DETERMINANTS OF OUTCOME IN EARLY ARTHRITIS

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Abstract

Ultrasound is useful in providing information on subclinical joint and tendon inflammation that is not clinically evident. Current ultrasound technology has very high sensitivity in detecting subtle ultrasound pathology even in healthy joints. In order to use ultrasound effectively it is therefore important to understand the extent of ultrasound findings in healthy individuals, particularly in age ranges where rheumatological disease presents. I have developed a large collaborative network of units experienced in musculoskeletal ultrasound in order to investigate the extent of these ultrasound changes (i.e. synovial hypertrophy, Power Doppler, effusion, osteophytes and erosion) in a large cohort of healthy individuals. Healthy individuals exhibit ultrasound changes in the small joints of the hands, wrists and feet. There is an age-dependent effect of these changes. Synovial effusion is common across all age groups.

Ultrasound has also been established as a predictive tool to identify early arthritis patients who will progress to persistent clinical synovitis. The predictive potential of joint synovitis as measured by ultrasound is well documented. The predictive potential of tendon inflammation measured by ultrasound is not clear. I have investigated the utility of tendon ultrasound variables in the prediction of rheumatoid and persistent arthritis development. Finger flexor tendon showed very promising capacity to predict both rheumatoid arthritis (RA) and persistent arthritis in patients who present within 12 weeks of symptom onset. This is even after taking into account conventional predictors such as RA-related autoantibodies and ultrasound joint synovitis. Finger flexor tendons should thus be considered as a candidate variable when designing prediction algorithms for early arthritis patients.

The ability to predict those who will develop rheumatoid arthritis could allow clinicians to better identify those who require immunosuppressant therapy within the therapeutic window of opportunity. However, the duration of this therapeutic window of opportunity has never been prospectively investigated, particularly in the context of how mode of onset may affect treatment response. Mode of onset refers to how rapid the joint symptoms develop at initial presentation. In this thesis, I classified mode of onset as abrupt, acute or palindromic. I have studied the relationship between treatment response and symptom duration prior to starting DMARDs treatment, taking into account the mode of onset. It appears the mode of onset has a measurable impact on treatment response in RA patients. This novel finding should be further assessed in a larger cohort.

This thesis is dedicated to my family.

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Abbreviations

ACR	American College of Rheumatology
ACPA	Anti-citrullinated protein/peptide antibodies
AS	Ankylosing Spondylitis
ASAS	Assessment of Spondyloarthritis International Society
APL	Abductor pollicis longus
BD	Twice daily
BEACON	Birmingham Early Arthritis Cohort
CASPAR	Classification for Psoriatic Arthritis
CRP	C-Reactive Protein
CSA	Clinically suspect arthralgia
DIP	Distal Interphalangeal joint
DMARDs	Disease modifying anti-rheumatic Drugs
EMS	Early morning stiffness
ESR	Erythrocyte sedimentation rate
EULAR	European League Against Rheumatism
ECRB	Extensor carpi radialis brevis
ECRL	Extensor carpi radialis longus
ECU	Extensor carpi ulnaris
EPB	Extensor pollicis brevis
EIP	Extensor indicis propius
EDM	Extensor digiti minimi
EDC	Extensor digitorum communis
EPL	Extensor pollicis longus
EHL	Extensor hallucis longus
EDL	Extensor digitorum longus
FAP	Fibroblast activation protein
FDL	Flexor digitorum longus
FHL	Flexor hallucis longus

GS	Grey-scale
HAQ	Health Assessment Questionnaire
HCQ	Hydroxychloroquine
HLA B27	Human leukocyte antigen B27
JAK	Janus kinase inhibitors
MTP	Metatarsophalangeal joint
MTX	Methotrexate
MRI	Magnetic Resonance Imaging
MCP	Metacarpophalangeal joint
NSAIDs	Non-steroids anti-inflammatory
OA	Osteoarthritis
OR	Odds Ratio
OW	Once weekly
PCA	Principal Component Analysis
PCR	Polymerase chain reaction
PD	Power Doppler
PT	Posterior Tibial
PsA	Psoriatic Arthritis
PIP	Proximal interphalangeal joint
PRF	Pulse repetition frequency
RA	Rheumatoid Arthritis
ReA	Reactive Arthritis
RF	Rheumatoid factor
SH	Synovial Hypertrophy
SpA	Spondyloarthropathy
SSZ	Sulfasalazine
SLE	Systemic lupus erythematosus
TA	Tibialis Anterior
TS	Tenosynovitis
US	Ultrasound

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1 General introduction

1.1 Background

Guillaume de Baillou (1538-1616), a French physician, first recognised arthritis in 1611, although this observation was not published until 1642 in which he gave the first description of gout in the post-humous publication of 'Liber de Rheumatisme et Pleuritide Dorsali' published in Paris (1) .

> *"Now what articular gout is in any limb, exactly so is rheumatism in the whole body, as regards pain, tension, and the 'feeling of burning heat,'- as I call it-others say 'sensation.' Both complaints are somewhat painful, but the gouty pain in the joint is repeated at definite times and periods.' Not so this rheumatism, unless it be in those who have sinned in their manner of living"*

In 1800, Augustin-Jacob Landre Beauvais, was the first to describe the clinical phenotype of rheumatoid arthritis (2). He called this disease 'primary asthenic gout'. He described the clinical characteristics of nine long-term patients at Salpêtrière Hospice, Paris, in his medical doctorate dissertation submitted in 1800. He noted that this disease had a predilection for female, tend to involve multiple joint at onset, associated with a chronic disease course and deterioration in general health. This was distinct from the clinical features of typical gout. In the last sentence of his dissertation, he suggested that this was a disease entity that was previously undescribed:

"...we must recognize the existence of a new form of gout under the designation primary asthenic gout".

Sir Alfred Garrod (1), a physician at University College Hospital, coined the name 'Rheumatoid Arthritis' to distinguish this from other joint diseases like gout. He proposed this term in his book 'The Nature and Treatment of Gout and Rheumatic Gout', published in 1859 (3).

> *"Although unwilling to add to the number of names, I cannot help expressing a desire that one might be found for this disease, not implying any necessary relation between it and either gout or rheumatism. Perhaps Rheumatoid Arthritis would answer the object, by which term I should wish to imply an inflammatory affection of the joints, not unlike rheumatism in some of its characters, but differing materially from it."*

Interestingly, Sir Garrod classified RA into acute, chronic and irregular types (4). The British Ministry of Health then implemented the term Rheumatoid Arthritis (RA) in 1922, and subsequently the American Rheumatism Association Arthritis in 1941 (1). This nomenclature has remained the same ever since.

1.2 Inflammatory arthritis

Inflammatory arthritis, today, is recognised as a group of joint disease that includes Rheumatoid Arthritis, Spondyloarthropathies (which includes Psoriatic Arthritis, Reactive Arthritis, Enteropathic Spondyloarthropathy, Ankylosing Spondylitis), crystal-related arthritis and Juvenile Idiopathic Arthritis.

1.2.1 Spondyloarthropathy

Spondyloarthropathy (SpA) is a nomenclature for a group of joint diseases that share common characteristics; inflammation involving the spine, sacroiliac joint and peripheral manifestations, mainly involving inflammation of entheses. The main conditions within this

spectrum are Ankylosing Spondylitis (AS), Reactive Arthritis (ReA), Psoriatic Arthritis (PsA), Enteropathic Arthritis, Juvenile SpA and Undifferentiated SpA. The common pathophysiology features in SpAs are spinal inflammation, enthesitis (inflammation at the ligament or bone insertional sites) and association with HLA B27. The main clinical features of SpAs are sacroiliitis, inflammatory neck and back pain, enthesopathy, dactylitis, uveal inflammation, aortitis (aortic valve disease) and sterile urethritis. Skin lesions that are included are psoriasis, balanitis and keratoderma (5). In 1974, Moll and colleagues were the first to propose that the individual joint diseases were in fact a clustered entity within the SpA spectrum (6). In 2009, the classification criteria to classify patients age less than 45 years old with inflammatory back pain was published by the ASAS (assessment of Spondyloarthritis International Classification Criteria) group. These classification criteria require sacroiliitis on imaging with at least one SpA feature. Alternatively, patients are required to have HLAB27 positivity with at least two SpA features. These classification criteria have a sensitivity of 82.9% and specificity of 84.4% (7) .

1.2.2 Ankylosing Spondylitis

The hallmark of Ankylosing Spondylitis (AS) is inflammatory spinal pain and stiffness. The prevalence amongst Caucasian is around 1%. It has a strong link to HLAB27 and usually develops around the late teenage or young adult years. The sex ratio of male to female is around 2.5:1 (8).

The natural history of AS is inflammation of the SI joints with inflammation ascending to the spine, leading to spinal ankylosis, kyphosis and restriction. The extra articular features are

iritis, cardiac conduction defect, aortic valve abnormality, cardiomegaly, inflammatory bowel disease, psoriasis or psoriaform-type skin lesions (8).

AS can be classified as *radiographic axial SpA*. Radiographs of sacroiliac joint and spine may be normal in early disease. As the disease progresses, sacroiliitis may present and can be bilateral on radiograph. The axial skeletal X-ray may show vertebral squaring during the early stages, The late stages radiograph may show ankylosis of vertebral segments with ossification of ligaments of spinal radiographs as well as erosion and ankylosis of the sacroiliac joint (4).

These radiographic changes are not apparent until the disease has matured after a few years or decades. Non-radiographic axial spondyloarthritis is an early phase of the disease where the sacroiliitis is only observed on MRI but not on radiographs (9). The 2009 ASAS criteria was welcomed as a big step towards improving the diagnosis of early axial AS as MRI features were recognised as a one of the classification criteria. . Delay in diagnosis of axial SpA is an ongoing challenge with an interval between symptom onset and diagnosis around 8-10 years (10, 11).

1.2.3 Psoriatic Arthritis

Psoriatic Arthritis (PsA) is a chronic autoimmune inflammatory joint disease in adults. The prevalence in psoriasis patients is 40% and in the general population up to 1% There is equal sex distribution with peak incidence between 35-55 years old (12). Clinical features include asymmetric or unilateral peripheral inflammatory joint disease with or without spinal pain. Dactylitis, or 'sausage digit', of the fingers or toes is one of the hallmark features of PsA present in around 40% of patients (13). Enthesitis, inflammation of the tendon and ligament insertional site into the bone, is also a regarded as a primary feature of PsA (14). Radiographic

features are normal in early PsA At a later stage, enthesophytes, juxta-articular new bone formation , 'flange' osteophytes in the DIP joints and soft tissue swelling of the dactylitis may be observed (12). In established disease, asymmetrical erosions may be seen in the DIP, PIP, MCP and carpus bone. In severe disease, gross osteolysis or 'pencil in cup' deformity may be observed (15).

The classification criteria of PsA around 50 years ago required the presence of three clinical features: inflammatory arthritis, skin psoriasis and negative Rheumatoid Factor (16). The current CASPAR criteria were published in 2006 (17). These criteria require established joint disease scoring at least three points from the following clinical features: current psoriasis or history of psoriasis, family history of psoriasis, dactylitis, new juxta-articular bone formation on radiograph, negative RF, and nail dystrophy. Current psoriasis scores two points and the remaining criteria score one point each. The CASPAR criteria has a sensitivity of 91% and specificity of 99% when compared against clinician opinion as a gold standard (17).

1.2.4 Reactive arthritis.

Reactive arthritis (ReA) is a non-infectious auto-immune arthritis occurring following an infection. Symptoms can start typically one to three weeks after the infection (18). It can occur at any age, although the mean age of onset is 25 to 35 years old (p). It is generally triggered by urogenital or enteric infections. Causative organism include *Campylobacter jejuni, Salmonella, Paratyphi B* and *C, Shigella, Chlamydia trachomatis* and *Yersinia pseudotuberculosis (12).*

Post-streptococcal arthritis is a reactive autoimmune disease but not generally clustered under the SpA spectrum. Lyme and viral arthropathies are also considered to be reactive arthritis but are neither within the SpA spectrum nor HLA B27-related (12).

Epidemiological studies have found it difficult to determine the true prevalence of disease as there are no clear cut diagnostic criteria, and also because some infections like *C trachomatis* are difficult to diagnose as they can be sub-clinical. The estimated prevalence is 0.03 to 1% with an incidence of 5-13 in 100,000 for post-urethritis and 5-14 in 100,000 for post-enteric reactive arthritis.

The clinical features include oligo-arthritis of large joints which are non-erosive. Dactylitis is seen in 16% of ReA patients. Limb enthesopathy can include heel pain, Achilles and patella ligament insertional pain in 30% of patients. Sacroiliitis is seen in 14-49%, and spondylitis in 12-26%. Eye manifestations include conjunctivitis, iritis, keratitis, episcleritis and corneal ulceration. Genitourinary symptoms can occur after *Chlamydia trachomatis* infection. This includes urethral pain and discharge, prostatitis, haemorrhagic cystitis and aseptic pyuria. 50% can get abnormal urine dipstick such as proteinuria, microhaematuria. Skin lesions can include circinate balanitis, palmar pustulosis, keratoderma and hyperkeratotic nails. Aortic valve disease is a rare and late complication (12).

There are no diagnostic or classification criteria for ReA, but in 1999 the ACR have published guidance after the International Workshop on Reactive Arthritis (19). They recommend that the nomenclature of ReA is **only used** if the clinical picture and infective agent involved are associated with spondyloarthritis and HLA B27. The ACR proposed the terminology of

'definite' of 'probable' ReA as follows. A 'definite' ReA if both major criteria and a corresponding minor criterion are fulfilled. A 'probable' ReA if both major criteria are fulfilled, with no corresponding minor criteria OR one major criterion with at least one minor criteria are met. The major criteria are 1) arthritis (with at least two features: asymmetrical, mono or oligo, lower limb involvement) and 2) preceding symptomatic infection (enteritis or urethritis within 3 day to 6 weeks prior to arthritis onset). The minor criteria are 1) evidence of infection trigger (i.e. positive urethral or cervical swab for *Chlamydia trachomatis* or positive stool culture for microbiome associated with ReA) and 2) persistent synovial infection defined as positive immunohistology or PCR for *Chlamydia trachomatis (19)*.

1.2.5 Crystal-related arthritis.

1.2.5.1 Gout

Gout is an inflammatory arthritis precipitated by crystallisation of monosodium urate crystals in joints or soft tissue. There is a broad spectrum of disease which includes, asymptomatic hyperuricaemia, acute gout, chronic tophaceous gout as well as urolithiasis and urate nephropathy although this is rare (12) .

The UK prevalence is 1.39% with a male to female ratio of 3.6:1. High serum uric acid is the main risk factor for developing gout. Other risk factors include central obesity, hypertension, loop and thiazide diuretics, excessive alcohol intake, and certain occupations including corporate executive or marine officers (12).

Gout can present either as acute or chronic. The first attack of acute gout are often mono-arthritis. The great toe MTP joint is affected in more than 50%. Other joints that are often

affected are weight-bearing joint, wrist, elbow and small joints of the hand. Attacks typically occur late at night or early morning. Sometimes patients report low grade fever and general tiredness. Triggers of an attack include surgery, dehydration or inter-current illness like infection. Gout attacks often last around five to seven days (12).

In chronic gout, a polyarticular pattern is often seen. The repeated gout flare up episodes become more frequent and last longer. This may result in joint deformity, reduced range of movement as well as chronic pain. Tophi are also often seen (20).

In 2015, the ACR and EULAR published gout classification criteria (21). The entry criterion is at least one episode of peripheral joint or bursal pain, tenderness or swelling. The criteria list eight domains. Two of these are imaging-detected gout-associated joint damage and urate deposition (based on US or dual-energy computed tomography appearance). Other domains include clinical joint involvement and laboratory features. The sensitivity and specificity of these 2015 criteria are 92% and 89% respectively. It is worth noting that patients who have MSU crystals in synovial fluid of a symptomatic joint, bursa or tophus are automatically classified as gout; and do not require scoring (22).

1.2.5.2 Pseudo-gout

Pseudo-gout is an inflammatory joint disease induced by calcium pyrophosphate disease (CPPD) crystal deposition in joint. It can present as acute or chronic arthropathy. The presence of CPPD crystals from synovial fluid on polarised light microscopy is considered the gold standard diagnosis. The prevalence increases with age and is more commonly observed in

women. Average age at presentation is 72. A number of attack triggers are recognised such as stroke, trauma and surgery. Plain radiograph may show chondrocalcinosis (12).

A mono-articular presentation is typical of acute pseudo gout. The knee, wrist, shoulder and elbow are often involved. Elderly patients can present with being generally unwell, with hypotension and confusion which can mimic systemic sepsis. Spinal involvement can also occur as 'crowned dens syndrome' which is acute attacks of neck pain associated with CPPDD deposition at the atlanto-axial joint (12).

1.3 Rheumatoid Arthritis

RA is a heterogeneous multi-system autoimmune joint disease. The aetiology is unknown. It typically manifests as symmetrical polyarthritis mainly affecting PIPs, MCPs, wrists and MTP joints. However any synovial joints can be affected. Fatigue is a common feature (12).

1.3.1 Clinical features

Early joint signs include soft tissue swelling, pain on active/passive joint movement and tenderness. The classic late signs include Boutonnière and swan-neck deformities of the fingers, Z thumb deformity, ulnar deviation and subluxation of the MCP and wrist joints. Rheumatoid nodules can be present in up to 20% of patients, usually in those with positive RF. Lower limb late signs include knee valgus and hind foot valgus with pes planus. Cock-up and hammer toe deformities are also late signs (23).

Radiographs of hands and feet may show soft tissue, swelling, reduced joint space, peri-articular osteopenia and erosions. However, radiographs are often normal during the early

disease phases. Bony erosions can be observed in around 80% of patients after 2 years of symptom onset (24).

1.3.2 Systemic impact of RA

Lymphoma incidence is increased around two-fold especially in longstanding disease (25), associated with prolonged high disease activity (26). RA is also associated with increased risk of cardiovascular disease such as myocardial infarction, cerebrovascular events and heart failure with a standardised mortality rate of 1.5 (27, 28). The increased rate is not accounted for by conventional risk factors of cardiovascular disease (29, 30), or the use of steroid or non-steroidal anti-inflammatory medications (31). In addition, the cardiovascular risk is increased during the early disease phases - which may reflect the subclinical inflammation in the arthralgia phases (32, 33). Pulmonary involvement can also be observed such as interstitial lung disease, pleurisy, pleural effusion and bronchiectasis.

Systemic inflammation in RA also impacts the brain causing fatigue and reduced cognitive function (31). Osteoporosis can occur even in the presence of modestly raised inflammatory markers or subclinical inflammation – and probably starts before the onset of joint disease (34-36). RA can also affect the exocrine glands causing secondary Sjogren's syndrome and muscle causing sarcopaenia (31) .

RA-associated interstitial lung disease (ILD) is the most common lung manifestation in RA patients. Estimates suggest that around a third of patients with RA have subclinical ILD which were detected by HRCT scans. The incidence of ILD remained stable despite advances in RA therapeutics (37) .

Rheumatoid vasculitis is the most serious extra-articular features in RA. However, the prevalence is now incredibly rare, likely due to therapeutic advances such as biologic therapy. It was previously a common cause of hospital admission in RA patients (38).

The classification criteria are based on clinical phenotype (39). In 2010, the ACR and EULAR established classification criteria with the entry criterion of a patient with at least one clinical clinically swollen joint not better explained by another disease. Patients are categorised as definite RA if they obtained at least 6 out of 10 scores from the following four domains; 1) number and site of joints involved, 2) inflammatory markers, 3) symptom duration and 4) presence of autoantibodies (39).

1.3.1 Pathophysiology of RA.

1.3.1.1 Synovitis

The hallmark of RA is joint inflammation alongside cartilage and bone damage (31). Synovitis is associated with both leukocyte infiltration within the synovial compartment and hyperplasia of resident cells. This leukocyte build-up is primarily a result of migration, as opposed to local proliferation. Cell migration is facilitated by activation of endothelium within the synovial micro-vessels, which increases the production of adhesion molecules and chemokines. Subsequently, neoangiogenesis occurs which is catalysed by the hypoxic micro-environment and presence of cytokines. In addition reduced lymphangiogenesis restricts cellular outflow from the synovial compartment. These environmental changes together with profound remodelling of synovial microarchitecture and local fibroblast activation result in thickening

of synovial tissue in RA (40). This build-up of synovial tissue then clinically manifests as joint swelling. Synovial hypertrophy can also be detected by imaging modalities such as US or MRI.

1.3.1.2 Tenosynovitis

Tenosynovitis is perhaps an under recognised pathology in RA. Rheumatology investigators have recently highlighted the importance of tendon inflammation in RA (41).

In 1950, Kellgren and Ball studied the incidence of tendon lesions in RA patients (42). They reviewed 100 consecutive RA patients at the Rheumatism Research Centre, Manchester University and the Manchester Royal Infirmary. They reported that 42 of 100 patients had tendon lesion. 37 of 42 patients had lesions of the finger flexors tendon. Other tendons affected were fingers extensors, ankle tendon and Achilles tendon. Their findings were consistent with that of their collaborator Edstrom which they also included in their Annals of Rheumatic Diseases paper. 188 out of 391 (48%) RA patients had tendon lesions. 125 out of 188 (65%) patients had tendon lesions involving the finger flexors tendon (42).

Interestingly, they performed therapeutic surgical procedures on the abnormal tendon for 18 of the RA patients and presented the cross section histology findings together with a detailed description of this surgical intervention. The spectrum of tendon findings was from 'dulling of the surface of tendon' to 'thickening of the tendon which looks yellow and streaked with grey gelatinous material'. Frequently nodules were also found varying in sizes – reportedly the severe nodules looked like 'large cauliflower-like masses' (42).

A modern histopathological study of tenosynovitis was reported in 2008 by Kaibara et al (43). They sought to assess the histological features of tenosynovitis compared to joint synovitis.

They reported that the synovial fibroblast harvested from the inflamed tenosynovium behaved in a similar manner to that isolated from joint synovium in terms of proliferation and inflammatory mediators production. Real-time polymerase chain reaction analysis showed similar mRNA inflammatory mediator expression in bothjoint synovitis. These findings suggested that the underlying synovitis and tenosynovitis are driven by similar pathogenesis in patients with RA (43).

1.3.2 Natural history of RA

There is increased recognition that inflammation and systemic autoimmunity precede the onset of joint disease in RA. EULAR has proposed a set of nomenclature for persons at risk to develop RA, in order to clearly a define specific patient population during the preclinical and early clinical phases of RA (44) Table 1-1.

Table 1-1 Terminology for each phase of the development of RA.

Phase	Terminology
A	Genetic risk factors
B	Environmental risk factors
C	Systemic autoimmunity
D	Arthralgia
E	Unclassified Arthritis
F	Rheumatoid Arthritis

These phases do not necessarily occur sequentially, as an individual may return to previous phases. In addition patients may not automatically go through all phases; for example seronegative RA patients may not go through phase C, although there may have been a transient phase of autoimmunity which is not detectable with current technology for autoantibody diagnostic tests (45) .

There is an interaction between genetic (46) and environmental risk factors in determining those who will develop into clinical RA. Environmental risk factors include smoking, obesity and even higher vagal tone increased the risk of RA development in those with RA-associated autoantibodies (40, 47). Other environmental risk factors are poor dental health (48, 49) and low level of oily fish consumption (50). This may be related to low level of n-6 polyunsaturated fatty acids (PUFA). It has been reported that erythrocyte membrane levels of the n-6 PUFA linoleic acid (LA) were inversely associated with risk of RA development in patients with pre-RA (51). The systemic autoimmunity phase can exist for some time prior to development of clinically apparent arthritis. RF and anti-CCP antibodies in peripheral blood was detected 5 years before onset of RA symptoms (median 4.5 years; range 0.1 to 13.8) (52). The risk of arthritis development in patients with ACPA and RF-positive arthralgia in those with positive was 40% (53).

In 1949, Swedish Rheumatologists reported in a study of 200 RA patients, 20% of which presented with 'arthralgia'. In their ARD paper, they defined arthralgia as 'pain without apparent objective joint changes' (54).

Data from the Leiden Early Arthritis cohort have shown that Rheumatologists had good clinical acumen to discriminate arthralgia patients who would progress to clinical RA vs those who do

not (OR 55) with sensitivity and specificity of 80% vs 93% respectively (55). This is based on clinical history, symptoms and signs with no diagnostic tests.

However, what constitutes 'inflammatory joint pain' can be difficult to define.

In 2017, a EULAR taskforce identified a combination of clinical characteristics that describe arthralgia patients at high risk developing clinical arthritis. The phrase was coined as 'clinically suspect arthralgia' (CSA). They identified five variables in the history domain and two variables in clinical examination domain that are associated with CSA (Table 1-2). The presence of at least three of these variables would identify those who would develop clinical arthritis with sensitivity and specificity of 90.2% and 74.4% respectively. The presence of at least four variables gives a sensitivity and specificity of 70.5% and 93.6% respectively.

Table 1-2 Variables for each domain for the clinically suspected arthralgia (CSA) description.

Domain	Variables
History	Joint symptom onset < 1 year. MCP joint symptoms. EMS of ≥60 min. Symptoms most severe in the early morning. First-degree relative with RA.
Clinical examination	MCP joints squeeze test positive. Difficulty making a fist.

Individuals with arthralgia may progress into developing clinical arthritis. However, the pattern of symmetrical polyarthritis may not develop immediately – especially in those with a more insidious disease onset.

1.4 Predictive algorithm to identify clinical arthritis development.

50% of patients with clinical synovitis of less than six weeks duration have resolving arthritis without therapy (56, 57). In the remaining patients, the disease progresses into persistent arthritis; some into RA whilst some would remain as unclassified arthritis or non-RA disease. It is important to distinguish those patients who will developed RA from those whose disease will remit spontaneously; so that aggressive immunosuppressant treatment can be targeted to the correct patient population as early possible (58).

In one of the early predictive studies, Tunn and Bacon reported RF positivity and ESR >30mmh as predictors of persistent synovitis in a cohort of patients with synovitis of 6 months duration. Green et al reported disease duration of greater than 12 weeks as the most important independent predictor of persistent arthritis (59).

In 2002, Rheumatology investigators from Leiden developed a rule to predict the development of persistent arthritis; with an area under the curve of 0.84 (60). This was based on scoring seven variables which were: 1) symptom duration, 2) anti-CCP positivity 3) RF positivity, 4) presence of radiographic erosions, 5) early morning stuff duration ≥1 hour (6) clinical arthritis in three or more joint areas; (7) positive bilateral compression pain in metatarsophalangeal (MTP) joints.

In 2007, the same group also developed a prediction rule to identify patients with unclassified arthritis who would then develop RA. This algorithm included nine clinical variables: 1) age, 2) sex, 3) site of symptoms, 4) early morning stiffness, 5) tender joint count, 6) swollen joint count, 7) CRP level, 8) RF positivity and 9) ACPA positivity (61).

This prediction rule gives a score ranging from 0 to 14. The higher the score, the higher the risk of RA development. E.g. 84% of patients with score of greater than 8.0 will develop RA and 91% of patients with a score of 6 or less will not develop RA. However, the utility of this algorithm is limited, as those who score 7-8 would have 50% risk of developing RA.

1.5 Main treatment strategies in RA.

1.5.1 Background

In the 1920s, physical treatment was the mainstay of RA therapy. Admitting patients with multiple active joints for investigation, rest and physical rehabilitation was common. Exercise was prescribed following rest to improve range of motion of inflamed joint as well as to strengthen the muscles (62). Massage and heat were also applied to improve circulation in affected joints with the aim to remove the local toxins. Joint casting and braces were applied to support the acutely inflamed joints and reduce contractures. Walking aids and orthoses were provided. In severely active joints, synovectomy was performed (62).

1.5.2 Current treatment strategies.

In 2020, although physiotherapy and orthotics are recommended, the mainstay of therapy is *disease modifying anti rheumatic drugs* (DMARDs).

The most recent NICE guidelines recommend methotrexate, leflunomide or sulfasalazine monotherapy as first line DMARD agent for newly diagnosed active RA patients (63). Hydroxychloroquine should be considered for first line therapy as alternative to oral methotrexate, leflunomide or sulfasalazine for mild or palindromic disease. A step up strategy with additional DMARDs should be considered when disease is not controlled on mono-therapy. The second DMARDs can be either oral methotrexate, leflunomide, sulfasalazine or hydroxychloroquine. Bridging therapy with oral, intra-muscular or intra-articular glucocorticoids should be considered whilst starting first line DMARDs (63).

Biological therapy should be prescribed if disease remains active despite DMARD combination. Active disease is defined by DAS28 greater than 5.1. The first line biological therapy that NICE recommends include adalimumab, etanercept, infliximab, certolizumab, golimumab, tocilizumab or abatacept. NICE recommends withdrawing biologic treatment if a moderate EULAR response is not achieved at 6 months. After a further six months , therapy should be withdrawn if moderate EULAR response is not sustained (63).

The treatment target should be to achieve remission, or if not possible low disease activity. The dose should be titrated as tolerated until treatment target is achieved. Remission should be a treatment target rather than low disease activity for patients who are at risk of radiographic progression, such as ACPA positive patients or those with erosive changes on baseline radiographs (63).

For analgesia, oral non-steroidal anti-inflammatory drugs (NSAIDs) should be considered for pain or stiffness symptomatic treatment. Other comorbidities such as renal, gastrointestinal,

liver and cardiotoxicity should be taken into account when prescribing NSAIDs. The lowest effective dose for the shortest time possible should be considered. Proton pump inhibitor (PPI) should be co-prescribed. If a patient is already on low dose aspirin, other forms of analgesia should be consider before adding NSAID (co-prescribe with PPI) (63).

In 2019, EULAR has updated the guideline for RA treatment (23). EULAR defined poor prognostic factors as follows; persistent moderate or high disease activity by composite measures despite synthetic DMARDs, high acute phase reactants, high swollen joint count, presence of RF and or ACPA, particularly if it is strongly positive, presence of early erosions, and failure of two or more synthetic DMARDS(23) . Patients with poor prognostic factor are recommended to start biologic or JAK inhibitor if inadequate response with methotrexate monotherapy. For those without poor prognostic factors, adding a second DMARD or switching to a different synthetic DMARD is recommended (23).

1.6 The 'window of opportunity' concept.

The concept of a therapeutic 'window of opportunity' is a widely recognised notion within the Rheumatology community. This paradigm first evolved when the concept of 'early and aggressive' therapy for RA replaced the traditional pyramid concept in which NSAIDs were the first-line of treatment and DMARDs introduced further down the line after the disease has progressed. The latter approach had been regarded as too little too late by the end of 1980s (64). The increased recognition that RA is no longer a benign disease, but rather a progressive systemic disease that results in substantial morbidity and mortality drove this change in treatment paradigm (20).

The hypothesis behind the 'window of opportunity' is that earlier disease phases are more amenable to therapy, and therefore that treatment instigated during them can alter the long-term disease trajectory. This is based on the understanding that synovitis, or the process underpinning synovitis, during the early disease phases is: a) ***quantitatively*** and b) ***qualitatively different,*** from that of established disease (65, 66).

A reduced 'synovitis load' or 'inflammation load' was thought to be more responsive to immunosuppressant compared to a higher disease load once the disease has progressed – similar to that of a primary cancer (65) .

There is an accumulation of evidence that the pathological process during the early disease process, particularly within the first three months of symptom onset is different (67). Synovial fluid from actively inflamed joints have a different cellular and cytokine fingerprint in early arthritis patients with symptom duration of less than 3 months who then progress to RA compared with those in whom arthritis resolves, and those with established disease (67-69) . Transient increases in distinctive cytokines from synovial biopsy taken in patients with RA compared to those whose disease is resolving have been described (68). In addition, synovial fibroblasts have long been acknowledged as one of the key players that drive persistence of inflammation in patients with RA and demonstrate functional and epigenetic differences in early and late phases of disease. Stromal marker fibroblast activation protein (FAP) is higher in patients with early RA compared with non-RA early arthritis patients. In addition, synovial fibroblasts from RA patients with a short symptom duration show a transient functional phenotype that encourages the migration of inflammatory infiltrates (70-73).

1.6.1 Evidence that earlier is better.

The concept of the 'therapeutic window of opportunity of RA is based upon the principle **that earlier treatment works better.** In other words, shorter symptom duration was found to be a predictor of therapeutic response, with therapy being 'any-type of treatment' – be it conservative, single DMARD or multiple DMARDs.

The first (reported) prognostic study that assessed predictors of treatment response in RA was described in Annals of Rheumatic Diseases in 1955 (74). Duthie and colleagues assessed the predictors of treatment response for 307 RA patients admitted to Northern General Hospital Edinburgh for in-patient rehabilitation for active RA between 1948 and 1951. The three phase treatment started with a two-week bed rest with 'skin-tight POP splints' to immobilise acute joints. This was followed by a tailored active and resisted exercise for the second phase. The third phase included strengthening exercise with weight-bearing activities and occupational therapy assessment to improve overall physical well-being (74).

The key message from these authors is in summarised in the following quote (74):

> *'The most important conclusion reached, on the basis of an analysis of the results of treatment on discharge and at follow-up, is that the immediate prognosis in patients admitted to hospital* ***within 1 year of the onset of symptoms is very materially better than it is in patients coming under treatment at a later stage of the disease****. This is in general agreement with the findings of other workers.'*

In this study, disease duration was classified as under one year, 1-5, 5-10 and over 10 years. Patients who had a disease duration of less than one year had better functional outcomes on

discharge and at follow-up. The authors also noted that duration of disease did not affect functional level on admission.

It is interesting to note that the remaining clinical predictors of treatment response assessed in that study were traditional clinical variables also studied in modern clinical studies. These were age, sex, disease severity on admission, ESR, anaemia, disability level, mode of onset (which was described as course of disease), and distribution of joint affected. In addition, socio-economic factors such as type of job (heavy, medium, light level of manual work) and occupation were also reported (74).

1.6.2 Evidence from observational studies and clinical trials.

In 1996, van der Heide et al designed a clinical trial specifically to test the 'window of opportunity' hypothesis. DMARD-naïve early RA patients were randomised to start DMARD immediately or at a later stage after inadequate response with NSAIDs. The radiological progression in both groups were similar at one year, although disease activity score after 6 and 12 months was reduced in those who started DMARD immediately (75).

In 2010, Van der Linden reported the clinical outcomes form the Leiden Early Arthritis cohort. The authors assessed the relationship between time-to-see first rheumatologists with these two clinical outcomes: 1) sustained DMARD-free remission, and 2) rate of joint disability at six year follow-up. There were 598 RA patients from the total 1674 early arthritis patients. One of the key findings was that RA patients who were reviewed within 12 weeks of symptom onset had 1) less joint damage and 2) a higher chance of DMARD-free sustained remission compared with those who presented with greater 12 weeks of symptom duration (76).

Finkh et al. reported a systematic review of clinical trials and observational studies that examined the association between treatment delay and radiographic joint damage in RA patients. Disease duration of less than 2 years was the main inclusion criterion. The pooled estimate effects from 12 studies showed that long term radiographic progression rates were lower with patients who were treated early compared to those who were treated later (77).

One of the main caveats when interpreting data from observation studies is confounding by indication (78). In the context of early arthritis, this is a bias that surfaces if the disease severity in RA drives the decision in terms of a) type of DMARD and/or, b) timing at which DMARD was initiated. To mitigate this issue, investigators from the Norfolk Arthritis Register used a propensity modelling technique to adjust for the relationship between time to start of DMARD therapy and radiographic progression – based upon the patients' likelihood (or propensity) to start DMARDs. The authors based their hypothesis on the assumption that the timing at which DMARD was initiated depended on the disease severity. In this study they compared 384 RA patients who had never been on DMARD with those who received DMARD *within* 6 months of symptom onset (early group), and with those *after* 6 months of symptom onset (delayed group) – with a view to assess radiographic progression over a 5-year period.

A simple comparison between those who received different treatment strategies would not be sufficient as the groups had different prognostic factors at baseline. After adjusting for disease severity, the increased level of disability (measured by HAQ) in those who started DMARD/steroid after 6 months of symptom onset compared with those who never had treatment persisted even after 5 years of follow-up (79).

In 2014, Van Nies et al. from Leiden undertook a systematic literature review of cohort and randomised controlled trials that reported outcome data of early RA patients in relation to symptom duration at start of treatment (80). This review concluded that prolonged symptom duration prior to starting first DMARDs was independently associated with a higher rate of radiographic progression and lower chance of DMARD-free sustained remission. A meta-analysis of more than 2000 patients within this review reported that shorter symptom duration was independently associated with DMARD-free sustained remission (80).

In 2019, Burgers et al. summarised the evidence on placebo-controlled clinical trials in early versus late treatment in early RA patients. The authors reported that eleven RCTs were published between 1988 and 2003 for RA patients with disease duration of less than two years (81). The intervention arm in these studies had various DMARDs including gold, sulfasalazine, hydroxychloroquine and oral prednisolone. Nine of these trials reported radiographic progression as one of the clinical outcomes. Out of these, nine RCTs reported favourable radiographic outcome in the early treatment groups. Four RCTs showed a statistically non-significant benefit of early treatment compared to late treatment and one showed no difference in radiographic outcome between the two groups (81).

1.6.3 Linear vs non-linear relationship between therapeutic response and time-to-therapy.

So far, we have seen compelling evidence that earlier treatment is superior to later treatment. However, the concept of a window of opportunity suggests that there is a specific time frame – after which treatment is not as beneficial as when treatment was initiated within the

window. Therefore, there is effectively a cut-off time-point that marks the 'closing of this window of opportunity'.

The fundamental question that may provide evidence for this concept is whether there is ***a linear or non-linear*** relationship between therapeutic response and time-to-therapy.

If this a *non-linear relationship*, then there is a time-limited period after the onset of symptoms during which the rate of therapeutic response is *significantly different*, compared to that of after this cut-off time. This is the duration that is referred as the '**therapeutic window of opportunity'.**

Figure 1-1 illustrates this hypothetical relationship graphically. Figure 1-1A shows a linear relationship between therapeutic response and time-to-therapy, and Figure 1-1B and C show a non-linear relationship.

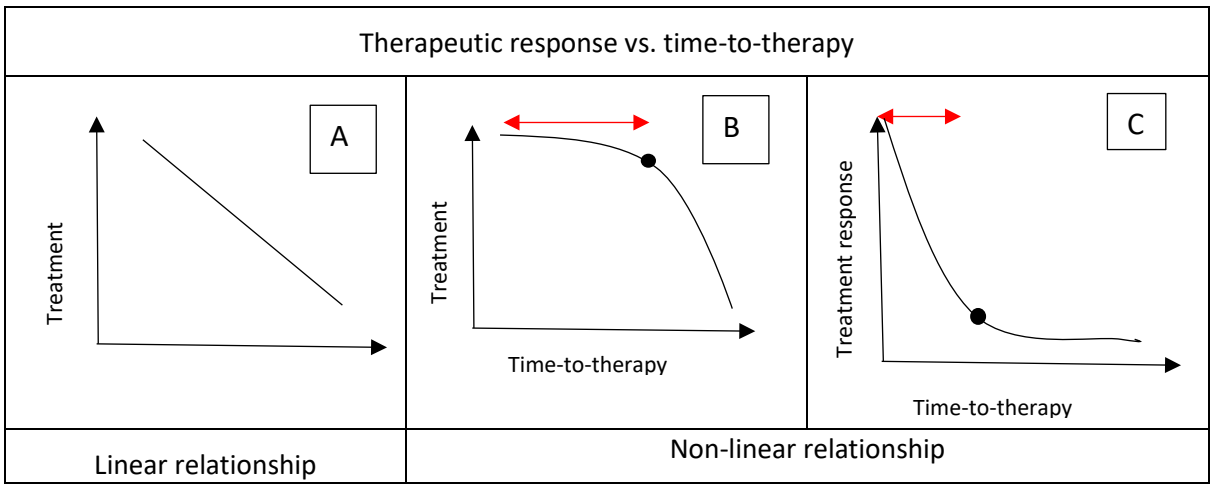


Figure 1-1 Hypothetical graphs of the relationship between therapeutic response and time-to-therapy. Black circle denotes the point of inflexion in the non-linear relationship; therapeutic response rate before and after this point is significantly different. The red arrows mark the therapeutic window of opportunity.

The ● in Figure 1-1 denotes the *point of inflexion* in the non-linear relationship graphs at which the *rate of therapeutic response before and after this point is significantly different*.

The red line in Figure 1-1 marks the '**therapeutic window of opportunity**.' This is the time-to-therapy up to the point of inflexion. There is still a treatment response after this window, but the rate of treatment response is disproportionately lower, compared to that of before the point of inflexion.

To clarify the conundrum whether the relationship between treatment response and symptom duration is linear vs non-linear, Nies et al. assessed the shape of the relationship between symptom duration with persistence of RA (82). Data of RA patients from two large longitudinal cohorts were analysed retrospectively; the Leiden Early Arthritis cohort (n=738), and French Early Arthritis cohort; ESPOIR n=533.

The relationship between **remission rate** and **symptom duration was not linear.** A time-dependent operator characteristic curve of symptom duration versus DMARD-free sustained remission was constructed. This showed that the best cut off in symptom duration that discriminated patients with DMARD-free sustained remission vs those with persistent disease was 14.9 weeks (95% CI 12.3 to 16) in the Dutch cohort. The corresponding figure in the French cohort was 19.1 weeks (96% CI 12.3 to 28.0) (82).

This has been the strongest suggestion to date, that indicates that there is a distinct period during the early months after symptom onset during which response to immunosuppressant therapy is greatest, leading to long-term improvement in clinical outcomes (83).

1.6.4 Limitations of current evidence

There are three major limitations which affect interpretation of evidence from existing studies. These are related to:

1. Varying definition of disease and symptom onset.
2. Different types of mode of onset.
3. Tool utilised to measure therapeutic response.

1.6.4.1 Definitions of disease and symptom onset

Definitions of the timing of disease onset have varied widely between studies, compromising the validity of systematic and meta-analytic studies (84).

In reported studies, 'disease onset' was defined as either

1. onset of symptoms which were suspected to be related to RA (77, 85-92).
2. onset of first joint swelling attributable to joint inflammation (79, 93).
3. date of the fulfilment of RA classification criteria (94).
4. date of when the clinical diagnosis was made (95-97).

These definitions represent (in most patients) chronologically separate points during the development of RA; from the very early symptomatic stages (without any clinical signs) to the stage when there is patient- or physician-observed multiple joint inflammation that fulfils RA classification (6). In some cases the patient-reported symptoms and patient-observed swelling occur simultaneously, which further complicates the interpretation of 'onset'.

Experts from the EULAR Study Group on Risk Factors for RA have highlighted the importance of *using clearly defined symptom and disease onset dates* in clinical studies (44, 84) in order to ensure generalisability of results.

In addition, 'symptoms' that can be associated with rheumatoid arthritis are not single attributes. Symptoms at the earliest phases of RA are diverse. Stack et al reported a synthesis of qualitative literature to summarise these symptoms complexes. At the early phases of RA, these symptoms were observed in five themes; 1) swelling, 2) pain and tenderness, 3) joint stiffness , 4) fatigue and weakness and 5) emotional impact (98).

A qualitative study was then performed in order to explore the symptomology of those with seropositive arthralgia and newly diagnosed RA patients. In this qualitative study, the collection of symptoms at the earliest disease phases was broad (99). These included joint pain, emotional distress, muscle cramps, numbness and tingling, stiffness, weakness, fatigue and sleep disturbances.

In summary, RA symptoms during the earliest phases of disease constitute a set of symptom complexes, rather than a single symptom attribute - this adds another layer of complexity when determining the 'symptom onset' in clinical studies. There is no define criteria of what constitutes 'inflammatory symptoms' - as the initial manifestation, which may indeed be extra-articular such as muscle weakness, fatigue and numbness and tingling.

1.6.4.2 Mode of onset was not defined

The second limitation of current evidence relates to how rapid the disease manifests at initial presentation. There is a spectrum of how quickly joint symptoms develop during early disease

phase. In this thesis, this is termed 'mode of onset'. The different modes of onset may have an impact on 1) measurement of disease duration and, 2) how quickly a patient presents. These factors are of key importance, if the window of opportunity in RA is to be determined, as they can introduce artefacts in disease duration measurements.

Rheumatologists recognise at least three distinct modes of disease onset. Patients with **abrupt onset RA** rapidly develop significant symptoms and signs over a period of hours to days as their first disease manifestation and may (or may not) present early to health services. Patients with **insidious onset RA** experience a gradual build-up of symptoms and joint swelling over weeks to months which may (or may not) delay presentation. Patients with **palindromic onset RA** experience intermittent short episodes of joint inflammation but return to normal between attacks. These different types of mode of onset are illustrated in Figure 1-2.

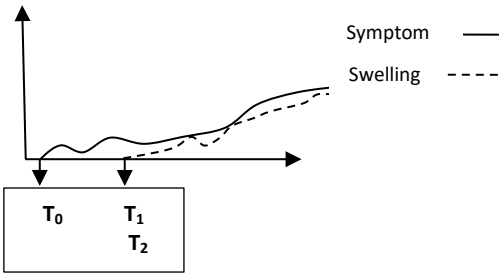
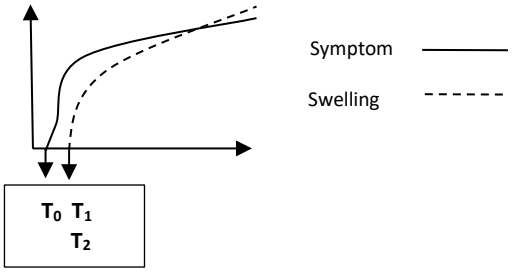
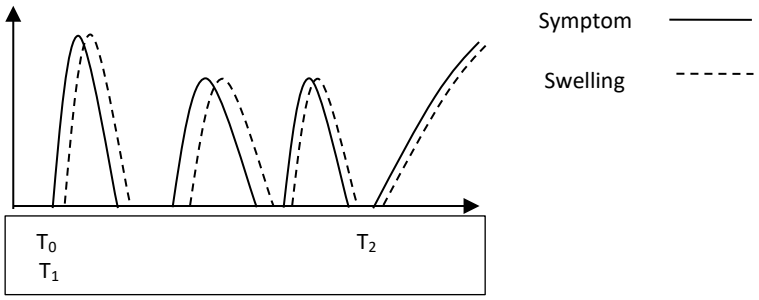
Insidious onset	Abrupt onset
	
Onset that begins gradually and reaches a maximum intensity within weeks or months.	Onset that begins abruptly and reaches a maximum intensity within hours or days.
Palindromic onset	
	A history or physical examination findings consistent with symptoms and/or synovial swelling that returns to normal between attacks.

Figure 1-2 Mode and timing of onset definitions. T0: Onset of current inflammatory symptoms. T1: Onset of any related inflammatory joint swelling. T2: Onset of current ongoing inflammatory joint swelling.

1.6.4.2.1 Literature review on mode of onset.

In 1944, Captain Boland from the US Army reported his observations of two different types of rheumatoid arthritis patients that presented to an Army General Hospital for the year 1942 (100). Out of 350 case of arthritis and related conditions, approximately 20% of these were RA cases. He noted that RA presentation amongst soldiers tend to be **subacute,** starting from the lower limbs. This was in contrast with **insidious onset** RA with early predilection of small joints in the fingers that was observed in rheumatoid arthritis patients amongst the general population.

In 1949, Swedish rheumatologists were interested to study the findings at onset further. I present a quote from their introduction section:

> *"Like Kahlmeter (1944), many people distinguish between two forms of rheumatoid arthritis: one, as described by Charcot, with insidious onset, symmetrical joint symptoms, and low temperature; and another with more acute onset, febrile reaction, and joint symptoms setting in violently."*

Intrigued by these differing modes of onset, this group then set out to systematically describe the 'Early Symptoms of Rheumatoid Arthritis' which was published in Annals of Rheumatic Diseases in 1949. They reviewed 200 case records of RA patients and concluded that there were six different types of onset, and provided a somewhat detailed description for each type of onset.

A short description with the proportion of each type is summarised as below:

Type 1: Slow and insidious onset and progressing course, with no fever. (37.5%)

Type 2: Acute onset on involving the small joints. Sometimes with fever. (14%)

Type 3: Similar to type 2, but onset was the large joints. (17%)

Type 4: 'atypical rheumatoid'; Affecting hands and feet for a considerable time. Can be asymmetrical, typically with low ESR and less likely to progress to full rheumatoid. (1%)

Type 5: Starts with arthralgia symptoms; defined as pain 'without apparent objective joint changes'. (20%)

Type 6: The remaining cases; small or large joints are involved, less symmetrical and may have extra-articular. (36%)

This was a cross-sectional study and therefore no long-term clinical outcome were reported. (54).

However, Duthie and colleagues in Edinburgh did report the long term outcome of RA patients according to mode of onset in 1955 (74). They classified mode of onset a 1) **slowly progressive,** 2) **remissions and exacerbations,** 3) **rapidly progressive** (74) . This was the longitudinal study in which RA patients had intensive in-patient rehabilitation. The authors reported that the *mode of onset had a prognostic value* in this cohort. Patients with a rapidly progressive course were more likely to have better functional capacity at follow up compared to those with slowly progressive and remission /exacerbation patterns. In this study they did not perform any regression analysis to control for both symptom duration and mode of onset. They also did not provide any description on each mode of onset.

The last notable study of mode of onset was reported by Jacoby and colleagues in the BMJ in 1973. This was a longitudinal study looking at 100 RA patient with 11 years follow-up. Interestingly, mode of onset was classified based upon patient recall. **Acute onset**, if the patient could recall the specific day, '**subacute onset'** if onset could be recall to the nearest week, and '**gradual onset'** if patient could recall to the nearest month. The authors reported that there was no significant difference in the functional ability at final follow-up according to the differing mode of onset groups (101). The most apparent limitation is that this definition of mode on onset was 1) vulnerable to recall bias and 2) open to interpretation of what onset it was dated for – was is symptom or joint swelling onset? These were not clarified in the study report.

The key issue that I wish to highlight is that – no study has assessed the relationship between symptom duration prior to treatment and outcome treatment whilst adjusting for the *mode of onset.*

The mode of onset can affect how soon a patient presents (102). In addition, disease severity and how much the symptoms are interfering with daily activities would also have an impact on how soon a patient seek medical advice. Presumably a soldier with low grade disease affecting the knees would have a need to present sooner to a clinician compared to an individual with a less physically demanding job. This is one of the potential confounders that has yet to be systematically addressed in studies looking at the timing of the window of opportunity in early RA.

1.6.4.3 Lack of robustness in measuring therapeutic response in early disease.

The final limitation is related to the clinical outcomes used to measure suppression of joint inflammation. Existing methods of measuring therapeutic response are limited by the use of subjective variables such as swollen and tender joint counts and patient or physician global indices. These are often combined as composite indices such as the disease activity score using 28 joints (DAS-28), which can be confounded by non-inflammatory co-morbidities such as nodal osteoarthritis, chronic pain and obesity (103). Ideally, measuring therapeutic response in clinical trials and routine practice requires a non-invasive point of care tool that can be used to accurately quantify inflammation at the joint level. Musculoskeletal ultrasound offers just such a tool and is increasingly being incorporated into clinical trials (104).

1.6.4.3.1 Role of ultrasound in early arthritis.

Over recent years, the use of musculoskeletal ultrasound has generated new insights into the onset of joint inflammation (105, 106) and evolution of erosive disease. Ultrasound is more sensitive and specific than clinical examination in the detection of joint inflammation (107), has been shown to be reliable and reproducible between trained observers (108, 109) and is sensitive to change in response to therapeutics (104, 110). This non-invasive imaging modality is an ideal disease-monitoring tool, particularly in the early phases of RA when clinical signs are frequently not overt (111). There is now a significant expansion in the number of qualified sonographers amongst specialists both in musculoskeletal Radiology and Rheumatology and increased availability of ultrasound equipment within Rheumatology departments (112). Integrating ultrasound as part of routine disease activity assessment in early RA will improve

the detection of response to therapy in addition to identifying residual inflammation during therapy which is otherwise not clinically detected.

1.6.5 Potential benefits to the NHS and patients.

Identifying the presence of therapeutic window of opportunity in RA would greatly benefit heath care providers and patients. This would identify the critical time frame within which GPs are required to refer patients with suspected inflammatory arthritis for a specialist opinion. This would enable health care providers to have a clear time-to-target definition in each mode of RA onset when designing public health and primary care information campaigns.

These outcomes would directly benefit patients. Short symptom duration is a predictor of DMARD-free sustained remission (113, 114),lower rate of radiological progression (76, 93), better functional outcomes (89) and a lower rate of joint replacement surgery (115). This translates to a better quality of life for RA patients.

Improved targeting of resources towards appropriate patients with clear knowledge of timelines is also likely to result in productivity gains for the economy. The NAO's economic modelling based on a crude estimate of the impact of delay suggested that increasing the proportion of RA patients treated within three months by ten percent could result in a gain of £31 million for the UK economy due to reduced sick leave and loss of employment, with a four percent increased in quality adjusted life years (QALY) (116).

Data on the window of opportunity will inform assessment for the health economic modelling of treating early arthritis within the time-to-therapy target in a real-world setting. This will

provide key data directing resources that should be leveraged for ongoing national initiatives to develop targeted public education and primary care campaigns aimed at delivering these benefits.

1.7 Ultrasound in Rheumatology

Ultrasound (US) imaging has become increasingly popular amongst rheumatologists over recent years and this is further encouraged by the falling cost of ultrasound units. US is an attractive point-of-care tool as it provides real-time image visualisation to both the operator and patient, which may enhance the clinical care experience. Furthermore it is safe, does not involve ionising radiation and can be easily repeated in subsequent visits. In addition, ultrasound can examine multiple regions in the same setting, unlike magnetic resonance imaging (MRI). Firstly, I summarise the principles which underpin the physics of US. Subsequently, I describe the musculoskeletal pathologies which are amenable to US examination. Lastly, I summarise the role of ultrasound according to disease area but especially in RA.

1.7.1 Physics of ultrasound

Ultrasound systems generate images based on the use of high frequency sound waves which usually range from 1 to 20MHz (i.e. 1 to 20 million vibrations per second). These sound waves are produced by mechanical oscillations of crystals within the ultrasound transducer as a result of electrical pulses. This is known as the *piezoelectric effect.* The sound waves reflected back from the tissues in turn vibrate the same crystals, generating an electrical impulse that is recorded by the system. The probe, therefore, acts as both emitter and receiver. Overall,

the ultrasound image generated by the system is the outcome of processed emitted and received waveforms that have been modified by passing through and reflected within biological tissues (117).

This process is described in more detail as follows. Ultrasound waves generated within the transducer propagate across the tissues, and some of these are lost through *scattering* to the surrounding tissues, whilst some are lost as heat energy. This process is called *attenuation*. The remaining of ultrasound waves are deflected back as *echoes* towards the transducer. The differential in density between two adjacent tissues dictates the magnitude of intensity of the returned echo. The ultrasound unit then processed these returning echoes to generate a two dimensional ultrasound image of the region of interest. Low density tissue such as fluid effusion is observed as a dark area on an ultrasound image. This dark area is called **anechoic**. Ultrasound waves cannot penetrate the cortical bone surface and most ultrasound waves are reflected back as echoes towards the transducer appearing as **hyperechoic** *(*i.e. bright white on an ultrasound image). Low density structures such as synovial hypertrophy may appear as **hypoechoic** structures *(dark-grey echogenicity)* (117). Anatomical structures with mixed density such as muscle and subcutaneous tissues appear as **isoechoic or mixed echogenicity** (similar to liver or thyroid parenchyma).

Given that ultrasound waves do not pass through structures such as bone, only tissues accessible by the **acoustic window** can be visualised by ultrasound scanning. **Acoustic shadowing** occurs when ultrasound waves are obstructed by a relatively high-density structure such as bone or calcification, resulting in an interruption of the ultrasound beam

pathway. Structures deprived of ultrasound waves appear as a dark shadow on the corresponding ultrasound image (117).

Higher frequency ultrasound waves have relatively short wavelengths which are easily attenuated and do not propagate into deep tissue. Therefore, high frequency transducers are suited to scan relatively superficial structures such as the small joints of the hand. Conversely, lower frequency transducers generate relatively long ultrasound wavelengths which propagate into the deeper tissues. Hence, low frequency transducers are used to visualise deeper structures such as the hip joint. The choice of transducer is crucial in generating high quality ultrasound images and largely depends on the type of joint and patient's body habitus. Table 1-3 gives a general guide on the range of ultrasound transducer in relation to the type of joint (117).

Table 1-3 The range of ultrasound transducer by joint.

3-7 MHz	7-12MHz	7-15 MHz	10-20 MHz
Hip	Knee	Elbow	MCP
	Shoulder	Ankle	MTP
	Hip	Knee	PIP
			DIP
			Wrist
			Ankle

The main utility of ultrasound is based upon the over-arching principle that ultrasound imaging improves the sensitivity and specificity of detecting joint and tendon inflammation compared to clinical examination. The mean detection rate of synovitis in the small joints of hand and foot using ultrasound is 2.18-fold greater than clinical examination (range: 0.55-8.96) (111).

1.7.2 Pathology identified by ultrasound examination

Ultrasound examination provides information on the articular bony surfaces, articular hyaline cartilage, bursae, joint recesses, tendons ligaments and entheses. It is imperative that the sonographer is familiar with the appearance of normal and abnormal tissues with the ultrasound scanner and probe used.

Synovial hypertrophy appears as non-displaceable or poorly compressible hypoechoic intra-articular tissue (relative to the subcutaneous fat and interstitial tissues) but can also appear as isoechoic under some circumstances (118) (119) (Figure 1-3).

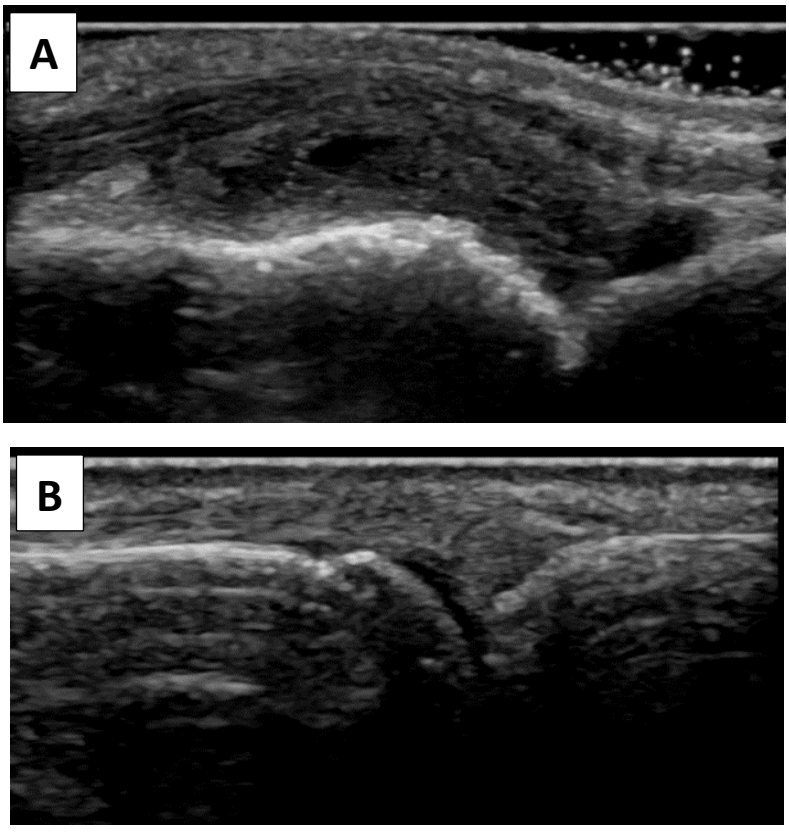


Figure 1-3 Ultrasound image of synovial hypertrophy (A) and normal appearance (B) of an MCP joint in longitudinal view.

Synovial effusion appears as abnormal anechoic intra-articular finding that is easily displaceable by the ultrasound transducer (120) (Figure 1-4).

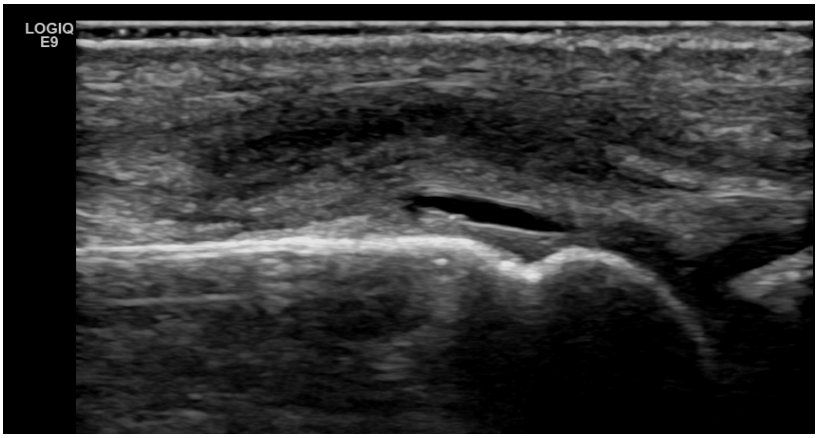


Figure 1-4 Ultrasound image shows synovial effusion with an MCP joint in longitudinal view.

Tenosynovial hypertrophy appears as abnormal hypoechoic (relative to tendon fibres) tissue within the tenosynovial sheath that is not displaceable and poorly compressible (Figure 1-5).

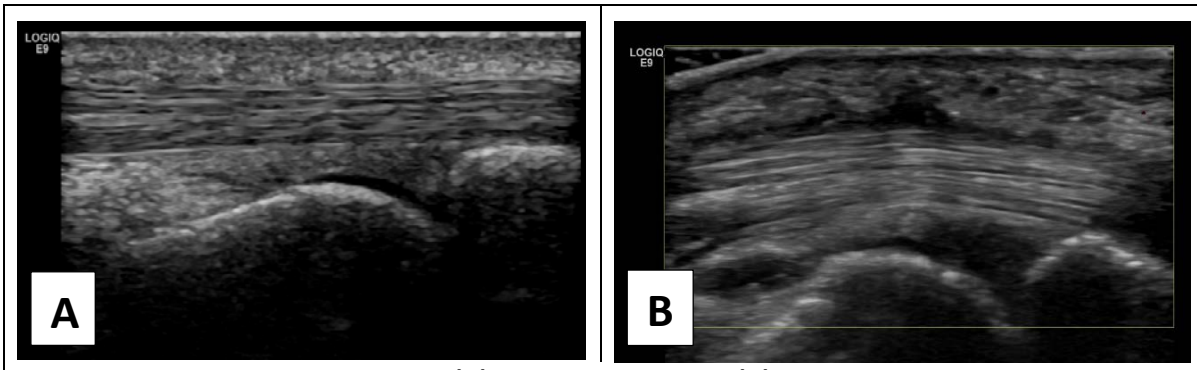


Figure 1-5 Ultrasound images show (A) normal tendon and (B) tenosynovial hypertrophy surrounding a finger flexor tendon in an RA patient on longitudinal view.

Tenosynovial effusion appears as abnormal anechoic or hypoechoic (relative to tendon fibres) displaceable areas within the synovial sheath, either localised (e.g. in the synovial sheath cul-de-sacs) or surrounding the tendon Figure 1-6 (108).

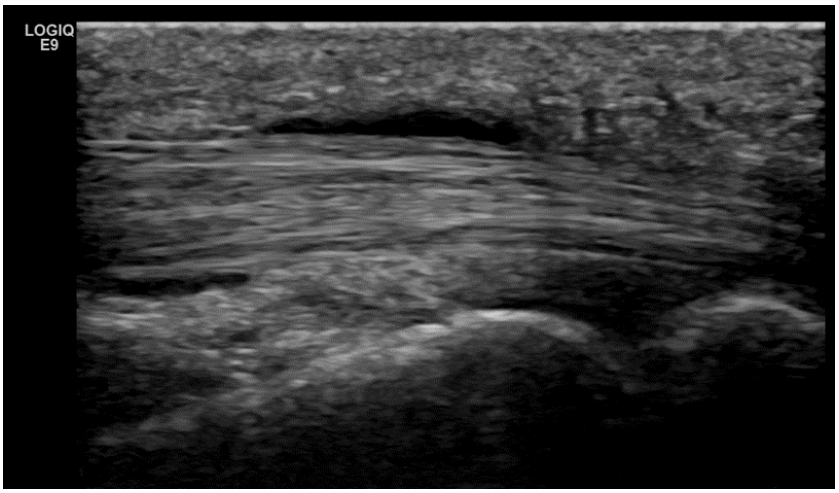


Figure 1-6 Ultrasound image illustrates tenosynovial effusion surrounding a finger flexor in an RA patient on longitudinal view.

Enthesitis appears as abnormal hypoechoic and/or thickened tendons or ligaments with loss of normal fibrillary architecture at the site of bony attachment, seen in two perpendicular planes. This may be associated with increased Doppler signal, hyperechoic foci consistent with calcification and/or bony changes, including enthesophytes, erosions or irregularity of the bony surface (120).

Erosion is a cortical break or defect which may be associated with an irregular floor, seen in two perpendicular planes (Figure 1-7).

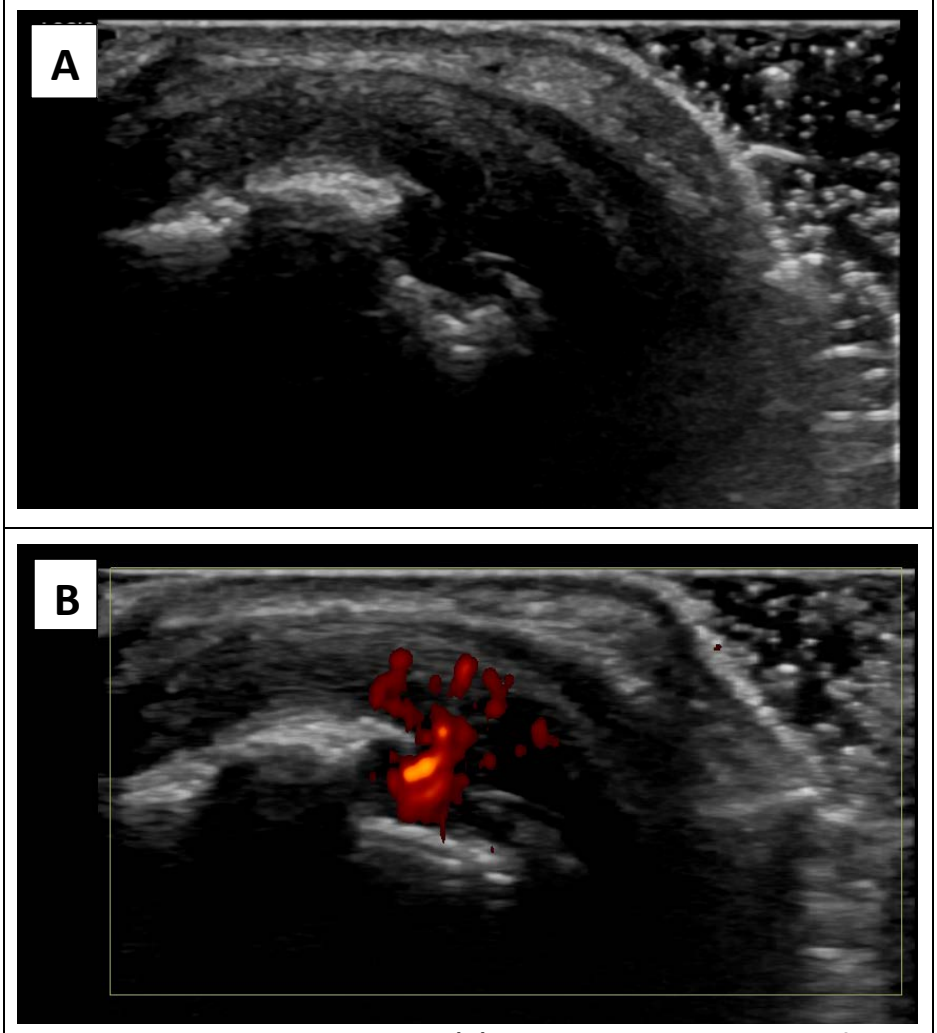


Figure 1-7 Ultrasound images show (A) an erosion in an MCP joint of an RA patient on longitudinal view, and (B) the corresponding Power Doppler (PD) image which shows PD activity within the erosion.

Osteophytes are seen as step-up bony prominences at the end of the normal bone contour, or at the margin of the joint seen in two perpendicular planes, with or without acoustic shadow (121)(Figure 1-8).

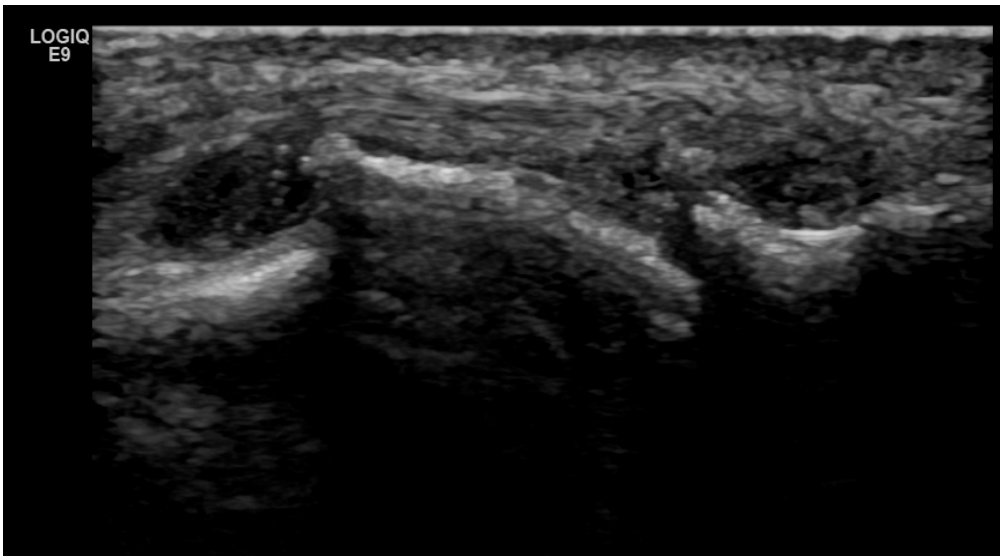


Figure 1-8 Ultrasound image shows an osteophyte at the bony margin of an MTP joint on longitudinal view.

Whilst grey-scale ultrasound provides information on based on tissue acoustic impedance which is dependent on tissue density, Doppler provides information on the extent of blood flow (hyperemia) within the region of interest Figure 1-9. The degree of hyperemia, shown as areas of Doppler enhancement within the synovial tissue of a joint, is closely correlated to active inflammation (122-124).

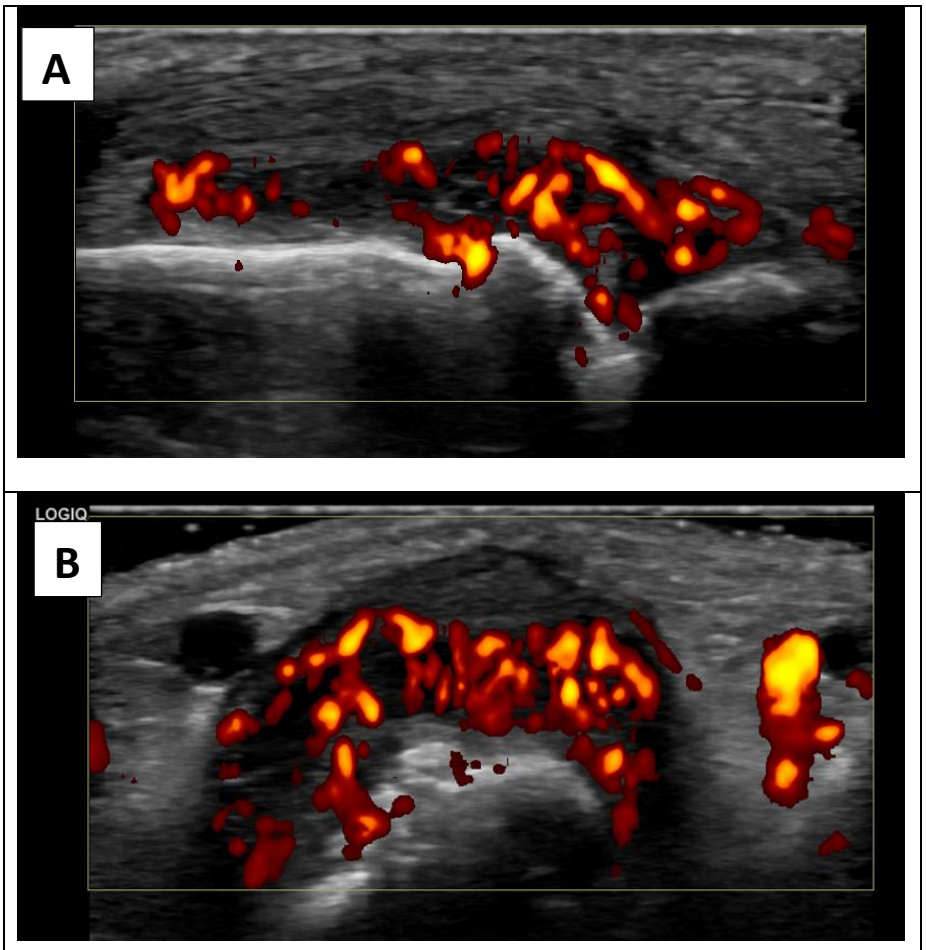


Figure 1-9 Ultrasound images show Power Doppler enhancement within the synovial hypertrophy area of an MCP joint in a patient with RA (A: longitudinal view; B: transverse view).

Similarly, active tenosynovial inflammation (or active tenosynovitis) is indicated by the presence of peri-tendinous Doppler signal within the synovial sheath, excluding normal feeding vessels (i.e. vessels at the mesotenon or vinculae or vessels entering the synovial sheath from surrounding tissues) only if the tendon shows peri-tendinous synovial sheath tissue hypertrophy on grey-scale (Figure 1-10)

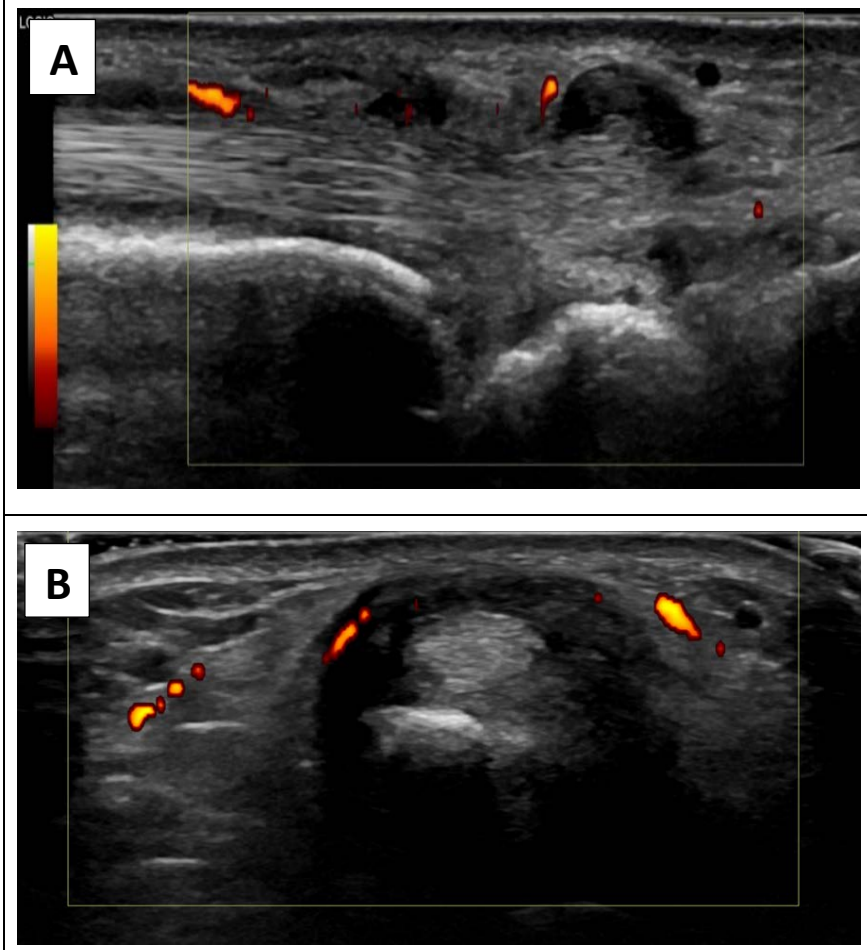


Figure 1-10 Ultrasound images show Power Doppler enhancement within the tenosynovial hypertrophy within the Extensor Carpi Ulnaris in a patient with RA (A: longitudinal view; B: transverse view).

Ultrasound examination cannot always distinguish between the underlying causes of imaging defined pathologies above. For instance synovitis caused by RA cannot be distinguished from synovitis caused by osteoarthritis using current technology. However differing patterns of involvement and pathognomic associated imaging features, for instance osteophytes in osteoarthritis (OA) or tophus in gout, can guide the diagnostic process. In addition, each of the ultrasound lesions should be observed in two perpendicular planes. As with other imaging modalities, ultrasound examination should be utilised as a part of an overall assessment; the interpretation should take into account the global picture including clinical, laboratory and other imaging assessments.

1.7.3 The role of ultrasound in improving diagnostic certainty in RA and prediction of arthritis development.

Ultrasound assessment may influence clinicians' clinical decisions with respect to diagnosis and planning therapy. Amongst newly-referred patients for inflammatory arthritis, ultrasound examination of small joints of the hand confirmed or changed the diagnosis in 76.3% of cases. Ultrasound assessment also influenced treatment decisions in 27% of follow-up patients (125). Matsos and colleagues have also shown that physician certainty of specific clinical findings improved significantly following ultrasound assessment [(synovitis; (9.7% vs. 38.7%), tenosynovitis (9.7% vs 46.8%), erosions (1.6% vs. 58.1%) and enthesitis (50.0 vs. 83.9%)]. In addition, ultrasound also influenced physicians' treatment decisions; in one study 89% of patients were planned to initiate DMARDs, but this figure fell to 48% following ultrasound assessment (126).

Multiple studies have demonstrated the value of power Doppler in prediction of inflammatory arthritis development. In patients with inflammatory hand symptoms for less than 12 weeks and negative auto-antibodies, the presence of power Doppler increased the certainty of developing inflammatory arthritis at 12 month follow-up (127). Similarly, Rakieh et. al. reported that the presence of PD enhancement on ultrasound is a risk factor to progression to inflammatory arthritis in a cohort of patients with new onset inflammatory symptoms and no clinical joint swelling (128).

In patients with unclassified inflammatory arthritis, the presence of power Doppler enhancement increased the likelihood of progression to RA (Odds Ratio (OR) of 9.9 with one positive joint, and 48.7 if three or more positive joints. In this cohort, the OR for high titre of ACPA or RF was 10.9 (129). Ultrasound-detected synovitis improves the prediction of RA progression above and beyond clinical predictors of RA development. Filer et. al reported that the sum of Doppler grades of MCP 2-3, wrists and MTP 2-3 significantly improved the prediction of RA development even after taking into account clinical variables such as presence of RF and/or ACPA, inflammatory markers and clinical joint counts. The inclusion criteria for this study were patients with at least one clinically swollen joint and symptom duration of less than 12 weeks (106).

Although the role of ultrasound-detected joint inflammation is well-described, the value of ultrasound-detected tendon inflammation in predicting disease development is under-reported. Lillegraven and co-workers reported that baseline ultrasound-detected extensor carpi ulnaris tenosynovitis was a predictor of erosive progression after one year (OR 7.18) and three years (OR 3.4)] in patients with early RA (130).

1.7.4 The role of ultrasound in the diagnosis of non-RA arthritides.

Ultrasound features may discriminate RA from other inflammatory arthritis. Tinazzi et al has reported that finger flexor tendon enthesopathy with enthesophyte was significantly more common in psoriatic arthritis (PsA) compared to RA and healthy subjects (p=0.001) (131). In addition, Zabotti et al. reported that the presence of at least one extra-synovial change on hand ultrasound was significantly associated with early PsA, rather than early RA. The combination of hand ultrasound features and dotted vessels observed on nail-fold dermatoscopy increased specificity for PsA to 90.5% (132). Ultrasound is also useful in the assessment in patients with established spondyloarthritis (SpA), particularly when discriminating from other diagnostic categories. Several studies have shown that ultrasound can differentiate SpA from RA (133-138). In addition, ultrasound assessment is useful to discriminate PsA from non-inflammatory musculoskeletal disease such as fibromyalgia (139-141). Power Doppler enhancement within plantar fascia enthesopathy and Achilles tendon inflammation were specific for PsA, and not for fibromyalgia patients. Importantly inflammatory changes in three or more sites had a high discriminating power between PsA and fibromyalgia (139).

In calcium pyrophosphate disease (CPPD)-related arthritis, ultrasound can visualise hyper echogenic crystal deposition within the cartilage layer, whilst in gout-related arthritis crystal deposition is seen on the surface of the cartilage (142). In osteoarthritic joints, ultrasound imaging can visualise abnormalities of articular cartilage during early disease phases, when irregularities and blurring of the cartilage margins are seen. As the disease progresses, there is loss of cartilage homogeneity and transparency. In long-standing disease, focal cartilage

thinning develops which may progress to diffuse thinning and complete destruction of cartilage leading to bony denudation. Joint effusion is common in osteoarthritis and when this is present overlying the upper surface of the cartilage it may create a false impression of a normal cartilage (pseudo thickening) (143). Overlying fluid must be carefully displaced to correct this artefact.

1.8 Limitation of current predictive algorithm.

Current predictive algorithms depend heavily on clinical and serological data as candidate variables. The natural history of clinical arthritis dictates that underlying inflammation that may not be clinically apparent, particularly during the early disease phases. Imaging modalities such as US and MRI have highlighted the limitation of clinical examination to detect joint inflammation. Szkudlarek and colleagues have demonstrated that clinical examination had only 40% and 43% of sensitivity for synovitis, compared to US and contrast-enhanced MRI, respectively (144) (107).

The ability of ultrasound to detect subclinical joint inflammation should be capitalised upon to improve predictive algorithms. Filer et al showed that the sum of Power Doppler grading of MCP 2-3, wrists and MTP 2-3 improved the prediction of RA development. The area under the curve value increased from 0.905 to 0.962 when the Power Doppler scores of those joints were combined with the Leiden prediction rule. The inclusion criteria for this study were patients with at least one clinically swollen joint and symptom duration of less than 12 weeks (106).

This clearly highlights that integrating ultrasound variables into clinical algorithms can enhance their predictive capacity. At present, no studies have included *tendon* ultrasound variables in the predictive algorithm of RA development. In addition, predictive algorithms for persistent arthritis development, so far, have only focused on *joint* ultrasound variables; rather than *tendon* US variables. This is a research area that is currently under-investigated. According to a report by Matsos et al, physician certainty of the presence of tenosynovitis increased from 9.7% to 46.8% following ultrasound assessment. Integrating tendon alongside joint US and other traditional variables in predictive algorithms may therefore improve the accuracy of identifying those who are destined to developed persistent arthritis or RA.

1.9 Limitation in rheumatology ultrasound.

One of the crucial aspects to consider when utilising ultrasound in clinical practice and trials is defining the threshold of normality at the joint and patient level. There has been a significant improvement in ultrasound technology over the last ten years, which has led to acquisition of higher image resolution. Subsequently, ultrasound can now detect subtle lesions even in healthy individuals with no joint symptoms. In one healthy subject study, Padovano et al. reported that 89% of healthy individuals (182 out of 207) had at least one ultrasound abnormality (24) . Although this number appears high, the ultrasound findings reported were only present in 9% of the joints examined. This was mostly in the feet, particularly in the first MTP joint (33% of the positive joints). Amongst the type of ultrasound pathology observed, synovial effusion was the most frequent ultrasound finding (68% of the positive joints), followed by synovial hypertrophy (31%). Nevertheless, the severity of these pathology was mild (grade 1 in average), regardless of the type of ultrasound pathology. These findings

indicate that the imaging boundary between normal joints (i.e. physiological changes) and those of early arthritis joints (i.e. early pathological changes) needs to be carefully studied (24). It is possible that a proportion of ultrasound-defined pathology observed in normal joints may be attributable to age-related changes or biomechanical factors (overuse for example). These issues highlight that it is crucial to describe the normal threshold at the joint level in each age group. This would then enable ultrasound investigators to define key thresholds to guide interventions, which includes defining ultrasound remission targets and escalation of therapy.

1.10 Hypotheses

In this thesis, I tested the following hypotheses.

1.10.1 Hypothesis 1

Ultrasound grading severity findings in healthy individuals are different across different age groups.

My objectives were to

1. create a study protocol based upon consensus scanning and grading techniques.
2. develop a large collaborative network of units experienced in musculoskeletal ultrasound.
3. study the ultrasound findings in a large cohort of healthy volunteers across the age range.

1.10.2 Hypothesis 2

Tendon ultrasound variables can add independent information to predict the development of

i. RA,
ii. persistent arthritis,

in combination with joint ultrasound, clinical and serological variables in patients with recent onset clinical synovitis.

My aims were to

1. assess the frequency and distribution of US-defined synovitis and tenosynovitis in patients with inflammatory arthritis of ≤3 months symptom duration.
2. investigate whether US-defined tenosynovitis provides predictive data over and above US-defined joint synovitis and other clinical and serological variables in the prediction of RA development.
3. compare the prevalence of US-defined synovitis and tenosynovitis in patients with persistent vs. resolving arthritis in a cohort of patients with inflammatory arthritis of ≤3 months symptom duration.
4. investigate whether US-defined tenosynovitis provides predictive data over and above US-defined joint synovitis and other clinical and serological variables in the prediction of persistent arthritis development.

1.10.3 Hypothesis 3

There is a defined therapeutic window of opportunity in the early phases of RA during which initiation of DMARD therapy results in a superior therapeutic response, compared to initiation after this window.

My aims were to

1. investigate the relationship between time-to-therapy and treatment response, taking into account different modes of disease onset.
2. identify the threshold of time from **first onset of episode of reported joint swelling** to DMARD initiation that results in a disproportionately better treatment response rate, compared to that achieved if DMARD was initiated after this time .

2 Defining the threshold of normality in musculoskeletal ultrasound.

2.1 Introduction

Improvement in ultrasound (US) technology over the recent years has led to acquisition of high resolution images in musculoskeletal US. As a result, previously undetected US lesions in asymptomatic healthy individuals have now become apparent on imaging (24) (145).

Consequently, defining the boundary of **normal vs abnormal** US changes in a given joint has become unclear. When do **normal physiological** US changes progress to **early pathological lesions**? Getting this right is crucial. Particularly in the context of using US to diagnose inflammatory joint disease during the arthralgia and early arthritis phases. Similarly, defining true remission becomes arbitrary as what constitutes normal findings in US remission is currently not clear.

Further complicating this matter is the fact that the US findings of normal vs abnormal may differ across different age groups**.** Some US lesions at a given joint level may be regarded as pathological in the older age group, but not in the younger population. For example, some Rheumatologists may interpret mild synovial hypertrophy in the older population as 'normal findings' or presumed to be age–related changes. Although the same lesion grading may represent early pathology changes (i.e. early inflammation) in a younger individual. At present, the extent of what is thought to be sonographic age-related changes is poorly defined.

Therefore, an important research question yet to be addressed is defining the '**threshold of normality'** in Rheumatology US. It is crucial to identify the **threshold** (or cut-off) that ***discriminates normal physiological vs. minimal pathological changes*** at each joint level. This

notion has been coined as '**ultrasound-detected minimal disease'** amongst ultrasound investigators.

This threshold of normality, subsequently, will be used to evaluate the **best sensitivity to detect changes in therapeutic response**, to **assess remission**, as well as to **facilitate the diagnosis of early disease in each joint.**

Defining normal reference value for a specific **age and sex range** at each **joint level** is a fundamental step towards defining the **threshold of US findings** which are **clinically important in inflammatory arthritis** patients.

Having a clear cut-off for normality in US would robustly improve the utility of US in both clinical trials and routine practice. Clear primary end-points for target US remission according to joint type and age group can be defined. Crucially, identifying the key US threshold that should trigger DMARD escalation in treat-to-target strategy trials can be improved. These are amongst the main methodological challenges when designing clinical trials using US as a guidance tool to escalate immunosuppression(146).

For the use in clinical practice, US experts have published pragmatic guidelines on the utility of ultrasound in clinical practice. At the same time, they have also highlighted that identifying normal threshold in US Rheumatology is a key research agenda (147).

The overarching aim is to define the threshold of ultrasound findings that should be considered **pathological at each joint level in patients with inflammatory arthritis** compared to the **age- and sex-matched normal population.**

The preliminary step towards this goal is first to ascertain whether **there are differences in the US pathology that are observed in healthy subjects and to that of early arthritis patients (Study A).** If there were differences in the type of US pathology found in these two population, the next step is to quantify **the extent of US findings in a large population of healthy individuals** (Study B).

In this thesis chapter, study A and B are presented sequentially.

Study A: Comparison of US findings between healthy subjects and early arthritis patients.

Study B: US findings in a large cohort of healthy subjects.

2.2 Objective - Study A: Comparison of US Findings between Healthy Subjects and Early Arthritis patients.

The specific objective is:

To describe US findings (i.e. synovial hypertrophy, synovial effusion and intra-synovial Power Doppler enhancement) in the hand and foot joints of healthy subjects and early arthritis patients, with a view to identify the

1. Type of joints in healthy subjects which are commonly involved in US pathology and to compare these to that of early arthritis patients.
2. US-findings which are typically observed in early arthritis patients that may also be present in healthy individuals.

2.3 Subjects and Methods - Study A.

This was a retrospective analysis of US findings from study participants from two separate cohorts: 1) Healthy subjects cohort from Paris, France, and 2) Early Arthritis Cohort from Birmingham, UK. The inclusion and exclusion criteria for study participants in each cohort are detailed in ***Table 2-1***.

Table 2-1 Inclusion and exclusion criteria of healthy subjects and early arthritis cohort.

Healthy Subjects Cohort Ambroise Pare Hospital, Boulogne-Billancourt, France	Early Arthritis Cohort City Hospital and Queen Elizabeth Hospital Birmingham, UK
Inclusion • Aged 18-90. • Able to consent. **Exclusion** • Personal history of RA Rheumatic inflammatory Degenerative joint disease • Previous history of joint trauma • Enteric and/or genitourinary infection •Joint pain during the last month with VAS <10/100. • Clinical joint inflammation.	**Inclusion** • ≥ 1 clinically swollen joint due to inflammatory arthritis as judged by a physician. • Symptom duration ≤3 months. • DMARD-naïve. **Exclusion** • Joint symptoms solely due to degenerative joint disease.

2.3.1 Recruitment strategy of healthy subjects

Healthy subjects were recruited between October 2013 and March 2014 at Ambroise Pare Hospital in Boulogne-Billancourt, France. The healthy recruits were from one of these categories: 1) medical or allied health care professionals or administrative staff members, 2) medical or nursing students who were at this hospital, 3) healthy relatives who were visiting or accompanying patients at this hospital, and 4) healthy volunteers who responded through online advertisement. Written consent was obtained. All healthy subjects completed a visual analogue score (0-100) to report level of joint pain for the last month. Healthy subjects were also asked on the use of analgesia for joint pain including NSAIDs over the last month. The following demographic data were recorded: age, sex, ethnicity and work (especially manual jobs). In addition, sport and leisure activities were recorded to identify activities that have high mechanical stress to the hands/and feet.

2.3.2 Clinical and US assessment of healthy subjects

All healthy subjects underwent a joint clinical examination assessed by an independent rheumatologist to exclude those with any tender or swollen joints. Joints clinically assessed were bilateral MCPs 1-5, PIPs 1-5, wrists and MTPs 1-5. Only healthy subjects with no tender or swollen joints and VAS score less than 10/100 were enrolled into this study.

US assessment was performed with a MyLab70 XVG (Esaote, Genoa Italy) and 6-18 MHz multi-frequency linear transducer. GS setting of 18 MHz was used. PD setting was a minimal of 11 MHz frequency with pulse repetition frequency (PRF) around 750 Hz; adjusted to detect small vessels with slow flow. PD gain was between 40-60% and adjusted to just below the

disappearance of background noise. The PD region of interest box included area of the whole joint up to the skin surface to avoid artefacts from superficial vessels reverberation. Table 2-2 shows the joint site, scanning approach and ultrasound lesion recorded for each health subjects.

Table 2-2 Joint site, scanning approach and ultrasound lesion recorded for each healthy subject.

Joint Site	Scanning approach	Ultrasound lesion
MCPs 1-5	MCP 1,3,4 : Dorsal MCP 2 & 5: Dorsal and lateral	Synovial hypertrophy Synovial effusion Synovial Power Doppler
PIPs 1-5	Dorsal	Synovial hypertrophy Synovial effusion Synovial Power Doppler
Wrist: Inter-carpal, radio-carpal, ulnar-carpal	Dorsal	Synovial hypertrophy Synovial effusion Synovial Power Doppler
MTPs 1-5	MTP 1 & 5: Dorsal and lateral MTP 2,3 & 4: Dorsal	Synovial hypertrophy Synovial effusion Synovial Power Doppler

Synovitis definition was defined as hypoechoic intra-articular tissue (i.e., Grey-scale synovial hypertrophy) which is non-displaceable and poorly compressible and which may exhibit intra-

synovial PD signal (120). Synovial effusion was defined as anechoic intra-articular area which is displaceable and compressible, and distinctly different form synovial hypertrophy (which is anechoic-hypoechoic) and does not exhibit any Doppler signal (120). A semi-quantitative grading system (grade 0-3) was used for each of the SH, PD and SE lesion Table 2-3 to Table 2-5 (148) .

Table 2-3 Synovial hypertrophy semi-quantitative grading system.

Synovitis defined as a non-compressible hypoechoic intra-capsular area (synovial thickening) (148).	Grade 0	No synovial thickening.
	Grade 1	Minimal synovial thickening [filling the angle between the peri-articular bones, without bulging over the line linking tops of the bones.
	Grade 2	Synovial thickening bulging over the line linking tops of the peri-articular bones but without extension along the bone diaphysis.
	Grade 3	Synovial thickening bulging over the line linking tops of the peri-articular bones and with extension to at least one of the bone diaphysis.

Table 2-4 Power Doppler semi-quantitative grading system.

Doppler flow defined by Power Doppler signal (148)	Grade 0	No flow in the synovium.
	Grade 1	Single vessel signals.
	Grade 2	Confluent vessel signals in less than half of the area of the synovium.
	Grade 3	Vessel signals in more than half of the area of the synovium.

Table 2-5 Joint effusion semi-quantitative grading system.

Joint effusion defined as compressible anechoic intra-capsular area (148)	Grade 0	No effusion.
	Grade 1	Minimal effusion.
	Grade 2	Moderate amount of joint effusion (without distension of joint capsule).
	Grade 3	Extensive amount of joint effusion (with joint capsule distension).

US assessment was performed by a competent sonographer (IP) with 2 years' experience of MSUS was based at Ambroise Pare Hospital in Boulogne-Billancourt, France. US examination was supervised by MADA. Each US assessment took approximately 30 minutes.

2.3.3 Recruitment strategy of early arthritis patients.

Patients were recruited from the Birmingham Early Arthritis Clinic based in Rheumatology Departments at Sandwell and West Birmingham Hospitals NHS Trust and University Hospitals

Birmingham NHS Foundation Trust, UK. All patients were referred by their GP to these two secondary care centres, which provide rheumatology service to a population of 1.3 million across Birmingham.

Consecutive DMARD-naïve patients with clinically-detected synovitis of at least one joint and inflammatory joint symptom duration (pain and/or stiffness and/or swelling) of three months or less were included. Patients who had joint symptoms attributed solely to degenerative joint disease were excluded. Ethical approval was obtained and all patients gave written informed consent.

2.3.4 Clinical and ultrasound assessment of early arthritis patients.

These clinical data were recorded: 68 tender and 66 swollen clinical counts, age, sex, symptom duration, early morning stiffness duration, medication, erythrocyte sedimentation rate, C-reactive protein, RF and ACPA status.

Final diagnostic outcomes were determined at 18 months follow-up. Patients were classified as having RA if they fulfilled cumulative 2010 ACR/EULAR criteria (39) by the 18-month visit. Non-RA patients were classified according to established classification criteria which were Psoriatic Arthritis, Systemic lupus erythematosus (SLE) and Ankylosing Spondylitis (17, 149, 150).

One experienced sonographer (AF) performed a blinded US assessment in a temperature controlled radiology suite within 24 hours of clinical assessment, A systematic multi-planar greyscale and power Doppler (PD) US examination of 19 bilateral joint sites and 16 bilateral

tendon compartments was performed based upon standard EULAR reference scans (108) using a Siemens Acuson Antares scanner (Siemens, Bracknell, UK) and multi-frequency (5–13 MHz) linear array transducers. The joint and tendon scanned are listed in Table 2-6 and Table 2-7, respectively. For PD assessments, the pulse repetition frequency (PRF) was adjusted to provide maximal sensitivity at the lowest possible value for each joint, resulting in PRFs of between 610 and 780. Examinations took around 60 minutes depending on disease extent and patient mobility.

Table 2-6 Synovial intra-articular recesses and peri-articular sites assessed by ultrasound.

Joint	Recess
MCP 1-5, PIP 1-5, MTP 2-5	Multi-planar scanning of dorsal recesses
	Lateral recess of MCP1,2,MTP5
Wrist	Intercarpal recess
	Radiocarpal recess
	Ulnarcarpal recess
	Volar carpal recess
Elbow	Anterior recess
	Humeroradial joint
	Humeroulnar joint
	Posterior recess
Shoulder	Posterior glenohumeral recess
Knee	Suprapatellar recess
	Medial parapatellar recess
	Lateral parapatellar recess
	Medial femorotibial joint line
	Lateral femorotibial joint line
Ankle	Anterior tibiotalar recess
	Medial tibiotalar recess
	Lateral tibiotalar recess

MCP, metacarpophalangeal joint; MTP, metatarsophalangeal joint; PIP, proximal interphalangeal joint.

Table 2-7 Tendon compartments evaluated by ultrasound

Tendon	Tendon Compartment
Digit	Flexor tendon 1-5
Wrist	Flexor tendon compartment
	Extensor compartment 1 (APL and EPB)
	Extensor compartment 2 (ECRL and ECRB)
	Extensor compartment 3 (EPL)
	Extensor compartment 4 (EDC and EIP)
	Extensor compartment 5 (EDM)
	Extensor compartment 6 (ECU)
Shoulder	Biceps tendon
Ankle	Anterior extensor compartment (TA, EHL, EDL)
	Peroneus longus and brevis
	Posteromedial compartment (PT, FDL, FHL)

APL: Abductor pollicis longus. EPB: extensor pollicis brevis. ECRL: extensor carpi radialis longus. ECRB: Extensor carpi radialis brevis. EPL: Extensor pollicis longus. EDC: extensor digitorum communis. EIP: Extensor indicis propius. EDM: Extensor digiti minimi. ECU: extensor carpi ulnaris. TA: Tibialis Anterior. EHL: Extensor Hallucis Longus. EDL: Extensor digitorum Longus. PT: Posterior tibial. FDL: Flexor digitorum longus. FHL: Flexor hallucis longus.

GS synovial hypertrophy in MCP, PIP an MTP joints was graded on a semi-quantitative scale from 0 – 3 ; modified from the grading system published by Szkudlarek et al (107, 148). This system reclassified the equivocal minimal synovial thickening as normal. Table 2-8 shows the details of the modified Szudlarek grading system.

Table 2-8 Modified Szudlarek grading system.

Synovial Hypertrophy	Grade 0	Equivocal 'minimal' thickening grade as normal.
	Grade 1	synovial thickening bulging over the line linking the tops of the peri-articular bones.
	Grade 2	Grade 1 plus extension to one bone diaphysis.
	Grade 3	Grade 1 plus extension to both bone diaphyses.

Synovial hypertrophy in wrist and other joints was graded as: 0, normal; 1, mild; 2, moderate; and 3, severe. Grade 1 indicates synovial thickening in excess of the mean plus 2 SD of normal range when available (151).

Synovial hyperaemia was measured by power Doppler in each recess and the maximal score graded according to Szkudlarek *et al as shown in* Table 2-9 *(148).* Synovial effusion for all joints was graded as absent or present. Effusion in the absence of synovial thickening was not classified as synovial hypertrophy.

Table 2-9 Power Doppler grading system.

Power Doppler	Grade 0	Absence
	Grade 1	Isolated signals.
	Grade 2	Confluent signals in less than half of the synovial area.
	Grade 3	Confluent signals in more than half of the synovial area.

2.3.5 Joint selection and statistical analysis

The main aim of this retrospective analysis was to identify whether there are any differences in the ultrasound changes of joints in healthy subjects and early arthritis patients at a given joint sub-set. The two cohorts were recruited and scanned independently for different studies at each centre. The joint selection for data analysis was based on the joint region scanned by both recruiting centres. The selected joints that overlapped at both centres were MCP 1-5, PIP 1-5, wrist and MTP 2-5. MTP 1 was not included as the Birmingham centre did not scan this joint. Ankle, elbow, shoulder and knee joints were not included as not scanned in the Healthy Subject study. Likewise, tendon ultrasound pathology was not included in the analysis as these were not scanned at Paris.

The US grading for SH, PD and SE was scored in a semi-quantitative grading system (grade 0-3) at Paris. For the joint analysis, the gradings were binarised into absent (grade 0) and Present (grade ≥ 1).

The US grading for each ultrasound SH and PD was scored in a semi-quantitative grading system (grade 0-3) at Birmingham. For the joint analysis, the gradings were binarised into absent (grade 0) and present (grade ≥ 1). The synovial effusion was scored as binary absent or present at Birmingham.

Statistical analyses were performed using IBM SPSS Statistics for Windows (Version 26.0. Armonk, NY: IBM Corp.) In the descriptive analysis, Fisher's exact test was used to compare proportions and Mann-Whitney test was used to compare non-parametric continuous variables.

2.4 Results- Study A: Comparison of Ultrasound Findings between Healthy Subjects and Early Arthritis patients.

2.4.1 Demographic characteristics of healthy subjects and early arthritis patients.

Healthy subjects were younger compared to the early arthritis patients (Table 2-10). Symptom duration of early arthritis patients were very short with a median of six weeks. 56 patients of early arthritis patients developed RA at 18 months (Table 2-11).

Table 2-10 Demographic characteristics of healthy subjects and early arthritis patients.

Characteristics	Healthy subjects	Early arthritis patients	p value
Sample size, n	206	107	NA
Number of joints examined	6177	3210	NA
Female, n (%)	146 (71)	60 (56)	<0.001[a]
Age, years, median (IQR)	32 (25-47)	51 (39-64)	<0.001[b]
Symptom duration, weeks median IQR	NA	6 (4-8)	NA

IQR; interquartile range. [a]Fisher's exact test. [b]Mann-Whitney test

Table 2-11 Final diagnoses of early arthritis patients.

Final diagnosis at 18 months, n	**107**
Rheumatoid Arthritis	56
Unclassified arthritis	24
Psoriatic Arthritis	9
Crystal arthropathy	4
Parvovirus arthropathy	4
SLE	3
Reactive arthritis	2
Sarcoidosis	2
Ankylosing Spondylitis	1
Post-streptococcal Arthritis	1
Infectious arthritis	1

2.4.2 Comparison of ultrasound findings between healthy subjects and early arthritis patients.

Table 2-12 shows the proportion of abnormal joints in these two separate cohorts. A total of 6177 healthy subject joints and 3210 early arthritis joints were included in the final analysis (Table 2-12). Seven percent of joints within the healthy subjects had at least one ultrasound abnormality. However, the proportion of abnormal joints was higher in the early arthritis patients compared to the healthy subjects (Table 2-13).

Table 2-12 Number of abnormal joint within the healthy subjects and early arthritis patients.

	Healthy Subjects	Early Arthritis Patients	p-value
Number of joints with at least one US abnormality, n (%)	403 (7)	971 (30)	
Number of joints with no US abnormality, n (%)	5774 (93)	2239 (70)	<0.001[a]
Total	6177	3210	

[a]chi-square test

Table 2-13 Number of joints examined for each groups.

Joint site	Number of joints examined

	Healthy Subjects N=206	Early Arthritis patient N=107
MCP 1	412	214
MCP2	412	214
MCP3	412	214
MCP4	412	214
MCP5	412	214
PIP1	412	214
PIP2	412	214
PIP3	412	214
PIP4	412	214
PIP5	412	214
Wrist	409*	214
MTP2	412	214
MTP3	412	214
MTP4	412	214
MTP5	412	214
Total	6177	3210
*Three wrists of three subjects were not scanned due to previous history of trauma (more than 15 years ago).		

Figure 2-1 shows the distribution of US pathology (synovial hypertrophy, synovial effusion and intra-synovial Power Doppler across the healthy subjects and early arthritis patients). Data were presented as proportion of joints with grade ≥ 1 ultrasound lesion; right and left joints

were counted as two separate joints (Table 2-12). One of the key findings was that Power Doppler was almost exclusively present in early arthritis subjects. Joint effusion, on the other hand, was mostly observed in healthy subjects, particularly within the MTP joints. The most common US pathologies in early arthritis patients were synovial hypertrophy and Power Doppler enhancement, and these were present throughout the MCP, PIP, wrist and MTP joints.

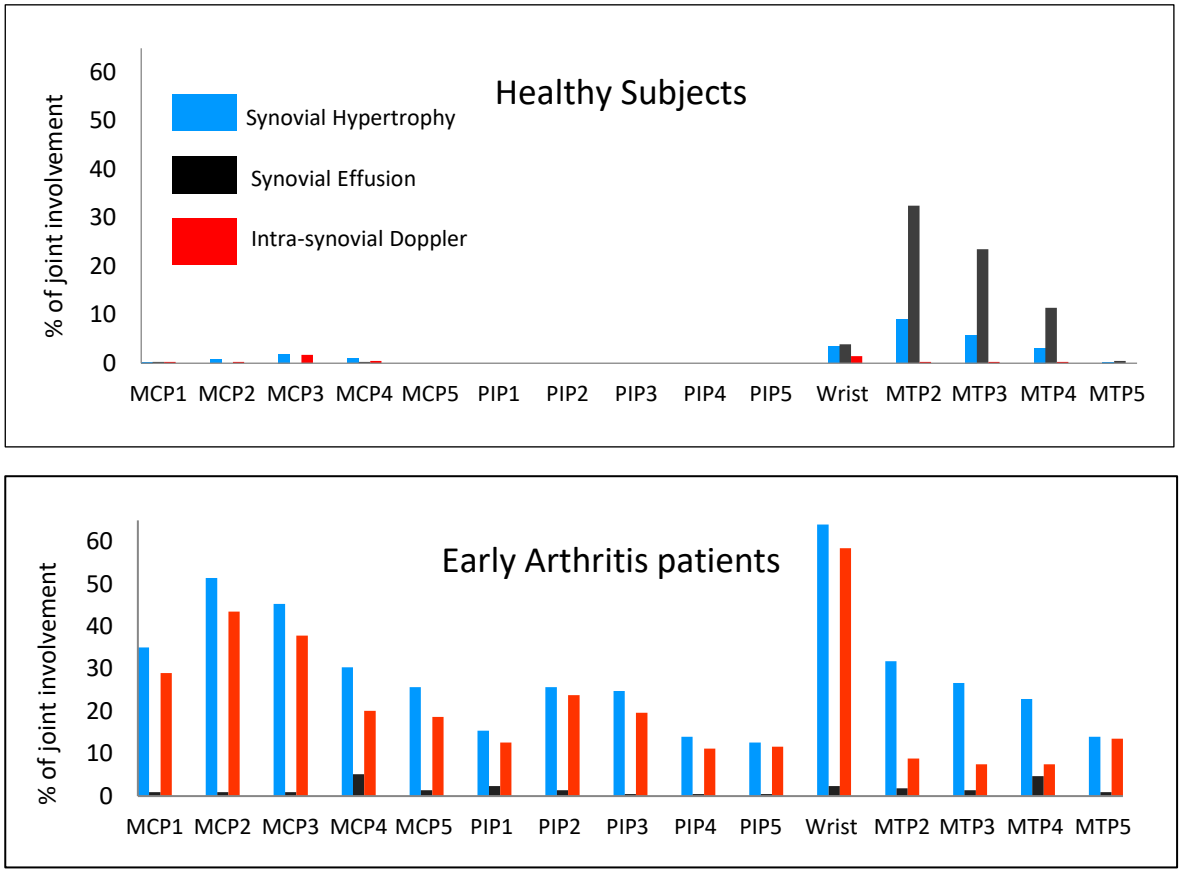


Figure 2-1 Synovial Hypertrophy, synovial effusion and intra-synovial Power Doppler comparison between healthy subjects and early arthritis patients. *Each bar represents the percentage of synovial hypertrophy (blue), synovial effusion (black) and intra-synovial Doppler (red) according to groups.*

Figure 2-2 shows the synovial hypertrophy by gradings at the joint level in healthy subjects and early arthritis patients. Data were presented as proportion of joints with right and left joints counted as two separate joints (Table 2-12). Synovial hypertrophy was rarely observed

in healthy subjects, and even when present it was mainly confined to lower gradings (grade 1 and 2) and present in very low proportions only. Synovial hypertrophy was more frequently present in early arthritis patients compared to healthy subjects. Importantly, when present, they were in both higher proportions and higher gradings (i.e. grade 2 and 3).

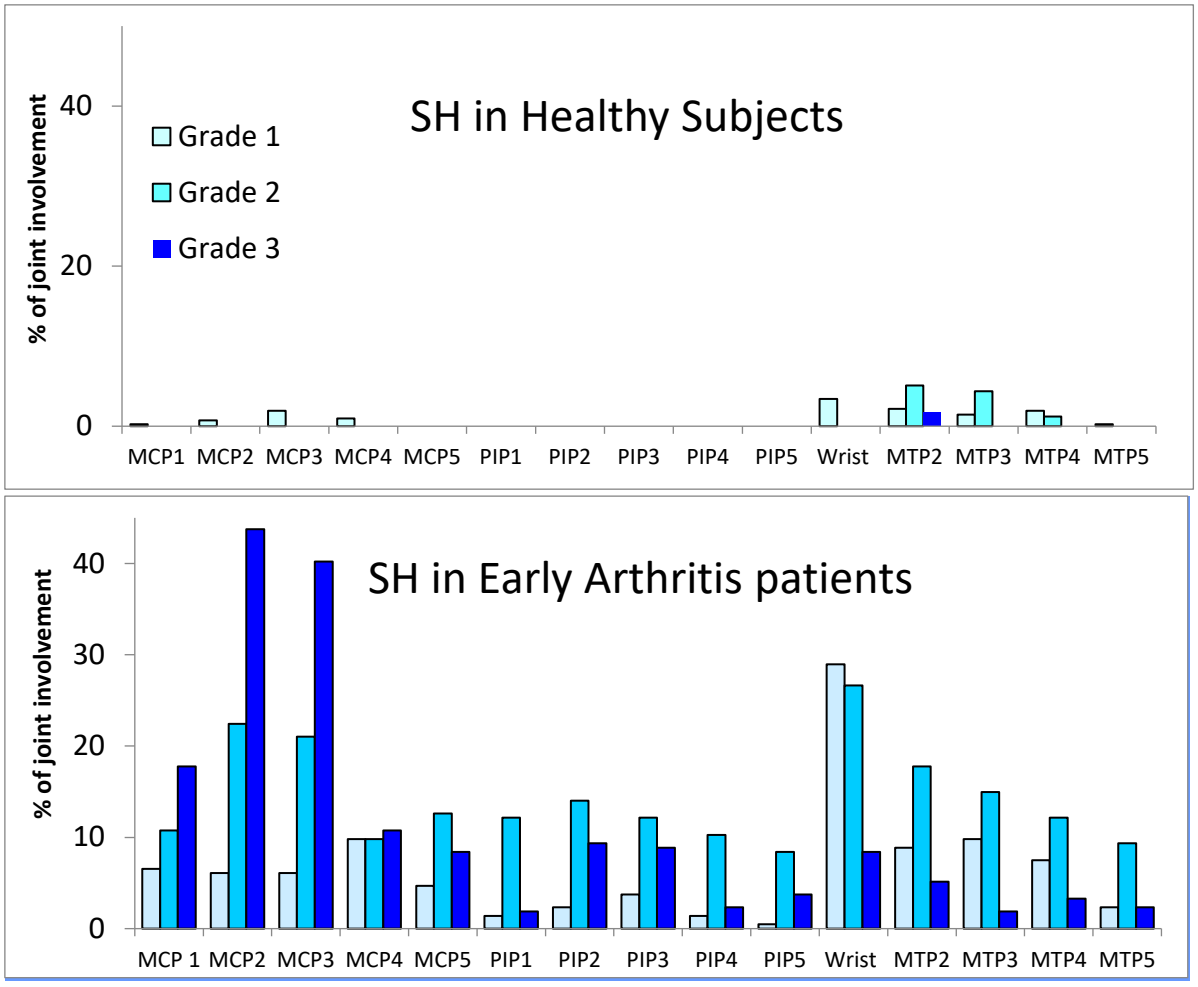


Figure 2-2 Synovial hypertrophy gradings in healthy subjects and early arthritis patients. *Each bar represents the proportion of joint involvement according to groups.*

Figure 2-3 shows the Power Doppler gradings at the joint level across the two cohorts. Data were presented as proportion of joints with right and left joints counted as two separate joints (Table 2-12). Intra-synovial Power Doppler was rarely observed in healthy subjects. When

present, these were mainly confined to grade 1 only. Conversely, intra-synovial Power Doppler were more frequent in early arthritis patients. When present, the higher gradings (i.e. grade 2 and 3) were more frequent.

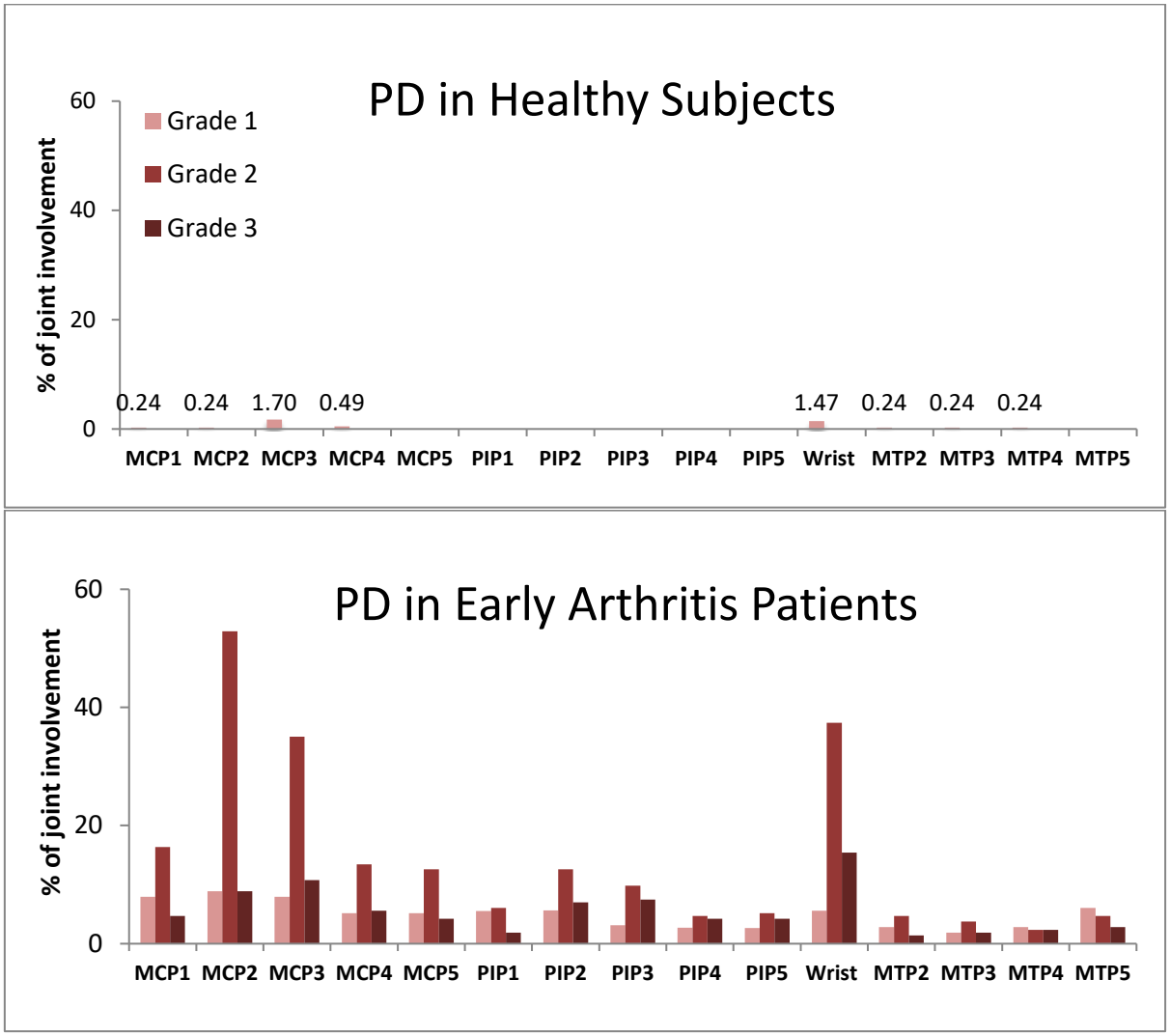


Figure 2-3 Power Doppler Gradings in healthy subjects and early arthritis patients. *Each bar represents the proportion of joint involvement according to groups.*

2.5 Discussion- Study A: Comparison of Ultrasound Findings between Healthy Subjects and Early Arthritis patients.

There are three important findings in this retrospective analysis. Firstly, synovial effusion was mainly confined to healthy subjects, with a predilection for MTP joints. Secondly, intra-synovial Power Doppler enhancement is almost exclusively present in early arthritis patients. Thirdly, synovial hypertrophy was mainly observed in joints of early arthritis patients, with the exception of mild changes (i.e. grade 1) in MCP 1 to 4.

These findings highlighted two fundamental issues. Firstly, certain US lesions can be observed in normal individuals but confined to a specific joint sub set (effusion in MTP joints). Secondly, some US pathology can be observed in both early arthritis and healthy subjects, but when present in the same joints there was a clear difference in the gradings with higher gradings more likely observed in early arthritis compared to that of healthy subjects.

This retrospective analysis has four main limitations. First is that the US data for healthy and early arthritis subjects were from two separate centres. Therefore, the data lacks generalisability. Furthermore, there was a lack of standardisation in terms of scanning protocol and/or grading between the two centres. In addition, healthy subjects were largely from a younger population (median age 32).

The final limitation relates to the inclusion criteria for the early arthritis cohort. Patients with early arthritis who also had co-existing hand osteoarthritis were not excluded. However, the healthy subjects with degenerative joint disease were excluded. Therefore, the differences that was observed in the ultrasound findings between the two cohorts could be attributed, to

some extent, to degenerative joint disease that may be present alongside early inflammatory changes. This limitation could be mitigated by excluding early arthritis patients who has concomitant hand osteoarthritis.

The strength of this study is that the early arthritis patients had very short symptom duration (median of six weeks), with patients with symptoms solely due to degenerative arthritis excluded. Therefore the US changes in the early arthritis cohort may represent early pathological changes. Study A was done as part of preliminary work for Study B. It was important to identify whether there was differences in the ultrasound findings at the joint level between these two groups, and if present , which ultrasound lesions it was. This guided our work when designing the scanning protocol for Study B.

Overall, this analysis highlights that there are fundamental differences in the US phenotype between healthy subjects and early arthritis both in term of a) types of pathology in each joint type, and also b) severity of each pathology.

2.6 Introduction - Study B: Ultrasound findings in a large cohort of healthy subjects.

The next stage required is assessing the **extent of US lesions across a wide range of age of healthy individuals**. This is to establish the frequency of each US pathology within each age group.

The EULAR-OMERACT consensus group has established the definition of US-detected synovitis with a clear definition of each component of US lesions: 1) synovial hypertrophy, 2) synovial effusion, and 3) synovial inflammation as measured by power Doppler enhancement (147). In

addition the grading of each US elementary lesion has been clearly defined and undergone reliability studies to ensure intra-observer consistency (148, 152).

As the definition of US pathology and gradings had been recently established, the extent of US changes in healthy subjects was measured using this new consensus grading system.

2.7 Objective

To systematically grade the US findings in joints of healthy asymptomatic individuals aged 18 and above, with a particular emphasis on the age of high RA prevalence (aged 45-65).

The grading definition is based on the OMERACT-EULAR consensus grading, see table Table 2-16 Grading system for joint and tendon sites Table 2-16 to Table 2-23 (118, 119).

2.8 Subject and Methods – Study B: Ultrasound findings in a large cohort of healthy subjects.

2.8.1 Recruitment strategy and centres

This is an observational cross-sectional multi-centre study of the 'Minimal Disease' task force within the Ultrasound Special Interest Group (SIG) which was developed following the OMERACT 2016 meeting. I drafted the initial protocol in July 2016 and the final version was agreed in June 2017 with Prof MA D'Agostino (Minimal Disease group mentor), and Dr A Filer, (Minimal Disease group lead). The MCP and PIP palmar views were excluded after a reliability exercise undertaken at EULAR 2017 which indicated that this view was highly vulnerable to inter-observer error. This is because the normal palmar joint recess may appear like a synovial

effusion. In addition, a small change in joint positioning resulted in a big change in the appearance of the palmar MCP joint recess. The decision was therefore made to exclude this view.

I presented the study invitation at the OMERACT ultrasound SIG meeting at EULAR 2017 with the details of the study requirements. Participating centres were required to recruit at least 30 healthy subjects within the recruitment window. A minimum recruitment figure was chosen to reduce inter-centre variability. I emailed the final invitations to all US OMERACT centres in July 2017. 33 centres expressed interest, 6 centres withdrew later and 4 centres did not reach target recruitment of 30 healthy subjects.

I sent the Protocol Version 1.0 in July 2017 to participating centres together with the patient, US record proforma and template of US excel). An updated protocol version 2.1 with minor edits were sent in Sept 2017. This protocol is attached in Appendix 1. The dateline for completing 30 healthy subjects was 31st Dec 2017.

Each centre was required to submit the US images of the first recruit. This was to ensure consistency of image quality and grading. I reviewed the first set of US images from each centre and communicated feedback accordingly. When reviewing the images, I ensured that the image quality was appropriate for grading, correct joint alignment and position of focus point. I also checked that the Doppler box covered the whole joint and up to the skin surface. In addition I checked that the gel layer above the skin surface was adequate. Finally I ensured that the grading scores were consistent with the images.

Seven centres had their grading rectified and fed back. Each centre could proceed to complete recruitment of 30 healthy subjects once their first images had been verified. For images with equivocal grading, particularly for MTP joint, images were reviewed and graded independently by Prof MADA and Dr Filer.

At OMERACT 2018, I presented the preliminary data for **594 healthy subjects** recruited from 23 centres. Anticipated recruitment was 691 healthy subjects at this stage. However, the recruitment age was skewed towards the younger population.

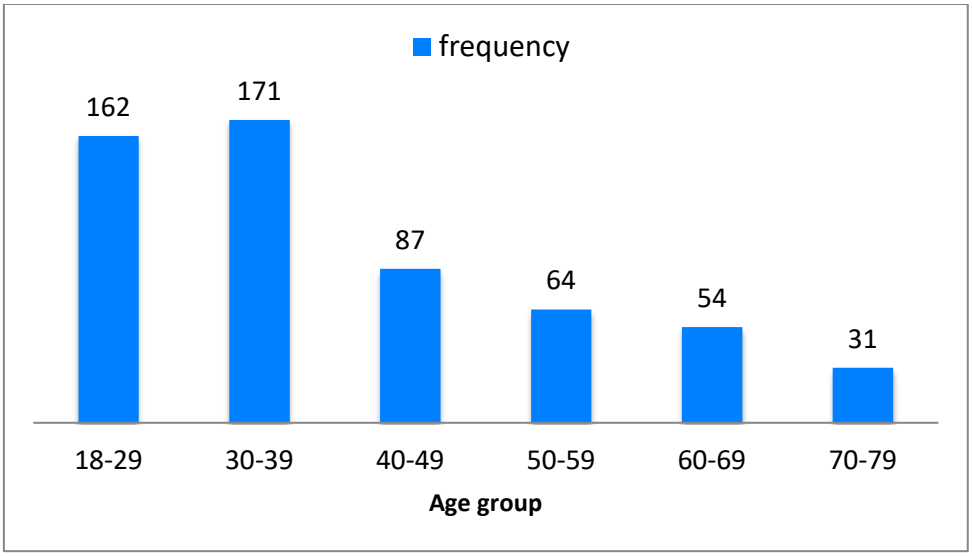


Figure 2-4 Age group distribution of preliminary data. Each bar shows the frequency of participants according to age groups.

Figure 2-4 shows the distribution of healthy subjects according to the age group at this time. The highly likely explanation was that healthy subjects were mainly recruited within the unit.

The decision was made, at that stage, to increase the number of healthy older subjects. One of the aims was to compare the US findings of healthy subjects to that of an age-match

population of RA (aged 45 and above). Therefore, having more healthy subjects above the age of 40 was crucial.

Each centre was then required to recruit an additional 10-15 healthy subjects in the age group of 40 and above. In total, 954 healthy subjects were recruited across 23 centres. One OMERACT US centre was keen to participate after EULAR 2019 and was therefore included. Final anticipated recruitment is **984 from 24 centres** upon study completion. Appendix 2 shows the list of recruiting centres.

2.8.2 Inclusion and exclusion criteria of healthy subjects.

Table 2-14 shows the inclusion and exclusion criteria of this study. Any individuals with history and/or signs of inflammatory, traumatic and degenerative joints disease were excluded. In addition, any individual who are at risk of Inflammatory Bowel Disease-related arthritis or infection associated with reactive arthritis were excluded. Individuals on medication that can affect US finding, such as steroids or NSAIDs were also excluded.

Table 2-14 Inclusion and exclusion criteria of healthy subjects.

Inclusion	Exclusion
Aged ≥ 18	• Previous/current inflammatory joint disease (including crystal arthropathy). • Visual Analogue Score (VAS) joint pain ≥ 10/100. • Joint trauma in the last month. • Hand osteoarthritis by the ACR criteria. • Clinical joint inflammation as identified by a physician. • Previous or current inflammatory bowel disease. • History of culture-proven enteric and/or genitourinary infection in the last month. • Current or previous corticosteroids use in the last 4 weeks. • Current non-steroidal anti-inflammatory use.

Hand osteoarthritis, as defined by the ACR criteria (153), was also an exclusion criterion. This is important as the main objective of this study was to identify the US findings observed in a 'normal joint' within each age group. Therefore it was important that clinically obvious hand OA was an exclusion criterion. Otherwise, the physiological 'age-related changes' on US scanning cannot be identified. The question whether patients in the older age population may have imaging-detected OA changes is a different research question. This important question is currently addressed by the OMERACT Hand osteoarthritis group. However, as the likelihood

of detecting subclinical sonographic OA is high; osteophyte lesions were included in the scanning protocol (see below).

2.8.3 Joint and tendon ultrasound assessment.

Table 2-15 shows the US assessment at the joint and tendon level. Two levels of US data acquisition were set for this study:

Level 1: Mandatory US lesion

Level 2: Optional US lesion

Table 2-15 Assessment of ultrasound lesion at the joint and tendon level.

Structure	Site	Ultrasound lesion (mandatory)	Ultrasound lesion (optional)
Joint	• MCPs 1-5 • PIPs 1-5 • Wrist:Inter-carpal, radio-carpal & ulnar-carpal • MTPs 1-5	• Synovial hypertrophy • Synovial effusion • Synovial Power Doppler	• Osteophyte • Erosion o MCP 2 & 5 o MTP 5
Tendon	• Finger flexor 1-5 • Extensor carpi ulnaris	• Tenosynovial hypertrophy • Tenosynovial effusion • Tenosynovial Power Doppler	

This joint and tendon subset was chosen as these structures are commonly involved in US-detected RA pathology. In addition, erosion was included as this can be observed in the joints of healthy individuals (154). Scanning for osteophyte and erosion were optional in order to cut own the scanning time for centers with limited time.

Table 2-16 shows the grey-scale and Power Doppler gradings at the joint and tendon level. The joint US elementary lesions recorded were **synovial hypertrophy, effusion and Power Doppler Enhancement**. Each US elementary lesion was graded according to the EULAR-OMERACT consensus definition (119, 147) as detailed in Table 2-16 to Table 2-23.

Table 2-16 Grading system for joint and tendon sites

Joint ultrasound lesion		**Grade**
Grey-scale	Synovial hypertrophy	Semi-quantitative 0-3
	Synovial effusion	Semi-quantitative 0-3
Power Doppler (PD)	PD grading	Semi-quantitative 0-3
Degenerative	Presence of osteophyte	Semi-quantitative 0-3
Erosion	Presence of erosion (only for MCP2, 5 and MTP5)	Yes/No
Tendon ultrasound lesion		**Grade**
Grey-scale	Tenosynovial hypertrophy	Semi-quantitative 0-3
	Tenosynovial effusion	Yes/No
Power Doppler (PD)	PD grading	Semi-quantitative 0-3

Table 2-17 Grading definition for joint synovial hypertrophy US elementary lesion (118, 119).

Joint synovial hypertrophy	Definition of grading
Abnormal hypoechoic **intra-articular tissue or** higher echoic **(relative to subdermal fat)** that is not or poorly displaceable, **and which may exhibit Doppler signal.**	**Grade 0** (none) No synovial hypertrophy independently of the presence of effusion.
	Grade 1 (minimal) **Minimal hypoechoic** synovial hypertrophy **up to the level of the horizontal line** connecting bone surfaces between the metacarpal head and the proximal phalanx.
	Grade 2 (moderate) **Moderate hypoechoic** synovial hypertrophy **extending beyond joint line but with the upper surface concave** (curved downwards) or **hypertrophy extending beyond** **the joint line but with the upper surface flat.**
	Grade 3 (severe) **Severe hypoechoic** synovial hypertrophy with or without effusion **extending beyond the joint line but with the upper surface convex (curved upwards).**

Table 2-18 Grading definition for Power Doppler US elementary lesion (118, 119).

Joint Power Doppler	Definition of grading
Abnormal vascularization detected within the hypoechoic synovial hyperplasia.	**Grade 0** (none) No Doppler signal
	Grade 1 (minimal) Up to **three single Doppler spots** OR up to **one confluent spot and two single spots** OR up to **two confluent spots.**
	Grade 2 (moderate) **Greater than Grade 1 but ≤50% Doppler signals in the total greyscale background.**
	Grade 3 (severe) **Greater than Grade 2 (>50% of the total greyscale background).**

Table 2-19 Grading definition for joint effusion US elementary lesion (155)

Joint effusion	Definition of grading
Abnormal anechoic or hypoechoic (relative to subdermal fat) intraarticular material that is easily displaceable**, but does not exhibit Doppler signal.**	**Grade 0** No effusion
	Grade 1 **Minimal** amount of joint effusion
	Grade 2 **Moderate** amount of joint effusion (little distension of the joint capsule)
	Grade 3 **Extensive** amount of joint effusion (with high distension of the joint capsule)

The **tendon** US elementary lesions recorded were **synovial hypertrophy, effusion** and **Power Doppler Enhancement**. Each ultrasound elementary lesion was graded according to the EULAR-OMERACT consensus definition (108) and detailed in Table 2-20 to Table 2-22.

Table 2-20 Grading definition for tenosynovial hypertrophy US elementary lesion (108)

Tenosynovial hypertrophy **(108)**	Definition of grading
Abnormal hypoechoic (relative to tendon fibres) tissue within the tenosynovial sheath that is not displaceable and poorly compressible **and seen in two perpendicular planes.**	**Grade 0** No abnormal hypoechoic within the tenosyovial sheath
	Grade 1 Minimal abnormal hypoechoic within the tenosyovial sheath
	Grade 2 Moderate abnormal hypoechoic within the tenosyovial sheath
	Grade 3 Severe abnormal hypoechoic within the tenosyovial sheath

Table 2-21 Grading definition for tenosynovial Doppler US elementary lesion (108)

Tenosynovial Doppler	Definition of grading
Presence of peritendinous Doppler signal within the synovial sheath, seen in two perpendicular planes, excluding normal feeding vessels (i.e. vessels at the mesotenon or vinculae or vessels entering the synovial sheath from surrounding tissues) only if the tendon shows peritendinous synovial sheath widening on B-mode	**Grade 0** No signal
	Grade 1* **Peritendinous focal signal** within the widened synovial sheath (i.e. signals in **only one area** of the widened sheath), seen in two perpendicular planes, excluding normal feeding vessels;
	Grade 2* **Peritendinous multifocal signal** within the widened synovial sheath (i.e. signals in **more than one area** of the widened sheath), seen in two perpendicular planes, excluding normal feeding vessels;
	Grade 3 **Peritendinous diffuse signal** within the widened synovial sheath (i.e. signals **filling most of the widened sheath**), seen in two perpendicular planes, excluding normal feeding vessels.

*If in addition to an abnormal peritendinous (i.e. intra-sheath) signal there was an abnormal intratendinous signal seen in two perpendicular planes (i.e. excluding intra tendinous small isolated signals that can correspond to normal feeding vessels detectable by US), then grades 1 and 2 would be increased by one point.

Table 2-22 Grading definition for tenosynovial effusion US elementary lesion (108)

Tenosynovial effusion	Definition of grading
Abnormal anechoic or hypoechoic (relative to tendon fibres) material within the synovial sheath, either localised (eg, in the synovial sheath cul-de-sacs) or surrounding the tendon that is displaceable and seen in two perpendicular planes.	**Absent** No abnormal displaceable hypoechoic region within the tenosyovial sheath
	Present Presence of at least minimal abnormal hypoechoic within the tenosyovial sheath

The recording of **osteophytes was optional** for this study. The definition and grading of osteophytes are detailed in Table 2-23.

Table 2-23 Definition and grading system of osteophytes (121, 156).

Osteophytes	Definition of grading
A step-up bony prominence at the end of the normal bone contour, or at the margin of the joint seen in two perpendicular planes, with or without acoustic shadow.	**Grade 0** No osteophytes, i.e. a smooth cortical surface.
	Grade 1 Small and distinct cortical protrusion(s) of the bony surface.
	Grade 2 Larger protrusion(s) which may have broad base(s).
	Grade 3 Very large protrusion(s) which may have very broad base(s).

The recording of **erosions** was optional for this study and only limited to MCP2, 5 and MTP. The definition and grading of the erosion are detailed in Table 2-24. These joints were screened for erosions as: a) RA are commonly found at these sites, b) presence of acoustic window to assess erosions at these joint sites.

Table 2-24 Definition of ultrasound erosion.

Erosion (157)	Definition of grading
A cortical "break" or defect with an irregular floor seen in longitudinal and transverse planes.	**Absent** No erosion
	Present Erosion present

The grading definition were detailed in the protocol sent to each recruiting centres, together with images representative US lesion of the MCP and PIP joints.

2.8.4 Ultrasound images record and data input

Each US image was recorded according to the recommendation published by the EULAR Working Group for Musculoskeletal Ultrasound 2017 (158). All structures were scanned using both longitudinal and transverse approaches. However, only longitudinal views were recorded for joint grading, and longitudinal and transverse views were recorded for tendon grading Table 2-25.

Table 2-25 Views to be recorded for joint and tendon core set.

Region	Joint and tendon core set	Views to be recorded
Hand	MCP 1-5 (dorsal)	Longitudinal
	PIP 1-5 (dorsal)	
	Flexor tendon 1-5	Longitudinal & transverse
Wrist	Inter-carpal, Radio-carpal, Ulnar-carpal	Longitudinal
	Extensor Carpi Ulnaris tendon	Longitudinal & transverse
Foot	MTP 1-5 (dorsal)	Longitudinal

Each centre was required to include the following details on the US image for each participant:

a. Date of visit
b. Participant research ID (with no personal ID i.e. anonymised)
c. Joint or tendon site using the abbreviations as shown in the Table 2-26.

Table 2-26 *Abbreviation for recorded US images.*

Right or left	Joint/tendon site	Number of position
R, L	MCP	1-5
	PIP	1-5
	MTP	1-5
	Wrist	IC/RC/ UC
	DF	1-5
	ECU	

Example:

i. L DF 1
ii. R MCP 5
iii. R ECU
iv. L wrist UC
v. L MTP 5

Upon study completion, each centre collated the US scores into the template excel spreadsheet that I had designed and provided with the protocol.

At the end of the study, US images of all healthy participants were transferred along with the US scores.

2.8.5 Joint position during scanning.

The hand was positioned flat on the table in a relaxed position as shown in Figure 4 below. Flexor tendon scanning was done at the level of the MCP joint. For foot scanning, the participant were placed on a couch with the knees flexed and foot flat on the couch Figure 2-5. Standardisation of joint position is important as different joint positioning may result in variability.

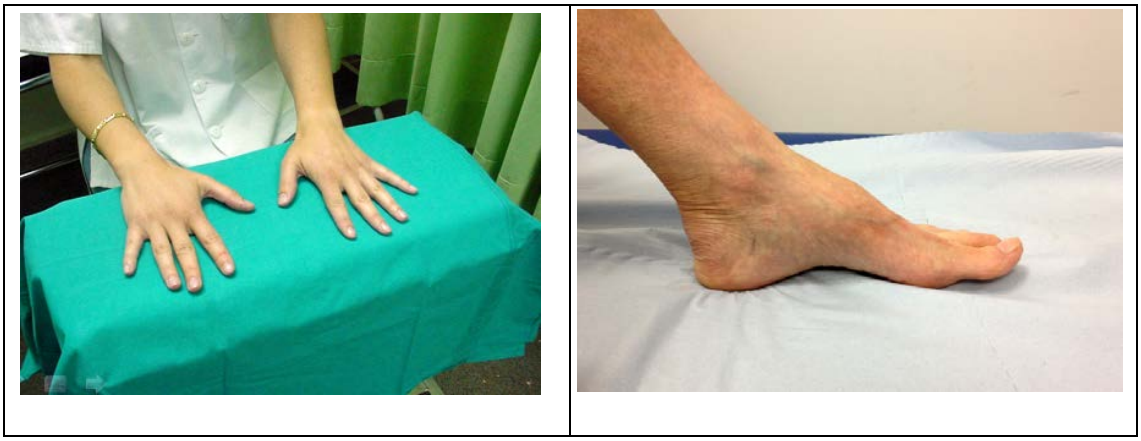


Figure 2-5 Hand and foot position during joint scanning.

2.8.6 Clinical Assessment

Clinical details were recorded for all participating healthy individuals on the clinical proforma provided. Section 2.8.6.1 details the patient demographic, medical history

and clinical examination that were recorded. At the end of the study, these clinical proforma were scanned and transferred along with the US scores and images.

2.8.6.1 History

i. Age

ii. Sex

iii. Personal history of skin psoriasis

iv. Family history of osteoarthritis, skin psoriasis, inflammatory arthritis, connective tissue disease, Inflammatory bowel disease.

v. Previous trauma/joint replacement (specify joint)

vi. Hobbies.

vii. Occupation; if retired previous occupation.

i. Current medication.

ii. Co-morbidities.

iii. Smoking status.

2.8.6.2 Examination

i. Visual analogue scale for overall joint pain.

ii. Clinical joint assessment: MCP 1-5, PIP 1-5, wrist, MTP 1- 5.

iii. Record presence of clinical MTP1 degenerative disease.

iv. Height and weight.

2.9 Results - Study B: Ultrasound findings in a large cohort of healthy subjects.

954 subjects were included in the analysis. Final anticipated figure recruitment is 984 healthy subjects across 24 US centres. US findings (i.e. synovial hypertrophy, joint effusion and Doppler enhancement) of MCP, PIP and MTP stratified by three age groups are presented. Osteophyte and erosion findings are also presented.

2.10 Demographic of healthy subjects.

Table 2-27 details the demographic characteristics of the healthy subjects. Right hand dominant data was collected during the second phase of recruitment.

Table 2-27 Demographics of healthy subjects.

Healthy subject demographics	N=954
Age, median (IQR)	43 (31 to 57)
Female, n (%)	681 (71.4)
Ethnicity group, n (%)	
Afro-Caribbean	16 (1.7)
Asian	59 (6.2)
Caucasian	818 (85.7)
Hispanic	2 (0.2)
Middle Eastern	5 (0.5)
Mixed	45 (4.7)
Not stated	9 (0.9)
Personal history of skin psoriasis$^{\alpha}$	19 (2)
Family history of psoriasis$^{\alpha}$	77 (8.1)
Family history of osteoarthritis$^{\alpha}$	347 (36.4)
Family history of inflammatory arthritis$^{\alpha}$	83 (8.7)
Family history of connective tissue disease$^{\alpha}$	17 (1.8)
Family history of inflammatory bowel disease$^{\alpha}$	30 (3.1)
Right hand dominant$^{\beta}$	124 (95)
Smoking status$^{\gamma}$	
Current	121 (12.7)
Ever	151 (15.8)
Never	678 (71.1)

$^{\alpha}$ Missing data=1, $^{\beta}$Missing data = 824, $^{\gamma}$ missing data= 4

Figure 2-6 shows the age distribution of healthy subjects. The age range was 18 to 80 years old. 549 healthy subjects above the age of 40 years old were recruited which is adequate number for these age groups.

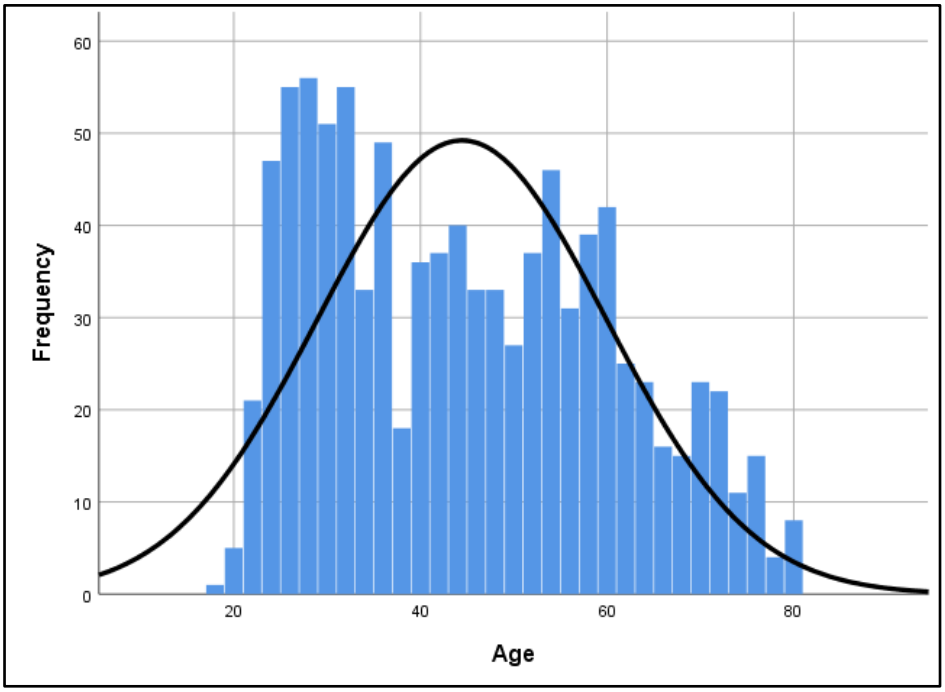


Figure 2-6 Age distribution of healthy subjects.

Data were stratified according to three age groups as shown in Table 2-28. Each tertile would encompass the young, middle and older population. This cut-off was chosen for ease of data interpretation. Around forty percent of healthy subjects recruited were below 40 years old and the remainder were between 40 and 80 years old. Just under a fifth of healthy subjects were in the older age groups.

Table 2-28 Proportion of healthy subjects according to age groups.

Age range (years)		n	Percentage
Young	18 to 39	405	42.5
Middle	40 to 59	363	38.1
Old	60 to 80	186	19.5
Total		**954**	**100.0**

2.11 Proportion of healthy subjects with ultrasound changes.

Table 2-29 shows the proportion of abnormal US parameters according to age groups. All, apart from one, older healthy subjects had at least one US abnormality in at least one joint. The prevalence of normal joints in the young and middle age healthy subjects was very low. Very few healthy subjects had completely normal examination findings in all joints. Overall, only three percent of healthy subjects had completely normal US findings.

Table 2-29 Proportion of abnormal ultrasound parameter according to age groups.

	Young (18-39 years old)	**Middle (40 to 59 years old)**	**Old (60 to 80 years old)**	**Total**
At least one abnormal US parameter, n (%)	389 (96.0)	351 (96.7)	185 (99.5)	954 (96.96)
No abnormal US parameter, n (%)	16 (4.0)	12 (3.3)	1 (0.5)	29 (3.0)
Total	**405**	**363**	**186**	**954**

2.12 Proportion of joints with ultrasound changes.

30,307 joints were scanned in this study. Table 2-30 shows the proportion of abnormal joints. 25% of these joints had at least grade 1 in at least one US parameter. Right and left MTP 1 had the two highest proportions of abnormal US parameters. Whilst right and left MCP 5 had the lowest proportion overall. At the MCP joint level, MCP 1 and 2 had the highest proportion, followed by MCP 3, 4 and 5. At the PIP level, PIP 1 had the highest proportion with approximately the same proportion for the remaining PIPs. At the MTP joint level, MTP 1 had the highest proportion followed by MTP 2, 3, 4 and 5. When compared at the overall joint level, MTP joint level had the highest proportion.

Table 2-30 Proportion of abnormal joints.

Joint	Total number of joints	All ultrasound parameters grade 0	Abnormal joints, n	Abnormal joints, %
R MCP 1	953	742	211	22
R MCP 2	953	702	251	26
R MCP 3	953	780	173	18
R MCP 4	953	836	117	12
R MCP 5	953	855	98	10
L MCP 1	954	756	198	21
L MCP 2	954	691	263	28
L MCP 3	954	806	148	16
L MCP 4	954	841	113	12
L MCP 5	954	858	96	10
R PIP 1	939	726	213	23
R PIP 2	954	798	156	16
R PIP 3	954	807	147	15
R PIP 4	954	828	126	13
R PIP 5	954	833	121	13
L PIP 1	939	768	171	18
L PIP 2	954	826	128	13
L PIP 3	954	821	133	14
L PIP 4	954	835	119	12
L PIP 5	954	840	114	12
R WRIST	936	688	248	26
L WRIST	936	693	243	26
R MTP 1	939	284	655	70
R MTP 2	939	422	517	55
R MTP 3	939	559	380	40
R MTP 4	939	654	285	30
R MTP 5	939	796	143	15
L MTP 1	939	311	628	67
L MTP 2	939	438	501	53
L MTP 3	939	576	363	39
L MTP 4	939	667	272	29
L MTP 5	939	813	126	13
	30307	**22850**	**7457**	

2.13 Joint findings in MCP, PIP and MTP joints stratified by age groups.

Figure 2-7 shows the proportion of abnormal US findings (synovial hypertrophy, Power Doppler and effusion) at the MCP joint level. Power Doppler enhancement was almost absent in all MCP joints across all age groups. When present at very low proportion in the older population, Power Doppler changes were mainly confined to grade 1. Synovial hypertrophy was observed at the MCP joint level in all age groups, although it was more common in the older population (up to 4 percent in MCP 1 in young population, and up to 9 percent in MCP 2 in older population). Synovial hypertrophy, when present at the MCP level, was mainly confined to grade 1 in healthy adults below 40 years old. Grade 2 was also observed in those above 60 years old but in very low proportion. At the MCP joint level, synovial hypertrophy at MCP 1-2 was more common compared to the remaining MCPs. Within each age group, MCP 5 had the lowest proportion of synovial hypertrophy and was almost limited to grade 1 only.

Synovial effusion was more common than synovial hypertrophy and Power Doppler at the MCP level. The prevalence was higher with increased age. The highest proportion was around 16 percent in MCP 1 & 2 in older population. Effusion when present was mainly limited to grade 1 only across all age groups. Within each age group, MCP 5 had the lowest proportion and MCP 1 and 2 tend to have the highest frequency. In summary, at the MCP level, joint effusion was most common followed by synovial hypertrophy. Power Doppler was almost absent across all age groups. Any US lesion, if present was mainly confined to grade one. MCP 2 were more likely to have any US findings compared to the remaining MCP joints. The frequency of effusion and synovial hypertrophy were higher with increased age.

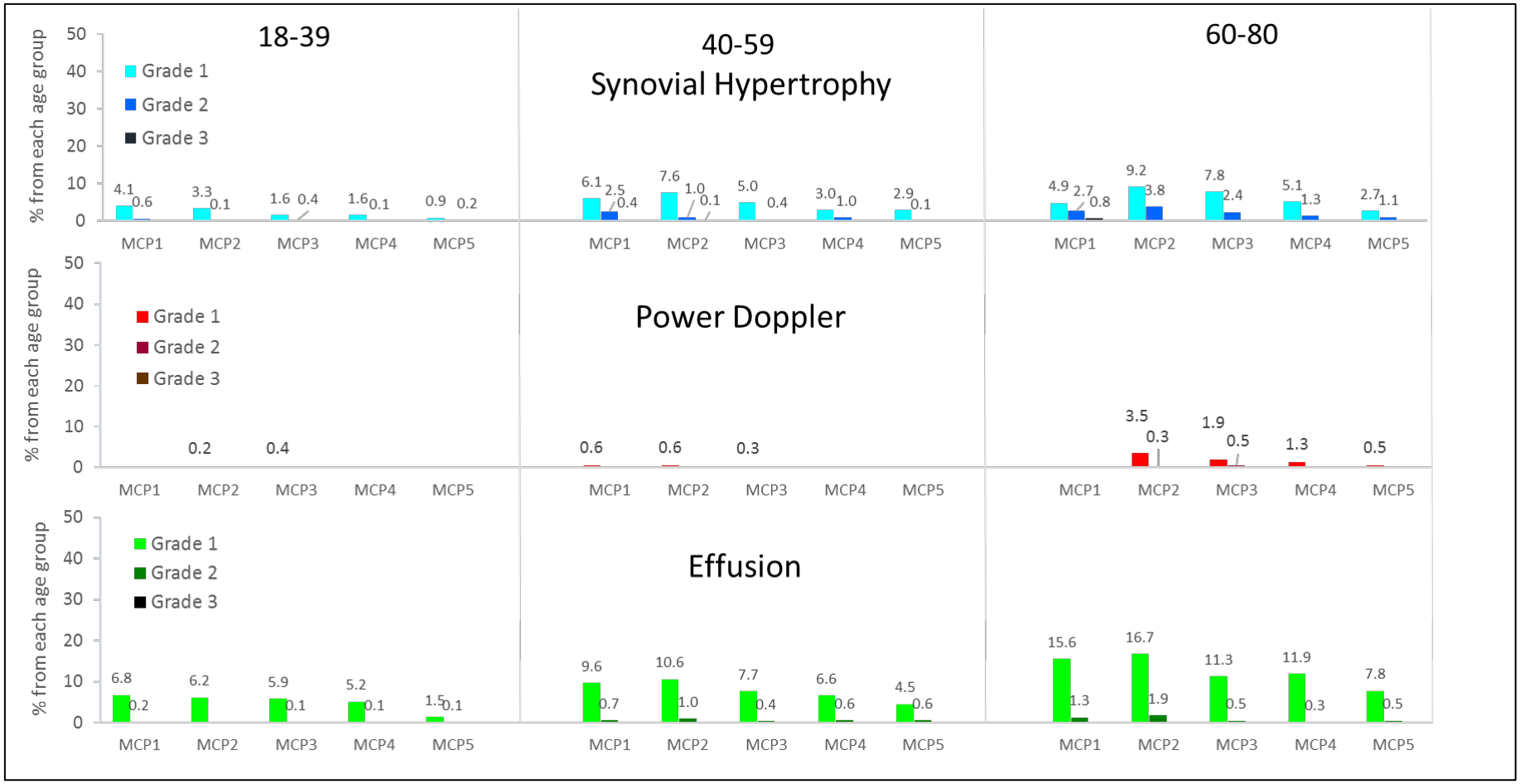


Figure 2-7 Proportion of ultrasound findings (synovial hypertrophy, Power Doppler and effusion) at the MCP level by age groups. Each bar represents the proportion of joint involvement according to age groups.

Figure 2-8 shows the proportion of synovial hypertrophy, Power Doppler and effusion at the PIP joint level across the three age groups. Power Doppler was almost absent across all age groups at the PIP level.

Synovial hypertrophy was more common than Power Doppler lesions at the PIP joints. The frequency of synovial hypertrophy increased with age. The severity was limited to Grade 1 and 2 only in those below the age of 60. Above this age cut off, both grade 1 and 2 were observed, but the prevalence was still very low (up to 3.5 percent for Grade 1 and 2.2 percent for Grade 2). Within each age group, PIP 5 abnormalities had the lowest prevalence (less than 1 percent for all age groups) compared to the other PIP joints.

Effusion was more common than synovial hypertrophy at the PIP joint level. Within each age group, PIP 1 was most common (up to 10-12 percent). Apart from PIP 4, prevalence of effusion did not increase with increasing age.

In summary, at the PIP joint level, Power Doppler was absent. Effusion was most common followed by synovial hypertrophy. Effusion was not dependent on age apart from PIP 4. PIP 1 had the highest frequency of any ultrasound findings compared to the other PIP joints. Any US findings when present were mainly confined to grade 1 severity only.

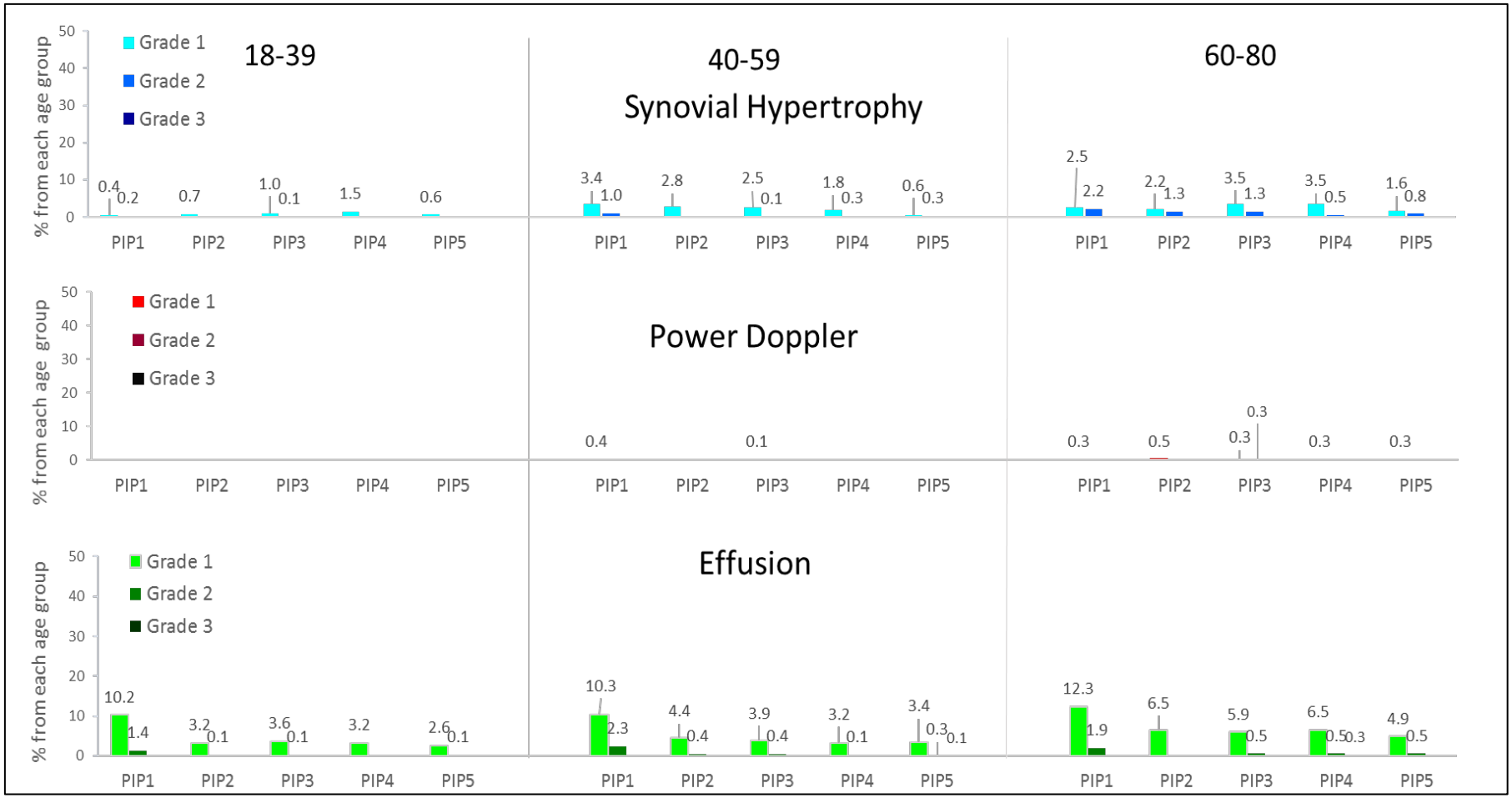


Figure 2-8 Proportion of ultrasound findings (synovial hypertrophy, Power Doppler and effusion) at the PIP level by age groups. *Each bar represents proportion of joint involvement according to age groups*.

Figure 2-9 shows the proportion of synovial hypertrophy, Power Doppler and effusion at the MTP joint level across the three age groups. Power Doppler was very rare across all age groups at the MTP level. Power Doppler was mainly limited to the MTP 1, even so it was observed in less than 4 percent in the older population.

Synovial hypertrophy was more common than Power Doppler. In general, the proportion of synovial hypertrophy increased with increasing age, particularly for MTP1. Severity was predominantly grade 1 -2 across all age groups. At the MTP level, MTP 5 had the lowest proportion within each age group.

Effusion was more common than synovial hypertrophy and Power Doppler at the MTP joint level. This was true for all age groups. The proportion did not increase with age. MTP 1 had the highest proportion within each age group (40 percent for below 40, 30 percent for 40-59 and 45 for above 60). Severity was predominantly grade 1 although grade 2 and to a less extent grade 3 was also observed. Table 2-31 shows the Kendall tau b correlation coefficient between grading severity and age.

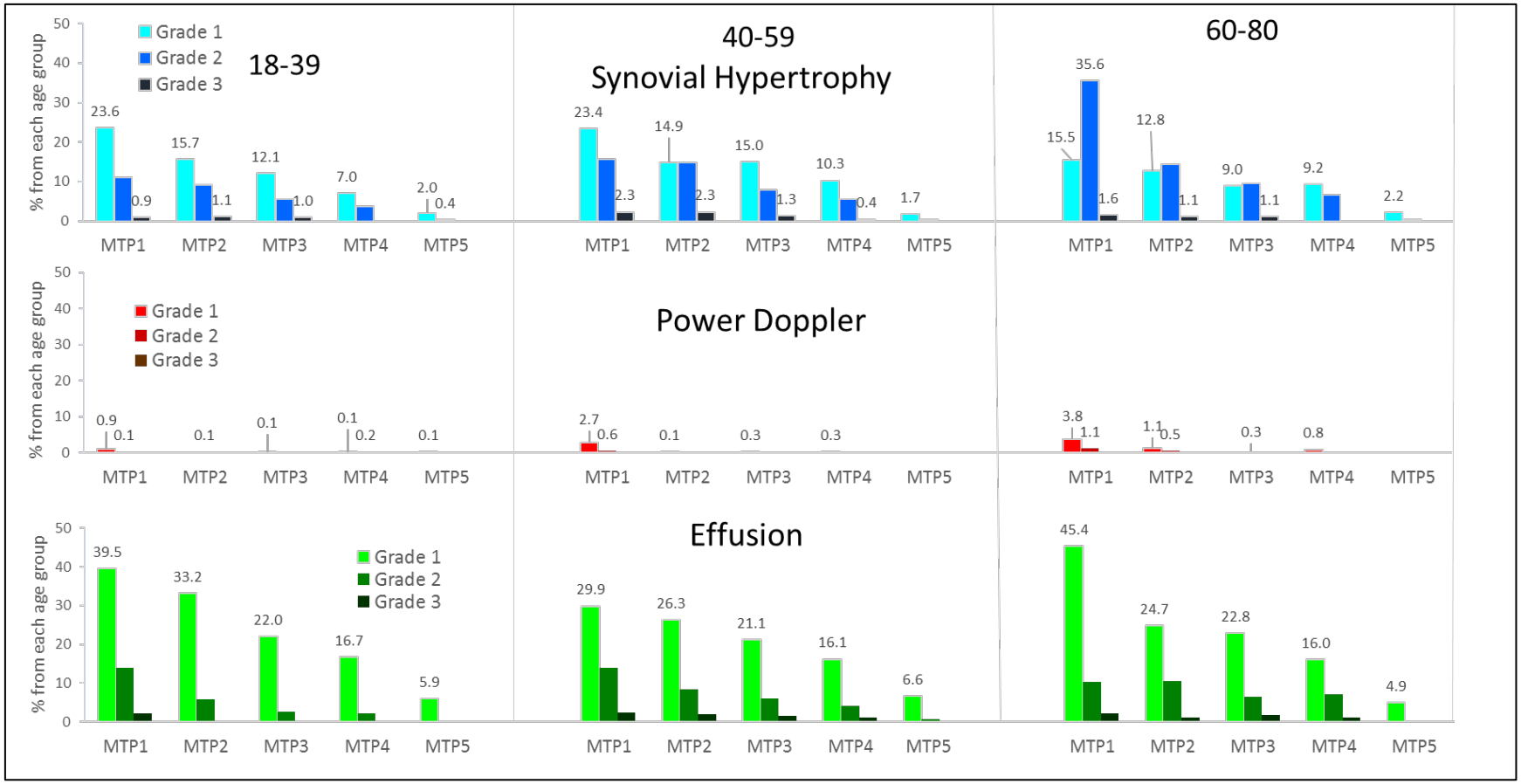


Figure 2-9 Proportion of ultrasound findings (synovial hypertrophy, Power Doppler and effusion) at the MTP level by age groups.

Each bar represents proportion of joint involvement according to age groups.

Table 2-31 Kendall-tau b correlation coefficient of MCP, PIP and MTP ultrasound variables and age groups.

Synovial Hypertrophy	Kendall tau-b	p-value	Power Doppler	Kendall tau-b	p-value	Effusion	Kendall tau-b	p-value
Rt MCP 1	0.073	0.017*	Rt MCP 1	0.019	0.542	Rt MCP 1	0.130	<0.001*
Rt MCP 2	0.171	<0.001*	Rt MCP 2	0.110	<0.001*	Rt MCP 2	0.131	<0.001*
Rt MCP 3	0.121	<0.001*	Rt MCP 3	0.064	0.036*	Rt MCP 3	0.052	0.088
Rt MCP 4	0.129	<0.001*	Rt MCP 4	0.080	0.009*	Rt MCP 4	0.081	0.008*
Rt MCP 5	0.092	0.003*	Rt MCP 5	NA	NA	Rt MCP 5	0.105	0.001*
Lt MCP 1	0.062	0.044*	Lt MCP 1	0.019	0.544	Lt MCP 1	0.087	0.004*
Lt MCP 2	0.092	0.003*	Lt MCP 2	0.091	0.003*	Lt MCP 2	0.148	<0.001*
Lt MCP 3	0.143	<0.001*	Lt MCP 3	0.068	0.027*	Lt MCP 3	0.091	0.003*
Lt MCP 4	0.049	0.107	Lt MCP 4	0.065	0.033*	Lt MCP 4	0.086	0.005*
Lt MCP 5	0.046	0.132	Lt MCP 5	0.065	0.033*	Lt MCP 5	0.134	<0.001*
Rt PIP 1	0.108	<0.001*	Rt PIP 1	0.019	0.532	Rt PIP 1	0.026	0.402
Rt PIP 2	0.085	0.006*	Rt PIP 2	0.046	0.132	Rt PIP 2	0.060	0.053*
Rt PIP 3	0.095	0.002*	Rt PIP 3	0.065	0.033*	Rt PIP 3	0.032	0.292
Rt PIP 4	0.050	0.105	Rt PIP 4	0.046	0.132	Rt PIP 4	0.032	0.298
Rt PIP 5	0.086	0.005*	Rt PIP 5	0.046	0.132	Rt PIP 5	0.041	0.183
Lt PIP 1	0.098	0.001*	Lt PIP 1	0.043	0.170	Lt PIP 1	0.028	0.366
Lt PIP 2	0.069	0.025*	Lt PIP 2	0.046	0.133	Lt PIP 2	0.046	0.137
Lt PIP 3	0.070	0.023*	Lt PIP 3	0.013	0.668	Lt PIP 3	0.054	0.080
Lt PIP 4	0.061	0.046*	Lt PIP 4	NA	NA	Lt PIP 4	0.087	0.004*
Lt PIP 5	0.019	0.540	Lt PIP 5	NA	NA	Lt PIP 5	0.058	0.059
Rt MTP 1	0.178	<0.001*	Rt MTP 1	0.116	<0.001*	Rt MTP 1	-0.010	0.724
Rt MTP 2	0.020	0.494	Rt MTP 2	0.035	0.262	Rt MTP 2	-0.005	0.881
Rt MTP 3	0.029	0.331	Rt MTP 3	-0.019	0.532	Rt MTP 3	0.069	0.023*
Rt MTP 4	0.048	0.117	Rt MTP 4	-0.003	0.925	Rt MTP 4	0.044	0.147
Rt MTP 5	0.007	0.818	Rt MTP 5	-0.033	0.288	Rt MTP 5	-0.008	0.801
Lt MTP 1	0.116	<0.001*	Lt MTP 1	0.062	0.044*	Lt MTP 1	-0.029	0.321
Lt MTP 2	0.067	0.026*	Lt MTP 2	0.090	0.004*	Lt MTP 2	-0.001	0.979
Lt MTP 3	0.035	0.253	Lt MTP 3	0.010	0.757	Lt MTP 3	0.056	0.063
Lt MTP 4	0.087	0.004*	Lt MTP 4	0.037	0.234	Lt MTP 4	0.062	0.043*
Lt MTP 5	-0.007	0.809	Lt MTP 5	NA	NA	Lt MTP 5	0.000	0.991

*. Correlation is significant at the 0.05 level

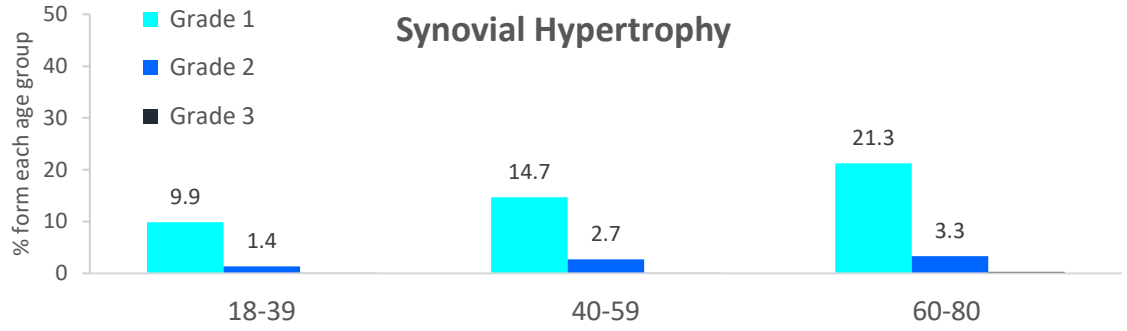
2.14 Joint findings in wrist joints stratified by age groups.

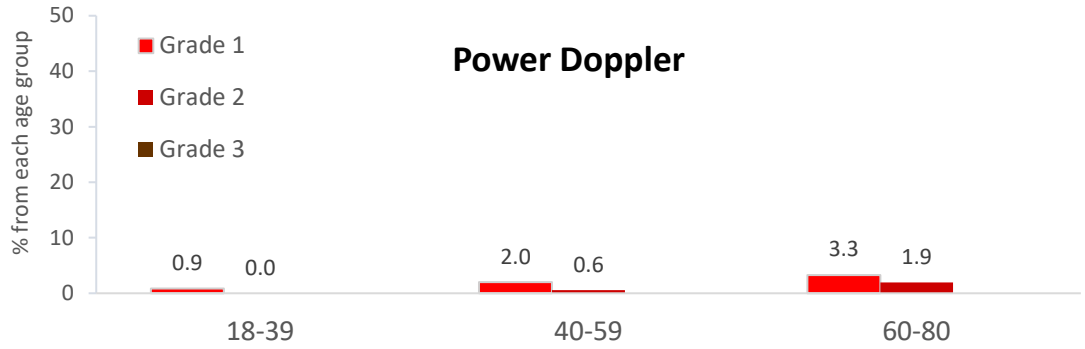
Figure 2-10 shows the proportion of synovial hypertrophy, Power Doppler, synovial effusion and osteophytes at the wrist level by age groups. Gradable Power Doppler was rarely observed at the joint level in all joint groups.

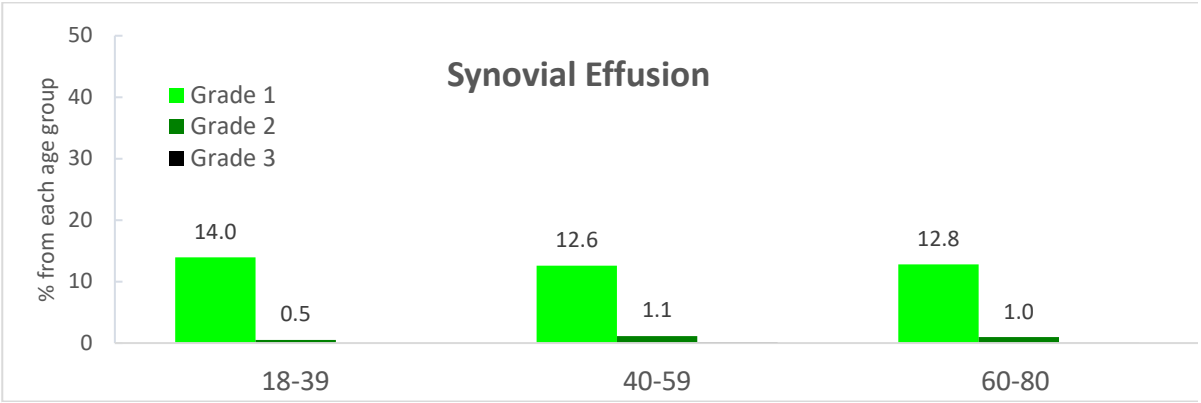
Synovial hypertrophy, however, was present across all age groups, with increasing frequency in the older age group. The proportion was just over twenty percent in the over 60 years old. When present however, the severity was predominantly grade 1. Grade 2 was present in very low proportions with up to 3.3% in the older age group. Synovial effusion was observed across all age groups at the wrist level. The frequency did not change significantly with increasing age. In addition, the severity was almost exclusively limited to grade 1.

Osteophytes at the wrist level were rare. When present the severity was limited to grade 1. The proportion increased in older population; 4.5% in the above 60 years old had osteophytes at the wrist level. Table 2-32 shows the Kendall-tau b correlation coefficient of wrist ultrasound variables and age groups.

Figure 2-10 Proportion of Synovial hypertrophy, Power Doppler , Synovial effusion and osteophytes findings at the wrist level by age groups.







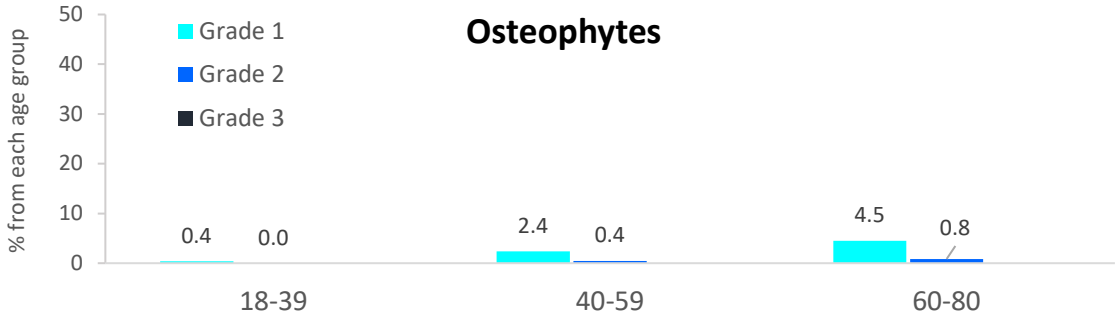


Table 2-32 Kendall-tau b correlation coefficient of wrist ultrasound variables and age groups.

Right wrist	Kendall tau-b	p-value	Left wrist	Kendall tau-b	p-value
Synovial Hypertrophy	.148**	.000	Synovial Hypertrophy	.105**	.001
Power Doppler	.107**	.001	Power Doppler	.070*	.023
Effusion	-.007	.833	Effusion	-.027	.382
Osteophytes	.148**	.000	Osteophytes	.113**	.000

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Figure 2-11 shows the proportion of osteophytes at the MCP level by age groups. The proportion and grading of osteophytes increased with age. Grade 2 osteophytes were only present in those above age 40, and grade 3 above age 60. Higher gradings even when present, the proportion was very low. Within each age group, MCP 2 followed by MCP 1 had the highest proportion compared to the remaining of MCP joints.

Figure 2-12 shows the proportion of osteophytes at the PIP level by age groups. The proportion and grading of osteophytes increased with increasing age. Only grade 1 was observed in the younger population. Grade 2 and 3 when present in the middle and older age groups were in low proportion.

Figure 2-13 shows the proportion of osteophytes at the MTP level by age groups. Osteophytes was more common in the MTP joints compared to the MCP and PIP joints. In general, the proportion and grading severity of osteophytes at the MTP joint level was higher with increasing age. MTP 1 had the largest proportion of osteophytes across all age groups. Grade 3 osteophytes were observed only in the MTP1 joint of those above the age of 40.

Table 2-33 shows the Kendall-tau b correlation coefficient between osteophytes and age groups. Apart from left MCP 5 and left MTP 3 the correlation between osteophytes grading severity increased with increasing age.

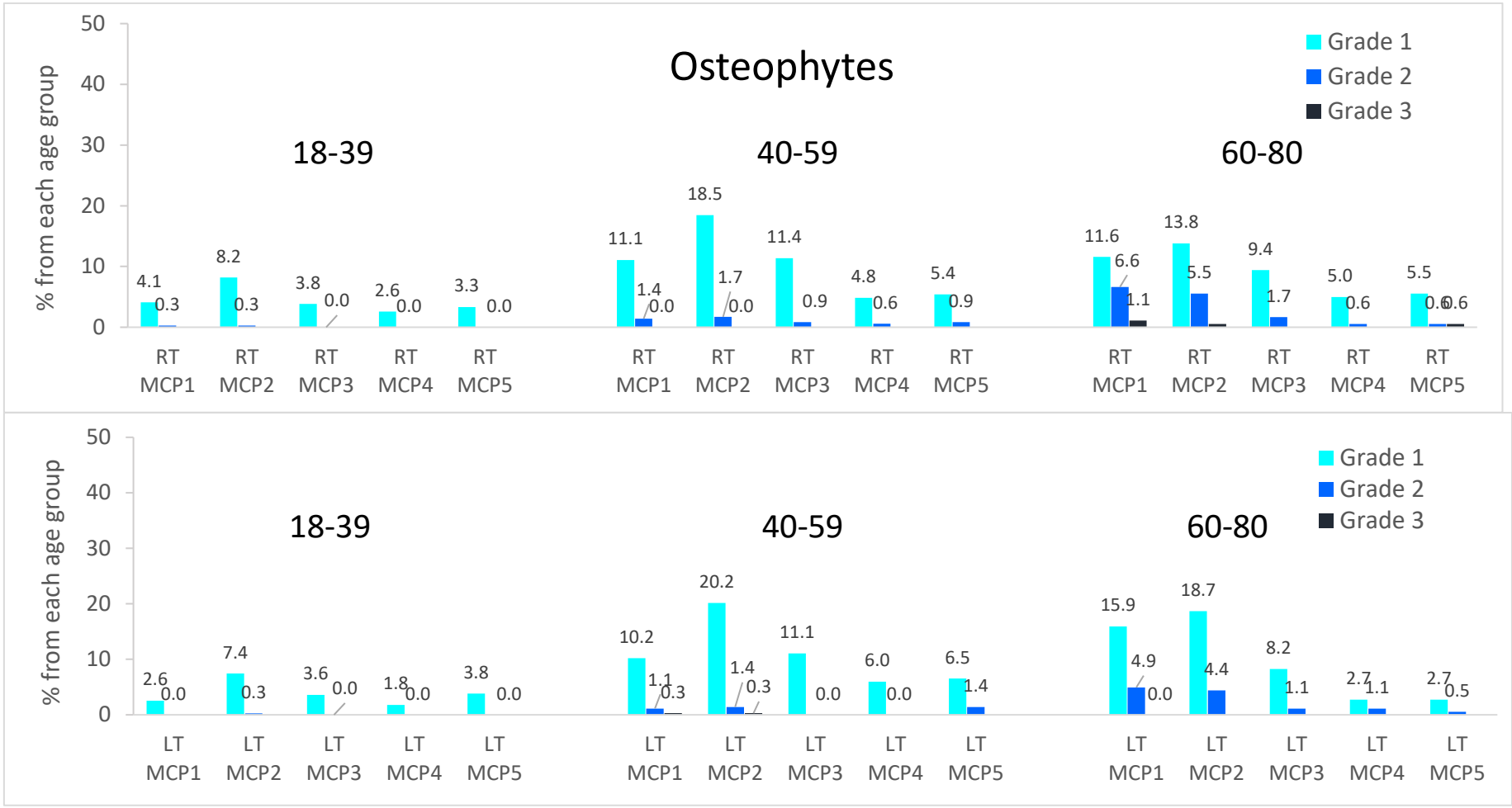


***Figure** 2-11 **Proportion of osteophytes at the MCP level by age groups.** Each bar represents proportion of joint involvement according to age groups.*

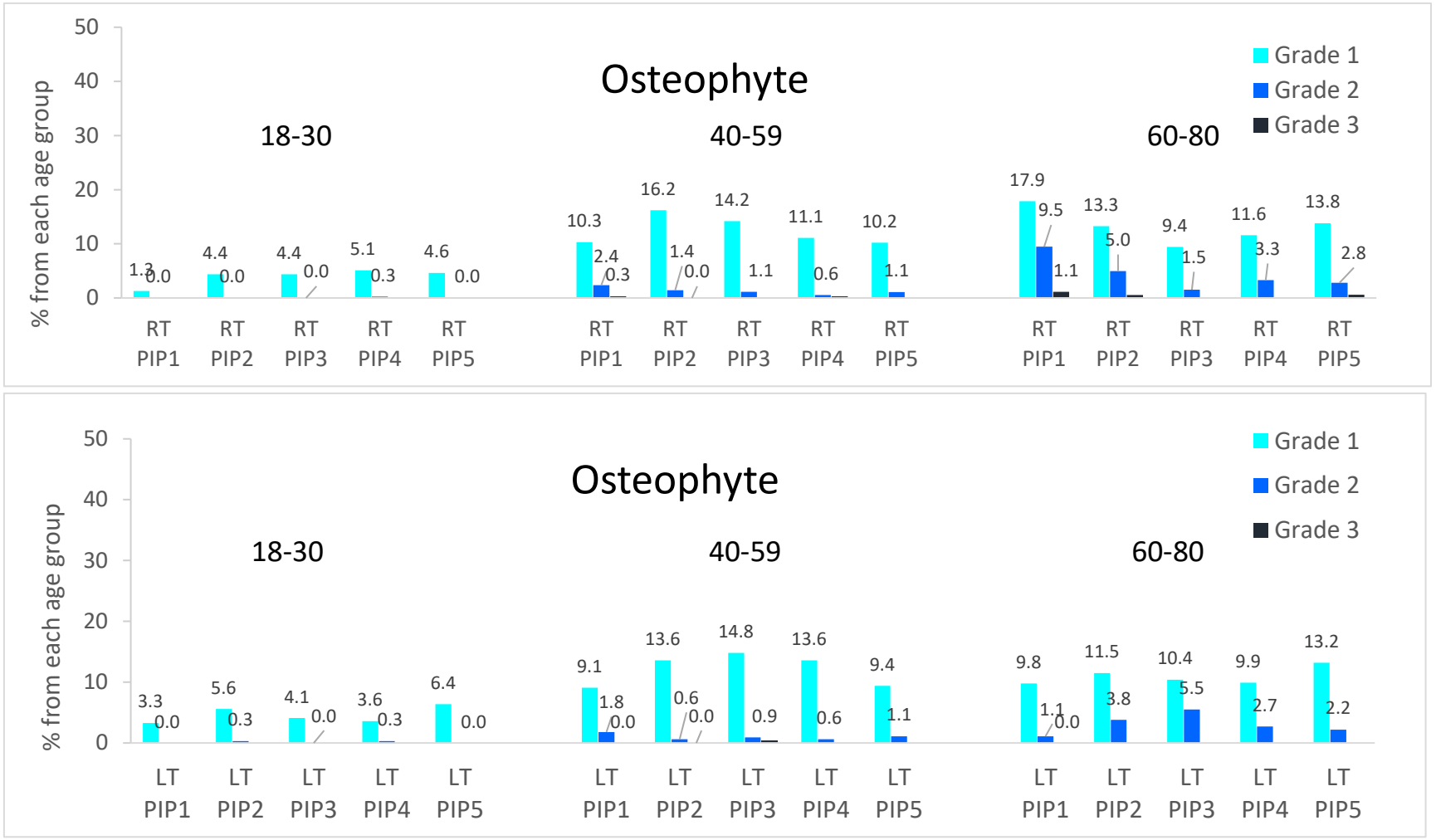


***Figure** 2-12 **Proportion of osteophytes at the PIP level by age groups.** Each bar represents proportion of joint involvement according to age groups.*

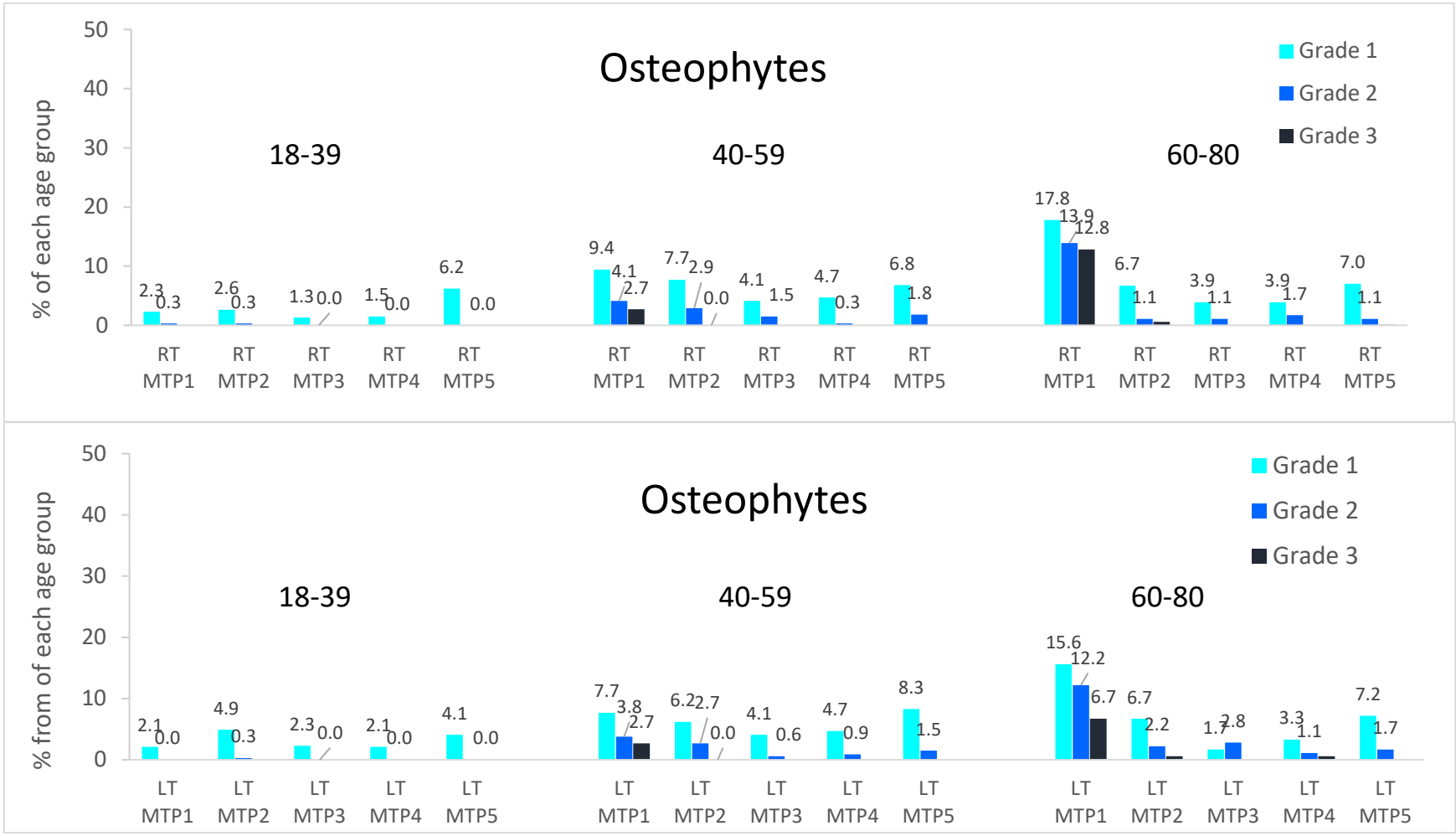


Figure 2-13 Proportion of osteophytes at the MTP level by age groups. *Each bar represents proportion of joint involvement according to age groups.*

Table 2-33 Kendall-tau b correlation coefficient for osteophytes and age groups.

Osteophytes	Kendall tau-b	p-value
Rt MCP 1	.181**	<0.001
Rt MCP 2	.143**	<0.001
Rt MCP 3	.119**	<0.001
Rt MCP 4	.063*	.044
Rt MCP 5	.063*	.044
Lt MCP 1	.221**	<0.001
Lt MCP 2	.178**	<0.001
Lt MCP 3	.105**	.001
Lt MCP 4	.064*	.041
Lt MCP 5	.022	.486
Rt PIP 1	.301**	<0.001
Rt PIP 2	.188**	<0.001
Rt PIP 3	.171**	<0.001
Rt PIP 4	.125**	<0.001
Rt PIP 5	.155**	<0.001
Lt PIP 1	.230**	<0.001
Lt PIP 2	.128**	<0.001
Lt PIP 3	.166**	<0.001
Lt PIP 4	.139**	<0.001
Lt PIP 5	.107**	.001
Rt MTP 1	.376**	<0.001
Rt MTP 2	.109**	.001
Rt MTP 3	.091**	.004
Rt MTP 4	.089**	.004
Rt MTP 5	.077*	.014
Lt MTP 1	.323**	<0.001
Lt MTP 2	.070*	.026
Lt MTP 3	.053	.091
Lt MTP 4	.071*	.024
Lt MTP 5	.086**	.006

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

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

Figure 2-14 shows the proportion of joints with ultrasound-detected erosions across the MCP 2, MCP 5 and MTP 5 across all age groups. Erosions were very rare across all joints scanned and across all age groups. Right MCP 2 in those above the age of 60 had the highest proportion (just under three percent).

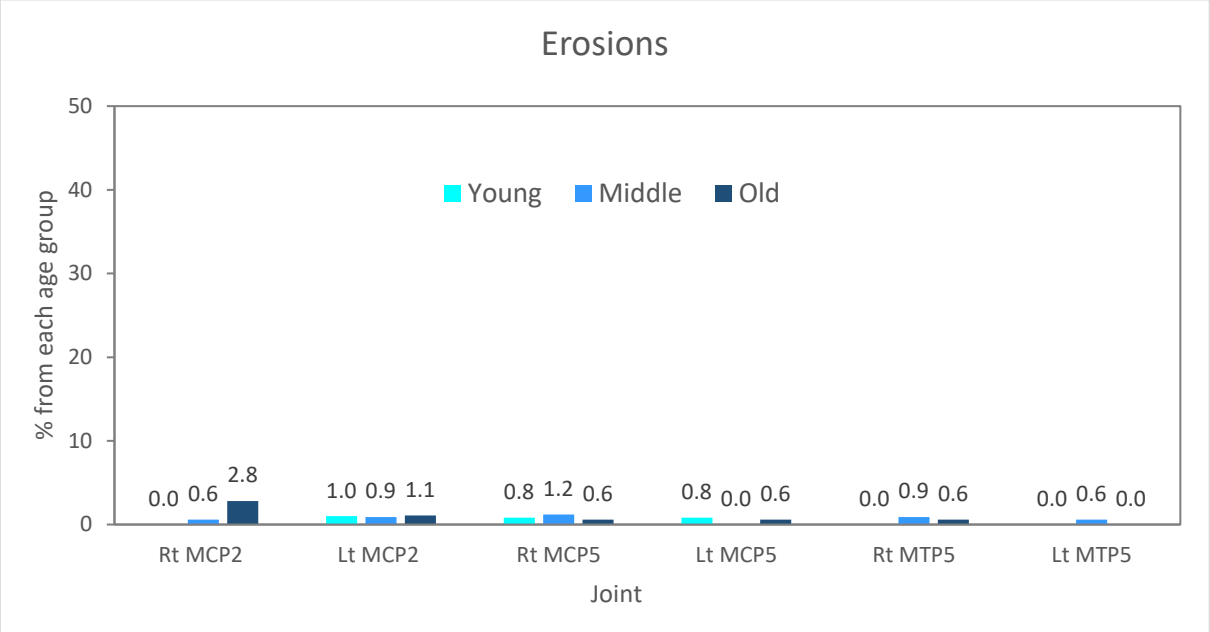


Figure 2-14 Proportion of joints with ultrasound-detected erosions within MCP 2, 5 and MTP 5. *Each bar represents joint involvement according to age groups. Young (18-39 years old), middle (40-59 years old), old (60-80 years old).*

Table 2-34 Kendall tau-b correlation coefficient for erosion and age groups.

Erosion	Kendall tau-b	p-value
Rt MCP 2	0.101	0.002*
Lt MCP 2	0.001	0.978
Rt MCP 5	0.001	0.972
Lt MCP 5	-0.027	0.403
Rt MTP 5	0.043	0.178
Lt MTP 5	0.020	0.535

*. Correlation is significant at the 0.05 level

2.15 Discussion

2.15.1 Interpretation of key findings.

These results give an indication of US lesions that should be regarded as 'non-inflammatory' changes in healthy individuals. Firstly, synovial effusion of lower grading in any joint of any group are more likely to be physiological. Synovial hypertrophy in the weight bearing joints such as MTP 1 and 2 are more likely to be physiological as well. Interestingly, mild synovial hypertrophy, particularly in the PIP joints may represent pathological changes and should not be dismissed especially in the younger population. In addition, the presence of erosions at the sites where RA-related erosions are often found (MCP 2 and 5, MTP 5) in healthy subjects should be regarded as abnormal.

Table 2-35 below summarises the US lesions that may be interpreted as abnormal, according to age groups, and grading threshold. The threshold of abnormality chose was 5%. In a normal population, 95% would fall within this threshold. This is the conventional cut-off level that is used to set threshold of normality in a given population.

Table 2-35 Proposed interpretation of ultrasound lesions according to age groups and pathology.

Young population (18 to 40 years old)		
PIP	SH	Any gradings likely abnormal
	PD	Any gradings likely abnormal
	Effusion	Grade 2-3 likely abnormal
MCP	SH	Grade 3 likely abnormal
	PD	Any gradings likely abnormal
	Effusion	Grade 2-3 likely abnormal
MTP	SH	Grade 3 likely abnormal
	PD	Any gradings likely abnormal
	Effusion	Grade 3 likely abnormal
Middle age (40 to 59 years old)		
PIP	SH	Any gradings likely abnormal
	PD	Any gradings likely abnormal
	Effusion	Grade 2-3 likely abnormal
MCP	SH	Grade 2-3 likely abnormal
	PD	Any gradings likely abnormal
	Effusion	Grade 2-3 likely abnormal
MTP	SH	Grade 3 likely abnormal
	PD	Any gradings likely abnormal
	Effusion	Grade 3 likely abnormal
Older population (60 to 80 years old)		
PIP	SH	Any gradings likely abnormal
	PD	Any gradings likely abnormal
	Effusion	Grade 2-3 likely abnormal
MCP	SH	Grade 2-3 likely abnormal
	PD	Any gradings likely abnormal
	Effusion	Grade 2-3 likely abnormal
MTP	SH	Grade 3 likely abnormal
	PD	Any gradings likely abnormal
	Effusion	Grade 3 likely abnormal

There was a high proportion of normal individuals with grade 1 synovial hypertrophy at the wrist level. Given this unexpected proportion, the wrist images are now being re-graded based on an US atlas that is provided centrally. The images that were included in the protocol was from the most recent EULAR consensus grading papers (118). Applying the synovial hypertrophy grading definition at the wrist joint level may not resulted in the over-scoring of grade 1 that was observed in the results. The normal wrist synovium almost always bulges over the bones surfaces. In addition, the definition of concave or convex for grade 2 and 3 is not really applicable for wrist as normal wrist may have these appearances. Defining the grading for wrists is further complicated by the fact that there are three recesses within the wrist joint – radio-carpal, inter-carpal and ulnar-carpal recesses. In addition, the size (or bulge) of these recesses can change significantly with slight wrist deviation. Furthermore, the presence of a (physiological) ligament within the radio-carpal joint can mimic grade 1 synovial hypertrophy-which may have led to the over scoring on the synovial hypertrophy at this joint level.

2.15.2 Strength of this study.

This is the largest reported study that used a consensus grading system applied to a huge population of healthy subjects in Rheumatology US. This has been a huge collaborative work between the US OMERACT centres spanning across four continents. This ensures external generalisability. A wide range of ethnic group and healthy subject demographics were included in this study.

In addition, the US lesions graded were carefully defined and standardised in the protocol that was provided to all centres. Images from the EULAR consensus gradings were provided together with the protocol. Importantly, the joint positioning was also standardised (image position provided in protocol), as variability in joint positioning is known to affect the gradings (159).

Only OMERACT centres with expertise in Rheumatology US were invited. To robustly ensure that the quality of image acquisition was high, the first set of US images were reviewed centrally prior to continuing recruitment. Finally, the proportion of healthy subjects within the RA age range (older than 45 years old) was high. This is crucial for the next stage of this study where age- match normal controls will be compared with RA. As a benefit for recruiting centres, they have a dataset of healthy subjects for their own comparison when publishing their own data. This study has created an incentive to recruit normal subjects.

2.15.3 Limitation of this study.

The main limitation is related to the fact that wrist US images need to be re-graded by all centres, based upon an atlas that is provided centrally.

2.15.4 Importance of normal threshold in clinical practice and trials.

A clear threshold of (ab)normality is important in clinical practice. This would allow clinicians to interpret the US findings within the clinical context appropriately. For example, one may detect mild synovial hypertrophy with effusion but without Power Doppler enhancement in an MCP2 joint in a 60 year old patient with what sounds like inflammatory arthralgia.

Although the US findings are 'positive', these changes are unlikely to represent early inflammation, but more likely to represent physiological changes. It is important that these subtle age-related changes are not regarded as inflammation. Otherwise, sonographers may interpret US findings as inflammation resulting in false-positive results. As a consequence, patients would then receive unnecessary immunosuppressant (or perhaps unnecessary follow-up without treatment) due to these so called inflammation based on US changes. This issue could be further confounded if there is concomitant fibromyalgia in patients with degenerative changes. These subtle non-clinically important US changes might be over-labelled as inflammation.

The utility of US in clinical trials is promising. However, its application in clinical studies may be of limited value without defining a clear threshold of normality.

An example is the application of US in a treat-to-target (T2T) strategy clinical trial. The clinically important US threshold that should trigger escalation of treatment to improve clinical outcomes is currently not defined. As a result, a key methodological challenge when designing US T2T clinical trial is identifying the appropriate US findings that should trigger a stepwise increase in immunosuppression(146).

Likewise in remission trials. True remission in different joint sub-sets in different age groups could be different. The remission criteria for primary end-point targets for clinical trials based on clinical measures such as DAS-28, CDAI and SDAI are clear.

However, using one definition of US as a remission target in clinical trials may not be appropriate. For example aiming for absence of synovial hypertrophy in all target joints and tendons may not be realistic – as some of the synovial hypertrophy in certain joint subsets may not be pathological. The applications of US within clinical trials would be more robust with a clear cut off definition of normal vs. abnormal.

The main caveat that one should bear in mind in interpreting these results is that – with any diagnostic tool – the overall clinical picture must be taken into account. The absence of inflammatory lesion at the point of scanning by all means does not exclude pathology findings. Nevertheless, the outcome of this study is hope to guide Rheumatologists to interpret US results.

3 Ultrasound as a prediction tool in early arthritis.

3.1 Introduction

Initiation of immunosuppressant therapy during the early phases of inflammatory arthritis alters the trajectory of disease progression (76). However, distinguishing individuals who are at risk of progressing to RA from those whose disease will regress amongst patients presenting with clinical arthritis within 12-weeks of symptom onset remains a challenge.

Many current predictive algorithms for RA and persistent arthritis progression are based on clinical joint involvement, alongside clinical and serological variables (60, 160).

Musculoskeletal US is a non-invasive and well-tolerated imaging technique and has been shown to improve the predictive ability of such algorithms (106, 161) due to the detection of subclinical synovitis (162).

Tenosynovitis (TS) is a well-recognised clinical feature of RA (43, 163-165) and US is a reliable tool to assess TS (108, 166). However, the ability of US-defined TS to add predictive data in patients with early clinically apparent synovitis is currently unknown.

3.2 Study objective

3.2.1 Prediction of RA

In this study, I sought to

1. assess the frequency and distribution of US-defined synovitis and TS in patients with inflammatory arthritis of ≤3 months symptom duration.
2. investigate whether US-defined TS provides predictive data over and above US-defined synovitis and other clinical and serological variables in the prediction of RA development.

3.2.2 Prediction of persistent arthritis.

In this study, I sought to

1. compare the prevalence of US-defined synovitis and tenosynovitis in patients with persistent vs. resolving arthritis in a cohort of patient with very early arthritis.
2. investigate whether US-defined TS provides predictive data over and above US defined synovitis and other clinical and serological variables in the prediction of persistent arthritis development.

3.3 Methods; Prediction of RA and persistent arthritis.

3.3.1 Patients and clinical assessments.

Patients were recruited from the Birmingham Early Arthritis Clinic based in Rheumatology Departments at Sandwell and West Birmingham Hospitals NHS Trust and University Hospitals Birmingham NHS Foundation Trust, UK. All patients were referred by their GP to these two secondary care centres, which provide rheumatology service to a population of 1.3 million across Birmingham.

Consecutive DMARD-naïve patients with clinically-detected synovitis of at least one joint and inflammatory joint symptom duration (pain and/or stiffness and/or swelling) of three months or less were included. Patients who had joint symptoms attributed solely to degenerative joint disease were excluded. Ethical approval was obtained and all patients gave written informed consent.

All consecutive patients who consented to this study were included in the analysis except for those who declined to continue follow-up before final diagnostic outcome data were available. All patients were reviewed at 1, 2, 3, 6, 12 and 18 months and detailed clinical data were recorded at all visits including DMARD treatments.

The following data were recorded at baseline: 68 tender and 66 swollen clinical counts, age, sex, symptom duration, early morning stiffness duration, medication, erythrocyte sedimentation rate, C-reactive protein, RF and ACPA status.

3.3.2 Classification at 18 months for prediction of RA.

Final diagnostic outcomes were determined at 18 months follow-up. Patients were classified as having RA if they fulfilled cumulative 2010 ACR/EULAR criteria (39) by the 18-month visit. Non-RA patients were classified according to established classification criteria which were Psoriatic Arthritis, Systemic lupus erythematosus (SLE) and Ankylosing Spondylitis (17, 149, 150).Patients were classified as having resolving disease at 18-month follow-up if they had no clinical evidence of joint synovial swelling, were not taking DMARDs and had not received steroid treatment (by any route) in the previous three months.

3.3.3 Classification at 18 months for prediction of persistent arthritis.

Patients were classified as having persistent arthritis or resolving arthritis at 18 month follow-up. Patients were classified as resolving disease at 18-month follow-up if they had no clinical evidence of synovial swelling, were not taking DMARDs and had not received steroid treatment for joint disease in the previous three months.

3.3.4 Sonographic assessment

Within 24 hours of clinical assessment, one experienced sonographer (AF) performed a blinded US assessment in a temperature controlled radiology suite. A second sonographer (IS) scanned a proportion of patients included in the persistent arthritis prediction study.

A systematic multi-planar greyscale and power Doppler US examination of 19 bilateral joint sites and 16 bilateral tendon compartments was performed based upon standard EULAR

reference scans (108) using a Siemens Acuson Antares scanner (Siemens, Bracknell, UK) and multi-frequency (5–13 MHz) linear array transducers. The joint and tendon sites scanned are listed in Table 3-1 and Table 3-2 respectively. For power Doppler (PD) examinations, the pulse repetition frequency (PRF) was adjusted to provide maximal sensitivity at the lowest possible value for each joint, resulting in PRFs of between 610 and 780. Examinations took between 50 and 60 minutes depending on disease extent and patient mobility.

Table 3-1 Synovial intra-articular recesses and periarticular sites assessed by ultrasound.

Joint	Recess
MCP 1-5, PIP 1-5, MTP 2-5	Multi-planar scanning of dorsal recesses
	Lateral recess of MCP1,2,MTP5
Wrist	Intercarpal recesses
	Radiocarpal recesses
	Ulnarcarpal recesses
	Volar carpal recesses
Elbow	Anterior recess
	Humeroradial joint
	Humeroulnar joint
	Posterior recess
Shoulder	Posterior glenohumeral recess
Knee	Suprapatellar recess
	Medial parapatellar recess
	Lateral parapatellar recess
	Medial femorotibial joint line
	Lateral femorotibial joint line
Ankle	Anterior tibiotalar recess
	Medial tibiotalar recess
	Lateral tibiotalar recess

MCP, metacarpophalangeal joint; MTP, metatarsophalangeal joint; PIP, proximal interphalangeal joint.

Table 3-2 Tendon compartments evaluated by ultrasound

Tendon	Tendon Compartment
Digit	Flexor tendon 1-5
Wrist	Flexor tendon compartment
	Extensor compartment 1 (APL and EPB)
	Extensor compartment 2 (ECRL and ECRB)
	Extensor compartment 3 (EPL)
	Extensor compartment 4 (EDC and EIP)
	Extensor compartment 5 (EDM)
	Extensor compartment 6 (ECU)
Shoulder	Biceps tendon
Ankle	Anterior extensor compartment (TA, EHL, EDL)
	Peroneus longus and brevis
	Posteromedial compartment (PT, FDL, FHL)

APL: Abductor pollicis longus. EPB: extensor pollicis brevis. ECRL: extensor carpi radialis longus. ECRB: Extensor carpi radialis brevis. EPL: Extensor pollicis longus. EDC: extensor digitorum communis. EIP: Extensor indicis propius. EDM: Extensor digiti minimi. ECU: extensor carpi ulnaris. TA: Tibialis Anterior. EHL: Extensor Hallucis Longus. EDL: Extensor digitorum Longus. PT: Posterior tibial. FDL: Flexor digitorum longus. FHL: Flexor hallucis longus.

US findings of Grey-scale synovial hypertrophy and PD positivity were defined according to consensus definitions (107, 151). GS and PD positivity in the metacarpophalangeal, proximal interphalangeal and metatarsophalangeal joints were graded from 0 to 3 as per consensus definition (106). Synovitis in other joints was graded as 0, normal; 1, mild; 2, moderate and 3, severe, as previously reported (106).

GS and PD tenosynovitis (TS) changes were defined and graded according to the OMERACT US Task Force consensus definitions (108). GS TS was defined as abnormal anechoic and/or

hypoechoic (relative to tendon fibres) tendon sheath widening which was related to tenosynovial abnormal fluid and/or hypertrophy. PD TS was defined as the presence of peritendinous Doppler signal within the synovial sheath, seen in two perpendicular planes, excluding normal feeding vessels. For the analysis, all GS and PD US variables were binarised into absent (grade=0) or present (grade ≥ 1).

3.3.4.1 Reliability analysis.

Intra-observer reliability was evaluated by blindly rescoring representative images of 20 patients for joint US assessments and analysed using κ statistics. A kappa value of 0-0.2 was considered poor, 0.21-0.40 fair, 0.41-0.6 moderate, 0.61-0.8 good, and 0.81 to 1 excellent.

3.3.5 Statistical analysis for prediction of RA.

3.3.5.1 Descriptive analysis

All data analyses were performed using IBM SPSS Statistics for Windows (Version 20.0. Armonk, NY: IBM Corp.) Baseline clinical variables between groups and the proportions of patients with US-defined synovitis and tenosynovitis between the outcome groups were compared using Kruskal–Wallis or Fisher's exact tests as appropriate. In descriptive analyses, $p \leq 0.017$ (0.05 divided by 3) was considered statistically significant after adjusting for the effect of multiple comparisons using the Bonferroni method.

3.3.5.2 Logistic Regression and Principal Component Analyses

The primary aim of these analyses was to identify the most parsimonious combination of US, clinical and serological variables that, when applied to a cohort of patients with early

arthritis, identified patients progressing to RA by 18 months. All GS and PD US variables were binarised into absent (grade=0) or present (grade ≥ 1). Univariate logistic regression analysis was performed to identify individual variables predictive of RA development. Secondly, principal component analysis (PCA) was used to identify which explanatory variables accounted for the majority of variance as well as to identify the US variables that highly correlated with each other.

Subsequently, multiple logistic regression analyses were performed to identify the relative independent predictive ability of the relevant variables. All independent clinical and serological variables were classified into categories as listed in Table 3-3.

Table 3-3. Classification of variables for the logistic regression analysis of RA prediction.

Variables	Category
Gender	Male*/female
Age	< 60 years*/ ≥60 years
ESR	Normal*/abnormal (by local standards)
CRP	Normal*/abnormal (by local standards)
Swollen joint count-66	1*, 2–5, 6–66
Tender joint count-68	1*, 2–5, 6–68
Early morning stiffness	<60 minutes* / ≥60 minutes
Rheumatoid factor (RF)	Normal* / low-positive[†] Normal* / high-positive[††]
Anti-cyclic citrullinated peptide (ACPA)	Normal* / low-positive[§] Normal* / high-positive[§§]

*Reference category. [†] RF > 20 IU/mL, [††] RF > 60 IU/mL, [§]ACPA >7 EU/ml, [§§]ACPA >21 EU/ml.

3.3.6 Statistical analysis for prediction of persistent arthritis.

3.3.6.1 Descriptive analysis

All data analyses were performed using IBM SPSS Statistics for Windows (Version 26.0; IBM Corp., Armonk, NY, USA). Baseline clinical variables between groups were compared using Mann Whitney or Fisher's exact tests as appropriate. The proportions of patients with US-defined synovitis and TS between the outcome groups were compared using Fisher's exact test. In descriptive analyses, a value of 0.05 was considered statistically significant.

3.3.6.2 Logistic regression and principal component analyses

The primary aim of this study was to identify the combination of US, clinical and serological variables that, when applied to a cohort of patients with early arthritis, identified patients who developed persistent inflammatory arthritis by 18 months. All GS and PD US variables were binarised into absent (grade = 0) or present (grade $\geqslant$1). Univariate logistic regression analysis was then performed to identify individual variables associated with the development of persistent arthritis. Secondly, principal component analysis (PCA) was used to assess the extent of clustering amongst US joint and tendon variables and then clinical and serological variables. The variables that had the highest loading factor within each component were then used in logistic regression analysis.

Further logistic regression modeling was then done by systematically changing the US joint variable within each regression question in order to confirm the independence of US-measured tendon and joint variables in prediction of persistent arthritis.

All independent clinical and serological variables were classified into categories as listed in Table 3-4 for persistent arthritis prediction.

Table 3-4 Classification of variables for the logistic regression analysis- persistent arthritis prediction.

Variables	Category
Gender	Male*/female
Age	< 60 years*/ ≥60 years
ESR	Normal*/abnormal (by local standards)
CRP	Normal*/abnormal (by local standards)
Swollen joint count-66	1*, 2–5, 6–66
Tender joint count-68	1*, 2–5, 6–68
Early morning stiffness	<60 minutes* / ≥60 minutes
Rheumatoid factor (RF)	Normal* / low-positive† / high-positive††
Anti-cyclic citrullinated peptide (ACPA)	Normal* / low-positive§ / high-positive§§

*Reference category. † RF > 20 IU/mL, †† RF > 60 IU/mL, §ACPA >7 EU/ml, §§ACPA >21 EU/ml.

3.4 Results; Prediction of RA.

3.4.1 Reliability analysis

The intra-observer reliability κ values for joint and tendon US scoring of GS and PD were excellent; κ value of 0.83 for joint GS, 0.97 for joint PD, 0.96 for tendon GS and 0.95 for tendon PD.

Table 3-5 Intra-observer reliability of joint ultrasound assessment.

Joint assessment	Greyscale	Power Doppler
Overall	**0.83**	**0.87**
PIP	0.85	0.91
MCP	0.85	0.91
Wrist	0.77	0.81
Elbow	0.81	0.82
Shoulder	0.86	0.65
Knee	0.77	0.70
Ankle	0.77	0.75
MTP	0.76	0.88

A kappa value of 0-0.2 was considered poor, 0.21-0.40 fair, 0.41-0.6 moderate, 0.61-0.8 good, and 0.81 to 1 excellent. Intra-observer reliability was evaluated by blindly rescoring representative images of 20 patients for joint US assessments and analysed using κ statistics.

Table 3-6 Intra-observer reliability of tendon ultrasound assessment.

Tendon assessment	Greyscale	Power Doppler
Overall	**0.96**	**0.95**
Digit	0.99	0.99
Wrist	0.97	0.95
Shoulder	0.94	0.86
Ankle	0.96	0.95
Tendon Compartments	**Greyscale**	**Power Doppler**
Digit flexor	0.99	0.99
Wrist flexor	0.97	0.97
Wrist extensor	0.97	0.94
Shoulder	0.94	0.86
Ankle anterior	0.97	0.96
Ankle posteromedial	0.99	0.99
Ankle peroneal	0.88	0.88

Intra-observer reliability was evaluated by blindly rescoring representative images of 20 patients for tendon US assessments, and analysed using κ statistics

3.4.2 Demographic and disease characteristics

107 patients were included in the analysis for the prediction of RA. Given that seropositivity for RF and/or ACPA is a strong predictor of RA, I analysed and presented results for the whole cohort and seronegative patients. I defined seronegativity as those who were both RF and ACPA negative.

Table 3-7 shows the baseline demographic and disease characteristics for the whole cohort and seronegative patients. Out of 107 patients, 46 patients (43%) developed persistent RA [referred to as RA hereafter], 17 patients (16%) developed non-RA persistent inflammatory arthritis and the remaining 44 (41%) had a resolving disease course, including 10 patients who fulfilled the 2010 ACR/ EULAR criteria for RA during the study period but whose disease had resolved by 18 months of follow up. Those who developed RA were more likely to be older, and had higher tender and swollen joint counts compared to those who had non-RA persistent arthritis and resolving arthritis.

76 patients were seronegative for both antibodies. Of these, 23 patients (30%) developed persistent seronegative RA [also referred to as RA hereafter], 14 patients (18%) developed seronegative non-RA persistent disease, and 39 (51%) developed seronegative resolving arthritis. Seronegative RA patients were more likely to have a higher swollen joint count compared to the other two groups at baseline.

Table 3-7 Baseline characteristics for all patients by diagnostic outcomes.

Diagnostic group	Persistent RA (RA)	Non-RA Persistent (NRAP)	Resolving (RES)	P RA vs NRAP	P RA vs RES
n (%)	46 (43)	17 (16)	44 (41)		
Age, years	61 (49-67)	39 (32-64)	44 (33-58)	0.019	0.003
Female, n (%)	24 (52)	11 (65)	25 (57)	NS	NS
Symptom duration, weeks	7 (5-9)	5 (4-8)	5 (3-7)	NS	<0.001
Morning stiffness, minutes	105 (60-180)	60 (10-180)	30 (0-60)	NS	NS
NSAID use, n (%)	33 (72)	13 (76)	27 (61)	NS	NS
RF positivity, n (%)	22 (48)	2 (12)	3 (7)	0.010	<0.001
ACPA positivity, n (%)	20 (43)	1 (6)	3 (7)	0.006	<0.001
ESR, mm/h	24 (12-39)	32 (11-59)	18 (5-32)	NS	NS
CRP, mg/l	13 (5-34)	24 (9-39)	10 (1-27)	NS	NS
Swollen joint count of 66	7 (3-14)	2 (1-7)	2 (1-5)	0.002	<0.001
Tender joint count of 68	11 (4-15)	5 (2-12)	4 (1-7)	NS	0.002
Presence of X-ray erosion$^{\Psi}$	1/46 (2.2)	1/16 (6.3)	1/39 (2.6)	NS	NS
Diagnostic group	**Seronegative Persistent RA (RA)**	**Seronegative Non-RA Persistent (NRAP)**	**Seronegative Resolving (RES)**	**P RA vs NRAP**	**P RA VS RES**
n (%)	23 (30)	14 (18)	39 (51)		
Age, years	60 (49-69)	39 (32-72)	43 (33-55)	NS	0.023
Female, n (%)	11 (48)	9 (64)	22 (56)	NS	NS
Symptom duration, weeks	7 (5-9)	6 (4-8)	5 (3-7)	NS	0.023
Morning stiffness, minutes	120 (60-240)	60 (8-180)	30 (5-60)	NS	0.001
NSAID use, n (%)	15 (65)	10 (71)	25 (64)	NS	NS
ESR, mm/h	19 (7-47)	36 (12-55)	18 (5-36)	NS	NS
CRP, mg/l	12 (0-26)	21 (5-36)	10 (1-29)	NS	NS
Swollen joint count of 66	7 (3-11)	2 (1-6)	2 (1-5)	0.005	0.001
Tender joint count of 68	12 (4-15)	5 (2-10)	5 (2-7)	NS	0.027

All variables are shown as median (IQR) unless otherwise specified. ACPA, anti-cyclic citrullinated peptide antibody; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; NSAID, non-steroidal anti-inflammatory drug; RF, rheumatoid factor. Ψ101 out of 107 patients had hand and/or foot X-ray.

Table 3-8 Final diagnoses for all patients by diagnostic outcomes.

Final diagnosis, n	RA, 46	Non-RA Persistent, 17	Resolving, 44
RA	46	0	10*
PsA	0	7	2
SLE	0	3	0
AS	0	1	0
Crystal arthropathy	0	0	4
Parvovirus arthropathy	0	0	4
Post-streptococcal	0	0	1
Reactive arthritis	0	1	1
Infectious arthritis	0	0	1
Sarcoidosis	0	2	0
Unclassified	0	3	21

*Of the 10 RA patients that were classified as resolving, six patients did not receive any DMARDs or corticosteroids; one patient received a single dose of intra-muscular methylprednisolone; one patient received a short course of prednisolone; one patient received a short course of prednisolone and hydroxychloroquine for three weeks; one patient received methotrexate monotherapy and hydroxychloroquine was added after two months, both DMARDs were withdrawn after six months.

Table 3-9 Final diagnoses for seronegative patients by diagnostic outcomes.

Final diagnosis, n	Seronegative Persistent RA, 23	Seronegative Non-RA Persistent, 14	Seronegative Resolving, 39
RA	23	0	8
PsA	0	5	2
SLE	0	3	0
AS	0	1	0
Crystal arthropathy	0	0	3
Parvovirus arthropathy	0	0	4
Post-streptococcal	0	0	1
Reactive arthritis	0	0	1
Infectious arthritis	0	0	1
Sarcoidosis	0	2	0
Unclassified	0	3	19

3.4.3 Distribution of US-defined joint synovitis

A total of 4066 joints (i.e. 19 bilateral joints in 107 patients) were included in the analysis. The distribution of US-defined joint synovitis is presented in Figure 3-1 . Compared to patients with resolving arthritis, RA patients were more likely to have GS and PD changes at PIP 1-5, MCP 1-5, wrist, elbow, MTP 3 and MTP 5 joints. In addition, RA patients were more likely to have MTP 2 PD changes, but not GS changes alone, compared to patients with resolving arthritis. The only US synovitis variable discriminative of RA from all non-RA patients was MCP 3 GS joint changes.

The distribution of US-defined joint synovitis for seronegative patients is presented in Figure 3-2 . Compared to patients with resolving arthritis, seronegative RA patients were more likely to have GS changes at the PIP 2, MCP 1, 2, 4 and 5 joints and PD changes at the PIP 2, 3, MCP 1, 2, 3, 5, wrist and MTP 2 joints.

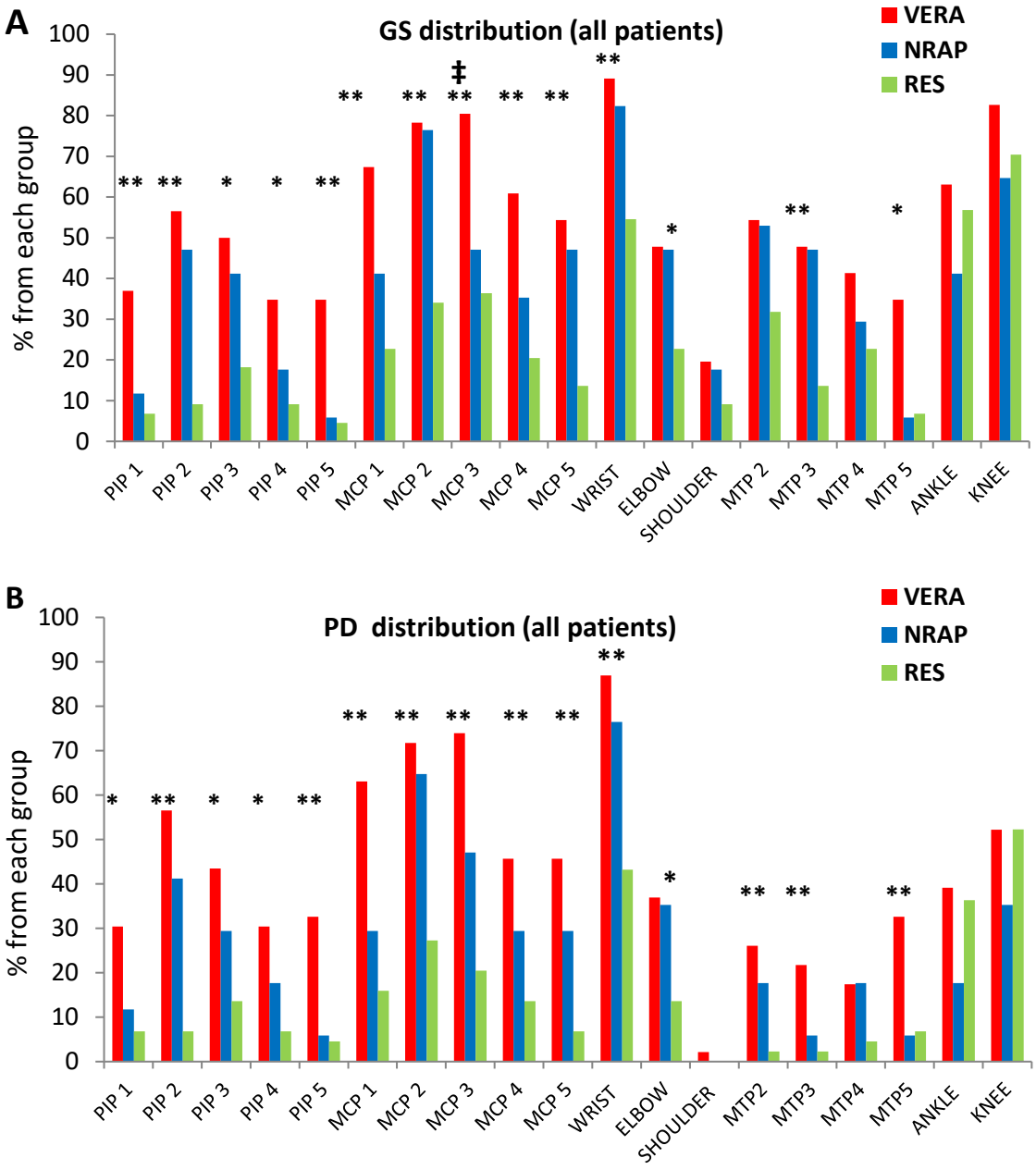


Figure 3-1 Distribution of joint US pathology in all patients.

Each bar represents the proportion of patients with US-defined joint synovitis involvement. P ≤ 0.017 (i.e. 0.05/3) was considered statistically significant as we adjusted for multiple comparisons using the Bonferroni method. VERA vs NRAP: †p≤0.017, ‡ p≤0.001. VERA vs RES: *p≤0.017, **p≤0.001. PIP: proximal interphalangeal joint. MCP: metacarpophalangeal joint. MTP: metatarsophalangeal joint.

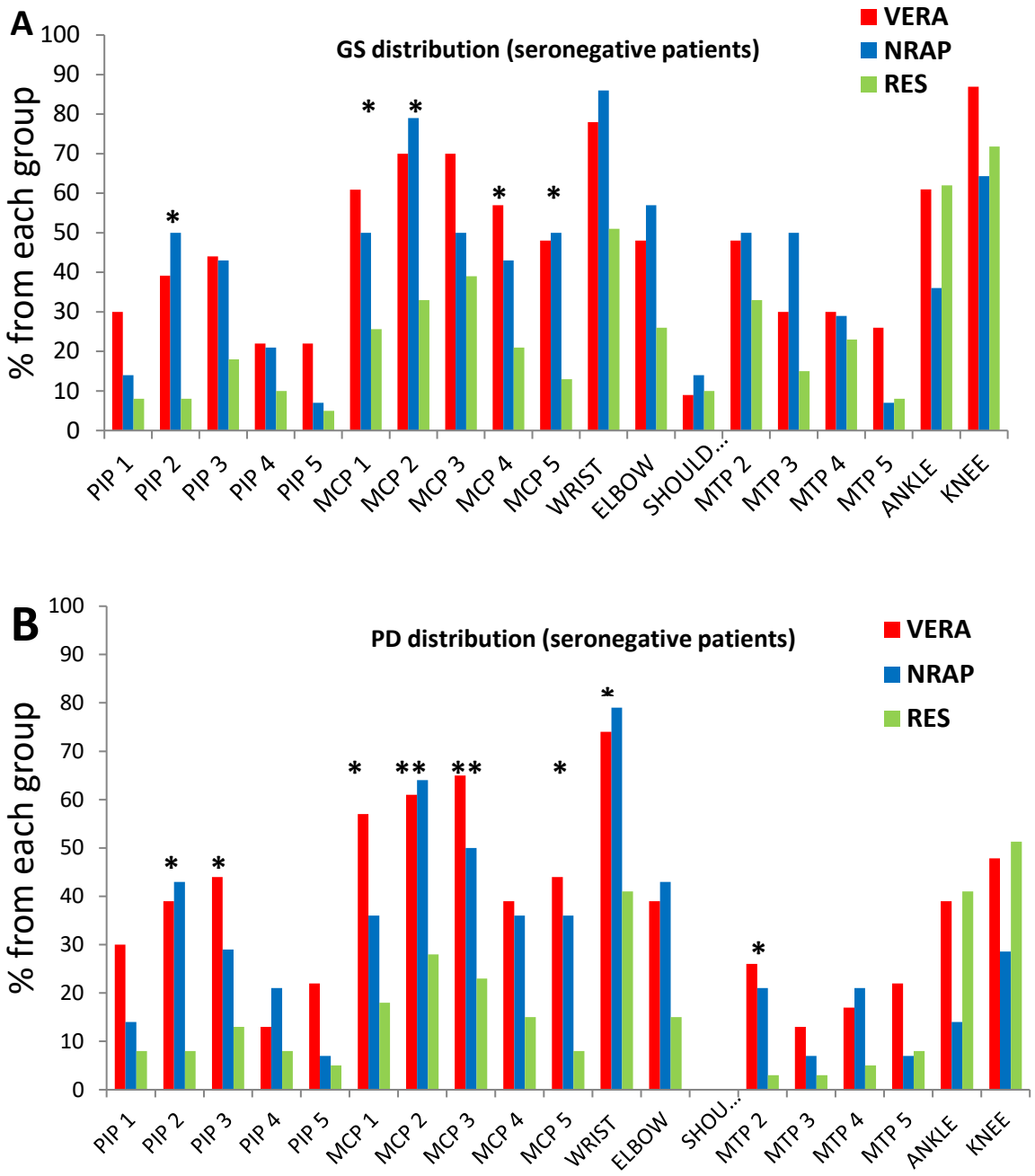


Figure 3-2 Distribution of joint US pathology in seronegative patients.
*Each bar represents the proportion of patients who had US-defined joint synovitis involvement. P ≤ 0.017 (i.e. 0.05/3) was considered statistically significant as we adjusted for multiple comparisons using the Bonferroni method. VERA vs RES: *p≤0.017, **p≤0.001. PIP: proximal interphalangeal joint. MCP: metacarpophalangeal joint. MTP: metatarsophalangeal joint.*

3.4.4 Distribution of US-defined tenosynovitis

3424 tendon compartments (i.e. 16 bilateral tendon compartments in 107 patients) were included in the analysis. All patient groups had evidence of US-defined tenosynovitis of at least one anatomical site at baseline (RA 85%, NRAP 71%, and Resolving 70%).

The distribution of US-defined tenosynovitis by tendon region for all patients is presented in Figure 3-3. Compared to patients with resolving arthritis, RA patients were more likely to have digit flexor and wrist extensor US-defined TS, with both GS and PD pathology.

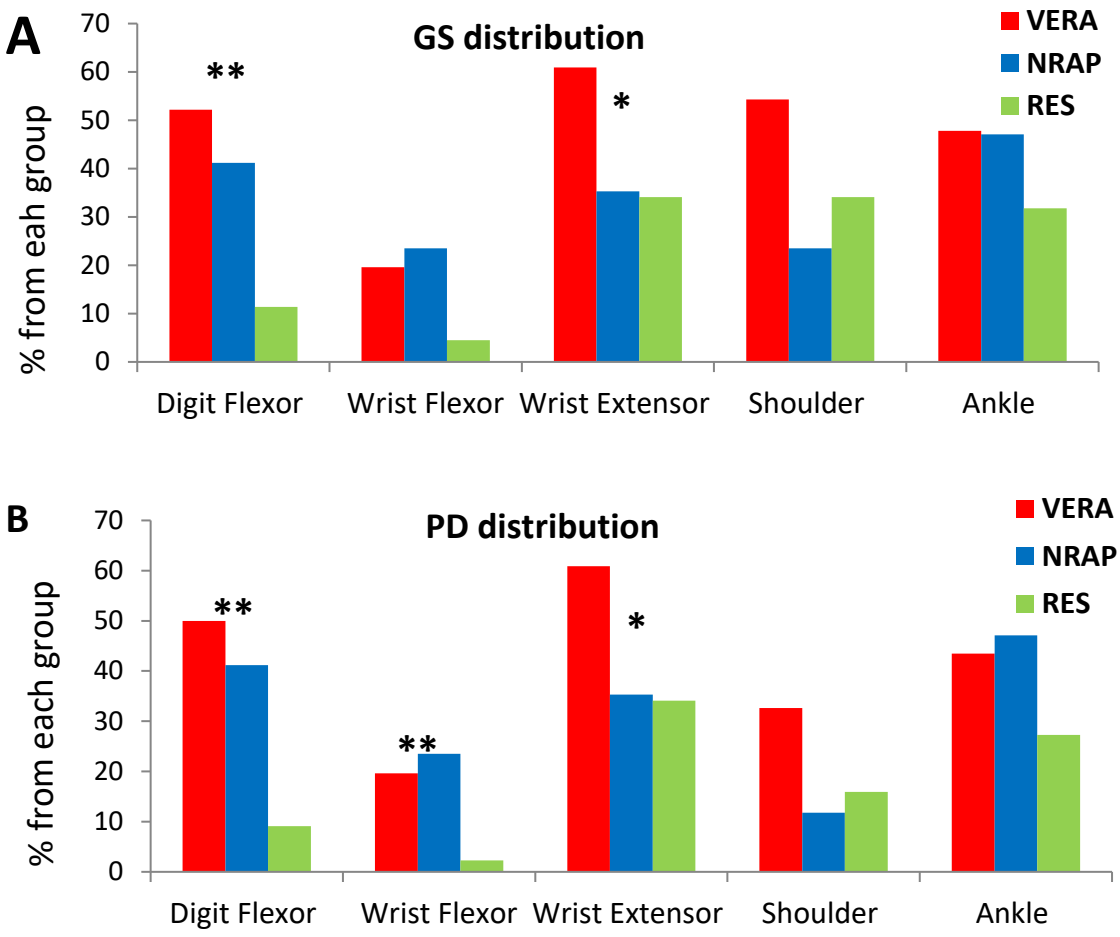


Figure 3-3 Distribution of US pathology by tendon region in all patients.
*Each bar represents the proportion of patients US-defined tenosynovitis involvement according to (A) and (B) tendon regions. P<0.017 (i.e. 0.05/3) was considered statistically significant as we adjusted for multiple comparisons using the Bonferroni method.VERA vs NRAP : † p≤0.017, ‡ p≤0.001. VERA vs RES *p≤0.017, **p≤0.001.*

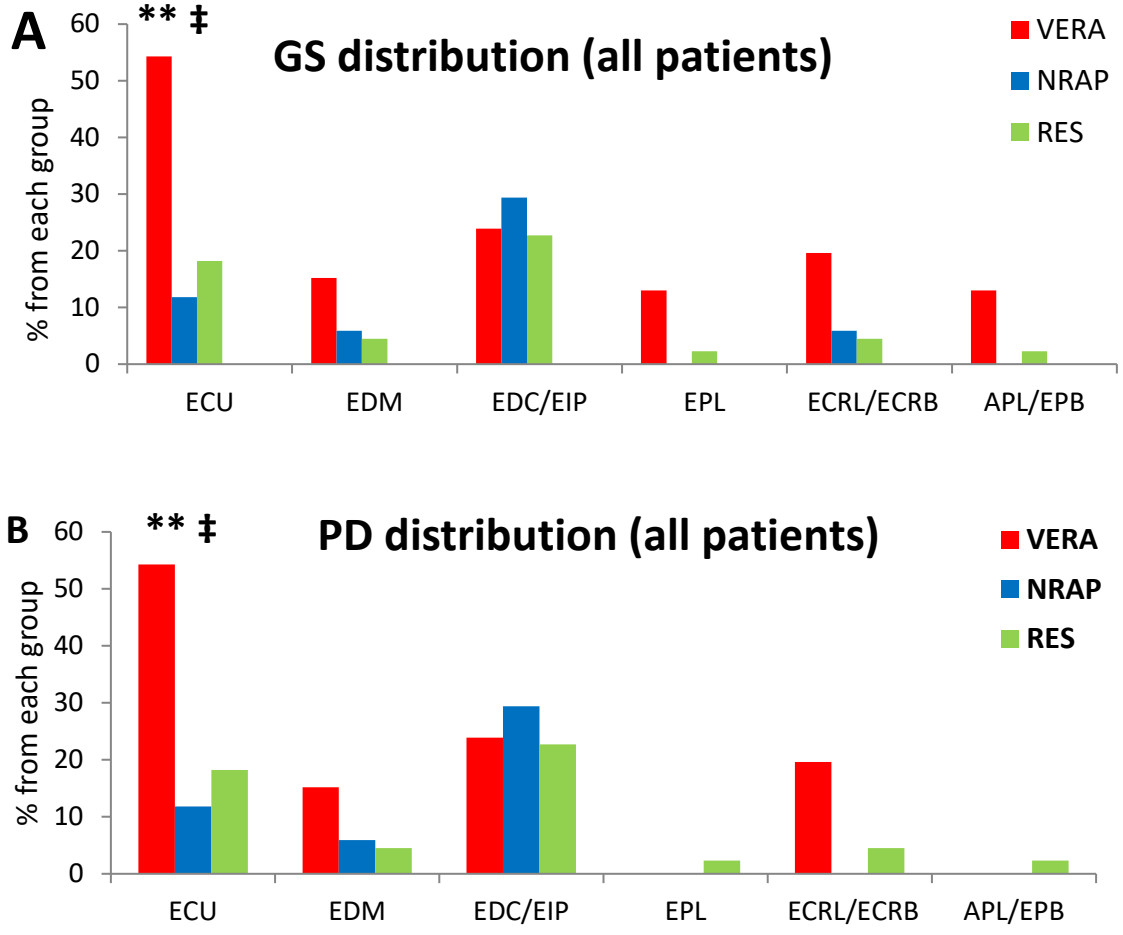


Figure 3-4 Distribution of tendon US pathology by wrist tendon compartment in all patients. Each bar represents the proportion of patients US-defined tenosynovitis involvement according to (A) and (B) wrist extensor compartments. P<0.017 (i.e. 0.05/3) was considered statistically significant as we adjusted for multiple comparisons using the Bonferroni method.VERA vs NRAP : † p≤0.017, ‡ p≤0.001. VERA vs RES *p≤0.017, **p≤0.001. APL: Abductor pollicis longus, EPB: extensor pollicis brevis, ECRL: extensor carpi radialis longus. ECRB: Extensor carpi radialis brevis. EPL: Extensor pollicis longus. EDC: extensor digitorum communis. EIP: Extensor indicis propius. EDM: Extensor digiti minimi. ECU: extensor carpi ulnaris.

US-defined disease across the six wrist extensor compartments is presented in Figure 3-4. Amongst the wrist extensor tendon compartments, US-defined extensor carpi ulnaris (ECU) TS was more prevalent in RA patients compared to both patients with resolving arthritis and non-RA patients. This was true for both GS and PD.

The distribution of US-defined TS by tendon region for seronegative patients is presented in Figure 3-5 US-defined digit flexor GS and PD TS were more prevalent in the RA group compared to the resolving arthritis group.

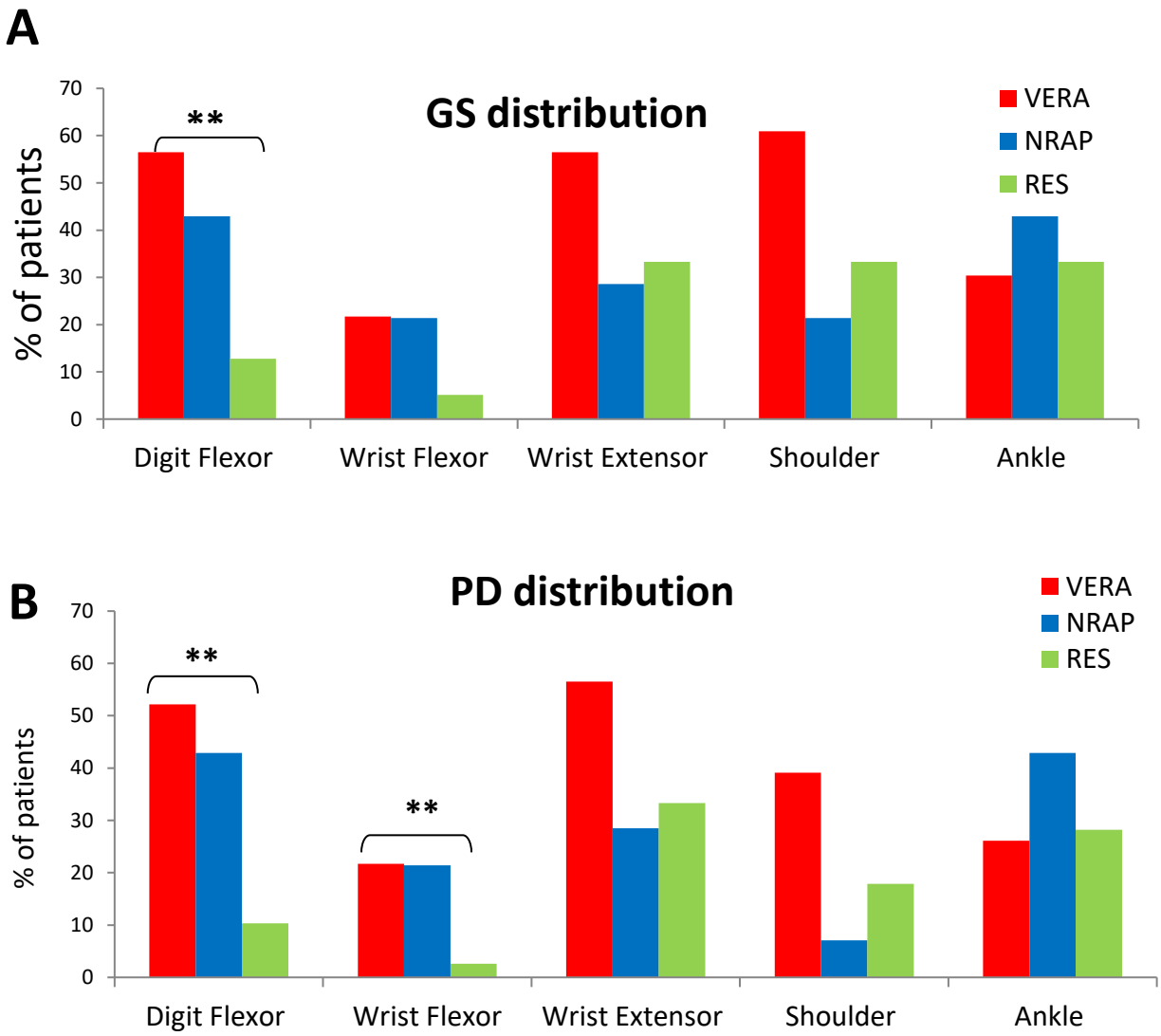
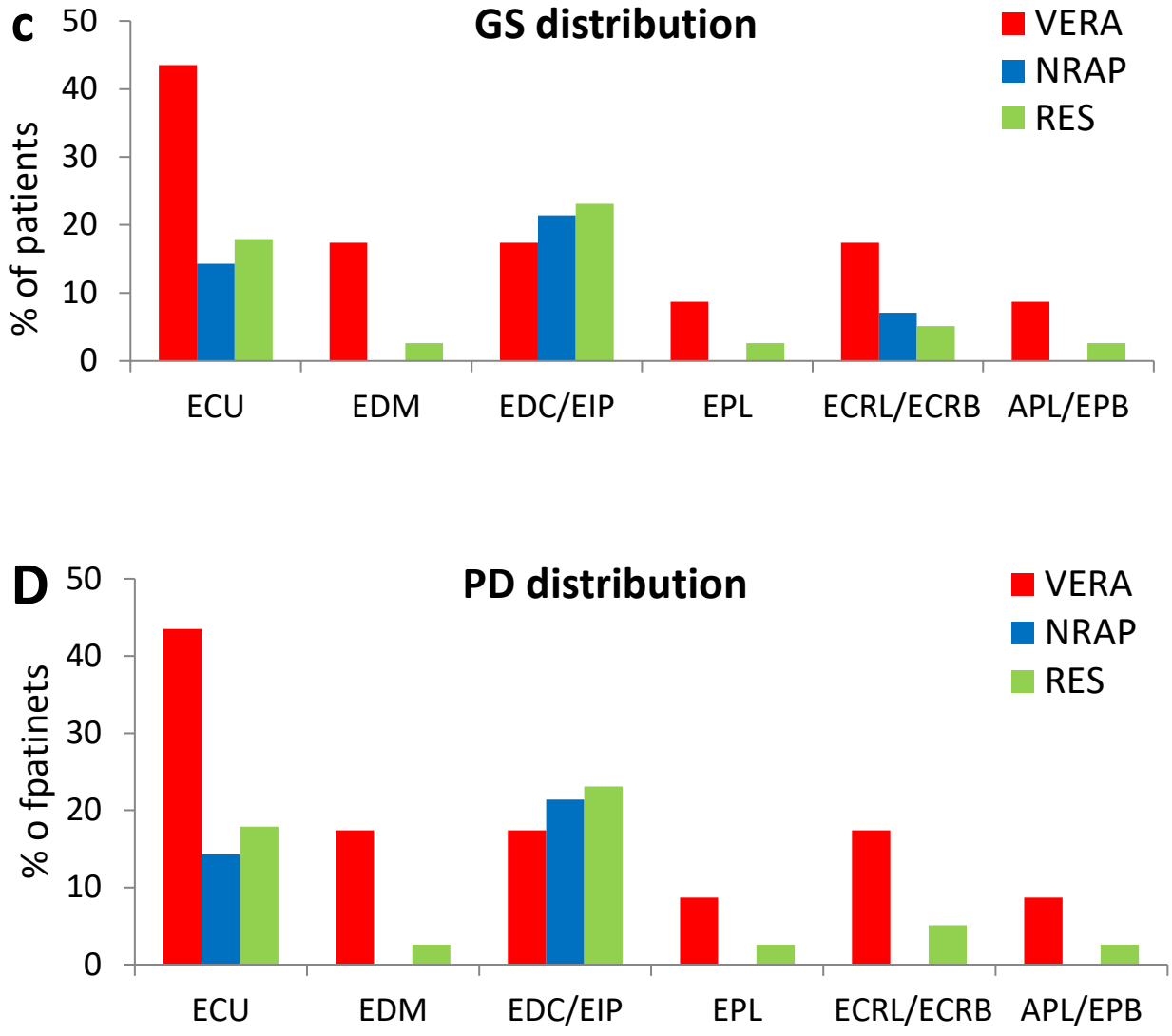


Figure 3-5 Distribution of tendon US pathology in seronegative patients. Each bar represents the proportion of patients who had US-defined tenosynovitis involvement according to tendon regions. P ≤ 0.017 (i.e. 0.05/3) was considered statistically significant as we adjusted for multiple comparisons using the Bonferroni method. VERA vs RES *p≤0.017, **p≤0.001.

Figure 3-6 shows the distribution of wrist tendon compartment US pathology in seronegative patients. There was no statistical difference between any of the groups in all tendon wrist compartments.

Figure 3-6 Distribution of wrist tendon compartment US pathology in seronegative patients.



Each bar represents the proportion of patients who had US-defined tenosynovitis involvement according to wrist extensor compartments. APL: Abductor pollicis longus, EPB: extensor pollcis brevis, ECRL: extensor carpi radialis longus. ECRB: Extensor carpi radialis brevis. EPL: Extensor pollicis longus. EDC: extensor digitorum communis. EIP: Extensor indicis propius. EDM: Extensor digiti minimi. ECU: extensor carpi ulnaris.

3.4.5 Univariate analysis of clinical and serological variables.

Univariate logistic regression analysis was performed with the clinical, serological and US predictors as independent variables, and RA versus non-RA outcome at 18 months as the dependent variable.

The clinical and serological predictors of RA for all patients on univariate analysis are shown in Table 3-10. Age ≥ 60 years, early morning stiffness duration of ≥ 60 minutes, swollen joint count-66 and tender joint count-68 ≥ 6 and symptom duration ≥ 6 weeks were significant predictors of RA on univariate analysis. In addition, RF and ACPA antibodies were also significant predictors of RA development on univariate analysis. The remaining clinical and serological variables were not predictive of RA on univariate analysis.

The clinical and serological predictors of seronegative RA on univariate analysis are shown in Table 3-11. In seronegative patients, only age ≥ 60 years, early morning stiffness duration of 60 minutes or more and swollen joint count-66 ≥ 6 were predictors of seronegative RA.

Table 3-10 Univariate analyses of clinical and serological variables at baseline for all patients in the prediction of RA.

Clinical variables	Odds Ratio	95% CI	p value
Age ≥ 60 years *	3.662	1.595 - 8.408	0.002
Age < 60 years	0.273	0.119 - 0.627	0.002
Female	1.320	0.610 - 2.855	0.481
Tender joint count: 1 joint	0.091	0.011 - 0.726	0.024
Swollen joint count: 1 joint	0.100	0.022 - 0.458	0.025
Tender joint count: 2-5 joints	0.897	0.393 - 2.046	0.796
Swollen joint count: 2-5 joints	0.969	0.448 - 2.095	0.936
Swollen joint count: ≥ 6 joints*	3.662	1.595 - 8.408	0.002
Tender joint count: ≥ 6 joints*	2.456	1.119 - 5.394	0.025
Early morning stiffness duration ≥ 60 mins*	3.972	1.677 - 9.408	0.002
Symptom duration ≥ 6 weeks*	2.878	1.286 - 6.445	0.010
Presence of radiographic erosion	0.589	0.052-6.710	0.670
Serological variables	**Odds Ratio**	**95% CI**	**p value**
Abnormal CRP	1.552	0.655 - 3.679	0.318
Abnormal ESR	1.341	0.601 - 2.991	0.474
ACPA positivity*	10.962	3.404 - 35.298	0.000
ACPA high-positivity*	9.161	2.832 - 29.635	0.000
RF positivity*	10.267	3.478 - 30.304	0.000
RF high-positivity*	17.293	3.740 - 79.951	0.000

**Denotes statistical significance at 0.05 level.*

Table 3-11 Univariate analyses of clinical and serological variables at baseline for seronegative patients in the prediction of RA.

Clinical and serological variables			
Clinical variables	**OR**	**95% CI**	**p value**
Age ≥ 60 years old*	3.727	1.316-10.553	0.013
Age < 60 years old	0.268	0.095-0.760	0.013
Female	0.651	0.243-1.740	0.392
Tender joint count: 1 joint	0.000	0.000-	0.999
Swollen joint count: 1 joint	0.000	0.000-	0.998
Tender joint count: 2-5 joints	1.250	0.454-3.439	0.666
Swollen joint count: 2-5 joints	1.292	0.483-3.455	0.610
Tender joint count: ≥ 6 joints	2.029	0.748-5.505	0.165
Swollen joint count: ≥ 6 joint*	3.727	1.316-10.553	0.013
Early morning stiffness duration ≥ 60 mins*	3.738	1.210-11.547	0.022
Symptom duration ≥ 6 weeks	2.266	0.822-6.247	0.114
Serological variables	**OR**	**95% CI**	**p value**
Abnormal CRP	1.338	0.448-3.999	0.602
Abnormal ESR	1.048	0.376-2.921	0.929

*Denotes statistical significance at 0.05 level.

3.4.6 Univariate analysis of joint US variables.

Table 3-12 shows the univariate analysis of the prediction of RA for all patients. GS and PD US synovitis of the MCP 1-5, PIP 1-5, wrist, MTP 3, and 5 joints were predictors of RA. In addition, MTP 2 PD joint synovitis, but not GS synovitis alone, was a predictor of RA.

Table 3-13 shows the univariate analysis of the prediction of seronegative RA. The GS joint US variables predictive of seronegative RA were MCP 1, 3, 4, 5, PIP 1, 5 and MTP 5. The PD joint US variables predictive of seronegative RA were MCP 1, 3, 5, PIP 1, 2, 3, 4 and MTP 2.

Table 3-12 Univariate analysis of joint US variables for all patients for RA prediction.

Joint US variables[a]	Odds Ratio (OR)	95% CI for OR	p value
MCP 1 GS*	5.349	2.326-12.299	0.000
MCP 1 PD*	6.966	2.918-16.627	0.000
MCP 2 GS*	4.243	1.790-10.055	0.001
MCP 2 PD*	4.194	1.839-9.567	0.001
MCP 3 GS*	6.338	2.599-15.455	0.000
MCP 3 PD*	7.333	3.091-17.398	0.000
MCP 4 GS*	4.770	2.078-10.949	0.000
MCP 4 PD*	3.818	1.594-9.144	0.003
MCP 5 GS*	3.997	1.739 – 9.186	0.001
MCP 5 PD*	5.565	2.167 – 14.289	0.000
PIP 1 GS*	6.566	2.200 - 19.592	0.001
PIP 1 PD*	4.900	1.615 - 14.863	0.005
PIP 2 GS*	5.308	2.248- 12.535	0.000
PIP 2 PD*	6.630	2.712 – 16.210	0.000
PIP 3 GS*	3.067	1.350 – 6.968	0.007
PIP 3 PD*	3.497	1.457 – 8.389	0.005
PIP 4 GS*	4.114	1.523 – 11.117	0.005
PIP 4 PD*	4.010	1.402-11.471	0.010
PIP 5 GS*	10.311	2.783-38.197	0.000
PIP 5 PD*	9.355	2.514-34.811	0.001
Wrist GS*	4.963	1.714-14.369	0.003
Wrist PD*	6.042	2.235-16.331	0.000
Shoulder GS	1.876	0.642-5.485	0.250
Shoulder PD	NA	NA	NA
Elbow GS	2.190	0.986-4.866	0.054
Elbow PD*	2.394	1.003-5.714	0.049
Ankle GS	1.546	0.708-3.378	0.275
Ankle PD	1.421	0.637-3.171	0.391
Knee GS	2.149	0.843-5.476	0.109
Knee PD	1.204	0.559 – 2.590	0.635
MTP 2 GS	1.967	0.904-4.280	0.088
MTP 2 PD*	5.029	1.502-16.844	0.009
MTP 3 GS*	3.077	1.340-7.065	0.008
MTP 3 PD*	8.194	1.698-39.536	0.009
MTP 4 GS	2.158	0.944-4.935	0.068
MTP 4 PD	2.358	0.717 – 7.757	0.158
MTP 5 GS*	7.600	2.332-24.770	0.001
MTP 5 PD*	6.895	2.105-22.586	0.001

[a]GS grading ≥ 1; PD grading ≥ 1; US pathology was present in at least unilateral joint.

**denotes statistical significant at 0.05 level.*

Table 3-13 Univariate analysis of joint US variables of seronegative patients for RA prediction

Joint US variables[a]	Odds Ratio	95% CI for OR	p value
MCP 1 GS*	3.294	1.192 - 9.106	0.022
MCP 1 PD*	4.442	1.561 - 12.638	0.005
MCP 2 GS	2.762	0.976 - 7.813	0.056
MCP 2 PD	2.567	0.940 - 7.011	0.066
MCP 3 GS*	3.221	1.135 - 9.138	0.028
MCP 3 PD*	4.336	1.534 - 12.259	0.006
MCP 4 GS*	3.621	1.298 - 10.103	0.014
MCP 4 PD	2.455	0.843 - 7.146	0.100
MCP 5 GS*	3.132	1.106- 8.868	0.032
MCP 5 PD*	4.327	1.418 - 13.207	0.010
PIP 1 GS*	4.200	1.168 - 15.099	0.028
PIP 1 PD*	4.200	1.168 15.099	0.028
PIP 2 GS	2.764	0.935 - 8.171	0.066
PIP 2 PD*	3.143	1.044 - 9.465	0.042
PIP 3 GS	2.367	0.841 - 6.663	0.103
PIP 3 PD	3.761	1.261 - 11.214	0.017
PIP 4 GS	1.825	0.512 - 6.503	0.353
PIP 4 PD	1.175	0.267 - 5.169	0.831
PIP 5 GS	4.630	1.003 - 21.367	0.050
PIP 5 PD	4.630	1.003 - 21.367	0.050
Wrist GS	2.362	0.761 - 7.339	0.137
Wrist PD*	2.728	0.931 - 7.996	0.067
Shoulder GS	0.746	0.139 - 4.007	0.733
Shoulder PD	NA	NA	NA
Elbow GS	1.782	0.658 - 4.827	0.256
Elbow PD	2.196	0.764 - 6.314	0.144
Ankle GS	1.287	0.475 - 3.488	0.619
Ankle PD	1.250	0.454 - 3.439	0.666
Knee GS	2.883	0.749 - 11.096	0.124
Knee PD	1.108	0.415 - 2.953	0.838
MTP 2 GS	1.512	0.563 - 4.066	0.412
MTP 2 PD*	4.324	1.087 - 17.189	0.038
MTP 3 GS	1.346	0.454 - 3.990	0.592
MTP 3 PD	3.825	0.594 - 24.630	0.158
MTP 4 GS	1.346	0.454 - 3.990	0.592
MTP 4 PD	2.021	0.489 - 8.345	0.331
MTP 5 GS*	4.324	1.087 17.189	0.038
MTP 5 PD	3.403	0.821 - 14.098	0.091

**GS grading ≥ 1; PD grading ≥ 1; US pathology was present in at least unilateral joint.*

3.4.7 Univariate analysis of tendon US variables.

Table 3-14 shows the univariate analysis of tendon compartment TS at baseline in the prediction of RA for all patients. US-defined digit flexor and wrist ECU were predictive of RA. The predictive ability of GS and PD variables for each tendon compartment was comparable. In addition shoulder biceps GS was also predictive of RA.

Table 3-14 Univariate analysis of tendon compartment TS at baseline for all patients in the prediction of RA

Tendon Compartment	Odds Ratio (OR)	95% CI for OR	p
Wrist ECU GS*	6.07	2.49-14.82	0.000
Wrist ECU PD*	6.07	2.49-14.82	0.000
Digit Flexor GS*	4.46	1.89-10.49	0.001
Digit Flexor PD*	4.55	1.90-10.87	0.001
Wrist Extensor GS	2.27	1.04-4.97	0.041
Wrist Extensor PD*	2.84	1.28-6.33	0.010
Wrist Flexor GS	2.23	0.73-6.80	0.158
Wrist Flexor PD	2.72	0.85-8.77	0.093
Shoulder Biceps GS*	3.35	1.47-7.605	0.004
Shoulder Biceps PD	2.80	1.09-7.15	0.032
Ankle Extensor GS	0.87	0.29-2.64	0.801
Ankle Extensor PD	0.71	0.22-2.26	0.557
Ankle Posterior Tibialis GS	1.42	0.64-3.17	0.391
Ankle Posterior Tibialis PD	1.50	0.65-3.45	0.340
Ankle Peroneal GS	2.42	0.86-6.85	0.095
Ankle Peroneal PD	2.42	0.86-6.85	0.095

**Denotes statistical significant at 0.05 level.*

Table 3-15 shows the univariate analysis of tendon compartment TS for the prediction of seronegative RA. Digit flexor and ECU remained as predictors of seronegative RA. The predictive ability of GS and PD for each tendon compartment was also comparable.

Table 3-15 Univariate of tendon compartment TS at baseline in seronegative patients for the prediction of RA.

Tendon Compartment	OR	95% CI	p
ECU GS*	3.76	1.26-11.21	0.017
ECU PD*	3.76	1.26-11.21	0.017
Digit Flexor GS*	4.97	1.72-14.30	0.003
Digit Flexor PD*	4.69	1.61-13.66	0.005
Wrist Extensor GS	1.94	0.71-5.28	0.194
Wrist Extensor PD	2.55	0.92-7.09	0.072
Wrist Flexor GS	2.67	0.69-10.32	0.155
Wrist Flexor PD	3.40	0.82-14.10	0.091
Shoulder Biceps GS*	4.79	1.68-13.61	0.003
Shoulder Biceps PD*	3.62	1.17-11.14	0.025
Ankle Extensor GS	0.536	0.11-2.75	0.454
Ankle Extensor PD	0.536	0.11-2.75	0.454
Ankle Posterior Tibialis GS	0.642	0.20-2.03	0.451
Ankle Posterior Tibialis PD	0.648	0.19-2.25	0.495
Ankle Peroneal GS	0.299	0.035-2.58	0.272
Ankle Peroneal PD	0.299	0.035-2.58	0.272

**Denotes statistical significant at 0.05 level.*

3.4.8 Principal component analysis

In this step, statistically significant variables from the univariate analysis were included in PCA analyses in order to identify the key variables that would account for the majority of the

explanatory variance observed as well as to identify the US variables that highly correlated with each other, in particular whether the joint and tendon US variables are clustered within the same component.

Two PCA analyses were performed; one for US and one for clinical and serological variables. The number of components extracted was based on eigenvalues with a cut-off of one and the rotation method adopted was according to the Varimax criteria with Kaiser Normalization.

The rotated factor loadings of the PCA for each clinical, serological and US variables are shown in Table 3-16 and Table 3-17. Three components were extracted from the clinical and serological PCA, whilst nine components were extracted from the joint and tendon US PCA Table 3-18 .

Table 3-16 Principal component analysis of clinical and serological variables for all patients

Clinical and serological variables	Component		
	1	2	3
ACPA low-positive§	0.925		
ACPA high-positive§§	0.912		
RF low-positive†	0.873		
RF high-positive††	0.864		
Swollen joint count ≥ 6		0.838	
Tender joint count ≥ 6		0.761	
Early morning stiffness duration ≥ 60 mins		0.616	
Symptom duration ≥ 6 weeks			0.783
Age ≥ 60 years old			0.744

Rotation method: Varimax with Kaiser Normalization. Factor loadings of <0.400 are suppressed to facilitate interpretation. The variable with the highest loading factor from each component is highlighted. §ACPA >7 EU/ml, §§ACPA >21 EU/ml, † RF > 20 IU/mL, †† RF > 60 IU/mL.

Table 3-17 Principal component analysis of US variables for all patients.

US variables	Component								
	1	2	3	4	5	6	7	8	9
MCP 2 PD	0.791								
MCP 3 PD	0.774								
MCP 3 GS	0.755								
MCP 1 GS	0.746								
MCP 1 PD	0.731								
MCP 4 GS	0.727								
MCP 2 GS	0.711								
MCP 4 PD	0.702								
PIP 2 GS		0.767							
PIP 3 GS		0.757							
PIP 2 PD		0.755							
PIP 5 GS		0.742							
PIP 3 PD		0.734							
PIP 5 PD		0.726							
PIP 4 PD		0.588	0.451						
PIP 4 GS		0.527	0.470						
PIP 1 PD			0.845						
PIP 1 GS			0.809						
Digit flexor tendon GS				0.850					
Digit flexor tendon PD				0.848					
MTP 3 PD					0.781				
MTP 2 PD					0.750				
MTP 3 GS					0.669				
ECU tendon PD						0.813			
ECU tendon GS						0.813			
Shoulder tendon PD						0.567			
Shoulder tendon GS						0.542			
MTP 5 PD							0.897		
MTP 5 GS							0.871		
WRIST GS								0.882	
WRIST PD								0.834	
MCP 5 GS									0.722
MCP 5 PD	.412								0.688

Rotation method: Varimax with Kaiser Normalization. Factor loadings of <0.400 are suppressed to facilitate interpretation. The variable with the highest loading factor from each component is highlighted.

Table 3-18 Components from the clinical, serological and US PCA.

PCA of clinical and serological variables

Components	1	2	3
Variables	ACPA positivity ACPA high-positivity RF positivity RF high-positivity	Swollen joint count-66 ≥ 6 Tender joint count-68 ≥ 6 Early morning stiffness duration ≥ 60 mins	Symptom duration ≥ 6 weeks Age ≥ 60 years old
% of variance explained	38.25	17.87	12.30

Cumulative % of variance explained = 68.41

PCA of US variables

Components	1	2	3	4	5	6	7	8	9
Variables	MCP1 MCP2 MCP3 MCP4	PIP2 PIP3 PIP4 PIP5	PIP1 PIP4	Digit Flexor	MTP2 MTP3	ECU Shoulder tendon	MTP 5	Wrist joint	MCP 5
% of variance explained	38.01	8.54	7.21	5.84	5.29	4.26	3.97	3.69	3.12

Cumulative % of variance explained = 79.93

3.4.9 Multi-variate logistic regression

In the final step, multiple logistic regression analyses were performed to identify the relative independent predictive ability of these variables. The variable with the highest loading factor from each component was extracted and made available as an independent variable in a forward step-wise multivariate logistic regression analysis, with RA outcome at 18 months entered as the dependent variable. These variables are listed in Table 3-19. This logistic regression analysis identified PIP1 PD, digit flexor GS and ACPA positivity as the variables which formed the final model in the prediction of RA, with the proportion of RA vs. non-RA correctly identified as 75.7% (Table 3-20).

In order to identify the combination of variables with the greatest potential to predict persistent RA, and in particular whether tendon US data enhanced predictive value, I systematically examined the impact of individual joint US variables in an exhaustive logistic regression model Table 3-20.

PIP 1 PD, ACPA and DF tendon formed the final model when all variables in Table 3.19 was made available in a forward step-wise multivariate logistic regression analysis. I then substituted PIP 1 GS variable and replaced it with one other joint variable in the same model to test whether DF tendon still provided independent predictor of RA. These results are shown in Table 3-20.

Amongst all the combination of variables, MCP3 PD, ACPA and DF tendon provided the highest predictive value, based on the highest proportion of patients correctly identified as RA vs non RA (Table 3-21).

In the model shown in Table 3-21, removing the digit flexor tendon from this model would result a lower overall accuracy from 80.4% to 73.8%. (i.e. % of patients correctly identified as RA vs. non-RA).

In other words, scanning the digit flexor tendon would increase the overall accuracy of classification by 6.6 out 100 patients, compared to that of by checking ACPA and scanning MCP 3 PD only. This accuracy figure is provided in a classification table alongside the regression equation when SPSS create the model. This figure is used to assess how well the model predicts the correct diagnosis, which in this case is RA development at 18 months. The classification criteria cut-off used in SPSS is 0.5 (i.e. default in SPSS).

Table 3-19 Variables included in logistic regression model

Clinical and serological variables	US variables
ACPA positivity	MCP 2 PD
Swollen joint count-66 ≥ 6	PIP 2 GS
Symptom duration ≥ 6 weeks	PIP 1 PD
	MTP 3 PD
	MTP 5 PD
	WRIST GS
	MCP 5 GS
	Digit flexor tendon GS
	ECU tendon PD

Table 3-20 Multi-variate regression of joint US variable with tendon US.

Combination of variable	Variable added	Nagelkerke R2	% patients correctly identified (VERA vs. non-VERA)
ACPA positivity DF tendon	GS positivity of MCP 1	0.464	78.5
ACPA positivity DF tendon	GS positivity of MCP 2	NS	NS
ACPA positivity DF tendon	GS positivity of MCP 3	0.424	79.4
ACPA positivity DF tendon	GS positivity of MCP 4	0.425	77.6
ACPA positivity DF tendon	GS positivity of MCP 5	0.417	77.6
ACPA positivity DF tendon	GS positivity of one or more MCP 1-5	0.451	78.5
ACPA positivity DF tendon	PD positivity of MCP 1	0.477	79.4
ACPA positivity DF tendon	PD positivity of MCP 2	NS	NS
ACPA positivity DF tendon	PD positivity of MCP 3	0.439	80.4
ACPA positivity DF tendon	PD positivity of MCP 4	NS	NS
ACPA positivity DF tendon	PD positivity of MCP 5	0.435	77.6
ACPA positivity DF tendon	PD positivity of one or more of MCP 1-5	0.463	78.5

Combination of variable	Variable added	Nagelkerke R2	% of patients correctly identified (VERA vs. non-VERA)
ACPA positivity DF tendon	GS positivity of PIP 1	0.436	75.7
ACPA positivity DF tendon	GS positivity of PIP 2	NS	NS
ACPA positivity DF tendon	GS positivity of PIP 3	NS	NS
ACPA positivity DF tendon	GS positivity of PIP 4	NS	NS
ACPA positivity DF tendon	GS positivity of PIP 5	NS	NS
ACPA positivity DF tendon	GS positivity of one or more PIP 1-5	0.415	78.5
ACPA positivity DF tendon	PD positivity of PIP 1	0.424	75.7
ACPA positivity DF tendon	PD positivity of PIP 2	0.415	77.6
ACPA positivity DF tendon	PD positivity of PIP 3	NS	NS
ACPA positivity DF tendon	PD positivity of PIP 4	NS	NS
ACPA positivity DF tendon	PD positivity of PIP 5	0.438	76.6
ACPA positivity DF tendon	PD positivity of one or more PIP 1-5	0.418	78.5

Combination of variable	Variable added	Nagelkerke R2	% of patients correctly identified (VERA vs. non-VERA)
ACPA positivity DF tendon	GS positivity of MTP 2	NS	NS
ACPA positivity DF tendon	GS positivity of MTP 3	NS	NS
ACPA positivity DF tendon	GS positivity of MTP 4	NS	NS
ACPA positivity DF tendon	GS positivity of MTP 5	0.436	75.7
ACPA positivity DF tendon	GS positivity of one or more MTP 2-5	NS	NS
ACPA positivity DF tendon	PD positivity of MTP 2	0.419	75.7
ACPA positivity DF tendon	PD positivity of MTP 3	NS	NS
ACPA positivity DF tendon	PD positivity of MTP 4	NS	NS
ACPA positivity DF tendon	PD positivity of MTP 5	0.418	76.6
ACPA positivity DF tendon	PD positivity of one or more MTP 2-5	NS	NS

Table 3-21 Logistic regression model.

Variable	OR	95% CI	p-value	Nagelkerke R^2	% of patients correctly identified (VERA vs. non-VERA)
ACPA positivity	10.973	3.031-39.730	0.000	0.439	80.4
MCP 3 PD positivity	4.066	1.444-11.444	0.008		
Digit Flexor tendon GS	3.078	1.047-9.046	0.041		
Variable	**OR**	**95% CI**	**p-value**	**Nagelkerke R^2**	**% correctly identified**
ACPA positivity	9.324	2.648-32.832	0.001	0.402	73.8
MCP 3 PD positivity	6.451	2.525-16.482	0.000		

3.5 Discussion; Prediction of RA.

Previous studies have reported that US-defined joint synovitis improves the prediction of RA above and beyond clinical and serological variables in early arthritis patients (106) and also improves the prediction of RA in seronegative unclassified arthritis patients (127).

In this study, I showed that US-defined TS, specifically digit flexor TS, provides additional predictive data alongside US-defined joint synovitis and other clinical and serological variables in a cohort of patients with early arthritis.

These findings are consistent with studies of gadolinium-enhanced MRI, in which digit flexor TS was a significant predictor of early RA in a cohort of patients with undifferentiated arthritis or clinically suspected RA with no joint swelling (167).

In agreement with our data, the authors concluded that MRI-defined digit flexor TS provided additional predictive data for patients in their cohort even in the presence of ACPA or RF. In addition, longitudinal data from the Leiden Early Arthritis Clinic showed that MRI-defined TS of the 5th ray flexor tendons was more common in early arthritis patients who later developed RA compared to those who did not (164).

Grassi et. al first described sonographic changes affecting the hand flexor tendon in RA patients. The authors reported that 90% (18/20) of RA patients had sonographic changes at either digit flexor and/or extensor tendons (168). Subsequent US studies described the distribution of tendon involvement in the hands and/or wrists of RA patients (169, 170).

This analysis is the first to describe the distribution of US-defined TS of multiple tendon sites, including the shoulder and ankle regions, in early arthritis. In addition, this study includes the most extensive US assessment to date, including the MCP, PIP, wrist, MTP, knee, ankle, elbow joints and digit, wrist, shoulder and ankle tendons.

One of the main challenges in US studies is identifying the minimal joint, or tendon, subset that will provide the maximal predictive ability for a given outcome (147). I undertook a PCA of joint and tendon US variables to identify overlapping US variables within a given patient population.

Importantly, in this study I showed that tendon US variables were not redundant with their corresponding regional joint US variables. For example, digit flexor tendon US variables were not placed within the same component as small joint synovitis variables of MCP or PIP joints

on PCA analysis. Similarly, wrist ECU tendon involvement did not share the same component as the wrist joint US variable. These key findings, which are reported for the first time in an early arthritis US study, suggest that tendon US variables provide additional predictive value alongside joint US variables in the context of early arthritis.

One of the strengths of this study is that it was undertaken prospectively in a real world setting. Consecutive patients were recruited from well-established Rheumatology Centres in the UK that had a wide catchment area. Patients from our cohort also had very short symptom durations with median symptom durations of between five to seven weeks. These findings suggest that US-detected TS alongside US-detected joint synovitis is a reliable imaging biomarker in the very early phase of arthritis, falling within the proposed 12 week therapeutic window of opportunity of early arthritis. (80)

Whilst several studies have assessed the tenosynovium in patients with RA compared with healthy controls, (163, 168) an additional strength of this study is that I assessed the predictive utility of TS assessment in a clinically meaningful context of an unselected early arthritis cohort. The comparator groups are patients with resolving and non-RA disease - patients frequently seen in early arthritis clinics and in relation to which management decisions have to be made on the basis of prediction of future outcomes.

This predictive study (chapter 3) was undertaken and published prior to the completion of the chapter 2 of this thesis where the threshold of normality of US was defined according to joint level and age group. In this predictive analysis, all US pathology of grade ≥ 1 or more was considered abnormal regardless of joint level and age. What would be interesting is to

assess whether setting the binary cut-off based on the threshold of normality reported in chapter 2 would change the overall predictive value of ultrasound. The main limitation of this study relates to the relatively small size of this initial cohort, necessitated by the extensive imaging performed per patient. A larger sample size is required in order to design weighted predictive algorithms and identify specific domains such as individual flexor tendons that provide the most useful predictive data in order to reduce scanning time.

Previous imaging studies illustrated that gadolinium enhanced MRI-defined digit flexor TS is an independent predictor of RA. My findings demonstrate similar findings for US, a more accessible point of care imaging tool. These data show that US-defined digit flexor TS provides independent predictive value for RA development in early arthritis patients. This finding should be further evaluated in a larger study and investigators testing imaging-based variables to within predictive algorithms for RA development should consider including this tendon component as a candidate variable.

3.6 Results; Prediction of persistent arthritis.

3.6.1 Demographic and disease characteristics.

150 patients were included in this analysis. At 18 months 100 (67%) had persistent arthritis and the remaining 50 patients (33%) had resolving disease. Baseline characteristics by prognostic outcomes are shown in Table 3-22. Patient with persistent arthritis were more likely to be older. In addition, persistent arthritis patients had longer symptom and early morning stiffness duration. They also had higher proportions with RF and ACPA antibodies and higher tender and swollen joint count. There were no difference in sex, mode of onset, NSAID use and inflammatory markers.

Table 3-22 Baseline characteristics of patients by prognostic outcome groups.

Prognostic outcome	Resolving Inflammatory Arthritis	Persistent Inflammatory Arthritis	p
N	50	100	
Age, years	45 (35-58)	57 (45-66)	0.006[b]
Female, n (%)	30 (60)	55 (55)	0.603[a]
Symptom duration, weeks	4 (5-8)	5 (7-9)	0.009[b]
Early morning stiffness*, mins	30 (0-68)	75 (30-180)	<0.001[b]
ACPA, n (%)			
Negative	48 (96)	64 (64)	
Low positive,	0 (0)	3 (3)	<0.001[a]
High Positive,	2 (4)	33 (33)	
RF, n (%)			
High Positive	2 (4)	26 (26)	<0.001[a]
Low Positive	1 (2)	16 (16)	
Negative	47 (94)	58 (58)	
Mode of onset[ϕ], n (%)			
Acute	36 (72)	60 (60)	
Insidious	9 (18)	32 (32)	0.210[a]
Missing	4 (8)	4 (4)	
NSAID use, n (%)	29 (58)	66 (66)	0.372[a]
ESR* , mm/h	18 (55-33)	23 (10-42)	0.113[b]
CRP* , mg/l	9 (1-26)	15 (5-32)	0.152[b]
Tender joint count of 68**	4 (1-7)	12 (3-19)	<0.001[b]
Swollen joint count of 66	2 (2-6)	6 (3-13)	<0.001[b]
Tender joint count of 28	2 (1-5)	7 (2-14)	<0.001[b]
Swollen joint count of 28	2 (1-4)	5 (2-11)	<0.001[b]
DAS-28 CRP*	3.35 (2.98-4.43)	4.78 (3.62-5.49)	<0.001[b]
DAS-28 ESR[δ]	3.82 (3.01-4.50)	4.91 (4.00-6.15)	<0.001[b]

All variables are shown as median (IQR) unless otherwise specified. [a] Fisher's exact test, [b] Mann-Whitney test, *n=148, **n=147, [ϕ]n=142, [δ]n=137, ACPA, anticyclic citrullinated peptide antibody; CRP, C-reactive protein; DAS28, disease activity score in 28 joints; ESR, erythrocyte sedimentation rate NSAID, non-steroidal anti-inflammatory drug; RF, rheumatoid factor.

Table 3-23 shows the final diagnoses of patients according to prognostic groups. RA patients had the highest proportion in the persistent arthritis group, whilst unclassified arthritis takes up the highest proportion in the resolving arthritis group.

Table 3-23 Final diagnoses according to prognostic outcome groups.

Diagnosis	Persistent Inflammatory Arthritis, n (%)	Resolving Inflammatory Arthritis, n (%)	Total, n
Rheumatoid Arthritis	73 (73)	12 (24)	85
Psoriatic Arthritis	12** (12)	2 (4)	14
Unclassified Arthritis	7 (7)	23 (46)	30
SLE	3 (3)	0 (0)	3
Sarcoidoisis	2 (2)	0 (0)	2
Palindromic Arthritis	1 (1)	0 (0)	1
Ankylosing Spondylitis	1 (1)	0 (0)	1
Peripheral SpA	1 (1)	0 (0)	1
Reactive Arthritis	0 (0)	2 (4)	2
Parvovirus Arthritis	0 (0)	5* (10)	5
Gout	0 (0)	3 (6)	3
Pseudo-gout	0 (0)	2 (4)	2
Septic Arthritis	0 (0)	1 (2)	1
	100 (100)	**50 (100)**	**150**

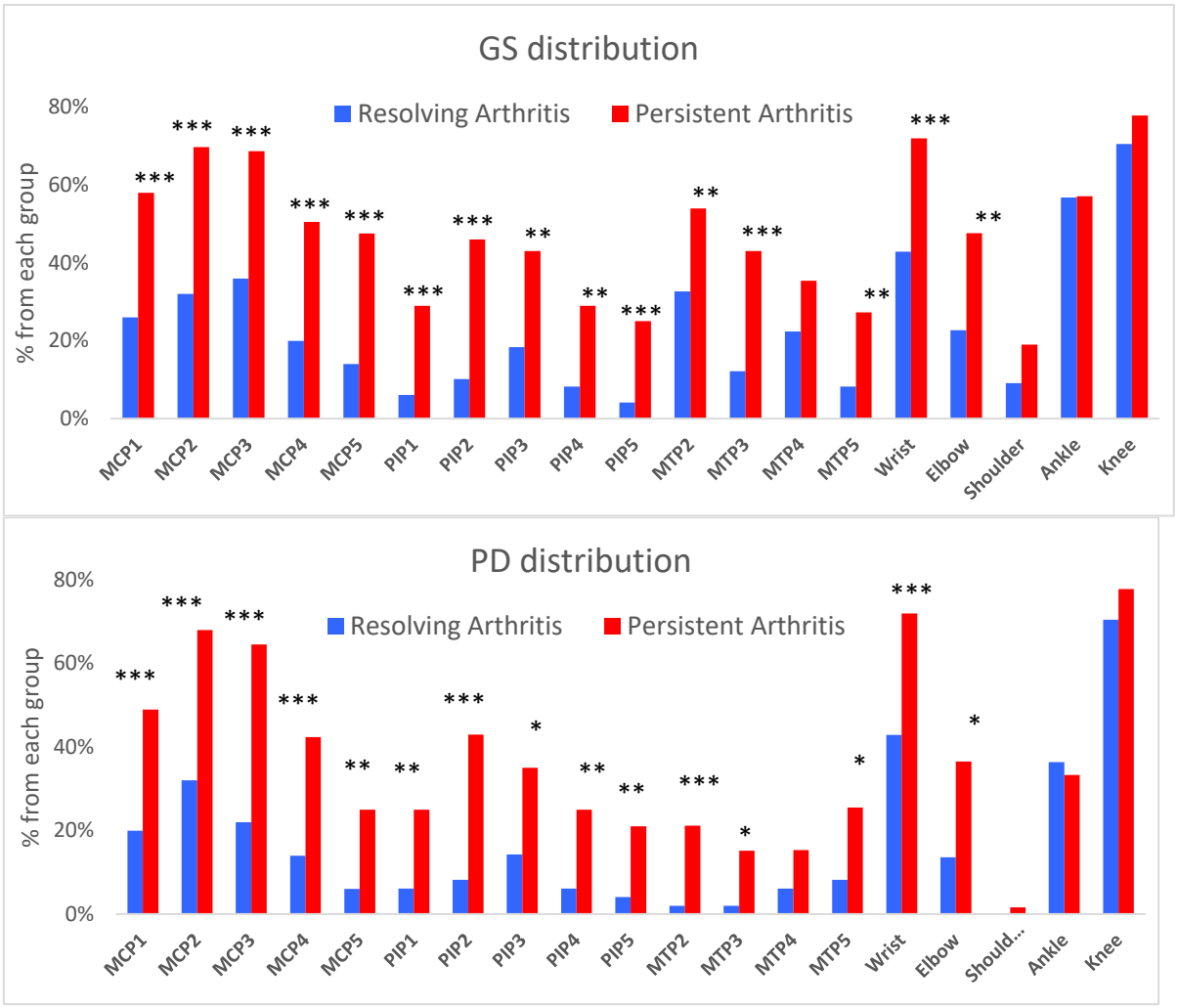
**One patient with parvovirus arthritis was on MTX for a different indication at final follow-up. **One patient with PsA had an initial diagnosis of reactive arthritis secondary to campylobacter at baseline.*

3.6.2 Distribution of joint ultrasound pathology.

GS distribution of joint US pathology is illustrated in Figure 3-7 There was a significant difference between in the distribution of joint US pathology between resolving and persistent arthritis in all joints apart from MTP4, shoulder, ankle and knee. This was true for

both GS and PD distribution. The greatest differences in proportion between persistent and resolving arthritis were MCP2 GS (Δ37.7%) and MCP3 PD (Δ42.2%).

Figure 3-7 Distribution of joint ultrasound pathology (GS and PD).



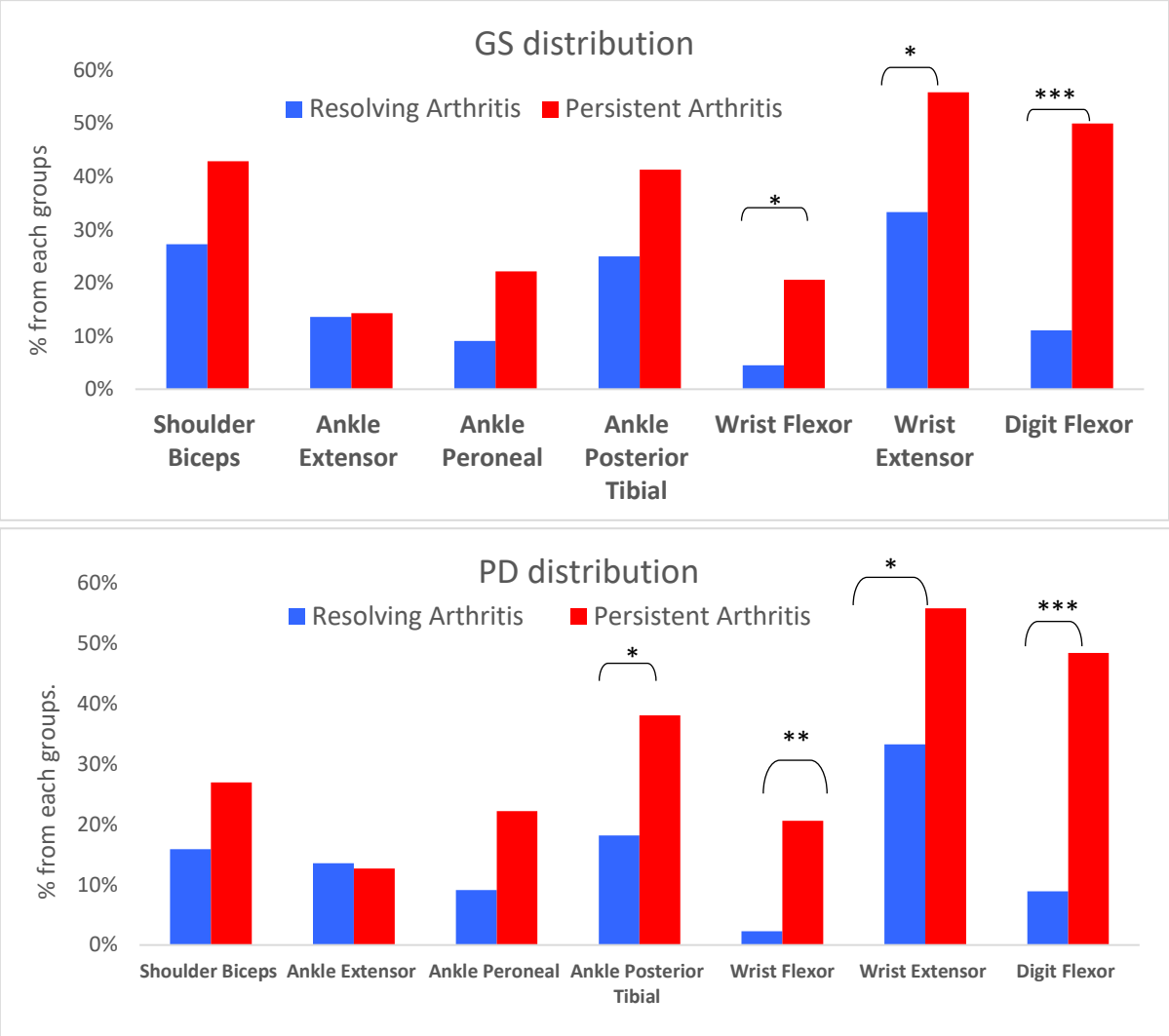
**p≤0.05, **p≤0.01, ***p≤0.001. Each bar represents the proportion of patients with US-defined synovitis involvement according to prognostic outcome groups. Missing data for GS, n=1 for MCP2-5, PIP2-5, MTP2-3, wrist; n=2 for MTP 4-5; n=42 for elbow, shoulder and ankle. Missing data for PD n=1 for MCP 3-4, PIP 1-5 and wrist; n=2 for MTP 2-3; n=3 for MTP4–5; N=43 for elbow, shoulder and ankle.*

3.6.3 Distribution of tendon ultrasound pathology

Distribution of tendon compartment pathology according to prognostic outcome group is shown in Figure 3-8. There was a significant difference in the proportion of US-defined

tenosynovitis in the wrist flexor, wrist extensor and digit flexor between the two prognostic outcome groups for both GS and PD. In addition, PD ankle posterior tibialis was more likely to be observed in the persistent arthritis group compared to the resolving group.

Figure 3-8 Distribution of tendon compartment US pathology (GS and PD).

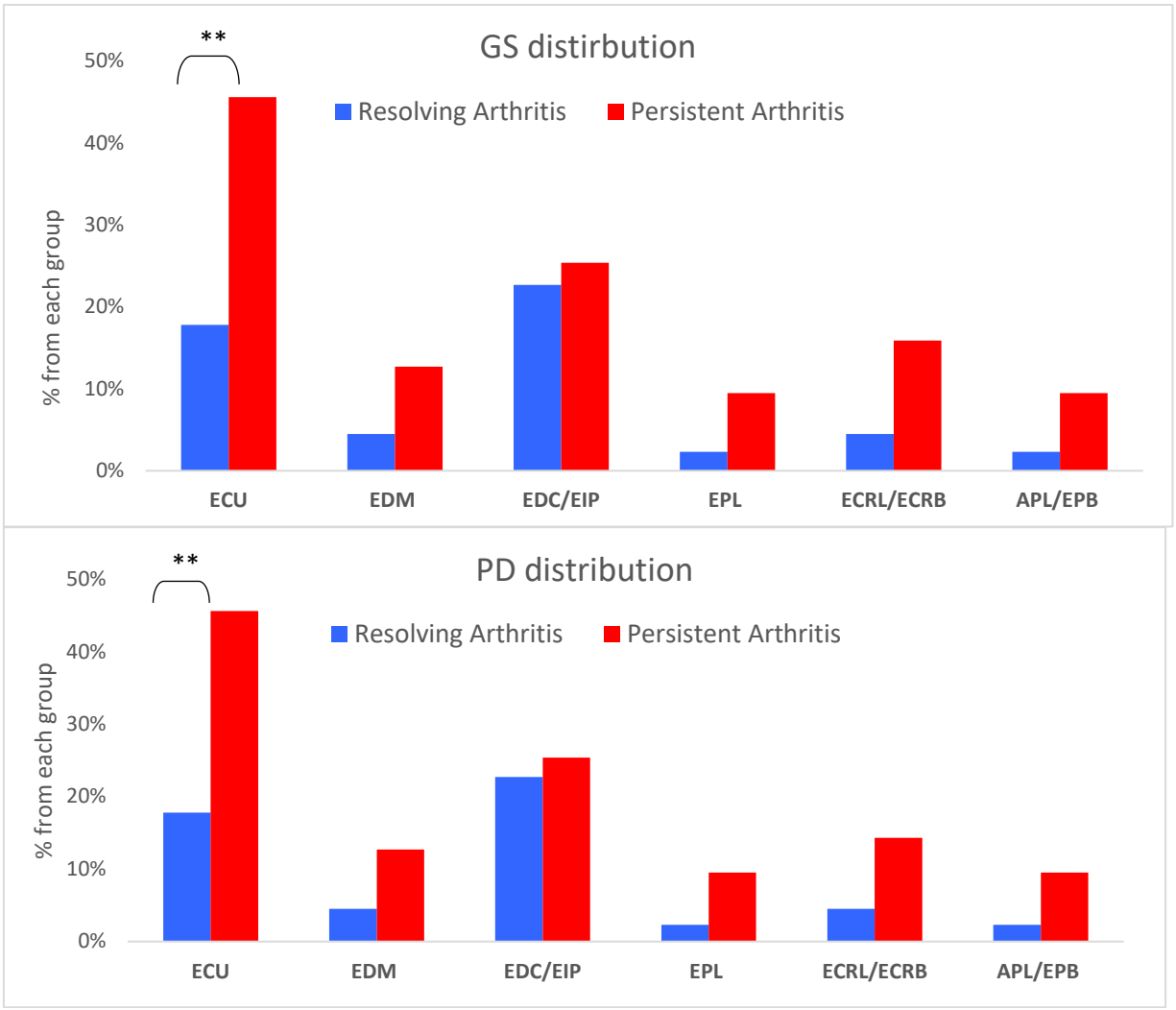


**p≤0.05, **p≤0.01, ***p≤0.001. Missing data n= 37 for wrist extensor, n= 39 for digit flexor, n=43 for shoulder biceps, ankle extensor, peroneal posterior tibial and wrist flexor.*

Wrist tendon compartment is made of six compartments. The distribution of individual wrist compartments is shown in Figure 3-9. Wrist ECU tendon compartment was more likely to be present in the persistent arthritis patients compared to resolving arthritis patients. This was

true for GS and PD. There was no difference in the proportion between the two prognostic outcome groups for the remaining wrist compartments.

Figure 3-9 Distribution of individual wrist tendon compartment US pathology (GS and PD).



**p≤0.05, **p≤0.01, ***p≤0.001. Each bar represents the proportion of patients' US-defined tenosynovitis involvement according to individual wrist extensor compartments. APL: abductor pollicis longus; EPB: extensor pollcis brevis; ECRL: extensor carpi radialis longus; ECRB: extensor carpi radialis brevis; EPL: extensor pollicis longus; EDC: extensor digitorum communis; EIP: extensor indicis propius; EDM: extensor digiti minimi; ECU: extensor carpi ulnaris. n=107 for all variables.*

3.6.4 Univariate analyses of clinical and serological variables.

Univariate analyses of clinical and serological variables measured at baseline to predict the development of persistent arthritis was performed. Results are listed in Table 3-24. Age greater than 60 years, tender or swollen joint count of at least 6 joints, symptom duration of 6 weeks or more and early morning stiffness duration of at least 60 minutes were significant predictors in the development of persistent arthritis on univariate analysis.

Table 3-24 Univariate analyses of clinical and serological variables at baseline in the prediction of persistent arthritis.

Clinical variables	P value	Odds Ratio	95% CI for OR	
Age ≥ 60 years old*	.013	2.675	1.229	5.820
Female	.560	.815	.409	1.624
Tender joint count: 0-1 joint	Ref			
Tender joint count: 2-5 joints	.060	2.786	.958	8.099
Tender joint count: ≥ 6 joints*	.002	4.622	1.722	12.404
Swollen joint count: 1 joint	Ref			
Swollen joint count: 2-5 joints	.513	1.366	.537	3.478
Swollen joint count: ≥ 6 joints*	.012	3.550	1.314	9.593
Mode of onset				
Acute	Ref			
Insidious	.080	2.133	.914	4.977
Symptom duration ≥ 6 weeks *	.026	2.199	1.099	4.402
Early morning stiffness duration ≥ 60 min*	.000	3.857	1.888	7.882
Serological variables	**p value**	**Odds Ratio**	**95% CI for OR**	
Abnormal CRP	.092	1.875	.902	3.898
Abnormal ESR	.361	1.384	.689	2.783
ACPA negative	Ref			
ACPA low- positivity	NA	NA	NA	.
ACPA high-positivity*	.001	12.375	2.830	54.120
RF negative	*Ref*			
RF low positivity	*.015*	*12.966*	*1.658*	*101.380*
RF high-positivity*	*.002*	*10.534*	*2.377*	*46.685*

** Denotes statistical significance at 0.05 level. Ref denotes reference value. OR; odds ratio.*

3.6.5 Univariate analysis of joint US variables

Univariate analysis of joint US variables measured at baseline to predict the development of persistent arthritis at final time point was performed Table 3-25. MCP 1-5, PIP 1-5, MTP 2, 3, 5, wrist, elbow joint GS US were predictors of persistent arthritis development. This was true for both GS and OD variables.

Table 3-25 Univariate analysis of joint US variables at baseline in the prediction of persistent arthritis.

Joint US variables	p	Odds Ratio	95% CI for OR	
MCP 1 GS*	.000	3.930	1.863	8.290
MCP 2 GS*	.000	4.887	2.349	10.169
MCP 3 GS*	.000	3.900	1.904	7.986
MCP 4 GS*	.001	4.082	1.839	9.058
MCP 5 GS*	.000	5.552	2.278	13.533
PIP 1 GS*	.004	6.263	1.803	21.755
PIP 2 GS*	.000	7.496	2.744	20.481
PIP 3 GS*	.004	3.353	1.470	7.646
PIP 4 GS*	.007	4.595	1.514	13.944
PIP 5 GS*	.007	7.833	1.773	34.609
MTP 2 GS*	.015	2.421	1.184	4.949
MTP 3 GS*	.000	5.406	2.109	13.860
MTP 4 GS	.113	1.889	.860	4.152
MTP 5 GS*	.011	4.219	1.385	12.855
Wrist GS*	.001	3.429	1.678	7.006
Shoulder GS	.164	2.353	.705	7.851

Joint US variables	p	Odds Ratio	95% CI for OR	
Elbow GS*	.010	3.091	1.306	7.313
Ankle GS	.973	1.013	.466	2.205
Knee GS	.392	1.468	.610	3.534
MCP 1 PD*	.001	3.843	1.733	8.520
MCP 2 PD*	.000	4.516	2.181	9.349
MCP 3 PD*	.000	6.483	2.955	14.225
MCP 4 PD*	.001	4.526	1.854	11.052
MCP 5 PD*	.010	5.222	1.493	18.261
PIP 1 PD*	.011	5.111	1.461	17.885
PIP 2 PD*	.000	8.487	2.835	25.409
PIP 3 PD*	.011	3.231	1.314	7.942
PIP 4 PD*	.011	5.111	1.461	17.885
PIP 5 PD*	.016	6.247	1.401	27.847
Wrist PD*	.001	3.429	1.678	7.006
Shoulder PD	NA	NA	NA	NA
Elbow PD*	.011	3.642	1.337	9.921
Ankle PD	.746	.875	.390	1.962
Knee PD	.636	.830	.384	1.794
MTP 2 PD*	.014	12.923	1.684	99.193
MTP 3 PD*	.040	8.571	1.098	66.921
MTP 4 PD	.122	2.771	.762	10.076
MTP 5 PD*	.018	3.853	1.258	11.795

*GS grading ≥ 1; PD grading ≥ 1; US pathology was present in at least unilateral joint. GS: Grey scale; PD: Power Doppler. *denotes statistical significant at 0.05 level.*

3.6.6 Univariate analysis of tendon US variables

Univariate analysis of tendon compartment measured at baseline were performed to identify predictors of persistent arthritis (Table 3-26; GS, Table 3-27; PD). Wrist flexor, wrist extensor, digit flexor and ECU tendon were predictors of persistent arthritis development. This was true for both GS and PD. Additionally, posterior ankle tendon PD variable also predicted persistent arthritis development.

Table 3-26 Univariate analysis of tendon compartment GS TS at baseline in the prediction of persistent arthritis.

Tendon Compartment (GS)	p	OR	95% CI for OR	
Shoulder Biceps GS	.102	2.000	.872	4.587
Ankle Extensor GS	.924	1.056	.347	3.213
Ankle Peroneal GS	.083	2.857	.872	9.364
Ankle Posterior Tibial GS	.084	2.108	.904	4.917
Wrist Flexor GS*	.031	5.460	1.166	25.576
Wrist Extensor GS*	.020	2.533	1.158	5.544
Digit Flexor GS*	.000	8.000	2.807	22.803
ECU GS*	.003	3.875	1.574	9.540
EDM GS	.172	3.055	.616	15.140
EDC/EIP	.751	1.157	.468	2.861
EPL	.169	4.526	.525	39.002
ECRL/ECRB	.086	3.962	.823	19.069
APL/EPB	.169	4.526	.525	39.002

*GS: Gray scale; PD: Power Doppler; TS: tenosynovitis. * denotes statistical significance at 0.05 level.*

Table 3-27 Univariate analysis of tendon compartment PD TS at baseline in the prediction of persistent arthritis.

Tendon Compartment (PD)	p	OR	95% CI for OR	
Shoulder Biceps	.181	1.953	.733	5.209
Ankle Extensor	.887	.921	.296	2.870
Ankle Peroneal	.083	2.857	.872	9.364
Ankle Posterior Tibial *	.030	2.769	1.104	6.945
Wrist Flexor *	.023	11.180	1.405	88.990
Wrist Extensor *	.020	2.533	1.158	5.544
Digit Flexor*	.000	9.647	3.102	29.999
ECU*	.003	3.875	1.574	9.540
EDM	.172	3.055	.616	15.140
EDC/EIP	.751	1.157	.468	2.861
EPL	.169	4.526	.525	39.002
ECRL/ECRB	.121	3.500	.718	17.066
APL/EPB	.169	4.526	.525	39.002

**Denotes statistical significance at 0.05 level.*

3.6.7 Principal Component Analysis (PCA).

Next, statistically significant variables from the univariate analysis were included in PCA analyses in order to identify the key variables that accounted for the majority of the explanatory variance observed. In particular, I wished to test the hypothesis that US-measured joint and tendon variables would cluster in separate components, indicating non-correlation.

Two PCA analyses were performed, one for clinical and serological variables (Table 3-28) and one for US (Table 3-29). The number of components extracted was based on eigenvalues with a cutoff of one and the rotation method adopted was according to the varimax criteria with Kaiser normalisation. The rotated factor loadings of the PCA for each clinical, serological and US variable are shown in Table 3-28 and Table 3-29. Three components were extracted from the clinical and serological PCA, whilst ten components were extracted from the joint and tendon US PCA.

Table 3-28 Principal component analysis of clinical and serological variables for all patients.

Variables	Component		
	1	2	3
Swollen joint count 66 (negative, low positive, high positive)	.854		
Tender joint count 68 (0, 1-5, ≥ 6)	.818		
Early Morning stiffness duration ≥ 60 minutes	.613		
Rheumatoid factor (negative, low positive, high positive)		.938	
ACPA (negative, low positive, high positive)		.932	
Symptom duration ≥ six weeks			.775
Age ≥ 60 years old			.759

Rotation method: Varimax with Kaiser Normalization. Factor loadings of <0.400 are suppressed to facilitate interpretation. The variable with the highest loading factor from each component is highlighted in bold. aACPA >7 EU/ml, bACPA >21 EU/ml, cRF > 20 IU/mL, d RF > 60 IU/mL.

Table 3-29 Principal component analysis of US variables for all patients

Rotated Component Matrix										
	Component									
	1	2	3	4	5	6	7	8	9	10
MCP 3 GS	.786									
MCP 3 PD	.778									
MCP 2 PD	.757									
MCP 1 GS	.742									
MCP 1 PD	.729									
MCP 2 GS	.697									
MCP 4 GS	.690									
MCP 4 PD	.647									
PIP 3 GS		.829								
PIP 3 PD		.807								
PIP 2 GS		.751								
PIP 2 PD		.721								
PIP 5 GS		.684		.411						
PIP 5 PD		.665		.422						
PIP 4 PD		.523		.522						
MTP 3 PD			.767							
MTP2 PD			.760							
MTP2 GS			.693							
MTP 3 GS			.675							
MCP 5 PD				.784						

	Component									
	1	2	3	4	5	6	7	8	9	10
MCP 5 GS				.603						
PIP 4 GS		.429		.528						
Digit flexor tendon GS					.875					
Digit flexor tendon PD					.872					
Ankle post tibial tendon PD										
PIP 1 PD						.857				
PIP 1 GS						.830				
MTP 5 PD							.876			
MTP 5 GS							.866			
Wrist ECU PD								.879		
Wrist ECU GS								.879		
Wrist joint PD									.888	
Wrist joint GS									.888	
Elbow GS										.895
Elbow PD										.873

Rotation method: Varimax with Kaiser Normalization. Factor loadings of <0.400 are suppressed to facilitate interpretation. The variable with the highest loading factor from each component is highlighted in bold. ECU: Extensor carpi ulnaris; GS: Gray scale; PD: Power Doppler.

Table 3-30 lists the clinical, serological and US variables that are clustered within the same component in the PCA analysis. The proportion of variance explained for each component is also listed. 68% of the variance observed can be explained by the three PCA components for the clinical and serological variables. Nearly 81% of the variance observed are from the 10 components of the US variables. The tendon and joint US variables are clustered separately; tendon variables in components 5 and 8, with the remaining components containing are joints US variables only. Wrist ECU and wrist joint were clustered separately in component 8 and 9. Component 1, 2, 3 have groupings of MCP, PIP and MTP joint respectively. The largest variance explained from an individual component was from component 1 which contains the MCP joint variables.

Table 3-30 Summary of PCA variables

PCA of clinical and serological variable

Components	1	2	3
Variables	Swollen joint count 66 (0, 1-5, ≥ 6) Tender joint count 68 (0, 1-5, ≥ 6) Early Morning stiffness duration ≥ 60 minutes	Rheumatoid factor (Negative, low positive, high positive) ACPA (Negative, low positive, high positive)	Symptom duration ≥ 6 weeks Age ≥ 60 years old
% of variance explained	30.599	21.866	15.644
Total variance explained	68.108		

PCA of US variables

Components	1	2	3	4	5	6	7	8	9	10
Variables	MCP 1 MCP 2 MCP 3 MCP 4	PIP 2 PIP 3 PIP 4 PIP 5	MTP 2 MTP 3	PIP 4 PIP 5 MCP 5	Digit flexor tendon	PIP 1	MTP 5	Wrist ECU	Wrist joint	Elbow
% of variance explained	37.189	7.480	6.645	5.969	5.027	4.445	4.167	3.660	3.292	2.991
Total variance explained	80.866									

3.6.8 Multivariate logistic regression

Subsequently, a multiple logistic regression model was developed using the variables identified by PCA. The variable with the **highest loading factor from each component** was extracted and made available as an independent variable in a forward step-wise multivariate logistic regression analysis, with persistent arthritis outcome at 18 months entered as the dependent variable. The variables which were included as independent variables in the logistic regression are listed in Table 3-31.

Table 3-31 variables included in the logistic regression model.

Clinical and serological variables	US variables
Swollen joint–66 (3 levels)	Joint
Rheumatoid Factor (3 levels)	MCP 3 GS
Symptom duration ≥ six weeks	MCP 5 PD
	PIP 1 PD
	PIP 3 GS
	MTP 3 PD
	MTP 5 PD
	Wrist joint PD
	Elbow GS
	Tendon
	Digit flexor tendon GS
	Wrist ECU PD

The logistic regression analysis identified wrist PD, digit flexor GS and RF positivity as the variables which formed the model for the prediction of persistent arthritis, with the proportion of persistent vs resolving arthritis identified as 73.8%

In order to robustly check that US-defined joint and tendon variables provided independent predictive value, a further regression analysis was performed Table 3-32 In this case, I systematically entered US joint variables identified in the univariate analysis into the logistic regression analyses. The most optimised model is based on the % of persistent vs resolving arthritis patients correctly identified.

Table 3-32 Multiple logistic regression.

Combination of variable	Variable added	P*	OR (95%)	Nagelkerke R^2	% patients correctly identified (Pers vs RES)
RF DF tendon GS	MCP 1 GS positivity	0.008	3.719 (1.414 to 9.780)	0.439	75.7
RF DF tendon GS	MCP 2 GS positivity	0.005	3.771 (1.506 – 9.441)	0.346	72.9
RF DF tendon GS	MCP 3 GS positivity	0.025	2.712 (1.081-6.799)	0.311	69.2
RF DF tendon GS	MCP 4 GS positivity	0.009	3.587 (1.376 – 9.348)	0.336	72.9
RF DF tendon GS	MCP 5 GS positivity	0.007	4.314 (1.482 – 12.555)	0.342	74.8
RF DF tendon GS	MCP 1 PD positivity	0.014	3.628 (1.305 – 10.086)	0.431	74.8
RF DF tendon GS	MCP 2 PD positivity	0.028	2.916 (1.120 – 7.596)	0.418	75.7
RF DF tendon GS	MCP 3 PD positivity	0.013	3.592 (1.307 – 9.873)	0.429	73.9
RF DF tendon GS	MCP 4 PD positivity	0.181	2.200 (0.693 – 6.982)	0.392	75.7
RF DF tendon GS	MCP 5 PD positivity	0.116	3.906 (0.713 – 21.402)	0.401	77.5
RF DF tendon GS	GS positivity of PIP 1	0.086	3.491 (0.836 – 14.577)	0.405	78.4

Combination of variable	Variable added	P	OR (95% CI)	Nagelkerke R2	% patients correctly identified (Pers vs RES)
RF DF tendon GS	GS positivity of PIP 2	0.005	5.975 (1.710 – 20. 874)	0.452	77.5
RF DF tendon GS	GS positivity of PIP 3	0.058	2.679 (0.969 – 7.408)	0.408	73.9
RF DF tendon GS	GS positivity of PIP 4	0.086	3.161 (0.848 – 11.781)	0.404	78.4
RF DF tendon GS	GS positivity of PIP 5	0.130	3.700 (0.681 – 20.107)	0.399	76.6
RF DF tendon GS	PD positivity of PIP 1	0.130	3.107 (0.715 to 13.489)	0.398	78.4
RF DF tendon GS	PD positivity of PIP 2	0.004	7.646 (1.928 – 30.326)	0.462	78.4
RF DF tendon GS	PD positivity of PIP 3	0.081	2.642 (0.886 – 7.882)	0.404	74.8
RF DF tendon GS	PD positivity of PIP 4	0.072	3.833 (0.887 – 16.556)	0.407	77.5
RF DF tendon GS	PD positivity of PIP 5	0.156	3.419 (0.626 – 18.661)	0.396	76.6
RF DF tendon GS	GS positivity of MTP 2	0.123	2.094 (0.819 – 5.351)	0.397	75.7
RF DF tendon GS	GS positivity of MTP 3	0.037	3.303 (1.074 – 10.152)	0.416	77.5
RF DF tendon GS	GS positivity of MTP 4	0.953	1.032 (0.358 – 2.978)	0.377	75.7

Combination of variable	Variable added	P	OR (95% CI)	Nagelkerke R2	% patients correctly identified (Pers vs RES)
RF DF tendon GS	GS positivity of MTP 5	0.008	2.798 (0.633-12.371)	0.394	74.8
RF DF tendon GS	PD positivity of MTP 2	0.037	10.176 (1.151 – 89.951)	0.433	77.5
RF DF tendon GS	PD positivity of MTP 3	0.103	6.538 (0.685 to 62.371)	0.407	76.6
RF DF tendon GS	PD positivity of MTP 4	0.152	3.550 (0.627 – 20.114)	0.397	75.7
RF DF tendon GS	PD positivity of MTP 5	0.339	2.100 (0.459 – 9.619)	0.385	75.7
RF DF tendon GS	Wrist GS positivity	0.005	4.158 (1.548 – 11.166)	0.448	75.7
RF DF tendon GS	Wrist PD positivity	0.005	4.158 (1.548 – 11.166)	0.360	71.0

3.6.9 Final logistic regression model.

The optimal combination identified was PIP 2 PD, digit flexor GS and RF positivity Table 3-33, with the proportion of persistent arthritis patients correctly identified in this cohort being 78.4%. Removing the digit flexor variable in this regression model results in the proportion of persistent vs resolving arthritis correctly identified falling from 78.4% to 69.1%.

Table 3-33 Final multi logistic regression model.

Variable	Odds Ratio	95% CI	p-value	Nagelkerke R2	% patients correctly identified (Pers vs RES)
RF				0.462	78.4
Negative	Reference				
Low Positive	11.143	1.19 -104.67	0.035		
High Positive	5.125	0.92 – 28.51	0.062		
PIP 2 PD positive	7.646	1.93 – 30.33	0.004		
Digit flexor tendon GS positive	**6.156**	**1.92 – 19.70**	**0.002**		

Variable	Odds Ratio	95% CI	p-value	Nagelkerke R2	% patients correctly identified (Pers vs RES)
RF				0 .308	69.1
Negative	Reference				
Low Positive	13.136	1.645-104.926	0.015		
High Positive	5.783	1.227 - 27.252	0.026		
PIP 2 PD positive	6.434	2.058 - 20.115	0.001		

3.7 Discussion; Prediction of persistent arthritis.

Digit flexor tendon predicts the development of persistent arthritis, even after taking into account joint US and clinical and serological variables.

This is a novel finding. Previous predictive studies on persistent arthritis emphasised on scanning the small joint of hands and feet (128, 171-173), rather than tendon scanning. One predictive study of persistent arthritis including digit flexor tendon. The authors reported that in a cohort of patients seronegative for RF and ACPA with musculoskeletal symptoms of less than 12 weeks, the presence of US score of GS3, or PD $\geqslant$1 or US erosion increased the risk of developing persistent arthritis. This study did not specifically assess the predictive utility of tendon and joint US separately (127). Other studies that included US in the predictive studies are listed in Table 3-34.

Digit flexor tenosynovitis in early RA is widely recognised (168-170). However, persistent arthritis patients included in our study also had diagnoses of PsA and SpA at final follow-up. How does one explain the digit flexor tenosynovitis as an independent predictor of these patients who are in the PsA/SpA spectrum as well?

Clinical dactylitis is one of the clinical features of PsA and SpA. In an MRI and US study reported by Olivieri et al, clinical dactylitis corresponded to flexor tenosynovitis for these patients (174). These results were subsequently replicated by two US studies in PsA patients (175, 176). In an MRI study of early RA and PsA there were no significant difference in the frequency of tenosynovitis between the two groups. Early disease defined as symptom

duration of ≤1 year. The distinguishing features between this two groups on MRI were enthesitis, subcutaneous oedema and extensive diaphyseal bone marrow oedema (177).

The strength of this study is the relatively large number of patients (n=150). In addition an extensive range of joint and tendon US variables were assessed. Both large and small joints were assessed. Similarly, tendons related to large and small joints were also investigated.

The limitation of this study was that the individual flexor tendons were not scored separately i.e. digit flexor tendon 1 to 5. In clinical practice, scanning only specific digit flexor tendons would cut down the scanning time. The current scanning protocol of the cohort scores each of the ten digit flexor tendon individually (bilateral digit flexor 1-5). The next stage of the predictive analysis will identify the specific digit flexor tendon that contributes to the prediction of persistent arthritis.

3.8 Conclusion; Prediction of RA and persistent arthritis.

US-defined digit flexor tendon tenosynovitis is an independent predictor of both RA and persistent arthritis – even after taking into account joint US and conventional clinical and serological variables. Investigators designing predictive algorithms for the development of RA and persistent arthritis should consider including digit flexor tendons as a candidate variable.

Table 3-34 Studies that assessed US variables in the prediction of persistent arthritis.

Paper Title	**Key findings**	**First author (year of publication)**	**n**	**Patient population**	**Joint sub-set scanned**	**Note on grading**
A diagnostic algorithm for persistence of very early inflammatory arthritis: the utility of power Doppler US when added to conventional assessment tools (127).	**PD** alongside clinical increased the certainty of inflammatory arthritis at **12M, in seronegative patients only.**	Freeston JE (2010)	50	Inflammatory hand symptoms (defined as EMS for at least 1 h in the hands **with or without clinical** synovitis) for ⩽12 weeks	Bilateral • MCP joints, • flexor tendons • wrists.	Each joint was scored for GS and power Doppler (PD) on a 0–3 semi-quantitative scale Presence or absence of flexor tenosynovitis was recorded. (but not graded). Variables were binarised, eg, any joint with a PD score ⩾1). A semi-quantitative cut-off of 3 was used for GS but ⩾1 for PD.

Paper Title	Key findings	First author (year of publication)	n	Patient population	Joint sub-set scanned	Note on grading
Predicting persistent inflammatory arthritis amongst early arthritis clinic patients in the UK: is musculoskeletal US required? (171)	**MSUS not predictive** of persistent disease arthralgia patients when **compared to the Visser score**.	Pratt AG (2013)	389	**New-onset arthralgia** (no clinically swollen joints)	16 peripheral **small joints** • 2^{nd}-4^{th} MCP joints bilaterally • 2^{nd}-4^{th} PIP joints bilaterally (dorsal and volar longitudinal planes, neutral and flexed position) • 1^{st}-2^{nd} MTP joints bilaterally (dorsal longitudinal plane only).	Synovial hypertrophy and synovial effusion were combined into a single score.

Predicting the development of clinical arthritis in anti-CCP positive individuals with non-specific musculoskeletal symptoms: a prospective observational cohort study (128).	**PD enhancement** increased the risk of arthralgia developing to inflammatory arthritis from when included in a model with these variables: **tenderness of hand or foot joints, EMS ≥30 min, high-positive autoantibodies.**	Rakieh C (2015)	100	New onset MSK symptoms **with no clinical evidence of inflammatory joint swelling with positive CCP.**	Wrists, MCPs and PIPs	• Binarised: PD ⩾1 Did not report any GS findings.
US findings predict progression to inflammatory arthritis in anti-CCP antibody-positive patients without clinical synovitis (172).	US features of joint inflammation may be detected in anti-CCP-positive patients without CS. US findings predict progression (and rate of progression) to IA, with the risk of progression highest in those with PD signal.	Nam JL (2016)	136	**MSK symptoms with a positive anti-CCP2.**	wrists, MCPs, PIPs and MTPs.	

Routine musculoskeletal US findings impact diagnostic decisions maximally in autoantibody-seronegative early arthritis patients (173).	The summed of US parameters from the seven-joint statistically significant but the size effect was very small. The effect of sonographer's impression contributed to the improvement of prediction of outcome after taking into account of other parameters alone in the ACPA-negative patients.	Iqbal K (2019)	831	**Unselected cohort of early arthritis clinic attendees**	most symptomatic: wrist, second/third MCPs and PIPs and second/fifth MTPs Sonographer's impression (definitely inflammatory, possibly inflammatory or non-inflammatory) were included as a predictive variable.	Semi-quantitative MSUS scoring

4 The therapeutic window of opportunity in RA.

4.1 Introduction

Although the "earlier the better" concept is widely accepted, what is not known from current evidence is the true duration of the distinct window during which initiation of immunosuppressant therapy can change the trajectory of disease progression, taking into account the different modes of onset at presentation.

Therefore, a systematic, large prospective study is required to define the time-to-therapy in RA which is associated with improved clinical outcomes, and its relationship with different modes of onset, in order to inform both clinical management and public health campaigns aimed at increasing early recognition of disease. Such a study has been identified as a research priority by The EULAR Study Group on Risk Factors for RA (44, 83).

The overarching aim is to identify the window of opportunity in DMARD-naïve RA patients during which first DMARD therapy results in proportionately better treatment response compared to that of after this window.

The hypothesis behind the ‘window of opportunity’ is that earlier disease phases are more amenable to therapy, and therefore that treatment instigated during that time can alter the long-term disease trajectory.

4.1.1 Primary objectives

1. To investigate the relationship between time-to-therapy and treatment response, taking into account different modes of disease onset.
2. To identify the threshold of time-to-therapy from **first onset of patient reported joint swelling** that results in a disproportionately better treatment response rate, compared to that of after this threshold.

In this study, I defined the **onset of symptoms and joint swelling** in relation to each **mode of onset.**

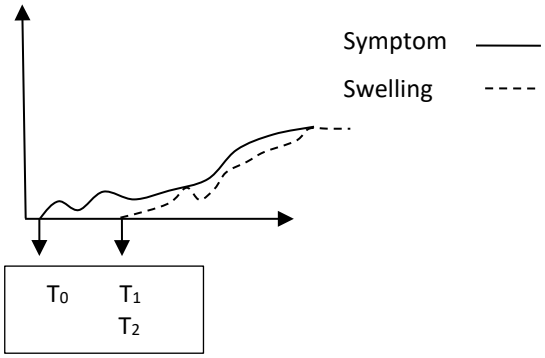
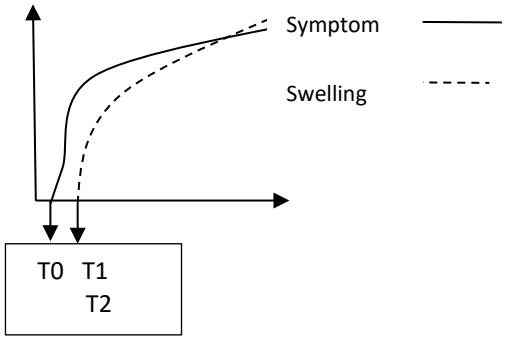
The **time-to-therapy** is defined from

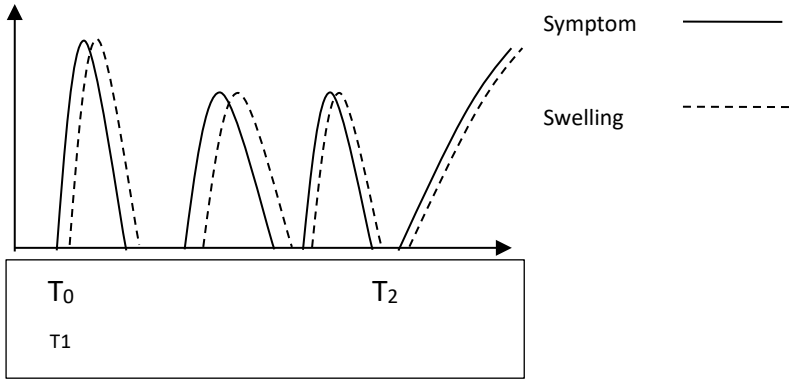
1. T0: onset of current inflammatory symptoms,
2. T1: onset of any related inflammatory joint swelling, and
3. T2: onset of current ongoing inflammatory joint swelling,

until first dose of methotrexate.

Mode of onset was classified as insidious, abrupt or palindromic. The definition of each mode of onset is detailed in Figure 4-1. Patients were shown this figure so that they could self-identify which mode of onset matched their symptoms best.

Figure 4-1 Mode and timing of onset definitions.

Insidious onset	Abrupt onset
	
Onset that begins gradually and reaches a maximum intensity within weeks or months.	Onset that begins abruptly and reaches a maximum intensity within hours or days.

Palindromic onset	
	A history or physical examination findings consistent with symptoms and/or synovial swelling that returns to normal between attacks.

Timing of onset definitions
T_0: Onset of current inflammatory symptoms.
T_1: Onset of any related inflammatory joint swelling.
T_2: Onset of current ongoing inflammatory joint swelling.

4.2 Methods/ Study protocol

4.2.1 Study design

This is a prospective observational cohort study of DMARD-naïve newly presenting RA patients.

I assessed therapeutic response by measuring

1. the suppression of clinical joint inflammation as measured by Disease Activity Score 28 joint counts (DAS-28),
2. the suppression of joint and tendon inflammation as quantified by high-resolution greyscale and Power Doppler US,
3. the improvement in functional outcome as measured by Health Assessment Questionnaire (HAQ) score,
4. the rate of radiographic progression of hand and foot radiographs as quantified by the modified Sharp van der Heijde Score.

4.2.2 Subject selection

4.2.2.1 Inclusion criteria

1. Age ≥ 18.
2. Able and willing to give written informed consent.
3. Patients fulfilling the 2010 ACR/EULAR criteria for RA.
4. Commencing methotrexate as first DMARD therapy.

4.2.2.2 Exclusion criteria

1. RA patients who have already started treatment with DMARDs excluding glucocorticoids.
2. Patients who have contraindications to methotrexate therapy ≥10mg weekly.

4.2.2.3 Recruitment centres

The TETRA study is a multi-centre study involving three centres in the West Midlands: University Hospitals Birmingham NHS Foundation Trust, Sandwell and West Birmingham Hospitals NHS Trust, and The Modality Primary Care Consortium. Figure 4-2 shows the catchment area of the Rheumatology service provided for these three sites. Patients who were enrolled to the Birmingham Early Arthritis Cohort (BEACON) and fulfilled the inclusion criteria were offered participation in this study.

I attended the EAC clinic at UHB and SWBH Rheumatology Department every week, to assess eligibility of potential TETRA patients. Eligible patients were invited to enrol to the study and commenced methotrexate within the same week. A number of patients at UHB were also referred directly from non-EAC clinics. These patients were offered a research baseline appointment within 1-2 weeks. All patients were consented at the baseline assessment visit and the discussion to start DMARD often happened at the same visit. However, some patients wished to return the following week for the DMARD discussion as the baseline research visit took up to 90 minutes. The TETRA study pathway is shown in Figure 4-2. Each patient was reviewed at baseline, 1, 2, 3, 4, 6 and 12 month time points.

Figure 4-2 Catchment area of Rheumatology services provided by the three recruiting centres.

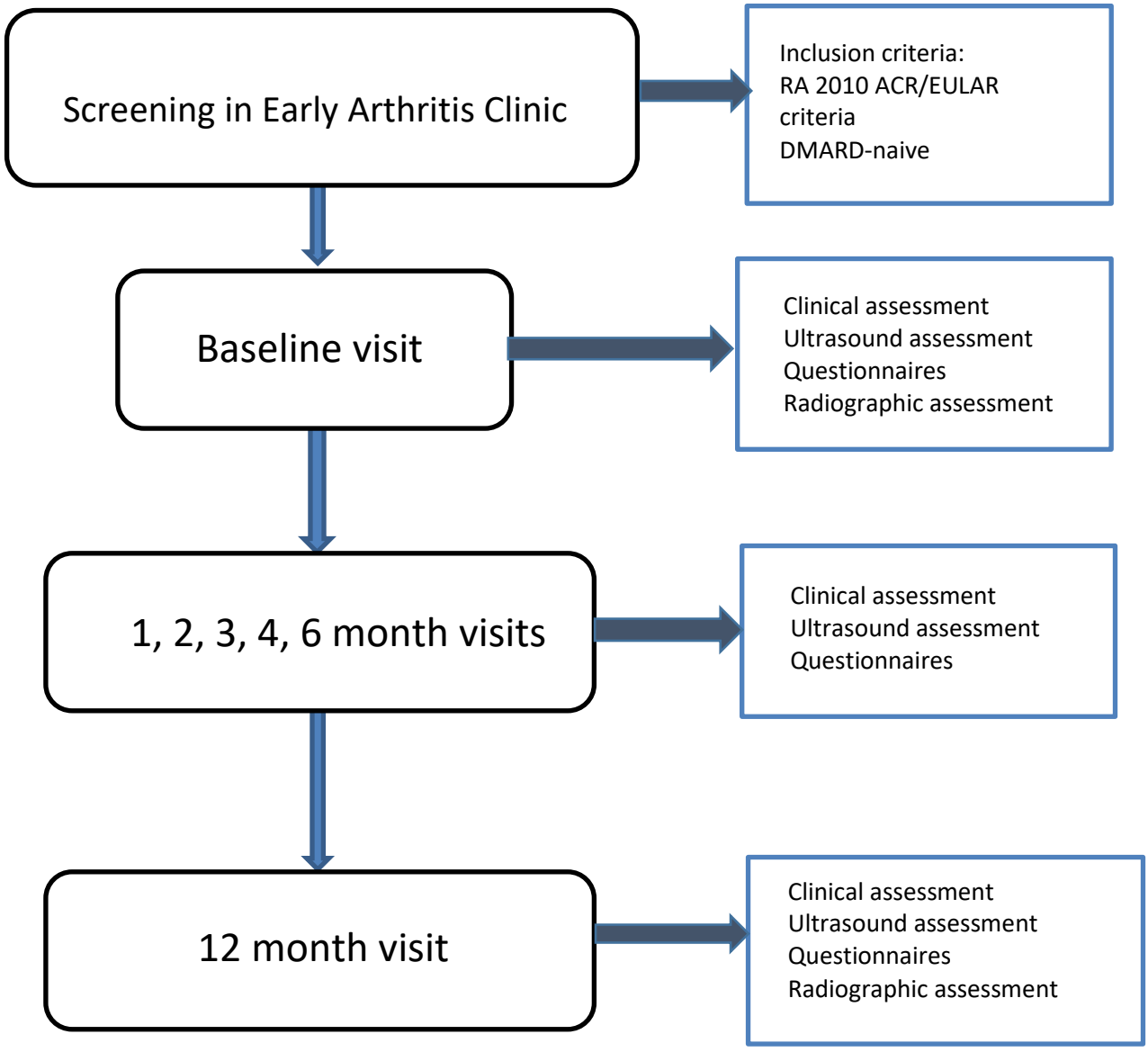
BEACON
BIRMINGHAM EARLY ARTHRITIS COHORT

SWBH

modality Partnership

UHB

Figure 4-3 The TETRA study patient pathway



4.2.3 Data Collection

4.2.3.1 Baseline visit

All patients provided written consent. The following clinical and functional measures were recorded:

1. Clinical history
    i. Dates of symptom onset (T0), of any related inflammatory joint swelling onset (T1) and of current ongoing inflammatory episode (T2) [Figure 4-1]
    ii. Mode of onset (abrupt, insidious or palindromic) [Figure 4-1]
    iii. Duration and severity of early morning stiffness,
    iv. Co-morbidities and concomitant medications.
    v. Disease activity measures
    vi. 68 tender and 66 swollen joint counts.
    vii. Patient-reported visual analogue scale (VAS) scores pain, global health, early morning stiffness severity and fatigue severity.
    viii. Physician-reported global health visual analogue scale scores.

2. Questionnaires
    i. Work Productivity and Activity Impairment Questionnaire: Rheumatoid Arthritis V2.0,
    ii. Symptoms in Persons at Risk of Rheumatoid Arthritis (SPARRA),
    iii. EQ-5D-5L and Health Assessment Questionnaire (HAQ).

3. Laboratory tests (standard of care)

i. Inflammatory markers: ESR, CRP.

ii. Autoantibody profiles: Rheumatoid factor (RF) and anti-citrullinated protein/peptide antibodies (ACPA).

4. Hand and foot radiographs (standard of care)

Radiographic imaging was performed at each site according to a standardised protocol. All radiograph images were anonymised. I will score the radiographs according to the modified Sharp score once all patients have completed the final follow-up.

4.2.3.2 Ultrasonography assessments

All patients underwent US imaging at baseline and follow-up visits. I scanned and scored all of the US assessments in real-time. I was blinded to the joint examination performed by the research nurses in order to avoid bias. At each visit, I scanned a core set of joints and tendons using a high frequency probe of at least 12MHz and high power Doppler sensitivity. The core set of joints and tendons scanned were bilateral metacarpophalangeal (MCP) 1-5 joints, proximal interphalangeal (PIP) 1-5 joints, metatarsophalangeal (MTP) 2-5 joints, extensor carpi ulnaris tendon and digit flexor (1-5) tendons at the level of the MCP joint. This joint core set was based on analysis of US data from the BEACON cohort (26). Grading of grayscale and power Doppler measurements was documented by applying semi-quantitative scales as per OMERACT consensus documents.

4.2.3.3 Follow-up visits

Patients were followed up at 1, 2, 3, 4, 6 and 12 months from the baseline visit. Research assessments were performed and recorded at each study visit Table 4-1. The increased frequency of visits between baseline and six months was important in order to record suppression of joint inflammation in line with escalation of DMARD therapies. The 12 month visit was important to assess disease progression and sustained clinical benefits in the medium term. Upon completion of study follow-up, participants were followed up according to normal practice in their referring centres. After completion of the above assessments, the management plan was initiated on the same day.

Table 4-1 Assessments at each research visit.

Assessments	BL	1M	2M	3M	4M	6M	12M
DMARDs dosing	X	X	X	X	X	X	X
Clinical Assessment i. 66 swollen & 68 tender joints ii. Patient & physician global visual analogue scale score	X	X	X	X	X	X	X
Inflammatory markers	X	X	X	X	X	X	X
Questionnaires i. Health Assessment Questionnaire (HAQ) ii. EQ-5D-5L iii. Work productivity and Impact Questionnaire V2.0	X	X	X	X	X	X	X
US assessment i. Joints: MCP, PIP, wrists ii. Tendons: Extensor carpi ulnaris, Digit flexor	X	X	X	X	X	X	X
Hand & foot radiographs	X						X

4.2.3.4 General principles of DMARDs strategy.

The overall treatment strategy and DMARD therapy escalation were protocolised to ensure DMARD therapy was standardised across recruitment centres. DMARD doses were titrated up to the maximal tolerated dose based on patients' age, disease severity, co-morbidities, risk of infection and baseline blood tests. All medication information were recorded on a medication sheet including start date, missing dose and reason for missing doses. Appendix shows the medication sheet template. Figure 4-4 summarises the TETRA study treatment pathway. In summary, all patients commenced methotrexate monotherapy at the start of the study according to NICE guideline recommendations. Those with an inadequate response to methotrexate mono-therapy and/or poor prognostic factors commenced a second DMARD. The initial choice of second DMARD was hydroxychloroquine. Sulfasalazine or leflunomide were alternative second DMARD choices. Patients who had active disease at 6 months despite combination therapy were escalated to biologic therapy.

Figure 4-4 The TETRA study treatment pathway.

Main inclusion criteria:

- RA according to ACR/EULAR 2010 classification criteria.
- DMARD-naïve.
- Starting MTX as first DMARD therapy.

Proposed treatment pathway:

Pathway 1: MTX +/- steroids[a] or Pathway 2: MTX and HCQ +/- steroids[a]

Pathway	Pathway 1: MTX monotherapy	Pathway 2[b]: MTX and HCQ[e] combination therapy		Prednisolone reducing course
Week	MTX[c]	HCQ		Prednisolone
1	10mg OW	NA		20mg OD
2	10mg OW	NA		15mg OD
3	15mg OW	NA		10mg OD
4^	15mg OW	NA		10mg OD
5	20mg OW	200mg OD		7.5mg OD
6	20mg OW	200mg BD		5mg OD
7	20mg OW	200mg BD		2.5mg OD
8^	20mg OW	200mg BD		NA
9	20mg OW	200mg BD[d]		NA
10	20mg OW	200mg BD		NA
11	20mg OW	200mg BD		NA
12^	20mg OW	200mg BD		NA
13	20mg OW	200mg BD		NA
14	20mg OW	200mg BD		NA
15	20mg OW	200mg BD		NA
16^	20mg OW	200mg BD		NA
17	20mg OW	200mg BD		NA
18	20mg OW	200mg BD		NA
19	20mg OW	200mg BD		NA
20	20mg OW	200mg BD		NA
21	20mg OW	200mg BD		NA
22	20mg OW	200mg BD		NA
23	20mg OW	200mg BD		NA
24^	20mg OW	200mg BD		NA

^Research visits a Steroid therapy is either IM depo-medrone or reducing oral prednisolone +/- intra-articular injections. b To consider MTX+HCQ combination therapy for patients with poor prognostic factors. c MTX: max dose of 25mg OW orally. To consider MTX SC if not tolerating orally. d To increase HCQ dose depending on patient's tolerability. Max dose of 5mg/kg. e

Sulfasalazine or leflunomide is an alternative second DMARD for those whim HCQ is contra-indicated.

4.2.4 Statistical analysis

Multiple linear regression analysis was used to assess the relationship between time-to-therapy and therapeutic response after adjusting for potential confounders (baseline disease activity score, presence of RF, presence of ACPA, mode of onset, gender, age).

Therapeutic response was defined as the change of disease activity score (DAS-28 CRP) between baseline and 12 months and was entered as the dependent variable. The increase in R squared (due to adding time-to-therapy into the model containing the six confounders) that could be detected with 80% power at the 5% significance level with a sample size of 100 ranges from **4.0% to 6.3%** as the initial R squared value (prior to adding time-to-therapy) varies from 50% to 20%.

I aimed to recruit 110 patients to allow for 10% dropout. I assessed the relationship between time-to-remission and time-to-therapy with a Cox-proportional regression model, after adjusting for the potential confounders (baseline disease activity score, presence of autoantibodies RF, presence of ACPA, mode of onset, gender, age).

Time-to-remission was defined from baseline to the time a patient reaches DAS-28CRP remission (DAS-28<2.6). Time-to-therapy was defined from symptom onset (T0), joint swelling onset (T1) and ongoing joint swelling (T2) to start of first DMARD. For each time-to-event outcome (i.e. time-to-remission), Kaplan-Meier survival curves were constructed and the log-

rank test was used to compare the difference between the curves for the different modes of onset. Patients with palindromic onset were excluded from the analysis due to very low numbers.

4.3 Results

4.3.1 Recruitment

125 patients were recruited between March 2017 and December 2019. 18 patients withdrew during the course of the study. 107 patients will be included in the final analysis of this study. 48 patients were included in the analysis for this thesis. These patients have completed 12-month follow-up by August 2019. All patients will have completed their 12 month visit by November 2020. Figure 4-5 shows the recruitment trajectory through the study period.

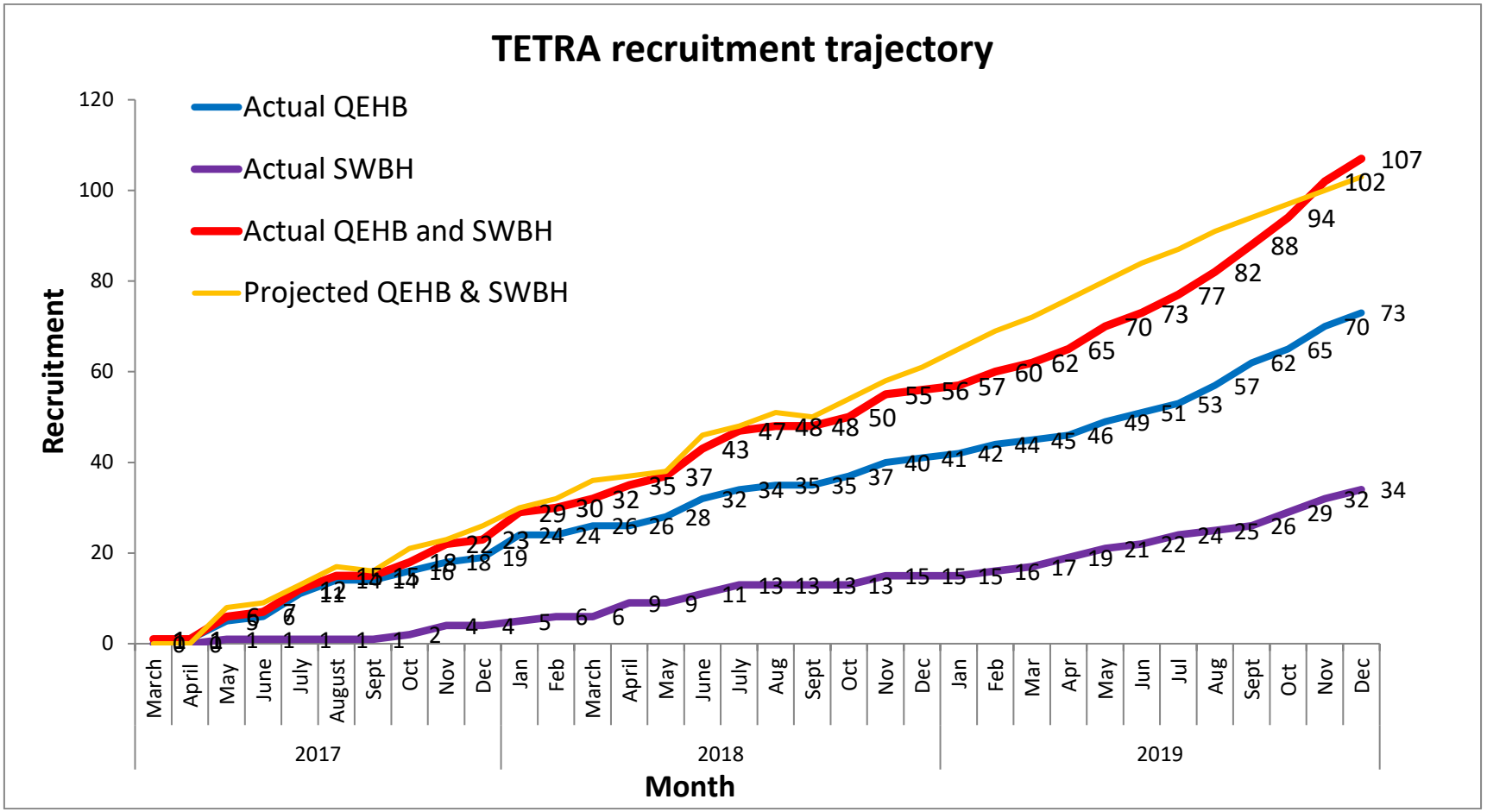


Figure 4-5 TETRA recruitment trajectory.

4.3.2 Baseline demographics

Table 4-2 shows the patients' and disease characteristics at baseline. The majority were female and white. Age at baseline assessment ranged from 18 to 87 years old with a median of 55 years old (Figure 4-6). Nearly 60% of patients were autoantibody positive (RF or anti-CCP). Patients had multiple swollen joints, long duration of early morning stiffness with high VAS scores across all four domains: pain, global health, severity of EMS and fatigue. The median DAS-28 score at baseline indicated high disease activity. There was approximately equal split between insidious and abrupt onset groups. Palindromic onset was the smallest group.

Figure 4-6 Histogram of age at baseline assessment.

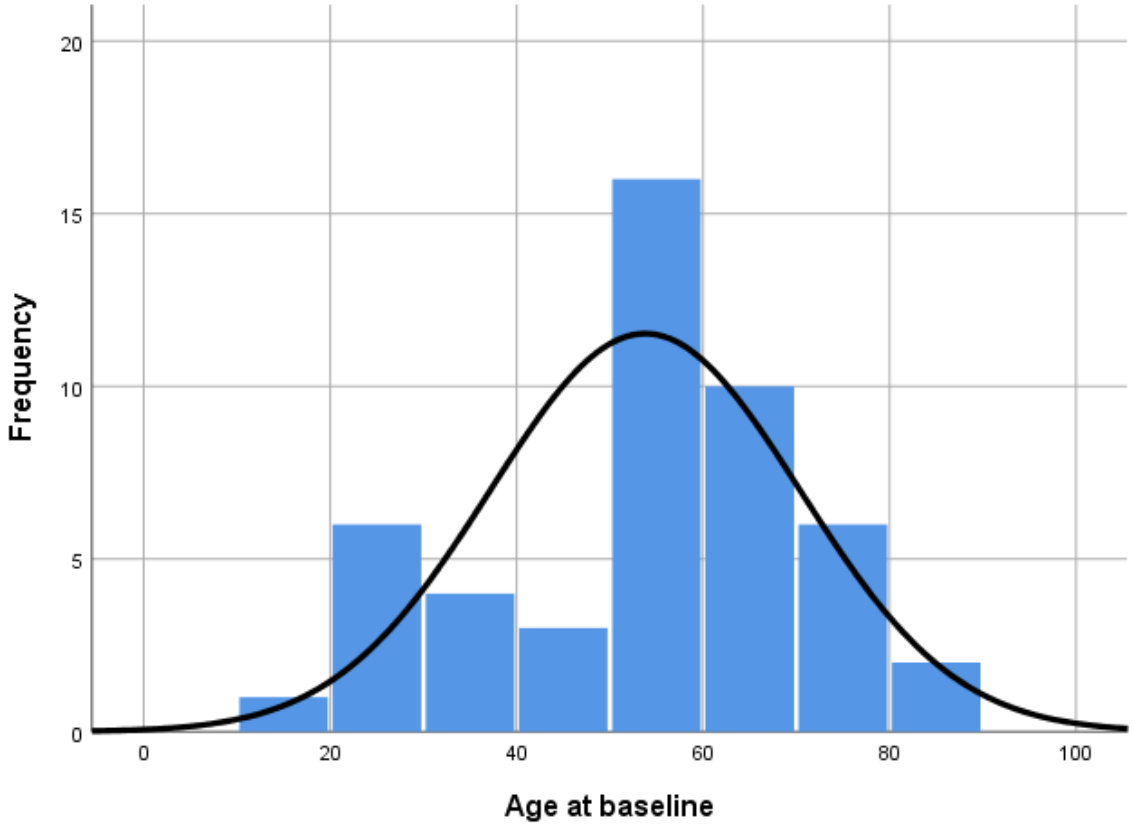


Table 4-2 Patient and disease characteristics at baseline.	
Patient characteristics	**N=48**
Age (years) at baseline assessment, median (IQR)	55 (45-67)
Age (years) at symptom onset, median (IQR)	54 (42-66)
Female, n (%)	38 (79.2)
Smoking status, n (%)	
current	9 (18.8)
ever	20 (41.7)
never	19 (39.6)
Smoking (in pack years)$^{\alpha}$	8 (0-27)
Alcohol intake (units/week)$^{\alpha}$	0 (0-4)
BMI (in units) , median (IQR)	27 (24-35)
Ethnicity, n (%)	
White British	40 (83.3)
White other	1 (2.1)
Black British - Caribbean	4 (8.3)
Asian British - Indian	2 (4.2)
Asian British - Bangladeshi	1 (2.1)
Disease characteristics at baseline	**N=48**
RF positivity, n (%)	28 (58.3)
Anti-CCP positivity, n (%)	27 (56.3)
Early morning stiffness duration (mins) $^{\beta}$, median (IQR)	60 (14-120)
DAS-28 CRP $^{\alpha}$, median (IQR)	5.04 (4.06 -5.83)
DAS-28 ESR $^{\gamma}$, median (IQR)	5.73 (4.24-6.33)
Tender joint count 68, median (IQR)	15 (9-26)
Swollen joint count 66, median (IQR)	7 (2-13)
Tender joint count 28, median (IQR)	11 (4-18)
Swollen joint count 28, median (IQR)	5 (2-10)
Patient-reported pain VASα (100mm), median (IQR)	56 (35-84)
Patient-reported global health VASα (100mm), median (IQR)	64 (39-79)
Patient-reported severity of early morning stiffness VASα (100mm), median (IQR)	74 (35-95)
Patient-reported fatigue VASα (100mm), median (IQR)	60 (31-85)
Physician-reported global health VASα (100mm)	64 (39-79)
Mode of onset	
Abrupt	25 (52)
Insidious	19 (40)
Palindromic	4 (8)

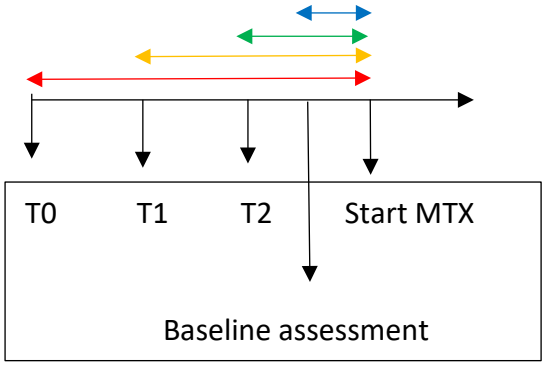
Missing data α, n=1; β, n=2; γ, n=4.

4.3.3 Symptom duration and mode of onset

The patient journey from T0 up to start of methotrexate therapy is illustrated in Figure 4-7.

T0 is defined as onset of inflammatory symptoms. **T1** is defined as onset of first joint swelling and **T2** is defined as onset of persistent joint swelling.

Figure 4-7 Patient timeline in relation to the time-to-therapy definition



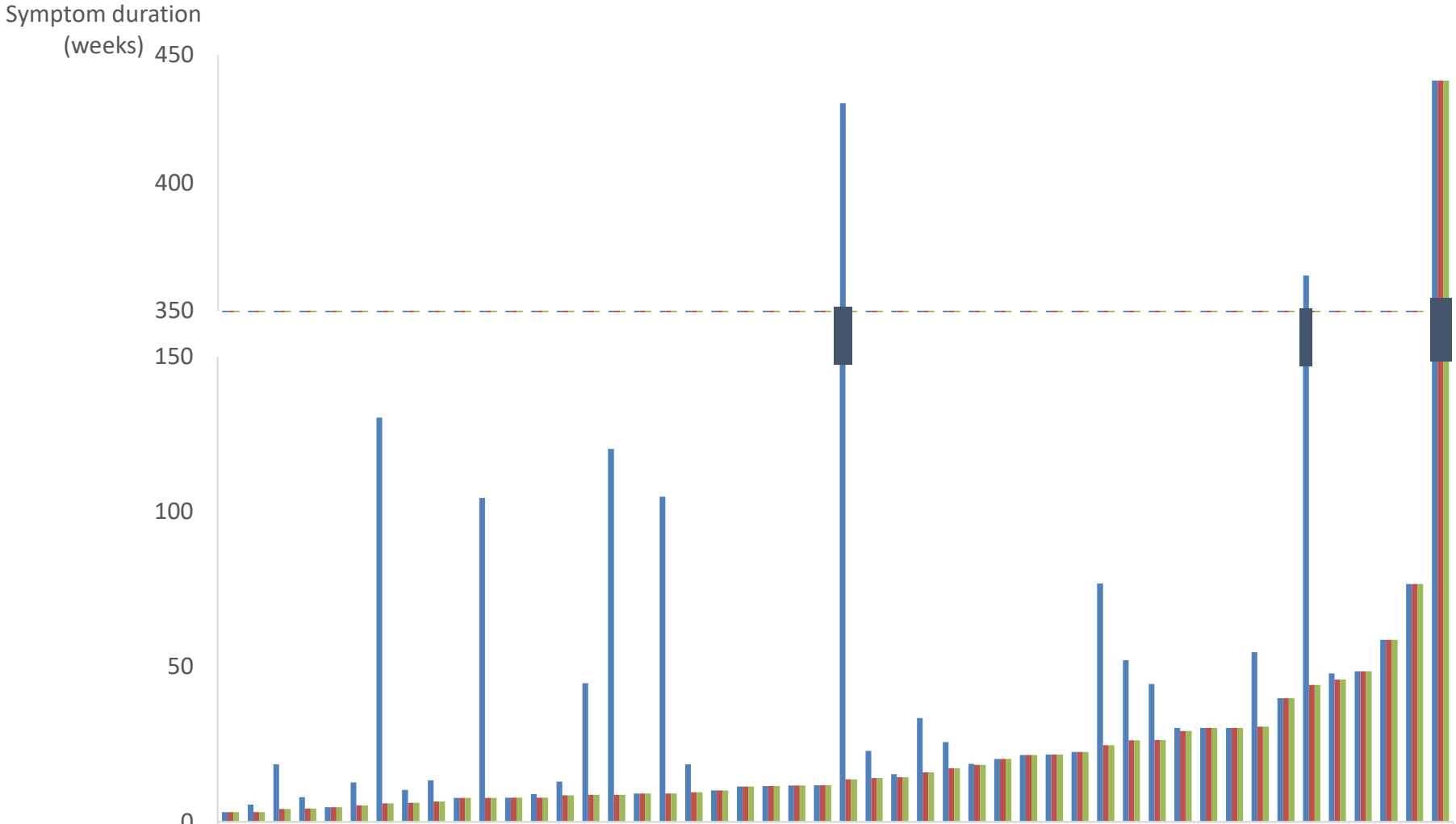
The red, yellow and green arrows indicate the time-to-therapy from T0, T1 and T2 respectively.

The blue arrow indicates the duration between baseline assessment and first dose of methotrexate. Symptom duration refers from T0, T1 and T2 to baseline assessment.

Figure 4-8 shows the symptom duration from T0, T1 and T2 to baseline assessment for the whole cohort. Median for symptom duration from T0 to baseline assessment was 22 weeks (IQR 12 to 51 weeks. The median (IQR) for symptom duration from T1 and T2 to baseline assessment were the same (Median 13 weeks; IQR 8 to 27 weeks). 24 out of 48 patients (50%) have symptom duration fromT0, T1 and T2 of </= 12 weeks.

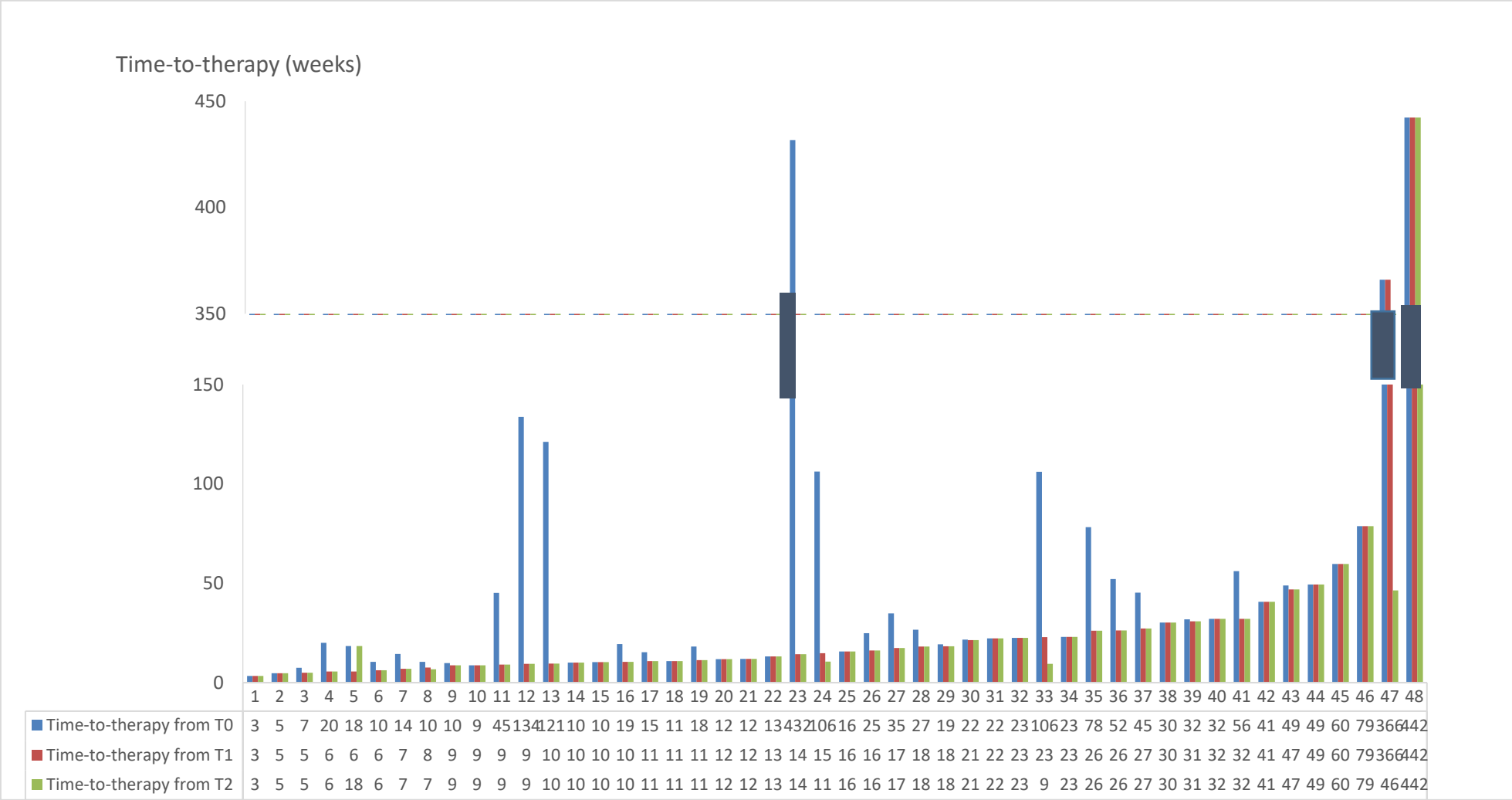
Figure 4-9 shows the time to first dose of methotrexate (referred to as time-to-therapy hereafter) from i) onset of symptoms (T0), ii) onset of first joint swelling (T1), iii) onset of persistent joint swelling (T2). The time-to-therapy from T0, T1 and T2 ranged from 3 to 442 weeks. The median for T0 was 22 weeks (IQR 12 to 52 weeks); the median for both T1 and T2 was 15 weeks (T1 IQR 9 to 27; T2 IQR 10 to 27 weeks).

Figure 4-8 Symptom duration from T0, T1 and T2 to baseline assessment. T0 is defined as onset of inflammatory symptoms. T1 is defined as onset of first joint swelling and T2 is defined as onset of persistent joint swelling.



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
From T0 to baseline	3	6	19	8	5	13	13	10	13	8	10	8	9	13	45	12	9	10	19	10	11	12	12	12	43	23	15	33	26	19	20	22	22	23	77	52	44	30	30	30	55	40	36	48	49	59	77	44
From T1 to baseline	3	3	4	4	5	5	6	6	7	8	8	8	8	9	9	9	9	9	10	10	11	12	12	12	14	14	14	16	17	18	20	22	22	23	25	26	26	29	30	30	31	40	44	46	49	59	77	44
From T2 to baseline	3	3	4	4	5	5	6	6	7	8	8	8	8	9	9	9	9	9	10	10	11	12	12	12	14	14	14	16	17	18	20	22	22	23	25	26	26	29	30	30	31	40	44	46	49	59	77	44

Figure 4-9 Time to therapy from T0, T1 and T2. T0 is defined as onset of inflammatory symptoms. T1 is defined as onset of first joint swelling and T2 is defined as onset of persistent joint swelling



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
Time-to-therapy from T0	3	5	7	20	18	10	14	10	10	9	45	134	121	10	10	19	15	11	18	12	12	13	432	106	16	25	35	27	19	22	22	23	106	23	78	52	45	30	32	32	56	41	49	49	60	79	366	442
Time-to-therapy from T1	3	5	5	6	6	6	7	8	9	9	9	9	10	10	10	10	11	11	11	12	12	13	14	15	16	16	17	18	18	21	22	23	23	23	26	26	27	30	31	32	32	41	47	49	60	79	366	442
Time-to-therapy from T2	3	5	5	6	18	6	7	7	9	9	9	9	10	10	10	10	11	11	11	12	12	13	14	11	16	16	17	18	18	21	22	23	9	23	26	26	27	30	31	32	32	41	47	49	60	79	46	442

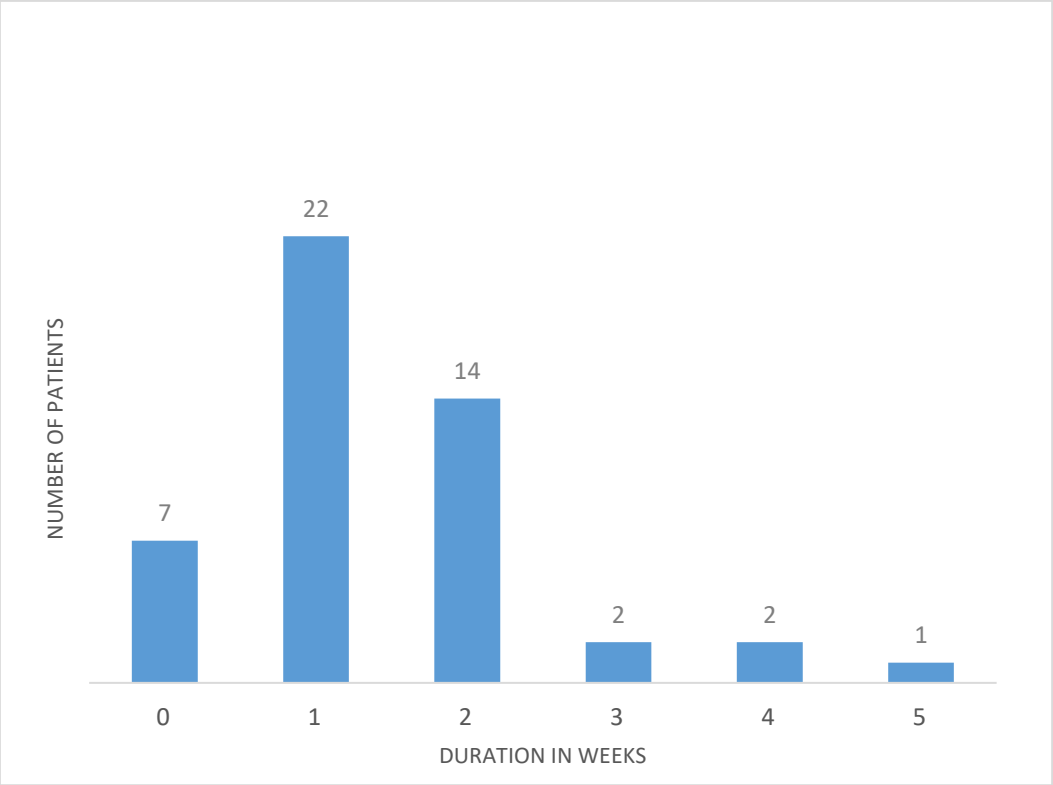


Figure 4-10 Frequency distribution of duration between baseline assessment and first dose of methotrexate.

Figure 4-10 shows the frequency distribution of duration between baseline assessment and first dose of methotrexate. 29 out of 48 patients started MTX within one week. 43 out of 48 patients started MTX within 2 weeks. The longest delay was 5 weeks; this was in a patient with lower respiratory tract infection.

4.3.4 Treatment strategy.

All patients commenced methotrexate as per inclusion criteria. 2 out of 48 patients' methotrexate treatment was stopped before 52 weeks for clinical reasons (i.e. persistently abnormal blood test).

Figure 4-11 shows the treatment duration of methotrexate therapy (prescribed and actual) over the 52 week study period. 45 out of 48 patients were on MTX therapy for ≥ 46 weeks.

The difference between prescribed and actual methotrexate treatment were missed doses. Reasons for missed doses were infection (n=19), abnormal blood test (n=6), surgery (n=3), missing tablets (n=1), holidays (n=1), mouth ulcers (n=1) and multiple traumatic fracture (n=1), where n refers to number of episodes. Each patient may have missed dose(s) due to more than one reason.

Figure 4-12 shows the treatment duration of hydroxychloroquine (prescribed and actual) over the 52 study period. All patients were prescribed 5mg/kg. 31 were on hydroxychloroquine as a second DMARD. 21 of these were on hydroxychloroquine for ≥ 35 weeks. 26 out of 31 patients were on treatment for ≥ 30 weeks. The difference between prescribed and actual were missed doses due to infection (n=4), concerns with visual symptoms (n=2). Three patients commenced hydroxychloroquine in week 3.

Figure 4-11 Treatment duration of methotrexate therapy (prescribed and actual). N=48

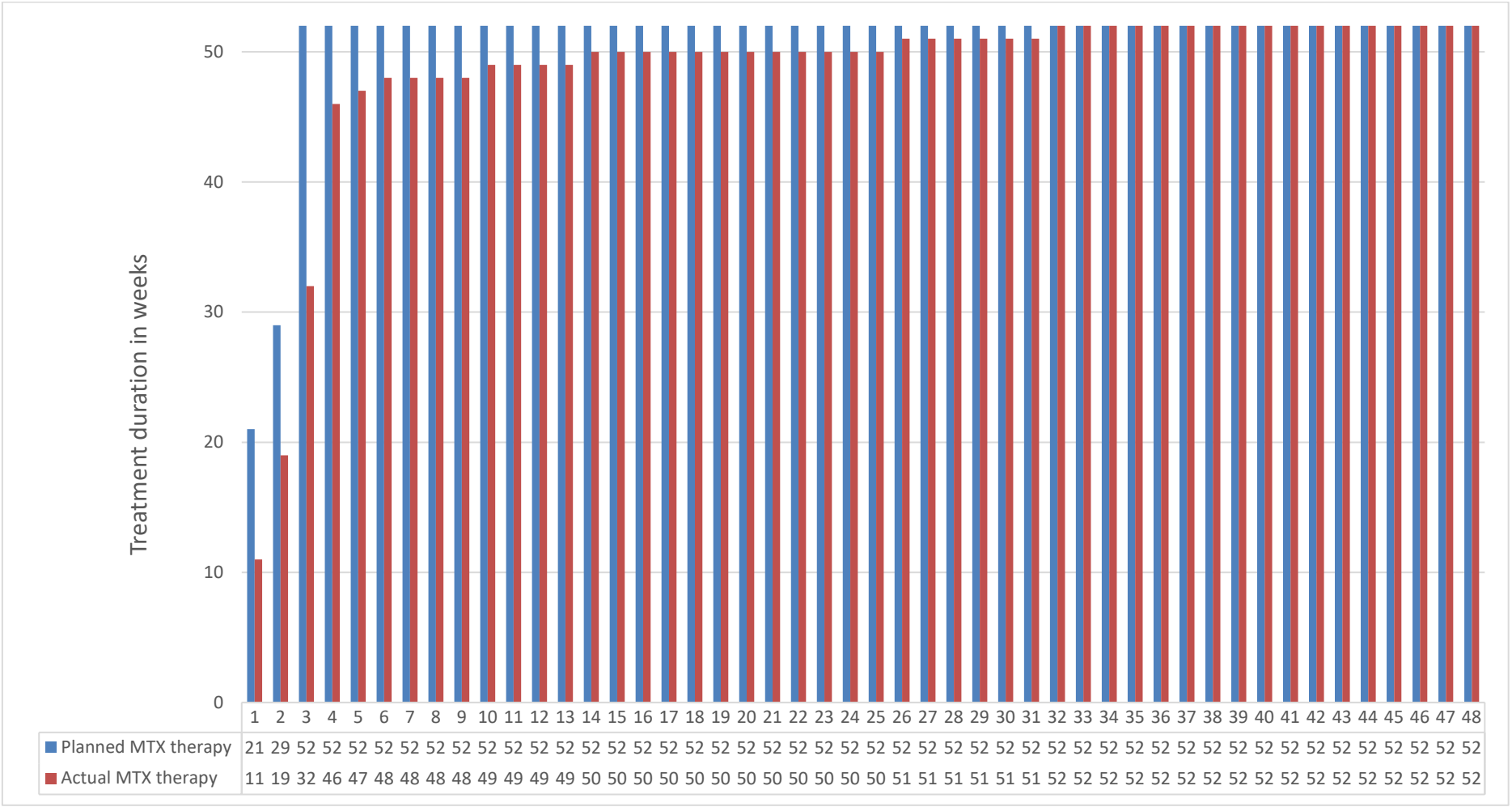
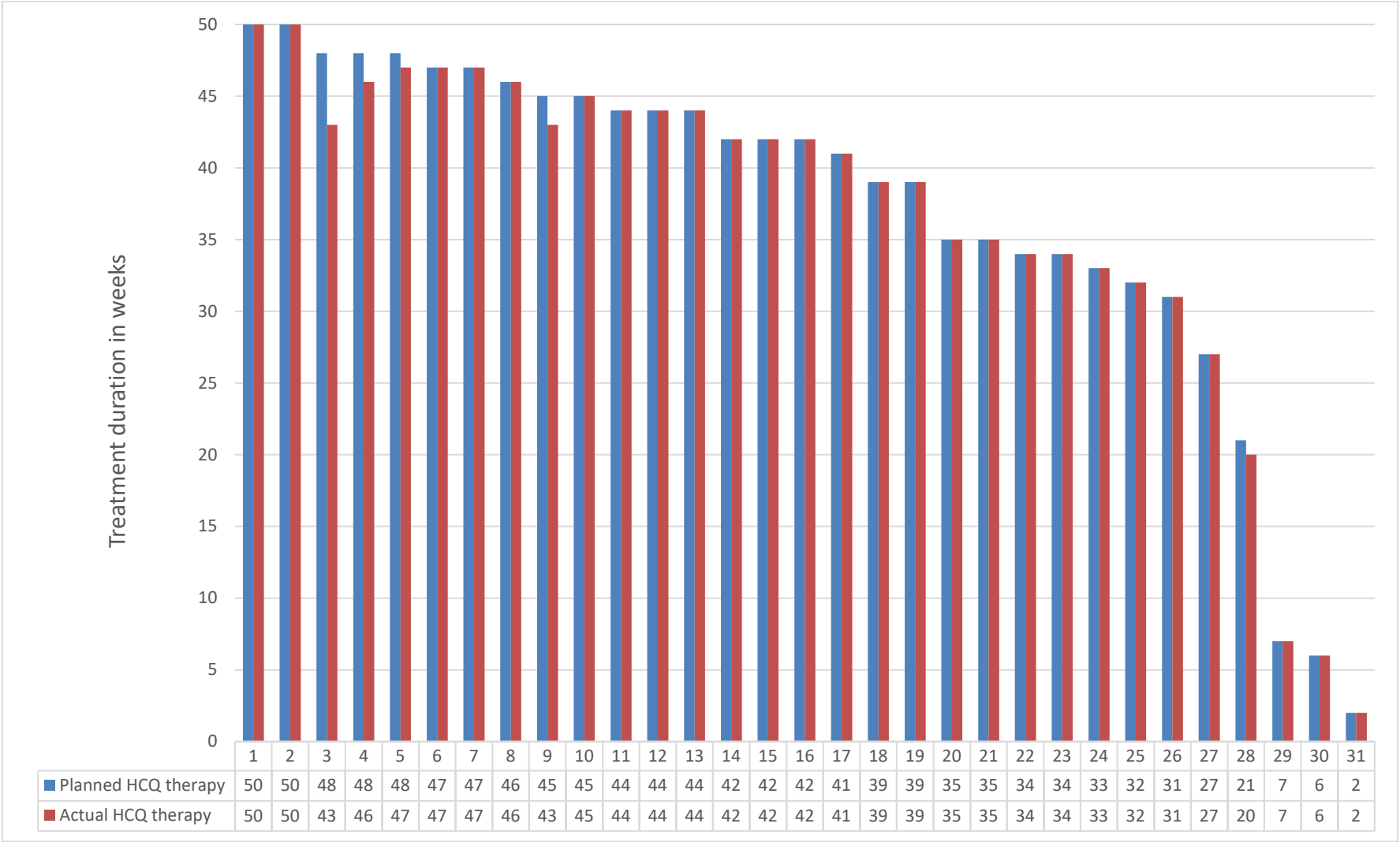


Figure 4-12 Treatment duration of hydroxychloroquine (prescribed and actual). N=31.



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Planned HCQ therapy	50	50	48	48	48	47	47	46	45	45	44	44	44	42	42	42	41	39	39	35	35	34	34	33	32	31	27	21	7	6	2
Actual HCQ therapy	50	50	43	46	47	47	47	46	43	45	44	44	44	42	42	42	41	39	39	35	35	34	34	33	32	31	27	20	7	6	2

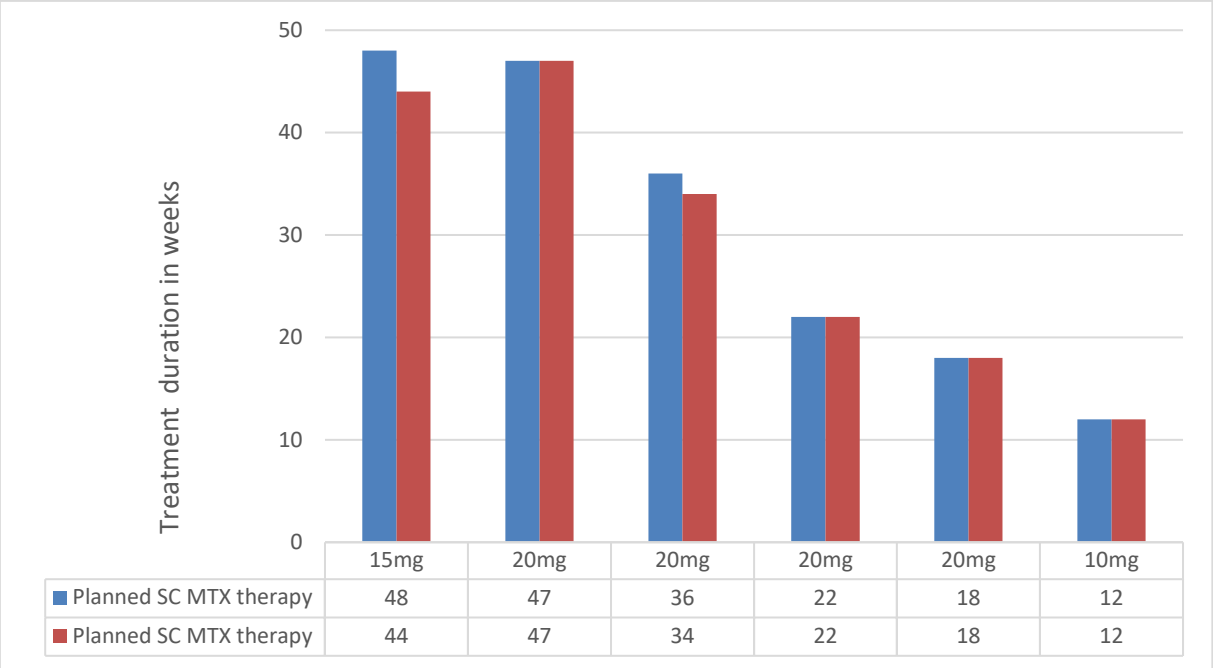


Figure 4-13 Treatment duration of SC methotrexate (prescribed and actual) and weekly dose. N=6

Figure 4-13 shows the treatment duration of SC methotrexate (prescribed and actual) over the 52 week study period. Six patients were on SC methotrexate. Reasons for switching were intolerable GI side effect with oral therapy (n=5) and to improve efficacy (n=1). Reason for missed SC MTX dose was infection (n=2). The maximum tolerated dose of SC MTX ranged from 10mg – 20mg/week. The duration of treatment was between 12-48 weeks.

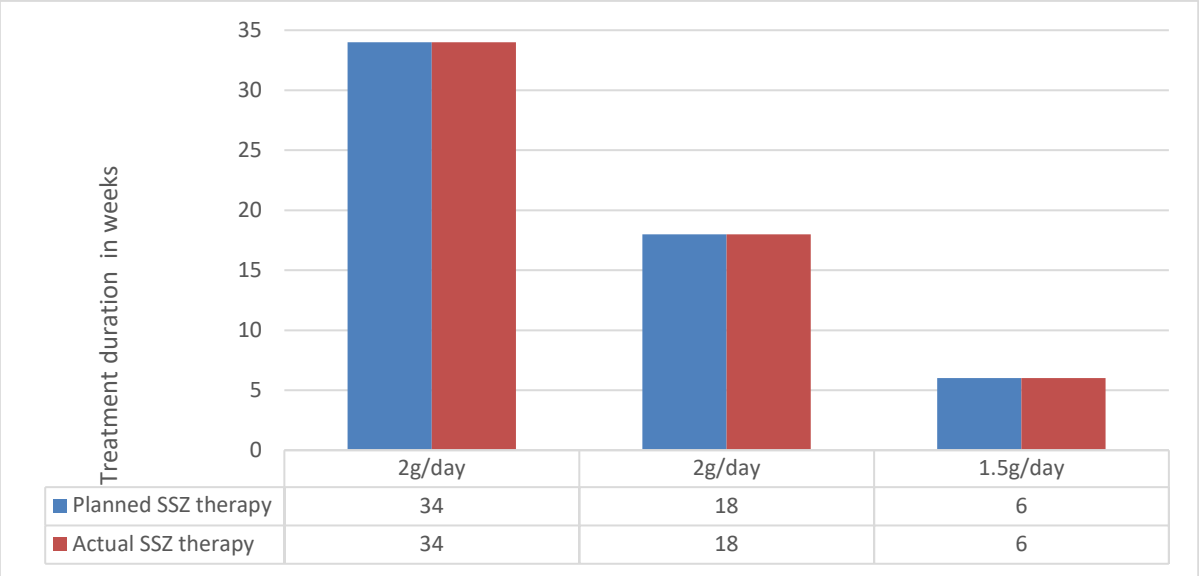


Figure 4-14 Treatment duration of Sulfasalazine (prescribed and actual). N=3.

Figure 4-14 shows the treatment duration of sulfasalazine (prescribed and actual) over the study period. Only three patients were prescribed sulfasalazine as an alternative second DMARD. The dose ranging from 1.5g-2g/day and treatment duration between 6 to 34 weeks.

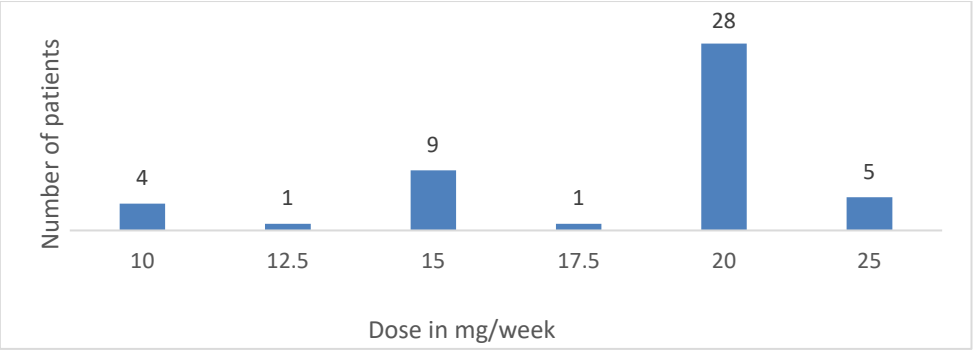


Figure 4-15 Distribution of oral methotrexate weekly dose. N=48.

Figure 4-15 shows the distribution of oral methotrexate weekly dose. This was the highest tolerated oral MTX dose for each patient. 33 out of 48 patients were on ≥ 20mg/week. 43 out of 48 patients were on ≥ 15mg/week.

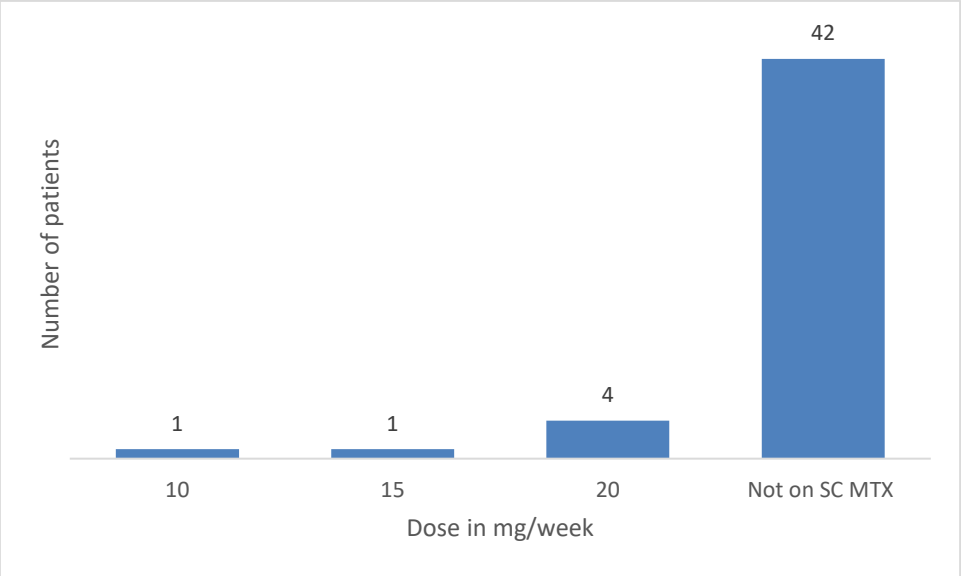


Figure 4-16 Distribution of SC methotrexate weekly dose.

Figure 4-16 shows the distribution of SC methotrexate highest tolerated weekly dose. Only six patients were prescribed SC methotrexate. The dose ranged from 10-20mg/week.

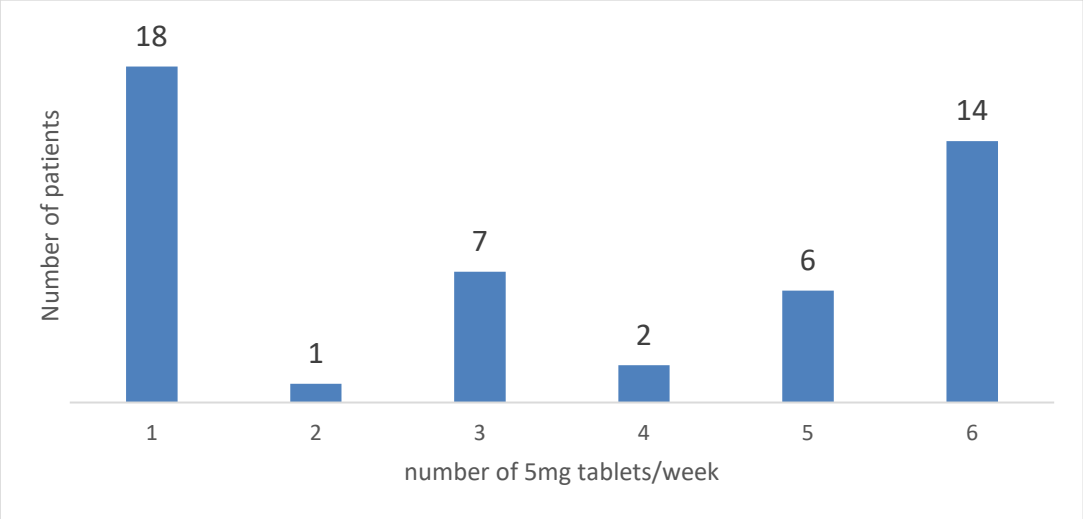


Figure 4-17 Distribution of folic acid weekly dose. N=48.

Figure 4-17 shows the distribution of folic acid weekly dose. The biggest proportion were once weekly, followed by six times/week. All patients were commenced on once weekly, then increased if they developed side effects (GI related, hair loss, mouth ulcer, fatigue, neutropenia).

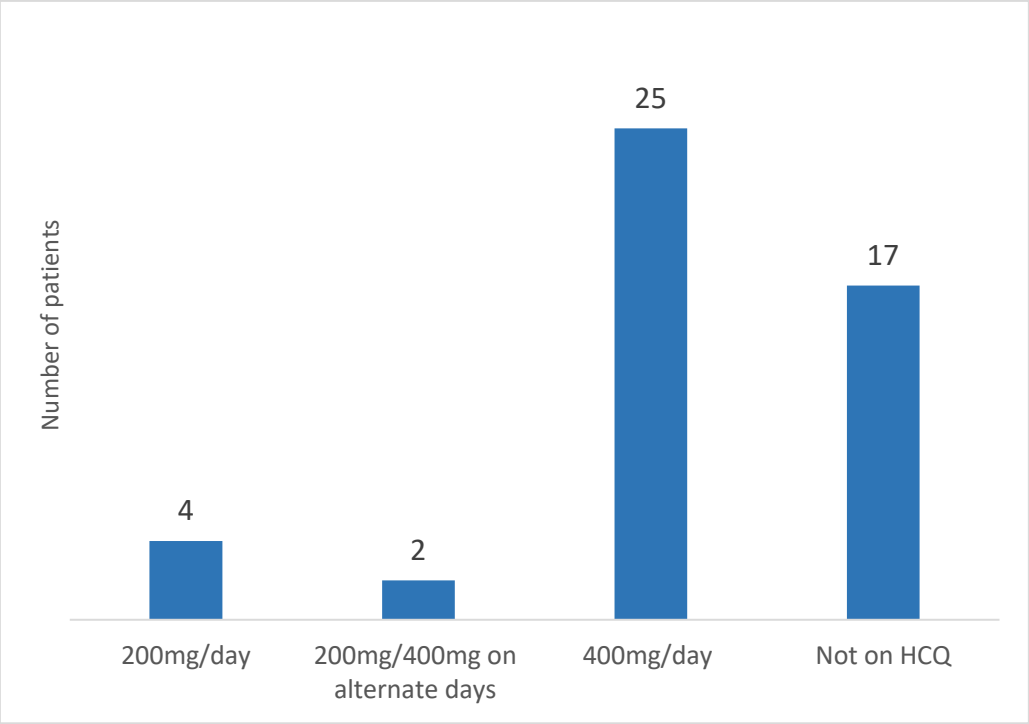


Figure 4-18 Distribution of hydroxychloroquine daily dose. n=31

Figure 4-18 shows the distribution of hydroxychloroquine daily dose. The majority of patients were on 400mg daily. The dose prescribed was 5mg/kg of ideal body weight.

Table 4-3 shows the percentage of patients who were on more than one disease-modifying agent. Nearly 60% of patients were on dual therapy and only one had triple therapy (MTX, HCQ and SSZ). The patient who was on triple therapy was also on biologic therapy. This was the decision of the responsible consultant.

Table 4-3 Number of DMARDs.

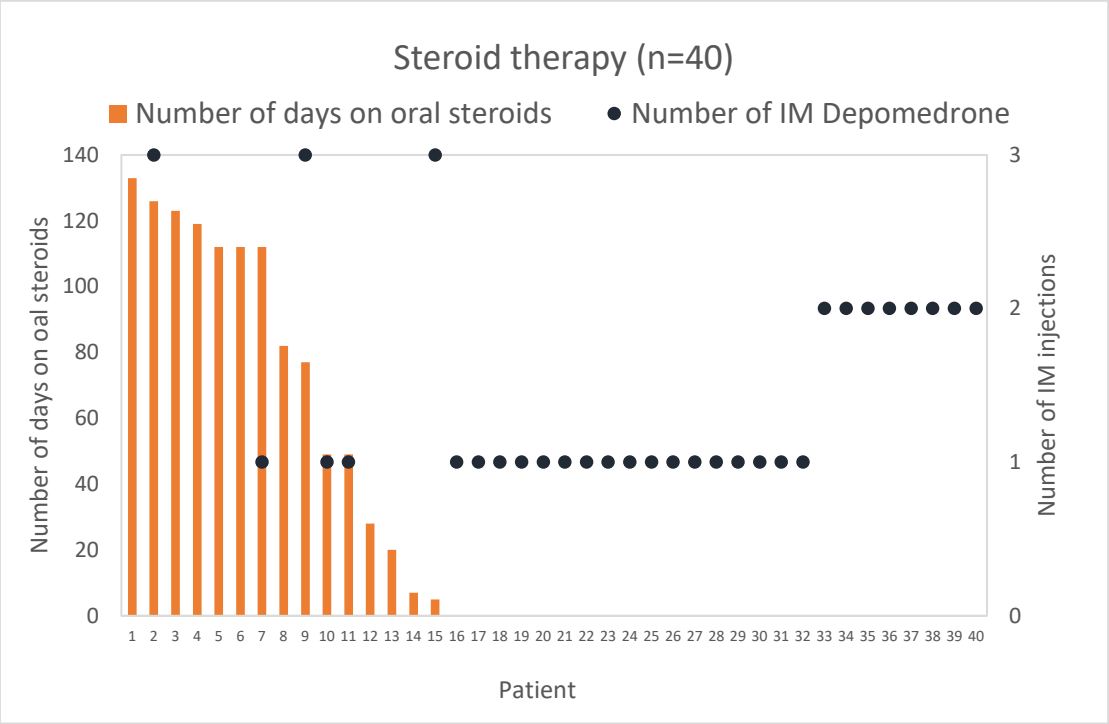
Number of DMARDs	N	Percentage
Monotherapy	19	40
Dual therapy	28	58
Triple therapy	1	2

Table 4-4 shows the details of biologic therapy in this cohort. Six patients were on biologic therapy. The patients who were on rituximab were also part of the STRAP clinical trial from W24.

Table 4-4 Number of patients of biological therapy.

Biologic agent	Number of patients	Week started on biologic therapy
Adalimumab	3	One patient at week 23. Two patients at week 32.
Rituximab	2	Week 29 and 30. One patient was on triple therapy as well.
Benapali	1	Week 32.

Figure 4-19 Steroid therapy.



The x-axis indicates each patient ID. The orange bar indicates the number of days on oral steroids for each patient (primary y-axis). The black dot indicates the number of IM Depomedrone injections for each patient (secondary y-axis).

Figure 4-19 shows the details of steroid therapy. 40 patients required steroid treatment. Number of IM Depomedrone was 0-3 injections per patient with a single dose ranging from 80mg to 160mg. One patient was on oral budesonide for inflammatory bowel disease. Two patients had a short course of prednisolone for COPD exacerbation. Three patients required intra-articular steroid injection (n=2 Depomedrone, n=1 with Kenalog] (not shown on graph).

Figure 4-20 shows both the cumulative dose of methotrexate and hydroxychloroquine over the study period. Cumulative dose of methotrexate per patient ranged from 1210mg to 110mg over one year, with a median of 955mg (IQR 744 to 990mg). The cumulative dose of hydroxychloroquine per patient ranged from 140,000mg to 2800mg. The median dose was 95,200mg (IQR 64,400 to 120,800mg).

I combined the cumulative dose of methotrexate and hydroxychloroquine in a single graph to identify whether those patients who were on lower cumulative dose of methotrexate had higher cumulative dose of hydroxychloroquine and vice-versa. A proportion of patients were able to tolerate high doses of MTX and HCQ, whilst some patients were able to tolerate high dose of one DMARD only.

Figure 4-20 Cumulative dose of methotrexate and hydroxychloroquine over 52 weeks.

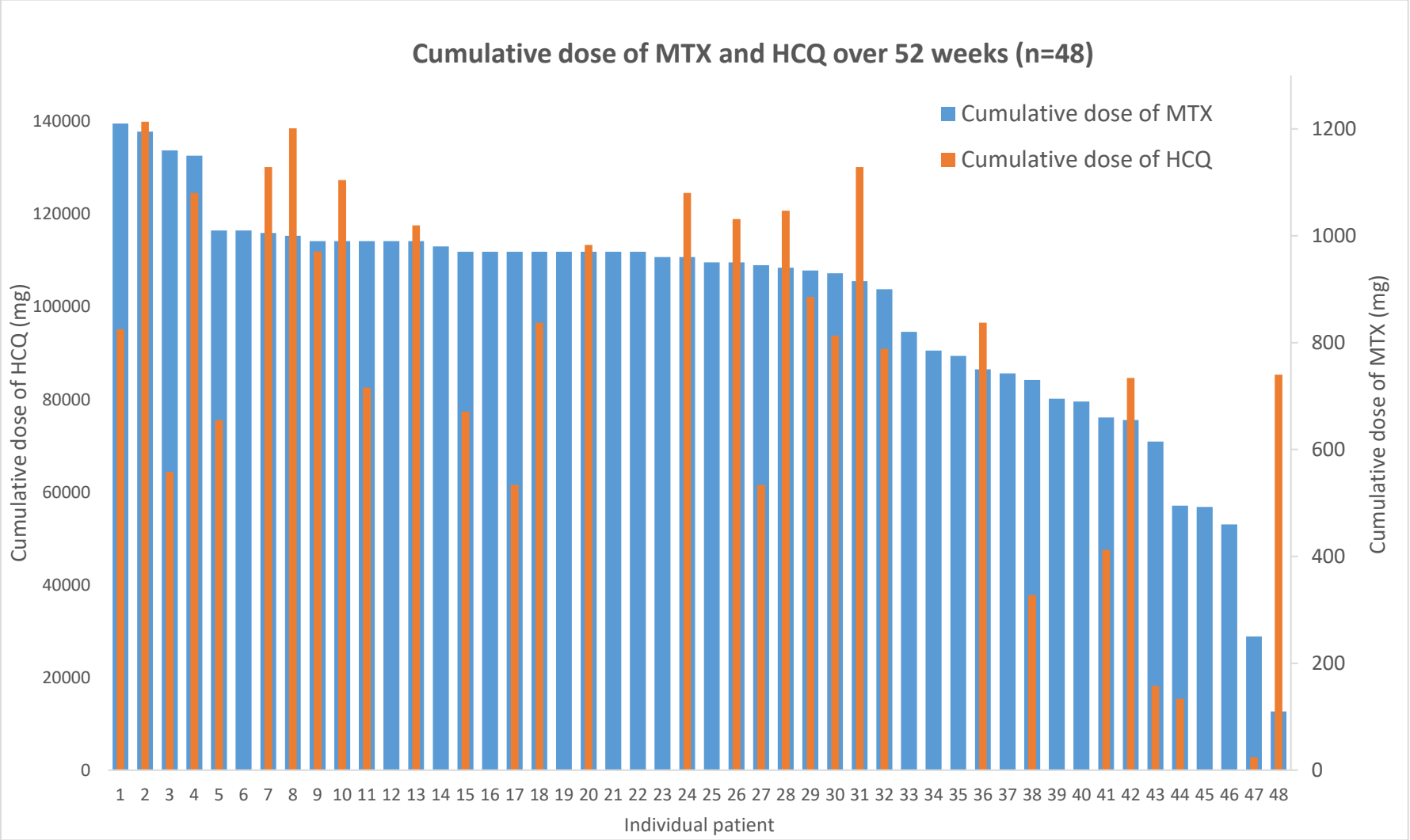


Figure 4-21 Cumulative dose of sulfasalazine over 52 weeks.

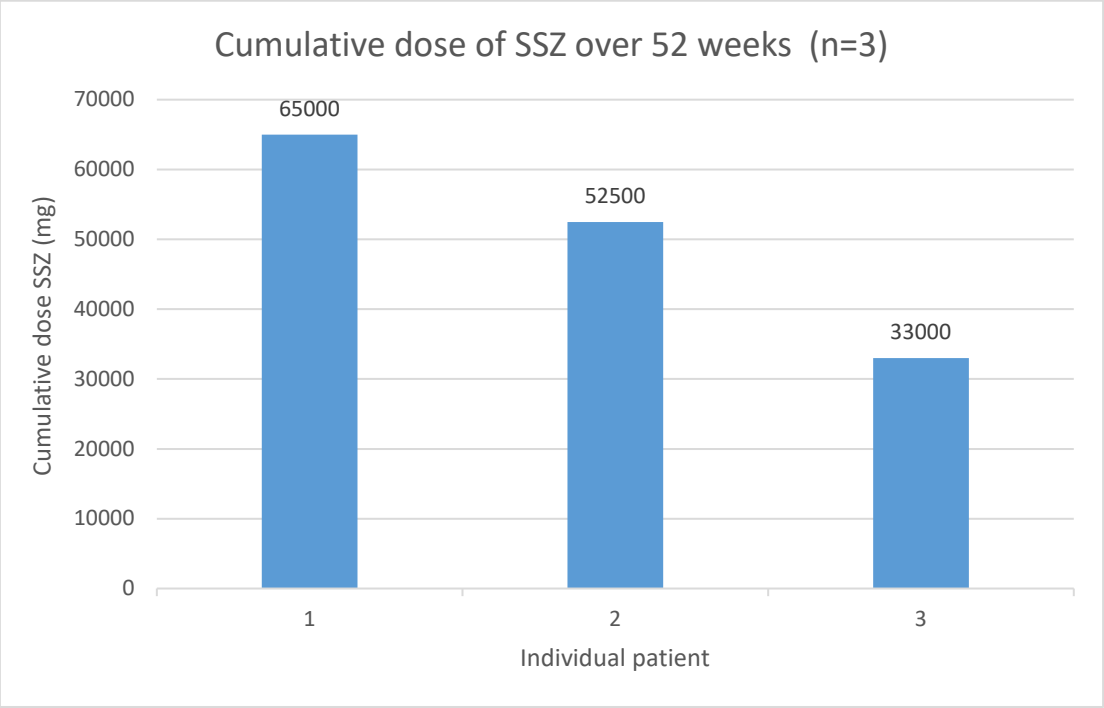


Figure 4-21 shows the cumulative dose of sulfasalazine over the study period. Only three patients were prescribed sulfasalazine as a second DMARD. The dose ranged 65,000mg to 33,000mg over 52 weeks.

Figure 4-22 Cumulative dose of steroid therapy in oral prednisolone equivalent dose.

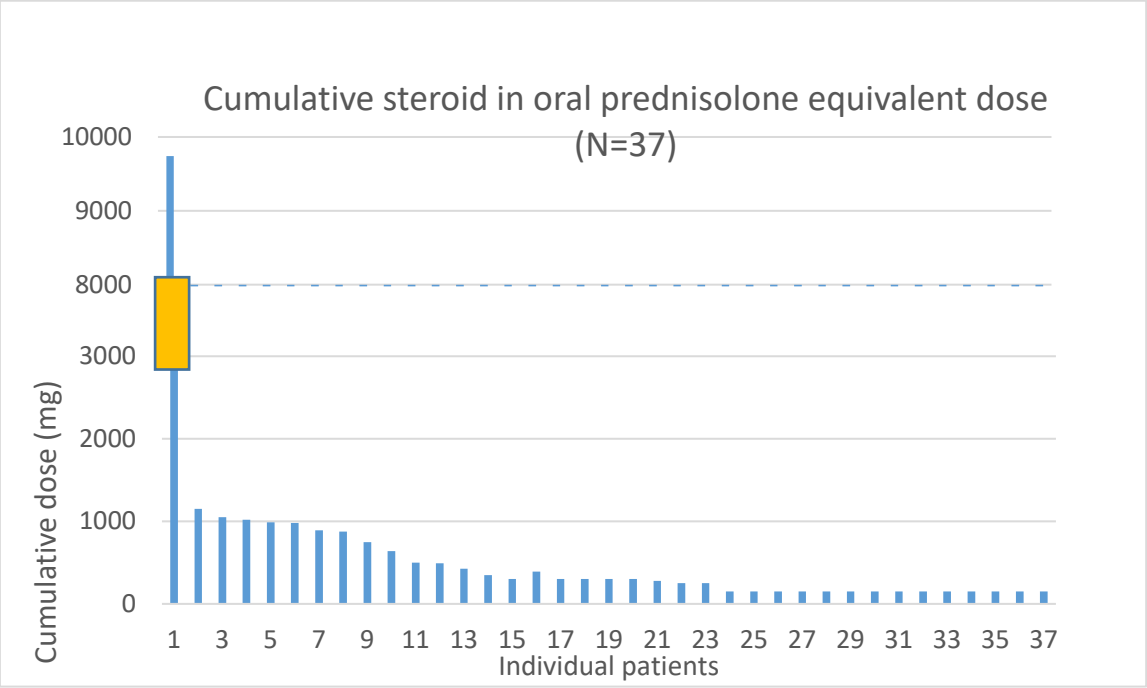


Figure 4-22 illustrates the overall steroid load. Patients had either intra-muscular Depomedrone, oral prednisolone or intra-articular injection. This was determined based on clinical need, although patient and physician's preferences play a significant role in this decision. Each patient had different permutations of steroid therapy (see Appendix). Hence, I took the pragmatic approach of converting the IM Depomedrone dose into the oral prednisolone equivalent. Patient 1 was on oral budesonide for 126 days for inflammatory bowel disease which was diagnosed during the study. This patient developed scleritis towards the end of the study (and was also on SC methotrexate and hydroxychloroquine).

Intra-articular steroids

Three patients had intra-articular (IA) steroids. One patient had 60mg of IA Depomedrone, one patient had 60mg of IA triamcinolone and one patients receive a total of 140mg IA Depomedrone. These are the sum of steroid dose for each patient (i.e. not dose per IA

injection). The intra-articular steroids are not included in the calculation for Figure 4-22. Appendix 4 summarises the treatment strategy of individual patients.

4.3.5 Therapeutic response and time-to-therapy.

Figure 4-23 shows the scatterplot of the relationship between therapeutic response and time-to-therapy from T0, T1 and T2 according to the mode of onset. Three patients with palindromic onset had very long time-to-therapy from T0 (T0>350 weeks). In addition, two and one patients had a very long time-to-therapy from T1 and T2, respectively (T1> 350 weeks and T2> 400 weeks). The palindromic patients are subsequently excluded from the linear regression analysis as they were outliers. Abrupt onset patients were more likely to have shorter symptom duration compared to insidious onset patients. This was true for T0, T1 and T2.

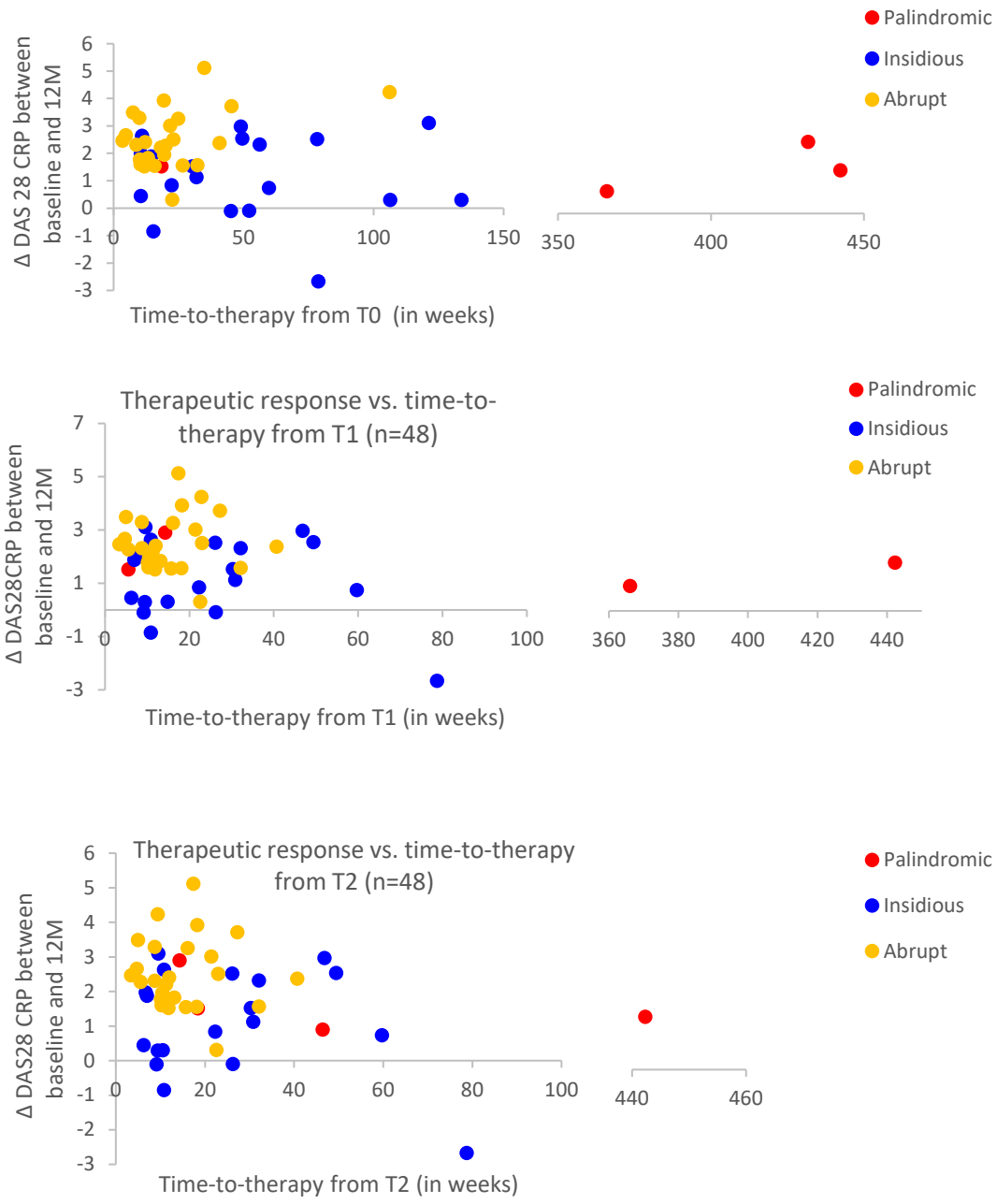
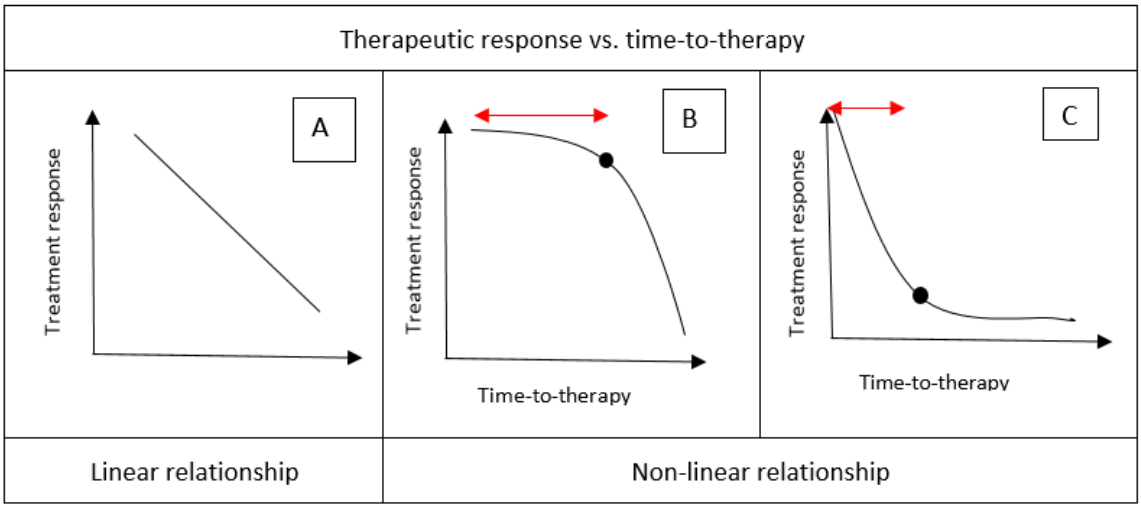


Figure 4-23 Scatterplot of therapeutic response vs. time-to-therapy by mode of onset.

4.3.6 Linear vs non-linear relationship between therapeutic response and time-to-therapy.

First, I assessed whether there was a **linear or non-linear relationship between therapeutic response and time-to-therapy.** It is important to understand this association in order to accurately assess the duration of the window of opportunity. If there was **a non-linear relationship** between treatment response and time-to-therapy**,** then the **'**end of therapeutic window of opportunity' would be at the point of inflexion as shown in the hypothetical figure Figure 4-24 B and C below.

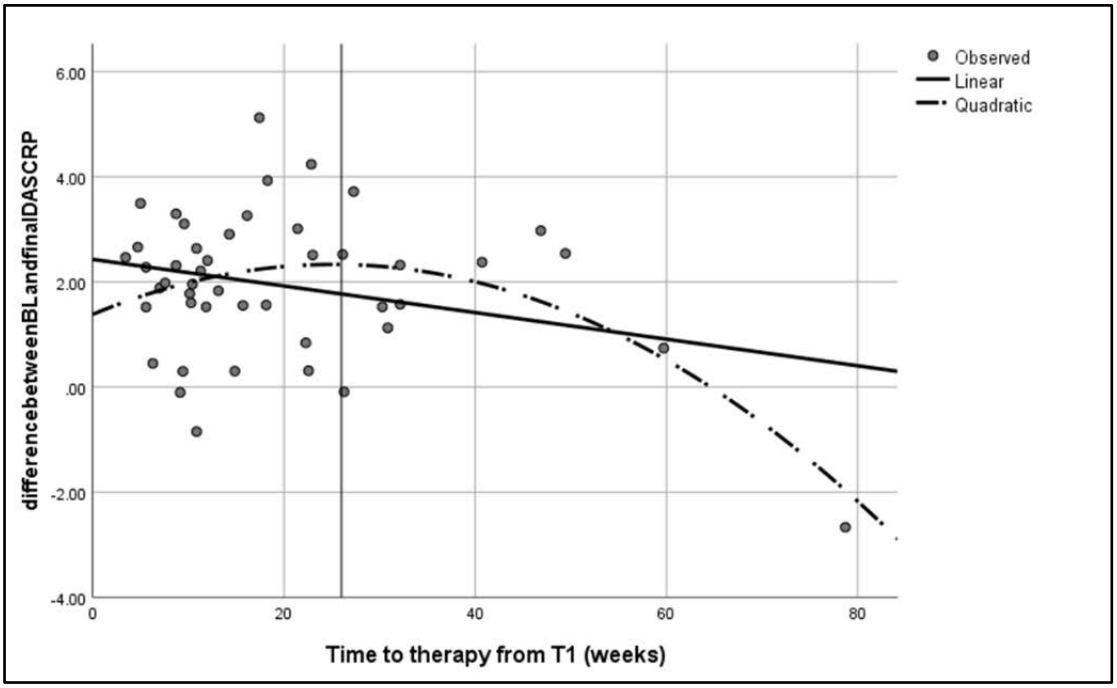
Figure 4-24 Hypothetical graphs to illustrate the relationship between therapeutic response and time-to-therapy.



The black circle on the graph denotes the point of inflexion in the non-linear relationship; therapeutic response rate before and after this point is significantly different. The red arrows mark the therapeutic window of opportunity.

In order to address this question, I modelled the relationship between DAS-28 CRP response between baseline and 12 months (as a measure of therapeutic response) and time-to-therapy from T1 in weeks (the primary outcome) with one linear model one non-linear model to assess the best model fit.

Figure 4-25 Association between therapeutic response and time-to-therapy from T1.



Grey circles represent each patient. The dotted line represents the non-linear model (quadratic equation);the black line represents the linear model.

Table 4-5 Linear and non-linear modelling.

Model	R squared	R squared change	F change	Sig F change
1[a]	0.078	0.078	3.733	0.060
2[b]	0.228	0.150	8.348	0.006

aPredictors: (Constant), Time to therapy from T1 (weeks)
bPredictors: (Constant), Time to therapy from T1 (weeks), T-2-T (weeks from T1) squared
Dependent Variable: Difference between baseline and final DASCRP

In the Table 4-5, model 1 is the linear relationship whilst model 2 is the non-linear relationship. This analysis showed that the non-linear element accounted for 15% of the model whilst the linear element accounted for only 7.8% (as shown by R-squared change). Therefore, the non-linear (i.e. quadratic) equation would explain 22.8% variance observed in this model. In other words, the time-to-therapy from T1 as non-linear relationship would account for nearly 23% of the therapeutic response, as measured in this sample. (The remaining 77% may be account

by other factors e.g., age, gender, treatment strategy, autoantibody profile or other unknown factors that affect treatment response).

In this model, the point of inflexion is marked by the grey vertical line at 26 weeks (Figure 4-25). However, this model was highly influenced by a single patient with a T-2-T of > 70. This patient was removed, on advice of the statistician, resulting in the linear model fitting better for this sample.

Figure 4-26 Linear and non-linear modelling of treatment response vs time-to-therapy with and without outlier.

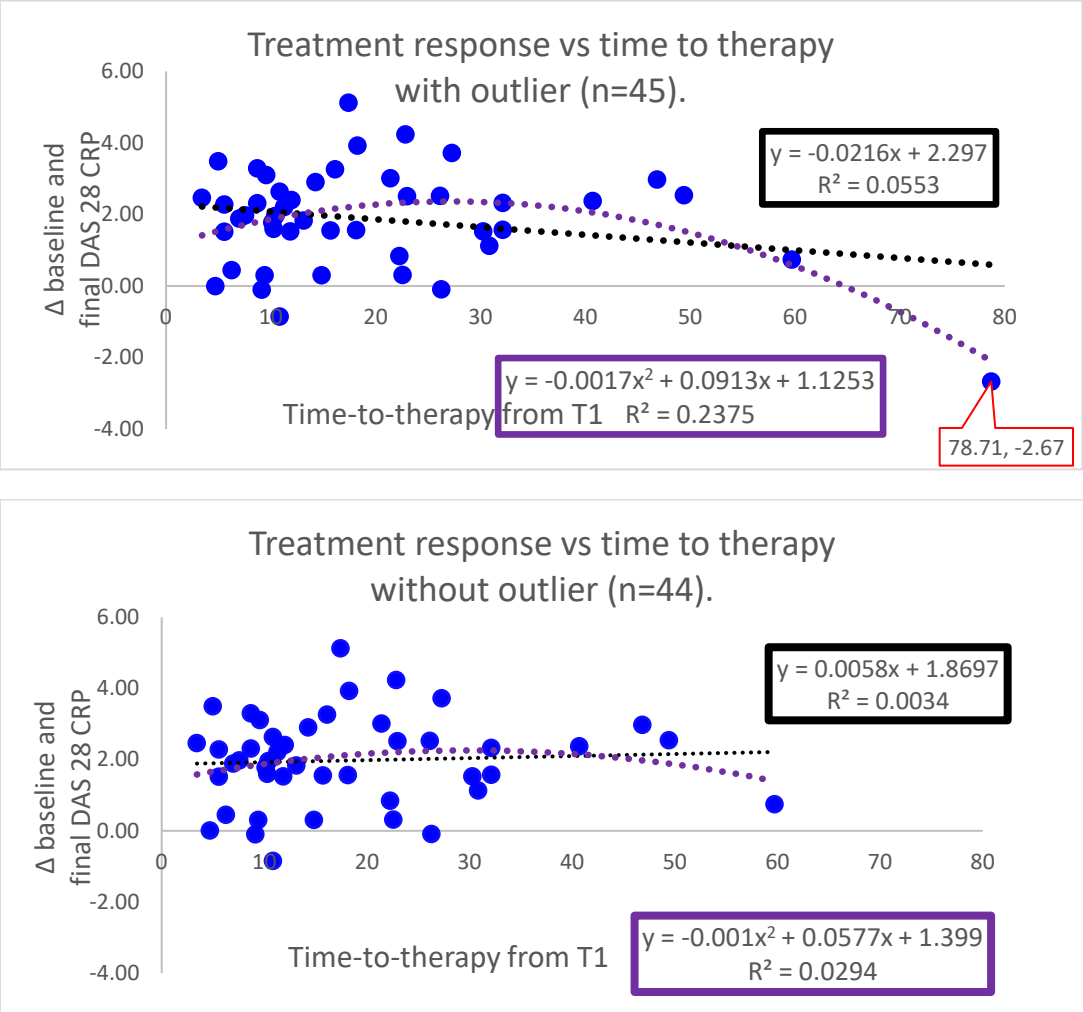


Figure 4-26 illustrates the effect of the outlier case graphically, showing the same analysis with and without the outlier. The equations within the purple box and black box denote the non-linear and linear models, respectively. The dotted purple line and black line denote the non-linear and linear models, respectively, between treatment response and time-to-therapy from T1. The time point highlighted in figure A by the red box is the outlier. Removal of the outlier (marked by the red box), caused a change of R squared of the non-linear equation from 0.2375 to 0.0294.

Nevertheless, this analysis highlights that more patients with longer symptom durations are required in order to elucidate whether the overall relationship is linear or non-linear. More observations, in particular with patients with time to therapy from T1 of >50 weeks would give us a clearer picture of this relationship.

For the rest of the analysis in this thesis, patients with very long time-to-therapy (i.e. outliers) were excluded from the analysis. Two patients had time-to-therapy for T1 as more 300 weeks, and one patient had T2T from T1 of more than 60 weeks.

4.3.7 Linear regression: Therapeutic response vs. time-to-therapy.

In order to assess the impact of time-to-therapy on therapeutic response, I took into account the factors that are known (or thought) to effect therapeutic response. In this analysis, I assessed whether these variables affect therapeutic response from each time-to-therapy (T0, T1 and T2). These variables were: a) mode of onset, b) baseline disease activity score, c) anti-CCP antibody status, d) age and sex.

4.3.7.1 Therapeutic response vs. time-therapy after adjusting for mode of onset.

Figure 4-27 Scatterplot of therapeutic response vs. time-therapy from T0, by mode of onset.

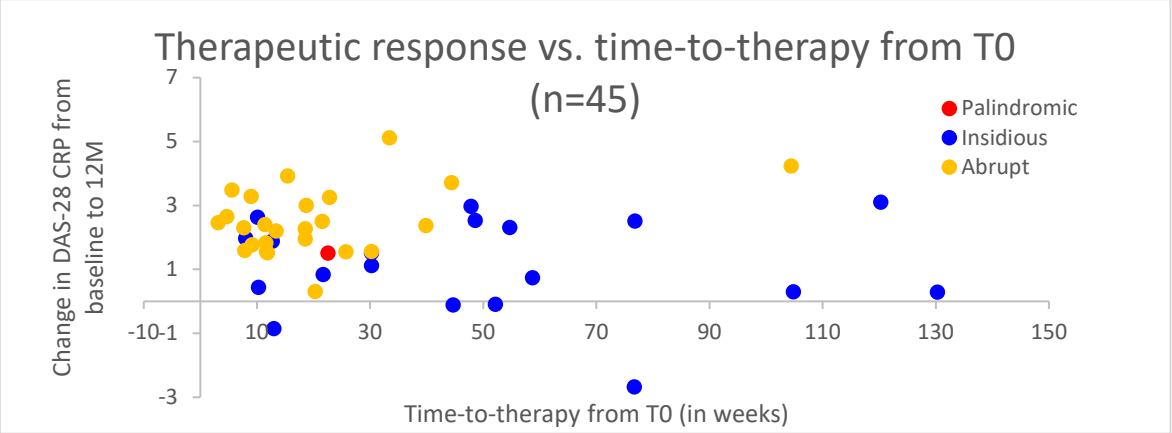


Table 4-6 Linear regression model for time-to-therapy from T0, adjusted for mode of onset.

Model	Independent predictors	B	95% CI for B	p	R square
Model 1	Time-to-therapy from T0	-0.006	-0.02 to 0.007	0.347	0.021
Model 2	Time-to-therapy from T0	0.004	-0.009 to 0.018	0.548	0.245[a]
	Mode of onset	1.502	0.631 to 2.373	0.001	

[a]Sig F change from model 1 to 2 is 0.001, n=44 after excluding palindromic arthritis and T2T from T0 > 150 weeks. Dependent variable was change in DAS-28 CRP from baseline to 12M. Mode of onset; 1 = abrupt.

This analysis illustrated the relationship between therapeutic response and time-to-therapy from T0, after taking into account mode of onset. If a patient had an abrupt onset RA, the difference in Δ DAS28-CRP from baseline to 12M was +1.50 unit, compared to a patient with insidious onset disease, at a given time-to-therapy from T0. Mode of onset alone accounted for 22.4% of the therapeutic response observed in this model. Note that the time-to-therapy from T0 variable was not statistically significant in either model

4.3.7.2 Therapeutic response vs. time-therapy from T1, adjusting for mode of onset.

Figure 4-28 Scatterplot of therapeutic response vs. time-therapy from T1, by mode of onset.

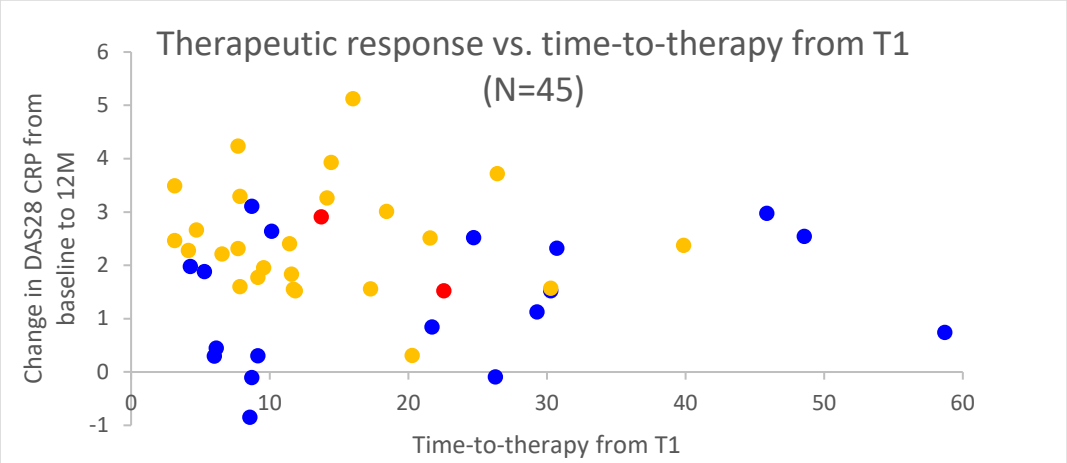


Table 4-7 Linear regression model for time-to-therapy from T1, adjusted for mode of onset.

Model	Independent predictors	B	95% CI for B	p	R square
Model 1	Time-to-therapy from T1	0.000	-0.030 to 0.031	0.980	0.000
Model 2	Time-to-therapy from T1	0.014	-0.014 to 0.042	0.322	0.239[a]
	Mode of onset	1.273	0.547 to 1.998	0.001	

[a]Sig F change from model 1 to 2 is 0.001. n=43 after excluding palindromic onset and T2T from T1 > 70 weeks. Dependent variable was change in DAS-28 CRP from baseline to 12M. Mode of onset; 1 = abrupt.

This model showed the relationship between therapeutic response and time-to-therapy from T1, taking into account the mode of onset. A patient with abrupt onset disease had a difference in ΔDASCRP from baseline to 12M by +1.27 unit, compared to a patient with insidious onset a given time-to-therapy from T1. Mode of onset alone accounted for 24% of the therapeutic response observed in this sample. Note that the time-to-therapy from T1 was not statistically significant in both models.

4.3.7.3 Therapeutic response vs. time-therapy from T2, adjusting for mode of onset.

Figure 4-29 Scatterplot of therapeutic response vs. time-therapy from T2, by mode of onset.

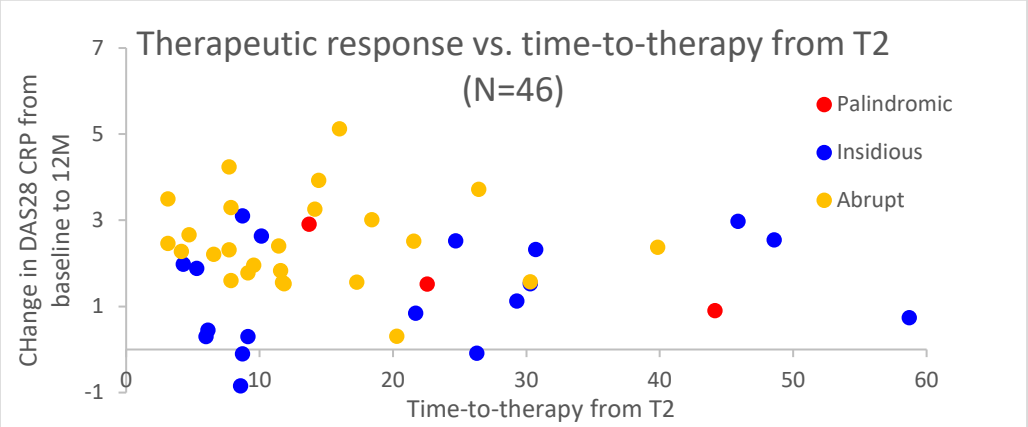


Table 4-8 Linear regression model for time-to-therapy from T2, adjusted for mode of onset.

Model	Independent predictors	B	95% CI for B	p	R square
Model 1	Time-to-therapy from T2	-0.003	-0.033 to 0.275	0.856	0.001
Model 2	Time-to-therapy from T2	0.0141	-0.017 to 0.038	0.440	0.232[a]
	Mode of onset	1.253	0.523 to 1.984	0.001	

[a]Sig F change from model 1 to 2 is 0.001. n=43 after excluding palindromic onset and T2T from T2 > 70 weeks. Dependent variable was change in DAS-28 CRP from baseline to 12M. Mode of onset; 1 = abrupt.

This model assessed the relationship between therapeutic response vs. time-to-therapy from T2 adjusting for mode of onset. Patients with an abrupt onset would have a difference in ΔDASCRP from baseline to 12M as +1.25 unit, compared to a patient with insidious onset group at a given time-to-therapy from T2. Interestingly, mode of onset accounted for 23% of the therapeutic response observed in this sample. Note that, again, the time-to-therapy variable from T2 was not statistically significant in either model.

Summary for linear regression model; mode of onset.

The time-to-therapy variables measured from T0, T1 or T2 appeared to make no significant additional contribution to the therapeutic response, whether mode of onset was taken into account or not. Importantly, mode of onset had a sizeable impact on therapeutic response (23.2% to 24.5%). This was true whether the time-to-therapy was measured from T0, T1 and T2. Patients with abrupt onset had a bigger change in DAS compared to those with insidious onset (range from +1.25 to +1.50) at a given time-to-therapy. Due to the small number of palindromic onset (n=4), this group was not included in the regression analysis.

4.3.7.4 Therapeutic response vs. time-therapy, adjusting for baseline DAS28.

Table 4-9 to Table 4-11 show the linear regression model with time-to-therapy from T0, T1 and T2 respectively, taking into account baseline DAS28 CRP. Baseline DAS-28 CRP was a significant predictor in the therapeutic response; for each increase of 1 unit of baseline DAS28 CRP, the difference in ΔDASCRP from baseline to 12M was +0.38 for T0 and+ 0.31 for T1 and T2. The time-to-therapy was not a statistically significant predictor. The baseline DAS28 CRP accounted for 15% in the therapeutic response observed in this sample.

Table 4-9 Therapeutic response vs. time-therapy from T0, adjusting for baseline DAS28.

Model	Independent predictors	B	95% CI for B	P value	R square
Model 1	Time-to-therapy from T0	-0.006	-0.020 to 0.008	0.008	0.018
Model 2	Time-to-therapy from T0	-0.002	-0.15 to 0.012	0.822	0.167[a]
	Baseline DAS28 CRP	0.378	0.092 to 0.663	0.011	

aSig. F Change=0.011, n=43 after excluding palindromic onset and T2T from T0 > 150 weeks. Dependent variable was change in DAS-28 CRP from baseline to 12M.

Table 4-10 Therapeutic response vs. time-therapy from T1, adjusting for baseline DAS28.

	Independent predictors	B	95% CI for B	P value	R square
Model 1	Time-to-therapy from T1	0.002	-0.029 to 0.033	0.912	0.000
Model 2	Time-to-therapy from T1	-0.002	-.031 to 0.028	0.919	0.132[a]
	Baseline DAS28 CRP	0.306	0.110 to 0.640	0.020	

. aSig. F Change=0.020, n=42 after excluding palindromic onset and T2T from T1 > 70 weeks. Dependent variable was change in DAS-28 CRP from baseline to 12M.

Table 4-11 Therapeutic response vs. time-therapy from T2, adjusting for baseline DAS28.

	Independent predictors	B	95% CI for B	P value	R square
Model 1	Time-to-therapy from T2	-0.002	-.032 to .029	0.920	0.000
Model 2	Time-to-therapy from T2	-0.004	-.033 to 0.025	0.378	0.134[a]
	Baseline DAS28 CRP	0.306	0.053 to .560	0.019	

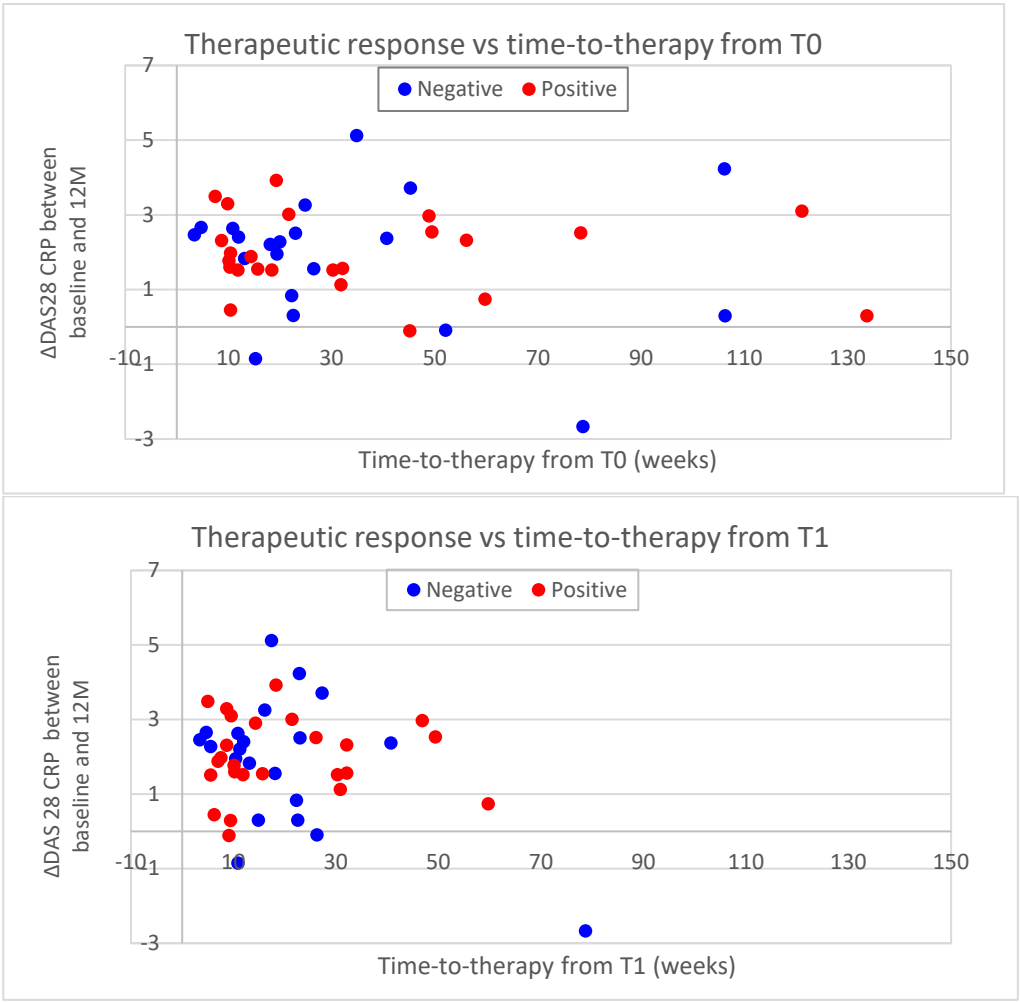
aSig. F Change=0.019, n=42 after excluding palindromic onset and T2T from T2 > 70 weeks. Dependent variable was change in DAS-28 CRP from baseline to 12M.

4.3.7.5 Therapeutic response vs. time-therapy, adjusting for anti- CCP antibody.

Figure 4-30 show the scatter plot of therapeutic response vs. time-to-therapy from T0, T1 and T2 by anti-CCP antibody status. Table 4-12 to Table 4-14 shows the linear regression analysis

between therapeutic response and time-to-therapy from T0, T1 and t2 respectively taking into account of anti CCP status. There was no impact of CCP positivity on therapeutic response whether time-to-therapy was from T0, T1 or T2.

Figure 4-30 Scatter plot of therapeutic response vs. time-to-therapy, by anti-CCP antibody status.



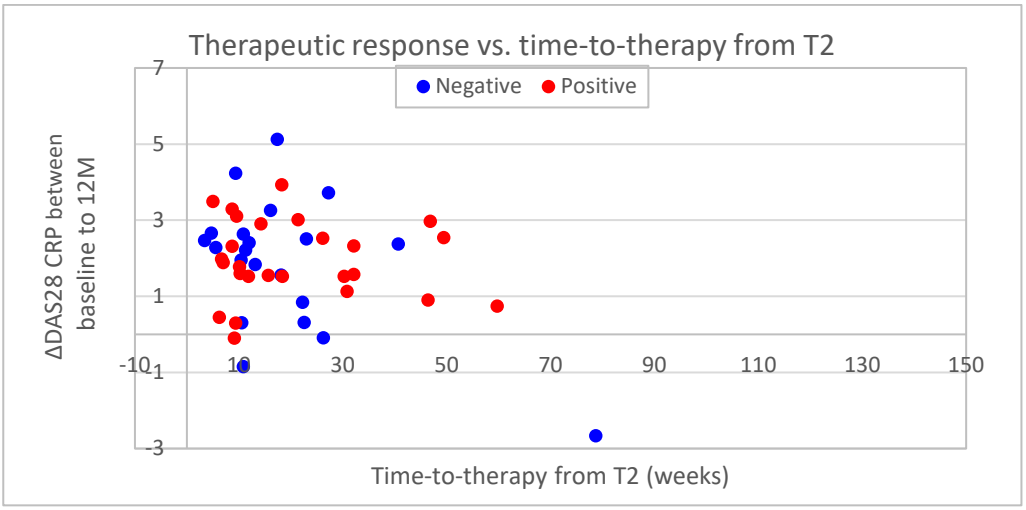


Table 4-12 Therapeutic response vs. time-therapy from T0, adjusting for anti-CCP status.

Model	Independent predictors	B	95% CI for B	p	R square
Model 1	Time-therapy from T0	-0.006	-0.020 to 0.007	0.354	0.020
Model 2	Time-therapy from T0	-0.006	-0.020 to 0.007	0.355	0.022[a]
	CCP positivity	0.11	-0.750 to 0.970	0.798	

aSig F change from model 1 to 2 is 0.798. n=45 after excluding T2T from T0 > 150 weeks. Dependent variable was ΔDAS-28 CRP from baseline to 12M. CCP status; coded 1 = positive.

Table 4-13 Therapeutic response vs. time-therapy from T1, adjusting for anti-CCP status.

Model	Independent predictors	B	95% CI for B	p	R square
Model 1	Time-therapy from T1	0.001	-0.029 to 0.030	0.958	0.000
Model 2	Time-therapy from T1	0.001	-0.029 to 0.031	0.936	0.002[a]
	CCP positivity	-0.096	-0.862 to 0.669	0.801	

aSig F change from model 1 to 2 is 0.801. n=45 after excluding T2T from T1 > 70 weeks. Dependent variable was ΔDAS-28 CRP from baseline to 12M. CCP status; coded 1 = positive

Table 4-14 Therapeutic response vs. time-therapy from T2, for anti-CCP status.

Model	Independent predictors	B	95% CI for B	p	R square
Model 1	Time-therapy from T2	-0.007	-0.035 to 0.021	0.624	0.006
Model 2	Time-therapy from T2	0.014	-0.035 to 0.023	0.672	0.007[a]
	CCP positivity	-0.104	-0.869 to 0.661	0.786	

[a]Sig F change from model 1 to 2 is 0.786. n=46 after excluding T2T from T2 > 70 weeks. Dependent variable was ΔDAS-28 CRP from baseline to 12M. CCP status; coded 1 = positive

4.3.7.6 Therapeutic response vs. time-therapy, adjusting for age and sex.

Figure 4-31 shows the scatterplot of therapeutic response from T0, T1 and T2 by age. The darker the blue marker dots the higher the age.

Figure 4-31 Therapeutic response vs. time-therapy from T0, T1 and T2, by age (n=48)

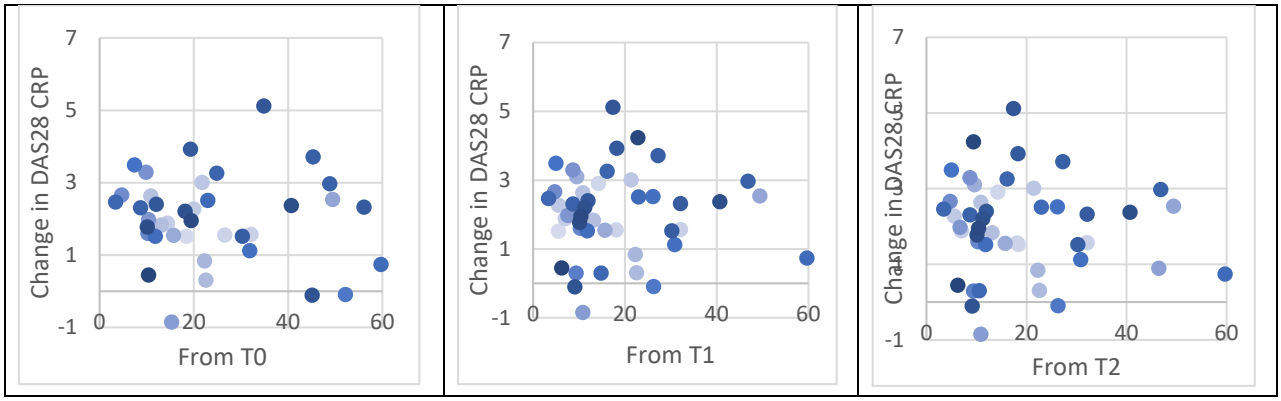


Table 4-15 to Table 4-17 show the linear regression analysis between therapeutic response and time-to-therapy from T0, T1 and T2 respectively, after taking into account age and sex. Note that the p values for time-to-therapy from T2 in model 2 and 3 indicate statistical significance; however the size effect was small and thus unlikely to be clinically meaningful. A one week increase in time-to-therapy from T2 would result in ΔDAS 28 CRP score by -0.03 unit (with 95% CI of -0.056 to -0.001.) Overall, there was no significant impact of age or sex on the therapeutic response at a given time-to-therapy, from all 3 time points.

Table 4-15 Therapeutic response vs. time-therapy from T0, adjusting for age and sex.

	Independent predictors	B	95% CI for B	P value	R square
Model 1	Time-to-therapy from T0	-0.006	-0.02 to 0.007	0.347	0.021
Model 2	Time-to-therapy from T0	-0.006	-0.02 to 0.008	0.365	0.021
	Age at onset	-0.001	-0.03 to 0.028	0.942	
Model 3	Time-to-therapy from T0	-0.006	-0143 to -0.898	0.375	0.021
	Age at onset	-0.001	-0.011 to -0.066	0.948	
	Sex	0.013	0.004 to 0.023	0.982	

n=44 after excluding palindromic. Dependent variable was ΔDAS-28 CRP from baseline to 12M.

Table 4-16 Therapeutic response vs. time-therapy from T1, adjusting for age and sex.

	Independent predictors	B	95% CI for B	P value	R square
Model 1	Time-to-therapy from T1	-0.026	-0.053 to 0.001	0.061	0.081
Model 2	Time-to-therapy from T1	-0.026	0.065 to -0.055	0.065	0.082
	Age at onset	0.002	0.861 to -0.025	0.861	
Model 3	Time-to-therapy from T1	-0.027	-0.055 to 0.002	0.066	0.084
	Age at onset	0.003	-0.025 to 0.032	0.816	
	Sex	0.146	-0.911 to 1.203	0.782	

n=44 after excluding palindromic. Dependent variable was ΔDAS-28 CRP from baseline to 12M.

Table 4-17 Therapeutic response vs. time-therapy from T2, adjusting for age and sex.

	Independent predictors	B	95% CI for B	P value	R square
Model 1	Time-to-therapy from T2	-0.028	-0.054 to -0.001	0.042	0.095
Model 2	Time-to-therapy from T2	-0.028	-0.056 to -0.001	0.046	
	Age at onset	0.002	0.861 to -0.025	0.882	0.095
Model 3	Time-to-therapy from T2	-0.028	-0.056 to 0.000	0.046	
	Age at onset	0.003	-0.025 to 0.031	0.836	0.097
	Sex	0.144	-0.905 to 1.193	0.782	

N=44 after excluding palindromic. Dependent variable was ΔDAS-28 CRP from baseline to 12M.

4.3.7.7 Impact of DMARD treatment on therapeutic response.

I constructed multiple linear regression models to assess whether DMARD treatments may impact treatment response. DMARDs treatment was measured as cumulative dose of methotrexate and hydroxychloroquine. DMARDs therapy did not affect the treatment response, as shown in models from Table 4-18 to Table 4-20. This is an important finding. DMARD treatment was not a confounder in treatment response for patients in this study.

Table 4-18 Therapeutic response vs. time-therapy from T0, adjusting for cumulative MTX and HCQ.

	Independent predictors	B	95% CI for B		P value	R square
Model 1	Time-to-therapy from T0	-.006	-.020	.007	.347	.021
Model 2	Time-to-therapy from T0	-.007	-.021	.007	.326	.026[a]
	Cumulative dose of MTX	.000	-.001	.002	.653	
Model 3	Time-to-therapy from T0	-.006	-.020	.008	.392	.033[b]
	Cumulative dose of MTX	.001	-.001	.003	.548	
	Cumulative dose of HCQ	-2.462E-6	.000	.000	.593	

aSig. F Change=0.653; bSig. F Change =0.593, n=44. Dependent variable was ΔDAS-28 CRP from baseline to 12M

Table 4-19 Therapeutic response vs. time-therapy from T1, adjusting for cumulative MTX and HCQ.

	Independent predictors	B	95% CI for B		P value	R square
Model 1	Time-to-therapy from T1	-.026	-.053	.001	.061	.081
Model 2	Time-to-therapy from T1	-.027	-.055	.000	.053	.091[a]
	Cumulative dose of MTX	.001	-.001	.002	.519	
Model 3	Time-to-therapy from T1	-.028	-.056	.000	.049	.107[b]
	Cumulative dose of MTX	.001	-.001	.003	.365	
	Cumulative dose of HCQ	-3.713E-6	.000	.000	.395	

aSig. F Change = 0 .519; bSig. F Change 0= ,.395 n= 44. Dependent variable was ΔDAS-28 CRP from baseline to 12M

Table 4-20 Therapeutic response vs. time-therapy from T2, adjusting for cumulative MTX and HCQ.

	Independent predictors	B	95% CI for B		P value	R square
Model 1	Time-to-therapy from T2	-.028	-.054	-.001	.042	.095
Model 2	Time-to-therapy from T2	-.029	-.056	-.002	.037	.104[a]
	Cumulative dose of MTX	.001	-.001	.002	.514	
Model 3	Time-to-therapy from T2	-.029	-.057	-.002	.036	.095[b]
	Cumulative dose of MTX	.001	-.001	.003	.369	
	Cumulative dose of HCQ	-3.530E-6	.000	.000	.415	

aSig. F Change = 0..514; bSig. F Change =0..415 n= 44. Dependent variable was ΔDAS-28 CRP from baseline to 12M

4.3.7.8 Survival analysis; Kaplan-Meier curve.

Kaplan-Meier survival analysis was performed to assess how soon patients reached clinical remission in this study. Clinical remission was defined as first time point when DAS28 CRP remission was achieved. Survival curves were stratified by mode of onset. Palindromic onset patients (n=4) were excluded in this analysis.

50% of abrupt-onset RA patients reached clinical remission at 16 weeks (IQR of 13 to 19 weeks). 50% of insidious-onset patients reached clinical remission at 20 weeks (13 to 27 weeks). There was no statistical difference between the two mode of onset groups; log rank test: p= 0.845 (Figure 4-32).

Overall, 29 out of 44 patients achieved clinical remission. 16 out of 25 patients with abrupt onset and 13 out of 19 of patients with insidious onset during the course of this 12 month study period (Table 4-21).

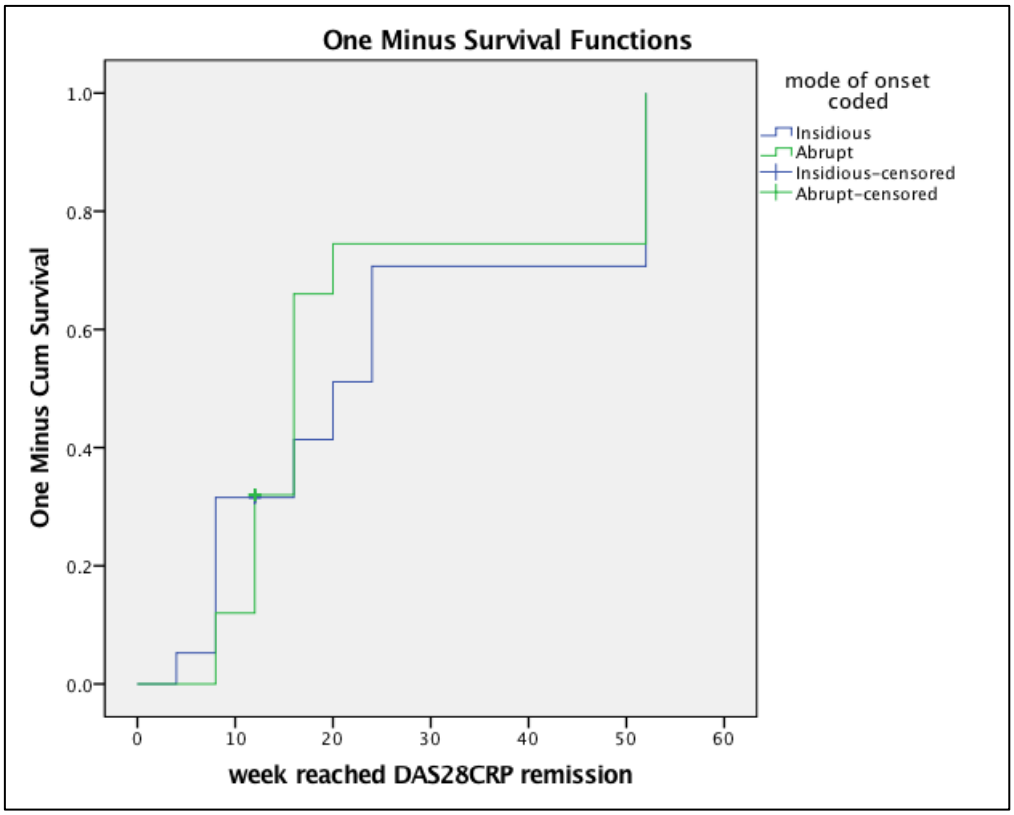


Figure 4-32 Kaplan-Meier survival analysis. 1-cumulative survival curve vs time to reach DAS28 CRP remission. Abrupt; median 16 weeks (IQR 13 to 19 weeks). Insidious onset; median 20 weeks (IQR 13 to 27 weeks). Log rank test: p= 0.845.

Table 4-21 Number of patients in clinical remission by 12 month time point.

Mode of onset	Total	Number of patients achieved clinical remission	Number & % of patients not achieved clinical remission	
Insidious	19	13	6	31.6%
Abrupt	25	16	9	36.0%
Overall	**44**	**29**	**15**	**34.1%**

4.3.8 Cox regression analyses

Cox proportional hazards regression was used to investigate clinical remission during the course of the study period. Clinical remission was defined according to DAS-28 CRP. N=44 after excluding patients with palindromic onset.

Mode of onset did not have an impact on the chance of achieving clinical remission during the first year (Table 4-22 to Table 4-24) Patients with a higher DAS28 CRP at baseline had a lower chance of reaching DAS 28 remission during the first year (Table 4-25 to Table 4-27).

Table 4-22 Cox regression analysis from time-to-therapy from T0, adjusting for mode of onset.

Independent predictors	Hazard ratio	95% CI for hazard ratio	P value
Time-to-therapy from T0	1.003	0.991 to 1.015	0.647
Mode of onset	1.121	0.515 to 2.443	0.773

Table 4-23 Cox regression analysis from time-to-therapy from T1, adjusting for mode of onset.

Independent predictors	Hazard ratio	95% CI for hazard ratio	P value
Time-to-therapy from T1	0.996	0.971 to1.021	0.757
Mode of onset	1.030	0.482 to 2.203	0.939

Table 4-24 Cox regression analysis from time-to-therapy from T2, adjusting for mode of onset.

Independent predictors	Hazard ratio	95% CI hazard ratio	P value
Time-to-therapy from T2	0.996	0.971 to 1.021	0.753
Mode of onset	1.027	0.479 to 2.204	0.945

Table 4-25 Cox regression analysis from time-to-therapy from T0, adjusting for baseline DAS28.

Independent predictors	Hazard ratio	95% CI for hazard ratio	P value
Time-to-therapy from T0	0.998	09.986 to 1.009	0.712
DAS 28 CRP at baseline	0.761	0.586 to 0.990	0.041

Table 4-26 Cox regression analysis from time-to-therapy from T1, adjusting for baseline DAS28.

Independent predictors	Hazard ratio	95% CI for hazard ratio	P value
Time-to-therapy from T1	0.995	0.973 to 1.017	0.659
DAS 28 CRP at baseline	0.776	0.611 to 0.986	0.038

Table 4-27 Cox regression analysis from time-to-therapy from T2, adjusting for baseline DAS28.

Independent predictors	Hazard ratio	95% CI for hazard ratio	P value
Time-to-therapy from T2	0.994	0.973 to 1.017	0.620
DAS 28 CRP at baseline	0.775	0.610 to 0.985	0.037

4.3.8.1 Correlation analysis

Abrupt onset RA patients were more likely to have better treatment response at one year compared to those with insidious onset at a given time-to-therapy. However, were abrupt onset RA patients more likely to present early compared to those with insidious onset disease? The answer is in Table 4-28.

Table 4-28 correlation co-efficient*

Time-to-therapy	**correlation co efficient, r_{PB}**	**p-value**
T0	0.378	0.003
T1	0.322	0.003
T2	0.328	0.003

***Point-biserial correlation coefficient was used as mode of onset is a dichotomous variable.**

There was a correlation between mode of onset and time-to-therapy from T0, T1 and T2. Patient with abrupt onset disease were more likely to present, and hence be treated early. Around 15% of the variability in the time-to-therapy from T0, T1 and T2 can be explained by the mode of onset.

Finally I explored whether patients with abrupt onset RA had more active disease when they present compared to those with insidious onset RA. Indeed there was a strong correlation between mode of onset and disease activity at baseline (Point biserial correlation coefficient =0.499, $p < 0.001$).

4.4 Discussion

4.4.1 What are the key findings?

This is the first study to have assessed the relationship between treatment response and time-to-therapy using well-defined onset of symptom and joint swelling recordings, and taking into account the different modes of onset. The key finding was that abrupt onset RA patient had a better therapeutic response at one year, compared to those with insidious onset disease at a given time-to-therapy. Importantly, the analysis suggested that time-to-therapy did not have an impact on treatment response at one year. This is true whether time-to-therapy was measured from onset of symptom, onset of first joint swelling or onset of ongoing joint swelling (as defined by T0,1 and T2 in this study). Interestingly, abrupt onset patients were

more likely to have a high baseline disease activity score and were also more likely to present quicker, compared to those with insidious onset.

In this analysis, the treatment strategy (as measured by cumulative dose of MTX and HCQ) does not have an impact on therapeutic response. In addition, age, sex and anti-CCP status did not impact on treatment response.

4.4.2 What do these findings mean?

These findings, albeit preliminary at this stage, challenge current dogma regarding early RA management.

The way a disease manifests is one of the key drivers that determines how soon a patient presents. An abrupt onset patient who is not managing their daily activity of living would likely present earlier, and perhaps these patients would in turn be referred earlier by the GP compared to a patient from the other end of the onset spectrum.

This single variable clusters together patients with specific disease characteristics. Acute onset patients have higher disease activity with short symptom duration at presentation. Insidious onset patients have lower disease activity at presentation with a longer symptom duration at presentation.

The effect of shorter symptom duration that has been observed in multiple studies may in fact reflect the patient population with abrupt onset disease – that have an intrinsically better

prognosis, therefore translating into better clinical outcomes. Conversely, those who have insidious onset disease have a tendency to present at a later stage, and have a poor prognosis

There is a wide spectrum of mode of onset. This has been described in the literature. Some may present with an acute on chronic picture, whilst some palindromic onset cases may mimic progressive insidious onset disease. In order to ensure the accurate classification of mode of onset in this study, I showed each patient the diagram of mode of onset during history taking to ensure that the correct classification was applied. Historical studies have defined mode of onset in different ways (54, 74, 100, 101). Perhaps the most controversial one is based upon patient recall of when they start having symptoms – to the nearest day, week and month (101). This method is highly vulnerable to recall bias, although one can argue there may be merits in using this definition. The different modes of onset influence outcomes. The raises the question whether the way the disease manifests represent two completely different RA subtypes.

4.4.3 Limitations

The first limitation relates to the small number of patients who were included in the analysis. I analysed data for 48 patients who have completed final follow-up at the time of writing this thesis. The total number of anticipated participants is 107 patients. The second limitation relates to the visit time points. With the benefit of hindsight, I would probably add a time point between 6 and 12 months follow-up period so that I can record at least three DAS scores with in the last three months. The final limitation relates to the fact that the number of patients

with a palindromic onset was very small. Therefore, these patients were not included in the linear regression analysis.

4.4.4 Future plans for this study.

The anticipated study completion is November 2020 with 15 patients completing study in the final month. Upon study completion, I will perform radiographic scoring to assess disease progression. I will also analyse DAS response based on US scores. I will also consider assessing whether mode of onset have an impact on the longer term outcomes of patients in this cohort.

4.4.5 Conclusion.

The window of opportunity in RA suggests that patients who are treated earlier do better in the longer term compared to those who are treated at a later stage of the disease. However, a key factor that drives early presentation - mode of onset - has never been assessed. This variable appears to be a strong prognostic marker in therapeutic response.

5 General Discussion and Future Work

US findings in the joints of healthy individuals have been reported (24, 178). However, there has been no attempt made to systematically identify the extent of these US changes across a wide range of age using a standardised consensus grading definition. In this thesis, US findings of healthy subjects from a wide range of age and demographic background have been presented.

The outcome of this study is a step forward towards defining the normal threshold cut-off at a specific age range in a given joint level in Rheumatology US. The next phase is to compare the US findings of healthy subjects with that of early arthritis patients in an age and sex match cross-sectional study in order to identify the overlapping pathology at a specified joint. This has been attempted in this thesis in a smaller group of early arthritis patients as a preliminary analysis. The limitation of this analysis was that the age of healthy controls was much younger compared to that of the early arthritis patients. Comparing each US pathology at a specified joint level in an age sex case-control study would allow us to identify the grading threshold at which the US changes progress from physiological to pathological. This endeavor is currently being planned.

Identifying the normal threshold of US according to joint and age will further enhance the utility of US in Rheumatology clinical practice and research studies. Particularly during the time when US imaging is increasingly being integrated in clinical practice and in predictive studies identifying clinical and serological markers that identify early arthritis patients who would develop RA and persistent arthritis.

However, predictive algorithms integrating US, so far, have been focusing on the predictive capacity of US *joint* synovitis (106).

Although the importance of *tendon* inflammation has been increasingly recognised in RA imaging studies (41), the potential of US-tenosynovitis as a candidate variable in predictive algorithms has not been reported. The results of this thesis and upcoming publications will add to this body of knowledge.

Tendon inflammation - as measured by US – predicts the development of RA and persistent arthritis. Specifically, finger flexor tendon inflammation predicts both the development of RA and persistent arthritis. This is even after taking into account conventional serological, clinical and joint US variables.

One of the potential reasons that the same tendon variable shows predictive value in the development of both RA and persistent arthritis is because there was a considerable number of RA patients in the persistent arthritis group. However, in persistent arthritis which includes the SpA/PsA spectrum of joint disease dactylitis is one of the hallmark. On imaging studies, finger flexor tendon inflammation is a component of dactylitis (179). Therefore, it is plausible that the finger flexor tendon inflammation was detecting subtle dactylitis.

The next stage is to identify which one of those ten finger flexor tendons (i.e. bilateral finger flexor 1-5) – would be the one to include as a candidate variable in predictive algorithms of RA and persistent arthritis. In the analysis presented, the ten finger flexor tendons were graded as a single US variable. The ideal finger tendon site will be those where there is no

sesamoid bone underneath creating an acoustic shadow to the underlying tissue. At present, all finger flexor tendons are scanned individually as part of the baseline US assessment for BEACON patients, enabling this analysis to be performed in future.

A larger multicenter study would be with standardised scanning proforma to validate these findings in an independent cohort would be a potential future work. It would be interesting to assess what is the optimal number of finger flexor tendons that would produce optimal predictive data. Amongst the MCP and PIP joints US variables, our group have shown that scanning MCP 2-3 and MTP 2-3 provides optimal data to predict RA development.

The reason for designing predictive algorithms to identify those who are destined to develop RA, or persistent arthritis, is so that early aggressive therapy can be targeted to the correct patient population. This is based upon the longstanding notion that earlier treatment works better for RA patients, particularly in the longer term as measured by progression of joint damage.

Patients who present earlier, and are therefore likely to be treated earlier have always fared better compared to those who present later. This appears to be true regardless what type of therapy is employed – ranging from conservative bed rest to synthetic or biologic DMARDs.

Although the mode of onset of RA has been recognised by Rheumatologists as far back as 1859 (3), the effect of mode of onset on treatment response has not been assessed. In addition, the impact of mode of onset on the measurement of symptom and/or disease duration has also not been studied. Furthermore, heterogeneity in the definition of symptom and/or disease

onset has further hampered the comparison between the studies that assessed this proposed therapeutic window of opportunity.

In my study, the mode of onset and duration from symptom and joint swelling onset were carefully recorded. Data analysis, albeit a preliminary one, suggested that mode of onset had a greater effect than symptom duration on therapeutic response. Furthermore, symptom duration did not appear to have a measurable impact on therapeutic response.

If confirmed, these data challenge the existing paradigm of the window of opportunity. Previously, mode of onset has never been considered as a prognostic marker in RA in longitudinal studies. Those who have abrupt disease onset do better than those with insidious onset, even after taking into account symptom duration. Important future work for this project is to complete patient follow up in order to meet the numbers required by my initial power calculation for this analysis. The follow-up for the current TETRA cohort is anticipated to complete November 2020.

Does the different mode of onset actually represent different clinical phenotypes of rheumatoid disease, rather than ends of the spectrum of the same disease? This would be an interesting mechanistic research question to address, particularly in a longitudinal cohort study which undertakes synovial tissue sampling pre-DMARDs.

Validating this data in an independent cohort, would be a potential future work. This may form a basis for future collaboration with other centres who have an ongoing inception cohort of early arthritis patients.

Another potential future work is to understand whether mode of onset is truly an independent prognostic marker from symptom duration on the treatment response, a stratified randomised clinical trial may be a potential study design to address this question. This way the effect of mode of onset and symptom duration can be independently assessed. Analysis of a longitudinal observational study never accurately adjust for different confounding effects. It would also be interesting to assess the long-term outcome of patients enrolled to the TETRA study to better assess the prognostic value of the mode of onset in RA patients.

ULTRASOUND-DETECTED MINIMAL DISEASE IN EARLY ARTHRITIS

ULTRASOUND WORKING GROUP

MINIMAL DISEASE SUB-TASK FORCE

STUDY PROTOCOL

Lead: Dr Andrew Filer
OMERACT US Fellow: Dr Ilfita Sahbudin
Mentor: Professor Maria Antonietta D'Agostino

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Background

Musculoskeletal ultrasound (MSUS) is more sensitive and specific compared to clinical examination in the detection of joint synovitis in inflammatory arthritis patients [1] and is increasingly utilised as a point-of-care tool in Rheumatology. MSUS is increasingly used to diagnose early arthritis, monitor treatment response [2] and detect subclinical disease in patients who are in apparent clinical remission [3].

However, the difference between ultrasound changes observed in normal joints (i.e. physiological changes) and those seen in early arthritis joints (i.e. early pathological changes) is poorly defined. In addition, there has been significant improvement in ultrasound technology over the last ten years which results in higher image resolution. As a result, MSUS is now able to detect minimal changes even in healthy individuals with no joint symptoms [4].

Defining the concept of ultrasound-detected 'minimal disease' is crucial in order to i) define the time of onset in early disease, ii) define minimal response to treatment, and iii) identify remission in patients with inflammatory arthritis.

Aim

The aim of this study is to define the threshold of at which ultrasound findings should be considered pathological at the joint level in patients with early arthritis. 3

Objective

To systematically document ultrasound findings (synovial hypertrophy, synovial effusion and Power Doppler) in the joints of healthy asymptomatic individuals who are above the age of 18.

Participant Criteria

Inclusion criteria

- Age ≥ 18.

Exclusion criteria

- Previous/current inflammatory joint disease (including crystal arthropathy).
- Visual analogue score (VAS) for joint pain > 10/100.
- Any history of joint trauma in the last month.
- Fulfilling hand osteoarthritis ACR criteria (Appendix 1).
- Any clinical joint inflammation as identified by a physician.
- Previous or current inflammatory bowel disease.
- History of culture-proven enteric and/or genitourinary infection in the last month.
- Current or previous corticosteroids use in the last 4 weeks.
- Current non-steroidal anti-inflammatory use.

Ultrasound Assessments

Joint and tendon sub-set

1. There are two levels of US data acquisition for this study (Table 1).
    a. Level 1: Mandatory ultrasound lesion
    b. Level 2: Optional ultrasound lesion

Table 1: List of mandatory and optional ultrasound lesion

Level	1 (mandatory)	2 (optional)
Ultrasound lesion	i. Joint synovial hypertrophy ii. Joint synovial effusion iii. Joint synovial Power Doppler iv. Tenosynovial hypertrophy v. Tenosynovial effusion vi. Tenosynovial Power Doppler	i. Osteophyte ii. Erosion (only for MCP 2 & 5, MTP 5)

2. The joint and tendon sites to be scanned are listed in Table 2 below.

Table 2: Joint and tendon sub-set

Structure	**Site**
Joint	i. MCPs 1-5 (dorsal) ii. PIPs 1-5 (dorsal) iii. Wrist Inter-carpal, Radio-carpal & Ulnar-carpal iv. MTPs 1-5 (dorsal)
Tendon	i. Finger flexor tendon 1-5 ii. Extensor carpi ulnaris tendon

3. The following grading of grey-scale and power Doppler will be documented for each joint:

Table 3: Grading system for joint sites

Grey-scale	Synovial hypertrophy	Semi-quantitative 0-3
	Synovial effusion	Semi-quantitative 0-3
Power Doppler (PD)	PD severity	Semi-quantitative 0-3
Degenerative	Presence of osteophyte	Semi-quantitative 0-3
Erosion	Presence of erosion (only for MCP2, 5 and MTP5)	Yes/No

4. The following grading of grey-scale and power Doppler will be documented for each tendon:

Table 4: Grading system for tendon sites

Grey-scale	Tenosynovial hypertrophy	Semi-quantitative 0-3
	Tenosynovial effusion	Yes/No
Power Doppler (PD)	PD grading	Semi-quantitative 0-3

Grading definitions

1. The **joint** ultrasound elementary lesions to be recorded **are synovial hypertrophy, effusion** and **Power Doppler Enhancement**. Each US elementary lesion should be graded according to the EULAR-OMERACT consensus definition [5, 6] as detailed in Table 5-7 below.
2.

Table 5: Grading definition for joint synovial hypertrophy US elementary lesion [5, 6]

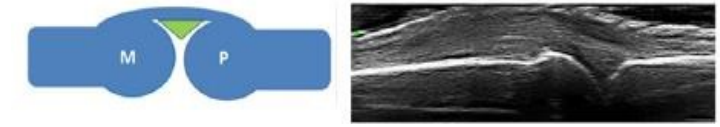
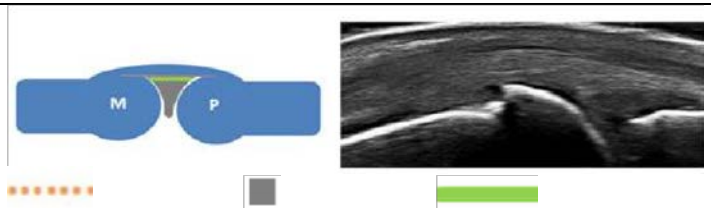
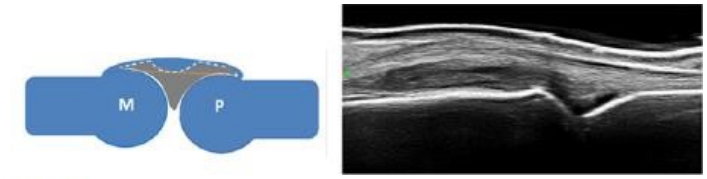
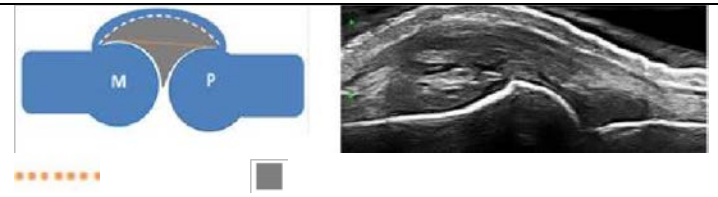
Joint synovial hypertrophy	Definition of grading	Representative schematic diagram and US images
Abnormal hypoechoic intraarticular tissue or **higher echoic** (relative to subdermal fat) **that is not or poorly displaceable,** and which may exhibit Doppler signal.	**Grade 0** (none) No synovial hypertrophy independently of the presence of effusion.	 Loose intra-articular connective tissue; M=metacarpal head; P=proximal phalanx.
	Grade 1 (minimal) **Minimal hypoechoic** synovial hypertrophy **up to the level of the horizontal line** connecting bone surfaces between the metacarpal head and the proximal phalanx.	 M=metacarpal head; P=proximal phalanx.
	Grade 2 (moderate) **Moderate hypoechoic** synovial hypertrophy **extending beyond joint line but with the upper surface concave** (curved downwards) or **hypertrophy extending beyond the joint line but with the upper surface flat.**	 Hypertrophy; M=metacarpal head; P=proximal phalanx.
	Grade 3 (severe) **Severe hypoechoic** synovial hypertrophy with or without effusion **extending beyond the joint line but with the upper surface convex (curved upwards).**	 M=metacarpal head; P=proximal phalanx.

Table 6: Grading definition for Power Doppler US elementary lesion [5, 6]

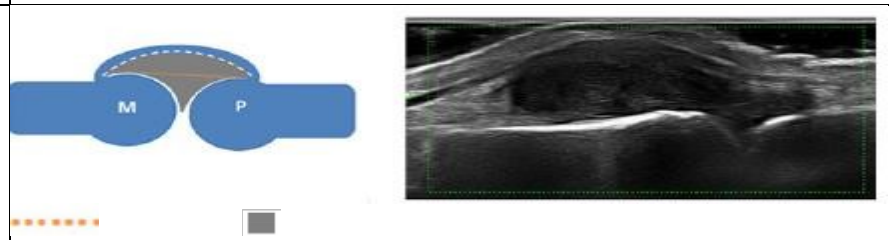
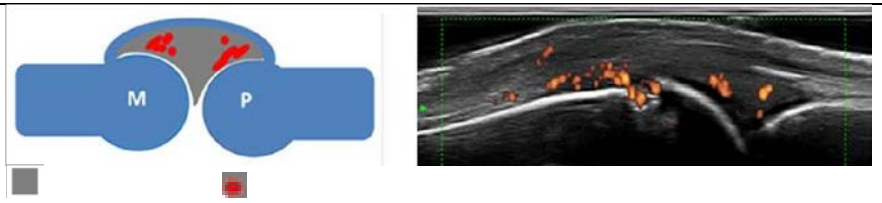
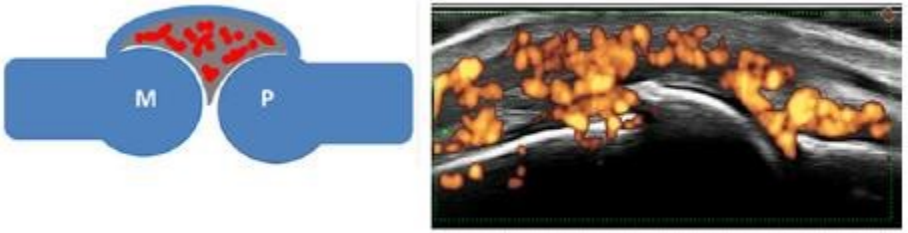
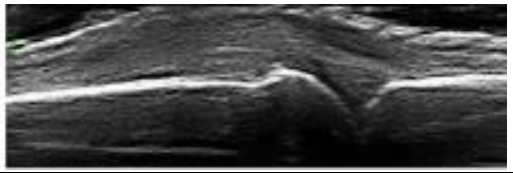
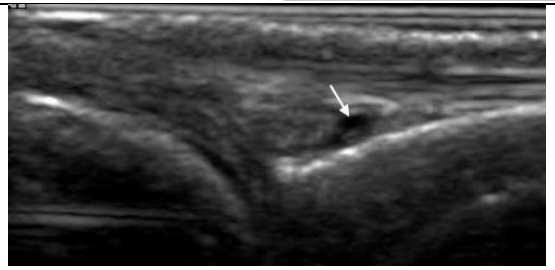
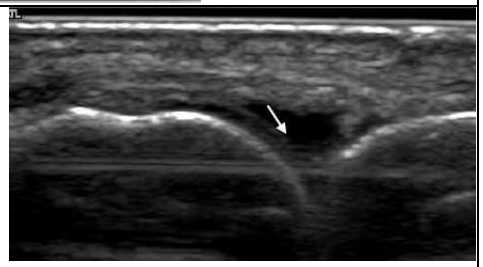
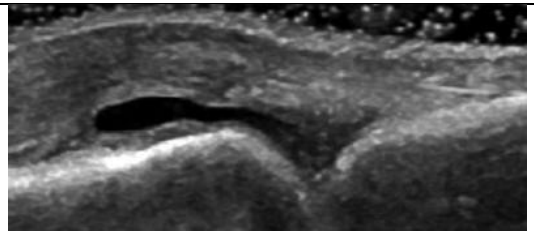
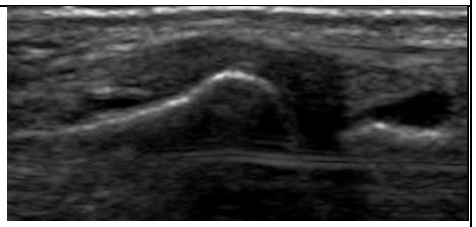
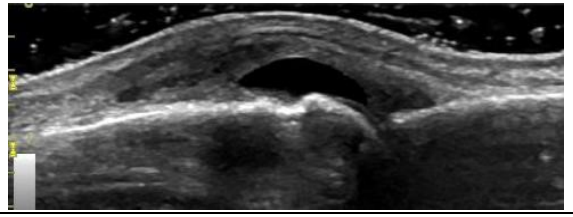
Joint Power Doppler	Definition of grading	Representative schematic diagram and US images
Abnormal vascularization detected within the hypoechoic synovial hyperplasia.	**Grade 0** (none) No Doppler signal	 Hypertrophy; M=metacarpal head; P=proximal phalanx.
	Grade 1 (minimal) Up to **three single Doppler spots** OR up to **one confluent spot and two single spots** OR up to **two confluent spots.**	 Hypertrophy; Intra-synovial Doppler; M=metacarpal head; P=proximal phalanx.
	Grade 2 (moderate) Greater than Grade 1 but **≤50% Doppler signals in the total greyscale background**.	
	Grade 3 (severe) Greater than Grade 2 **(>50% of the total greyscale background**).	 Hypertrophy; Intra-synovial Doppler; M=metacarpal head; P=proximal phalanx.

Table 7: Grading definition for joint effusion US elementary lesion [2]

Joint effusion	Definition of grading	Representative US images
Abnormal anechoic or hypoechoic (relative to subdermal fat) intraarticular material that is **easily displaceable**, but does not exhibit Doppler signal.	**Grade 0** No effusion	
	Grade 1 **Minimal** amount of joint effusion	 
	Grade 2 **Moderate** amount of joint effusion (little distension of the joint capsule)	 
	Grade 3 **Extensive** amount of joint effusion (with high distension of the joint capsule)	

3. The **tendon** ultrasound elementary lesions to be recorded **are synovial hypertrophy, effusion** and **Power Doppler Enhancement**. The grading system for each US elementary lesion is according to the EULAR-OMERACT consensus definition [7] and detailed in Table 8 below.

Table 8: Grading definition for tenosynovial hypertrophy US elementary lesion [7]

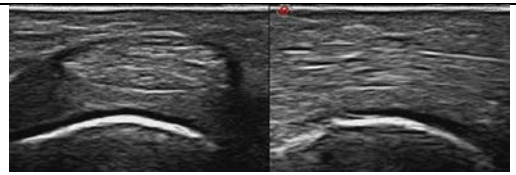
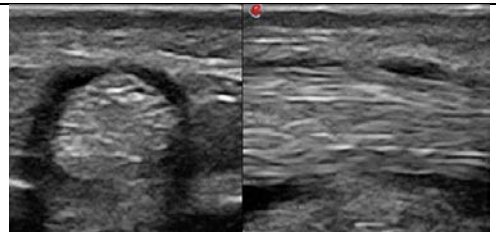
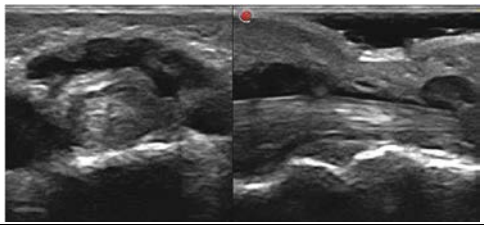
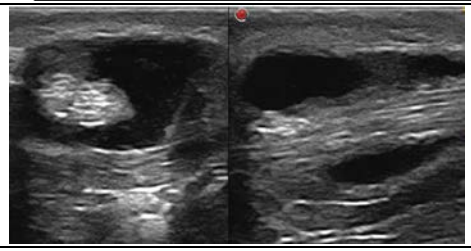
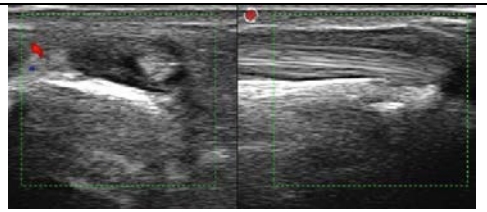
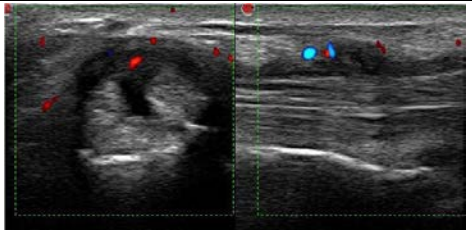
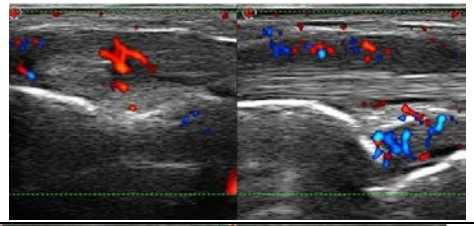
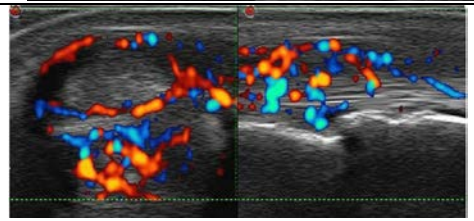
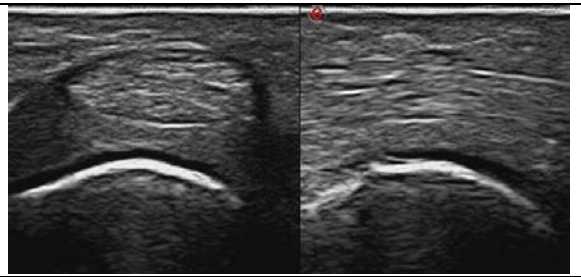
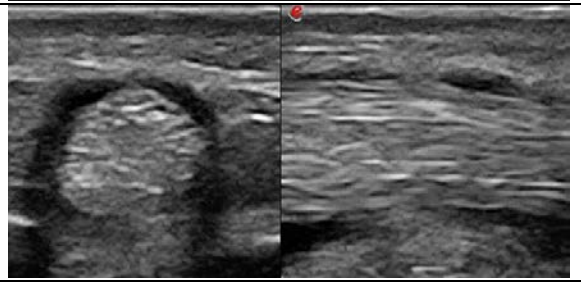
Tenosynovial hypertrophy [7]	Definition of grading	Representative US images
Abnormal hypoechoic (relative to tendon fibres) tissue within the tenosynovial sheath that **is not displaceable and poorly compressible** and seen in two perpendicular planes.	**Grade 0** No abnormal hypoechoic within the tenosyovial sheath	
	Grade 1 Minimal abnormal hypoechoic within the tenosyovial sheath	
	Grade 2 Moderate abnormal hypoechoic within the tenosyovial sheath	
	Grade 3 Severe abnormal hypoechoic within the tenosyovial sheath	

Table 9: Grading definition for tenosynovial Doppler US elementary lesion [7]

Tenosynovial Doppler	Definition of grading	Representative US images
Presence of peritendinous Doppler signal within the synovial sheath, seen in two perpendicular planes, excluding normal feeding vessels (i.e. vessels at the mesotenon or vinculae or vessels entering the synovial sheath from surrounding tissues) **only if the tendon shows peritendinous synovial sheath widening on B-mode**	**Grade 0** No signal	
	Grade 1* **Peritendinous focal signal** within the widened synovial sheath (i.e. signals in **only one area** of the widened sheath), seen in two perpendicular planes, excluding normal feeding vessels;	
	Grade 2* **Peritendinous multifocal signal** within the widened synovial sheath (i.e. signals in **more than one area** of the widened sheath), seen in two perpendicular planes, excluding normal feeding vessels;	
	Grade 3 **Peritendinous diffuse signal** within the widened synovial sheath (i.e. signals **filling most of the widened sheath**), seen in two perpendicular planes, excluding normal feeding vessels.	

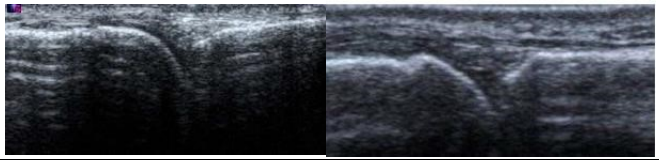
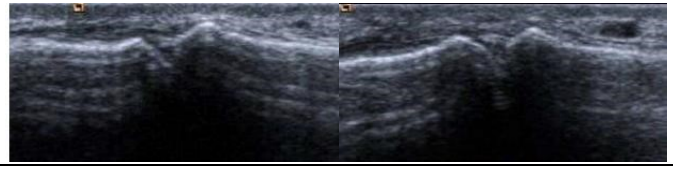
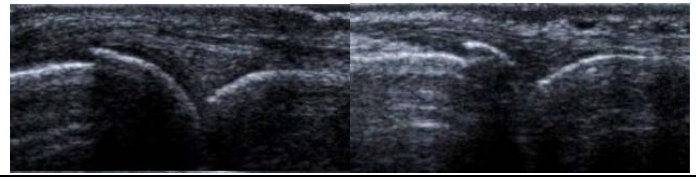
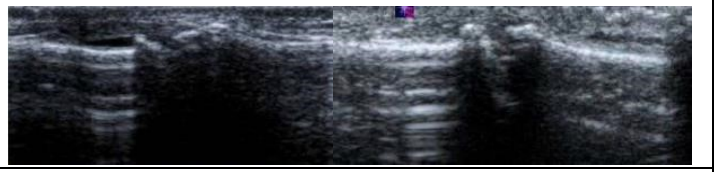
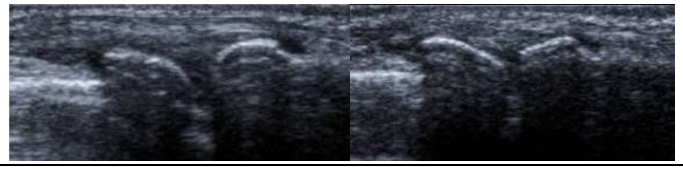
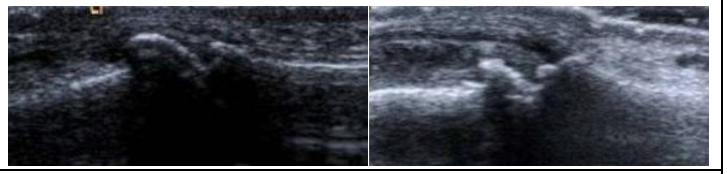
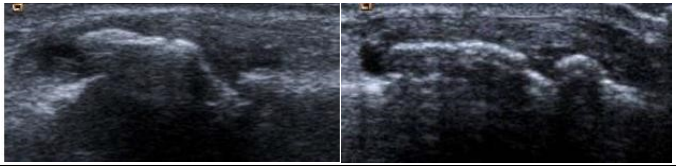
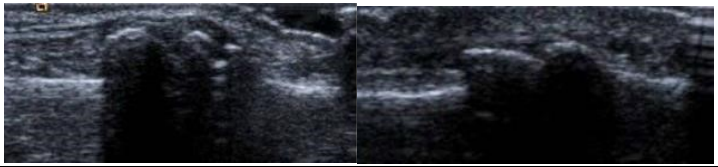
***If **in addition to an abnormal peritendinous** (i.e. intra-sheath) signal there was an **abnormal intratendinous signal** seen in two perpendicular planes (i.e. excluding intra-tendinous small isolated signals that can correspond to normal feeding vessels detectable by US), **then grades 1 and 2 would be increased by one point.**

Table 10: Grading definition for tenosynovial effusion US elementary lesion [7]

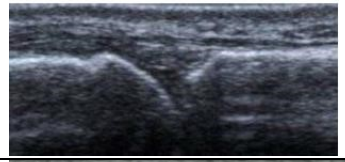
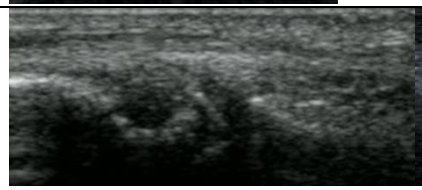
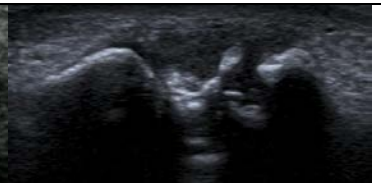
Tenosynovial effusion	Definition of grading	Representative US images
Abnormal anechoic or hypoechoic (relative to tendon fibres) material within the synovial sheath, either localised (eg, in the synovial sheath cul-de-sacs) or surrounding the tendon **that is displaceable** and seen in two perpendicular planes	**Absent** No abnormal displaceable hypoechoic region within the tenosyovial sheath	
	Present Presence of at least minimal abnormal hypoechoic within the tenosyovial sheath	

4. The recording of **osteophytes is optional** for this study. The definition and grading of osteophytes are detailed as below.

Table 11: Definition and grading system of osteophytes [8, 9].

Osteophytes	Definition of grading	Representative MCP joint  US images	Representative PIP joint  US images of
A step-up bony prominence at the end of the normal bone contour, or at the margin of the joint seen in two perpendicular planes, with or without acoustic shadow.	**Grade 0** No osteophytes, i.e. a smooth cortical surface.		
	Grade 1 Small and distinct cortical protrusion(s) of the bony surface.		
	Grade 2 Larger protrusion(s) which may have broad base(s).		
	Grade 3 Very large protrusion(s) which may have very broad base(s).		

3. The recording of **erosions** is optional for this study and only limited to MCP2, 5 and MTP 5. The definition and grading of the osteophytes are detailed as below.

Erosions [10]	Definition of grading	Representative US images
A cortical "break" or defect with an irregular floor seen in longitudinal and transverse planes.	**Absent** No erosion	
	Present Erosion present	 

US images record and data input

1. All images should be recorded according to the EULAR Working Group for Musculoskeletal Ultrasound 2017 recommendation [11].
2. All structures should be scanned using both longitudinal and transverse approaches.
3. However, only longitudinal views are recorded for joint scanning, and longitudinal and transverse views are recorded for tendon scanning (see Table 13).

Table13: Views to be recorded for joint and tendon core set

Region	Joint and tendon core set	Views to be recorded
Hand	MCP 1-5 (dorsal)	Longitudinal
	PIP 1-5 (dorsal)	
	Flexor tendon 1-5	Longitudinal & transverse
Wrist	Inter-carpal, Radio-carpal, Ulnar-carpal	Longitudinal
	Extensor Carpi Ulnaris tendon	Longitudinal & transverse
Foot	MTP 1-5 (dorsal)	Longitudinal

4. All images and US scores from the first participant should be compiled onto a power point presentation and emailed to Dr Sahbudin (i.sahbudin@bham.ac.uk) as soon as feasible.

5. At the end of the study, all images must be anonymised and recruiting centres will contact Dr Ilfita Sahbudin (i.sahbudin@bham.ac.uk) to liaise the transfer of the US images of all healthy participants along with the US scores. The US scores should be compiled onto the excel spreadsheet that has been provided.

6. The following details should be recorded on the US images for each participant a. Date of visit

   b. Participant research ID (i.e. anonymised)

   c. Joint or tendon site using the abbreviations as shown in the table 14:

Table 14: Abbreviation for recorded US images

Right or left	Joint/tendon site	Number of position
R, L	MCP PIP MTP Wrist	1-5 1-5 1-5 IC/RC/ UC
	DF ECU	1-5

Example:

i. L DF 1

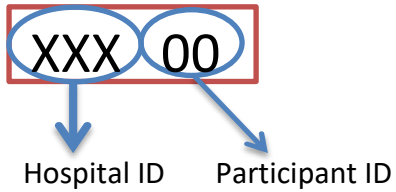
ii. R MCP 5

iii.R ECU

iv. L wrist UC

v. L MTP 5

7. Each centre should assign a unique research ID for each healthy subject participant in the following format:



E.g. The 30 healthy participants recruited at University Hospital Birmingham, UK, would have the following consecutive research IDs:

UHB 01 – UHB 30.

Joint positioning

The hand should be positioned flat on the table in a relaxed position as shown in Figure 1 below. Flexor tendon scanning should be done at the level of the MCP joint. For foot scanning, the participant should be placed on a couch with the knees flexed and foot flat on the couch (Figure 2).

Figure 1: Hand position during joint scanning

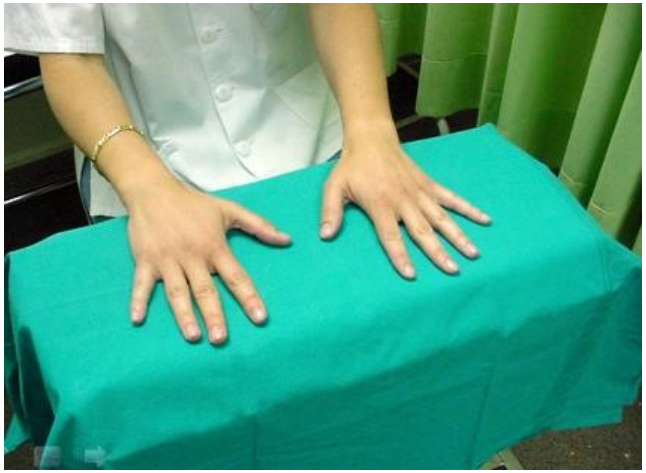
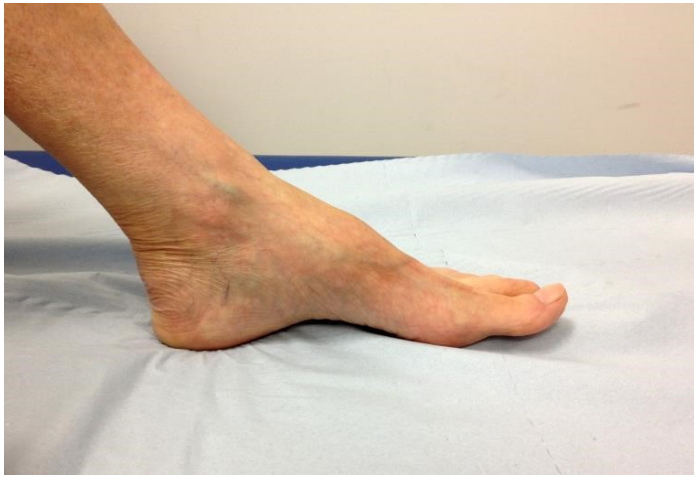


Figure 2: Foot position during joint scanning



Clinical Assessment

1. Clinical details from section 5.1 and 5.2 will be recorded for all participating healthy individuals on the clinical proforma (provided).
2. At the end of the study, all clinical proforma should be scanned and transferred to Dr Sahbudin along with the documents listed in appendix 2.

History

a. Age
b. Sex
c. Personal history of skin psoriasis
d. Family history of
    i. osteoarthritis,
    ii. skin psoriasis,
    iii. inflammatory arthritis, iv. connective tissue disease,
    v. Inflammatory bowel disease.
e. Previous trauma/joint replacement (specify joint)
f. Hobbies
g. Occupation; if retired previous occupation
h. Current medication
i. Co-morbidities
j. Smoking status

Examination

a. Visual analogue scale for overall joint pain
b. Swollen and tender joint assessment: MCP 1-5, PIP 1-5, wrist, MTP 1- 5
c. Record presence of clinical MTP1 degenerative disease
d. Height and weight

Recruitment and Sample Size

This is a cross-sectional study with the main recruitment centres at University Hospital Birmingham, UK and Ambroise Pare Hospital, BoulogneBillancourt, France. Additional OMERACT recruitment centres will be involved by invitation. The target recruitment is 200 healthy individuals.

Appendices

Appendix 1

The ACR criteria for the classification of osteoarthritis of the hand*

Hand pain, aching, or stiffness, and

3 or 4 of the following features:

- Hard tissue enlargement of 2 or more of 10 selected joints
- Fewer than 3 swollen MCP joints
- Hard tissue enlargement of 2 or more DIP joints
- Deformity of 1 or more 10 selected joints

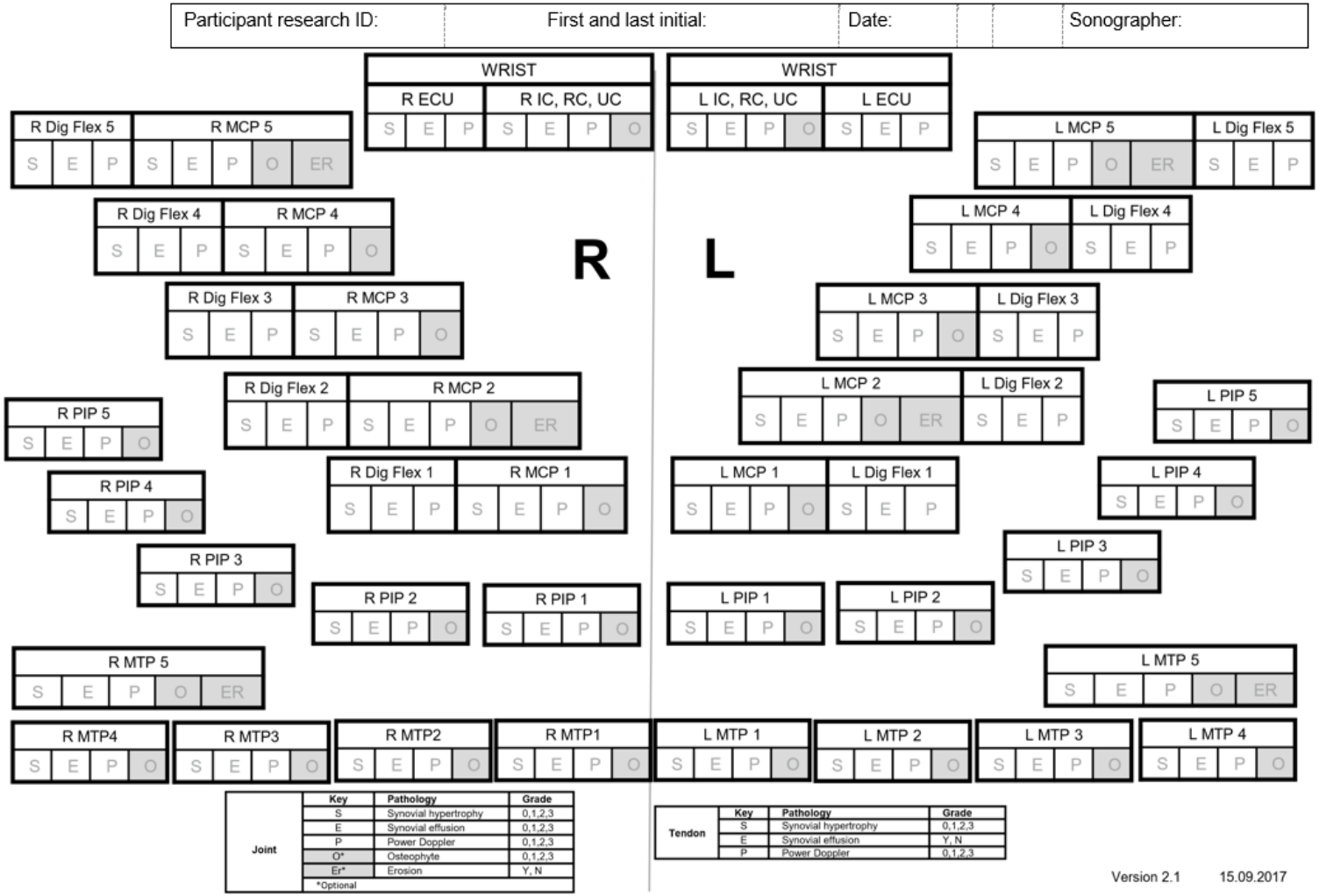
*The 10 selected joints are the second and third DIP, the second and third proximal interphalangeal, and the first carpometacarpal joints of both hands.

Appendix 2

Upon completion of recruitment and data collection, these documents should be transferred to Dr Sahbudin (i.sahbudin@bham.ac.uk).

1. Anonymised US images for all healthy participants.
2. Compiled US gradings for all healthy participants in excel spreadsheet.
3. Scanned copies of individual clinical proforma.

Ultrasound record sheet.



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Appendix 2 Recruitment centres

Alessandra Bortoluzzi University of Ferrara, Cona, Italy
Annamaria Iagnocco Università degli Studi di Torino, Italy
Carlos Pineda Instituto Nacional de Rehabilitacion, Mexico
Cesar A. Sifuentes-Cantú Hospital Universitario Fundación Jiménez Díaz, Spain
Coziana Ciurtin University College London Hospital, UK
Cristina Reategui-Sokolova Instituto Nacional de Rehabilitacion, Mexico
Hilde Berner Hammer Diakonhjemmet Hospital, Oslo, Norway
Daniela Fodor"Iuliu Hatieganu" University of Medicine and Pharmacy, Romania
Esperanza Naredo Hospital Universitario Fundación Jiménez Díaz, Madrid, Spain
Ellen-Margrethe Hauge Aarhus University Hospital, Aarhus, Denmark
Florentin Vreju University of Medicine and Pharmacy Craiova, Romania
Garifallia Sakellariou University of Pavia, Italy
George Bruyn MC Groep, Lelystad, The Netherlands
Georgios Filippou University of Ferrara, Cona, Italy
Giuliana La Paglia Hospital Universitario Fundación Jiménez Díaz, Madrid, Spain
Gustavo Leon Instituto Nacional de Rehabilitacion, Mexico City, Mexico
Hélène Gouze Hôpital Ambroise Paré, Paris France
Helen Keen University of Perth, Australia
Ilaria Tinazzi "Sacro Cuore" Hospital, Verona, Italy
Irene Azzolin Università degli Studi di Torino, Turin, Italy
Jacek Fliciński Pomeranian Medical University, Szczecin, Poland
Jeanette Trickey University of Birmingham, Birmingham, UK
Kei Ikeda Chiba University Hospital, Japan
Lene Terslev Copenhagen University Hospital, Denmark
Mads Ammitzball Danielsen Copenhagen University Hospital, Denmark
Mads Nyhuus Bendix Rasch Aarhus University Hospital, Denmark
Marcin Milchert Pomeranian Medical University, Szczecin, Poland
Maria Stoenoiu Cliniques Universitaires Saint-Luc, Bruxelles, Belgium
Marion Kortekaas Leiden University Medical Center, The Netherlands
Marwin Gutierrez Instituto Nacional de Rehabilitacion, Mexico City, Mexico
Mihaela Maruseac Cliniques Universitaires Saint-Luc, Bruxelles, Belgium
Mohamed Mortada Zagazig University, Egypt
Philippe Carron Ghent University Hospital, Belgium
Rositsa Karalilova University Hospital “Kaspela", Plovdiv, Bulgaria
Ruth Wittoek Ghent University Hospital, Belgium
Sarah Ohrndorf Charité Universitätsmedizin Berlin, Germany
Takeshi Suzuki Japanese Red Cross Medical Center, Tokyo , Japan
Teodora Șerban Ospedale La Colletta, Genoa, Italy

Appendix 3 Medication record sheet.

TETRA ID: _____QETETRA **EAC ID: _____QEEAC**

Study visit	**0 Baseline BEACON**	**1**	**2**	**3**
Time point	**-1 W**	**0 W**	**4 W**	**8 W**
Range of time point	0W	+ 2W	+/- 2W	+/- 2W
Study visit	**4**	**5**	**6**	**7**
Time point	12 W	16 W	26 W	52 W
Range of time point	+/- 2W	+/- 2W	+/- 4W	+/- 4W

		mg		x5mg					
W	Date	MTX OW dose	Route	F.A OW Dose	MTX omitted code	HLQ daily dose	HCQ omitted code	Steroid dose	Route
1									
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3									
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51									
52									

Date of first MTX dose _____

Deviated from pathway? **Y N**

Reason for omitting / stopping MTX/ HCQ

1) Infection
2) Abnormal blood test
3) Intolerant to side effects
4) Non-adherence
5) Other - Please state _____

Reason for deviating from proposed escalation pathway

1) Abnormal blood test
2) Abnormal CXR or lung function test
3) Intolerant/side effects
4) Physician preference
5) Patient preference
6) Non-adherence
7) Infection
8) Other: Please state _____

Appendix 4 Summary of DMARDs and steroid therapy for the whole cohort (n=48). Basic demographic and baseline and final DAS28-CRP were included.

Age/ Sex	T2T from T1 (wks)	BL DAS28 CRP	Deviation from DMARD escalation protocol. If yes reason	Number of weeks/wkly dose of MTX	Number of weeks /daily dose of HCQ	Cumulative dose of MTX /year (mg)	HCQ per year mg/year (mg)	Reason for missed dose of DMARDs/ biologic therapy	Cumulative dose of steroids	Cumulative dose of 3rd DMARD	Final DAS 28 CRP
23/F	32	3.41	N	52 /20mg	Nil	970	0	Nil	240mg IM Depomedrone	Nil	1.84
26/F	6	5.87	N	PO: 5/15mg SC:47/20 mg	39 / 200/400mg alt days	970	77400	GI side effect	693g of Budesonide for IBD 400mg IM Depomedrone	Nil	3.64
60/F	16	5.74	Y/Abnormal blood test	52/15mg	35/400mg	750	96600	Nil	748mg oral Prednisolone	Nil	2.40
56/F	12	7.49	Y/Abnormal blood test	49/20mg	Nil	820	0	Holiday	120mg IM Depomedrone	Nil	5.97
52/F	5	NA	Y/Abnormal blood test	19/15mg	2/200mg	250	2800	Infection	280 IM Depomedrone 150mg oral prednisolone	SSZ: 6W/ 500mg TDS/ cumulative dose 52500mg	3.71
53/F	6	4.13	Y/ Physician preference	52/20mg	44/400mg	990	117600	Nil	240mg IM Depomedrone	Benapali; standard dose for 21 weeks.	2.89
61/M	15	5.58	Y/Abnormal blood test	52/20mg	34/400mg	930	93800	Nil	120mg IM Depomedrone	Nil	5.28
59/F	3	5.81	N	52/20mg	42/400mg	935	102200	Nil	490mg oral Prednisolone	Nil	3.35
70/F	9	1.65	N	50/20mg	47/400mg	915	130200	abnormal blood test	892.5mg oral Prednisolone	Nil	1.75
45/F	10	1.58	N	PO: 4/10mg SC:44/15 mg	42/ 200mg/400mg altdays	655	84700	Infection	120mg IM Depomedrone	Nil	1.13
67/F	32	4.45	N	49/20mg	43/400mg	950	119000	infection, abnormal blood test	120mg IM Depomedrone	Nil	2.13

54/M	9	2.63	N	51/20mg	46/400mg	990	127400	infection	280mg oral Prednisolone	Nil	2.33
52/F	9	6.02	N	52/20mg	47/400mg	1005	130200	Nil	200mg IM Depomedrone	Nil	2.35
57/F	60	4.51	Y/Abnormal blood test	48/15mg	20/400mg	660	47600	infection, abnormal blood test	120mg IM depomedrone	Nil	3.85
29/F	21	4.90	Y/ Physician preference	49/25mg	44/200mg	945	61600	abnormal blood test	120mg IM Depomedrone	Rituximab given at W29	1.89
73/F	11	6.50	Y/ Physician preference	51/20mg	Nil	960	0	Surgery	120mg IM Depomedrone	Nil	4.35
74/M	41	5.83	N	51/20mg	Nil	980	0	infection	120mg IM Depomedrone	Nil	3.46
74/F	10	3.20	Physician preference	51/15mg	Nil	742.5	0	infection	1050mg oral Prednisolone	Nil	2.11
56/F	26	4.67	Y/ Physician preference	51/20mg	47/400mg	940	120800	infection	120mg IM Depomedrone	Nil	2.15
67/F	27	5.69	N	52/20mg	Nil	1010	0	Nil	0	Nil	1.97
54/F	366	3.34	Y/ Physician preference	50/15mg	Nil	785	0	infection	0	Nil	2.44
68/M	18	5.40	Y/ Physician preference	48/15mg	Nil	695	0	Infection, surgery	0	Nil	1.47
75/F	23	6.35	N	50/20mg	Nil	950	0	Surgery	982.5mg oral Prednisolone	Nil	2.12
57/M	23	4.15	N	52/25mg	34/400mg	1210	95200	Nil	200mg IM Depomedrone 140mg IA Depomedrone	Nil	1.79
24/F	7	2.87	N	50/20mg	Nil	970	0	infection	0	Nil	0.99
53/F	10	3.12	Y/ Physician preference	52/15mg	Nil	775	0	Nil	120mg IM Depomedrone	Nil	1.52
50/F	442	5.79	N	52/20mg	32/400mg	1010	75600	Nil	945mg oral Prednisolone, 60mg IA triamcinalone	Nil	4.04
52/F	8	1.82	Y/Patient & physician preference	52/10mg	7/400mg	615	18200	infection	0	Humira; standard dose for 30 weeks	1.16

54/F	5	5.49	Y/ Physician preference	52/20mg	Nil	990	0	Nil	120mg IM Depomedrone	Nil	2.00
30/F	14	7.83	N	51/20mg	42/400mg	990	112000	infection	875mg oral prednisolone	SSZ: 34W/ 1g BD/cumulative dose 65000mg. Rituximab given at W30.	4.93
55/F	31	5.15	N	49/20mg	Nil	970	0	infection	250mg oral Prednisolone	Nil	4.03
80/F	6	2.99	Y/Abnormal blood test	47/10mg	Nil	492.5	0	infection	350mg oral Prednisolone, 60mg of IA Depomedrone	Nil	2.54
40/F	22	5.85	N	PO:16/20 mg SC:34/20 mg	41/400mg	970	113400	infection	90mg oral Prednisolone, 240mg of IM Depomedrone	Humira; standard dose for 21 weeks.	5.01
23/F	18	6.67	Y/ Patient preference	52/20mg	39/400mg	990	82600	Nil	120mg IM Depomedrone	Humira; standard dose for 21 weeks.	5.16
45/F	49	3.91	N	50/20mg	Nil	970	0	abnormal blood test	0	Nil	1.40
39/F	23	5.88	N	50/25mg	43/400mg	1150	124600	infection	700mg of oral Prednisolone, 360mg of IM Depomedrone.	Nil	5.53
64/F	47	5.70	N	52/25mg	50/400mg	1195	140000	Nil	120mg IM Depomedrone	Nil	2.63
53/F	26	4.82	N	50/17.5mg	Nil	690	0	infection	0	Nil	4.91
87/F	79	2.86	N	50/20mg	44/400mg	970	61600	lost tablets	120mg IM Depomedrone	Nil	5.53
46/M	16	3.34	N	PO: 30/20mg SC: 22/20mg	50/400mg	1000	138600	Nil	988mg of oral prednisolone	Nil	2.17

61/F	9	5.64	N	PO: 32/20mg SC: 18/20mg	35/400mg	970	96600	mouth ulcers	0	Nil	3.33
50/M	11	4.11	Y/Infection	48/20mg	33/400mg	900	91000	infection, run out of tablets	0	Nil	4.96
69/M	17	7.32	Y/Abnormal blood test	50/20mg	45/400mg	960	124600	infection	240mg of IM Depomedrone, 80mg of IA Depomedrone	Nil	2.20
36/F	13	6.53	Y/Abnormal blood test	PO: 20/20mg SC: 12/10mg	Nil	460	0	infection	0	Nil	4.70
74/M	10	4.42	Y/Lung disease	50/15mg	27/200mg	730	37800	infection	0	Nil	2.64
34/F	11	5.77	N	52/25mg	46/200mg	1160	64400	Nil	490mg of oral Prednisolone, 120mg of IM Depomedrone.	Nil	2.40
68/F	12	6.12	Y/ Physician preference	46/12.5mg	6/400mg	495	15400	infection, abnormal blood test	240mg IM Depomedrone	SSZ: 18W/1g BD/ cumulative dose 33000mg	3.72
66/M	30	4.10	Y/Multiple traumatic fracture	11/10mg	31/400mg	110	85400	multiple traumatic fracture	240mg IM Depomedrone	Nil	2.58

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