort, we elected not to report or include it as a covariate in the regression models. More complete disease duration data would certainly have strengthened this study, especially when comparing PAM and non-PAM cases in terms of HAQ and axial radiographic disease, both of which are known to be dependent on disease duration. We attempted to adjust for this by including age at clinical / radiographic assessment in the regression models for HAQ and axial radiographic disease, respectively. The magnitude and direction of potential confounders should be similar in PAM and non-PAM cases, therefore have less impact on comparisons between the two groups.

As will be discussed in chapter 9, the chronology of pathological events leading to osteolysis in PAM has never been described. The radiographic scoring conducted in this study included domains for osteolysis, erosion, joint space narrowing, osteoproliferation, periostitis, osteopaenia and tuft resorption. This thesis chapter only reports the natural history of PAM based on change in osteolysis score. Within the constraints of this study design and data available, the type of pathological changes that occur before the onset of osteolysis in a particular joint will be investigated. Anecdotal observations during radiograph scoring is of progressive joint space narrowing before onset of osteolysis, rather than progressive erosions or osteopaenia. However, after the onset of osteolysis, bone remodelling appeared to occur in the form of osteoproliferation, perhaps as a compensatory mechanism to correct abnormal joint loading and movement. Periostitis seemed very rare in the PAM cases.
7.6.2 Conclusions

To conclude, PAM is a rare but severely destructive subphenotype of PsA. Further research is needed to investigate the pathogenesis of PAM, serum-soluble and/or genetic biomarkers of PAM, and whether anti-TNF or anti-resorptive agents may offer therapeutic efficacy.
7.7 PUBLICATIONS ARISING FROM THE WORK OF THIS THESIS

CHAPTER

PEER-REVIEWED PAPERS


CONFERENCE ABSTRACT (oral presentation)


CONFERENCE ABSTRACT (poster presentation)

8 CHAPTER VIII: SERUM-SOLUBLE BONE-TURNOVER BIOMARKERS IN PSORIATIC DISEASE

8.1 ABSTRACT

**Background:** Psoriatic spondyloarthritis (PsSpA), peripheral-only psoriatic arthritis (pPsA) and ankylosing spondylitis (AS) are characterised by differing patterns and degrees of pathological new bone formation (osteoproliferation) and bone loss (resorption), which may be reflected by differing levels of serum-soluble bone-turnover biomarkers. Our study objectives were to investigate four potential serum-soluble bone-turnover biomarkers (Dkk-1, M-CSF, MMP-3 and OPG) as predictors of: (i) PsSpA occurrence, by comparison with pPsA, AS, psoriasis-only (PsC) cases and healthy controls (HC); (ii) axial radiographic disease severity / morphology in PsSpA and AS patients; (iii) psoriatic arthritis mutilans (PAM) occurrence in a cohort of PsA patients.

**Methods:** A prospective cross-sectional study was conducted recruiting spondyloarthritis (SpA; pPsA, PsSpA and AS) patients from a single-centre, followed by clinical and radiographic assessment, and peripheral blood sampling. Serum samples were obtained from two other centres of clinically characterised PsC patients, and sex / age-matched HC subjects. Serum samples were tested with commercially available ELISA kits to determine concentrations of Dkk-1, M-CSF, MMP-3 and OPG. Statistical comparisons were made between serum concentrations and disease phenotypes.

**Results:** OPG appears to be a biomarker of axial disease in patients with SpA; concentrations were significantly lower in SpA patients with axial disease than in those without (adjusted odds ratio, OR$_{\text{adj}}$ 0.20 per ng/ml increase in concentration; 95% CI 0.05, 0.80; p=0.02), independently of having psoriasis.

Dkk-1 appears to be a biomarker of axial disease in patients with SpA, with a pattern for increasing concentration along a spectrum of increasing axial involvement. Dkk-1 concentrations were significantly higher in SpA patients with axial disease than in those without (OR$_{\text{adj}}$ 1.22; 95% CI 1.05, 1.42; p=0.01), independently of having psoriasis, and significantly lower in PsSpA compared with AS (OR$_{\text{adj}}$ 0.85; 95% CI 0.74, 0.98; p=0.02).

M-CSF appears to be a biomarker of arthritis. Compared with HC, M-CSF concentrations were significantly lower in pPsA (OR$_{\text{adj}}$ 0.14; 95% CI 0.06, 0.32; p<1x10$^{-5}$), PsSpA (OR$_{\text{adj}}$ 0.07; 95% CI 0.03, 0.17; p<1x10$^{-5}$), and AS (OR$_{\text{adj}}$ 0.37; 95% CI 0.16, 0.85; p<1x10$^{-7}$). Similarly, M-CSF concentrations were significantly lower in PsA compared with
PsC (OR_{adj} 0.44; 95% CI 0.24, 0.82; p=0.01) patients. However, M-CSF concentrations didn’t differentiate various forms of SpA, i.e. pPsA, PsSpA and AS.

MMP-3 appears to be a biomarker of arthritis. Compared with HC, MMP-3 concentrations were significantly higher in pPsA (OR_{adj} 1.06; 95% CI 1.01, 1.10; p=0.02), PsSpA (OR_{adj} 1.06; 95% CI 1.01, 1.11; p=0.02), and AS (OR_{adj} 1.06; 95% CI 1.01, 1.11; p=0.01). Similarly, MMP-3 concentrations were significantly higher in PsA compared with PsC (OR_{adj} 1.02; 95% CI 1.01, 1.03; p=0.0004) patients. However, MMP-3 concentrations didn’t differentiate various forms of arthritis, i.e. pPsA, PsSpA and AS.

Only OPG significantly and positively correlated with radiographic severity (adjusted p=0.03) and osteoproliferation burden (adjusted p=0.01), as measured by the Psoriatic Arthritis Spondylitis Radiology Index (PASRI); although only in PsSpA cases.

Concentrations of each of the four biomarkers were no different in PsA cases with and without PAM.

**Conclusion:** OPG and Dkk-1 appear to be biomarkers of axial disease in patients with SpA (pPsA, PsSpA and AS), independently of cutaneous psoriasis status. Both M-CSF and MMP-3 appear to be biomarkers of arthritis, although don’t differentiate various forms of SpA. Only OPG has shown signals to be a biomarker of radiographic severity / morphology, but the clinical significance of this remains be determined. No biomarkers for the occurrence of PAM in PsA cases were found. The high concentration of Dkk-1 in AS and PsSpA compared with HC, supports previous reports that Dkk-1 may be dysfunctional in SpA. Since bone mineral density was not measured, it is difficult to determine if these serum biomarkers are reflecting SpA-related pathology in trabecular bone, or vertebral corners and sacroiliac joints.
Psoriatic arthritis (PsA) and ankylosing spondylitis (AS) are chronic inflammatory conditions of the musculoskeletal system belonging to the family of spondyloarthritis (SpA). They are characterised by: bone loss (resorption) in the form of erosion and bone mineral density (BMD) loss; and new bone formation (osteoproliferation) in the form of sacroiliac joint (SIJ) ankylosis (bony bridging across the joint), syndesmophytes, and periostitis (inflammation of the bone cortex). AS is the prototypical SpA, and its pathogenesis has been studied in far more detail than either PsA or psoriatic spondyloarthritis (PsSpA). It is likely however, that AS and PsSpA share common pathogenic processes. In AS the primary site of inflammation is the enthesis and subchondral bone marrow, with bone marrow oedema, lymphocytic infiltrates, increased osteoclast density, and increased microvessel density being typical findings during acute inflammation [260]. In AS, joint fusion is thought to be the result of increased endochondral cartilage formation followed by bone formation (ossification), which allows bone formation and bridging of the joint space [261, 262]. After an initial phase of bone resorption, to counteract the now abnormal load bearing of the deformed joint, joints are thought to ‘respond’ through a marked anabolic remodelling of bony architecture and form syndesmophytes, which are bony appositions originating from the juxta-articular periosteal lining [263]. These bone appositions are formed via endochondral ossification, and take the form of bridging / non-bridging syndesmophytes and / or SIJ ankylosis. AS patients also suffer bone loss, especially in trabecular bone, resulting in osteoporosis and fractures. The prevalence of osteoporosis in AS is estimated between 19 - 62 per 100 AS cases, and vertebral fractures at 9 - 42 per 100 AS cases [264-266]. The risk of osteoporosis is also higher in PsA patients compared with the general population [267, 268]. Markers of bone loss and formation may therefore vary with disease phenotype, pathological phase, disease activity and hold prognostic value. The paradox in SpA is that these diametrically opposite pathologial processes of bone resorption and formation occur concurrently. The interpretation of circulating serum bone-biomarkers can therefore be challenging.

The systematic review of bone and cartilage-turnover markers in PsA and PsSpA detailed in Chapter 4, identified four bone-turnover markers to be of interest in PsA, although data were limited. These four candidate biomarkers will therefore be investigated further in this chapter of the thesis, using the cohorts of PsSpA, pPsA and AS identified and characterised in Chapters 5 and 6 of this thesis. Three biomarkers promote bone resorption: macrophage colony stimulating factor (M-CSF), dickkopf 1 (Dkk-1), and matrix metalloproteinase 3 (MMP-3). Osteoprotegerin (OPG) promotes new bone formation by
inhibiting bone resorption. A more detailed description of the four biomarkers is given in turn below, and the key pathways and effects of these biomarkers are summarised in Figure 8.1.
Figure 8.1. Metabolic bone pathways involving Dkk-1, OPG, M-CSF and MMP-3

[original figure, compiled from various sources. Green cross indicates induction effect along the pathway. Red dash indicates inhibition effect along the pathway]
8.2.1 Dickkopf 1 (Dkk-1)

The dickkopf (Dkk) gene family encodes secreted proteins and consists of four main members in vertebrates (Dkk-1, -2, -3, and -4). Human dickkopf related protein 1 (Dkk-1) is a 40 kDa glycosylated protein [269, 270]. Dkk proteins contain two conserved cysteine-rich domains separated by a linker region [269, 270]. The C-terminal domain contains a colipase fold with a conserved pattern of ten cysteine residues, and is necessary and sufficient for Wnt inhibition [271]. A distant Dkk family member is soggy (sgy) and is also known as Dickkopf-like protein 1 (DkkL1). The hallmark of the Dkk-1, Dkk-2 and Dkk-4 family is their ability to modulate Wnt signaling. Mostly their effect is inhibitory, but there is evidence that Dkk-2 can also activate Wnt signaling [269, 270]. Dkk-1 is primarily an antagonist of the Wnt signaling pathway (Figure 8.1), and was discovered by its ability to block Wnt signaling during early Xenopus (aquatic frogs native to sub-Saharan Africa) embryogenesis, which is required for head induction [272], and later in many other cell types and vertebrate species [270]. Dkk-3 and sgy are divergent members of the Dkk family, which do not seem to function in Wnt signaling [270].

The Wnt family of growth factors are essential in numerous processes during embryological development, and in physiological and pathological processes [273]. In the normal state Wnt proteins bind to a co-receptor complex, which comprises low-density lipoprotein receptor-related protein 5 (LRP5) or LRP6, and a member of the Frizzled (Fz) family of proteins. This leads to an increase in intracellular beta-catenin levels through inhibition of the beta-catenin degradation complex. Beta-catenin triggers a series of events that lead to osteoblastogenesis, and ultimately new bone being formed and laid down (accretion) (Figure 8.1). Wnts can be inhibited and induced by several effectors acting intracellularly, e.g. modulating components of the signal transduction machinery, or extracellularly, e.g. modulating ligand receptor interactions [270]. Five families of extracellular Wnt antagonists have been identified and include: the Dkk family of secreted proteins, the secreted frizzled-related protein (sFRP), Wnt inhibitory factor 1 (Wif1), Wise, and Xenopus Cerberus [270]. Wnts trigger three pathways that employ Wnt receptors of the frizzled seven transmembrane class: Wnt/b-catenin pathway (canonical pathway); the planar cell polarity pathway (PCP), which does not involve b-catenin but recruits small GTPases of the rho/cdc42 family to activate Jun kinase; and the Wnt/Ca2+ cascade which may overlap with the PCP pathway [270]. Dkks specifically affect the Wnt / beta-catenin cascade, by binding and modulating Wnt co-receptors LRP5 and LRP6, which are indispensable for routing the Wnt signal to the b-catenin pathway. Wnt engages a receptor complex composed of the Fz proteins, LRP5 and LRP6 [274]. Dkk-1 antagonises Wnt by forming ternary complexes of LRP5/6 with Kremen1 (Krm1) or Kremen2 (Krm2) [274].
Internalisation of the Dkk-1/LRP6/Krm2 complex down-regulates Wnt signaling [274]. Krm1 and Krm 2 greatly potentiate the ability of Dkk-1 to block Wnt signaling. Krm2 forms a ternary complex with Dkk-1 and LRP6, and induces rapid endocytosis and removal of LRP6 from the plasma membrane, thereby blocking beta-catenin signaling [270]. While Dkk-1 acts as a pure inhibitor of Wnt / beta-catenin signaling, Dkk-2 can either activate or inhibit the pathway depending on its host cell and environment [270]. Dkk-1 and Dkk-2 may also have beta-catenin-independent functions [270]. These pathways are shown in Figure 8.2.

![Figure 8.2. Model of Dkk-1 interaction with the Wnt/beta-catenin pathway.](image)

[a] Wnt forms ternary complexes with the Frizzled (Fz) and low-density lipoprotein receptor-related protein 5 and 6 (LRP5 and LRP6), which promotes stabilisation of beta-catenin, thereby activating the pathway. [b, c] Dkk-1 binding to LRP6 blocks signal transduction preventing Wnt-Fz binding [b] and/or LRP6 endocytosis in the presence of the Dkk-1 co-receptor Kremen (Krm) [c]. [d] Dkk-2 can activate Dkk-2 LRP6. [Figure taken from Neihrs et al. 2006]

The Wnt/b-catenin pathway is pivotal in bone formation; increasing bone mass by various mechanisms including renewal of stem cells, osteoblast proliferation, promoting osteoblastogenesis and inhibiting osteoblast apoptosis and osteocyte apoptosis, as shown in Figure 8.2 [275]. During embryogenesis Dkk-1 antagonises Wnt-7a during limb development and keratinocytes [276, 277]. In children, Dkk-1 is expressed mainly by osteoblasts and osteocytes, when its homeostasis with Wnt determines bone formation [278]. Insufficient Dkk-1 activity secondary to the deletion of one Dkk-1 allele in a mouse model resulted in increased bone density [278]; whilst excess Dkk-1 activity resulted in decreased bone density [279]. There is emerging evidence that high Dkk-1 expression is pathogenic in erosive bone disorders such as SpA [121, 260, 280-286], rheumatoid arthritis (RA), Paget’s disease, and glucocorticoid-induced osteoporosis [89, 287, 288]. Dkk-1 levels have been investigated in PsA [121] and in AS [260, 280-286]. More details are given later in this Chapter. Pathological overexpression of Dkk-1 in human myeloma
cells is associated with increased bone erosion and osteolytic bone lesions [289]. In humans, LRP5 loss-of-function mutations lead to osteoporosis-pseudoglioma syndrome, characterised by low bone density [290], whilst LRP5 gain-of-function mutations are associated with high bone mass [291]. Dkk-1 has a physiological role in the skin complexion of the palm of the hand. Palmo-plantar fibroblasts secrete Dkk-1, which inhibits melanocyte growth and differentiation, explaining the lighter pigmentation of the palm compared with the dorsum of the hand [277].

Diarra et al. used a neutralising monoclonal antibody (mAb) to mouse Dkk-1 (anti-Dkk-1) in a transgenic mouse model of RA [287]. They found that Dkk-1 plays a key role in the remodeling of joints by two mechanisms. In RA, Dkk-1 prevents compensatory bone-formation following pathological bone degradation by osteoclast-forming inflammatory tissue. Secondly, through inhibition of the Wnt-pathway-related osteoblastogenesis, Dkk-1 reduces OPG secretion by osteoblasts. Increased serum levels of OPG were demonstrated upon mAb inhibition of Dkk-1. A mutual inverse relationship between OPG and Dkk-1, exists, allowing Dkk-1 to have an indirect influence on osteoclastogenesis-mediated bone resorption (Figure 8.1). Dkk-1 therefore has a dual action; regulating anabolic and catabolic pathways in both health and disease. These processes are summarised in Figures 8.3 and 8.4.

Tumour necrosis factor-alpha (TNF-alpha) promotes destruction of bone by decreasing the number of osteoblasts that form bone (in part through the Dkk-1/Wnt pathway), and increasing the number of osteoclasts that resorb bone (in part through the OPG/RANKL and M-CSF pathways) (Figure 8.1 and Figure 8.4). TNF-alpha therefore has an overall bias toward bone resorption [292]. TNF-alpha also induces the expression of MMP-3, which degrades the extra-cellular matrix of bone and cartilage (Figure 8.1).

Dkk-1 expression is also tightly linked to sclerostin expression. Sclerostin is another Wnt antagonist, and has been associated with syndesmophyte formation [293]. Wnt agonists such as R-spondin have been shown to induce bone spur formation along inflamed joints [294].
Wnt/β-catenin signaling regulates osteogenesis through multiple mechanisms, but leading ultimately to more bone formation and less bone resorption. (i) Wnts promote osteoblast differentiation, proliferation, and mineralisation activity. (ii) Wnts inhibit mesenchymal differentiation pathways such as adipocyte and chondrocyte differentiation. (iii) Wnts inhibit osteoblast apoptosis. (iv) Osteoblasts secrete OPG, and therefore Wnt-induced osteoblasts increase the ratio of OPG. RANKL is an inducer of osteoclastogenesis leading to increased bone resorption. However, since, OPG acts as a decoy receptor to RANKL, Wnt pathways lead to increased OPG:RANKL ratio, thereby inhibiting osteoclastogenesis, thus decreased bone resorption. (The ‘green + signs’ indicate positive effects of Wnt; the ‘red – signs’ indicate inhibitory effects of Wnt. Dlx5, distal-less homeobox 5; MSC, mesenchymal stem cell; Msx2, msh homeobox homolog 2; Osx, osterix; Runx2, runt-related transcription factor 2.)

**Figure 8.3. Downstream cellular effects of Wnt/β-catenin signaling**

(Figure taken from Krishnan et al. 2006 [275])

(a) In a physiological state, cortical bone formation and resorption next to joints are in balance. (b) Inflammatory arthritis such as rheumatoid arthritis leads to an imbalance between bone formation and resorption. Bone formation is hampered by TNF-mediated expression of DKK-1, which suppresses Wnt signals, whereas bone resorption is enhanced by expression of RANKL. (c) Blockade of DKK-1 relieves Wnt signaling from DKK-1-mediated suppression and induces bone formation resulting in osteophytes. Moreover, Wnt proteins induce OPG expression, which blocks RANKL-mediated bone resorption.

**Figure 8.4. Dkk-1 is critical for joint remodeling**

(Figure taken from Diarra et al. 2007 [287])
8.2.1.1 Evidence that Dkk-1 is dysfunctional in ankylosing spondylitis

One study by Daoussis et al., has in particular given detailed insight into the role and function of Dkk-1 in rheumatological disease [280]. As the findings of that study are so pertinent to the Dkk-1 results of this chapter of the thesis, the methods and findings of Daoussis et al. will be presented in some detail here, and revisited in the Discussion section of this chapter. In 45 AS, 45 RA, 15 PsA, and 50 HC subjects, Daoussis et al. measured serum total Dkk-1, serum functional Dkk-1, and performed Dkk-1 function assays using Jurkat T cells [280]. Serum total Dkk-1 concentrations were higher in AS patients compared with HC (2.73 vs. 2.38 ng/ml; p=0.04), AS compared with PsA (2.73 vs. 2.44 ng/ml; p=0.05), and AS compared with RA (2.73 vs. 1.85 ng/ml; p=0.02) patients (unadjusted for anti-TNF use). Although Dkk-1 concentrations were not associated with disease duration, disease activity, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), or synthetic disease modifying anti-rheumatic drug (DMARD) use in any patient group, nor the presence of peripheral arthritis in AS patients, an association with anti-TNF use was found. Once adjusted for anti-TNF use, serum total Dkk-1 levels were similar between patients with AS, RA, and HC (p=0.62 by one-way analysis of variance). AS patients on anti-TNF therapy (n=20) had significantly higher serum Dkk-1 concentrations compared with AS patients not on anti-TNF (n=25) (3.13 vs. 2.42 ng/ml; p=0.01). Disease activity (BASDAI) was not identified as a confounder in this comparison; sub-analyses showed that patients on anti-TNF and in clinical remission had similar Dkk-1 levels to those using anti-TNF and with active disease (BASDAI ≥4). In contrast, RA patients on anti-TNF (n=20) had significantly lower serum Dkk-1 levels than those not on anti-TNF (1.41 vs. 2.20 ng/ml; p=0.02), again not confounded by disease activity as measured by the DAS28. Daoussis et al. showed that in AS, longitudinally-measured Dkk-1 rises following anti-TNF therapy, the converse relationship was found in RA patients, whereby longitudinally-measured Dkk-1 levels decreased following anti-TNF therapy. This is contrary to what might be expected given that TNF-alpha is an inducer of Dkk-1 expression, so one might expect anti-TNF agents to decrease serum Dkk-1 levels.

To evaluate the functional integrity of Dkk-1, Daoussis et al. used ELISA to measure the amount of Dkk-1 binding to receptor LRP6 [280]. Microtiter plates were coated with 3 mcg/ml of human LRP6-Fc chimera, then serum samples were added. The amount of serum Dkk-1 that bound to LRP6 was measured using human anti-Dkk-1 antibody, and regarded as the amount of functional Dkk-1. Functional Dkk-1 levels were lower in AS compared with HC (n=5) (133 vs. 249 pg/ml; p=0.0001), and in AS compared with anti-TNF-naïve RA patients (133 vs. 390 pg/ml; p=0.0001). In AS patients, the amount of Dkk-1 bound to LRP6 was no different before or after anti-TNF use (133 vs. 125 pg/ml;
p=0.33). Taken together with the study’s other findings, it would seem that whilst serum total Dkk-1 levels are no different in AS compared with HC and RA patients (adjusted for anti-TNF use), Dkk-1 binds less avidly to LRP6 (as measured by the level of functional Dkk-1) in AS compared with both HC and RA. In addition, whilst only tested in 8 AS patients, the results indicate that although serum total Dkk-1 rises after anti-TNF therapy, the amount of Dkk-1 causing biological consequence (i.e. functional Dkk-1) is unchanged.

Daoussis et al. investigated further by testing the functional capacity of Dkk-1 to directly antagonise Wnt signaling using ex-vivo lithium-treated Jurkat T cells (of leukaemic rather than osteoblastic origin) by Western immunoblotting [280]. Dephosphorylated beta-catenin is the active form of beta-catenin, and is thus a direct marker of the degree of Wnt pathway activation. Lithium chloride (LiCl) reduces beta-catenin degradation, thus stabilises beta-catenin. Addition of LiCl to Jurkat cells leads to an increase in intracellular beta-catenin levels. Addition of HC sera (n=10) to LiCl-treated Jurkat cells had a moderate/minimal effect on beta-catenin levels. Whereas the addition of AS serum (n=10) led to a significant increase in intracellular active beta-catenin levels (no differences found between AS patients on anti-TNF and those not). These experiments indicate that in AS serum, despite the increased circulating levels of total Dkk-1 (a Wnt-inhibitor), at an ex-vivo cellular level, the net effect (stimulatory plus inhibitory) of AS serum on the Wnt pathway is stimulatory (abnormal), as compared to inhibitory (normal) in HC serum. One explanation as proposed by Daoussis et al., is that in AS serum, Dkk-1 is not mediating the induction of Wnt-pathways, instead AS serum contains abnormal levels of other soluble molecules e.g. growth factors, that are inducing the Wnt-pathway. A second hypothesis proposed is that an autoantibody or a soluble receptor against Dkk-1 is present in the serum of AS patients, compromising Dkk-1 function. An alternative explanation, proposed by Daoussis et al. is that Dkk-1 is dysfunctional in AS serum and is therefore unable to inhibit the Wnt-pathway, which in turn has excessive activity. Higher Dkk-1 levels are found in AS patients because the body tries progressively harder to inhibit the Wnt-pathway by expressing ever more Dkk-1, in an attempt to compensate for the dysfunctional Dkk-1. This hypothesis is fundamental to explaining our own results, as will be described later in this chapter.

To directly test the hypothesis that Dkk-1 is dysfunctional in AS patients, a mAb specifically blocking the interaction of Dkk-1 with LRP6 was added to sera [280]. This addition to HC sera led to an increase in active beta-catenin levels, indicating that Dkk-1 is acting physiologically in HC sera, acting an inhibitor of the Wnt pathway. However, the addition to sera from AS patients did not increase active beta-catenin levels, suggesting that the function of Dkk-1 in patients with AS may be compromised and thus pathological.
The authors concluded that in AS patients Dkk-1 is dysfunctional, perhaps with impaired Dkk-1 binding to LRP6, resulting in negligible Dkk-1-mediated inhibition of the Wnt pathway, and consequent excess activity of bone-formation pathways [280].

8.2.2 Osteoprotegerin (OPG)

Bone formation and resorption are normally tightly coupled, and osteoprotegerin (OPG) and receptor activator of nuclear factor kappa-B ligand (RANKL) have key regulatory roles. OPG is a 55 kDa glycoprotein and a member of the TNF receptor family [292]. OPG is secreted by osteoblasts, and its expression is increased by Wnt-signaling, IL-4, interferon-gamma (IFN-gamma), bone morphogenetic protein (BMP) and parathyroid hormone (PTH) (Figure 8.1) [295]. Importantly, Wnt-pathway mediated osteoblastogenesis creates osteoblasts that in turn express OPG. Through inhibition of the Wnt-pathway, Dkk-1 reduces osteoblastogenesis and Dkk-1 is therefore an inhibitor of OPG [275]. However, OPG also regulates Dkk-1 through a negative feedback loop (Figure 8.1) [296].

Receptor activator of nuclear factor kappa-B (RANK) is a receptor present on the surface of osteoclasts [292]. Osteoclasts are responsible for physiological, and in the abnormal state pathological resorption of bone. The binding of RANK to its ligand, RANKL, leads to the promotion of osteoclastogenesis, which in turn increases bone resorption. RANKL is mainly produced by osteoblasts and activated T cells, and OPG is predominantly secreted by osteoblasts [292]. RANKL expression is increased by TNF, interleukin-1 (IL-1), IL-6, IL-13 and IL-17, all of which are abnormally up-regulated in inflammatory arthritis and appear to have differentially-weighted influence on RANKL [292]. However, OPG is a serum-soluble decoy receptor to RANKL, preventing the binding of RANKL to RANK. OPG therefore inhibits osteoclastogenesis, and ultimately leads to reduced bone resorption [292] (Figure 8.1). Fujita, et al. found that Dkk-1 can inhibit OPG secretion, and promotes osteolytic lesions in vivo by enhancing RANKL-mediated osteoclastogenesis [297].

Serum OPG levels have been investigated in PsA [121] and in AS [260, 281, 282, 284, 285, 295, 298-304]. More details as they relate to our own study findings, are given in the Discussion section of this Chapter. Dysregulation of the OPG-RANKL axis has been more thoroughly studied in RA, where the excess production of RANKL relative to OPG, by activated T cells, likely leads to bone and cartilage destruction [305]. Kim et al. showed that whilst OPG levels were no different in AS and HC subjects, RANKL levels were increased in AS, resulting in a higher RANKL:OPG ratio in AS compared with HC [302].
Synovial tissue from PsA has been shown to express very low quantities of OPG and high levels of RANKL, possibly resulting in excessive bone resorption, and providing a plausible mechanism for bone erosions in PsA [306]. OPG has shown a role in the formation of osteolytic bone lesions in multiple myeloma [307]. Given that mAbs that inhibit RANKL (e.g. Denusomab) have shown significant clinical efficacy in the management of osteoporosis [308], OPG may also have some utility in the treatment of osteoporosis associated with increased osteoclast activity [309]. Since TNF stimulates osteoblastic cells to express RANKL and M-CSF, anti-TNF agents may inhibit bone resorption [295]. It has not yet been determined if OPG promotes osteoproliferation in the form of syndesmophytes or osteophytes. Osteophytes can form independently of the OPG-RANKL pathway, through anabolic bone pathways mediated by Dkk-1 [296].

In brief, OPG reduces bone resorption by acting as a decoy receptor in the RANK/RANKL pathway (Figure 8.1). OPG expression is reduced by Dkk-1 through inhibition of the Wnt-pathway.

8.2.3 Macrophage-colony stimulating factor (M-CSF)

Macrophage-colony stimulating factor (M-CSF) is a four-α-helical-bundle cytokine, and has several isoforms of varying size. M-CSF is secreted by fibroblasts, activated macrophages, bone marrow stromal cells, vitamin D-stimulated osteoblasts, activated endothelial cells and endometrial secretory epithelium [310-314]. M-CSF receptor is expressed on monocytes and tissue macrophages, and is responsible for mediating M-CSF endocytosis and transducing the pleotropic effects of M-CSF. M-CSF promotes differentiation of several cells, including bone osteoclasts [310-315] and macrophages [310]. Parathyroid hormone (PTH) induces the release of M-CSF from osteoblasts, which in turn binds to receptors on osteoclasts, and induces osteoclast differentiation, increased bone resorption and subsequent increased plasma calcium levels (Figure 8.1). M-CSF receptor is also expressed on osteoblasts where it down-regulates RANKL production, thus allowing M-CSF to also inhibit osteoclast production [316]. M-CSF is the primary regulator of macrophage differentiation, proliferation and survival, and is essential for macrophage-related functions such as bone resorption [310]. M-CSF regulates the release of cytokines and other inflammatory modulators from macrophages and monocytes, and stimulates chemotaxis and pinocytosis, which in-turn cause inflammation [310, 312].

A unique characteristic of M-CSF, setting it apart from other pro-inflammatory cytokine in rheumatological diseases, it that M-CSF only affects cells of the monocyte lineage [317].
Animal-models deficient in M-CSF are resistant to the development of collagen-induced arthritis [318]. M-CSF-deficient mice become deficient in macrophages and subsequently develop osteopetrosis (hardening and increased density of bones), and have failure of teeth eruption due to insufficient differentiation of bone-resorbing osteoclasts [311-313]. Serum M-CSF levels have been investigated in PsA [121] and in AS [295, 317, 319, 320]. More details are given in the Discussion section of this Chapter. M-CSF expression has shown to be increased in inflammatory bowel disease [310, 311, 313]. The expression of M-CSF receptor on cancer cells is thought to facilitate metastasis to the bone by chemotaxis toward osteoblast-produced M-CSF, and by promoting osteolysis [321].

8.2.4 Matrix metalloproteinase 3 (MMP-3)

Matrix metalloproteinases (MMPs) are zinc and calcium dependent endopeptidases that degrade the extracellular matrix (ECM) of bone and cartilage during normal physiological processes such as embryogenesis, morphogenesis, reproduction and tissue remodelling [322]. However, MMPs can also degrade extracellular matrix in a pathological manner in arthritis, cancer and cardiovascular disease [323]. Most MMPs are secreted as inactive pro-proteins, and later activated by extracellular proteinases through cleavage. MMP-3 has a molecular weight of 54 kDa, and active-MMP-3 can cleave collagen (types III, IV, IX and X), aggrecan, fibronectin, laminin, IGFBP-3, serpins, and IL-1β. Active-MMP-3 also has a role in activating other MMPs, such proMMP-1, -8, -9, and -13, giving MMP-3 a pivotal role in connective tissue remodelling [324]. MMP-1 (aka. interstitial collagenase 1) is important in the degradation of articular cartilage, and MMP-9 (aka. gelatinase B) digests gelatin and several types of collagens [325].

The MMP3 gene is part of a cluster of MMP genes which localise to chromosome 11q22.3. The synthesis of MMP is determined at the level of transcription; whilst the proteolytic activity of existing MMP is controlled by the activation of pro-enzymes and the inhibition of active enzymes by endogenous inhibitors such as α- tissue inhibitors of metalloproteinases. MMP-3 is expressed following stimulus in osteoblasts, fibroblasts, chondrocytes, keratinocytes, endothelial cells, macrophages, and vascular smooth muscle cells [323]. Stimuli include IL-1, TNF-alpha, epidermal growth factor (EGF), and platelet-derived growth factor (PDGF) (Figure 8.1). Conversely, glucocorticoids, oestrogen, progesterone, tumour derived growth factor-beta (TGF-beta) and retinoic acid inhibit MMP-3 synthesis. MMP-3 is a peptide that has several domains, that are cleaved-off at various biological stages: a signal peptide cleaved during cellular secretion; a pro-domain cleaved during activation; a catalytic domain containing a conserved zinc-binding site, a hemopexin domain, and a hinge domain [326]. Pro-MMP-3 is secreted into the ECM in its
inactive form, and activated by the removal of the pro-peptide by the action of serine proteases, plasmin and trypsin [326].

Serum levels of MMP-3 have been found to be elevated in RA patients compared with osteoarthritis patients [327], shown to correlate with disease progression in RA patients [328], and baseline levels have been shown to be prognostic markers of radiographic progression in RA [329]. Serum MMP-3 levels have been found to be elevated in PsA [121]. Serum MMP-3 levels are elevated in AS and correlate with AS-related outcome measures [295, 301, 303, 317, 330-338]. More details are given in the Discussion section of this Chapter, specifically as they relate to our own study results.

8.2.5 Summary

The bone forming and resorbing phenotypes of PsSpA, pPsA and AS make this group of SpAs good candidates to investigate serum-soluble bone-turnover biomarkers. Although some research has performed in AS patients, very little had been conducted in PsA and PsC, and none in PsSpA or psoriatic arthritis mutilans (PAM) patients. This chapter of the thesis aims to do exactly that. Comparisons will be made with the existing literature, to either corroborate or challenge the results of previous studies, and where possible explain why there may be disparity. Through a better understanding of serum bone-turnover biomarkers in SpA, it is hoped that patients can be better classified, monitored and managed using the principles of stratified medicine.
8.3 STUDY OBJECTIVES

The objectives of this study chapter were to:

(i) investigate four potential serum-soluble bone-turnover biomarkers (Dkk-1, M-CSF, MMP-3 and OPG) as predictors of PsSpA occurrence, by comparison with pPsA, AS, psoriasis-only (PsC) cases and healthy controls (HC).

(ii) investigate four potential serum-soluble bone-turnover biomarkers (OPG, Dkk-1, MMP-3 and M-CSF) as predictors of axial radiographic disease severity / morphology in patients with PsSpA and AS.

(iii) To investigate four potential serum-soluble bone-turnover biomarkers (OPG, Dkk-1, MMP-3 and M-CSF) as predictors of PAM occurrence, by comparison with non-PAM PsA cases.
8.4 METHODS

A comprehensive description of general, clinical and radiographic methods, and identification of PAM patients are detailed in Chapters 4, 5, 6 and 7 of this thesis, and will therefore not be repeated in this chapter. Specific methods for this component of the study are described below.

8.4.1 Samples

Cases were enrolled and characterised using the methods described in Chapters 4 to 7. Each patient provided a blood sample on the day of clinical assessment for serum-soluble biomarker testing and high-sensitivity CRP testing. Serum samples were provided by 201 AS cases and 200 PsA cases enrolled in the study. One PsA patient had insufficient venepuncture to provide serum, and was not willing to reattend. The facilities of the Bath Institute for Rheumatic Diseases (BIRD) laboratory were used to process and test samples. Blood samples were centrifuged at 2,600 r.p.m. for ten minutes to separate the serum and plasma. Four aliquots of serum, each of 1 ml were stored at -80 degrees Celsius for testing later.

After inception of the original study protocol, it was thought that the study would be further strengthened by comparison of serum biomarkers levels with a healthy control (HC) group and psoriasis-only (PsC; without clinical evidence of arthritis) cases, as reference groups. Fifty HC serum samples were sourced from Health Survey England (HSE) part of the Health and Social Care Information Centre (HSIC). These were age, sex and ethnicity matched to the 201 PsA cases in this study. Subjects had been screened by the HSIC using a questionnaire and deemed to be healthy. A further 200 PsC samples were sourced from Professor James Elder (Department of Dermatology, University of Michigan Medical School, Michigan, USA). These samples had been collected as part of their ongoing research of psoriasis, stored at -80 degrees Celsius, and had associated clinical details including sex, age, disease duration (from psoriasis onset to blood sampling), anti-TNF or synthetic DMARD use, and Psoriasis Area and Severity Index (PASI). These PsC samples were not matched to the PsA cohort.
8.4.2 Biomarkers tested

Commercially available enzyme-linked immunosorbent assay (ELISA) kits were purchased to measure the absolute concentration of four biomarkers (MMP-3, M-CSF, Dkk-1 and OPG) in 200 PsA, 201 AS cases, 200 PsC and 50 HC cases using the facilities of the BIRD laboratory. DJ performed the laboratory work, under the supervision of Dr. Juliet Dunphy (Biomedical Scientist and Head of Laboratories), and Professor Mark Lindsay. Due to the closure of the BIRD laboratory in October 2014, OPG was tested a couple of months later using the Pharmacology Laboratory of the University of Bath, by DJ and Ms Hui Lu, under the supervision of Professor Mark Lindsay and Dr. Juliet Dunphy.

Quantikine ELISA kits were sourced from the R&D systems (Abindgon, U.K.) for Dkk-1, MMP-3, and M-CSF. ELISA kits were sourced from Biorbyte (Cambridge, U.K.) for OPG. All kits have been validated for use in clinical research and have cited use by other research groups for these biomarkers. The ELISA kits used in this experiment measured serum total MMP-3 (pro-MMP-3 and active-MMP-3), total Dkk-1 (functionally active and inactive Dkk-1), total OPG and total M-CSF. For brevity, the methods employed to test one biomarker (Dkk-1) are detailed below. The methods were similar for the other three biomarkers.

8.4.3 Sample storage and general methods

Serum were stored at -80 degrees Celsius, and to avoid repeated freeze-thaw cycles altering biomarkers level [339], each biomarker was tested using a different aliquot, thawed on the morning of testing. One biomarker was tested per laboratory day. Four plates comprising 96 wells were tested in a semi-automated fashion using a machine to distribute samples and reagents, and read the optical density of the wells. Subject serum samples were barcoded to minimise the risk of handling error. All cases, standards and controls were tested in duplicate. With some variation between biomarker kits, this allowed approximately 156 serum samples, one negative standard, five to seven positive standards, four inter-plate, and three to six intra-plate samples to be tested. The control serum was that used routinely by BIRD laboratory for service and research, and comprised pooled serum from cases with known systemic lupus erythematosus.

Data was output as optical density, mean optical density of the duplicate samples, sample serum concentration derived from the Standard Curve, mean serum concentration, and co-efficient of variation percentage (CV%) between duplicate samples. A CV% of ≤15%
was considered acceptable. Case samples not achieving a CV% of ≤15 were repeated until the target CV% was attained.

8.4.4 Methods for the Dkk-1 assay

The Quantikine Human Dkk-1 immunoassay is a 4.5 hour solid phase ELISA designed to measure human total Dkk-1 in serum, plasma and cell culture supernates. The kit contains Sf 21-expressed recombinant human Dkk-1, and has been shown to accurately quantify the recombinant factor. The kit has been validated for use to determine relative mass values of naturally occurring human Dkk-1. The kits measures serum total Dkk-1 (both functionally active and inactive Dkk-1).

8.4.4.1 Principle of the assay

A quantitative sandwich enzyme immunoassay technique is employed by the kit. Microplates are pre-coated with a monoclonal antibody specific for human Dkk-1. Dkk-1 present in the standards and samples pipetted into the wells is bound by the immobilised antibody. Unbound substances are washed away and an enzyme-linked polyclonal antibody specific for Dkk-1 is added to the wells. Unbound antibody-enzyme reagent is washed away, a substrate solution is added to the wells and colour develops proportional to the amount of Dkk-1 bound in the initial step. Colour development is terminated and the intensity of the color indicates the concentration of Dkk-1 in that sample or standard.

8.4.4.2 Procedures followed to optimise the assay

• Foaming was avoided when mixing or reconstituting protein solutions.
• Pipette tips were changed between additions of each standard level, between sample additions, and between reagent additions, to avoid cross-contamination. Separate reservoirs for used for each reagent.
• Plate sealers were used during incubation steps.
• Since we used an automated plate washer, a 30 second soak period following the addition of wash buffer was programmed.
• Substrate solution was protected from light until the moment of use.
• Stop solution was added to the plate in the same order as the substrate solution to ensure uniformity of incubation time.
8.4.4.3 Materials and storage

Dkk-1 Microplate
96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against Dkk-1. Stored at 2-8 °C in a foil pouch for ≤1 month.

Dkk-1 Standard
20 ng recombinant human Dkk-1 in a buffered protein solution with preservatives; lyophilized. Stored at 2-8 °C for ≤1 month.

Dkk-1 Conjugate
21.5 mL of a polyclonal antibody against Dkk-1 conjugated to horseradish peroxidase with preservatives. Stored at 2-8 °C for ≤1 month.

Assay Diluent (RD1W)
11 mL buffered protein solution with preservatives. Stored at 2-8 °C for ≤1 month.

Calibrator Diluent (RD5-24)
21 mL buffered protein solution with preservatives. Stored at 2-8 °C for ≤1 month.

Wash buffer concentrate
21 mL 25-fold concentrated solution of buffered surfactant with preservative. Stored at 2-8 °C for ≤1 month.

Color Reagent A
12 mL stabilised hydrogen peroxide. Stored at 2-8 °C for ≤1 month.

Color Reagent B
12 mL stabilised chromogen (tetramethylbenzidine). Stored at 2-8 °C for ≤1 month.

Stop Solution
6 mL of 2 N sulfuric acid. Stored at 2-8 °C for ≤1 month.

Adhesive plate sealers.

Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm.

Pipettes and pipette tips.

Deionised water.

Automated microplate washer.

Horizontal orbital microplate shaker at 500 r.p.m.

Polypropylene test tubes for dilution of standards and samples.

8.4.4.4 Sample preparation

Serum samples required an eight-fold dilution. 50 µL of sample was added to 350 µL of calibrator diluent RD5-24 (1:2).
8.4.4.5 Reagent preparation

All reagents were brought to room temperature before use.

Wash Buffer
20 mL of wash buffer concentrate was diluted into deionised water to prepare 500 mL of wash buffer.

Substrate Solution
Color reagents A and B were mixed together in equal volumes within 15 minutes of use, and protected from light. 200 µL of the resultant mixture was pipetted into each well.

Calibrator Diluent RD5-24 (1:2)
Calibrator diluent RD5-24 (1:2) was prepared by mixing 6 mL of calibrator diluent RD5-24 to 6 mL of deionised water.

Dkk-1 Standard
Dkk-1 standard was reconstituted with 1.0 mL of deionised water to produce a stock solution of 20,000 pg/mL. The standard was thoroughly mixed to ensure complete reconstitution. The standard was allowed to sit for ≥30 minutes with gentle agitation prior to making dilutions.

Polypropylene tubes were used to pipette 900 µL of calibrator diluent RD5-24 (1:2) into the 2000 pg/mL tube. 500 µL of calibrator diluent RD5-24 (1:2) was pipetted into the remaining tubes. The stock solution was used to produce a dilution series (Figure 8.5), mixing each tube thoroughly before the next transfer. The 2000 pg/mL standard served as the high standard. Calibrator diluent RD5-24 (1:2) served as the zero standard (0 pg/mL).

![Figure 8.5. Preparation of the standards](image-url)

[900 µL of calibrator diluent was added to each of the tubes. 100 µL of standard was then added to the first test tube. 500 µL of the resultant mixture was then added to the 2nd tube, and so on. This made a series of the required standards from the original Dkk-1 Standard]
8.4.4.6 Assay procedure

All reagents and samples were brought to room temperature before use. All standards, samples, and controls were assayed in duplicate. The following procedure was performed:

1. 100 µL of assay diluent RD1W was added to each well.
2. 100 µL of standard, control, or sample was added to each well. The microplate was covered with an adhesive strip, and incubated for two hours at room temperature on a horizontal orbital microplate shaker set at 500 r.p.m.
3. Each well was aspirated and washed with wash buffer (400 µL) using an auto-washer; repeated three times for a total of four washes. Complete removal of liquid at each step was ensured to maintain good assay performance.
4. 200 µL of Dkk-1 conjugate was added to each well, covered with a new adhesive strip, and incubated for two hours at room temperature on the shaker.
5. The aspiration / wash as in step 5 were repeated.
6. 200 µL of substrate solution was added to each well, incubated for 30 minutes at room temperature on the bench-top and protected from light.
7. 50 µL of stop solution was added to each well, and gently shaken for 30 seconds to ensure thorough mixing.
8. The optical density of each well was determined immediately using a microplate reader set to 450 nm with wavelength correction set to 540 nm to optimise accuracy.

8.4.4.7 Calculation of results

Duplicate readings for each standard, control, and sample were averaged. The average zero standard optical density was then subtracted from each of those readings. A standard curve was created by reducing the data using Zenit computer software, generating a four parameter logistic (4-PL) curve-fit. Since samples were diluted, the concentration read from the standard curve was multiplied by the dilution factor of eight.

8.4.4.8 Sensitivity, linearity, calibration and specificity of the Dkk-1 assay

The manufacturer kit insert states that 179 assays were evaluated and the minimum detectable dose (MDD) of Dkk-1 ranged from 0.94-15.6 pg/mL. The mean MDD was 4.2 pg/mL. The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration. To assess the linearity of the assay, samples containing and/or spiked with
high concentrations of natural Dkk-1 were serially diluted by the manufacturer with the appropriate calibrator diluent to produce samples with values within the dynamic range of the assay. Assay sensitivities and ranges according to the manufacturers’ information are summarised in *Table 8.1*.

**Table 8.1. Summary of assay sensitivities and ranges according to the manufacturers' information**

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Sensitivity (ng/ml)</th>
<th>Assay range (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dkk-1</td>
<td>0.015</td>
<td>0.031, 2.000</td>
</tr>
<tr>
<td>M-CSF</td>
<td>0.047</td>
<td>0.078, 5.000</td>
</tr>
<tr>
<td>MMP-3</td>
<td>0.045</td>
<td>0.156, 10.000</td>
</tr>
<tr>
<td>OPG</td>
<td>&lt;0.002</td>
<td>0.016, 1.000</td>
</tr>
</tbody>
</table>

Dkk-1: Dickkopf 1  OPG: osteoprotegerin  MMP-3: matrix metalloproteinase 3  M-CSF: macrophage colony stimulating factor

The manufacturer states that this immunoassay is calibrated against a highly purified Sf 21-expressed recombinant human Dkk-1 produced at R&D Systems. The assay recognises oligomeric forms of natural and recombinant human Dkk-1. Factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant human Dkk-1 control were assayed for interference. No significant cross-reactivity or interference was observed with recombinant human: β-Catenin; Cerberus; Dkk-3; Dkk-4; Kremen-1; Kremen-2; LRP-6; Soggy-1; WIF-1.

### 8.4.4.9 Intra-assay reliability

The concentration of each biomarker was measured in duplicate for 651 samples. The coefficient of variation (CV) percentage for each sample was calculated by dividing the duplicate standard deviation (SD), by the duplicate mean, then multiplying by 100 [340, 341]. Any sample with a CV >15% was re-assayed until the CV was ≤15%. The intra-assay CV% for one experiment was calculated as the mean of the individual CV%’s for that one experiment. The mean intra-assay CV% for a biomarker was calculated as the mean CV% for each experiment [340, 341]. These formulae are summarised below.

\[
\text{CV\% for each sample} = \left[ \frac{\text{duplicate SD}}{\text{duplicate mean}} \right] \times 100
\]

Intra-assay CV% for one experiment = mean CV% for that experiment

Intra-assay CV% for a biomarker = mean CV% for each experiment

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8.4.4.10 Inter-assay reliability

To monitor plate-to-plate variation, one control sera was used as an internal-control and run in duplicate on every plate run for that biomarker. The inter-assay CV was calculated using the following formula [340, 341]:

\[
\text{Inter-assay CV} = \left( \frac{\text{SD of plate means}}{\text{mean of plate means}} \right) \times 100
\]

1.1.1 Statistical analysis

A variety of statistical methods were used to analyse the data, determined by the nature of the data and the comparison being made. Therefore specific statistical methods are detailed at the beginning of each results section. Data are presented as mean, median, inter-quartile range (IQR), standard deviation (SD), odds ratio (OR), adjusted OR (OR_{adj}), 95% confidence interval (95% CI), and p-value, as appropriate to the data. The alpha-level for statistical significance was set at 0.05.

Data were analysed by Ms. Amelia Jobling (AJ; PhD Research Assistant, Department of Pharmacy and Pharmacology, University of Bath) using 'R' [252], under the guidance of Dr. Gavin Shaddick (GS; Reader, Department of Mathematical Sciences, University of Bath). Apart from the multinomial logistic regression, all other analyses were subsequently repeated by DJ using STATA 12.1 (2011 Texas, USA) to ensure familiarity with the methods and rationale for the statistical tests.
RESULTS

Demographic and clinical characteristics of the HC and disease groups are detailed in Table 8.2. Statistical comparisons were not made between groups, as these data are for descriptive purposes only. The HC group was sex and age-matched to the PsA cohort and this is reflected in the data. The parameters varied between the groups, and since the literature suggests that some of these parameters can influence biomarker concentrations, we proceeded to compare biomarkers concentrations with these parameters, to guide our choice of covariates to include in the regression models.

Table 8.2. Demographic and clinical characteristics of the healthy control and disease groups

<table>
<thead>
<tr>
<th>At blood sampling</th>
<th>HC (n=50)</th>
<th>PsC (n=200)</th>
<th>pPsA (n=127)</th>
<th>PsSpA (n=117)</th>
<th>AS (n=157)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Median (IQR)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>59.93 (51.44, 67.56)</td>
<td>54 (42, 65)</td>
<td>58.45 (50.31, 66.63)</td>
<td>59.53 (59.56, 66.54)</td>
<td>54.70 (44.52, 63.41)</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>n/a</td>
<td>24 (16, 36.5)</td>
<td>15 (7, 26)</td>
<td>18 (9, 27)</td>
<td>22 (10, 31)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>n/a</td>
<td>n/a</td>
<td>29.04 (26.30, 32.84)</td>
<td>28.56 (26.06, 31.98)</td>
<td>27.07 (24.58, 30.13)</td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>n/a</td>
<td>n/a</td>
<td>2 (0, 5)</td>
<td>3 (2, 7)</td>
<td>4 (1, 10)</td>
</tr>
<tr>
<td>ASDAS (units)</td>
<td>n/a</td>
<td>n/a</td>
<td>2.1 (1.3, 2.7)</td>
<td>2.2 (1.5, 2.9)</td>
<td>2.1 (1.6, 2.9)</td>
</tr>
<tr>
<td>PASI (units)</td>
<td>n/a</td>
<td>4.8 (2.4, 8.8)</td>
<td>0.8 (0.0, 2.6)</td>
<td>0.8 (0.0, 2.8)</td>
<td>n/a</td>
</tr>
<tr>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Male sex</td>
<td>26 (52.00)</td>
<td>102 (51.00)</td>
<td>66 (51.97)</td>
<td>74 (63.25)</td>
<td>118 (75.16)</td>
</tr>
<tr>
<td>Anti-TNF used</td>
<td>n/a</td>
<td>26 (13.00)</td>
<td>47 (37.01)</td>
<td>53 (45.30)</td>
<td>59 (37.58)</td>
</tr>
<tr>
<td>HLA-B27 positive</td>
<td>n/a</td>
<td>n/a</td>
<td>9 (7.09)</td>
<td>47 (40.17)</td>
<td>140 (89.17)</td>
</tr>
</tbody>
</table>

HC: healthy control
PsC: psoriasis without arthritis
pPsA: peripheral PsA
PsSpA: psoriatic spondyloarthropathy
AS: ankylosing spondylitis
n/a: not available / applicable
n: number / proportion
IQR: interquartile range
BMI: body mass index
HLA: human leucocyte antigen
DMARD: synthetic disease modifying anti-rheumatic drug

All samples (HC and disease) were run in duplicate. The majority of Dkk-1, M-CSF and MMP-3 duplicate sample achieved a CV ≤15% on the first run, and very few needed to be re-assayed to achieve a CV ≤15%. With the OPG duplicate samples, despite up to four runs, a sample CV ≤15% was not achieved in 48 subjects. Therefore OPG serum
concentrations were available for analysis in 603/651 subjects (41/50 HC, 143/157 AS, 108/127 pPsA, 117/118 PsSpA, and 194/200 PsC subjects).

8.5.1 Example standard curve for Dkk-1

An example standard curve from one Dkk-1 experiment is shown in Figure 8.6. The curve is straight, the duplicate standards show good precision, and the standards used cover the range of concentrations expected (based on the literature review) for Dkk-1 in our HC and disease samples. These observations support the reliability of the experiment.

Figure 8.6. Standard curve for Dkk-1 in 47 PsA cases

8.5.2 Intra-assay and inter-assay reliability

Each of the four biomarkers were within the accepted intra-assay CV% of ≤10%, and within the accepted inter-assay CV% of ≤15% (Table 8.3) [340, 341].

Table 8.3. Intra- and inter-assay reliability of each biomarker

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Intra-plate assay CV% (SD)</th>
<th>Inter-plate assay CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dkk-1</td>
<td>3.22 (0.58)</td>
<td>4.9</td>
</tr>
<tr>
<td>M-CSF</td>
<td>3.88 (1.01)</td>
<td>7.1</td>
</tr>
<tr>
<td>MMP-3</td>
<td>2.17 (0.23)</td>
<td>6.3</td>
</tr>
<tr>
<td>OPG</td>
<td>7.56 (2.11)</td>
<td>9.2</td>
</tr>
</tbody>
</table>

CV%: coefficient of variation percentage  SD: standard deviation  Dkk-1: Dickkopf 1  OPG: osteoprotegerin  MMP-3: matrix metalloproteinase 3  M-CSF: macrophage colony stimulating factor

8.5.3 Biomarker concentrations in the cohort

Serum concentrations of the biomarkers in the HC and disease groups, as well as for the entire cohort, as detailed in Table 8.4. Serum concentrations were not normally distributed
(all positively skewed, at both group and cohort level) (Figure 8.7). Therefore a logarithmic transformation was applied prior to comparison of concentrations across groups.

**Table 8.4. Biomarker concentrations in the cohort**

<table>
<thead>
<tr>
<th>Group</th>
<th>Dkk-1 (ng/ml)</th>
<th>M-CSF (ng/ml)</th>
<th>MMP-3 (ng/ml)</th>
<th>OPG (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>IQR (range)</td>
<td>Median</td>
<td>IQR (range)</td>
</tr>
<tr>
<td>HC</td>
<td>3.52</td>
<td>2.72, 4.460</td>
<td>0.81</td>
<td>0.41, 0.99</td>
</tr>
<tr>
<td></td>
<td>(0.24, 9.58)</td>
<td></td>
<td>(0.12, 2.65)</td>
<td>(4.57, 33.19)</td>
</tr>
<tr>
<td>PsC</td>
<td>2.50</td>
<td>1.87, 3.42</td>
<td>0.62</td>
<td>0.25, 0.88</td>
</tr>
<tr>
<td></td>
<td>(0.37, 23.01)</td>
<td></td>
<td>(0.05, 2.60)</td>
<td>(4.49, 329.55)</td>
</tr>
<tr>
<td>pPsA</td>
<td>3.03</td>
<td>1.93, 3.69</td>
<td>0.29</td>
<td>0.16, 0.68</td>
</tr>
<tr>
<td></td>
<td>(0.28, 33.37)</td>
<td></td>
<td>(0.05, 2.40)</td>
<td>(4.28, 131.19)</td>
</tr>
<tr>
<td>PsSpA</td>
<td>3.34</td>
<td>2.43, 4.44</td>
<td>0.27</td>
<td>0.17, 0.64</td>
</tr>
<tr>
<td></td>
<td>(0.57, 12.92)</td>
<td></td>
<td>(0.02, 1.46)</td>
<td>(1.28, 113.62)</td>
</tr>
<tr>
<td>AS</td>
<td>3.51</td>
<td>2.92, 4.58</td>
<td>0.32</td>
<td>0.17, 0.58</td>
</tr>
<tr>
<td></td>
<td>(1.73, 40.67)</td>
<td></td>
<td>(0.05, 2.21)</td>
<td>(3.01, 1141.27)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Median</th>
<th>IQR</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC</td>
<td>3.12</td>
<td>2.23, 3.98</td>
<td>0.24, 40.67</td>
</tr>
<tr>
<td></td>
<td>0.40</td>
<td>0.20, 0.78</td>
<td>0.02, 2.65</td>
</tr>
<tr>
<td></td>
<td>16.78</td>
<td>11.13, 24.60</td>
<td>1.28, 1141.27</td>
</tr>
<tr>
<td></td>
<td>0.165</td>
<td>0.121, 0.224</td>
<td>0.038, 3.882</td>
</tr>
</tbody>
</table>

HC: healthy control  
PsC: psoriasis-only  
pPsA: peripheral PsA  
PsSpA: psoriatic spondyloarthropathy  
AS: ankylosing spondylitis  
Dkk-1: Dickkopf 1  
M-CSF: macrophage colony stimulating factor  
MMP-3: matrix metalloproteinase 3  
OPG: osteoprotegerin  
IQR: interquartile range

**Figure 8.7. Biomarker concentrations across the five groups**

(security concentrations were not normally distributed; all positively skewed, at both group and cohort level)

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8.5.4 Biomarker levels in healthy controls compared with disease groups

8.5.4.1 Analysis of covariates

Analyses were performed to determine if the serum concentrations of biomarkers are affected by the following covariates: sex, age at blood sampling, disease duration at blood sampling, anti-TNF use, body mass index (BMI) and HLA-B27 positivity. Since all serum concentrations were positively skewed a logarithmic transformation was applied before using linear regression to test the effect of the continuous covariates (e.g. age, disease duration and BMI) followed by multiple linear regression to adjust for the other nine covariates. Logistic regression was used for binary covariates (e.g. sex and anti-TNF use), both unadjusted and adjusted for the other nine potential covariates.

On multivariate analyses, the concentration of MMP-3 (regression coefficient, beta 0.01; p=0.04) and OPG (beta 0.01; p<0.0001) increased with each year of age (Table 8.5). M-CSF and Dkk-1 were not influenced by age at blood sampling. Sex had a significant effect on MMP-3 concentrations, with men likely to have lower MMP-3 concentrations than women (adjusted OR, OR_{adj} 0.91; 95% CI 0.83, 0.99; p<0.0001) (Table 8.5). No other biomarkers were influenced by sex. Disease duration at blood sampling, anti-TNF use (at cohort level), and BMI were not associated with serum concentrations of any of the four biomarkers (Table 8.5).

A few studies in AS have shown that anti-TNF use influences biomarker concentrations (in particular Dkk-1, MMP-3 and M-CSF), both when comparing anti-TNF users to non-users cross-sectionally [282, 342], and when comparing patients before and after commencing anti-TNF longitudinally [260, 285, 295, 317, 332]. One study showed that the effect of anti-TNF use can be differential between diseases, e.g. anti-TNF causes Dkk-1 to rise in AS patients, but fall in RA patients [280]. Given that these findings have not been consistent across studies, we compared concentration of each of the biomarkers in anti-TNF users and non-users, separately in the four disease groups. Univariate analyses of each of the four disease groups, demonstrated no statistically significant difference in biomarkers concentrations in patients using and not-using anti-TNF therapy (Table 8.6).

HLA-B27 status data was available for AS, PsSpA and pPsA cases, but not for PsC or HC subjects. HLA-B27 status was not associated with serum concentrations of any of the four biomarkers, when analysed for each of the three disease groups separately, using both univariate and multivariate analyses (Table 8.7). Therefore, in order to minimise noise and as it was not available in all groups, HLA-B27 was not included in the regression models comparing HC with disease, or disease with disease.
**Table 8.5. Association of potential covariates with serum concentrations of the biomarkers**

<table>
<thead>
<tr>
<th></th>
<th>Dkk-1</th>
<th>M-CSF</th>
<th>MMP-3</th>
<th>OPG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unadjusted</td>
<td>Adjusted</td>
<td>Unadjusted</td>
<td>Adjusted</td>
</tr>
<tr>
<td><strong>Beta (SEM)</strong></td>
<td><strong>p-value</strong></td>
<td><strong>p-value</strong></td>
<td><strong>Beta (SEM)</strong></td>
<td><strong>p-value</strong></td>
</tr>
<tr>
<td>Age</td>
<td>0.72  (0.27, 10.74)</td>
<td>0.56*</td>
<td>0.59  (0.07, 4.73)</td>
<td>0.62**</td>
</tr>
<tr>
<td>Disease duration</td>
<td>0.44  (0.06, 3.29)</td>
<td>0.42*</td>
<td>4.45  (0.54, 36.41)</td>
<td>0.16***</td>
</tr>
<tr>
<td>BMI</td>
<td>1.38  (0.54, 3.53)</td>
<td>0.90*</td>
<td>0.87  (0.35, 2.18)</td>
<td>0.77****</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td><strong>p-value</strong></td>
<td><strong>p-value</strong></td>
<td>OR (95% CI)</td>
<td><strong>p-value</strong></td>
</tr>
<tr>
<td>Sex</td>
<td>0.94  (0.71, 1.25)</td>
<td>0.68*</td>
<td>1.07  (0.78, 1.46)</td>
<td>0.68*</td>
</tr>
<tr>
<td>Anti-TNF use</td>
<td>1.38  (1.01, 1.89)</td>
<td>0.05^</td>
<td>1.17  (0.97, 1.73)</td>
<td>0.43**</td>
</tr>
</tbody>
</table>

* linear regression model (unadjusted)  
** reverse-stepwise linear regression model (reduced model adjusted for: MMP-3, anti-TNF use, BMI, and disease duration)  
*** reverse-stepwise linear regression model (reduced model adjusted for: age and BMI)  
**** reverse-stepwise linear regression model (reduced model adjusted for: MMP-3, age, disease duration, and ASDAS)  
^ reverse-stepwise logistic regression model (unadjusted)  
^^ reverse-stepwise logistic regression model (reduced model adjusted for: MMP-3)
### Table 8.6. Biomarker concentrations in anti-TNF non-users vs. users, in each of the four disease groups

<table>
<thead>
<tr>
<th></th>
<th>Dkk-1</th>
<th>M-CSF</th>
<th>MMP-3</th>
<th>OPG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>p-value*</td>
<td>OR (95% CI)</td>
<td>p-value*</td>
</tr>
<tr>
<td>AS</td>
<td>1.04 (0.95, 1.14)</td>
<td>0.36</td>
<td>0.71 (0.28, 1.80)</td>
<td>0.47</td>
</tr>
<tr>
<td>PsSpA</td>
<td>1.14 (0.95, 1.36)</td>
<td>0.17</td>
<td>0.61 (0.18, 2.03)</td>
<td>0.42</td>
</tr>
<tr>
<td>pPsA</td>
<td>0.84 (0.62, 1.12)</td>
<td>0.23</td>
<td>1.20 (0.49, 2.97)</td>
<td>0.69</td>
</tr>
<tr>
<td>PsC</td>
<td>0.97 (0.78, 1.22)</td>
<td>0.82</td>
<td>0.72 (0.30, 1.71)</td>
<td>0.45</td>
</tr>
</tbody>
</table>

Dkk-1: Dickkopf 1  
M-CSF: Macrophage colony stimulating factor  
MMP-3: Matrix metalloproteinase 3  
anti-TNF: anti-tumour necrosis factor-alpha  
OPG: osteoprotegerin  

* linear regression model (unadjusted)

### Table 8.7. Biomarker concentrations in HLA-B27 negative vs. positive cases, in each of the three SpA disease groups

<table>
<thead>
<tr>
<th></th>
<th>Dkk-1</th>
<th>M-CSF</th>
<th>MMP-3</th>
<th>OPG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>p-value*</td>
<td>OR (95% CI)</td>
<td>p-value*</td>
</tr>
<tr>
<td>AS</td>
<td>0.89 (0.31, 2.58)</td>
<td>0.83</td>
<td>0.74 (0.39, 1.42)</td>
<td>0.37</td>
</tr>
<tr>
<td>PsSpA</td>
<td>2.44 (0.85, 7.16)</td>
<td>0.12</td>
<td>0.96 (0.61, 1.52)</td>
<td>0.86</td>
</tr>
<tr>
<td>pPsA</td>
<td>1.01 (0.31, 3.32)</td>
<td>0.99</td>
<td>1.07 (0.48, 2.41)</td>
<td>0.86</td>
</tr>
</tbody>
</table>

### Adjusted OR (95% CI) p-value

<table>
<thead>
<tr>
<th></th>
<th>Dkk-1</th>
<th>M-CSF</th>
<th>MMP-3</th>
<th>OPG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>p-value*</td>
<td>OR (95% CI)</td>
<td>p-value*</td>
</tr>
<tr>
<td>AS</td>
<td>0.86 (0.30, 2.44)</td>
<td>0.77**</td>
<td>0.91 (0.44, 1.87)</td>
<td>0.79**</td>
</tr>
<tr>
<td>PsSpA</td>
<td>2.92 (0.82, 7.16)</td>
<td>0.12***</td>
<td>0.75 (0.42, 1.34)</td>
<td>0.33***</td>
</tr>
<tr>
<td>pPsA</td>
<td>1.01 (0.31, 3.32)</td>
<td>0.99***</td>
<td>1.07 (0.48, 2.41)</td>
<td>0.86***</td>
</tr>
</tbody>
</table>

anti-TNF: anti-tumour necrosis factor-alpha  
Dkk-1: Dickkopf 1  
M-CSF: Macrophage colony stimulating factor  
MMP-3: Matrix metalloproteinase 3  
OPG: osteoprotegerin

* linear regression model (unadjusted)  
** reverse-stepwise linear regression model (reduced model adjusted for disease duration and BMI)  
*** reverse-stepwise linear regression model (reduced model adjusted for sex, age, disease duration, and OPG)  
**** reverse-stepwise linear regression model (reduced model adjusted for sex, age, and disease duration)  
* linear regression model (reduced model did not require any adjustment for covariates)
8.5.4.2 Comparison of biomarkers concentrations with C-reactive protein

Linear regression was performed to determine if C-reactive protein (CRP; a validated biomarker of inflammation) correlates with each of the four biomarkers, and should therefore be included as a covariate in the regression model. Analyses were performed separately for each arthritis disease group (AS, PsSpA and pPsA; CRP was not available in the PsC cases) and are shown in Table 8.8, and plotted in Figure 8.8. In AS patients, CRP was positively associated with Dkk-1 (beta 1.22; standard error of the mean, SEM 0.25; p=2x10^{-6}), M-CSF (beta 16.49; SEM 2.92; p=1x10^{-7}), and MMP-3 (beta 0.04; SEM 0.01; p=1x10^{-6}), and negatively associated with OPG (beta -25.61; SEM 8.74; p=0.004). In PsSpA patients, CRP was positively associated with MMP-3 (beta 0.18; SEM 0.05; p=0.001) only. In pPsA patients, CRP was positively associated with MMP-3 (beta 0.16; SEM 3.51; p=0.001) only. In summary, MMP-3 was the only biomarker associated with CRP, across all three arthritis disease groups. Dkk-1, M-CSF, and OPG were associated with CRP only in the AS group.

Table 8.8. Relationship between biomarker concentration and CRP concentration, in each of the three SpA groups

<table>
<thead>
<tr>
<th></th>
<th>Dkk-1 (beta (SEM))</th>
<th>M-CSF (beta (SEM))</th>
<th>MMP-3 (beta (SEM))</th>
<th>OPG (beta (SEM))</th>
</tr>
</thead>
<tbody>
<tr>
<td>AS</td>
<td>1.22 (0.25)</td>
<td>16.49 (2.92)</td>
<td>0.04 (0.01)</td>
<td>-25.61 (8.74)</td>
</tr>
<tr>
<td>PsSpA</td>
<td>0.52 (0.41)</td>
<td>4.67 (2.96)</td>
<td>0.18 (0.05)</td>
<td>3.91 (5.31)</td>
</tr>
<tr>
<td>pPsA</td>
<td>-0.01 (0.27)</td>
<td>2.51 (2.08)</td>
<td>0.16 (3.51)</td>
<td>-1.27 (1.74)</td>
</tr>
</tbody>
</table>

beta: regression coefficient
M-CSF: Macrophage colony stimulating factor
MMP-3: Matrix metalloproteinase 3
OPG: osteoprotegerin
SEM: standard error of the mean

*D linear regression model (unadjusted)
Figure 8.8. Chart showing the relationship between biomarker concentration [see legend box on right] and CRP concentration [x-axis] in each of the SpA groups [pPsA top-row; PsSpA middle-row; AS bottom-row]

8.5.4.3 Comparison of biomarkers concentrations with ASDAS

Linear regression was performed to determine if the Ankylosing Spondylitis Disease Activity Score (ASDAS; a validated measure of axial arthritis disease activity) correlates with each of the four biomarkers, and should therefore be included as a covariate in the regression model. Analyses were performed separately for each arthritis disease group (AS, PsSpA and pPsA; CRP was not available in the PsC cases) and are shown in Table 8.9, and plotted in Figure 8.9.

In AS patients, ASDAS was positively associated with Dkk-1 (beta 0.07; SEM 0.02; p=0.003), M-CSF (beta 0.87; SEM 0.25; p=0.001), and MMP-3 (beta 0.001; SEM 0.001; p=0.05), and negatively associated with OPG (beta 1.66; SEM 0.76; p=0.03). In PsSpA patients, ASDAS was positively associated with M-CSF (beta 0.61; SEM 0.31; p=0.05) only. In pPsA patients, ASDAS was not significantly associated with any of the four biomarkers. Since ASDAS is a more encompassing measure of axial disease activity than CRP, it was included in the regression model when comparing biomarker concentrations...
in two arthritic disease groups, to ensure that axial disease activity was not confounding the results.

**Table 8.9. Relationship between biomarker concentration and axial disease activity (ASDAS), in each of the three SpA groups**

<table>
<thead>
<tr>
<th></th>
<th>Dkk-1 beta (SEM)</th>
<th>M-CSF beta (SEM)</th>
<th>MMP-3 beta (SEM)</th>
<th>OPG beta (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p-value*</td>
<td>p-value*</td>
<td>p-value*</td>
<td>p-value*</td>
</tr>
<tr>
<td>AS</td>
<td>0.07 (0.02)</td>
<td>0.87 (0.25)</td>
<td>0.001 (0.001)</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>0.003</td>
<td>0.001</td>
<td>0.05</td>
<td>-1.66 (0.76)</td>
</tr>
<tr>
<td></td>
<td>(0.25)</td>
<td>(0.001)</td>
<td>(0.76)</td>
<td>0.03</td>
</tr>
<tr>
<td>PsSpA</td>
<td>0.04 (0.04)</td>
<td>0.61 (0.31)</td>
<td>0.05 (0.01)</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>0.40</td>
<td>0.05</td>
<td>0.51</td>
<td>0.56 (0.56)</td>
</tr>
<tr>
<td></td>
<td>(0.32)</td>
<td>(0.01)</td>
<td>(0.56)</td>
<td>0.32</td>
</tr>
<tr>
<td>pPsA</td>
<td>0.0001 (0.32)</td>
<td>0.28 (0.25)</td>
<td>0.26</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>0.26</td>
<td>0.49</td>
<td>-0.30 (0.21)</td>
</tr>
<tr>
<td></td>
<td>(0.32)</td>
<td>(0.25)</td>
<td>(0.21)</td>
<td>0.15</td>
</tr>
</tbody>
</table>

* linear regression model (unadjusted)

**Figure 8.9. Chart showing the relationship between biomarker concentration and axial disease activity (ASDAS) [x-axis] in each of the SpA groups [pPsA top-row; PsSpA middle-row; AS bottom-row]**

beta: regression coefficient
M-CSF: Macrophage colony stimulating factor
MMP-3: Matrix metalloproteinase 3
OPG: osteoprotegerin

SEM: standard error of the mean
Dkk-1: Dickkopf 1
beta: regression coefficient
SEM: standard error of the mean
Dkk-1: Dickkopf 1
8.5.4.4 Biomarker concentrations in healthy controls compared with each of the four disease groups

Multinomial logistic regression was used to compare each of the disease groups (PsC, pPsA, PsSpA and AS) against the reference HC group, in order to test the null hypothesis that biomarker concentrations are similar across each group, i.e. there are no differences in biomarker concentrations between HC and pPsA cases. All models were adjusted for sex, age at blood sampling, and other biomarkers using a reverse-stepwise method. Univariate and reduced-multivariate models are shown in Table 8.10, and the results are summarised in turn below.

Dkk-1 concentrations were significantly lower in PsC cases compared with HC (OR_{adj} 0.72; 95% CI 0.61, 0.86; p=2x10^{-4}) (Table 8.10). Dkk-1 concentrations were statistically no different in HC compared with pPsA, PsSpA or AS cases.

M-CSF concentrations were significantly lower in pPsA (OR_{adj} 0.14; 95% CI 0.06, 0.32; p<1x10^{-5}), PsSpA (OR_{adj} 0.07; 95% CI 0.03, 0.17; p<1x10^{-6}), and AS (OR_{adj} 0.37; 95% CI 0.16, 0.85; p<1x10^{-7}) cases compared with HC. M-CSF concentrations were statistically no different in HC compared with PsC cases.

MMP-3 concentrations were significantly higher in pPsA (OR_{adj} 1.06; 95% CI 1.01, 1.10; p=0.02), PsSpA (OR_{adj} 1.06; 95% CI 1.01, 1.11; p=0.02), and AS (OR_{adj} 1.06; 95% CI 1.01, 1.11; p=0.01) cases compared with HC, with a trend approaching statistical significance for higher MMP-3 concentrations in PsC (OR_{adj} 1.05; 95% CI 1.00, 1.09; p=0.06) compared with HC.

There was a trend approaching statistical significance for higher OPG concentrations in pPsA (OR_{adj} 25.77; 95% CI 0.72, 913.42; p=0.07) compared with HC. OPG concentrations were statistically no different in HC compared with PsC, PsSpA or AS cases. There was high variance in OPG concentrations, and concentrations were not available on several HC subjects. This resulted in very wide confidence intervals. These analyses are likely to have been underpowered to detect a difference.

Whilst multinomial logistic regression was used to compare each disease group against the HC group, in order to determine whether there were any differences in the distributions of each biomarker across all five groups the ‘test for homogeneity’ was used. The homogeneity p-value indicates whether the biomarkers have significantly different effects when modelling the patient groups separately, whilst controlling for the covariates.
sex, age at blood sampling and the other biomarkers. MMP-3 (homogeneity p-value 4x10^{-4}) and OPG (homogeneity p-value 5x10^{-5}) concentrations were significantly different across the five groups, but Dkk-1 and M-CSF concentrations were not (Table 8.10).

**Table 8.10. Serum bone-turnover biomarkers in the four disease groups compared with the reference healthy control group**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Unadjusted *</th>
<th>Adjusted **</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR per ng/ml increase</td>
<td>95% CI</td>
</tr>
<tr>
<td><strong>Dkk-1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PsC</td>
<td>0.71</td>
<td>0.61, 0.84</td>
</tr>
<tr>
<td>pPsA</td>
<td>0.84</td>
<td>0.72, 0.99</td>
</tr>
<tr>
<td>PsSpA</td>
<td>0.98</td>
<td>0.87, 1.11</td>
</tr>
<tr>
<td>AS</td>
<td>1.04</td>
<td>0.94, 1.16</td>
</tr>
<tr>
<td><strong>M-CSF</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PsC</td>
<td>0.68</td>
<td>0.39, 1.17</td>
</tr>
<tr>
<td>pPsA</td>
<td>0.19</td>
<td>0.09, 0.39</td>
</tr>
<tr>
<td>PsSpA</td>
<td>0.12</td>
<td>0.05, 0.27</td>
</tr>
<tr>
<td>AS</td>
<td>0.17</td>
<td>0.05, 0.27</td>
</tr>
<tr>
<td><strong>MMP-3</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PsC</td>
<td>1.02</td>
<td>0.99, 1.06</td>
</tr>
<tr>
<td>pPsA</td>
<td>1.04</td>
<td>1.01, 1.08</td>
</tr>
<tr>
<td>PsSpA</td>
<td>1.04</td>
<td>1.01, 1.08</td>
</tr>
<tr>
<td>AS</td>
<td>1.05</td>
<td>1.01, 1.08</td>
</tr>
<tr>
<td><strong>OPG</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PsC</td>
<td>2.85</td>
<td>0.15, 55.08</td>
</tr>
<tr>
<td>pPsA</td>
<td>8.87</td>
<td>0.46, 169.37</td>
</tr>
<tr>
<td>PsSpA</td>
<td>3.17</td>
<td>0.15, 66.27</td>
</tr>
<tr>
<td>AS</td>
<td>1.14</td>
<td>0.05, 26.82</td>
</tr>
</tbody>
</table>

OR: odds ratio per 1 ng/ml increase in concentration  
95%CI: 95% confidence interval  
Dkk-1: Dickkopf 1  
M-CSF: macrophage colony stimulating factor  
MMP-3: matrix metalloproteinase 3  
OPG: osteoprotegerin (n=143 AS, 41 HC, 108 pPsA, 194 PsC, 117 PsSpA)  
PsC: psoriasis without arthritis  
pPsA: peripheral-only PsA  
PsSpA: psoriatic spondyloarthritis  
AS: ankylosing spondylitis

* Multinomial logistic regression: Homogeneity p-value tests for no difference in the concentration of each biomarker across all 5 groups (adjusted for: sex, age at blood sampling, and the other biomarkers)  
* Multinomial logistic regression comparing HC with each of the 4 disease groups (unadjusted)  
** Multinomial logistic regression comparing HC with each of the 4 disease groups (adjusted for: sex, age at blood sampling, and other biomarkers)
These analyses indicate that Dkk-1, M-CSF and MMP-3 have different concentrations in health compared with disease, and may therefore be biomarkers of pathology. OPG concentrations were different across the five groups, as determined by the test of homogeneity. It is possible that due to our difficulties achieving a CV% ≤15% in some OPG samples, thereby reducing the number of HC and cases with OPG results to 41/50 and 562/601, respectively, that our analyses comparing each of the disease groups with HC were underpowered to detect a difference in concentrations. However, taken together these data justify proceeding to compare biomarker concentrations in different disease states, as is presented below.

8.5.4.5 Biomarker concentrations in PsSpA compared with AS cases

Logistic regression analyses adjusted for covariates (sex, age at blood sampling, disease duration from diagnosis to blood sampling, anti-TNF use at blood sampling, most recent BMI and ASDAS at blood sampling) were used to compare each of the four biomarker concentrations in PsSpA and AS cases, to determine if any of these biomarkers can be used to predict if a patient has PsSpA or AS. Univariate and reduced-multivariate results are presented in Table 8.11.

PsSpA cases had significantly lower Dkk-1 concentrations than AS cases (OR_{adj} 0.85 per ng/ml increase in concentration; 95% CI 0.74, 0.98; p=0.02) (Table 8.11). Expressed differently, for every 1 ng/ml increase in Dkk-1 concentration, the probability of a test case being classified as having PsSpA decreases by 15%. Concentrations of M-CSF, MMP-3 and OPG were statistically no different in PsSpA and AS cases (Table 8.11).

Table 8.11. Biomarker concentrations in PsSpA vs. AS cases

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted</th>
<th>Adjusted *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR per ng/ml increase</td>
<td>95% CI</td>
</tr>
<tr>
<td>Dkk-1</td>
<td>0.87</td>
<td>0.77, 0.99</td>
</tr>
<tr>
<td>M-CSF</td>
<td>0.86</td>
<td>0.36, 2.02</td>
</tr>
<tr>
<td>MMP-3</td>
<td>1.00</td>
<td>0.99, 1.00</td>
</tr>
<tr>
<td>OPG</td>
<td>4.63</td>
<td>0.65, 33.30</td>
</tr>
</tbody>
</table>

OR: odds ratio per 1 ng/ml increase in biomarker concentration
95% CI: 95% confidence interval
Dkk-1: Dickkopf 1
M-CSF: macrophage colony stimulating factor
MMP-3: matrix metalloproteinase 3
OPG: osteoprotegerin

*Multivariate logistic regression (reduced model adjusted for: sex, age, and disease duration)
8.5.4.6 Biomarker concentrations in PsSpA compared with pPsA cases

Logistic regression analyses adjusted for covariates (sex, age at blood sampling, disease duration from diagnosis to blood sampling, anti-TNF use at blood sampling, most recent BMI, and ASDAS at blood sampling) were used to compare each of the four biomarker concentrations in PsSpA (PsA with axial disease) and pPsA (PsA without axial disease) cases, to determine if any of these biomarkers can be used to predict if a PsA patient has axial disease. Univariate and reduced-multivariate results are presented in Table 8.12.

There was a trend approaching statistical significance for M-CSF concentrations to be lower in PsSpA compared with pPsA cases (OR_{adj} 0.50 per ng/ml increase in concentration; 95% CI 0.22, 1.11; p=0.09) (Table 8.12). There was also a trend approaching statistical significance for OPG concentrations to be lower in PsSpA compared with pPsA cases (OR_{adj} 0.28 per ng/ml increase in concentration; 95% CI 0.06, 1.18; p=0.08). Lower M-CSF and OPG concentrations may therefore predict a higher likelihood of having axial disease in PsA. Concentrations of Dkk-1, and MMP-3 were statistically no different in PsSpA compared with pPsA cases (Table 8.12).

### Table 8.12. Biomarkers concentrations in PsSpA vs. pPsA cases

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted</th>
<th>Adjusted *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR per ng/ml increase</td>
<td>95% CI</td>
</tr>
<tr>
<td>Dkk-1</td>
<td>1.08</td>
<td>0.95, 1.23</td>
</tr>
<tr>
<td>M-CSF</td>
<td>0.55</td>
<td>0.25, 1.21</td>
</tr>
<tr>
<td>MMP-3</td>
<td>1.01</td>
<td>0.99, 1.02</td>
</tr>
<tr>
<td>OPG</td>
<td>0.30</td>
<td>0.08, 1.15</td>
</tr>
</tbody>
</table>

OR: odds ratio per 1 ng/ml increase in biomarker concentration  
95% CI: 95% confidence interval  
Dkk-1: Dickkopf 1  
M-CSF: macrophage colony stimulating factor  
OPG: osteoprotegerin  
MMP-3: matrix metalloproteinase 3  

*Multivariate logistic regression (reduced model adjusted for: ASDAS)

8.5.4.7 Biomarker concentrations in PsC compared with PsA cases

Biomarker concentrations in PsC and PsA cases were compared, to determine if any of these biomarkers can be used to determine if a psoriatic patient has inflammatory arthritis. PsSpA (n=117) and pPsA (n=127) cases were combined into one group comprising patients with both psoriasis and arthritis (PsA, n=244) and compared with 200 PsC cases with psoriasis but no arthritis. Logistic regression analyses adjusted for covariates (sex, age at blood sampling, disease duration at blood sampling, anti-TNF use at blood
sampling, and PASI at blood sampling; but not BMI as this was not available for the PsC cohort) were used to compare concentrations of each of the four biomarkers in PsC and PsA cases. As both groups have psoriasis, skin disease should not be a confounder in this model. Univariate and reduced-multivariate results are presented in Table 8.13.

MMP-3 concentrations were significantly higher in PsA (median 17.44; IQR 11.79, 26.88) compared with PsC (median 13.13; IQR 9.52, 18.37) cases (ORadj 1.02 per ng/ml increase in concentration; 95% CI 1.01, 1.03; p=0.0004) (Table 8.13). In other words, the probability of a test case being classified as PsA rather than PsC increases by 2% for every 1 ng/ml increase in MMP-3 concentration. PsA cases (median 0.28; IQR 0.16, 0.67) had significantly lower M-CSF concentrations than PsC (median 0.62; IQR 0.25, 0.88) cases (ORadj 0.44 per ng/ml increase in concentration; 95% CI 0.24, 0.82; p=0.01). PsA cases (median 3.11; IQR 2.02, 3.75) demonstrated a non-significant trend for higher Dkk-1 concentrations than PsC (median 2.50; IQR 1.86, 3.42) cases (ORadj 1.14 per ng/ml increase in concentration; 95% CI 0.99, 1.31; p=0.07). OPG concentrations were statistically no different in PsA compared with PsC cases.

Table 8.13. Biomarker concentrations in PsA vs. PsC cases

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Unadjusted</th>
<th>Adjusted *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR per ng/ml increase</td>
<td>p-value</td>
</tr>
<tr>
<td>Dkk-1</td>
<td>1.16</td>
<td>1.03, 1.32</td>
</tr>
<tr>
<td>M-CSF</td>
<td>0.23</td>
<td>0.13, 0.39</td>
</tr>
<tr>
<td>MMP-3</td>
<td>1.01</td>
<td>1.00, 1.03</td>
</tr>
<tr>
<td>OPG</td>
<td>2.36</td>
<td>0.80, 6.92</td>
</tr>
</tbody>
</table>

OR: odds ratio per 1 ng/ml increase in biomarker concentration
95% CI: 95% confidence interval
Dkk-1: Dickkopf 1
M-CSF: macrophage colony stimulating factor
MMP-3: matrix metalloproteinase 3
OPG: osteoprotegerin

*Multivariate logistic regression (reduced model adjusted for: age, disease duration, and PASI)

8.5.4.8 Biomarkers of radiographic axial disease, and determination of the influence of psoriasis

Biomarker concentrations in cases with and without radiographic axial disease (RAD) were compared. The influence of psoriasis (presence or absence) as a covariate in the model was also assessed, in order to test the classification system used in this cohort (PsSpA: RAD with psoriasis; and AS: RAD without psoriasis). PsSpA (n=117) and AS (n=157) cases both have RAD and were therefore combined into one group (RAD; n=274) and compared with the 127 pPsA cases without RAD (non-RAD). Logistic regression analyses adjusted for covariates (psoriasis, sex, age at blood sampling, disease duration
at blood sampling, anti-TNF use at blood sampling, most recent BMI, and ASDAS at blood sampling) were used to compare each of the four biomarker concentrations in RAD and non-RAD cases. Univariate and reduced-multivariate results are presented in Table 8.14.

Dkk-1 concentrations were significantly higher in RAD (median 3.42; IQR 2.75, 4.49) compared with non-RAD (median 3.03; IQR 1.93, 3.69) cases (OR_{adj} 1.22 per 1 ng/ml increase in concentration; 95% CI 1.05, 1.42; p=0.01) (Table 8.14). In other words, the probability of a test case being classified as RAD increases by 22% for every 1 ng/ml increase in Dkk-1 concentration. OPG concentrations were significantly lower in RAD (median 0.16; IQR 0.11, 0.22) compared with non-RAD (median 0.19; IQR 0.12, 0.28) cases (OR_{adj} 0.20 per 1 ng/ml increase in concentration; 95% CI 0.05, 0.80; p=0.02). The concentrations of M-CSF and MMP-3 were statistically no different in RAD compared with non-RAD cases.

Of note, cutaneous psoriasis was not a significant covariate in any of these models; suggesting that Dkk-1 and OPG are biomarkers of RAD irrespective of whether a patient has psoriasis or not, and therefore Dkk-1 and OPG can be used as biomarkers of RAD in spondyloarthritis (SpA) as a whole.

Table 8.14. Biomarker concentrations in RAD vs. non-RAD cases

<table>
<thead>
<tr>
<th></th>
<th>OR per ng/ml increase</th>
<th>95% CI</th>
<th>p-value</th>
<th>OR per ng/ml increase</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dkk-1</td>
<td>1.23</td>
<td>1.06, 1.44</td>
<td>0.01</td>
<td>1.22</td>
<td>1.05, 1.42</td>
<td>0.01</td>
</tr>
<tr>
<td>M-CSF</td>
<td>0.60</td>
<td>0.31, 1.17</td>
<td>0.13</td>
<td>0.64</td>
<td>0.32, 1.26</td>
<td>0.19</td>
</tr>
<tr>
<td>MMP-3</td>
<td>1.01</td>
<td>1.00, 1.02</td>
<td>0.20</td>
<td>1.00</td>
<td>1.00, 1.01</td>
<td>0.30</td>
</tr>
<tr>
<td>OPG</td>
<td>0.17</td>
<td>0.05, 0.62</td>
<td>0.01</td>
<td>0.20</td>
<td>0.05, 0.80</td>
<td>0.02</td>
</tr>
</tbody>
</table>

OR: odds ratio per 1 ng/ml increase in biomarker concentration
95% CI: 95% confidence interval
Dkk-1: Dickkopf 1
M-CSF: macrophage colony stimulating factor
MMP-3: matrix metalloproteinase 3
OPG: osteoprotegerin

* Multivariate logistic regression (reduced model adjusted for: sex, age, and disease duration. Psoriasis, anti-TNF use, ASDAS & BMI were not significant covariates in the model).

8.5.4.9 Biomarkers of psoriatic arthritis mutilans

Of the 201 PsA cases in ADIPSA cohort, it is known from Chapter 7 of this thesis that 19/201 cases have peripheral radiographic evidence of psoriatic arthritis mutilans (PAM) and 182/201 do not have PAM (non-PAM). In Chapter 7 it was found that RAD is far more common in PAM compared with non-PAM cases. The concentrations of each of the biomarkers were therefore compared in cases with PAM and non-PAM, using a logistic
regression adjusted for covariates (RAD, sex, age at blood sampling, disease duration at blood sampling, anti-TNF use at blood sampling, most recent BMI, and ASDAS at blood sampling). Univariate and reduced-multivariate results are presented in Table 8.15.

Concentrations of each of the four biomarkers were statistically no different in PAM compared with non-PAM cases (Table 8.15).

### Table 8.15. Biomarker concentrations in PAM vs. non-PAM cases

| Biomarker | Unadjusted | | Adjusted * | | |
|-----------|------------|----------------------|----------------------|----------------------|
|           | OR per ng/ml increase | 95% CI | p-value | OR per ng/ml increase | 95% CI | p-value |
| Dkk-1     | 0.78       | 0.52, 1.16         | 0.22       | 0.71       | 0.45, 1.13 | 0.15 |
| M-CSF     | 0.96       | 0.24, 3.84         | 0.96       | 0.64       | 0.13, 3.19  | 0.58 |
| MMP-3     | 1.01       | 0.98, 1.03         | 0.68       | 0.99       | 0.97, 1.02  | 0.56 |
| OPG       | 0.70       | 0.11, 4.33         | 0.70       | 0.35       | 0.004, 25.95 | 0.63 |

OR: odds ratio per 1 ng/ml increase in biomarker concentration  
95% CI: 95% confidence interval  
Dkk-1: Dickkopf 1  
M-CSF: macrophage colony stimulating factor  
MMP-3: matrix metalloproteinase 3  
OPG: osteoprotegerin  
* Multivariate logistic regression (reduced model adjusted for: radiographic axial disease and disease duration)

8.5.5 Relationship between biomarker concentration and radiographic axial disease severity / morphology

8.5.5.1 Relationship between biomarker concentration and a composite radiographic severity index (PASRI)

Analyses were performed to determine if biomarker concentrations correlate with radiographic axial disease (RAD) severity, as measured by the composite Psoriatic Arthritis Spondylitis Radiology Index (PASRI), which scores for both osteoproliferation (new bone formation) and erosion (bone resorption) in the spine and sacroiliac joints. To determine this, flexible modelling of the changes in concentrations in relation to disease severity was performed using generalised additive models (gam), using penalised splines by Ms Amelia Jobling (AJ). Unlike a linear regression model or Spearman rank correlation, a GAM model allows for non-linear relationships to be tested, and chooses the best fit model (linear, exponential, quadratic etc.). Unadjusted and adjusted (potentially for sex, age at blood sampling, disease duration at blood sampling, anti-TNF use at blood sampling, most recent BMI, ASDAS at blood sampling, and other biomarkers) analyses are presented in Table 8.16.
Figure 8.10 show plots of the modelled relationship between the predicted value of the PASRI score as a function of OPG, MMP-3 and M-CSF concentrations in PsSpA patients (plots created by AJ). The effects of OPG, MMP-3 and M-CSF concentrations in PsSpA patients remained significant when considered independently (p=0.03, p=0.01, and 0.02, respectively) and when adjusted for other covariates in a reduced model (sex, anti-TNF use and ASDAS) (p=0.03, p=0.002, and 0.01, respectively) (Table 8.16). GAMs do not indicate the strength of correlation (e.g. like a Spearman rank correlation Rho value) because the correlation can be non-linear (Figures 8.10). However, on further inspection of these plots, we see that in Figure 8.10 PASRI score increases with increasing OPG concentration along a curved line; a correlation pattern that is clinically meaningful and therefore useful. However, in Figure 8.10 the relationship between PASRI and MMP-3 is initially a sinusoidal line, which in clinical practice is neither meaningful nor clinically useful because it predicts that a patient with an OPG of 2 units can have the same PASRI score as a patient with an OPG of 3.8 units, but a lower PASRI score than a patient with an OPG of either 1 unit or 2.8 units. Similarly, in Figure 8.10 the relationship between PASRI and M-CSF is initially a sinusoidal line, which again in clinical practice is neither meaningful nor clinically useful.

Table 8.16. Relationship between biomarker concentration and radiographic severity (PASRI), in PsSpA and AS cases

<table>
<thead>
<tr>
<th></th>
<th>Dkk-1</th>
<th>M-CSF</th>
<th>MMP-3</th>
<th>OPG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p-value</td>
<td>Adjusted p-value</td>
<td>p-value</td>
<td>Adjusted p-value</td>
</tr>
<tr>
<td>PsSpA</td>
<td>0.10</td>
<td>0.45 *</td>
<td>0.02</td>
<td>0.01 *</td>
</tr>
<tr>
<td>AS</td>
<td>0.61</td>
<td>0.33 **</td>
<td>0.34</td>
<td>0.96 **</td>
</tr>
</tbody>
</table>

Dkk-1: Dickkopf 1
M-CSF: macrophage colony stimulating factor
MMP-3: matrix metalloproteinase 3
PsSpA: psoriatic spondyloarthritis
AS: ankylosing spondylitis

Generalised additive model (GAM)
* GAM (reduced model adjusted for: sex, anti-TNF use and ASDAS)
** GAM (reduced model adjusted for: sex, age, disease duration, and BMI)
In AS cases, the multivariate GAM demonstrated no correlation between any of the four biomarkers and radiographic severity (PASRI) score (Table 8.16). Correlations between the PASRI and biomarker concentrations in PsSpA (and AS separately) were also tested using a linear regression model. No statistically significant associations were demonstrated (data not shown).

### 8.5.5.2 Bone-formation biomarker correlation with osteoproliferation burden

Analyses were performed to determine if biomarker concentrations correlate with vertebral osteoproliferation burden; as measured by the sum of the facet-joint, bridging syndesmophyte, and non-bridging syndesmophyte scores of the PASRI. This was performed for PsSpA and AS cases separately. Multivariate GAM were used, allowing for the possibility of non-linear relationships between biomarker concentrations and osteoproliferation score, and adjusted for covariates (sex, age at blood sampling, disease severity...).
duration at blood sampling, anti-TNF use at sampling, most recent BMI, ASDAS at blood sampling, and other biomarkers). Figure 8.11 shows plots of the modelled relationship between the predicted value of the osteoproliferation burden score as a function of OPG, MMP-3 and M-CSF concentrations in PsSpA patients. The effects of OPG, MMP-3 and M-CSF concentrations in PsSpA patients remained significant when considered independently (p=0.01, p=0.01, and 0.08, respectively) and when adjusted for other covariates (reduced model adjusted for sex, anti-TNF use and ASDAS; p=0.01, p=0.002, and 0.01, respectively) (Table 8.17). However, on further inspection of these plots, it is seen that osteoproliferation burden increases with increasing OPG concentration along a curved line; a correlation pattern that is clinically meaningful and therefore useful. However, in Figure 8.11 the relationship between osteoproliferation burden and MMP-3 is initially sinusoidal, which in clinical practice is neither meaningful nor clinically useful. In the same Figure, the relationship between osteoproliferation burden and M-CSF is also initially sinusoidal, which in clinical practice is neither meaningful nor clinically useful. No significant correlation was demonstrated between any of the biomarkers and osteoproliferation burden in AS (Table 8.17).

**Table 8.17. Relationship between biomarker concentration and osteoproliferation burden, in PsSpA and AS cases**

<table>
<thead>
<tr>
<th></th>
<th>Dkk-1</th>
<th>M-CSF</th>
<th>MMP-3</th>
<th>OPG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p-value</td>
<td>Adjusted p-value</td>
<td>p-value</td>
<td>Adjusted p-value</td>
</tr>
<tr>
<td>PsSpA</td>
<td>0.77</td>
<td>0.53 *</td>
<td>0.08</td>
<td>0.01 *</td>
</tr>
<tr>
<td>AS</td>
<td>0.82</td>
<td>0.39 **</td>
<td>0.59</td>
<td>0.34 **</td>
</tr>
</tbody>
</table>

Dkk-1: Dickkopf 1  
M-CSF: macrophage colony stimulating factor  
MMP-3: matrix metalloproteinase 3  
OPG: osteoprotegerin  
PsSpA: psoriatic spondyloarthritis  
AS: ankylosing spondylitis

Generalised additive model (GAM)

* GAM (reduced model adjusted for: sex, anti-TNF use and ASDAS)

** GAM (reduced model adjusted for: sex, age, disease duration, and BMI)
Multivariate GAM (reduced model adjusted for sex, anti-TNF use and ASDAS)
PsSpA: psoriatic spondyloarthritis  Osteoproliferation: osteoproliferation severity  OPG: osteoprotegerin
MMP-3: matrix metalloproteinase 3  M-CSF: macrophage colony stimulating factor

Figure 8.11. Relationship between osteoproliferation severity and serum biomarker concentrations

GAM output plot of the relationship between osteoproliferation severity (PASRI-subdomain) score and OPG [top left], MMP-3 [top right] and M-CSF [bottom left] concentration, in PsSpA cases

8.5.5.3 Bone-resorption biomarker correlation with erosion burden

M-CSF, MMP-3 and Dkk-1 are promoters of bone resorption (e.g. erosion). OPG is an inhibitor of bone resorption. M-CSF, MMP-3, Dkk-1 and OPG concentrations were compared with vertebral erosion burden (as measured by the grade 1-subscore of the PASRI) in PsSpA and AS cases separately. Since vertebral erosions were infrequent in both PsSpA and AS cases, there were few PASRI-erosion scores >0. PASRI-erosion scores were therefore grouped into two categories: 0 (n=112 in PsSpA; n=150 in AS); and ≥1 (n=6 in PsSpA; n=7 in AS). Logistic regression analyses were used to compare scores in PsSpA and then AS cases, adjusted for covariates (sex, age at blood sampling, disease duration at blood sampling, anti-TNF use at sampling, most recent BMI, ASDAS at blood sampling, and other biomarkers).
In PsSpA cases, Dkk-1 concentration was significantly lower in patients with an erosion score ≥1 (OR_adj 0.28 for every 1 ng/ml increase in concentration; 95% CI 0.10, 0.80; p=0.02) (Table 8.17). No significant correlations were demonstrated between Dkk-1, M-CSF, MMP-3 or OPG and erosion burden AS cases (Table 8.18).

**Table 8.18. Biomarker concentration correlation with erosion burden, in PsSpA and AS cases**

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>PsSpA Unadjusted *</th>
<th>PsSpA Adjusted **</th>
<th>AS Unadjusted *</th>
<th>AS Adjusted ***</th>
</tr>
</thead>
<tbody>
<tr>
<td>OR (95% CI)</td>
<td>p-value</td>
<td>OR (95% CI)</td>
<td>p-value</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Dkk-1</td>
<td>0.45 (0.20, 1.00)</td>
<td>0.05</td>
<td>0.28 (0.10, 0.80)</td>
<td>0.02</td>
</tr>
<tr>
<td>M-CSF</td>
<td>2.06 (0.06, 66.41)</td>
<td>0.68</td>
<td>3.00 (0.06, 158.73)</td>
<td>0.59</td>
</tr>
<tr>
<td>MMP-3</td>
<td>0.97 (0.89, 1.05)</td>
<td>0.41</td>
<td>0.94 (0.85, 1.03)</td>
<td>0.17</td>
</tr>
<tr>
<td>OPG</td>
<td>13.80 (0.01, 13149)</td>
<td>0.45</td>
<td>0.12 (0.00, 28814)</td>
<td>0.74</td>
</tr>
</tbody>
</table>

OR: odds ratio per 1 ng/ml increase in biomarker concentration
95% CI: 95% confidence interval
PsSpA: psoriatic spondyloarthritis
AS: ankylosing spondylitis
Dkk-1: Dickkopf 1
M-CSF: macrophage colony stimulating factor
MMP-3: matrix metalloproteinase 3
* logistic regression model (unadjusted)
** logistic regression model (reduced model in PsSpA adjusted for: age at blood sampling)
*** logistic regression model (reduced model in AS did not have any significant covariates)

The key results presented so far are summarised below in Figure 8.12.
8.6 DISCUSSION & CONCLUSIONS

8.6.1 Discussion of MMP-3 results

8.6.1.1 Relationship between MMP-3 and disease groups

Compared with HC, MMP-3 concentrations were significantly higher in AS (OR\textsubscript{adj} 1.06; 95% CI 1.01, 1.11; p=0.01), PsSpA (OR\textsubscript{adj} 1.06; 95% CI 1.01, 1.11; p=0.02), pPsA (OR\textsubscript{adj} 1.06; 95% CI 1.01, 1.10; p=0.02) groups, and approached statistical significance to be higher in the PsC (non-significant trend OR\textsubscript{adj} 1.05; 95% CI 1.00, 1.09; p=0.06). Further investigation showed that MMP-3 concentrations were significantly higher in PsA compared with PsC cases (OR\textsubscript{adj} 1.02; 95% CI 1.01, 1.03; p=0.0004). However, MMP-3 concentrations were statistically no different in PsSpA compared with AS cases, PsSpA compared with pPsA cases, or RAD compared with non-RAD cases. Collectively, these results indicate that MMP-3 is a biomarker of disease, perhaps more specific to arthritis rather than psoriasis, and therefore could potentially be used to differentiate psoriatic patients with and without arthritis. However, MMP-3 appears not to differentiate different forms of arthritis, e.g. peripheral versus axial arthritis, or AS versus PsSpA.

Our results are supported by a study by Chandran et al., who also found that MMP-3 concentrations are higher in PsA compared with HC (OR\textsubscript{adj} 1.40; 95% CI 1.12, 1.75; p=0.003), a trend for MMP-3 concentrations to be higher in PsC compared with HC (OR\textsubscript{adj} 1.15; 95% CI 0.98, 1.35; p=0.09), and that MMP-3 concentrations are higher in PsA compared with PsC patients (OR\textsubscript{adj} 1.28; 95% CI 1.02, 1.60; p=0.03) [88]. Two other studies have also shown MMP-3 concentrations to be higher in PsA compared with HC [147, 148], and one other study has shown higher MMP-3 concentrations in PsA compared with HC [148]. Our results are more reliable that those of Shibata et al. as their study had very few female PsA patients, and so comparisons were mainly between male PsA patients and HC. Of note, we are the first study to compare MMP-3 concentrations in PsSpA patients and HC.

Other studies have compared MMP-3 concentrations in AS and HC. Our results are consistent with four other studies that have shown MMP-3 levels to be higher in AS patients compared with HC [301, 330, 331]. Two studies found no difference in MMP-3 concentrations in AS patients and HC [147, 317]. However, we feel that our statistical analyses are more robust than those by Ribbens et al. and better powered to detect a difference, given that they dichotomised MMP-3 concentrations as being normal or abnormal using a cut-off based on two standard deviations from the mean in the HC.
group. We log-transformed the data then performed adjusted multinomial logistic regression. The study by Yang et al. may have been underpowered to detect a difference, given that they only had 41 AS and 28 HC subjects [317].

8.6.1.2 Relationship between MMP-3 and radiographic severity

MMP-3 concentrations were significantly and positively associated with radiographic severity (PASRI total; adjusted p-value 0.002), and osteoproliferation burden (PASRI domain; adjusted p=0.002). However, a plot of the relationship found it to be an initially sinusoidal relationship, and therefore not clinically useful. We are the first reported study in both AS and PsSpA to compare MMP-3 levels with axial radiographic severity as measured by the PASRI, osteoproliferation burden and erosion burden. Almodovar et al. used a related axial radiographic severity score, the BASRI-total (spine and hip), and found no cross-sectional correlation with MMP-3 levels in AS patients [337]. A longitudinal study of 36 AS patients followed over 22 weeks found that baseline MMP-3 concentration does not predict either axial radiographic progression as measured by the mSASSS, or new syndesmophyte formation [334]. However, an earlier longitudinal study by Maksymowych et al. found that baseline MMP-3 level predicted radiographic progression as measured by the mSASSS, whilst other clinical parameters such as CRP, sex, age, disease duration and baseline mSASSS did not [303].

8.6.1.3 Relationship between MMP-3 concentration and anti-TNF use

Our exploratory analyses showed that MMP-3 concentrations are not altered by anti-TNF therapy use, either when the four disease groups were analysed separately, or when analysed together. We are the first study to make this comparison in PsSpA, pPsA and PsC patients. Longitudinal studies in PsA show that MMP-3 levels fall after the commencement of anti-TNF therapy [125, 343]. Longitudinal studies in AS patients have shown that MMP-3 concentrations decrease within a few weeks of anti-TNF therapy commencement [295, 317, 336], and in both clinical responders and non-responders [332], although not in axial-only AS patients without peripheral arthritis [335]. Our cross-sectional rather than longitudinal study design likely explains the conflicting results. A longitudinal study is the ideal study design, and our exploratory analyses were mainly to guide covariates to include in the multivariate models.
MMP-3 results in the context of MMP-3 biology

MMP-3 is produced by chondrocytes, macrophages and fibroblasts in synovial tissue, as well as some non-articular tissues [326]. MMP-3 levels have been shown to be four times higher in synovial fluid compared with serum in the same AS patients with peripheral involvement [317], and a thousand times higher in synovial tissue compared with serum samples from the same SpA patients with peripheral involvement [344]. MMP-3 may therefore be a biomarker more specific to peripheral synovial-based arthritis, than to axial non-synovial (fibrocartilage) arthritis. Studies have shown that in axial SpA patients, MMP-3 levels are higher in those with peripheral arthritis compared with those without peripheral arthritis [331, 344, 345]. In peripheral SpA patients, synovial sub-lining MMP-3 protein expression best differentiated anti-TNF responders from non-responders, and anti-TNF users from non-users [346].

In AS patients, MMP-3 has been shown to decrease after the use of anti-TNF therapy [295, 317, 332, 335, 336]. However, the results of Arends et al. suggested that this phenomena may be more specific to mixed axial-peripheral AS patients rather than axial-only AS patients [336]. In addition, in studies where response criteria were based on axial rather than peripheral disease, MMP-3 levels decreased in both anti-TNF clinical responders and clinical non-responders [332]. One might surmise that reductions in MMP-3 levels may reflect improvement in peripheral synovial inflammation, rather than non-synovial axial disease. Current peripheral arthritis on examination (tender and / or swollen joint) was present in 76% of our PsA and 47% of our AS patients. Whilst our data included the number of clinically swollen and tender joints, no measure of joint size or degree of inflammation was recorded. Higher proportions, may have had a history of peripheral arthritis, especially in the PsA group. Therefore, adjusting for peripheral arthritis in regression models comparing, for example AS with PsSpA, may be inappropriate and misleading. In part, this may also explain why MMP-3 levels did not correlate in a meaningful manner with axial radiographic severity or osteoproliferation burden in our AS or PsSpA cases. In our study, very few AS and PsSpA cases had axial erosions, and tests for correlation with MMP-3 were probably underpowered.

However, it still seems sensible to have investigated MMP-3 as a biomarker of disease in axial SpA (AS and PsSpA) since MMP-3 has been shown to be highly expressed in fibroblasts of the annulus fibrosus of intervertebral discs, and in macrophages present in granulation tissue of herniated discs [347]. Macrophage infiltrates have been demonstrated in the subchondral bone marrow of pathological hypervascular spinal facet joints in immunohistochemical studies [348]. Axial skeletal tissue obtained at spinal
surgery in AS and osteoarthritis patients and analysed using immunohistochemistry, showed invasion of bone at entheseal sites by MMP-1-expressing cells, but not by MMP-3 expressing cells [349]. In murine models, IL-1 and IL-6 induced MMP-3 expression appeared to have a role in bone matrix degradation leading to bone resorption [350].

Disparity of study results may in part be explained by the analysis kit used. For example, Ribbens et al. [147] and Yang et al. [317] used kits that measured three components of MMP-3 (pro-MMP-3, active-MMP-3 and MMP-3/TIMPs complex), whereas our kit and that used by Chen et al. measured two components (pro-MMP-3 and active-MMP-3). Active-MMP-3 levels may be the best surrogate of MMP-3 activity. As suggested by data from Chen et al., the ratio of MMPs and tissue inhibitors of matrix metalloproteinases (TIMPs) may determine whether MMPs degrade ECM to cause joint damage, and therefore be more informative than total-MMP-3 [330]. Some assays measure activity of MMP-3 using a substrate. The cleavage capacity of MMP-3 is regulated by three phases: synthesis, activation and inhibition. Since only active-MMP-3 is able to cleave ECM proteins, the ability to measure just active-MMP-3 rather than total-MMP-3 may be more informative, particularly in terms of imbalance in the latent / active axis, and abnormal binding between active-MMP-3 and TIMPs in pathological states [338]. In addition, since pro-MMP-3 levels are usually much higher than active-MMP-3 levels, measuring total MMP-3 may not detect subtle pathological differences in active-MMP-3 levels. Currently available commercial assays cannot distinguish the pro- and active- forms of MMP-3. Sun et al. recently reported developing a highly sensitive and reproducible assay for measuring active-MMP-3 concentrations in human sera [338]. In that cross-sectional study of both AS and RA patients, active-MMP-3 levels correlated with CRP and ESR, but not mSASSS or BASDAI, and levels of active-MMP-3 significantly decreased after anti-TNF use mirroring clinical improvement. One further complicating factor is that active-MMP binds to carrier proteins found in high levels in human serum, e.g. TIMPs and alpha-2 macroglobulin [351]. Alpha-2 macroglobulin can capture active-MMP and shield it from detection by monoclonal antibody assays [352], and so serum active-MMP levels may falsely appear lower than reality.

Our results in the context of clinical practice and future research, will be discussed in more detail in the next chapter of this thesis, Discussion of Thesis.
8.6.2 Discussion of M-CSF results

8.6.2.1 Relationship between M-CSF and disease groups

Compared with HC, M-CSF concentrations were significantly lower in pPsA (OR_adj 0.14; 95% CI 0.06, 0.32; p<1x10^{-5}), PsSpA (OR_adj 0.07; 95% CI 0.03, 0.17; p<1x10^{-5}), and AS (OR_adj 0.37; 95% CI 0.16, 0.85; p<1x10^{-7}) cases. These results indicate that M-CSF is a biomarker of disease compared with health. Further exploration showed that M-CSF concentrations were significantly lower in PsA compared with PsC cases (OR_adj 0.44; 95% CI 0.24, 0.82; p=0.01), suggesting that M-CSF may be a biomarker of arthritis in patients with psoriasis. M-CSF concentrations were statistically no different in PsSpA compared with pPsA, PsSpA compared with AS cases, or RAD compared with non-RAD cases; indicating that whilst M-CSF is a biomarker of arthritis, it does not differentiate different forms of SpA, e.g. peripheral (pPsA) from axial PsA (PsSpA), or PsSpA from AS. M-CSF may not differentiate these diseases because it is in fact a biomarker of a pathological process common to these three forms of arthritis, e.g. enthesal disease or bone resorption.

Generally in our study, M-CSF concentrations were lower in disease than in HC, and in PsA compared with PsC. Along with other covariates, our analyses were adjusted for age and disease duration at blood sampling which should have adjusted for patients being in different phases (early, middle, or late) of their disease, and adjusted for anti-TNF use given that TNF is a known inducer of M-CSF release by osteoblasts. SpA, especially AS, is characterised by increased bone resorption in trabecular bone resulting in osteoporosis, and one might therefore expect higher M-CSF levels in SpA. However, in SpA paradoxical abnormal new bone formation at enthesal sites resulting in syndesmophytes and SIJ ankylosis is also observed. The latter phenomena might be explained by an imbalance in the metabolic bone axis, with either less bone resorption, more bone formation, or both pathological processes simultaneously. Lower M-CSF levels in our SpA patients compared with HC and PsC subjects, suggest SpA patients have less M-CSF-related bone resorption. This may translate into the typical bone-forming phenotype of SpA. We did not measure bone mineral density as a surrogate for osteoporosis in our study, and so cannot test the effect of low M-CSF on trabecular bone in our cohort. It is possible that in SpA, M-CSF is a less important mediator of trabecular bone resorption than other molecules such as OPG and RANKL, and therefore serum M-CSF concentration is not a biomarker of trabecular bone resorption.

Few data have been published on M-CSF concentrations in PsA compared with HC. Using the same R&D ELISA kits, Dalbeth et al. found higher concentrations of M-CSF in...
PsA compared with HC (p<0.01), and PsA compared with PsC (p<0.01), but no difference in concentrations between PsC and HC [126]. They did not sub-categorise PsA into pPsA and PsSpA. Whilst Dalbeth et al. argued that their data suggests M-CSF-induced bone loss at peripheral joint sites (M-CSF is a promoter of osteoclast-mediated bone resorption), no association was found between M-CSF concentration and the extent of systemic bone loss in PsA as measured by bone mineral density. Their results therefore do not follow-through on their biological hypothesis. Another hypothesis proposed by Dalbeth et al. was that serum M-CSF might promote the survival or release of circulating osteoclast precursor cells, rather than differentiation or activity of osteoclasts.

In AS patients, data on serum M-CSF is conflicting; one study showed higher M-CSF concentrations in AS compared with HC [319], and another study showed no difference [317]. A study from the University of California by C. Yang et al. found no difference in M-CSF levels in 41 AS and 28 HC subjects [317]. They used the same ELISA kits as we did, but their AS cohort was quite different to ours: all AS cases were anti-TNF-naïve; had lower mean age than ours (mean 25 vs. median 53 years, respectively); had shorter disease duration than ours (mean 6 vs. median 22 years, respectively); and had higher disease activity than ours (median BASDAI 5.25 vs. 3.20, respectively). Overall their cases appear to have been recent-onset younger AS cases in the initial active stage of their disease, compared with our more established cases with a spectrum of disease activity and anti-TNF use. Compared with our study, C.Yang et al. had a smaller sample size for both cases and HC, and their range of measured M-CSF concentrations in AS cases was larger (0.40 to 3.83 ng/ml). Both factors increase the likelihood that their analyses had higher statistical error and lower power to detect a difference, than our study. Data published in the form of a letter in Annals of the Rheumatic Diseases by P-T. Yang et al. from China, showed M-CSF concentrations to be higher in AS compared with HC (2.89 vs. 0.18 ng/ml) as part of a secondary sub-analysis [319]. The primary objective of the pilot study was determine if M-CSF levels differed in active AS patients taking thalidomide or not. The study had a very small sample size (29 AS cases, 10 HC subjects), AS patients were male, of young age (approximately 23 years) and of low disease duration (approximately 1.8 years). AS patient sampling was selective (all were required to have active disease, BASDAI ≥3), and the allocation of thalidomide to patients was determined by a desire not to have children, rather than being randomised. All HCs were male (age-matched to AS cases) and sampling methods not detailed. Overall, it therefore seems inappropriate to compare the results of these studies with ours.

Koths reported that M-CSF has several isoforms, and so ELISA kits using different target sites may be prone to interaction with other molecules [353]. The M-CSF ELISA kits we
used (R&D Systems, Minnesota, USA) are reported to have no significant cross-reactivity with other molecules. This may in part explain disparity between some of our results and those of some other studies.

8.6.2.2 Relationship between M-CSF and radiographic severity

We are the first study to compare M-CSF levels with radiographic axial radiographic severity, osteoproliferation burden and erosion burden, in PsSpA and AS. Whilst there was a statistically significant correlation in PsSpA cases between M-CSF concentrations and radiographic severity (PASRI; \( p=0.01 \)) and osteoproliferation burden (PASRI subdomain; \( p=0.01 \)), plots of these relationships showed them not to be clinically useful. Instead of using a generalised additive model (GAM), when forced into a more linear model using linear regression, these associations were no longer evident. We found no association between M-CSF and erosion burden (PASRI subdomain), in either PsSpA or AS cases.

8.6.2.3 Relationship between M-CSF concentration and anti-TNF use

Our exploratory analyses showed that M-CSF concentrations are not altered by anti-TNF therapy use, either when the four disease groups were analysed separately, or when analysed together. We are the first study to make this comparison in PsSpA, pPsA and PsC patients. In AS patients, one longitudinal study testing M-CSF concentrations before and after anti-TNF therapy commencement supports our findings [317], but one similarly designed study found that M-CSF concentrations decreased after 12 weeks of anti-TNF therapy [295].

Our results in the context of clinical practice and future research, will be discussed in more detail in the next chapter of this thesis, Discussion of Thesis.
8.6.3 Discussion of OPG results

8.6.3.1 Relationship between OPG and disease groups

OPG concentrations were not significantly different in HC compared with the four disease groups. Our findings of no difference in OPG concentrations in HC compared with pPsA is supported by the results of Dalbeth et al. [126], although different to two studies that found higher levels in PsA (not sub-categorised into pPsA and PsSpA) compared with HC [88, 141]. Just as we found, two other studies also found no difference in OPG concentrations in PsC compared with HC [126]. Our findings of no significant difference in OPG concentrations in AS compared with HC, is consistent with two other studies [281, 302]. Other studies show very mixed results, with three studies showing lower OPG concentrations in AS compared with HC [282, 299, 342], and four studies showing higher OPG concentration in AS compared with HC [141, 285, 298, 300, 301]. It is possible that our OPG analyses were underpowered to detect a difference between HC and disease groups. The 95% confidence intervals for comparisons were wide, indicating high statistical uncertainty, most likely related to our small sample of HC subjects (n=41); lower than the other biomarkers due to some difficulty obtaining satisfactory CV% in duplicate samples.

In our study, OPG concentrations were lower in RAD compared with non-RAD cases (OR_{adj} 0.20; 95% CI 0.05, 0.80; p=0.02), and on the same spectrum a trend approaching statistical significance for OPG levels to be lower in PsSpA compared with pPsA cases (OR_{adj} 0.28; 95% CI 0.06, 1.18; p=0.08). To our knowledge, we are the first study to make these comparisons. In our study, OPG concentrations were no different in PsSpA compared with AS cases, or PsA compared with PsC cases. The latter finding is supported by the results of Dalbeth et al. [126]. The general direction of OPG levels being lower in patients with axial SpA (RAD vs. non-RAD, and PsSpA vs. pPsA), is in keeping with the broad theme demonstrated by three studies showing significantly lower OPG levels in AS compared with HC, in particular the well-designed large sample-size (264 AS cases and 240 age-matched HC) study by Franck et al. [282, 299, 342]. Franck et al. also found evidence that bone loss (e.g. osteoporosis as measured by low bone mineral density) in AS patients was associated with low serum levels of OPG, sex steroid hormones, and inflammatory activity; with and lack of compensatory age-related increase of OPG serum levels. Their results, were consistent with data from studies of RA patients, where low serum OPG levels were considered inappropriately low to counteract enhanced bone resorption [354].
8.6.3.2 Biology of the OPG results

Our results showing OPG concentrations to be lower in RAD compared with non-RAD cases, and in PsSpA compared with pPsA cases make biological sense. Bone resorption in SpA occurs at several different sites: axial and peripheral articular sites in the form of erosion and osteolysis; and the general skeleton in the form of bone mineral density loss resulting in osteoporosis. Compared with the general population, there is an excess burden of osteoporosis in both AS patients [264-266], and PsA patients [267, 268], with similar prevalence between disease groups. Through the inhibition of RANKL-mediated osteoclastogenesis, high OPG levels would reduce bone resorption. If OPG levels are low, osteoclastogenesis would be less impeded, and result in more bone resorption in trabecular bone (i.e. osteoporosis) and possibly at entheseal insertions into cortical bone (e.g. entheseal erosions and / or vertebral corner erosions). The lower levels of OPG we found in axial SpA patients (RAD compared with non-RAD, and PsSpA compared with pPsA) might manifest as osteoporosis and increasing entheseal erosions characteristic of SpA. In fact, synovial tissue from PsA patients has been shown to express very low quantities of OPG and high levels of RANKL, and therefore increased OPG/RANKL-mediated osteoclastogenesis has been proposed as a possible mechanism for bone erosions in PsA [306]. In our study, this translated as would be expected to lower circulating serum concentration of OPG. A study of synovial tissue from RA and AS patients found higher RANKL expression in tissues with active synovitis than normal tissues, and lower OPG expression in RA patients (although not in AS patients) with active synovitis than normal tissues [355]. The authors therefore proposed RANKL-mediated osteoclastogenesis as a mechanism for synovial-based erosions.

Some authors argue that OPG should be higher in diseases characterised by axial and / or peripheral osteoproliferation and / or periostitis [88]. The counter-argument is that although OPG inhibits bone resorption, this does not necessarily mean that OPG’s effects translate further to cause increased entheseal and articular bone formation in the form of osteoproliferative syndesmophytes and SIJ ankylosis. High OPG levels may simply lead to increased trabecular bone density and subsequent high bone mass. Whilst Grisar et al. found elevated levels of OPG in AS and PsA cases compared with HC, they could not convincingly explain the biological mechanism for these results [141]. They cited osteoblast-upregulation as a reaction to increased bone resorption, and / or reactive TNF-mediated induction of OPG in inflammatory arthritis as possible explanations. Their own uncertainty was evidenced by their comment to interpret their results with caution.
Unfortunately, we were unable to measure serum RANKL, as currently available assays are not sufficiently reliable. Our study might have been improved had we also measured serum RANKL levels, as there is some evidence that the ratio of RANKL:OPG is more indicative of axis dysregulation than either biomarker alone [300, 302, 342]. Kim et al. showed that whilst OPG is no different in AS compared with HC subjects, the ratio of RANKL:OPG was higher in AS due to higher RANKL in AS patients [302]. The imbalance of OPG and RANKL, rather than the absolute serum level of each biomarker, may be pathogenic in SpA. In addition, we may have found a stronger association between OPG and radiographic progression had we tested for correlation using the ratio of OPG:RANKL. In a study of RA patients, peripheral radiographic progression was weakly associated with RANKL concentrations, not associated with OPG concentrations, but strongly associated with the ratio of OPG:RANKL [356].

8.6.3.3 Relationship between OPG and radiographic severity

We found a statistically significant and clinically meaningful positive correlation in PsSpA cases, whereby OPG levels increased with increasing radiographic severity as measured by the PASRI (GAM p=0.03), and with increasing osteoproliferation burden as measured by the PASRI (GAM p=0.01). Such correlations were not demonstrated in AS cases. We are the first study to test for correlation between OPG levels and radiographic severity in PsSpA cases, and the first to do so using the PASRI total, osteoproliferation burden domain of the PASRI and erosion burden domain of the PASRI. We are the first study to use the PASRI for this purpose in AS patients. Maksymowych et al. used the related radiographic severity score, mSASSS, in AS patients and found no correlation between OPG and two-year radiographic progression in a multivariate model [303].

However, from a biological perspective our results are somewhat difficult to explain. Firstly, when comparing HC with disease groups it was concluded that OPG might be a biomarker of increased trabecular bone resorption in axial SpA, rather than enthesal / articular osteoproliferation. Consequently, there seems to be no biological reason for circulating serum OPG to correlate with axial radiographic severity (PASRI total) or osteoproliferation burden. As mentioned before, there was limited axial bone erosion, for that to be confounding the apparent correlation with radiographic severity (PASRI total). Other than the pathogenesis of AS and PsSpA being differentially mediated by OPG, which seems unlikely, there appears little reason for a correlation to be seen in PsSpA but not in AS. Collectively, this suggests that the correlation of OPG with radiographic severity in PsSpA may simply be a chance finding.
8.6.3.4 Relationship between OPG concentration and anti-TNF use

Our exploratory analyses showed that OPG concentrations are not altered by anti-TNF use, either when the four disease groups were analysed separately, or when analysed together. This is somewhat surprising given that TNF-alpha induces Dkk-1, which indirectly inhibits osteoblastogenesis-related OPG expression (Figure 8.1). In addition, TNF-alpha induces osteoblast expression of RANKL, and one might therefore expect a reactive down-regulation of OPG expression in anti-TNF users. We are the first study to make this comparison in PsSpA, pPsA and PsC. Our results are supported by Chandran et al. who demonstrated no longitudinal change in OPG levels after the commencement of anti-TNF in PsA patients [343]. The AS literature is conflicting with some reports of OPG levels being lower in anti-TNF-treated patients [282, 285, 342], and other studies reporting no difference [295, 304]. The disparity in study findings is likely related to both cross-sectional and longitudinal study designs being used, and a variety of statistical methods used to test for correlation and / or association. Many studies did not adjust for confounders as analyses were secondary objectives.

Given the negative feedback loop between OPG and Dkk-1 (Figure 8.1), we performed exploratory analyses but found no correlation between OPG and Dkk-1 concentrations in either AS, PsSpA or pPsA cases (results not shown; tested using univariate and multivariate linear regression, and Spearman rank correlation).

Our results in the context of clinical practice and future research, will be discussed in more detail in the next chapter of this thesis, Discussion of Thesis.

8.6.4 Discussion of Dkk-1 results

8.6.4.1 Dkk-1 levels in healthy controls compared with disease groups

Compared with HC, the odds ratios (ORs) for Dkk-1 concentrations steadily increased from PsC, to pPsA, to PsSpA to AS following the clinical characteristic of progressively increasing axial disease. There was no statistically significant difference in Dkk-1 concentrations in HC compared with either PsSpA or pPsA cases, but there was a trend approaching statistical significance for Dkk-1 to be higher in AS compared with HC (OR_{adj} 1.10; 95% CI 0.97, 1.25; p=0.13). This latter finding is consistent with the results of three other studies, also using R&D ELISA kits, that showed higher Dkk-1 levels in AS compared with HC: Daoussis et al. (2.73 vs. 2.38 ng/ml; p=0.04) [280]; Klingberg et al.
(2.89 vs. 2.66 ng/ml; p=0.06) [281]; and Wang et al. (4.04 vs. 3.20 ng/ml; p=0.13) [260]; and also potentially by a further study that compared 52 SpA cases (number of AS cases not stated) with 26 HC (5.79 vs. 3.09 ng/ml; p<0.0001) [282]. Two studies challenge our results. Firstly, Taylan et al. showed Dkk-1 concentrations to be no different in AS cases and HC, although their Dkk-1 concentrations were very low compared with all other reports (median 0.01 ng/ml in AS, and 0.12 ng/ml in HC); and contrary to other studies they found that Dkk-1 concentrations were higher in AS patients using synthetic DMARD(s) compared with non-users, but no different in AS patients using anti-TNF compared with non-users [342]. Both issues question the reliability of the Taylan et al. results. A second paper, by Kwon et al. found Dkk-1 concentrations to be lower in AS compared with HC (12.31 vs. 20.81 ng/ml; p<0.0001) [285]; although that study had fewer AS (n=56) and HC (n=40) cases, and the disease duration of their AS patients was much lower than in our study (median 7 vs. 22 years, respectively).

We measured serum total Dkk-1, rather than serum functional Dkk-1 (a measure of the amount of serum Dkk-1 binding to its receptor LRP6). In a cross-sectional study, Yucong et al. found no difference in serum total Dkk-1 in AS compared with HC, but did find a lower concentration of serum functional Dkk-1 in AS compared with HC (9.15 vs. 17.24 pg/ml; p<0.001) [283].

Our findings of no difference in Dkk-1 concentration in pPsA cases compared with HC, is broadly supported by Daoussis et al. who compared PsA with HC [280]. Dalbeth et al. found higher Dkk-1 concentrations in the PsA cases compared with HC, but were unable to explain the differential direction of Dkk-1 concentrations in PsC (lower) and PsA (higher) cases compared with HC, when one might expect the same direction in these two related diseases. Several factors might explain the disparity between our results and those of Dalbeth et al. Dalbeth et al. did not subcategorise their 38 PsA cases into pPsA and PsSpA cases (n=127 and n=118 in our study, respectively), and they had only 12 HC compared with our 50 HC. Dalbeth et al. used the less validated R&D Duoset kits which requires coating wells with Dkk-1 antibody in-house, compared with the more validated R&D Quantikine kits that we used, that had pre-coated wells. In addition, Dalbeth et al. stored serum at -20 degrees Celsius, rather than the standard procedure of storing at -80 degrees Celsius, as we did, to avoid biomarker degradation.
8.6.4.2 Dkk-1 levels across disease groups

RAD cases had significantly higher Dkk-1 than non-RAD cases (OR\textsubscript{adj} 1.22; 95\% CI 1.05, 1.42; \(p=0.01\)). Similarly, a general pattern was seen for Dkk-1 to progressively higher along a spectrum of increasing axial disease, for example: AS cases had significantly higher Dkk-1 than PsSpA cases (OR\textsubscript{adj} 1.18; 95\% CI 1.02, 1.35; \(p=0.02\)); PsSpA cases showed a trend for higher Dkk-1 than pPsA cases (OR\textsubscript{adj} 1.06; 95\% CI 0.95, 1.19; \(p=0.28\)); and PsA cases showed a trend for higher Dkk-1 than PsC cases (OR\textsubscript{adj} 1.14; 95\% CI 0.99, 1.31; \(p=0.07\)). Collectively these results suggest that Dkk-1 concentrations increase with progressively increasing axial SpA. Daoussis \textit{et al.} found higher Dkk-1 concentrations in AS compared with PsA cases (2.73 vs. 2.44 ng/ml; \(p=0.05\)) [280]. We are the first study to compare Dkk-1 concentrations in PsSpA and AS, and RAD and non-RAD cases. Alongside clinical history, examination and imaging, these results indicate that Dkk-1 levels could potentially be used in a clinical setting to help differentiate PsSpA from AS, SpA cases with and without RAD, and possibly PsA from PsC. Of note, cutaneous psoriasis was not a significant covariate in the regression model that showed Dkk-1 levels to be higher in RAD compared with non-RAD cases. This indicates that Dkk-1 may be a biomarker specific to RAD in SpA, independently of cutaneous psoriasis, and therefore gives Dkk-1 more clinical utility.

It seems unlikely that significant baseline temporal fluctuations in serum Dkk-1 levels may explain the disparity between the results of different studies. Heiland \textit{et al.}, found that serum functional Dkk-1 levels were relatively stable over the three time-points spanning a three-year period [286]. If found to be a useful biomarker, this characteristic would add to the utility of Dkk-1 as a biomarker.

8.6.4.3 Biological explanation of results showing higher Dkk-1 levels in axial-SpA

Given that induction of the Wnt pathway promotes osteoblastogenesis, resulting in increased new bone formation, and since Dkk-1 is an inhibitor of the Wnt pathway, one might expect Dkk-1 concentrations to be progressively lower along a spectrum of diseases with increasing new bone formation (\textit{e.g.} from PsC, to pPsA, to PsSpA to AS). Whilst our Dkk-1 results are contrary to what we might expect from a simple understanding of biological pathways, our results are mostly consistent with the results of studies in AS and one study in PsA.

As described in detail in the Introduction section of this chapter, Dkk-1 levels may be higher in SpA, particularly AS, because Dkk-1 is pathologically dysfunctional. Daoussis \textit{et al.}
found that whilst serum total Dkk-1 levels are higher in AS compared with HC and PsA subjects (unadjusted for anti-TNF use), Dkk-1 binds less avidly to LRP6 (as measured by the level of functional Dkk-1) in AS compared with both HC and RA [280]. Their experiments also showed that in AS serum, despite the increased circulating levels of total Dkk-1, at an ex-vivo cellular level, the net effect (stimulatory plus inhibitory) of AS serum on the Wnt pathway is stimulatory (abnormal), as compared to inhibitory (normal) in HC serum. Daoussis et al. also showed that the addition of an anti-Dkk-1 monoclonal antibody to sera from HC led to an increase in active beta-catenin levels, indicating that Dkk-1 is acting physiologically to inhibit the Wnt pathway. The same increase in beta-catenin was not observed in AS sera, suggesting that the function of Dkk-1 in AS patients may be compromised and thus pathological. The authors concluded that Dkk-1 is dysfunctional in AS serum, and is therefore unable to inhibit the Wnt-pathway, which in turn has excessive bone-forming activity. Their study conclusions explain the progressively higher Dkk-1 levels along our spectrum patients with increasing axial-SpA. Our AS compared with HC subjects, RAD compared with non-RAD cases, and PsSpA compared with pPsA cases appear to be expressing more Dkk-1 in an attempt to compensate for the dysfunctional Dkk-1. Dysfunctional Dkk-1 is not inhibiting Wnt-pathways as it should, allowing Wnt-related bone-forming pathways to flourish unimpeded, possibly translating to the osteoproliferative phenotype of AS and RAD characterised by axial syndesmophytes and ankylosis.

In mouse-models, increased Dkk-1 levels have been associated with increased bone resorption in terms of osteopaenia [357], decreased levels with new bone formation in terms of trabecular number / thickness and cortical thickness [279], and anti-Dkk-1 monoclonal antibodies (mAbs) have been shown to induce fusion of sacroiliac joints in mice transgenic for TNF [89]. Diarra et al. used a mAb to inhibit Dkk-1, thereby reversing the bone-destructive pattern of a mouse model of RA to the bone-forming pattern of osteoarthritis. Bone erosion was halted and bony growths (osteophytes) typical of osteoarthritis formed. They identified TNF as a key inducer of Dkk-1 in their mouse inflammatory arthritis model. These data give further credence to the hypothesis that serum Dkk-1 is dysfunctional in our SpA patients, thereby explaining the reactive abnormally high serum total Dkk-1 levels detected by our study.

8.6.4.4 Dkk-1 levels in PsC

In our study, Dkk-1 concentrations were significantly lower in PsC compared with HC (OR_{adj} 0.72; 95% CI 0.61, 0.86; p=0.0002), which is consistent with the results of Dalbeth et al. who found a trend for the same [126], but different to results by Seifert et al. who
found Dkk-1 to be no different in PsC and HC [358]. Seifert et al. had much fewer PsC (n=46) and HC (n=25) cases than our study and may therefore have been underpowered to detect a difference.

We also found a non-significant trend for higher Dkk-1 concentrations in PsA compared with PsC cases (ORadj 1.14; 95% CI 0.99, 1.31; p=0.07), which is consistent with the findings of Dalbeth et al. (p<0.001) [126]. Dkk-1 levels may be higher in PsA compared with PsC, because Dkk-1 is dysfunctional in PsA but not in PsC, and therefore the body reacts by increasing expression of serum total Dkk-1 in attempt to compensate for biologically-inactive dysfunctional Dkk-1.

As has been presented so far, Wnt-proteins are regulators of cell proliferation / differentiation and potentially have a role in innate immune pathway signaling. For this reason it may be hypothesised that Wnt-pathways may be involved in non-bone pathways, particularly in diseases such as psoriasis, characterised by keratinocytes hyperproliferation and altered innate immunity. There is emerging evidence that Wnt-related beta-catenin is increased in lesional suprabasal psoriatic epidermis [359], and that Wnt16B has a role in mediating keratinocyte proliferation [360]. As mentioned earlier, Dkk-1 through Wnt-pathways regulates skin pigmentation and thickness [277]. Studies have demonstrated increased expression of Wnt5a mRNA and Wnt-5a protein psoriatic skin lesions [361, 362]. Given that Wnt pathways are known to induce angiogenesis by increased synthesis of vascular endothelial growth factor (VEGF) [363], Seifert et al. hypothesised that increased Dkk-1 expression in non-lesional psoriatic skin may inhibit angiogenesis by antagonising Wnt signaling thus preventing psoriatic plaque formation [358]. The Dkk-1 gene is pro-apoptotic, and it has therefore been hypothesised that altered Dkk-1 gene expression may lead to lower Dkk-1 levels in psoriatic lesions, resulting in less cellular apoptosis and keratinocyte hyperproliferation [358].

8.6.4.5 Relationship between Dkk-1 and radiographic severity

In PsSpA cases we found that Dkk-1 concentrations were lower in patients with vertebral erosions than those without erosions (ORadj 0.28; 95% CI 0.10, 0.80; p=0.02). No association was found in AS cases. Vascular proliferation in the form of pannus extension into articular cartilage and bone, is a critical part of erosion formation in inflammatory arthritis. In human embryonic stems cells, Dkk-1 has been shown to play an important role in the promotion of synovial angiogenesis, that could potentially lead to inflammatory pannus formation, and subsequent erosion [364].
We did not find a correlation in either PsSpA or AS cases, between Dkk-1 concentrations and radiographic severity as measured by the PASRI-total, or between Dkk-1 concentrations and osteoproliferation burden severity as measured by the PASRI-osteoproliferation domain. These results are unsurprising given that the PASRI-total encompasses diametrically opposite pathologies; both osteoproliferation and erosion. When analysed separately, a signal was detected for Dkk-1 to be lower in PsSpA cases with vertebral erosions than without. However, that result must be taken with caution given the low frequency of vertebral erosion in both PsSpA and AS cases.

No other studies have investigated PsSpA cases in terms of Dkk-1 correlation with axial radiographic severity, axial osteoproliferation burden or axial erosion burden. Dalbeth et al. compared Dkk-1 concentrations with peripheral radiographic severity in PsA patients; finding no correlation between Dkk-1 concentrations and the van Heijde composite score, joint space narrowing score, peripheral erosion score, number of joint with osteoproliferation, or number of joints with osteolysis [126].

Supporting our results in AS patients, Daoussis et al. found no correlation between Dkk-1 concentrations and axial radiographic severity using the mSASSS, nor did they find a correlation with a peripheral radiographic severity score (the modified Sharp score) [280]. Klingberg et al. found significantly lower Dkk-1 concentration (2.78 vs. 3.27 ng/ml; p=0.003) in the fourth quartile (mSASSS ≥20) compared with the first quartile (mSASSS = 0) of the mSASSS severity score [281]. This statistical difference was only found by categorising the mSASSS into quartiles, rather than treating it as continuous variable, as we did for the PASRI. There are no validated severity categories for the mSASSS or PASRI, so the use of quartiles was simply for the purpose of statistical modeling, rather than being clinically meaningful. We would therefore stand by our decision to compare Dkk-1 with PASRI as a continuous variable.

We measured serum total Dkk-1, rather than serum functional Dkk-1. Yucong et al. found that serum functional Dkk-1 levels in AS were significantly and negatively associated with the mSASSS (r = -0.39; p=0.03), and the New York grading score for sacroiliitis (r = -0.24; p=0.04), i.e. Dkk-1 concentration was lower in AS cases with higher mSASSS and higher sacroiliitis score [283]. In a longitudinal study by Heiland et al. of 65 TNF-naïve AS cases from the German Spondyloarthritis Inception Cohort (GESPIC) cohort, followed with axial radiographs at baseline, 1 and 2 years, reported that functional Dkk-1 concentration (mean of the three time-points) was significantly lower in patients with syndesmophyte growth (n=12) than in patients without (n=37) syndesmophyte growth (4.13 vs. 6.78 pg/ml; p=0.03) [286]. Serum total Dkk-1 was no different in patient with and without...
syndesmophyte growth. The authors proposed that patients with lower baseline functional Dkk-1 have less inhibition of the Wnt pathway, and therefore more osteoproliferation in the form of syndesmophytes.

8.6.4.6 Relationship between Dkk-1 concentration and anti-TNF use

Given that TNF-alpha is an inducer of Dkk-1 expression by fibroblastic synovial cells and neighboring chondrocytes (Figure 8.1), one might predict that anti-TNF agents would reduce serum Dkk-1 levels in patients using these agents. However, our exploratory analyses showed that Dkk-1 concentrations are not altered by anti-TNF use, either when the four disease groups were analysed separately, or when analysed together. One might surmise that dysfunctional Dkk-1 in SpA does not decrease following anti-TNF therapy, like it does in RA, because its dysfunctional structure responds differently to attempted induction by TNF-alpha. The published literature has conflicting results. Daoussis et al. showed that in AS, cross-sectionally measured Dkk-1 is higher in anti-TNF treated than non-treated cases, and when measured longitudinally, Dkk-1 rises following anti-TNF therapy [280]. Both findings are contrary to what one might expect given that TNF is an inducer of Dkk-1 expression. In the same study, the converse relationship was found in PsA and RA patients, whereby Dkk-1 was lower in anti-TNF treated cases, and Dkk-1 levels decreased following anti-TNF therapy in RA patients [280]. Daoussis et al. attempted to explain their Dkk-1 results in AS as being a “counter-balancing mechanism to attenuate Wnt signaling, which is turned on following resolution of inflammation in AS”. No difference in Dkk-1 concentrations in anti-TNF users and non-users was found in two other cross-sectional studies [282, 342], and one longitudinal study [285] of AS cases. The role of TNF-alpha in the pathogenesis of AS is not yet understood. Whilst patients with AS and PsSpA patients do respond to anti-TNF in terms of pain, stiffness and fatigue, data on the effects of anti-TNF therapy on radiographic progression in AS is conflicting, with some indicating inhibition of syndesmophyte progression over five years [236], and some data suggesting no effect over a shorter period of two years [237, 238]. Perhaps this is because the TNF-related Dkk-1 / Wnt pathway is one of several independent pathways mediating the pathogenesis of SpA. Pathways such as the transforming growth factor-beta (TGF-beta) [365] and bone morphogenetic protein (BMP) [281, 366] pathways have also been implicated in the pathogenesis of SpA. Lories et al. reported evidence for uncoupling of inflammation and joint remodeling in SpA, where anti-TNF did not affect the severity or incidence of joint ankylosis in a mouse model of SpA [367]. They proposed therefore that the process of entheseal osteoproliferation may be independent of TNF. The ‘TNF-alpha brake hypothesis’ proposes that TNF-alpha may act as a molecular brake, perhaps through the Wnt-pathway, to inhibit new bone formation [368]. Therefore
anti-TNF therapy may actually lead to induction of Wnt signaling in AS. However, in humans it has not yet been robustly tested or proven clinically that anti-TNF therapy translates to progression of osteoproliferation in SpA.

8.6.5 Biomarkers of PAM

None of the four biomarkers had different concentrations in PsA cases with (n=19) and without (n=182) psoriatic arthritis mutilans (PAM). This is the first reliable reported study to compare serum soluble bone and cartilage-turnover biomarkers in cases with and without PAM. Given the severe and extensive bone-remodelling observed in PAM, characterised by peripheral articular deep bone resorption resulting in osteolysis, and by extensive osteoproliferation (as described in Chapter 7), one might expect bone biomarker concentrations to be different in PAM compared with non-PAM. This was not found to be the case. This may be explained by one or both of the following reasons. One explanation is that the amount of bone remodelling taking place in the relatively small articular joints of the hands and feet is insufficient to alter circulating serum levels of bone biomarkers. Another explanation is that these patients were sampled during the refractory phase of PAM, when the destruction of PAM had passed, and only small-scale remodelling in keeping with general PsA was happening. Serum biomarker concentrations were therefore in keeping with general PsA. The results from Chapter 7, showing rapidly progressive osteolysis soon after the onset of PAM, followed by a tapering of activity, would certainly support the second hypothesis. One final, albeit less likely explanation, is that the PAM is mediated by metabolic bone pathways not involving Dkk-1, M-CSF, OPG or MMP-3.

Only one other study has reported on serum biomarkers in PAM, but their data seem unreliable [126]. In a cohort of 38 PsA cases Dalbeth et al. identified some cases that had pencil-in-cup deformity on plain radiographs of the hands and feet. Pencil-in-cup deformity is a radiographic marker of PAM, and is formed by the combination of osteolysis and osteoproliferation either side of an articular surface. Dalbeth et al. reported that M-CSF (Spearman r=0.36; p<0.05) and RANKL (Spearman r=0.41; p<0.05) concentrations positively correlated with the number of joints affected by pencil-in-cup deformity. Concentrations of Dkk-1 and OPG were not found to correlate with the number of joints affected by pencil-in-cup deformity. They did not report the number of cases with PAM. Given that PAM has an estimated prevalence of 5-6% in PsA cohorts (see Chapter 7), one would estimate that only two of their PsA cases had PAM; unless their cohort was unselected, and would therefore add bias to their data. In addition, since PAM cases have
on average 1-4 osteolytic joints (see Chapter 7), and with such low numbers of PAM cases, it is unclear how Dalbeth et al. were able to meaningfully correlate the number of joints affected by pencil-in-cup deformity with serum biomarkers concentrations. These observations question the reliability of their results.

8.6.6 General discussion of serum biomarkers in SpA

8.6.6.1 Reasons for disparity in study findings

Disparity between study findings will likely be explained by differences in study cohorts, design, and analysis methods. Most studies were cross-sectional which is appropriate for HC comparisons with disease groups, and other studies were longitudinal which is more appropriate for assessment of biomarker correlation with disease activity. Most studies had patients with recent-onset disease, rather than a mixture of both newly diagnosed and established disease patients as we did. Almost all of our SpA patients were blood sampled in the morning, which may influence some biomarker levels, whilst other studies did not state the timing of their venepuncture. We stored samples at -80 degrees Celsius and minimised the number of free-thaw cycles, whereas other studies did not. Studies used different ELISA kits used, some less validated, and there was varying stringency for assay quality control.

Disparity in study findings may in part be explained by a lack of multivariate analyses adjusted for potential confounders such as sex, age, disease duration, disease activity, pathological phase of disease, medication use, and body mass index. The prospective nature of our study allowed us to collect parameters thought to be relevant to biomarker testing, and significantly strengthened our analyses. The majority of studies never stated a power calculation for their primary analysis, and were likely underpowered to detect a difference, particularly for their secondary analyses. Through clinical and biological driven a priori hypotheses, we limited the number of comparisons that we made, and therefore did not adjust for multiple testing. Many studies have performed a lot of statistical comparisons without an a priori hypothesis, and made no adjustment for this. This may explain some of the false positive associations in the literature, that we did not corroborate.

The concentrations of biomarkers in our large study were not normally distributed. However, several studies, even very small studies such as that by Chandran et al. [88], reported means and standard deviations for their biomarker concentrations, and proceeded to perform parametric tests. Had the authors stated the distribution of their
concentrations, then the appropriateness of using parametric tests would have been confirmed. A further difference is that we compared biomarker concentrations as a continuous variable in different groups. Other studies dichotomised biomarkers as being normal or abnormal, on the basis of being two standard deviations from the mean in the healthy control group [143, 147]. This approach does not seem robust given that the HC sample was often small and perhaps not reflective of the general population. To use such a method requires a normally distributed HC sample, which seems unlikely especially if small. In addition, dichotomising data in such a way reduces the power of analyses to detect a difference between groups, compared with using data in its original continuous form.

The phase of disease when serum biomarkers are measured is a potential important consideration. Considering AS as a predominantly bone-forming disease, RA as a predominantly bone-resorbing disease, and PsA (both pPsA and PsSpA) as having both bone-forming and bone-resorbing characteristics, may be over-simplifying the situation and not taking into consideration different stages of disease, or concurrent processes in the musculoskeletal system. In AS vertebrae, the erosive process at vertebral corners (Romanus lesions) can be followed by osteoproliferative events such as vertebral sclerosis, vertebral squaring and syndesmophyte formation. In the SIJs, sclerotic changes are followed by erosive changes, and then osteoproliferation in the form of SIJ ankylosis. Heiland et al. found higher Dkk-1 levels in AS patients with progressive syndesmophytes compared with those non-progressive syndesmophytes over a two-year period [286]. Research has not yet established whether the syndesmophyte-forming phase happens at the same point after disease onset in all AS patients, at multiple time-points, or even whether it can be detected by changes in ASDAS or CRP. Our AS and PsSpA cases generally had high disease activity, as measured by the ASDAS, BASDAI and PaGA. AS patients in other studies had lower, similar and higher disease activity than those in other studies. This may explain some of the disparity in study results. It is yet to be established whether these activity indices reflect erosive disease, osteoproliferative disease, or both. If erosion is the first step in the formation of osteoproliferation, then perhaps ASDAS is reflecting global-burden of erosive processes, that will ultimately lead to osteoproliferation. Therefore statistical modelling adjusted for these covariates can never completely adjust for phase in axial pathology, such as syndesmophyte formation.

In AS and PsSpA there exists a paradox of both osteoproliferation in the axial spine, and bone resorption characterised by vertebral erosions, and osteoporosis. Bone loss can occur soon after AS onset, with even young AS patients experiencing osteoporotic fragility fractures [369]. Therefore, measuring some circulating serum biomarkers e.g. Dkk-1 and
M-CSF, may not be sufficiently tissue-specific to indicate pathology at axial tissue-level. This may explain why some serum biomarker results do not appear to align with anticipated biological pathways.

8.6.6.2 Study critique

This study has several strengths, in particular: the very large sample size compared with previously reported studies in PsA and in particular for PAM; the robust case classification system employed; no missing data for three of the biomarkers, albeit some missing data for OPG; HC samples being age and sex-matched to the PsA cohort; and the quality control and rigour of our laboratory experiments. These aspects have improved the study’s power, and the reliability of the results. All PsA and AS clinic attendees were invited to participate in the study, thereby minimising selection bias, and giving us a broad spectrum of recent-onset / established-disease patients, clinically active / inactive patients, and younger / older patients. In addition, our hospital is a secondary referral centre, rather than a centre receiving high volumes of specialist tertiary-centre referrals. This makes our study results more generalisable to SpA patients in other centres, and our findings translatable to real-world clinical practice. This is an important tenant for biomarker research, especially if intended to be used in clinical practice, rather than just for research.

This study has its limitations. The cross-sectional design of this study limits the interpretation of our analyses correlating biomarker concentrations with outcomes such as CRP and ASDAS. This would have been better investigated through a longitudinal study with biomarkers and outcomes indices measured at several time-points and then tested for correlation. However, doing so would have been more time-consuming, costly, and enrolment of patients to a study requiring multiple attendance for venepuncture and clinical assessment would have hampered recruitment.

Our fifty HC samples were obtained from Health Survey England, and whilst subjects contributing these samples were screened for disease and medication use, the reliability of these screening methods can not be verified. A higher proportion of our HC samples (18%), compared with our disease samples (1-15%) had missing OPG data because intra-sample variation (CV%) of ≤15% was difficult to attain. This meant that comparisons of OPG levels in HC compared with disease groups may have been less well-powered than for other biomarkers. Reassuringly, missing OPG data was relatively proportional across the disease groups, likely resulting in similar magnitude and direction of confounding. Some of our analyses e.g. comparison of biomarker levels with axial erosion...
burden, had too few patients with erosions to make robust comparisons, especially adjusted comparisons. Caution when interpreting those results is therefore recommended.

Our pPsA, PsSpA and AS samples were only subject to one cycle of freeze-thaw between venepuncture and ELISA testing, and were always stored at -80 degrees Celsius. However, as HC and PsC samples were sourced from other centres, it is possible that they may have been subject to several freeze-thaw cycles, and not always stored at -80 degrees. This may have resulted in some biomarker degradation in the HC and PsC samples.

One must also consider the presence of uncontrolled confounding. As mentioned in Chapter 5, despite our best attempts with the data collected, time-varying variables such as disease activity (ASDAS), BMI and medications can not been fully adjusted for in cross-sectional studies such as this. BMI and medication use change over time. It is not known how long these factors impact on biomarker levels, if there is a differential effect once certain thresholds of BMI are reached, or if certain anti-TNF agents differentially affect some biomarkers. On balance, for these two time-varying variables, the direction and magnitude of their confounding is unlikely to be significantly different across the four disease groups, and therefore their effect on the results of this study may be minimal.

We also acknowledge that there are unmeasured confounders, of which some we may not even be aware. For example, corticosteroid medications have been reported to alter MMP-3 levels [147]. We did not collect data on corticosteroid use, as it is difficult to comprehensively do so because it is time-varying variable with a variety of doses, modes of administration and durations of therapy used. Corticosteroids are less likely to have been used in HC, PsC and AS patients compared with pPsA and PsSpA patients. This may have confounded the comparisons of MMP-3 levels in these groups. Similarly, oral contraceptive pill use, phase of menstrual cycle, diet / meal composition, and timing of sample after ingestion have been shown to alter some serum biomarkers [150, 339]. We did not collect these parameters. The AS and HC subjects were generally younger than the other three groups, so the females in the AS and HC groups may have been more likely to have used the oral contraceptive pill, or still be menstruating. This may have resulted in differences both within and between groups. The PsC samples were collected from patients living in Michigan, who may have had a different diet and / or lifestyle to the SpA patients living in south-west England. This may have confounded our comparisons of biomarker levels in PsC versus HC, and PsC versus PsA.
As mentioned earlier in the Discussion, our results would have been strengthened and provided more biological insight had we also measured active-MMP-3 rather than total MMP-3, measured functional Dkk-1 and/or Dkk-1 biological activity rather than just total Dkk-1, and measured bone mineral density.

8.6.7. Conclusions

We have compared a large cohort of well-characterised PsSpA, pPsA, AS and PsC cases with a group of matched HC to determine biomarkers of axial/peripheral inflammatory arthritis occurrence, and radiographic severity. OPG appears to be a biomarker of axial disease in patients with SpA (pPsA, PsSpA and AS). It can therefore be used to differentiate PsSpA from pPsA, and axial-SpA from peripheral-only SpA patients independently of psoriasis status. Dkk-1 appears to be a biomarker of axial disease in SpA; with a pattern for increasing concentrations along a spectrum of increasing axial involvement. It can therefore be used to differentiate PsSpA from AS patients, axial-SpA from peripheral-only SpA patients irrespective of psoriasis status, and potentially PsSpA from pPsA patients. Both M-CSF and MMP-3 appear to be biomarkers of arthritis, differentiating SpA (PsSpA, pPsA and AS) from HC and PsC. However, neither M-CSF nor MMP-3 appear to differentiate various forms of SpA, i.e. pPsA, PsSpA and AS. Only OPG has shown signals to be a biomarker of radiographic severity and osteoproliferation burden, although only in PsSpA, and the clinical significance of this remains be determined. No biomarkers for the occurrence of PAM in PsA cases were found. The high concentration of Dkk-1 in AS and PsSpA compared with HC, supports previous reports that Dkk-1 may be dysfunctional in SpA. Since bone mineral density was not measured, it is difficult to determine if these serum biomarkers are reflecting SpA-related pathology in trabecular bone, or vertebral corners and sacroiliac joints. In the next chapter, ‘Discussion of Thesis’, the wider implications of our results in the context of clinical practice and future biomarkers research, will be discussed in greater detail.
8.7 PUBLICATIONS ARISING FROM THE WORK OF THIS THESIS

CHAPTER

Manuscript for submission in preparation.
9 CHAPTER IX: DISCUSSION OF THE THESIS

The aims of this thesis were to determine the prevalence and characteristics of psoriatic spondyloarthritis (PsSpA) and psoriatic arthritis mutilans (PAM) in a cohort of psoriatic arthritis (PsA) patients, and to determine biomarkers of these PsA phenotypes. In the order that they relate to the ‘Aims and Objectives’ of this thesis, the key findings of the studies comprising this thesis will now be highlighted and discussed, in particular their wider meaning in the context of clinical practice and how they may inform future research. The Discussion section of each of the earlier chapters have already critiqued the studies in this thesis and compared our results with the published literature. Those discussions will therefore not be repeated unless specifically relating to a particular theme.

9.1 DISCUSSION OF THESIS OBJECTIVE 1: Prevalence and characteristics of psoriatic spondyloarthritis, and a comparison with ankylosing spondylitis

Objective 1(a) of this thesis was to determine the prevalence and characteristics of axial involvement (PsSpA) in a PsA cohort, using patient reported outcome measures (PROMs), clinical examination and radiographic indices. Objective 1(b) was to compare the pattern of axial involvement in PsSpA with that of an ankylosing spondylitis (AS) cohort, using PROMs, clinical examination and radiographic indices. A prospective single-centre cross-sectional study was conducted to achieve these objectives. The key findings of the study comparing the clinical characteristics of PsSpA, AS and peripheral-only PsA (pPsA) are summarised and discussed below.

• A significant proportion of SpA patients had PsSpA (48.16%). PsSpA therefore represents a common and important PsA phenotype to diagnose and manage. The robust radiographic and clinical classification system employed in this study strengthens the reliability of our estimate of the prevalence of PsSpA in SpA, especially when compared with the varied estimates in the published literature.

• PsSpA was often symptomatically-silent but radiographically-evident (25.42%). This finding may prompt and justify the baseline and interval screening of PsA patients for PsSpA as part of routine clinical care. A prospective longitudinal study investigating the natural clinical and radiographic history of such patients is needed, especially to determine if there is progression to axial symptoms, restricted metrology, function and
or disability. This clinical approach would be further justified if future research demonstrates that pharmacological and physical therapies halt or temper the progression of asymptomatic but radiographically-evident PsSpA.

• A significant proportion of SpA patients fulfilled diagnostic / classification criteria for two diseases. The modified New York (mNY) diagnostic criteria for AS were fulfilled by 48/201 (23.88%) of PsA cases, and the Classification of Psoriatic Arthritis (CASPAR) criteria were fulfilled by 49/201 (24.38%) of AS cases. These results question the validity of the CASPAR and mNY criteria for use in PsSpA. To prevent the misclassification of PsSpA cases as PsA or AS, thereby altering their course of management and eligibility for more specialist biological therapies, the inception of PsSpA-specific classification criteria should be a high research and clinical priority. As discussed in previous chapters, the variety of classification systems used for PsSpA has led to varied and conflicting results when comparing the clinical, radiographic and genetic characteristics of PsSpA with pPsA and AS. An international consensus classification system for PsSpA can only serve to improve clinical care and future research efforts. As discussed in chapter 5, the establishment of a ‘SpA clinic’, rather than separate PsA, AS and general rheumatology clinics, may improve the ‘patient journey’, facilitating correct diagnosis, and both appropriate and timely instigation of specialist therapies. Our data does not detail exactly how the PsSpA cases accessed anti-TNF therapy, and whether their original diagnoses were reconsidered according to clinical need.

• Clinical predictors for a PsA patient developing axial disease (i.e. having PsSpA rather than pPsA) included: **HLA-B27** positivity, presence of inflammatory bowel disease (IBD), younger age at arthritis symptom onset, and higher psoriatic nail disease severity as measured by the mNAPSI, in particular nail onycholysis severity. Male PsA cases were no more likely to be classified as PsSpA than female patients. As discussed in chapter 5, these clinical predictors are biologically plausible, thereby adding to the credence of these results. **HLA-B27** testing is readily available and inexpensive in most hospitals in the U.K.. The other clinical parameters are easily determined through history taking and clinical examination. The clinical predictors of PsSpA occurrence identified by this study are therefore attainable and feasible in clinical practice, and should prompt the identification of PsSpA in symptomatic patients and / or screening of asymptomatic patients. The positive predictive value of these clinical predictors should be tested during any future inception of a classification system for PsSpA. The high prevalence of IBD in PsSpA compared with pPsA patients should trigger the investigation and early management of PsA patients with compatible
gastrointestinal symptoms. The markedly different prevalence of HLA-B27 positivity in PsSpA, pPsA and AS cases does imply that this class I receptor has a pathogenic role in PsSpA, but not in pPsA, and perhaps a slightly different pathogenic role in AS. Further investigation is warranted, and as will be discussed later in this chapter, the role of HLA- and non-HLA genetic variants in PsSpA will be studied as part of post-doctoral research.

- Function as measured by the BASFI, was poorer in AS compared with PsSpA cases. However, AS and PsSpA cases had similarly high disease activity (as measured by the ASDAS, BASDAI, and PaGA), similarly poor metrology (as measured by the BASMI), and similar disability (as measured by the HAQ). Collectively, these results indicate that PsSpA has just as significant a clinical impact on patients as does AS. The management of AS has been the focus of much research over the last few decades. There has been a dearth of clinical trials for PsSpA. Much has therefore been borrowed from AS, in terms of pharmacological and physical therapies, to manage PsSpA. This may not be entirely appropriate in light of the clinical, radiographic, genetic and serum biomarker differences this thesis has shown exist between the two conditions. The clinical impact of PsSpA on patients that we have demonstrated, will hopefully serve to emphasise the need and prompt clinical trials specifically investigating PsSpA. These results also underline the pressing clinical need of PsSpA patients for more intensive therapies, such as biologics, at a level on par with AS. The health economics of using such costly agents needs further investigation, but will likely be similarly justified.

PsSpA and AS patients were compared in terms of axial radiographic features and severity. The specific objectives were to compare: (i) the pattern of radiographic axial disease in PsSpA and AS; (ii) the morphological (osteoproliferation and erosion) features of radiographic axial disease in PsSpA and AS; (iii) axial radiographic severity in PsSpA and AS. The key findings of this radiographic comparison, and their wider impact on the clinical care and research of PsSpA are detailed below.

- A significant proportion of PsSpA cases had spondylitis without sacroiliitis (33.05%), whilst 38.14% had sacroiliitis with spondylitis, and 28.81% had sacroiliitis without spondylitis. The majority of PsSpA cases with spondylitis-alone were symptomatic (56.41%); although were less likely to be symptomatic than PsSpA cases with sacroiliitis with / without spondylitis. These results are important in the broader context of PsA for several reasons. Firstly, spondylitis without sacroiliitis is important clinically if the majority of such patients are symptomatic; it is not an incidental finding with no
clinical consequence. Secondly, this may indicate that even within PsSpA, axial
disease can have varying degrees of expression radiographically and in terms of
symptoms. A prospective longitudinal study with interval radiographs and PROMs is
needed to clarify if patients with spondylitis-alone, develop sacroiliitis and / or become
symptomatic. Perhaps most importantly, any international consensus classification
system for PsSpA developed in the future would need to capture PsSpA patients with
spondylitis alone. Our results indicate that only classifying PsA patients with sacroiliitis
as having PsSpA would overlook a significant proportion of symptomatic individuals
with genuine inflammatory spinal disease.

- **HLA-B27** positivity was significantly more likely in PsSpA cases with both sacroiliitis
  and spondylitis, compared with PsSpA cases with spondylitis-alone. Broadly this
  suggests that **HLA-B27** has a differential pathogenic role in PsSpA, and may be more
  involved in pathways leading to sacroiliitis rather than spondylitis. As will be discussed
  later in this chapter, I will investigate the role of **HLA**- and non-**HLA** genetic variants on
  the radiographic and clinical phenotype expression of PsSpA as part of post-doctoral
  research.

- AS cases were more likely than PsSpA cases to have complete ankylosis of the SIJs,
  and vertebral bridging syndesmophytes. These radiographic features may be more
  useful than paramarginal syndesmophytes, in both clinical and research settings to
distinguish PsSpA from AS cases [59, 191]. As was discussed in chapter 6,
  subsequent studies [58] found paramarginal syndesmophytes to not be uncommon in
  AS. In addition, paramarginal syndesmophytes can be difficult to distinguish from the
degenerative osteophytes of osteoarthritis. Our study therefore offers two unique and
morphologically distinct radiographic features to differentiate PsSpA from AS. These
findings along with the results on radiographic severity below, give a unique insight
suggesting that PsSpA does not have the same degree of osteoproliferation as AS;
with PsSpA-related osteoproliferation less likely to completely bridge the SIJ or
intervertebral disc space.

- Both PsSpA and AS cases tended to have bilateral symmetrical-grade sacroiliitis, with
  no difference between the two groups in the occurrence of SIJ sclerosis, erosion or
  partial ankylosis, or in terms of cervical vertebrae spondylitis, lumbar vertebrae
  spondylitis, or cervical facet joint fusion. Unlike several previous studies [58, 59, 191],
  our analyses adjusted for both disease duration and age, which allowed us to
  accommodate for potentially different ages at onset of radiographic axial disease in
  PsSpA and AS cases. Previous studies have proposed that unilateral grade 2
sacroiliitis is common in PsSpA and can therefore be used to classify SpA patients as PsSpA. Our results would challenge that position, and favour using a definition of sacroiliitis in PsSpA that is consistent with AS (i.e. bilateral sacroiliitis of grade ≥2, or unilateral sacroiliitis of grade ≥3). Our results could therefore inform the radiographic criteria of any future classification systems for PsSpA.

- Radiographic axial disease was more severe in AS compared with PsSpA, as measured by the Psoriatic Arthritis Spondylitis Radiology Index (PASRI), modified Stoke Ankylosing Spondylitis Spinal Score (mSASSS), and osteoproliferative burden, although not erosion burden. Despite the differences, radiographic axial disease severity in PsSpA was not mild; it was clinically important and should be actively treated. This is the first study to compare radiographic severity in PsSpA and AS using the more PsA-specific PASRI score, and the mSASSS. Our exploratory analyses showing moderate-to-good correlation between metrology (BASMI) and axial radiographic indices (PASRI and mSASSS) both globally and regionally. However, it is notable that this difference in radiographic severity between the two conditions did not translate into worse metrology (BASMI), disability (HAQ) or disease activity (ASDAS, BASDAI, or PaGA) in AS compared with PsSpA patients. As has been shown to be the case in osteoarthritis of the hip and knee [370-372], in PsSpA and AS there also appears to be some disassociation between radiographic severity and clinical impact. This may in part be explained by the differential impact of pharmacological and physical therapies on clinical symptoms compared with radiographic structural damage. Unlike magnetic resonance imaging (MRI), plain radiographs do not indicate new or active structural damage. As has been discussed in previous chapters, patients also have differing degrees of physical, psychological and pain-management adaptations to their disease, thereby differentially circumventing their radiographic structural damage. In clinical practice, these study results should remind the clinician to modify clinical care in PsSpA and AS patients according to clinical symptoms and examination, rather than necessarily on the basis of radiographic severity.

9.1.1 Clinical and radiographic research of spondyloarthritis

Correct classification of cohorts is important for research, particularly for biomarker and prognosis research. Unlike rheumatoid arthritis which has a more homogenous phenotype, PsA is a heterogenous disease, with several distinct phenotypic expressions. Given the diversity of diseases under the umbrella term of SpA, further insight into SpA will only be learnt if cohorts are carefully characterised clinically, and through imaging and biomarkers. The Classification for Psoriatic Arthritis (CASPAR) criteria are a notable
example whereby the introduction and uptake of a classification system for PsA, has homogenised cohorts and improved the quality of international research. Much has been published debating whether clinicians and researchers should be ‘combining’ or ‘splitting’ cohorts. Grouping SpAs in a simple manner such as peripheral-SpA and axial-SpA, can only impede research of this complex collection of overlapping but distinct diseases. ‘Combining’ may be appropriate for management pathways where there is much overlap in treatment strategies, and homogenising cohorts allows more uniform access to treatments, and diagnostic nomenclature is more interpretable for patients and non-specialists. However, for biomarker and prognosis research I would advocate ‘splitting’ and refining cohorts into phenotypes and subphenotypes. When investigating specific metabolic bone pathways relating to new bone formation, it would seem sensible not to ‘combine’ patients who are bone formers with those who are bone resorbers into one category, and have a mixed group of quite different subphenotypes.

It could be argued that studies to date have not clinically characterised cohorts well enough, and have therefore had limited success in biomarker research. Therein lies the strength of this current study, with a highly clinically- and radiographically-characterised cohort. Four decades ago, HLA-B27 variants were found to be more common in PsSpA compared with pPsA [110]. The results of this thesis, and of two other recent studies suggest that the phenotypic expression of PsSpA itself, may be determined by HLA-variants and / or their haplotypes [102, 106]. However, relatively little is still known on how HLA-B27 variants translate biologically into the phenotype of PsSpA. Such research would be impeded by combining SpA cohorts with different phenotype and genotype expressions.

An interesting concept that should guide future research of PsA, in particular PsSpA research, was proposed by Chandran et al. [61]. Chandran et al. proposed that radiographic damage, nail dystrophy, and periostitis might indicate severity of the disease, instead of being markers of pathogenic events. As disease severity increased, more structures were involved, and so in fact, patients with peripheral arthritis plus axial arthritis, periostitis, and nail dystrophy represented the complete PsA disease spectrum, whilst patients with a few manifestation represented incomplete PsA [61].

The knowledge gained from the clinical experience of looking after SpA cases longitudinally should not be underestimated, as many characteristics and end-points do not lend themselves well to observational medical research. Gladman et al. demonstrated disparity between patient-reported (such axial stiffness / pain) and clinician-
measured clinical indices (such as axial range of movement), and their potential limitation for reliability when used in longitudinal studies of PsSpA [34].

9.2 DISCUSSION OF THESIS OBJECTIVE 2: Prevalence, characteristics and natural radiographic history of psoriatic arthritis mutilans

Objective 2 of this thesis had three key aims: (i) to determine the prevalence of psoriatic arthritis mutilans (PAM) in a cohort of PsA cases; (ii) to compare the clinical characteristics of PAM and non-PAM cases in a cohort of PsA cases using PROMs, clinical history and radiographic indices; (iii) to determine the natural radiographic history of PAM. These objectives were met through the performance of a retrospective cohort study of all PsA patients attending a single centre, with a review of all PsA patients’ hands and feet radiographs, followed by scoring of sequential radiographs of PAM cases. The key study findings are described below.

• Of 610 PsA cases, 36/610 (5.9%) had PAM.
• PAM was more likely in PsA cases with psoriatic nail dystrophy, and younger age at diagnosis of PsA. Nail dystrophy also predicted more severe osteolysis.
• Compared with non-PAM cases, PAM cases had more disability (as measured by the HAQ), and radiographic axial disease, especially radiographic sacroiliitis.
• PAM cases were more likely than non-PAM cases to have used a synthetic DMARD, but not an anti-TNF agent. Given that 29/33 PAM cases had initiated a synthetic DMARD and 4/13 had initiated anti-TNF prior to first demonstration of PAM, within the constraints of this study’s design and this being a secondary analyses, it appears neither agent prevents the onset of PAM.
• During follow-up, PAM tended to be progressive from monoarticular to polyarticular involvement. Certain joints were more frequently affected.
• The majority of PAM-related damage occurs soon after the onset of osteolysis, with a monophasic pattern of activity. Osteolysis was initially rapid and progressive in the hands and feet, tapering later during disease course.

These results in the context of previous research, have already been discussed in chapter 7 and will therefore not be repeated here. Further research is needed to investigate particular aspects of this rare but severely destructive subphenotype of PsA. In particular, little is known of the pathogenesis of PAM, serum-soluble and genetic biomarkers of PAM, and whether anti-TNF or anti-resorptive agents may offer therapeutic efficacy. Serum
soluble bone-biomarkers of PAM have been investigated in chapter 8 of this thesis, and the results are summarised below. Genetic biomarkers of PAM and the chronology of pathological events leading to PAM have yet to be determined, and I plan to undertake this as part of post-doctoral research (see below under heading 9.5). The efficacy of anti-TNF or anti-resorptive agents for PAM can only be reliably determined through a clinical trial. However, given the rarity of PAM and it relatively narrow monophasic period of activity, much research may initially need to performed in proof-of-concept animal models of PAM. Knowledge of abnormal immune pathways involved in the pathogenesis of PAM, through serum and genetic biomarker studies, as provided by this thesis, would certainly guide and facilitate that research.

9.3 DISCUSSION OF THESIS OBJECTIVE 3: Serum-soluble bone-turnover biomarkers of psoriatic spondyloarthritis

9.3.1 Systematic review of the literature on serum-soluble bone and cartilage-turnover biomarkers in PsA and PsSpA

A systematic review of the literature on serum-soluble bone and cartilage-turnover biomarkers in PsA and PsSpA was performed. The key findings of the systematic review are described below.

- No studies have investigated serum bone and cartilage-turnover biomarkers in PsSpA; either as a primary objective, or as part of secondary analyses. Only ten studies have directly compared serum bone and cartilage-turnover biomarkers in PsA and healthy controls (HC). Some of these studies proceeded to compare the same biomarkers in PsA and psoriasis-only patients without arthritis (PsC).
- These studies have used a variety of study designs, classification systems for PsA patients, patient selection methods, laboratory methods, clinical end-points, and analysis techniques. Meta-analysis of the study results was therefore not possible. The majority of studies have had small sample size, and have likely been underpowered to detect a difference.
- Several serum bone and cartilage-turnover biomarkers have shown signals for being able to differentiate PsA from HC, PsA from PsC, and PsC from HC. Some biomarkers have shown signals for association with demographic, clinical, radiographic and laboratory parameters (Figure 2.2).
- The systematic review identified four candidate biomarkers that warrant further investigation for association with PsA and PsSpA in the first instance, and
subsequently for association with hard end-points such as radiographic outcome. These included: Dickopff 1 (Dkk-1), macrophage colony-stimulating factor (M-CSF), osteoprotegerin (OPG), and matrix metalloproteinase-3 (MMP-3).

The results of this systematic literature review guided our methods and choice to investigate four candidate biomarkers in subjects (AS, PsSpA, pPsA, PsC and HC) enrolled in our study, as detailed in chapter 8 of this thesis. The systematic literature review has been published in a high-impact peer-reviewed journal, and it is hoped that this synthesis of the existing canon of knowledge and identification of areas of limited evidence, will inform and promote future serum biomarker research in the broader SpA research community.

9.3.2 OBJECTIVE 3a: To investigate four candidate serum-soluble bone-turnover biomarkers as predictors of PsSpA occurrence, by comparison with PsA, AS, PsC cases and HC subjects

Chapters 5 and 6 of this thesis showed that PsSpA is characterised by pathological new bone formation (osteoproliferation) and bone loss (resorption), with a differing pattern and magnitude to that seen in pPsA and AS. We hypothesised that this may be reflected by differing levels of serum-soluble bone-turnover biomarkers in PsSpA, pPsA, AS, PsC and HC. We therefore compared a large cohort of 651 well-characterised PsSpA, pPsA, AS and PsC cases with a group of matched HC to determine biomarkers of axial / peripheral inflammatory arthritis occurrence, and radiographic severity. The key findings of this study are listed below.

• OPG appears to be a biomarker of axial disease in patients with SpA (pPsA, PsSpA and AS). It can therefore potentially be used to differentiate PsSpA from pPsA, and axial-SpA from peripheral-only SpA patients irrespective of psoriasis status.
• Dkk-1 appears to be a biomarker of axial disease in SpA; with a pattern for increasing concentrations along a spectrum of increasing axial involvement. It can therefore potentially be used to differentiate PsSpA from AS patients, axial-SpA from peripheral-only SpA patients irrespective of psoriasis status, and potentially PsSpA from pPsA patients.
• Both M-CSF and MMP-3 appear to be biomarkers of arthritis, differentiating SpA (PsSpA, pPsA and AS) from HC and PsC. However, neither M-CSF nor MMP-3 appear to differentiate various forms of SpA, i.e. pPsA, PsSpA and AS.

These results in the context of clinical practice and future research will now be discussed. The results of this study indicate that Dkk-1 and OPG are biomarkers of axial disease in
SpA. In a clinical setting this may be useful for several purposes: to identify asymptomatic PsA patients with PsSpA; and to risk-stratify PsA patients with non-specific but suggestive inflammatory axial symptoms and / or subtle axial radiographic changes, who are likely to develop more florid PsSpA. Of note, in statistical models demonstrating these associations, cutaneous psoriasis was not found to be a significant covariate, indicating that the association of OPG and Dkk-1 with axial disease in SpA, is independent of cutaneous psoriasis.

Dkk-1 also appears to differentiate PsSpA from AS, with Dkk-1 levels being higher in AS patients. Following the establishment of sensitivities and specificities for different concentrations, this may be helpful to differentiate patients with PsSpA from those with AS and coincidental psoriasis. These data also fuel the debate on whether PsSpA and AS are separate clinical entities determined by different pathological pathways, or the same disease determined by the same pathological pathway, with an altered phenotype expression due to genetic and / or environmental factors.

Both M-CSF and MMP-3 appear to be biomarkers of arthritis. In a clinical setting this may be useful in several situations. Firstly, it not uncommon for PsA patients to initially present with non-specific symptoms, limited clinical signs of inflammatory arthritis, normal plain radiographs and / or normal acute-phase response. M-CSF and MMP-3 may help differentiate individuals with and without inflammatory arthritis in keeping with PsA. Secondly, if testing for these two laboratory biomarkers were readily available and inexpensive, they may be more appealing for use in clinical practice than further imaging with Magnetic Resonance Imaging (MRI) or ultrasound, which can be less readily available and dearer. Thirdly, M-CSF and MMP-3 could be investigated further in terms of their sensitivity and specificity for detecting subclinical arthritis in PsC patients; either in PsC patients with suggestive but non-specific symptoms, or as a wider screening tool for arthritis in all PsC patients. The early detection of subclinical arthritis in PsC patients, and potential for intervention to halt the full expression of PsA, is currently very topical in the SpA research community. In fact, this forms a component of one work-stream of investigation in the National Institute for Health Programme Grant ‘PROMPT’ study being led by the Royal National Hospital for Rheumatic Diseases in Bath.

As reviewed in the Discussion section of chapter 8, some of the directions of associations were surprising at first glance. In particular, the higher concentration of serum total Dkk-1 along the spectrum of increasing axial disease. One might expect serum Dkk-1 levels to be lower in AS and PsSpA, since both are diseases characterised by osteoproliferative changes, and a mouse model has shown that blockade of Dkk-1 results in sacroiliac joint
fusion [89]. However, my review of the AS literature, which is more extensive than the PsA literature, showed that serum total Dkk-1 levels are in fact higher in AS than HC, PsA and RA subjects. Our results can be explained from a biological perspective by the findings of a study by Daoussis et al. who found that Dkk-1 is dysfunctional in AS, in terms of its binding to the LRP6 receptor and its induction of beta-catenin-mediated intracellular events, that would normally have led to inhibited osteoblastogenesis and decreased bone formation. Unfortunately, corroborating this hypothesis by measuring functional Dkk-1 levels and downstream effects of Dkk-1 using cell-cultures is beyond the remit of this thesis. However, this would certainly be a highly interesting avenue of further research.

Similarly, from a biological perspective one might have expected OPG to be higher in axial-SpA, due to the osteoproliferative characteristics of these conditions. We found the converse to be true, with low OPG levels in axial-SpA compared with peripheral-only-SpA patients. The low serum OPG levels may be allowing unimpeded RANKL-mediated trabecular bone resorption. The circulating serum OPG measured in this study therefore likely reflects the more large-scale processes leading to bone mineral density loss (i.e. osteoporosis) often seen in AS, rather then smaller-scale osteoproliferative changes in the relatively small axial joints.

9.3.3 OBJECTIVE 3b: To investigate four potential serum-soluble bone-turnover biomarkers as predictors of disease severity in patients with PsSpA

Objective 3(a) of this thesis was to investigate four potential serum-soluble bone-turnover biomarkers (OPG, Dkk-1, MMP-3 and M-CSF) as predictors of disease severity in patients with PsSpA. Axial radiographs of the PsSpA and AS patients were scored using the PASRI, and compared with serum concentrations of each of the four biomarkers, separately for PsSpA and AS. The results of this comparison are summarised below.

- Only OPG showed signals to be a biomarker of radiographic severity as measured by the PASRI-total score, and osteoproliferation burden as measured by the PASRI-osteo proliferation domain score. However, this correlation was only demonstrated in PsSpA, and not in AS cases.
- Levels of Dkk-1, M-CSF, and MMP-3 did not correlate with axial radiographic severity or morphology.

The clinical significance and biological plausibility of these results remains to be determined, particularly since OPG showed correlation only in PsSpA patients, and because OPG levels were lower in patients with axial disease compared to those without axial disease and HC.
A biomarker of axial radiographic severity would be particularly useful. As discussed in the Introduction chapter of this thesis, a few clinical, laboratory and genetic prognostic markers of disease severity have been identified in PsA. Radiographic severity is a hard end-point, and therefore a good surrogate of overall disease severity. Patients with poor prognostic biomarkers could be targeted for more intensive pharmacological therapy in the form of biologics (e.g. anti-TNF), and closer clinical and radiographic monitoring for disease progression. Patients with few poor prognostic biomarkers could be saved the inconvenience and potential side-effects of intensive therapies. From a public health perspective, the use of dearer biologic therapies and more frequent healthcare utilisation, is more justified in patients with poor prognostic biomarkers, if these interventions are ultimately shown to alter disease course.

Correlation of a serum biomarker with disease severity, but in particular a morphological feature such as osteoproliferation or erosion, may indicate the pathway leading to that pathology. For example, this may give insight into the biological pathways leading to osteoproliferation, and thus potential candidates within that pathway that may be amenable to pharmacological alteration, in order to correct the pathological imbalance in that axis. Just as when anti-TNF therapy was first introduced for the treatment of inflammatory arthritis, caution must be taken with this approach, as not to inadvertently cause side-effects such as cancer or tuberculosis. Altering one cytokine or chemokine in a complex biological pathway with multiple mediators, could have unwanted downstream effects. For example, altering the OPG-RANKL axis to inhibit osteoporosis, could inadvertently cause pathological high bone mass elsewhere.

9.3.4 OBJECTIVE 3c: To investigate four potential serum-soluble bone-turnover biomarkers as predictors of PAM occurrence, by comparison with non-PAM PsA cases

Given the severe and extensive bone-remodelling observed in PAM, characterised by peripheral articular severe deep bone resorption resulting in osteolysis, and by extensive osteoproliferation, one might expect bone biomarker concentrations to be different in PAM compared with non-PAM. We tested this hypothesis, and found that:

- None of the four biomarkers tested were predictors for the occurrence of PAM in the PsA cases.

As discussed in more detail in chapter 8, this is most likely explained by a combination of two reasons. Firstly, the amount of PAM-related bone remodelling taking place in the relatively small articular joints of the hands / feet is insufficient to alter circulating serum
levels of bone biomarkers. Secondly, the PAM patients had blood sampling performed during the refractory phase of PAM, once the monophasic destruction of PAM had passed, and only small-scale remodelling in keeping with general PsA was active. Our results from chapter 7, showing rapidly progressive osteolysis soon after the onset of PAM, followed by a tapering of activity, would certainly support this reasoning. The search for a biomarker of PAM occurrence would have been improved by a longitudinal rather than cross-sectional study design. However, given the rarity and natural history of PAM as determined in chapter 7 of this thesis, such a study would require two-yearly interval serum biomarker testing and imaging over a period spanning 20-40 years. This is certainly beyond the remit of the current thesis, and such a study would be logistically challenging and costly to perform. However, this observation does underline the importance of our serum biomarker study in PAM. Few other centres have as many well-characterised robustly-classified PAM cases as in this study, and therefore no other study has compared serum bone-biomarker levels in PAM and non-PAM PsA cases. Unlike time-varying variables such as serum biomarkers, genetic variants do not change over time and therefore do not necessarily require a longitudinal study to investigate them as biomarkers for PAM occurrence. Haroon et al. took advantage of this fact and showed an association between osteolysis, a radiographic surrogate of PAM, and a variant of the HLA-C*02:02:02 locus [106]. As will be discussed under subheading 9.5 of this thesis chapter, we also plan to compare both HLA- and non-HLA variants in our PAM and non-PAM PsA cases. Compared with our serum analyses that comprised 19 PAM and 182 non-PAM cases, our genetic analyses will comprise up to 38 PAM cases, and up to 480 non-PAM PsA. The analyses will therefore be better powered to detect differences.

Given the ferocity of destruction resulting in hand / feet deformity and disability, as shown in chapter 7, the identification of a biomarker of PAM occurrence in PsA would have been particularly useful clinically. PsA patients with serum biomarker levels heralding the onset of PAM could be targeted for more intensive therapies to either halt or impede PAM progression. Biomarkers could also prompt the need for closer clinical and radiographic monitoring. Although not designed to address the specific question, the results of chapter 7 indicate that anti-TNF therapy does not prevent the onset of PAM. Given the rarity of PAM, few clinical trials have robustly reported the clinical efficacy of pharmacological therapies in PAM. As discussed in chapter 7, the pathogenesis of PAM has yet to be determined. A serum biomarker of PAM occurrence may give insight into the pathology of PAM, inform proof-of-concept pharmaceutical trials for PAM, and potentially serve as a biomarker for disease monitoring in clinical practice and / or clinical trials.
9.3.5 The importance of biomarker research in spondyloarthritis

During the process of testing potential covariates to include in the regression models comparing serum biomarker levels in the different groups, our exploratory analyses demonstrated that concentrations of some of the four serum biomarkers correlated with axial disease activity as measured by the ASDAS. The conclusions that can be drawn from these exploratory analyses are limited by the cross-sectional nature of this study. The best way to explore the relationship would be through a longitudinal study with biomarker concentrations and ASDAS measured at several time-points, both during a period of no treatment alteration, and also perhaps before and after the commencement of anti-TNF therapy. This would give insight into three other potential utilities of biomarkers: monitoring disease, monitoring treatment response, and predicting response to treatment. The identification of biomarker predictors of treatment response is currently an important research agenda in SpA and rheumatology in general. Up to 30-40% of PsA and AS patients do not respond to biological therapies [25, 26, 334, 373], and even the six-month period trialing a biologic-therapy before assessing efficacy, can be costly. C-reactive protein (CRP) does not hold the same value for monitoring disease activity in PsA and AS, as it does in rheumatoid arthritis (RA). In PsA and AS it is often within the normal range, and is less responsive to change in axial disease activity [48]. An alternative serum biomarker to CRP to monitor disease activity, progression, and treatment response would therefore be very valuable in both clinical and research settings. Such a serum biomarker would hopefully be more available and cheaper than magnetic resonance imaging, and be more sensitive to change than plain radiographic progression which can take several years.

Our analyses of covariates to include in the regression model for the primary analyses indicate that there is: a significant and positive correlation between ASDAS and M-CSF in AS and PsSpA; a significant and positive correlation between ASDAS and both MMP-3 and Dkk-1 in AS; and a significant and negative correlation between ASDAS and OPG in AS. In addition, our analysis of covariates also found that there is: a significant and positive correlation between CRP and MMP-3 in pPsA, PsSpA and AS; a significant and positive correlation between CRP and both M-CSF and Dkk-1 in AS; and a significant and negative correlation between CRP and OPG in AS. Whilst these exploratory analyses suggest that some of these biomarkers are promising biomarkers of axial disease activity, more investigation is required to estimate the clinical usefulness of these biomarkers. Correlation expressed as a regression coefficient (beta) or correlation coefficient (Spearman rho) is not a measurement of the clinical usefulness of a candidate biomarker. The same patients would need to be tested longitudinally at multiple time-points with
paired serum biomarker and ASDAS measurements, and an estimation made of the sensitivity, specificity, and positive and negative predictive values of the test. Since CRP comprises one domain of the ASDAS and is already used as a biomarker for SpA, any new biomarker must be compared against CRP using standard receiver operating characteristic (ROC) plots. The area under the curve (AUC) of these plots is a measure of the information provided by the candidate biomarker. Since four of the five domains comprising the ASDAS, are completed by the patient and therefore subjective, characteristics of patient populations is an important consideration. Therefore, to validate the new biomarker test for use in other centres or countries, the same study methods would need to be repeated in independent cohorts nationally and internationally.

Chen et al. assessed correlation between MMP-3 and axial disease activity as measured by the BASDAI [330]. Rather than using MMP-3 concentrations from healthy controls as threshold cut-offs in AS patients, they used the cut-off value selected by ROC analysis and the median value as the threshold. Their statistical modeling was based on methods used in large-scale epidemiology studies in cardiology, showing that subjects with high-sensitivity CRP levels, previously thought to be normal based on thresholds in the general population, can also pose an excess risk of cardiovascular disease [374].

Even if the concentration of a biomarker is no different in the diseased compared with healthy state, the biomarker can still have utility in clinical practice. For example, serum CRP concentrations in apparently healthy individuals, are used as a prognostic marker for future ischaemic heart disease [375]. Our results showing no difference in M-CSF concentrations in HC and PsC cases, but difference in concentrations in PsC compared with PsA, gives M-CSF clinical utility in PsC. Similarly, M-CSF could still be used to monitor response to anti-TNF therapy in a longitudinal study with serial M-CSF measurements before and after the commencement of anti-TNF therapy.

9.3.6 Other promising serum-soluble biomarkers in PsSpA

Several other biomarkers warrant further investigation in PsSpA. Once reliable commercially available assays for RANKL are available, serum RANKL levels, as well as the ratio of RANKL:OPG may be more indicative of axis dysregulation than either biomarker alone [300, 302, 342]. Neoepitopes of type 2 collagen formation (CPII) and degradation (C2C), and in particular the ratio of CPII:C2C have shown promise as useful biomarkers in PsA [88] and AS [376]. Sclerostin is another Wnt antagonist, and its expression is closely linked to Dkk-1 expression. In AS patients, low levels of sclerostin have been shown to be associated with syndesmophyte formation and progression [86,
293, 377]. During the systematic literature review of serum bone and cartilage-turnover biomarkers, it was noted that sclerostin has never been investigated in PsA or PsSpA. R-Spondin 1 is a Wnt agonist, and has been shown to protect against inflammatory bone damage by modulating the Wnt pathway in a mouse model of arthritis [294]. In vivo, R-Spondin 1 promoted osteoblast differentiation and bone formation while blocking osteoclast development, thereby contributing to the integrity of joints during inflammatory arthritis. Both sclerostin and R-Spondin 1 therefore warrant further investigation as biomarkers in PsSpA. TGF-β is involved in bone and matrix turnover as well as new bone formation in AS, and has shown some utility in the assessment of treatment efficacy in AS [376, 378], and may also be a useful biomarker in PsSpA.

9.3.7 Systemic-circulating versus tissue-specific biomarkers

The measurement of circulating serum-soluble biomarkers, may be too simplistic an approach for biomarker research, and not sufficiently take into consideration the complex downstream interplay of signaling molecules such as cytokines and chemokines. Tissue-specific biomarkers levels may be far more informative than circulating serum biomarker levels. For example, serum OPG may primarily be a surrogate of OPG levels in large bones, rather than small-scale OPG-mediated pathology in the entheses and vertebral corners. Therefore, OPG levels in enthesal structures may be far more useful to measure. In SpA, elevated serum MMP-3 may primarily be a consequence of peripheral arthritis, rather than axial arthritis. Therefore any comparison between diseases, e.g. AS with PsSpA, may need adjustment for the presence and degree of peripheral arthritis. Adjusting for the degree (severity) of peripheral arthritis is difficult and may not even be possible due to the varying sizes of joints and grades of arthritis activity. However, without such adjustment MMP-3 levels may falsely appear no different in AS and PsSpA cases. The measurement of MMP-3 levels in axial tissue would circumvent the need to adjust for peripheral arthritis presence and severity. In addition, the influence of a biomarker on osteoblasts and osteoclasts at tissue level may be different to that at a more systemic level.
9.4 SUMMARY OF THE KEY FINDINGS OF THIS THESIS

The work of this thesis has found differences and similarities between PsSpA, AS and pPsA patients, as well as between PAM and non-PAM patients. These are summarised below in Figures 9.1 to 9.4.

**Figure 9.1. The similarities and differences between PsSpA and AS**

- **Differences**
  - In PsSpA compared with AS:
    - lower prevalence of HLA-B27 variants.
    - males and females equally likely to be affected.
    - lower prevalence of current or past history of axial inflammatory symptoms.
    - better function as measured by the BASFI.
    - less restriction of cervical and lumbar forward flexion.
    - lower frequency of complete SIJ ankylosis and vertebral bridging syndesmophytes.
    - lower axial radiographic severity, as measured by the PASRI, mSASSS, and osteoproliferation burden.
    - lower serum Dkk-1 concentrations, and higher serum OPG concentrations.

- **Similarities**
  - PsSpA and AS found to be no different in terms of:
    - disability as measured by the HAQ.
    - disease activity as measured by the ASDAS, BASDAI and PaGA.
    - metrology as measured by the BASMI.
    - frequency of extra-articular manifestations and family history of SpA.
    - proceeding to anti-TNF therapy.
    - prevalence of cervical and/or lumbar spondylitis, and cervical facet joint fusion.
    - symmetry of sacroiliitis grade.
    - SIJ sclerosis, erosion and/or partial ankylosis.
    - vertebral non-bridging syndesmophytes and erosion.
    - serum M-CSF and MMP-3 concentrations.

**Figure 9.2. The similarities and differences between PsSpA and pPsA**

- **Differences**
  - In PsSpA compared with pPsA:
    - higher prevalence of HLA-B27 variants.
    - higher prevalence of IBD.
    - younger age at arthritis onset.
    - more severe nail disease, especially nail onycholysis severity.
    - fewer tender peripheral joints.
    - more likely to be associated with PAM.
    - higher serum Dkk-1 concentrations, and lower serum OPG concentrations.

- **Similarities**
  - PsSpA and pPsA found to be no different in terms of:
    - male to female distribution.
    - age at psoriasis onset.
    - swollen joint counts.
    - extra-articular manifestations: skin severity as measured by the PASI; enthesitis burden as measure by the LEI; personal history of dactylitis, enthesitis or uveitis.
    - family history of SpA, psoriasis, uveitis and IBD.
    - disability as measured by the HAQ.
    - proceeding to anti-TNF agent use.
    - serum concentrations of M-CSF and MMP-3.
Figure 9.3. The similarities and differences between PAM and non-PAM cases

In PAM compared with non-PAM PsA cases:
- earlier age at PsA diagnosis.
- more disability as measured by the HAQ.
- more prevalent nail dystrophy
- more prevalent radiographic axial disease and sacroiliitis.

In PAM, the rate of osteolysis is higher in earlier disease, then tapers over time, especially in the hands.
Nail dystrophy is a biomarker of more severe osteolysis.
DMARDs and anti-TNF appear not to prevent PAM occurrence.

Figure 9.4. Serum biomarkers of spondyloarthritis phenotypes

**Biomarkers of axial disease**

**Dkk-1**
- can differentiate SpA patients with and without radiographic axial disease, independently of psoriasis status.
- can differentiate PsSpA from AS.

**OPG**
- can differentiate SpA patients with and without radiographic axial disease, independently of psoriasis status.
- can differentiate PsSpA from AS.

**Biomarkers of arthritis**

**M-CSF and MMP-3**
Can differentiate patients with and without arthritis:
- PsA from PsC
- pPsA from HC
- PsSpA from HC
- AS from HC
9.5 CURRENT DEVELOPMENTS & FUTURE RESEARCH WORK

Experts on serum biomarker research suggest that either a panel of biomarkers or the ratio of biologically-related biomarkers, rather than a single biomarker, may be most informative [379-381]. The regression models used in chapter 8 did test for interaction between serum biomarkers, and some signals were detected. However, the ability of a panel of biomarkers to differentiate between the five subject groups was not formally tested. I plan to do this after seeking advice on the mathematical methods to achieve this.

Receiver operating characteristic (ROC) curves were used by Chandran et al. to determine the serum concentration cut-off for optimal sensitivity and specificity to differentiate PsA from PsC [88]. I plan to do the same. For example, to determine the best concentration cut-off for Dkk-1 / M-CSF to differentiate PsSpA from AS, and M-CSF / MMP-3 to differentiate PsA from PsC.

As discussed above, the measurement of tissue-specific biomarkers appears to offer several significant advantages over systemic-circulating biomarkers. My longer-term research strategy is therefore to investigate tissue-specific biomarkers as predictors of SpA-subphenotype occurrence, severity and disease activity. I have made some progress in this research through the acquisition of skills in musculoskeletal ultrasound, taking ultrasound-guided synovial biopsy, and involvement in international multi-centre studies investigating synovial tissue biomarkers in rheumatoid arthritis. I intend to apply these research skills and principles to synovial and / or enthesal tissue biomarker research in SpA.

This thesis has focused on axial disease in SpA. Almost all the PsA cases also have peripheral arthritis. This period of research towards a PhD was part funded by an Investigator Initiated Research Grant (iCRP-2013) from Pfizer Limited. Part of the remit of that research grant was to investigate serum-soluble bone-biomarkers of peripheral radiographic disease. During the last year I have therefore scored the most recent hands and feet radiographs of the 201 PsA patients enrolled in the ADIPSA1 study, using the modified van der Heijde scoring tool [145] and Ratingen scoring tool [249], both of which have been validated for use in PsA. I will therefore investigate the relationship between Dkk-1, OPG, M-CSF, MMP-3 and these peripheral radiographic scores in terms of overall severity, as well as severity of erosion, osteoproliferation, and joint-space narrowing.

As mentioned earlier, a key unknown aspect of PAM is the chronology of pathological events leading to the osteolysis of PAM. The radiographic scoring conducted in the study
included domains for osteolysis, erosion, joint space narrowing, osteoproliferation, periostitis, osteopaenia and tuft resorption. Chapter 7 only reports the natural history of PAM based on change in osteolysis score. Within the constraints of this study design and data available, I plan to investigate the type of pathological changes that occur before the onset of osteolysis in a particular joint. My anecdotal observations having read the radiographs longitudinally, is that there is progressive joint space narrowing before onset of osteolysis, and perhaps no progressive erosion or osteopaenia. However, after onset of osteolysis bone remodelling does appear to occur in the form of osteoproliferation, perhaps as a compensatory mechanism to correct abnormal joint loading and movement. Periostitis seemed very rare in the PAM cases. This will need to be tested mathematically.

A review of the literature on the genetics of PsSpA and PsA is detailed in the Introduction chapter of this thesis. Apart from variants of \textit{HLA-B27}, other genetic biomarkers have not been reported in this thesis. This will be undertaken as part of my post-doctoral research. The 402 patients enrolled in the ADIPSA1 study were consented for DNA collection and testing. As mentioned in chapter 4, these patients’ DNA has been sequenced for \textit{HLA-B} and \textit{HLA-C} variants, in more detail than the \textit{HLA-B27} data presented in this thesis. Over the forthcoming year, this genetic sequencing data of these 118 PsSpA, 127 pPsA and 157 AS cases will be compared to determine if specific \textit{HLA} genotypes or haplotypes associate with PsSpA, or its radiographic and / or clinical phenotype expression. Comparison of our results will be made with two recently published investigating the same [106, 382]. In addition, 35 of the 36 PAM patients identified in chapter 7 of this thesis, also consented through the LOPAS1 [38] study to have their DNA sequenced for \textit{HLA-B} and \textit{HLA-C} variants. A recent study showed an association between osteolysis (a marker of PAM) and \textit{HLA-C02:02:02} [106]. \textit{HLA}- genotypes and haplotypes will therefore be tested for association with PAM in our cohort.

As mentioned above, this period of research towards a PhD was part funded by the iCRP-2013 grant from Pfizer Limited, as part of the ADIPSA2 study. Part of the remit of that research grant was to investigate genetic biomarkers of axial and peripheral radiographic disease. During the last year I have therefore scored the most recent axial, hands and feet radiographs of 515 PsA patients (201 PsA patients enrolled in the ADIPSA1 study and 314 PsA patients enrolled in the LOPAS1 study) using the PASRI (for axial disease), mSASSS (for axial disease), modified van der Heijde scoring tool (for peripheral disease) [145] and Ratingen scoring tool (for peripheral disease) [249]. Patients enrolled in ADIPSA2 study consented (through ADIPSA1 and LOPAS1) to have their DNA genotyped. This was performed in November 2014 by Professor Anne Barton’s team at the Arthritis Research U.K. Epidemiology Unit in Manchester (U.K.) using the Illumina
Infinium HumanCoreExome BeadChip, which genotypes 500,000 single nucleotide polymorphisms (SNPs) in the exome and inter-genic regions. Informed by the published literature, a candidate gene approach will be used to identify genetic loci associated with PsSpA, radiographic axial disease severity, peripheral radiographic morphology (erosion and osteoproliferation) and peripheral radiographic severity. Some preliminary analyses have already been undertaken, and will continue in the forthcoming year as part of post-doctoral research. An abstract of some of this work has been accepted for poster presentation at the American College of Rheumatology Annual Congress in November 2015.
9.6 FINAL CONCLUSIONS

To conclude, the work of this thesis has identified several clinical biomarkers, imaging biomarkers, serum-soluble biomarkers and genetic biomarkers that differentiate PsSpA from pPsA and AS. Given that PsSpA has its own discrete clinical and biomarker signature, its clinical management and research should be tailored from that of pPsA and AS. PsSpA affects a significant proportion of PsA patients. PsSpA is not simply AS with coincidental psoriasis, nor is PsSpA a milder version of AS. This thesis has shown that PsSpA involvement is as disabling and clinically impactful as AS; perhaps in part because PsSpA progresses for a long time period, and brings with it a burden of peripheral arthritis [201]. PAM was found to be associated with PsSpA, and in addition, this thesis identified clinical biomarkers of PAM occurrence and radiographic progression. Taken together, this thesis indicates that PsSpA is on a spectrum of musculoskeletal disease, in between pPsA and AS; with PsSpA comprising a continuum itself, and with a phenotype expression related to disease duration. It is hoped that recognition of these facts will prompt the inception of an international consensus classification system for PsSpA, for which there is burning need. The advent of new imaging techniques, laboratory markers, and epidemiological studies on PsSpA should facilitate this process. Ultimately this may further the effort for stratified and personalised medicine. Gaps in the current canon of knowledge on PsSpA and PAM have been identified by this body of work and will hopefully inform future research. Some of these will be addressed in my future post-doctoral research as described above. Other identified topics will hopefully be investigated by readers of this thesis and the associated published papers.
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APPENDIX 1: ADIPS A1 DATA COLLECTION PROFORMA

Royal National Hospital for Rheumatic Diseases
ADIPS A study

ADIPS A Outcome Measures

BASDAI

Please place an ‘X’ in the box to indicate your answer to each question relating to the past 1 week.

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

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

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

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

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

6. How long does your morning stiffness last from the time you wake up?
Please place a 'X' in the box to indicate your level of ability with each of the following activities during the LAST 1 WEEK.

1. Putting on your socks or tights without help or aids (e.g. sock aid).
   
   0 1 2 3 4 5 6 7 8 9 10
   EASY

2. Bending forward from the waist to pick up a pen from the floor without an aid.
   
   0 1 2 3 4 5 6 7 8 9 10
   EASY

3. Reaching up to a high shelf without help or aids (e.g. helping hand).
   
   0 1 2 3 4 5 6 7 8 9 10
   EASY

4. Getting up out of an armless dining room chair without using your hands or any other help.
   
   0 1 2 3 4 5 6 7 8 9 10
   EASY

5. Getting up off the floor without help from lying on your back.
   
   0 1 2 3 4 5 6 7 8 9 10
   EASY

6. Standing unsupported for 10 minutes without discomfort.
   
   0 1 2 3 4 5 6 7 8 9 10
   EASY

7. Climbing 12-15 steps without using a handrail or walking aid.
   
   0 1 2 3 4 5 6 7 8 9 10
   EASY

8. Looking over your shoulder without turning your body.
   
   0 1 2 3 4 5 6 7 8 9 10
   EASY
9. Doing physically demanding activities (e.g. physiotherapy exercises, gardening or sports).

10. Doing a full day's activities, whether it be at home or at work.

BACK PAIN

1. Based on your assessment, please place a “X” in the box to indicate what is the amount of back pain at night that you experienced during the last week?

2. Based on your assessment, please place an “X” in the box to indicate what is the amount of back pain at any time that you experienced during the last week?

PATIENT GLOBAL ASSESSMENT

1. Please place an 'X' in the box to indicate your overall assessment of your disease activity over the last week.
Section 1 – Health Assessment questionnaire

We are interested in learning how your illness affects your ability to function in daily life. Please feel free to add any comments at the end of this form.

*Please tick the one response which best describes your usual abilities over the past week.*

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<tr>
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<th>Without ANY difficulty</th>
<th>With SOME difficulty</th>
<th>With MUCH difficulty</th>
<th>Unable to do</th>
</tr>
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<tbody>
<tr>
<td>1) Dressing and Grooming</td>
<td>Are you able to:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Dress yourself including shoelaces and doing buttons?</td>
<td>☐</td>
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<td>☐</td>
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<tr>
<td>- Shampoo your hair?</td>
<td>☐</td>
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<tr>
<td>2) Rising</td>
<td>Are you able to:</td>
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<tr>
<td>- Stand up from an armless straight chair?</td>
<td>☐</td>
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<tr>
<td>- Get in and out of bed?</td>
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<td>3) Eating</td>
<td>Are you able to:</td>
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<tr>
<td>- Cut your meat?</td>
<td>☐</td>
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<tr>
<td>- Lift a full cup or glass to your mouth?</td>
<td>☐</td>
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<tr>
<td>- Open a new carton of milk (or soap powder)?</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>4) Walking</td>
<td>Are you able to:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Walk outdoors on flat ground?</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>- Climb up 5 steps?</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

Please tick any AIDS or DEVICES that you usually use for any of these activities:

- Walking stick
- Walking frame
- Crutches
- Wheelchair
- Devices used for dressing (button hook, zipper pull, long handled shoe horn etc)
- Built-up or special utensils
- Special or built-up chair
- Other (specify)

Please tick any categories for which you usually need ASSISTANCE FROM ANOTHER PERSON

- Dressing and Grooming
- Rising
- Eating
- Walking

Please turn over
Please tick the one response which best describes your usual abilities over the past week.

1) **Hygiene**
   Are you able to:
   - Wash and dry your entire body?  
   - Take a bath?  
   - Get on and off the toilet?

2) **Reach**
   Are you able to:
   - Reach and get down a 5 lb object (e.g. bag of potatoes) from just above your head?  
   - Bend down to pick clothing from the floor?

3) **Grip**
   Are you able to:
   - Open car doors?  
   - Open jars which have previously opened?  
   - Turn taps on and off?

4) **Activities**
   Are you able to:
   - Run errands and shop?  
   - Get in and out of a car?  
   - Do chores such as vacuuming, housework or light gardening?

Please tick any AIDS or DEVICES that you usually use for any of these activities:

- Raised toilet seat
- Bath seat
- Jar opener (for jars previously opened)
- Bath Rail
- Long-handled appliances for reach
- Other (specify)

Please tick any categories for which you usually need HELP FROM ANOTHER PERSON:

- Hygiene
- Reach
- Gripping and opening things
- Activities

For office use only

- Dressing & grooming
- Rising
- Eating
- Walking
- Hygiene
- Reach
- Grip
- Activity
THE FOLLOWING WILL BE PERFORMED
WITH THE RESEARCHER

ASDAS

CRP [ ] mg/dl (today or <1 week) [ ] CRP requested

BASMI

1. Tragus to wall distance [ ] cm

2. Lateral spinal flexion
   - LEFT
     - 1st [ ]
     - 2nd [ ]
   - UPPER
     - [ ]
   - LOWER
     - [ ]
   - DIFFERENCE
     - [ ]
   - BEST LEFT [ ] cm
   - BEST RIGHT [ ] cm

3. Modified Schober test [ ] cm (e.g. 15/19.2=4.2)

4. Cervical rotation
   - LEFT [ ] Degrees
   - RIGHT [ ] Degrees

5. Intermalleolar distance [ ] cm

Chest expansion

Chest expansion [ ] cm

LEI (Leeds Enthesitis Index)

Please place a 'X' for any site that is tender.

Right Left

Lateral epicondyle humerus [ ] [ ]

Medial femoral condyle [ ] [ ]

Achilles tendon insertion [ ] [ ]

Score [ ]
# mNAPSI (modified Nail Psoriasis Severity Index)

<table>
<thead>
<tr>
<th>Score</th>
<th>Orycholysis</th>
<th>Pitting</th>
<th>Crumbling</th>
<th>Leukonychia</th>
<th>Splinter Haem</th>
<th>Hyperkeratosis</th>
<th>Red spot Lunula</th>
<th>Cumulative Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>0 (Range 0-140)</td>
</tr>
<tr>
<td>1</td>
<td>1-10% nail affected</td>
<td>1-10% pits</td>
<td>1-25% of nail crumbled</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>11-30% nail affected</td>
<td>11-49 pits</td>
<td>26-49% of nail crumbled</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>&gt;30% nail affected</td>
<td>≥50 pits</td>
<td>≥50% of nail crumbled</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>3</td>
</tr>
</tbody>
</table>

### Global assessment of nails - PATIENT

- **0**
  - Normal Nails
- **100**
  - Very bad Nails

### Global assessment of nails - RESEARCHER

- **0**
  - Normal Nails
- **100**
  - Very bad Nails
### PASI (Psoriasis Area Severity Index)

<table>
<thead>
<tr>
<th>Extent</th>
<th>Extent score</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10%</td>
<td>1</td>
</tr>
<tr>
<td>10-29%</td>
<td>2</td>
</tr>
<tr>
<td>30-49%</td>
<td>3</td>
</tr>
<tr>
<td>50-69%</td>
<td>4</td>
</tr>
<tr>
<td>70-89%</td>
<td>5</td>
</tr>
<tr>
<td>90-100%</td>
<td>6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Extent</th>
<th>Erythema</th>
<th>Desquamation</th>
<th>Infiltration</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - no symptoms</td>
<td>No redness</td>
<td>No scaling</td>
<td>0mm No elevation</td>
</tr>
<tr>
<td>1 - slight</td>
<td>Pink or light red</td>
<td>Fine white scale</td>
<td>0-0.5mm elevation</td>
</tr>
<tr>
<td>2 - moderate</td>
<td>Darker pink or red</td>
<td>Moderate scale</td>
<td>0-0.75mm elevation</td>
</tr>
<tr>
<td>3 - severe</td>
<td>Very red</td>
<td>Thick white scale</td>
<td>1mm elevation</td>
</tr>
<tr>
<td>4 - very severe</td>
<td>Deep red or purple</td>
<td>Very thick scale</td>
<td>1.25mm elevation</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Skin area</th>
<th>Extent</th>
<th>Score</th>
<th>Total Extent</th>
<th>Erythema</th>
<th>Desquamation</th>
<th>Infiltration</th>
<th>SEVERITY</th>
<th>Total Extent x Severity</th>
<th>Total Extent x</th>
<th>Total severity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head</td>
<td>x 0.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arms</td>
<td>x 0.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trunk</td>
<td>x 0.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Legs</td>
<td>x 0.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[
PASI (PsA Skin Index) = \text{extent} \times \text{severity} \]

\[
\text{(max 72)}
\]
Extra-Articular features

Has the patient experienced any of the following?

- Inflammatory neck pain
- Inflammatory thoracic pain
- Inflammatory back pain
- Inflammatory buttock pain
- Chest wall pain
- Enthesitis
- Plantar fasciitis
- Dactylitis
- Iritis
- Psoriasis
- Crohn’s / Ulcerative Colitis
- Family History of SpA (Ank Spond, PsA or uSpA)

**Definition**

- Inflammatory pain = Pain with stiffness that is worse first thing after waking or after sitting down for an hour; it improves later in the day with some activity / exercise. Lasts for >30 min each time.
- Chest wall pain = Pain / swelling either side of the breast bone
- Enthesitis = Pain / swelling where ligaments join bone e.g. tennis elbow, golfer’s elbow, Achilles tendon, front of knee cap
- Plantar fasciitis = Pain on the sole of the foot near the heel base
- Dactylitis = When a finger / toe swells up along its entire length “like a sausage”, with a shiny stretched skin appearance
- Iritis = Red painful eye that affects vision. Lasts for a week or more. Often needs steroid eyedrops to settle.
- Crohn’s / Ulcerative colitis = Bloody diarrhoea and weight loss for several weeks / months.

**Modified New York Criteria for the Diagnosis of Ankylosing Spondylitis**

Having excluded spinal fracture, intervertebral disc disease & fibromyalgia; complete the following:

**Clinical criterion**

1. Inflammatory low back pain & stiffness >3m that
   - improves with exercise
   - not relieved by rest

2. Reduced motion of the lumbar-spine in both sagittal & frontal planes

3. Limited chest expansion

---

**End of researcher completed Proforma**

- 2. Yes / No
- 3. Yes / No
### PASRI (Psoriatic Arthritis Spondylitis Radiology Index)

#### Scoring Key for PASRI & mSASSS

<table>
<thead>
<tr>
<th>Joint</th>
<th>Vertebral</th>
<th>Posterior elements</th>
</tr>
</thead>
<tbody>
<tr>
<td>SIJ</td>
<td>0 = Normal</td>
<td>0 = No abnormality</td>
</tr>
<tr>
<td></td>
<td>1 = Suspicion of sacroiliitis</td>
<td>1 = Erosions, sclerosis, or squaring</td>
</tr>
<tr>
<td></td>
<td>2 = Sclerosis either side of joint</td>
<td>2 = Non-bridging syndesmophyte</td>
</tr>
<tr>
<td></td>
<td>3 = Joint space widening, erosions or partial ankylosis</td>
<td>3 = Bridging syndesmophyte</td>
</tr>
<tr>
<td></td>
<td>4 = Complete joint ankylosis</td>
<td>4 = No fusion</td>
</tr>
</tbody>
</table>

#### SI joints (0-4)

<table>
<thead>
<tr>
<th></th>
<th>Right</th>
<th>Left</th>
<th>Total</th>
</tr>
</thead>
</table>

#### Cervical spine (0-3 each, lateral views only)

- Lower C2
- Upper C3
- Lower C3
- Upper C4
- Lower C4
- Upper C5
- Lower C5
- Upper C6

(C) TOTAL (range: 0-24)

#### Lumbar spine (0-3 each, using AP & lateral views)

- Lower T12
- Upper L1
- Lower L1
- Upper L2
- Lower L2
- Upper L3
- Lower L3
- Upper L4
- Lower L4
- Upper L5
- Lower L5
- Upper S1

TOTAL (range: 0-36)

#### Posterior Cervical elements (0-1 each level, lateral views only)

- C2-C3
- C3-C4
- C4-C5
- C5-C6

TOTAL (range: 0-4)

#### Posterior Lumbar elements

- T12-L1
- L1-L2
- L2-L3
- L3-L4
- L4-L5
- L5-S1

TOTAL (range: 0-6)

#### FINAL SCORE (range: 0-78)
mSASSS  (modified Stoke Ankylosing Spondylitis Spinal Score)

Cervical spine  (0-3 each anterior site, lateral views only)
- Lower C6
- Upper C7
- Lower C7
- Upper T1

THIS TOTAL  (range 0-12)

(CS) TOTAL (from PASRI)  (range 0-24)

TOTAL  (range 0-36)

Lumbar spine
Use score from PASRI; from Lower T12 - Upper S1

TOTAL  (range 0-36)

FINAL SCORE  (range 0-72)

Modified New York Criteria for the Diagnosis of Ankylosing Spondylitis ...

Radiological criterion

Has radiographic sacroiliitis
- No
- Grade ≥ 2 bilaterally
- Grade ≥ 3 unilaterally

Does patient fulfill N.Y. criteria for A.S.?
(Radiological criterion  1 clinical criterion)
- Yes
- No

Hip Impingement

Radiographic evidence
- Right
- Left
PATIENT INFORMATION SHEET

Study Title:
Axial Disease in Psoriatic Arthritis (ADIPSA)

You are being invited to participate in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information and ask for anything that is unclear to be explained.

• Part 1 tells you the purpose of the study and what will happen to you if you take part.
• Part 2 gives you more detailed information about the conduct of the study.

Part 1

What is the purpose of the study?
Individuals with Psoriatic Arthritis or Ankylosing Spondylitis can experience spinal symptoms and damage. Although the study title refers to ‘Psoriatic Arthritis’, we are also studying patients with Ankylosing Spondylitis so that we can compare and contrast.

• Firstly, the ADIPSA study will investigate if people with Psoriatic Arthritis or Ankylosing Spondylitis have similar / different symptoms, X-ray changes, altered spinal movement, impact on lifestyle or damage to the nails, skin or joints of the hands and feet.

• The second aim of the ADIPSA study is to investigate how proteins dissolved in the blood (soluble serum biomarkers) or genes (molecules contained in all the body’s cells that help determine how we look or if we will develop certain diseases) contribute to this.

Overall, this study will help us understand the similarities and differences between Psoriatic Arthritis and Ankylosing Spondylitis, so that we may care for patients in the best possible way.

Why have I been chosen?
You have been chosen to participate as you have a diagnosis of either Psoriatic Arthritis or Ankylosing Spondylitis.

Do I have to take part?
It is up to you to decide. You do not have to take part. If you do not want to take part, it will not affect the care you receive now or in the future. However, if you do decide to take part, you can keep this sheet and be asked to sign a consent form. You are still free to withdraw at any time without giving a reason. Your participation will not interfere with the standard of care you receive.

What will happen to me if I take part?
Your participation will involve the following:
(i) Agreement to have routine measurements of your spine, hands, feet & skin performed.
(ii) Agreement to complete four questionnaires about how your arthritis affects you.
(iii) Agreement to have a blood sample taken for further testing.
(iv) Agreement for a member of the ADIPSA research team to look at your medical notes or X-rays that you have had previously, to look for evidence of damage to your joints.

It will take approximately 30-45 minutes to complete these tasks, immediately before your next routine outpatient clinic appointment.
What do I have to do?

- If you agree to take part in the study, a member of the ADIPSA research team (Doctor, Nurse or Physiotherapist) will ask you to sign a consent form, perform the measurements, give you the questionnaires and arrange for a blood sample to be taken.
- Most of these tests would be performed as part of the treatment that you would receive anyway, regardless of whether or not you choose to participate in this study.
- No extra X-rays will be taken.

What will the blood sample be tested for?
The blood sample will be tested for markers of inflammation in the blood (soluble serum biomarkers), which are sometimes present in patients with Psoriatic Arthritis or Ankylosing Spondylitis. The blood sample will also be tested for genes that may make you more / less likely to experience particular symptoms, for example inflammatory back pain.

What are the possible benefits of taking part?
We cannot promise that the results of the study will help you directly, but the information we get might improve the treatment of people with Psoriatic Arthritis and Ankylosing Spondylitis.

Will the research influence the treatment I receive?
The research does not alter the treatment you receive. Your specialist will start and stop treatments as determined by your clinical condition.

Will my taking part in the study be kept confidential?
Yes. We follow ethical and legal practices and all the information about your participation in this study will be kept confidential. The details are included in Part 2.

Who is organising and co-ordinating the study?
The study is being co-ordinated by Professor. Neil McHugh, Dr Raj Sengupta and Dr Ellie Korendowych.

Who is funding the study?
Pfizer Limited are funding this study. Pfizer do not have any commercial interest in the study outcome.

This completes Part 1 of the information sheet.
If the information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making any decision.

Part 2

What will happen if I don't want to carry on with the study?
If you withdraw from the study, we will destroy all of your identifiable samples, but we will need to use the data collected up until your withdrawal.

Will my taking part in the study be kept confidential?
All information which is collected about you during the course of the research will be kept strictly confidential. You will be assigned a unique ADIPSA study number (ASIN), so that your name is kept confidential and separate from the tests, measurement or questionnaires you complete.

Clinical information about you will be collected and compared with genetic data. The individuals performing the genetic tests at the Arthritis Research U.K. centre in Manchester, will have no access to any personal identifiable information apart from an anonymised identification number, year of birth and gender.
What will happen to the blood sample I give?
The blood sample will be tested either immediately or stored in the Bath Institute for Rheumatic Diseases for further testing as part of this study.

- DNA (a molecule that contains your genes) will be extracted and sent to the Arthritis Research U.K. centre in Manchester for further genetic testing. The DNA will be analysed but the tests conducted will not produce results with clinical or familial significance. Samples will be stored with a code, so that they will be anonymous to researchers conducting the DNA analysis. However, we know which code belongs to which participant, so that they can be linked if needed.
- The blood sample will be tested on-site for proteins (soluble serum biomarkers) that may affect the type of disease you have.

What physical measurements will be taken?
The researcher will count the number of tender / swollen joints you have, if some of the sites where tendons attach to your bone are tender, look to see if your nails / skin are affected and perform measurements of your back. The back measurements include the distance between your ear and a wall, how far you can bend forward / side-ways, how far you can turn your head, how deep you can breath in and the distance between your ankles when spread apart.

What will happen to the results of the research study?
Results of the ADIPSA study will be published in scientific journals and presented at national and international rheumatology / genetics meetings. You will never be identified.

Who has reviewed the study?
All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by the Frenchay Research Ethics Committee.

For more information please contact:
The study co-ordinator:  
Dr Deepak Jadon  
on 07870 221111 or 01225 465941 ext 269  
or email: Deepak.Jadon@nhhrd.nhs.uk

Professor. Neil McHugh on 01225 465941  
Dr Raj Sengupta on 01225 473442  
Dr Ellie Korendowych on 01225 465941

Thank you for taking time to read the information sheet, which you should keep for future reference.
CONSENT FORM
Axial Disease in Psoriatic Arthritis
(ADIPSA)

Patient Study Number (ASIN):

Researcher’s Name:

Please Initial the Boxes

1. I confirm that I have read and understand the Patient Information Sheet dated 24/04/2012 (version 2) for the ADIPSA study.

2. I understand that my participation is voluntary and that I am free to withdraw at any time up to the point at which my data is anonymised 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 regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records, but understand that strict confidentiality will be maintained. The purpose of this is to check that the study is being carried out correctly.

4. I agree to having a blood sample taken.

5. I agree to having my blood sample stored at the Bath Institute for Rheumatic Diseases for the period of the ADIPSA study, to be used to investigate genetic & serum biomarkers.

6. I agree for my blood sample to undergo DNA analysis.

7. I agree to take part in the ADIPSA study.

Patient Name __________________________ Signature __________________________ Date

Name of researcher taking consent __________________________ Signature __________________________ Date

ADIPSA Consent Form, v2, 24/04/2012
Health Research Authority

NRES Committee South West - Frenchay
Bristol Research Ethics Centre
Level 3, Block B
Whitefriars
Lewins Mead,
Bristol
BS1 2HT

Telephone: 0117 342 1334
Facsimile: 0117 342 0445

08 May 2012

Dr Deepak Jadon
Research Fellow
Royal National Hospital for Rheumatic Diseases NHS Foundation Trust
Upper Borough Walls
Bath
BA1 1RL

Dear Dr Jadon

Study title: Axial Disease in Psoriatic Arthritis (ADIPSA)
REC reference: 12/SW/0110
Protocol number: Version 2, 01/12/2011

Thank you for your letter of 24 April 2012, responding to the Committee’s request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

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.

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/HS R&D office prior to the start of the study (see “Conditions of the favourable opinion” below).

Conditions of the favourable opinion

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

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

Management permission (“R&D approval”) should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

A Research Ethics Committee established by the Health Research Authority
Guidance on applying for NHS permission for research is available in the Integrated Research Application System 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 approvals from host organisations.

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).

Approved documents

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

<table>
<thead>
<tr>
<th>Document</th>
<th>Version</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Covering Letter</td>
<td></td>
<td>13 March 2012</td>
</tr>
<tr>
<td>Investigator CV</td>
<td></td>
<td>13 March 2012</td>
</tr>
<tr>
<td>Letter from Sponsor</td>
<td></td>
<td>06 March 2012</td>
</tr>
<tr>
<td>Letter from Statistician</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other: CV Professor McHugh</td>
<td></td>
<td>12 September 2011</td>
</tr>
<tr>
<td>Other: Letter and research agreement from funder</td>
<td></td>
<td>10 August 2011</td>
</tr>
<tr>
<td>Other: CV Dr Tillett</td>
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<tr>
<td>Other: CV Dr Sengupta</td>
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<tr>
<td>Other: CV Dr Korendowych</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other: CV Mrs Griffith</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other: CV Mrs Waldron</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other: CV Miss Olstain</td>
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<tr>
<td>Other: Data protection act research form</td>
<td></td>
<td>14 March 2012</td>
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<tr>
<td>Other: Letter of invitation to participant - PsA</td>
<td>2</td>
<td>24 April 2012</td>
</tr>
<tr>
<td>Other: Letter of invitation to participant - AS</td>
<td>2</td>
<td>24 April 2012</td>
</tr>
<tr>
<td>Participant Consent Form</td>
<td>2</td>
<td>24 April 2012</td>
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<tr>
<td>Participant Information Sheet</td>
<td>2</td>
<td>24 April 2012</td>
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<tr>
<td>Protocol</td>
<td>2</td>
<td>01 December 2011</td>
</tr>
<tr>
<td>Questionnaire: ADIPS A Outcome Measures</td>
<td></td>
<td>24 April 2012</td>
</tr>
<tr>
<td>REC application</td>
<td></td>
<td>13 March 2012</td>
</tr>
<tr>
<td>Response to Request for Further information</td>
<td></td>
<td>24 April 2012</td>
</tr>
</tbody>
</table>

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

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

Further information is available at National Research Ethics Service website > After Review

12/SW/0110 Please quote this number on all correspondence

With the Committee’s best wishes for the success of this project

Yours sincerely

Dr Mike Shale Chair

Email: uhb.tr.SouthWest5@nhs.net

Enclosures: "After ethical review – guidance for researchers" – sent via e-mail

Copy to: Jane Carter, Royal National Hospital for Rheumatic Diseases NHS Foundation Trust
Dear Deepak

RBB 376 – ADIPSA

Thank you for your application for approval of the above project, which was considered at the R&D Committee meeting on the 15th May 2012. I am pleased to inform you that the R&D Committee approved the project and the Trust will act as sponsor.

Details of this project will be entered onto the RNHRD database. The reference number for your project is RBB 376 and this should be used in all correspondence. A short progress report will be required annually or at the end of the project, whichever occurs first.

All research approved by the R&D Committee should follow good clinical practice and adhere to the systems in place for Research Governance. All Principal Investigators must undertake Good Clinical Practice training and are responsible for ensuring that their research staff have received appropriate training.

You are responsible for ensuring that, all participants sign informed consent (whenever applicable) and that the protocol agreed by the local research ethics committee is adhered to by yourself and any co-workers.

You are required to provide us with information about any amendments to the protocol, changes in funding, personnel or end date and any research-related adverse events. Any staff working on this study at this site must be issued with a contract with RNHRD (honorary or substantive) or Letter of Access before they commence work on the study at this site. Please make sure that the RNHRD is acknowledged on all academic papers which may be written as a result of this research.

In addition, other information may be requested from time to time and a lay summary of the results will be requested from you at the end of the study. This study may be subject to audit by the R&D Office.

We wish you well with this research.

Yours sincerely

[Signature]

Jane Carter, R&D Manager
APPENDIX 4: PSORIATIC ARTHRITIS MUTILANS STUDY DATA COLLECTION

PROFORMA

Please place patient label here

Date Of Birth / / 
Completed By DJ WT

BPSA:

OSTEOPAENIA (mSTB)      OSTEOLYSIS (own)      EROSION (mSS)      JSN (mSS)
Hands L R Hands L R Hands L R Hands L R
IP   DIP2 DIP3 DIP4 DIP5 PIP2 PIP3 PIP4 PIP5 MCP1 MCP2 MCP3 MCP4 MCP5
MCP1 MCP2 MCP3 MCP4 MCP5
Wrist-global 1st CMCJ (0-6)
Distal radius (0-6)
Distal Ulna (0-6)
Wrist global (0-1)

Feet L R Feet L R Feet L R Feet L R
IP   MTP1 MTP2 MTP3 MTP4 MTP5
MTP1 MTP2 MTP3 MTP4 MTP5
MTP5

RNHRD: 4803036903
## Proliferation (PARS)

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## Periostitis juxta-articular

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## Periostitis wrist

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## Tuft resorption

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### Foot Positions

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APPENDIX 5: LOPAS1 ETHICAL APPROVAL LETTER

Royal United Hospital Bath

BATH LOCAL RESEARCH ETHICS COMMITTEE
Direct tel/fax: 01225 825725 e-mail: research.ethics@ruh.bath.swnhs.uk

3 August 2000

Dr E Korendowych
Research Fellow
Royal National Hospital for Rheumatic Diseases
NHS Trust
Upper Borough Walls
Bath BA1 1RL

Dear Dr Korendowych

BA74/00-01 (please quote this reference on all correspondence)
Prognostic markers of severe outcome in Psoriatic Arthritis

At the meeting held on 27 July 2000, the Committee considered your application for approval of the above study, comprising the following documents:

- Completed South & West application form
- Protocol
- Patient Information Sheet, Patient Consent Form
- Questionnaire
- CV

This study was approved subject to amendment of the information sheet. The following points were made:

1. The information sheet should have a suitable introduction, for example: ‘You are a person who suffers from psoriatic arthritis and this information sheet …’

2. There should be a statement to explain that the patient may decline to participate without giving reasons and without this affecting the quality of the treatment he/she receives in the future.

3. As the study involves genetic analysis, a number of issues would need to be addressed, for example, patients will need to be given information as to whether their blood will be stored for future use and confidentiality issues about the disclosure of results would need to be fully explained.

4. It was felt that the in paragraph 3, sentence 3 should be amended to read ‘…it may help us to ensure that the right patients get the right treatments.’

5. It was asked that the information sheet should follow the Committee’s standard guidelines for writing patient information sheets.

In order to help you revise this, please find attached a copy of a new leaflet produced by CERES (Consumers for Ethics in Research) ‘Generic Research and You’, the Committee’s standard guidelines for writing patient information, together with a sample patient information sheet produced for a study involving genetic research. Full approval will be given upon receipt of a suitably revised patient information sheet.

For all research being undertaken within provider trusts, approval must be obtained from the Culver Lead prior to commencement of the study. This Committee is organised and operates according to ICH/GCP Guidelines and the applicable laws and regulations. Any changes or extensions to the protocol, or additional investigators should be notified to the Committee for approval. Serious and unexpected adverse events should also be notified to the meeting. May we remind you of the Data Protection Act 1984 and the need to conduct the trial in accordance with the Good Clinical Practice Guidelines.

The Committee is required to audit progress of research and to produce a yearly report to the Avon Health Authority and Department of Health. You are therefore required to provide a brief yearly report and a short final report.

Yours sincerely

Dr Peter Rudd
Chairman