Phenotyping DAS28 and Refractory Rheumatoid Arthritis

John Francis Fitton

Supervisors:

Dr Richard Wakefield

Professor Maya Buch

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The candidate confirms that the work submitted is his own, except where work which has formed part of jointly authored publications has been included. The contribution of the candidate and the other authors to this work has been explicitly indicated below. The candidate confirms that appropriate credit has been given within the thesis where reference has been made to the work of others.

The following paper features in part in chapters 7 (Refractory Rheumatoid arthritis and JAK inhibitor use at Leeds Teaching Hospitals) and 8 (Discussion).

Fitton J, Melville AR, Emery P, Nam JL, Buch MH. Real-world single centre use of JAK inhibitors across the rheumatoid arthritis pathway. Rheumatology (Oxford). 2021 Sep 1;60(9):4048-4054.

The Candidate collected the data, performed the statistical analysis and wrote the paper.
A Melville assisted in data collection, P Emery, J Nam and M Buch were involved in clinical supervision and data collection and assisted with editing of the final paper.

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Abstract

Objectives

To identify homogeneous clusters of patients with different DAS28, ultrasound and combined clinical and imaging phenotypes in a population of established RA patients, identify differences between these groups at baseline and track response to targeted therapy to determine if differential responses exist. To establish if the presence of baseline ultrasound synovitis or baseline phenotypic cluster predicts response to therapy. To determine if different Targeted Therapy response trajectories exist within a group of established RA patients. To classify Refractory RA, determine its extent within the local population and establish the efficacy of JAK inhibitors in refractory RA.

Methods

Two-hundred established RA patients were investigated using clinical assessment, Musculoskeletal ultrasound and Patient reported outcome measures. Latent profile analysis was carried out using combinations of these variables to identify disease clusters which were compared using Kruskal-Wallis or Chi^2 for continuous or categorical indicators respectively. Follow up was performed at 3 and 6 months, with repeat MUS at 6 months, to assess response. Multinomial logistic regression models were used to compare responders to non-responders to determine whether latent profile or baseline synovitis predicted response. Longitudinal mixed modelling was used to identify differences in response over time between clusters and Growth mixture modelling was used to identify disease response trajectories. A local RefRA cohort was assessed for patterns of TT switching and to assess JAKi efficacy in RefRA.

Results

Equivalent 3 and 4 class solutions were identified using DAS28 components as indicator variables, with 3 class solutions identified using only MUS variables or a combination of clinical and MUS indicators. In all models, differences in objective DAS28 elements and GS and PD synovitis/tenosynovitis were identified. Neither baseline synovitis nor baseline disease cluster predicted response to targeted therapy. However, distinct patterns of change over time in a two component DAS28 score in response to therapy were noted between clusters. Four response trajectories, including a RefRA trajectory were identified. >10% of the Leeds Teaching Hospitals cohort have RefRA, ~ 6% of this group exhibit multiple primary failures. JAKi showed efficacy across the RA treatment pathway, including in those with RefRA.

Conclusions

Different phenotypes of established RA exist. Neither baseline synovitis or disease cluster predicts response, but clear differential improvements in MUS synovitis/tenosynovitis are observed, highlighting the complexity of treatment response.

Publications and Presentations obtained during PhD period

Publications

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Fitton J, Giollo A, Buch MH. GP2015 as a promising therapy for rheumatoid arthritis. Expert Opin Biol Ther. 2018 Apr;18(4):477-481.

*Denotes publication that contains work included within this thesis.

Poster presentations

***Abstract.** John Fitton, Kamran Naraghi, Jacqueline Nam, Shouvik Dass, Paul Emery, Maya H Buch. 089 Single centre cohort of refractory rheumatoid arthritis also identifies a rare subgroup of multiple targeted therapy class non-response, *Rheumatology*, Volume 58, Issue Supplement_3, April 2019, kez106.088, https://doi.org/10.1093/rheumatology/kez106.088

***Abstract.** John Fitton, Shouvik Dass, Paul Emery, Jacqueline Nam, Maya H Buch, 087 Janus kinase inhibitors demonstrate effectiveness in a real-world multi-biologic DMARD refractory rheumatoid arthritis population, *Rheumatology*, Volume 58, Issue Supplement_3, April 2019, kez106.086, https://doi.org/10.1093/rheumatology/kez106.086

***Abstract.**John Fitton, Andrew Melville, Shouvik Dass, Paul Emery, Jacqueline Nam, Maya Buch. THU0169 JANUS KINASE INHIBITORS DEMONSTRATE EFFECTIVENESS IN A REAL-WORLD MULTI-BIOLOGIC DMARD REFRACTORY RHEUMATOID ARTHRITIS POPULATION. Annals of the Rheumatic Diseases Jun 2019, 78 (Suppl 2) 358-359; DOI: 10.1136/annrheumdis-2019-eular.7246

***Abstract.**John Fitton, Andrew Melville, Kamran Naraghi, Jacqueline Nam, Shouvik Dass, P aul Emery, Maya Buch. FRI0090 SINGLE CENTRE COHORT OF REFRACTORY RHEUMATOID ARTHRITIS ALSO IDENTIFIES A RARE SUBGROUP OF MULTIPLE TARGETED THERAPY CLASS NON-RESPONSE. Annals of the Rheumatic Diseases Jun 2019, 78 (Suppl 2) 708; DOI: 10.1136/annrheumdis-2019-eular.7444

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of the Rheumatic Diseases Jun 2020, 79 (Suppl 1) 303-304; DOI: 10.1136/annrheumdis-2020-eular.4534

Courses and skills.

Musculoskeletal ultrasound

A large part of this study is based on musculoskeletal ultrasound in RA patients in order to phenotype RA patients and assess treatment response. As a result, I have become proficient in musculoskeletal ultrasound. I have attended EULAR Basic ultrasound (Amsterdam 2018) and EULAR Intermediate ultrasound courses (Madrid 2019) (I was unable to attend the Advanced course due to the COVID-19 pandemic). I have carried out a regular MUS session as part of the Leeds Teaching Hospitals early arthritis clinic during my PhD period.

Medical statistics

This thesis contains a number of complex medical biostatistics models. In order to improve my knowledge in this area I have attended a number of courses on medical statistics including university courses on SPSS, Introductory Medical statistics course, Heart and Lung institute, Imperial college (November 2019), Statistical horizons, Latent class analysis course, Lanza and Brey (December 2020).

Glossary of abbreviations

ABT	Abatacept
ACPA	Anti-citrullinated protein antibodies
ACR	American college of rheumatology
ADA	Adalimumab
ADAb	Anti-drug antibody
AE	Adverse event
AFF3	AF4/FMR2 Family member 3
AIC	Akaike Information Criterion
ANA	Anti-Nuclear Antibody
ANKRD55	Ankyrin repeat domain-55
bDMARD	Biologic disease modifying anti-rheumatic drug
BIC	Bayesian Information criterion
BLK	B lymphocyte kinase
BMI	Body mass index
BSRBR	British society of rheumatology biologics register
CD	Cluster of differentiation
CDAI	Clinical disease activity index
CRF	Case Report Form
CRP	C reactive protein
csDMARD	Conventional synthetic disease modifying anti-rheumatic drug
CT	Computed tomography
CTLA4	Cytotoxic T-lymphocyte associated protein 4
CXCL13	Chemokine ligand 13
CXR	Chest X-Ray
CZP	Certolizumab pegol
DAS	Disease activity score
DAS-P	Disease activity score – Patient reported components
DAS28	Disease activity score 28 joints
DAS28-CRP	Disease activity score 28 joints – C reactive protein
DAS28-ESR	Disease activity score 28 joints – erythrocyte sedimentation rate
DAS28CRP-2C	Disease activity score 28 joints C reactive protein – 2 component
Df	Degrees of Freedom
DMARD	Disease modifying anti-rheumatic drug
DNA	Deoxyribose nucleic acid
EAM	Extra-articular manifestation

EBV	Epstein Barr virus
ERAN	Early Rheumatoid arthritis network
ESR	Erythrocyte sedimentation rate
ETN	Etanercept
EU	European union
EULAR	European league against rheumatism
FAB	Fragment antigen binding
FACIT	Functional Assessment of Chronic Illness Therapy
FACT	Functional Assessment of Cancer Therapy
FBC	Full Blood Count
Fc	Fragment crystallisable
FcGR	Fc Gamma receptor
FDA	Federal drug administration
FLS	Fibroblast like synoviocytes
FM	Fibromyalgia
GCs	Glucocorticoids
GE	General Electric
GH	General health
GI	Gastrointestinal
GLM	Golimumab
GM-CSF	Granulocyte macrophage -colony stimulating factor
GMM	Growth Mixture Modelling
GS	Grey scale
HADs	Hospital Anxiety and Depression Scale
HAQ	Health assessment questionnaire
HAQ-DI	Health assessment questionnaire – disability index
HCQ	Hydroxychloroquine
HLA	Human leucocyte antigen
IA	Inflammatory arthritis
IBD	Inflammatory bowel disease
ICAM1	Intracellular adhesion molecule 1
IFN	Interferon
IFX	Infliximab
Ig	Immunoglobulin
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M

IHD	**I**schemic heart disease
IL	Interleukin
IL6ST	Interleukin-6 cytokine family signal transducer
ILD	Interstitial lung disease
IQR	Interquartile Range
IR	Inadequate response
IV	Intravenous
JAK	Janus Kinase
JAKi	Janus Kinase inhibitor
K-W	Kruskal-Wallis
Kg	Kilogram
LCA	Latent Class Analysis
LCMM	Latent Class Mixture Modelling
LEF	Leflunominde
LFT	Liver Function Test
LMM	Longitudinal mixture modelling
LPA	Latent Profile Analysis
LTA	Latent Transition Analysis
LTHT	Leeds Teaching Hospitals Trust
MCP	Metacarpophalangeal
MED15	Mediator complex subunit 15
Mg	Milligram
MHz	MegaHertz
MLS	Macrophage like synoviocytes
MMP	Matrixmetalloproteinase
MRI	Magnetic resonance imaging
MTP	Metotarsophalangeal Joint
MTX	Methotrexate
MUS	Musculoskeletal ultrasound
NET	Neutrophil extracellular trap
NF1A	Nuclear factor 1 A
NHS	National Health Service
NICE	National institute for health and care excellence
NK	Natural killer
NSAID	Non-steroidal anti-inflammatory drug
NSIP	Non-specific intersitial pneumonia
OA	Osteoarthritis

OD	Once daily
OMERACT	Outcome measures in rheumatoid arthritis clinical trials
OPG	Osteoprotegrin
OR	Odds ratio
PAD	Peptidyl-arginine deaminase
PD	Power doppler
PDUS	Power Doppler Ultrasound
PEG	Polyethylene glycol
PIP	Proximal interphalangeal
PIPJ	Proximal interphalangeal joint
PRL	Prolactin
PRO	Patient reported outcome
PROM	Patient reported outcome measure
PsA	Psoriatic arthritis
PTPN22	Protein tyrosine phosphatase, non-receptor 22
PTPRC	Protein Tyrosine kinase receptor type c
RA	Rheumatoid arthritis
RADAR	Rheumatoid Arthritis DiseAse Research
RANK	Receptor activator of nuclear factor Kappa B
RANKL	Receptor activator of nuclear factor Kappa B Ligand
RCT	Randomised controlled trial
RefRA	Refractory Rheumatoid arthritis
RF	Rheumatoid factor
RRR	Relative risk ratio
RTX	Rituximab
SAR	Sarilumab
Sc	Subcutaneous
SD	Standard Deviation
SDAI	Simplified disease activity index
SF-36	Short form-36
SH	Synovial hypertrophy
SHS	Sharp-Van der Heijde score
SJC	Swollen joint count
SNP	Single nucleotide polymorphism
SpA	Spondyloarthritis
SSA	Sulfasalazine
STAT	Signal transduction and activator of transcription

T2T	Treat to target
TB	Tuberculosis
TCZ	Tocilizumab
TJC	Tender joint count
TNF	Tumour necrosis factor
TNF1A	Tumour necrosis factor receptor 1 A
TNFAIP3	Tumour necrosis factor, alpha-induced protein 3
TNFi	Tumour necrosis factor inhibitor
tsDMARD	Targeted synthetic disease modifying anti-rheumatic drug
TSS	Total Sharp score
TT	Targeted therapy
TYK2	Tyrosine kinase 2
U+E	Urea and Electrolytes
UIP	Usual interstitial pneumonia
UK	United Kingdom
US	Ultrasound
USA	United States of America
VAS	Visual analogue scale
VAS-GH	Visual analogue score – general health
VTE	Venous thromboembolism

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1 Introduction and literature review

1.1 Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease. It is the prototypical inflammatory arthritis and is characterised by joint pain, stiffness and swelling. It affects between 0.3-1% of the population in western countries (1) which makes it one of the most common inflammatory diseases. Sub-optimally treated RA can damage joint structures, erode periarticular bone and degrade cartilage, leading to progressive joint destruction with consequent deformity and disability. Although joint inflammation is the defining feature, RA should be recognised as a syndrome which may have multiple systemic manifestations (2). These include anaemia, myalgia, anorexia, and low-grade pyrexia. In addition, a number of serious complications such as vasculitis or pulmonary fibrosis can occur (3). RA leads to an excess in mortality, mainly through increased cardiovascular disease (4) and an increased susceptibility to infections (5). In 2009 The National Audit Office estimated that RA costs the UK National health service (NHS) £560 million in direct health care costs annually (6). In addition work disability and sick leave are major consequence of RA, particularly in western countries(7), causing an estimated further £1.8 billion cost to the UK economy(6).

Over recent decades, an increased understanding of the molecular pathology of RA, particularly in relation to the importance of inflammatory cytokines and autoreactive B and T lymphocytes, has allowed the development of therapies directly targeted at these key inflammatory mediators. The use of targeted therapies (TT) has revolutionised the treatment of RA over the last 20 years. Starting with the introduction of the first generation of TNF inhibitors (TNFi) in the late 1990s, the biologic disease modifying anti-rheumatic drug (bDMARD) repertoire has expanded regularly to include five different TNFis; the fusion protein

etanercept, the monoclonal antibodies infliximab, adalimumab, golimumab and the pegylated monoclonal Fab fragment certolizumab pegol, two monoclonal antibodies directed against IL-6; tocilizumab and sarilumab, the anti-CD20 monoclonal antibody rituximab and a CTLA4-Ig fusion protein abatacept which modulates T cell co-stimulation. Biosimilar versions of etanercept, infliximab, adalimumab and rituximab are also now available. In addition, the small molecule Janus Kinase inhibitors (JAKi), which block one or more of the molecules of the JAK-STAT intracellular signalling cascade common to multiple inflammatory cytokines, have recently become available, further expanding the treatment armamentarium, and providing an alternative mechanism to treat RA. Tofacitinib and baricitinib were licenced for use in late 2017 with 2 further JAKis, upadacitinib and filgotinib the most recent drugs to be approved for the treatment of RA, with a number of others in development or the regulatory pipeline.

Despite this treatment revolution, over the last few years a growing cohort of patients who have tried and failed most or even all the available targeted therapies have begun to emerge and provide a challenge in the rheumatology clinic. This problem is now recognised to be such an important issue that it has been declared to be a key research priority in the field (8) . The proportion of RA patients which fall into the category of refractory RA (RefRA) is unclear and varies depending on how refractoriness is defined. Until recently, there was no agreed definition of refractory or difficult to treat RA and no clear evidence about how to proceed when patients fail more than one targeted treatment. EULAR introduced its definition of difficult to treat RA in late 2020 (9). This provides a broad definition of difficult to treat disease and allows a more standardised approach for research in the area. However, a distinction can be drawn between those with difficult to treat RA, who may cycle through treatments due to a combination of inefficacy, adverse reactions and co-existing or emerging co-morbidity, and those patients who fail to respond to therapy due to biological resistance of their disease. The proportion of patients with multiple primary inefficacies; ‘true’ refractory disease, is likely to be a small proportion of the overall difficult to treat cohort. Whether refractoriness is inherent in

these patients or evolves as a consequence of inadequate therapy or incorrect targeting of drugs in early disease is yet to be elucidated.

RA is a complex multi-system disease, with multiple genetic and environmental factors involved in its development, evolution and response to treatment. The reasons for recurrent drug failure are likely to be equally complex with pharmacokinetic and pharmacodynamic factors, patient adherence to therapy and the burden of psychological and physical comorbidity all playing a role in treatment response.

The pathological hallmark of RA is synovitis, the suppression of which can be said to be the primary focus of RA therapy. In European practice, response to therapy is measured indirectly using the DAS28 score, a composite score calculated using tender and swollen joint counts, a laboratory determined marker of inflammation and a patient's global assessment of their own disease activity and general health. It is well recognised that pain, co-morbidity, and psychological factors can influence the tender joint count (TJC) and Visual analogue scale (VAS) scores, as these DAS components have a more subjective assessment than swollen joint count (SJC) and a laboratory measured marker of inflammation. This may influence a patients disease activity score irrespective of the presence or severity of synovitis. As a result, patients with the same DAS28 score may have differing levels of joint inflammation and thus, it is likely that for a given DAS28 score there exists a number of differing RA phenotypes, whose response to a given therapy cannot be assumed to be the same. Emerging evidence in early RA suggests that these different clusters of patients exist (10), but there is no evidence base for this in advanced RA.

The purpose of this thesis is therefore to assess the extent of RefRA within our population and to identify whether a cohort of true refractory RA patients exists within it. It is also to identify whether clusters of RA patients exist within an advanced RA population, to use musculoskeletal ultrasound to assess for the presence of absence of synovitis and to assess

the response of these patients to targeted therapy to see if RA phenotype affects response to therapy. This will help to take a step towards a more personalised approach to the management of advanced RA.

1.2 Rheumatoid Arthritis

1.2.1 History

The first recognised modern description of RA was made by the French physician Augustin Jacob Landre-Beauvais in 1800 (11). He identified a small group of patients whose joint pain and swelling differed from other forms of 'rheumatism' recognised at the time. Unlike gout, it was more common in females and predominantly affected poor people. As a result it was likely to be ignored by physicians of the time (12). Landre-Beauvais named the condition "Primary Asthenic Gout". The English physician Alfred Garrod (1919-1907) made the next important contribution to the study of RA (13). He differentiated gout from other forms of arthritis by identifying high levels of uric acid in the blood of gout patients, but not in those with other forms of arthropathy. In his "Treatise on Nature of Gout and Rheumatic Gout" he described RA as a distinct condition which he named "Rheumatic Gout". His son, Archibald Garrod, was the first to coin the term 'Rheumatoid Arthritis' in 1890 (12).

There has been much debate regarding the historical origins of RA, with some authors speculating that RA is a relatively modern disease. Others have suggested that it was present in the Americas, but not in Europe until the discovery of the new world in the 1500s. However, descriptions of clinical signs and symptoms consistent with RA in historical medical texts date back as far as Hippocrates. This combined with depictions in art and literature and evidence from the paleo-biological records, suggest RA has likely been present in the population since antiquity (12).

1.2.2 Epidemiology of Rheumatoid Arthritis

The incidence and prevalence of RA varies across different populations and between groups of the same ethnicity living in different geographical areas (1, 14), highlighting the role of both genetic and environmental factors in its development. RA affects all ethnic groups. In North America and Northern Europe RA is estimated to have an incidence of approximately 20-50 cases per 100,000 people with a prevalence varying from 0.3 to 1.1% of the population (15). It appears to be more common in Northern than southern Europe, where the incidence has been estimated to be 9-24 cases per 100,000 (15). Epidemiological data from developing countries is lacking. Studies have suggested very low levels of RA in rural areas of Africa (16) and levels approaching those seen in western countries in urban Kinshasa, Democratic Republic of the Congo (17). A prevalence as high as 5% in some North American Indian tribes has been observed (18).

RA is up to 4 times more common in females than males prior to the menopause and twice as common at the age of 60 with the sex ratio becoming more even in the elderly population (19, 20). RA disease activity and progression also tends to be more severe in women than in men (21). The onset of RA can occur at any age, with the peak incidence occurring within the fourth and fifth decades of life (22).

1.2.3 Pathophysiology of Rheumatoid Arthritis

1.2.3.1 Genetics

Rheumatoid arthritis is a heterogeneous condition with multiple genetic and environmental factors involved in its development, progression, severity and response to therapy (2). There is a well-established heritable element to RA. The relative risk of disease development in the

first degree relatives of affected individuals is approximately two (23). Twin studies have indicated that the genetic contribution to RA pathogenesis is around 60% (24). However, disease concordance in identical twins of only 15% indicates that there must be other environmental susceptibility factors involved (24). RA can be subclassified into two forms based on the presence or absence of associated autoantibodies in the sera of affected patients (Anti-Citrullinated protein antibodies - ACPA and Rheumatoid factor - RF). Approximately 70% of patients have one or both autoantibodies and are classified as having seropositive RA with the other 30% classified as being seronegative. Genome wide studies have produced estimates of the genetic contribution to ACPA positive RA of around 55%, with a lower estimate for ACPA negative RA of approximately 20%(25).

More than 100 loci associated with RA risk have been identified in genome wide association studies using single nucleotide polymorphisms (SNPs). These are spread widely across the genome, with an important concentration in genes associated with innate and adaptive immune responses (26). The largest genetic risk factor is found within the human leukocyte antigen (HLA) class II region encoding the HLA-DRB1 molecule. The specific alleles of HLA-DRB1 associated with RA share a conserved sequence of 5 amino acids lying within the binding groove of the HLA molecule (27). This is known as the shared epitope, and strongly implicates peptide binding and antigen presentation in the pathogenesis of RA. The shared epitope is particularly associated with seropositive RA, with a much smaller association with seronegative disease (27). Several other genetic differences between ACPA positive and ACPA negative disease have also been identified. Whereas variants in HLA-DRB1, PTPN22, BLK, ANKRD55 and IL6ST associate with both types of RA, SNPs in AFF3, CD28 and TNFAIP3 genes are found only in seropositive disease and SNPs in PRL and NF1A genes are only found in the seronegative variant (28, 29).

Epigenetic differences may also play a role in RA pathogenesis. For example, analysis of DNA methylation in T cells in patients with RA has revealed global hypo-methylation when

compared to controls (23). In addition, an altered DNA methylome signature in synovial fibroblasts derived from RA patients has been identified in several studies, with hypomethylated genes clustering in gene pathways related to cell adhesion, migration and interaction with extracellular matrix (30, 31). However, it is not known definitively when these epigenetic changes take place or whether they are an initiator of disease, or a consequence of the prolonged inflammatory environment found in RA.

1.2.3.2 Risk factors

Several lifestyle and environmental triggers have been associated with RA. Smoking is a significant risk factor for both RA development and for severe disease. Current smokers have been shown to have an odds ratio (OR) of 1.87 for developing RA , with a lower, but still elevated OR of 1.76 in past smokers (32). The risk is particularly high in Rheumatoid factor positive males (32) or ACPA positive patients with at least 1 copy of the shared epitope (33). It is estimated that smoking accounts for up to 30% of the environmental risk for RA (34). Smoking is also directly related to both periodontal and lung disease, which are themselves identified as risk factors for RA development (35). It has been proposed that smoking leads to increased citrullination of proteins, particularly in the lungs, and in genetically susceptible individuals, leads to the generation of ACPA (36).

Inhalation of dusts, including silica (37) and particulate air pollution have been indicated in the development of RA (35, 38). Indeed, dust containing silica, cement, glass, asbestos and other materials inhaled by 1st responders to the September 11th 2001 attacks in New York has been shown to increase the risk of RA as well as other autoimmune diseases in this group (39). Occupational exposure to dust created during the manufacture of textiles has also been suggested to be a risk factor for RA development (40).

Obesity has also been linked to the development of RA. Using the United States Nurses health survey, which enrolled female nurses from 1976 onwards, Lu et al (41) showed that being obese or overweight gave a hazard ratio of 1.76 for the development of RA. It also increased the risk of developing RA before the age of 55. Conversely, a healthy diet has been suggested to reduce the risk of developing RA in females before age 55 (42). No individual dietary factor has been linked conclusively with RA development. Low intake of vitamin D, high consumption of red meat, sodium, sugar and iron have all been suggested as possible risk factors (35, 43-46), whereas high intake of omega 3 fatty acids has been suggested to be protective against RA development in several studies (47).

Like many other autoimmune diseases, RA is more common in females. Women are around 4 times more likely to develop RA than men prior to menopause and twice as likely thereafter (20). This higher frequency has been attributed to the role of female sex hormones on inflammation, but the exact mechanism by which they influence RA risk is unclear. The postmenopausal stage, early menopause, the postpartum period, and the use of anti-oestrogen drugs have all been associated with an increased risk of RA development (20).

Several microorganisms and viruses, including both pathogens and microorganisms within an individual's microbiome, have been implicated in RA development. Multiple viral and bacterial infections including Epstein-Barr virus (EBV), Parvovirus, Proteus and a variety of mycobacterial infections have been inconclusively linked (48, 49). There is increasing epidemiological evidence linking periodontal disease to rheumatoid arthritis. It has been hypothesised that a number of micro-organisms associated with periodontal infection, particularly Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans, may be involved in the generation of ACPA (50). The gut microbiome has also been implicated in the pathogenesis of RA. A number of studies have identified differences between the microbiome of RA patients and healthy controls (51). Evidence of a reduced risk of developing RA in the

2 years post antibiotics for urinary or gastrointestinal infections has also been shown, possibly because of the antibiotics effect on the microbiome (52).

1.3 Pathology

The interaction of this multitude of genetic and environmental risk factors, coupled with the presence or absence of autoantibodies in different subtypes of RA show that it is a pathologically heterogeneous condition. The exact mechanisms by which immune tolerance breaks down in RA are yet to be fully understood. In many seropositive RA patients' ACPA are detectable in the sera several years before signs and symptoms of RA develop. Thus, seropositive RA can be said to begin with a genetically determined high risk or susceptibility stage and, through the interaction of genetic risk factors with triggers in the environment, evolve through a pre-clinical arthritis stage and finally into established disease (28). It is likely that seronegative disease has a similar evolution.

1.3.1 Autoantibodies

An important role for B lymphocytes in the development of RA is suggested by the presence of specific autoantibodies in the majority of patients with RA. These antibodies may be present many years before the development of arthritis. Anti-citrullinated protein antibodies (ACPA) are a collection of autoantibodies of different isotypes (IgA, IgM, IgG) with specificity for the amino-acid citrulline within proteins (53). Citrullination is the irreversible post-translational modification of the amino acid arginine to citrulline, by the peptidyl-arginine deaminase enzymes (PAD). PADs are a highly conserved family of enzymes involved in normal functioning of the immune system and in reproduction. PAD2 and PAD 4 are expressed on leucocytes that migrate to inflamed joints in RA and are therefore most implicated in the development of ACPA (54). These PAD expressing leukocytes release large amounts of enzyme into the inflamed synovial tissues, which in turn, produces large concentrations of different citrullinated proteins, including fibrin, vimentin and fibrinogen, in the synovial fluid

(54). The process of citrullination reduces the amount of positively charged amino acids within a peptide and thus decreases the protein's ability to form hydrogen bonds. This may lead to changes within the peptides structure and a consequent increase in immunogenicity, leading to the production of autoantibodies (53). A number of experimental studies have suggested that the ACPA formation may first begin at mucosal surfaces in the lungs or oral mucosa in response to inflammation, with the development of localised IgA ACPA (55). This has led to the hypothesis that, like RF, ACPA generation has a physiological role, but the mechanism by which this transition into the systemic generation of IgM/IgG class ACPA occurs has not been definitively elucidated.

The presence of ACPA in RA is associated with more aggressive disease, more rapid radiological progression (56) and an increased risk of complications such as interstitial lung disease (57). This suggests a potential role for ACPA in the pathogenesis of the disease. Indeed, ACPA have been shown to enhance tissue injury in murine models of inflammatory arthritis (58). There are a number of potential mechanisms by which ACPA can activate the immune cascade in RA. ACPA has been shown to activate macrophages in the synovium via Fc receptors and stimulate them to produce TNF alpha (59). Immune complexes of ACPA and citrullinated proteins can activate the compliment cascade (60). It has also been suggested that ACPA can cause the formation of Neutrophil extracellular Traps (NETosis), a process of neutrophil cell death involving the extrusion of the intracellular material, resulting in creation of cellular trap, which further drives the inflammatory response (53). Furthermore, ACPA has been shown to be directly involved in osteoclast activation and bone erosion formation by binding to osteoclast precursors and activating them in a TNF medicated autocrine mechanism (61).

Rheumatoid factor is an autoantibody directed at the Fc portion of IgG. Different isotypes of RF can be present, but IgM is the most common. RF is not specific to RA and is found in many

inflammatory diseases, although it is said to undergo affinity maturation in RA, unlike other inflammatory conditions. High titres of IgM (and IgA) RF are said to be more specific for RA (62). The role of RF in the pathology of RA is not clear. It is capable of forming immune complexes which can deposit into joint and other tissues, promoting an inflammatory response.

1.3.2 Synovitis

The pathological hallmark of RA is synovitis (63); inflammation of the lining of synovial joints. In a normal joint the synovium consists of a thin membrane made up of 1or 2 layers of cells. These consist of fibroblast-like synoviocytes (FLS) (type B synoviocytes) and macrophage-like synoviocytes (MLS) (type A synoviocytes) which are supported by a sub-lining of connective tissue, fibroblasts, blood and lymphatic vessels and nerves (64, 65). Synovium lines joint cavities, tendon sheaths and bursae. It secretes synovial fluid, which nourishes joint cartilage and maintains joint homeostasis (66). In RA the synovium becomes inflamed and thickened due to an influx of inflammatory cells including dendritic cells, mast cells and B and T lymphocytes. Neo-angiogenesis and the proliferation of resident FLS and macrophages also contribute to synovial thickening (64). These cells secrete inflammatory cytokines and chemokines leading to the further recruitment, activation and retention of inflammatory cells into this inflamed tissue. The thickened synovium, known as pannus, can fill the joint cavity and invade cartilage, bone and tendons, directly damaging joint structures, inhibiting function and causing the joint swelling classical of RA.

1.3.3 Inflammatory cytokines

The secretion of inflammatory cytokines and chemokines in the RA joint is crucial to the initiation and maintenance of the inflammatory process. A number of inflammatory cytokines are found in high concentrations in the rheumatoid joint, including amongst others, Tumour necrosis factor α (TNFα), members of the IL-1 family, interleukin (IL)-6, interferons and

Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF) (67). The success of targeted drugs that directly inhibit some of these cytokines, as well as those directed against auto reactive lymphocytes, in part responsible for their production, show their importance in inflammatory conditions. In particular, the success of drugs directed against TNFα and IL-6 show the significance of these particular cytokines in the clinical syndrome of RA (68).

The relative failure of targeted therapies directed against IL-1 and IL-17 in RA underlines the complexity of this cytokine biology as they are known to be key mediators of the inflammatory response. IL-1 is elevated in the RA joint. However, its functions may be shared by other cytokines. It is thus not a 'pivotal' cytokine in the maintenance of inflammation in the synovium (67). Recent advances in the understanding of cytokine biology have suggested that the hierarchical dominance of certain cytokines may change over time and that the cytokines involved in the initiation of disease, may differ from those that are responsible for its maintenance and thus the chronicity of RA (69). IL-6, IL-21, IL-23 and IL-17 likely drive the immune activation and loss of tolerance in pre-clinical RA, whereas distinct cytokines, including TNFα and IL-6 may be responsible for the transition to, and maintenance of, chronicity (67). It is therefore possible that IL-17 blockade may be useful in pre-clinical disease, but less efficacious in established RA. Similarly, a small subset of RA patients with an autoinflammatory phenotype may respond to anti-IL-1 treatment (70).

Another cytokine, receptor activator of nuclear factor Kappa-B (RANK) is involved in the activation and differentiation of osteoclasts. In the RA synovium high levels of RANK Ligand (RANKL) and low levels of the competitive inhibitor osteoprotegrin (OPG) lead to activation and differentiation of osteoclasts which cause bone resorption at the synovium-bone interface leading to bone erosions (71). RANKL is secreted by activated FLS and T cells within the synovium in response to stimulation by TNF, IL-1 and other inflammatory cytokines (71). Activated FLS have also been shown to be directly involved in joint damage. FLS secrete enzymes such as matrixmetalloproteinases (MMP) and others capable of degrading cartilage

extracellular matrix and have been shown to be capable of directly invading joint cartilage (65). These processes lead to bone erosions and cartilage destruction which contribute to the joint degeneration that characterises advanced RA.

1.4 Clinical features

1.4.1 Presentation

Joint pain and soft tissue swelling are the classical signs and symptoms of RA, but its presentation and progression can be varied and there is no single set of clinical features that definitively differentiate it from other types of Inflammatory arthritis (IA), without further investigation. RA is usually preceded by a long pre-clinical phase where autoantibodies, inflammatory cytokines and a raised inflammatory response may be present in the sera before symptoms develop. Disease onset is often insidious, progressing from fatigue, stiffness and arthralgia to frank IA over a period of months, although a more rapid, dramatic onset can also occur. Stiffness, particularly early morning stiffness or stiffness after periods of inactivity, is an important symptom. Significant stiffness lasts more that 30 minutes and can last several hours, only resolving after a period of activity.

RA is classically described as a symmetrical polyarthropathy, with multiple joints, most commonly in the hands, wrists and feet, involved bilaterally at onset. However, oligo- and occasionally mono-arthropathies can also occur at presentation. Therefore, patients may be classified as having undifferentiated arthritis until the full clinical syndrome of RA develops over time (72). In older patients a presentation involving limb girdle pain and stiffness resembling polymyalgia rheumatica is well recognised (73). A further subset of patients develops a condition known as palindromic rheumatism, where joint pain, swelling and stiffness appear rapidly and last from hours to a few days before resolving and then recurring regularly. Around 50% of patients with palindromic rheumatism progress to persistent RA over time (74).

1.4.2 Joint involvement

Inflammation of the synovium leads to joint swelling, pain, stiffness and immobility. Involved joints are swollen and tender to palpation, may be erythematous and have a restricted range of movement. Any synovial joint can be affected in RA but those most commonly involved include the proximal interphalangeal (PIP) joints, metacarpophalangeal (MCP) joints, wrists and the small joints of the feet, as well as the elbows, shoulders, knees and ankles (75). The relative sparing of the distal interphalangeal joints of the hands and the axial skeleton help to distinguish RA from the seronegative spondyloarthropathies. The exception to this is tendency for RA to involve the C1-C2 atlantoaxial joint in the cervical spine. Inflammation leading to stretching of the transverse ligament of C1 and erosion of the C2 odontoid peg can lead to atlanto-axial subluxation, compression of the cervical cord and a cervical myelopathy.

Inadequately treated RA leads to progressive joint damage and deformity. Periarticular bone erosion and cartilage degradation leads to the development of secondary osteoarthritis. As well as direct joint involvement, peri-articular structures such as joint capsule, tendons, ligaments, and facia can become inflamed, stretched or directly invaded by pannus. This process can lead to some of the classical deformities of poorly controlled RA, such as Swan-neck deformity, boutonnieres deformity, ulnar deviation and joint subluxation. Tenosynovitis in tendon sheaths is common and can contribute to tendon rupture. Although enthesitis, inflammation around the insertion sites of tendons and ligaments, is a pathological hallmark of spondyloarthritis, imaging studies of inflamed joints comparing SpA to RA suggest it can also be a feature of RA (76) and is thus responsible for some of the pain and stiffness in the rheumatoid joint.

1.4.3 Extraarticular manifestations

RA is a complex, multisystem autoimmune disease which can affect multiple sites beyond the joints. Extra-articular sequelae are common, particularly in patients with sub-optimally controlled disease and tend to occur late, although conditions such as pulmonary fibrosis may be the presenting feature (77). Extra-articular manifestations (EAM) of RA occur in 18-41% of RA patients, with a higher incidence reported in Northern European countries, again suggesting that both genetic and environmental factors may play a role in their development (78). Fatigue, pyrexia and anorexia, occasionally with significant weight loss can occur alongside a number of organ specific manifestations (28).

1.4.4 Rheumatoid nodules

Rheumatoid nodules are lesions, usually of greater than 5mm, which mainly develop in the subcutaneous tissue, but can also form in joints and in major organs such as the lungs and pericardium. They can affect up to 30% of RA patients and are far more common in RF positive patients (79), possibly suggesting a role for immune complex deposition in their aetiopathogenesis, but this is yet to be definitively defined. They are also associated with smoking and shared epitope positivity (78). Mature rheumatoid nodules have a classic multi-layered structure with a necrotic centre containing deposits of fibrinoid material, surrounded by a layer of palisaded mononuclear cells with an outer fibrous layer consisting of vascular connective tissue infiltrated by inflammatory cells (80). In the skin they occur at areas that are subject to pressure or irritation such as the elbows, occiput, fingers and heels, but can form at other sites. Treatment with Methotrexate (MTX) can trigger or worsen nodulosis in some RA patients (78).

1.4.5 Vasculitis

Inflammation of the blood vessels in the rheumatoid synovium is an important feature of RA pathology, but vascular inflammation is not confined to the joint tissue. Both autopsy and blind biopsy studies have shown evidence of widespread vascular inflammation in RA patients. This is predominantly asymptomatic but can contribute to significant end organ damage (81). Skin involvement is the most common manifestation (~ 90%), where it can cause petechiae, purpura and even skin ulceration, particularly on the lower limbs. The next most common site for vasculitis is the peripheral nerves. Vasculitis of the vasa nervorum can lead to mononeuritis multiplex or peripheral sensory neuropathy (81). Central nervous system vasculitis is rare, although eye involvement is reported to be relatively common with retinal vasculitis estimated in some reports to affect 1% of patient with established RA (82). Involvement of other end organs such as the kidneys, gut or lungs is rare in RA and is more suggestive of other forms of vasculitis.

1.4.6 Pulmonary complications

Lung involvement in RA is common and comprises several complications including interstitial lung disease (ILD), bronchiectasis and pleural disease as well as an increased susceptibility to respiratory infection. Pulmonary fibrosis is the most serious complication and is estimated to affect around 10% of RA patients and account for 10-20% of the mortality associated with RA (83). Usual interstitial pneumonia (UIP) is the most common subtype, accounting for up to 66 % of cases (83), followed by non-specific interstitial pneumonia pattern (NSIP). Other forms of ILD, including organising pneumonia and lymphocytic interstitial pneumonia are seen less often. Pleural disease is common, with autopsy studies showing a prevalence rate of 50-70%, although these studies pre-date the biologic therapy era. Symptomatic pleural disease is present in less than 5% (84).

1.4.7 Cardiac complications

EAM in the heart are also well recognised. All cardiac structures can be affected. Cardiac complications include pericarditis, myocarditis, myocardial fibrosis, valvular disease, pulmonary hypertension, cardiac arrhythmia due to conduction system involvement, coronary artery disease and ischemic cardiomyopathy. Pericardial disease is the most common manifestation with a prevalence of 30-50% in echocardiographic and autopsy studies, although less than 10% of patients with severe RA show clinical signs of pericarditis (85). Rheumatoid arthritis patients are at increased risk of ischemic heart disease (IHD) and stroke. This increase in risk is only partially explained by an increase in the prevalence of other risk factors for IHD in the RA population, with persistent inflammation in RA an independent risk factor for IHD (86). Longer disease duration is associated with more significant coronary artery disease identified on coronary artery CT (87). Heart failure, particularly diastolic dysfunction, is around twice as prevalent in the RA population than in those without the disease and is only partially explained by the increase in IHD (85).

1.4.8 Other organ manifestations

A number of other organs can also be involved in RA, including the eye, nervous system, kidneys and blood. The most common ocular manifestation of RA is keratoconjunctivitis sicca, which affects between 18-90% of RA patients, with moderate to severe symptoms seen in up to 50% of patients. Episcleritis is the next most common affecting 1-5% of patients, followed by scleritis and peripheral ulcerative keratitis, which can lead to corneal ulceration, scaring and even perforation (88).

Most of the neurological manifestations of RA are due to nerve compression by joint synovitis. The classical examples of this are compression of the median nerve within the carpal tunnel or atlantoaxial subluxation causing compression of the cervical spinal cord (89). Rheumatoid

meningitis is a rare complication which may present with headaches and stroke like episodes (90).

Although renal disease is common in RA patients, renal disease caused by RA pathology is relatively rare. Renal amyloidosis due to prolonged poorly controlled inflammation or glomerulonephritis are the most common complications, but more often than not renal complications are related to drugs, such as NSAIDs, or the increased vascular risk associated with RA (91).

Haematological manifestations are common, particularly an anaemia of chronic disease and thrombocytosis. Felty's syndrome is a rare complication of RA and is characterised by splenomegaly and neutropenia in RA patients. The risk of both Hodgkin's and non-Hodgkin's lymphoma is approximately doubled in patients with RA (92).

1.4.9 Mortality in rheumatoid arthritis

Mortality rates in patients with RA are higher than in the general population. The median standardised mortality ratio for RA patients compared to the general population is in the region of 1.5-1.6 (93). The most common cause of death is cardiovascular disease, which occurs at a similar rate to the general population, but on average occurs at an earlier age in RA patients (93). Other causes of death include infections, renal disease, gastrointestinal disease and malignancy. RA was associated with a mean loss of 4.97 years of life expectancy in one longitudinal cohort (94).

1.5 Diagnostic criteria

Diagnostic criteria for RA were developed in 1983 and updated in 2010 in a collaboration between the American college of Rheumatology (ACR) and the European league against

Rheumatism (EULAR) (95) (**Table 1.1**). The criteria were primarily devised to standardise diagnosis for the purposes of clinical trials. They are designed to be applied to patients with at least 1 swollen joint, in whom other causes of synovitis are excluded. The presence of an alternative diagnosis excludes RA. Furthermore, the presence of classical joint erosions on imaging is diagnostic for RA and doesn't require the below criteria to be met. A score of 6 or more allows a diagnosis of RA to be made.

Criteria	**Score**
Joint distribution	
1 large joint	0
2-10 large joints	1
1-3 small joints (large joints not counted)	2
4-10 mall joints (large joints not counted)	3
> 10 joints (at least 1 small joint)	5
Serology	
Negative RF and negative ACPA	0
Low positive RF OR low positive ACPA	2
High positive RF OR high positive ACPA	3
Symptom duration	
< 6 weeks	0
≥ 6 weeks	1
Acute phase reactants	
Normal CRP AND normal ESR	0
Abnormal CRP OR abnormal ESR	1

***Table 1.1**: ACR/EULAR 2010 diagnostic criteria for RA*

RF – Rheumatoid factor, ACPA – Anti- Citrullinated Protein antibody, CRP-C-Reactive Protein, ESR- Erythrocyte sedimentation rate.

1.6 Disease activity indices and response criteria

1.6.1 Disease Activity Score, Disease Activity Score 28 and European League Against Rheumatism response criteria

There are a number of measures of disease activity and general health used to assess disease severity and response to treatment in RA (96). The DAS28 score is the tool most used in standard clinical practice. The original DAS score was developed in 1990 (97) as a means to standardise the monitoring of disease activity and treatment response. As there is no one laboratory indicator of disease activity in RA, and no one sign for a clinician to monitor, the DAS score was developed as a composite score of a number of indicators of disease activity, including the earlier Ritchie index of joint tenderness (98) a 44 swollen joint count, ESR and a patient assessment of disease activity (visual analogue score (VAS), scored on a scale of 0-100). The component variables in the DAS are either patient reported, and therefore represent symptomatic disease (TJC,VAS), clinician assessed (SJC), which although also symptomatic, represents a more objective disease marker of activity, or laboratory measured (ESR); which more directly addresses inflammation. Subsequent versions of the DAS score, using a 28 joint count (DAS28-ESR) and using CRP instead of ESR (DAS28-CRP) have been developed and validated (99, 100). The DAS28-CRP scores are generally around 0.2 points lower than those provided by the DAS28-ESR (101) meaning that the scores are not directly interchangeable in an individual patient.

In the UK the DAS28 is used to define eligibility for starting a targeted therapy and defines the level of response required for continuing treatment (102-104). The DAS28 is a validated composite score comprising a 28 tender joint count, 28 swollen joint count, and ESR or CRP and a patient visual assessment score. It is calculated using the following formulae:

DAS 28-ESR = (0.28 X √SJC) + (0.56 x √TJC) + (0.70 x ln(ESR) + 0.014 X GH)

DAS 28-CRP = (0.28 X √SJC) + (0.56 x √TJC) + (0.36 x ln(CRP+1) + 0.014 X GH) + 0.96

The DAS and DAS 28 scores have been used to define the EULAR response criteria which give a measure of response to therapy outlined in **table 1.2** (105-107) and can be used to classify patients as either good, moderate or non-responders to treatment. They are also used to classify disease activity states from high disease activity to remission (**table 1.3**) (108).

DAS at endpoint	DAS 28 at endpoint	Improvement in DAS or DAS 28 at endpoint		
		≤1.2	>0.6 and ≤1.2	≤0.6
≤2.4	≤3.2	Good	Moderate	None
>2.4 and ≤3.7	>3.2 and ≤5.1			
>3.7	>5.1			

Table 1.2*: EULAR response criteria*

DAS score	DAS 28 score	Disease activity category
<1.6	<2.6	Remission
≥1.6 and ≤2.4	≥2.6 and ≤3.2	Low disease activity
>2.4 and ≤3.7	>3.2 and ≤5.1	Moderate disease activity
>3.7	>5.1	High disease activity

Table 1.3*: EULAR disease severity indices*

1.6.2 Two component Disease Activity Score 28

The DAS28 scores were all developed before the widespread use of imaging techniques such as musculoskeletal ultrasound, which have been able to show a discrepancy between clinical assessment and the presence of active synovitis. Patients with DAS28 defined remission can still have active subclinical synovitis visible on musculoskeletal imaging and can still develop

radiological progression (109, 110). Baker et al found that only swollen joint count and acute phase reactants were independently associated with active synovitis on MRI (111). Hensor et al (112, 113) further investigated this by studying the results of ultrasound in 3 early arthritis cohorts and found the same result. They calculated a 2 component DAS28 score which was able to better reflect active synovitis than the traditional 4 component model using the following formulae:

2C-DAS28CRP = $\sqrt{}$SJC28+(0.6 x ln(CRP+1))

2C-DAS28ESR = $\sqrt{}$SJC28+(0.32 x ln(ESR))

1.6.3 Simplified disease activity index (SDAI)

The SDAI score was designed as a simplified disease activity score, calculated by summing together 5 indicators of disease activity, which can be done in clinic without the complex calculation required to perform a DAS 28 score. It is validated in RA patients and shows good correlation with the DAS28 (114). It is calculated by summing together a 28 tender joint count, 28 swollen joint count, patient global assessment of disease activity on a 0-10 scale, physician assessment of disease activity on a 0-10 scale and the CRP in mg/dl. The level of disease activity can be interpreted as remission (SDAI ≤3.3), low (3.3 < SDAI ≤ 11), moderate (11 < SDAI ≤ 26), or high (SDAI >26) (115). A change in SDAI score of 16 corresponds to a change of 1.2 in the DAS28 (96).

1.6.4 Clinical disease activity index (CDAI)

The CDAI is analogous to the SDAI, except it excludes the CRP and can thus be calculated without requiring a blood test or in clinic before blood results are available. It is calculated by summing together a 28 tender joint count, 28 swollen joint count, patient global assessment of disease activity on a 0-10 scale and a physician assessment of disease activity on a 0-10 scale. It correlates well with DAS 28 score (116). The level of disease activity can be

interpreted as remission (CDAI ≤2.8), low (2.8 < CDAI ≤ 10), moderate (10 < CDAI ≤ 22), or high (CDAI >22). As it does not include an acute phase reactant it may be particularly appropriate to use in patients on IL-6 blockade, which will mechanistically normalise CRP independent of its effects on disease activity (117).

1.6.5 American college of Rheumatology response rates (ACR20/50/70)

The ACR20 response rate was devised in the early 1990s to provide a standardised measure of disease improvement in order to allow comparison of clinical trial outcomes in Rheumatology, which was lacking prior to this (118). It is based on the ACR core set of disease activity measures (119). There are 7 core disease activity measures: a tender joint count, swollen joint count, patient's assessment of pain, patient's and physician's global assessments of disease activity, patient's assessment of physical function, and laboratory evaluation of either CRP or ESR. The ACR 20 score is a 20% improvement in tender and swollen joint counts and a 20% improvement in 3 of the other 5 core activity measures. ACR 50 and ACR 70 response rates represent 50% and 70% improvement. In 2007 an amendment to the measure was proposed by the ACR which retained the information obtained from ACR20/50/70 and combined it with a mean percent improvement in core set measures (120).

1.7 Principles of management of Rheumatoid arthritis

1.7.1 Treating Rheumatoid arthritis to target

Alongside the targeted therapy revolution, the most important advance in the management of RA over the last 2 decades has been the implementation of treat to target (T2T) strategies. Treating patients aggressively by rapidly escalating therapy with the target of inducing disease remission reduces the risk of structural damage and improves outcomes in RA patients (103,

121). The principles of T2T highlight the importance of early diagnosis to enable rapid initiation of csDMARD, usually MTX first line, in combination with corticosteroid treatment followed by regular review and, when remission is not achieved, escalation of therapy to combination csDMARD and on to TT rapidly. It is recommended that treatment should be escalated at least every 3 months to achieve remission or low disease activity when remission cannot be achieved (121). T2T guidelines highlight the importance of shared decision making between patients, clinicians and the multi-disciplinary team in optimising patient care and ensuring adherence to therapy.

In addition to pharmacological treatments exercise, physiotherapy, smoking cessation, and the maintenance of a healthy diet are all important interventions in RA management, but for the purpose of this thesis I will focus primarily on the pharmacological management of RA.

1.8 Treatment of Rheumatoid arthritis

1.8.1 Corticosteroids

Glucocorticoids (GCs) have been a mainstay of RA therapy for decades. Indeed, the first patient treated with glucocorticoid therapy in 1948 suffered from severe RA (122). GCs are naturally occurring hormones with myriad biological functions. Named for their effects on carbohydrate metabolism, they also have a role in the regulation of metabolism, development and crucially, inflammation (123). GCs have several anti-inflammatory effects, including the down regulation of the production of inflammatory cytokines, including TNFα, IL-6 and IL-1. CGs are important for the development of T and B cells and can inhibit the function of T cells and NK cells and, at high doses, inhibit antibody production (122). They also reduce the production of matrix-metalloproteinases by fibroblasts (122). In RA CGs have disease modifying effects and can supress disease activity at low to moderate doses in both early and

late RA (124). If used in early disease GCs can help to suppress bone erosion (125). Their rapid onset of action allows them to be used as a bridging therapy at the initiation of treatment whist awaiting the onset of csDMARDs (124). They can be administered orally or parenterally. The use of glucocorticoids is limited by their significant side effect profile. Long-term use of CGs can lead to, amongst other things, diabetes, osteoporosis, cataracts, hypertension and increase risk of infections (122).

1.8.2 Conventional Synthetic Disease Modifying Anti-Rheumatic Drugs (csDMARDs)

1.8.2.1 Methotrexate

Methotrexate (MTX) is a cornerstone drug in the management of RA. It is efficacious as a monotherapy or in combination with other csDMARDs or TTs, where it increases TT efficacy and reduced the risk of anti-drug antibody formation. It is administered either orally or parenterally at doses of between 7.5mg and 25-30mg once weekly. MTX was first developed in the 1940s as a treatment for haematological malignancies, where it is administered at significantly higher doses. MTX inhibits folate metabolism, and it is by this mechanism that it treats malignancy, but the exact mechanism of action by which it treats RA is not completely clear. The inhibition of adenosine signalling, reduction in immune cell chemotaxis, inhibition of pro-inflammatory cytokine production and generation of reactive oxygen species have all been proposed as possible mechanisms of action in RA (126).

The first reports of its efficacy of low dose MTX in patients with IA date back to the 1960s (127), but the first placebo controlled clinical trials were not performed until the 1980s. Weinblatt et al published one of the first placebo controlled clinical trial of MTX in RA patients (128). Thirty-five patients were randomised to receive either MTX or placebo. In the MTX arm statistically significant improvement in tender and swollen joint count, ESR, patient and

clinician global assessment and early morning stiffness were observed. Patients were crossed over at 12 weeks and in those who switched from MTX to placebo, flares in disease activity were generally observed at between 3 and 6 weeks. Similar results were observed in several contemporary studies (129-131). Long term extension studies confirmed the sustained efficacy of MTX in RA patients (132). Treatment with MTX has been shown to reduce radiographic progression when used as a first line csDMARD (133, 134). Methotrexate was shown to be more efficacious than several other csDMARDs that were licensed for use in RA at the time of its approval, including azathioprine (135) and gold (136). More recent analysis has suggested that MTX is more efficacious than all the available csDMARDs, other than Leflunomide which has similar efficacy (137).

MTX is generally safe and well tolerated. The main side effects are gastrointestinal disturbance, myelotoxicity, derangement of liver function, renal impairment and rarely pneumonitis. The co-administration of folic acid has been shown to reduce some of the side effects of MTX therapy (138).

1.8.2.2 Sulfasalazine

Sulfasalazine (SSA) has been shown to be safe and efficacious in RA. For a number of years, it was used as a first line therapy in the management of RA in many centres, although it is now more commonly used in combination with MTX or after MTX intolerance. The mechanism of action of SSA in RA is unclear, nor is it clear whether it is the drug itself or its metabolites (sulphapyridine and mesalazine) that are responsible for its DMARD effect. Administration of SSA in vitro has been shown to reduce the concentrations of several inflammatory cytokines including TNFα. It has also been shown to reduce chemotaxis of inflammatory cells, including neutrophils (139). It has been shown to be efficacious in the management of RA in placebo-controlled trials (140-143). Meta-analysis of clinical trials into csDMARDs available for management of RA prior to the emergence of TTs , suggested that SSA may be as efficacious

as MTX, gold and penicillamine and better than hydroxychloroquine and azathioprine (144). The combination of SSA and etanercept (ETN) has been shown to be efficacious in one clinical trial, but not more so than ETN alone (145). SSA is safe and well tolerated. The most common side effects are gastrointestinal disturbances, followed by rash and headache and more rarely myelotoxicity and deranged liver function. Oligospermia in males leading to reduced fertility is common on SSA, but reversible on stopping treatment (139).

1.8.2.3 Hydroxychloroquine

Hydroxychloroquine (HCQ) is an antimalarial drug that has been found to have some efficacy in RA. There is limited evidence relating to its use as a monotherapy (146) and it is generally felt to be of lower efficacy than the other available csDMARDs, but its good safety and tolerability profile make it a useful choice in patients with mild disease. HCQ is immunomodulatory rather than immunosupressive and therefore doesn't increase the risk of infection or malignancy. The most common side effects are gastrointestinal upset. Retinopathy is a rare complication associated with prolonged use and high cumulate dose and screening by opticians/ophthalmology is recommended (147). Data suggests that it is useful in combination with other csDMARDs, particularly MTX in the management of RA and may also help to improve metabolic profiles in patients with RA (146).

1.8.2.4 Leflunomide

Leflunomide (LEF) is an isoxazole derivative and acts to inhibit de novo pyrimidine synthesis and thus inhibits lymphocyte proliferation, which is the mechanism by which it is felt to treat RA. LEF has been shown to be efficacious in the management of RA when compared to placebo (148-150) and have similar efficacy to MTX in reducing RA disease activity and radiographic progression (151, 152). Like MTX, LEF can cause deranged liver function and myelosuppression and in addition, LEF is associated with elevation in blood pressure in some patients. It's very long half-life and teratogenicity mean that it requires washing out with

cholestyramine before pregnancy can be considered. This limits is use in women of child baring age (153).

1.8.2.5 Other conventional synthetic DMARDs

There are several other medications which have shown to be of use in the management of RA, but the use of which is limited either due to their poorer safety profile or relative lack of efficacy. The dawning of the targeted therapy era has meant that they have a limited role in the management of RA in clinical practice as those patients responding poorly to MTX and the other more commonly used csDMARDs are escalated rapidly to TTs, although the emergence of patients who are refractory to or intolerant of TTs may warrant their consideration. Ciclosporin has been shown to improve disease activity and reduce radiological progression in RA (154) as has tacrolimus (155). Older drugs such as injectable gold (sodium aurothiomalate), oral gold (Auranofin) and penicillamine are available, but no longer commonly used.

1.8.2.6 Combination conventional synthetic DMARDs.

The combination of 2 or more csDMARDs, with and without steroid therapy, has been shown to increase the efficacy of treatment. O'Dell et al (156) showed that treatment with "triple therapy" of MTX, SSA and HCQ was more efficacious than either MTX monotherapy or combination therapy of SSA and HCQ. Triple therapy was also shown to be more efficacious than either combination of MTX and SSA or MTX and HCQ (157). The results of the BeST trial suggest the addition of corticosteroids to combination MTX/SSA at baseline leads to better outcomes than the step wise escalation of csDMARD (158). Combination MTX and LEF has also been shown to be effective in treating patients with an inadequate response to MTX monotherapy (159).

1.8.3 Targeted therapies

The development of targeted therapies over the last two decades has revolutionised the treatment of RA (160). A total of 11 TTs were available for the treatment of RA at the time that this thesis was initiated. These include 5 TNFis (Infliximab, etanercept, adalimumab, certolizumab and golimumab), two drugs directly targeting IL-6 (tocilizumab and sarilumab), one B cell depleting therapy (Rituximab), one inhibitor of T cell co-stimulation (Abatacept) and two Janus kinase inhibitors (tofacitinib and baricitinib). These will be discussed in turn. Two further JAKis (upadacitinib and filgotinib) have been introduced after data for this thesis had been collected.

1.8.3.1 Biological disease modifying anti-rheumatic drugs (bDMARDs)

1.8.3.1.1 Anti-TNFα therapy

Tumour necrosis factor α (TNFα) plays a central role in the cytokine cascade involved in the pathogenesis of RA. It recruits inflammatory cells into the RA joint, induces the production of other inflammatory cytokines (including IL-1 and IL-6) and the production of matrix metalloproteinases and adhesion molecules by synovial fibroblasts. It also helps to stimulate bone resorption by stimulating osteoclast differentiation (161). Therapeutic blockade of TNFα has been shown to be efficacious in a wide range of inflammatory diseases including RA. All 5 licensed TNFis are available for the treatment of RA.

1.8.3.1.1.1 Infliximab

Infliximab (IFX) is a chimeric monoclonal antibody directed against TNFα. It comprises 75% human and 25% murine protein, with the murine portion containing the variable region and the human region responsible for effector function (162). It avidly binds soluble, and membrane bound TNFα. In RA it is administered by intravenous infusion at a licensed dose of 3mg/kg of body weight at 0, 2 and 6 weeks and then every 8 weeks and is given in combination with MTX. The dose may be increased up to a maximum of 7.5mg/kg every 8 weeks or 3mg/kg every 4 weeks in the event of declining response (163). It was licenced for use in the UK in 2000 making it one of the first biologic DMARDs and the first monoclonal antibody available for the treatment of RA. Infliximab was first licenced under the trade name Remicade®. Several biosimilar versions of comparable efficacy and tolerability (Remisma®, Inflectra®) are now available (164).

IFX has been shown to be efficacious in both early and established RA. The first studies of IFX in RA date back to 1992. Patients with active RA, despite treatment with MTX, were given intravenous doses of IFX ranging from 1-20mg/kg of body weight. These were the first studies to show that blocking TNF was efficacious in RA (161, 165). The ATTRACT study was pivotal in showing the efficacy of IFX in managing the clinical symptoms of RA (166). Patients suffering from active RA, despite treatment with MTX were randomised into 5 groups to receive either 3mg/kg or 10mg/kg IFX every 4 or 8 weeks, in combination with MTX or MTX and placebo. ACR20 response at 30 weeks was achieved in 51.8% of the IFX treated patients, but only 17% of those treated with placebo (166). IFX plus MTX was also shown to significantly slow the progression of structural damage. Radiological data showed a higher rate of bone erosion at week 54 in the group given placebo, than in the IFX treated groups (167). After 2 years patients receiving IFX and MTX continued to have a good clinical response and inhibition of progressive joint damage. They also experienced improvement in physical function and health related quality of life (168).

IFX has also been shown to be efficacious in early RA (169). In the ASPIRE trial 1004 MTX naïve patients with early RA (>3months, <3 years) were treated with either IFX 3mg/kg or 6mg/kg in combination with MTX. After 54 weeks ACR20, ACR50 and ACR70, HAQ and van der Heijde–Sharp scores of bone erosion were all significantly better in the IFX arms than in those treated with MTX alone (170). The BeST study showed similar results (158). Patients were allocated to one of four treatments, sequential csDMARD monotherapy, step up csDMARD combination therapy, combination therapy with csDMARD and steroid and combination therapy with MTX and IFX. Initial combination with IFX and MTX resulted in earlier functional improvement and less radiological progression than either combination DMARDs or monotherapy.

IFX is generally safe and well tolerated. The most commonly occurring adverse event in those treated with IFX are infections, with respiratory tract infections the most common. Reactivation of tuberculosis is a recognised complication of IFX and the other TNFis and screening for latent TB is required before drug initiation. The development of autoantibodies is observed in some patients treated with infliximab. In the ATTRACT trial 24% of patients developed a positive ANA. Other adverse events include infusion reactions and, more rarley, demyelinating disease (161).

1.8.3.1.1.2 Etanercept

Etanercept (ETN) is a fully humanised dimeric fusion protein consisting of two extra-cellular ligand binding domains of the 75kDa TNF receptor linked to the Fc portion of human IgG1. It binds with high affinity to both TNFα and TNFβ (lymphotoxin-α) and exerts its biologic effects by competitively inhibiting the binding of TNFα to its cell surface receptor, rendering it inactive (171). The biological significance of TNFβ in RA is unknown. ETN is administered subcutaneously from a pre-filled syringe at a dose of either 50mg weekly or 25mg twice weekly, with both doses shown to be equivalent (163, 172). It was first licenced in the USA for

the treatment of RA in 1998 (173, 174) and has been approved in Europe since 2000 (175). Reference ETN was licenced under the name Enbrel® but several biosimilar versions of comparable safety and efficacy are now available (Benipali®, Erezli®) (173, 176).

The efficacy of ETN in RA was first demonstrated in phase II trials in 1997 (177). ETN was subsequently shown to be efficacious in patient with suboptimal disease control despite MTX therapy in phase III trials. Weinblatt et al randomised 89 patients on stable doses of MTX (15-25mg) to receive either ETN 25mg or placebo subcutaneously twice weekly. The primary endpoint was an improvement in ACR20 response after 24 weeks; 71% of patients in the combined ETN and MTX treatment group achieved this versus 27% in the placebo arm, 39% in the combined arm met an ACR50 response vs 3% in the placebo arm (178). In the TEMPO trial 684 patients with established RA (average 6-year disease duration) were randomised to receive MTX 20mg weekly as monotherapy, ETN 25mg twice weekly as monotherapy or combination ETN and MTX. Combined ETN and MTX produced advantages in ACR20, 50 and 70 responses over either monotherapy. The primary efficacy endpoint was the numeric index of the ACR response (ACR-N) area under the curve (AUC) over the first 24 weeks, which showed a statistically significant advantage for combination therapy over either monotherapy. Combination therapy was also significantly reduced radiological progression (179). As a result of this it is recommended that when possible ETN should be used in combination with MTX. However, the results of the ADORE trial, where MTX-IR were randomised to either add ETN to their MTX therapy or replace MTX with ETN monotherapy, showed comparable results for improvement disease activity measures and in HAQ and global VAS. This suggests important improvement in disease activity and quality of life for patients treated with ETN monotherapy (180).

ETN has also been studied for use and shown to be efficacious in early RA. Bathon et al studied 632 patients who were randomised to receive twice weekly subcutaneous ETN (10mg or 25mg) or a rapidly escalating dose of oral MTX. The ETN 25mg group showed more rapid

improvement with significantly more patients obtaining ACR 20/50/70 responses at 6 months. Although at 12 months there was no difference between disease activity between the groups, the ETN group had less radiological progression, highlighting the importance of rapid disease control (181). The COMET study compared DAS 28 remission criteria and radiographic progression between a group of patients with early RA receiving combination ETN and MTX and a group on MTX monotherapy. 50% of the combination group achieved DAS28 remission versus 28% in the MTX monotherapy group. There was also a significant advantage in radiological progression (182). Sub analysis of the COMET data suggested a particular advantage in very early disease (<4 months) (183), although this hasn't been confirmed in a subsequent RCT (184).

1.8.3.1.1.3 Adalimumab

Adalimumab (ADA) was the first fully human monoclonal antibody introduced for the treatment of RA. It consists of human heavy and light chain variable regions and human IgG1 constant regions engineered using phage display technology (185). It binds with high affinity to membrane bound and soluble TNFα. It was first authorised for use in EU in 2003. ADA is administered subcutaneously from a pre-filled pen device at a dose of 40mg every 2 weeks, which can be increased to weakly in the event of a decreased response (163). Adalimumab was first licensed under the name Humira®. Since 2018 five different biosimilar versions of adalimumab have become available in Europe (Amgevita™, Imraldi ™, Hulio™, Hyrimoz™ and Idacio™)(186).

The ARMADA trial was the first phase III trial demonstrating the efficacy of adalimumab in the treatment of RA (187). 271 patients with inadequate response to csDMARDs, including MTX, were randomised to receive MTX plus ADA 20, 40 or 80mg or MTX and placebo. Statistically significant advantages in ACR 20, 50 and 70 were seen, as well as significant improvement

in HAQ and acute phase reactants. A 4-year extension study showed that in the 147 patients who completed 4 years of treatment, these advantages were maintained. 42% of patients achieved DAS 28 remission and 22% had no abnormality in physical function (HAQ 0) (188). Similar results were obtained in a study by Keystone et al, in which 619 RA patients were randomised to receive either ADA 40mg every other week, 20mg weekly or placebo, plus MTX. At week 52 there was a statistically significant improvements in disease activity, but also a significant reduction in radiographic progression in the ADA 40mg group over the placebo group. Moreover, 72% of the patients treated with adalimumab showed no radiographic progression at all (189).

ARMARDA and Keystone et al confirmed that, like other TNFis, ADA in combination with MTX is highly effective. Evidence of efficacy in combination with other csDMARDs was provided by several studies. The ReACT study was a large open label study of ADA therapy in 6610 patients who were inadequately controlled on standard therapy with multiple different csDMARDs. The cohort also included patients who had previously failed either IFX or ETN. Overall, 69% achieved an ACR 20 response at 12 weeks. In the TNFi exposed cohort only a slightly lower proportion of patients (60% vs 69%) also achieved an ACR 20 response. Responses were maintained over a 52-week extension (190). The STAR trial also showed efficacy in patients taking up to 3 csDMARDs. Six-hundred and thirty-six patients with inadequate disease control were randomised to receive either ADA 40mg every other week or placebo in combination with their existing therapy. Statistically significant advantages in ACR 20, 50 and 70 were shown, but with no difference in adverse events between the 2 cohorts (191).

The DE011 clinical trial demonstrated that in patients with inadequate response to or intolerance of MTX, adalimumab can be effective as a monotherapy, showing a statistically significant advantage over placebo (192). In the PREMIER trial patients with early aggressive RA, naïve to MTX were treated with either MTX monotherapy, ADA monotherapy or ADA in

combination with MTX. Combination therapy was superior to both monotherapy groups for all end points. There was no statistically significant difference in disease activity scores for ADA monotherapy over MTX, but there was less radiological progression in the ADA group (193). This demonstrates that combination therapy is more efficacious, but also, like other TNFis, ADA is effective in the management of early RA. This has been confirmed in subsequent studies (194).

1.8.3.1.1.4 Certolizumab Pegol

Certolizumab Pegol (CZP) is a novel TNFi, consisting of a Fab fragment targeted against TNFα, bound to a 40kDa Polyethylene glycol (PEG) moiety. The addition of the PEG element increases the half-life of the Fab fragment and allows CZP to be given 2-4 weekly (195). CZP lacks an Fc region and therefore doesn't induce complement or antibody dependent cell lysis, as has been observed in vitro with other TNFi, suggesting that this mechanism is not key to the efficacy of TNFi in inflammatory diseases (196). The lack of an Fc portion also means that there is very minimal transfer across the placenta, making CZP safe to continue throughout pregnancy (197). CZP was approved for use in Europe in 2009 and approved for use in patients with severe RA, who have failed csDMARDs in the UK by NICE in 2010. CZP is administered subcutaneously at a dose of 400mg at weeks 0, 2 and 4 and then at a dose of 200mg every 2 weeks thereafter (163). It is sold under the trade name Cimzia®.

The efficacy of CZP in treating RA was shown in 3 pivotal phase III trials. The RAPID 1 and 2 trials were multicentre, placebo-controlled trials testing the efficacy and safety of CZP in patients with an inadequate response to MTX (198, 199). RAPID 1 was a study of 982 patients using a lyophilised CZP over a 52-week period, whereas RAPID 2 was a 24-week trial of 619 patients using a liquid formulation of CZP. In both trials patients were randomised 2:2:1 to receive CZP 400mg at weeks 0, 2 and 4 followed by either 200mg or 400mg every 2 weeks, plus MTX or placebo and MTX. The primary endpoint for both studies was ACR 20 response

at 24 weeks, with mean change in modified total sharp score (TSS) at week 52 a co-primary end point in RAPID 1. In both trials both CZP doses showed statistically significant advantages over placebo in ACR 20 as early as week 1, with the advantage peaking at week 12 and being maintained throughout the duration of both studies. Mean change in TSS was also significantly better in the CZP groups than placebo. Treatment with CZP and MTX also gave significantly better DAS 28 responses, pain VAS, HAQ and SF-36 scores than MTX and placebo.

The FAST4WARD (Efficacy and safety of CZP – 4 weekly dosage in RA) trial investigated the efficacy of CZP as a monotherapy in RA (200). 220 patients with adult-onset RA who had failed cs DMARDs were randomised to receive either CZP 400mg four weekly or placebo. The CZP treated group achieved statistically significant improvements in ACR 20 over placebo as early as week 1 with the advantage maintained throughout the 24-week study period. As were improvements in ACR 50, 70, DAS 28 and patient reported outcomes.

CZP has been shown to be efficacious in patients with prior TNFi failure(201, 202) and has also been trialled and shown benefit in patients with moderate disease activity, as defined by DAS 28 score (203).

1.8.3.1.1.5 Golimumab

Golimumab (GLM) is a human IgG1 monoclonal antibody targeted against TNFα (204). It binds with high affinity to both soluble and membrane bound TNF and thus blocks its interaction with its receptor. It is administered subcutaneously from a pre-filled injection pen at a dose of 50mg every 4 weeks, although in patients weighing more than 100kg a dose of 100mg may be used in those who do not respond adequately to 50mg (163). It was approved by the FDA in 2009 under the brand name Simponi® and approved for use, in combination with MTX, in the UK by NICE in 2011 (205).

The clinical efficacy of GLM in RA has been shown in a number of phase III clinical trials and has been shown to efficacious and well tolerated in patients naïve to MTX, poorly responsive to MTX and in those who have failed previous TNFi. The GO-BEFORE trial randomised a cohort of active RA patients 1:1:1:1 to receive either GLM and placebo, MTX and placebo, GLM 50mg and MTX, and GLM 100mg and MTX. The study did not reach its primary end point of showing an advantage in ACR50 of GLM in combination with MTX over MTX alone, but a modified intention to treat analysis and analysis of other endpoints, including response/remission criteria for DAS28, did show an advantage. It was concluded that the efficacy of GLM plus MTX is better than, and the efficacy of GLM alone is similar to, the efficacy of MTX alone in reducing RA signs and symptoms in MTX-naive patients (206).

In the GO-FORWARD study patients inadequately controlled on MTX were randomised to receive either MTX and placebo, GLM 100mg and placebo, GLM 50mg and MTX, and GLM 100mg and MTX. A statistically significant improvement in both ACR20 and HAQ-DI scores was shown in the GLM and MTX combination groups, showing that in patients poorly controlled on MTX monotherapy the addition of GLM significantly reduces the signs and symptoms of RA (207). The GO-AFTER study evaluated the efficacy of GLM in patients who had an inadequate response to a previous TNFi (IFX, ADA, ETN) due to intolerance or lack of efficacy. Patients were randomised to receive either placebo, GLM 50mg or GLM 100mg. There was a statistically significant improvement in ACR20 response in patients receiving both doses of GLM, showing it to be efficacious in patients previously treated with an alternative TNFi (208, 209).

The GO-FURTHER study showed that not only does GLM improve disease outcome measures in patients' refractory to MTX, but GLM in combination with MTX reduces radiological progression of RA at weeks 24 and 52 with no significant excess of adverse events up to 12 months (210, 211). Pooled safety data from 5 phase III trials of GLM in RA, PsA and ankylosing spondylitis show that GLM is generally well-tolerated. Overall, in these trials, upper-

respiratory infections, nasopharyngitis, elevated aminotransferase levels and hypertension were the most common adverse events. TB, lymphoma and opportunistic infection was more common in the 100mg dose groups than the 50mg group (212).

1.8.3.1.2 Anti-Interleukin-6 therapy

IL-6 is another key cytokine involved in the initiation and propagation of the persistent inflammatory response that drives RA. IL-6 is mainly produced by neutrophils and monocytes in response to activation of toll like receptors. It is present in high concentrations in the serum and synovial fluid of RA patients (213) where it leads to the recruitment and activation of inflammatory cells and the activation of resident fibroblasts and macrophages which secrete further inflammatory cytokines. IL-6 stimulates B cell differentiation and activation and as a result, directly effects the production of antibodies. It stimulates T cells to differentiate into T Helper 17 cells (214). It also acts on hepatocytes and stimulates them to produce CRP (215). There are two drugs licenced for use in RA that target IL-6: tocilizumab and sarilumab.

1.8.3.1.2.1 Tocilizumab

Tocilizumab (TCZ) is a recombinant, fully humanised IgG1 monoclonal antibody directed against membrane bound and soluble IL-6 receptor (216). It binds competitively with the receptor preventing IL-6 from binding and thus blocks its action. Originally licensed in Japan in 2005 for the treatment of Castleman's disease and then in 2008 for RA, the intravenous formulation was approved for use in the EU for RA in 2009 (216). Subcutaneous TCZ was approved in 2014 (163) after studies showed comparable efficacy to the IV formulation (217). TCZ can be administered as either an intravenous infusion at a dose of 8mg/kg body weight (up to a maximum dose of 800mg) every 4 weeks or as a subcutaneous injection at a dose of 162mg weekly. It is licenced under the name RoActemra® in Europe and Actemra® in USA.

The clinical efficacy of tocilizumab has been demonstrated in an extensive clinical trials programme, a number of post marketing trials and several observational studies of real-world experience. Several trials have shown the efficacy of TCZ in patients with established RA. It has been demonstrated in MTX-IR (218-222) and those with inadequate response to csDMARDs (223-225). In both the LITHE (218) and ACT-RAY (221) trials TCZ in combination with MTX was also shown to reduce radiographic progression when compared to MTX and placebo. In the ACTION study (224), Choy et al compared treatment with either TCZ or a TNFi as a first line TT in csDMARD IR patients. TCZ had a statistically significant advantage over TNFi in both DAS 28 and CDAI remission at week 52 of the trial.

TCZ has also been shown to be efficacious in patients who have previously failed a TNFi in several trials (217, 225-227). In the RADIATE trial 499 patients with inadequate response to 1 or more TNFi were randomised to receive either TCZ 8mg/kg, 4mg/kg or placebo. ACR 20 response rates were achieved in 50% of those on 8mg/kg, 30.4% on 4mg/kg and only 10% of those in the placebo arm. ACR 50 and 70 and DAS28 remission was also significantly higher in the 8mg/kg arm (226).

As in advanced disease, TCZ has been shown to be efficacious in very early RA (228-230). In the FUNCTION study 1162 patients with a mean disease duration of 5 months were randomised 1:1:1:1 to receive TCZ 4mg/kg +MTX, TCZ 8mg/kg + placebo, TCZ 8mg/kg + MTX and MTX + placebo. The primary outcome measure was DAS28 remission at week 24. There was a statistically significant advantage in all 3 treatment groups over MTX and placebo. In the TCZ 8mg/kg+ MTX group there was also a statistically significant reduction in radiological progression over placebo (229). In U-ACT-Early csDMARD naïve patients with a disease duration of less than 1 year were randomised to receive either TCZ 8mg/kg plus MTX, TCZ 8mg/kg monotherapy or MTX monotherapy. In the three arms a sustained 2-year DAS28 remission was achieved by 86%, 84%, and 44% of patients, respectively (230).

The results of the U-ACT-Early trial suggests comparable efficacy of TCZ monotherapy to combination therapy with MTX. This has also been shown in several further clinical trials. The AMBITION trial demonstrated the head-to-head superiority of a TCZ monotherapy over MTX in RA patients who had not previously failed MTX or bDMARD. TCZ 8 mg/kg was better than MTX monotherapy according to all primary outcomes at 24-weeks, including ACR20, 50 and 70, and DAS28 remission (228). This advantage in efficacy was maintained for up to 264 weeks in a long-term extension trial (231). In the ACT-RAY trial of MTX-IR, adding TCZ to MTX or switching to TCZ monotherapy was comparable according to both clinical and radiographic endpoints (221). The ADACTA trial (222) was the first RCT head-to-head comparing two anti-cytokine agents with different mechanisms of action as monotherapy. TCZ was superior to adalimumab in the 24-week primary end point of mean change from baseline in DAS28, with a similar incidence of adverse events.

Pooled analysis of adverse events from clinical trials in patients treated with TCZ suggest that infections, particularly upper respiratory tract infections, nasopharyngitis, pneumonia, and cellulitis are the most common adverse effects of treatment with TCZ. Common laboratory abnormalities, including decreased neutrophil counts, elevated liver enzymes, and increase in serum lipid levels have also been identified as common side effects (232). Gastrointestinal perforation, particularly lower GI perforation associated with diverticular disease has been shown to be a particular concern with TCZ and, as a result it is recommended that TCZ is avoided in patients with a history of diverticulitis (233).

1.8.3.1.2.2 Sarilumab

Sarilumab (SAR) is a fully human IgG1 monoclonal antibody targeted against soluble and membrane bound IL-6 receptor. In light of the success of TCZ, sarilumab was developed to bind to IL-6R with higher affinity. It has been shown to have a 20-fold higher affinity for IL6-Rα than TCZ (234). SAR was licenced for use in the UK in 2017. It is administered as a

subcutaneous injection from a pre-filled pen device at a dose of 200mg every 2 weeks. The dose can be reduced to 150mg in the event of adverse effects (125). It is licenced under the trade name Kevzara®.

The clinical efficacy of SAR was established in a number of phase II and III clinical trials. The MOBILITY trial was designed as both a phase II and phase III study and performed in 2 parts. MOBILITY part A was a double-blind placebo-controlled phase II portion of the study (235). Three hundred and sixty-six eligible patients (18-75 years of age, with active RA for a duration of at least 3 months, despite a stable dose of MTX 15-25mg for at least 6 weeks, with no prior bDMARD failures) were randomised receive SAR in addition to MTX at a range of doses (100mg weekly, 100mg 2 weekly, 150mg weekly, 150mg 2 weekly, 200mg 2 weekly). All doses at or above 150mg every 2 weeks showed a statistically significant improvement in ACR20 response and DAS 28 CRP reduction. In MOBILITY B (phase III) (236) Patients were randomized 1:1:1 to receive placebo, sarilumab 150 mg every 2 weeks, or sarilumab 200 mg every 2 weeks, in combination with MTX. Both sarilumab doses showed statistically significant improvement in ACR20 response at week 24 (58.0% and 66.4% respectively), change in Health Assessment Questionnaire-Disability Index (HAQ-DI) at week 16 and radiographic progression of structural damage assessed by change in the modified Sharp van der Heijde score at week 52. Positive results were also achieved for several secondary end points, including DAS28-CRP remission, CDAI score and ACR50 and 70 response rates.

In the TARGET trial (237), Fleischmann et al randomised 546 RA patients, who had previously failed at least 1 TNFi, to receive either SAR 150mg every 2 weeks, SAR 200mg every 2 weeks or placebo every 2 weeks, in combination with cs DMARDs. Statistically significant improvement in the ACR20 response was observed in patients receiving sarilumab 150 mg or 200mg over placebo (55.8% and 60.9% vs 33.7%), regardless of the number of prior TNFis. Similar benefits were observed for secondary clinical outcomes, including ACR50 and 70 response, and mean change from baseline in DAS28-CRP score.

In MONARCH (238), a multicentre, randomized, active-controlled, double-blind, double-dummy, Phase III superiority trial, sarilumab 200mg every 2 weeks was compared to adalimumab monotherapy (40mg every 2 weeks) in patients with active RA who were unable to tolerate MTX or were MTX-IR. The primary endpoint was mean change from baseline DAS28-ESR at week 24. SAR achieved significantly bigger improvements than ADA (-3.28 vs -2.20). Similarly, SAR monotherapy demonstrated superiority compared to ADA monotherapy in all secondary endpoints (DAS28-ESR remission rate, ACR20/50/70 responses, HAQ-DI score, SF-36 score, and FACIT-F score).

SAR is safe and well tolerated, with analysis form clinical trials showing the most common AEs are infections, neutropenia and deranged LFTs. SAR has been shown to have a similar safety profile to TCZ (234).

1.8.3.1.3 B cell therapy

The identification of autoantibodies in RA patients highlights the fact that disorders in the humoral immune response play a role in its pathogenesis. B cells are a key effector cell in the adaptive immune response. (239). In RA activated, autoreactive B cells are present in the synovium and peripheral blood. These autoreactive B cells are not eliminated and are thus able to mature into ACPA and RF producing plasma cells and present antigen to T cells, leading to T cell driven inflammation (239). They also have a role in the innate immune system and are responsible for the production of a number of inflammatory cytokines including IL-12, IL-23, IL-17 and both TNFα and IL-6 (240); two cytokines that are also therapeutic targets in RA. B cells were thus identified as an important target in the management of RA. To date there are a number of biotherapeutic drugs capable of depleting B cells, but the only drug licensed for this purpose in RA is rituximab, giving it a unique role in the RA treatment armamentarium.

1.8.3.1.3.1 Rituximab

Rituximab (RTX) is a chimeric monoclonal antibody directed against CD20; a molecule found on the cell surface of a subset of cells from the B cell lineage. RTX is 20% murine and 80% human protein (241). RTX depletes susceptible B cells through a number of mechanisms including complement dependent cell lysis and antibody dependent cytotoxicity (242). RTX depletes mature B cells and pre-B cells through to memory B cell stages, but does not deplete stem cells, pro-B cells, most terminally differentiated plasma cells, or plasmablasts as they do not express CD20 (242). In RA, treatment with IV rituximab results in almost complete depletion of peripheral B cells, but variable levels of depletion in the synovium and lymphoid tissue (243) with clinical response correlating to with both peripheral and, to a lesser extent, synovial B cell depletion (243, 244). Reconstitution of B cells can lead to relapse of clinical disease. RTX is administered as an intravenous infusion and licensed for use in combination with MTX in patients with prior TNFi failure. The recommended dose is two infusions of 1000mg given 2 weeks apart (245). This can be reduced to 500mg in patients with recurrent infection and/or low immunoglobulin levels. RTX was licensed for use in RA in 2006. RTX was first licensed under the trade name Mabthera®. Two biosimilar versions of comparable efficacy and safety have more recently been approved for use (Truxima™, Rixathon™) (246).

The first reports of the efficacy of RTX for the management of RA were published in 2001(247). Since then, several phase III trials investigating the efficacy of RTX in the management of RA have been published (248-256). Several of these studies showed the efficacy to RTX in patients who had failed csDMARDs. In the DANCER trial (252) 265 patients with longstanding RA refractory to csDMARD therapy (and some with prior bDMARD-IR) were randomised to receive either RTX 500mg (2 doses) or RTX 1000mg (2 doses) or placebo (2 doses), in combination with MTX. At week 24 a statistically significant proportion of patients in the RTX treated arms achieved ACR 20, 50 and 70 responses compared to the placebo arm. In the MIRROR trial (250) 346 MTX-IR patients were treated at baseline and 6 months with three

different treatment regimens: 2 doses of 500mg at baseline and 6 months, 2 doses of 500mg at baseline and 2 doses of 1000mg at 6 months or 2 doses of 1000mg at baseline and 6 months. ACR responses were similar between all 3 arms, but EULAR moderate/good response was higher in those treated with 2 doses of 1000mg than those treated with 500mg. At week 48 those patients who received 2 cycles of 1000mg plus 1000mg, maintained or improved their response. All 3 arms had similar safety profiles. Similar results were obtained for the SERENE study (249) where treatment with either RTX 500mg or 1000mg was shown to more efficacious than placebo in MTX-IR patients.

RTX has been shown to be efficacious in patients with prior TNFi failure. In the REFLEX trial (251) 520 patients with longstanding RA, who had failed at least 1 TNFi were randomised to receive either two 1000mg doses of RTX or placebo, in combination with MTX. ACR 20, 50 and 70 responses were all statistically significantly more likely in the RTX treated arm. At 2 years radiological progression was significantly lower in the RTX group. In the SUNRISE trial (253) 559 patients in TNFi-IR were given an open label treatment with RTX (2 doses of 1000mg) in combination with MTX. At 6 months, those who had failed to achieve remission defined by DAS 28 score were randomised to receive either a further cycle of RTX or placebo. Those who received RTX had a significant improvement in DAS 28, suggesting that not only is RTX efficacious in TNFi-IR, but in those who get a suboptimal response, retreatment with RTX at 6 months is associated with improved efficacy. In the RESET trail (256) Haraoui et al treated 112 TNFi-IR patients with RTX and then retreated them on relapse. 69% required retreatment during the study period with an average time between treatments of 8.5 months. Clinical response was retained after retreatment, with RF positive patients showing a superior responsiveness to seronegative patients.

Finally, the IMAGE trial (254) showed that RTX therapy can also be efficacious in early RA in patients naïve to MTX. This study recruited 748 patients with early RA (less than 2 years duration) and randomised them to receive either two doses of RTX 500mg, two doses of RTX

1000mg or two doses of placebo, all in combination with MTX. 80% required retreatment by week 30. Both does of RTX showed a statistically significant advantage in ACR 50 response by week 52 when compared to MTX alone. Only the RTX 1000mg treatment arm showed a significant reduction in radiological progression, although the study was not powered to directly compare RTX doses.

RTX is safe and generally well tolerated. Meta-analysis of data from IMAGE, DANCER and REFLEX showed no significant increase in serious infections above those experienced in patients who received placebo (257). Pooled data from the clinical trials programme and drug registries suggests that the most common AE is infusion reaction, which affects up to 25% at the first infusion and decreases with subsequent infusions (258). The risk of infection appears to be low, with pneumonia being the most common serious infection observed followed by upper respiratory infections, nasopharyngitis, urinary tract infections, bronchitis, sinusitis, diarrhoea, and gastroenteritis (241). TB, reactivation of hepatitis B and progressive multifocal leukoencephalopathy secondary to JC virus infection have been observed as rare events in patients receiving RTX (241, 259). Although RTX does not affect plasma cells, the development of hypogammaglobulinemia and subsequent infection is a concern with long-term RTX use. In the pooled analysis of long-term rituximab safety 22.4% developed a low IgM level and 3.5% a low IgG level lasting more than 4 months after RTX treatment (260).

1.8.3.1.4 Inhibition of T cell co-stimulation

Activated T cells are abundant in the rheumatoid synovium. The association between seropositive RA and HLA DRB1, along with other genetic susceptibility factors associated with T cell function (PTPN22, CTLA4) suggests that they likely have a pivotal role in RA pathogenesis (261). Activated CD4+ T cells secrete proinflammatory cytokines that attract and activate other inflammatory cells in the joint and are responsible for driving the formation of

ectopic lymphoid tissue in the RA synovium (261). T cells also play an important role in activating B cells and stimulating them to produce autoantibody (261).

T cell activation requires two signals, the initial recognition of antigen by the T cell receptor and co-stimulation via the binding of CD80 and/or CD86 on the surface of antigen presenting cells, to CD28 on the surface of the T cell. Shortly after activation, T cells begin to express CTLA4, which binds CD80/86 with more avidity than CD28 and thus functions as a negative regulator of T cell activation (262). It is this negative regulation of T cell activation that is a useful therapeutic target in RA.

1.8.3.1.4.1 Abatacept

Abatacept (ABT) is a recombinant fusion protein comprising the extracellular domain of CTLA-4 linked to the Fc portion of human IgG1. ABT competitively binds to CD80/86 and thus inhibits T cell co-stimulation and activation (262). ABT was approved for use in Europe in 2007. ABT can be given either as an intravenous infusion or subcutaneously, with either route having comparable efficacy(263). The IV preparation is given at doses of 500mg for an individual weighing less than 60 Kg, 750mg for patients between 60 and 100Kg and 1000mg for patients over 100Kg. It is given as a loading dose at weeks 0, 2 and 4 and then every 4 weeks. The subcutaneous preparation is administered from a pre-filled syringe device at a dose of 125mg weekly (163). ABT is licensed under the trade name Orencia®.

A number of phase III trials have shown the efficacy of ABT in patients with an inadequate response to csDMARDs (263-266) or TNFi (267, 268). In the AIM trial (264) 652 MTX-IR patients were treated with either IV ABT 10mg/Kg or placebo in combination with MTX. At 6 months 67.9% of ABT treated patients achieved an ACR 20 response versus 39.7% in the placebo arm. After 1 year of treatment ABT had significantly slowed radiological progression vs placebo. In the ATTEST trial (265), Schiff et al demonstrated that ABT plus MTX was more

effective than MTX monotherapy and had a similar efficacy to IFX by day 85 and had significantly better response data by 1 year. However, it was not powered to show superiority of ABT over IFX. The AMPLE study obtained similar results, comparing ABT to ADA (266). 646 patients with MTX-IR were randomised to receive either sc ABT or sc ADA. At the end of the study period 64.8% of the ABT treated group vs 63.4% of the ADA treated group had an ACR 20 response. Genovese et al showed ABT to be efficacious in patients who had previously failed at least 1 TNFi (267). Patients were randomised to receive either ABT or placebo and after 6 months 50.4% of patients in the ABT had achieved ACR 20 response rates vs 19.5% in the placebo arm. Statistically significant advantages in ACR 50 and 70 were also observed as was improvement in health-related quality of life measured by HAQ.

ABT has also been shown to be efficacious in early arthritis in patients naïve to MTX. In the AGREE trial (269), MTX naïve patients with poor prognostic markers (sero-positive, baseline erosions, high inflammatory response) were randomised to receive either ABT 10mg/kg or placebo in combination with an escalating dose of MTX. After 1 year the ABT and MTX group had a significantly higher rate of DAS-28 CRP remission, lower radiological progression and significant advantages in ACR 20, 50, 70 and 90 responses. In the second year of the study all patients were treated received open label ABT and MTX (270). The remission rate in those initially randomised to receive ABT was maintained and in those originally treated with MTX monotherapy a remission rate of 44.5% was observed. In the AVERT trial (271) MTX naive patients with sero-positive RA and active disease (DAS 28 >3.2) were randomised to receive sc ABT 125mg weekly plus MTX, MTX alone or ABT alone. After 12 months patients with a DAS-28 CRP of less than 3.2 were eligible to enter a withdrawal period where ABT was stopped and MTX and steroids were gradually withdrawn. Patients whose disease flared after 3 or more months could restart open label ABT plus MTX. The primary end point was DAS28-CRP of <2.6. This was achieved in 60.9% of the combination therapy arm and 45.2% of the MTX monotherapy arm ($p=0.01$) at 12 months (42.5% of the ABT monotherapy arm achieved

remission). Significantly more patients in the ABT and MTX combination arm remained in remission after treatment withdrawal.

ABT is safe and well tolerated. In pooled analysis of placebo-controlled trials adverse events were reported in 51.8% of ABT recipients and 46.4% of patients who received placebo. The most common adverse events were headache, nausea and URTI. In AGREE and AVERT the tolerability of ABT and MTX was comparable with that of MTX alone (262).

1.8.3.2 Targeted Synthetic Disease Modifying Anti-Rheumatic Drugs (tsDMARDs)

1.8.3.2.1 Janus Kinase inhibitors

Janus Kinases (JAK) are intracellular transduction molecules that act down-stream of the receptors of type1 and type 2 cytokines. The Janus Kinase/Signal Transduction and Activation of Transcription (JAK/STAT) pathway thus plays a role in many inflammatory conditions. Multiple different inflammatory cytokines with potential roles in the pathogenesis of RA signal via the JAK/STAT pathway, most notably IL-6, the blockade of which is an important therapeutic target in RA. The role of other cytokines which signal via this route in RA are less well established (272). In mammals there are 4 JAKs, JAK1, JAK2, JAK3 and Tyrosine Kinase 2 (TYK2). Each cytokine receptor recruits a specific combination of JAK/STATs and thus, inhibition a specific JAK subtype can impede the actions of multiple cytokines. Binding of cytokine leads to a conformational change in its receptor which activates two JAK molecules, which then interact to auto/trans phosphorylate each-others tyrosine residues and phosphorylate downstream signalling molecules in the STAT (signal transduction activation of transcription) family. STAT phosphorylation leads to its translocation to the nucleus where they regulate the transcription of target genes (273). A number of molecules have been

designed to inhibit differing JAK molecules with varying avidity, and to date four drugs have been licensed for use in RA in the UK, tofacitinib, baricitinib, upadacitinib and filgotinib, with a number of others either licensed elsewhere or in the regulatory pipeline.

1.8.3.2.1.1 Tofacitinib

Tofacitinib was the first small molecule JAK inhibitor to be licensed for use in RA. In vitro tofacitinib potently inhibits JAK1, 2 and 3 and to a lesser extent TYK2, but in vivo tofacitinib preferentially inhibits cytokine receptors associated with JAK1 and JAK3 (274, 275). Tofacitinib is administered as an oral tablet in combination with MTX at a licensed dose of 5mg BD in RA. The 10mg preparation is not licenced for use in Europe due to its worse safety profile. Tofacitinib was approved for use in the UK in 2017 and is licenced under the trade name Xeljanz®.

The ORAL clinical trials programme investigated the efficacy of tofacitinib and was one of the most extensive clinical trials programme of any drug used in the treatment of RA. It has shown to be efficacious in MTX naïve patients (276), patients with inadequate responses to MTX (277-279) or other csDMARDs (280, 281) and in patients with prior exposure to other TTs (282, 283). The ORAL Start trial (276) tested the efficacy of tofacitinib in early arthritis, in patients naïve to MTX. Nine hundred and fifty-eight patients were randomised to receive tofacitinib 5mg twice daily, 10mg twice daily or MTX at a dose that was gradually increased up to 20mg over a period of 8 weeks. In both tofacitinib arms patients were significantly more likely to gain an ACR 70 score than in those treated with MTX (25.5% for tofacitinib 5mg vs 12% for MTX) and were significantly less likely to have radiological progression at 6 months. This suggests that tofacitinib is more efficacious as a first line therapy than the anchor drug, MTX. However, tofacitinib is not licensed for use in MTX naïve patients.

Five trials investigated the efficacy of tofacitinib in patients who has failed MTX or csDMARD. In ORAL standard (278) 717 MTX-IR patients were randomly assigned to receive either tofacitinib 5mg BD, 10mg BD, adalimumab 40mg sc every 2 weeks or placebo in addition to their MTX. Statistically significant advantages in ACR20 response, DAS 28 remission and HAQ were seen for both doses of tofacitinib and adalimumab over placebo. Numerically similar results were observed between tofacitinib and ADA. The ORAL scan trial (277) was specifically designed to look for structural preservation in MTX-IR patients started on tofacitinib. Once again patients were randomised to receive either of 2 doses of tofacitinib or placebo in addition to their baseline MTX. Patients receiving either dose of tofacitinib showed significantly less radiological progression over the 12-month trial period. In ORAL solo (281) 611 patients with inadequate response to csDMARD were randomised 4:4:1:1 to tofacitinib 5mg BD monotherapy, 10mg BD monotherapy, placebo for 3 months followed by tofacitinib 5mg BD or placebo for 3 months followed by tofacitinib 10mg BD. At 3 months ACR 20 response was statistically significantly better in the tofacitinib arms than the placebo groups, although there wasn't a statistically significant advantage in DAS28 remission. A similar design was uses in the ORAL sync trial (280), although in this case patients continued their csDMARD rather than using tofacitinib as a monotherapy. Once again ACR20 rates were significantly higher in the tofacitinib groups and DAS28 remission and HAQ score improvement was numerically higher. Finally, ORAL strategy (279) was designed to compare the efficacy of tofacitinib 5mg BD as monotherapy to tofacitinib 5mg BD and MTX combination therapy and ADA and MTX combination therpay. 1146 MTX-IR patients were randomised 1:1:1 to receive one of these treatment strategies. Tofacitinib in combination with MTX was non-inferior to ADA in combination with MTX. Non-inferiority was not declared for monotherapy against either combination arm. This study was not powered to demonstrate superiority of any of the treatments.

Tofacitinib is also efficacious in patients with prior bDMARD failures. In ORAL step (282) patients with inadequate responses to a TNFi were randomised to receive either available

dose of tofacitinib or placebo, in combination with MTX. ACR20 responses were statistically more likely in the tofacitinib arms than placebo (41.7% vs 21.4%) suggesting that tofacitinib is efficacious in TNFi-IR patients. Post Hoc analysis of the phase II and phase III trials programme shows tofacitinib to be efficacious in patients who have failed bDMARDs other than TNFi (283).

The most common adverse effects associated with tofacitinib are infections, most commonly upper respiratory tract infections, nasopharyngitis and gastrointestinal infection (274). Shingles secondary to reactivation of herpes zoster is more common in patients taking tofacitinib with an incidence rate of 2.4 in Europe and up to 8.4 in Japan. The risk is elevated further when using concomitant steroid therapy (284). There may be an increased risk of venothromboembolism (VTE) in patients taking tofacitinib (285).

1.8.3.2.1.2 Baricitinib

Baricitinib is a novel small molecule that selectively and reversibly inhibits JAK1 and JAK 2 and to a lesser extent TYK2 (286). Baricitinib is administered as an oral tablet at a licensed dose of 4mg once daily or 2mg daily in patients over 75 years of age or in those at high risk of infection (287). It is approved either in combination with MTX or as a monotherapy. It was first approved for use in 2017 and is sold under the trade name Olumiant®.

Baricitinib has been shown to be efficacious in early RA patients naïve to MTX. In RA BEGIN (288) 588 patients with active RA, who had not been exposed to csDMARD (8% had 3 or fewer doses of MTX prior to the start of the trial) were randomised 4:3:4 to receive either MTX monotherapy, baricitinib 4mg OD monotherapy or baricitinib in combination with MTX for 52 weeks. Baricitinib monotherapy was found to be statistically significantly more effective than monotherapy with MTX at producing an ACR 20 response by week 24 (77 vs 62%). Combination therapy was similarly effective. Improvement was noted as early as week 1 in

the baricitinib arms of the trial. Both baricitinib monotherapy and combination therapy reduced radiographic progression, although only combination therapy reached statistical significance. These advantages were maintained at week 52.

Two trials studied the effects of baricitinib in patients with inadequate response to csDMARDs. In RA BUILD (289) 684 patients with active RA, who had failed 1 or more csDMARDs, but not been exposed to bDMARD, were randomised to receive either baricitinib 2mg, 4mg or placebo in addition to their csDMARD therapy. By week 12 both baricitinib groups had statistically significant advantages in ACR20, DAS28 and SDAI remission and improvement in HAQ score. These advantages were numerically higher in the 4mg group. By week 24 both doses of baricitinib had reduced radiological progression. RA BEAM (290) was a 52-week, double blind placebo and active control trial which assessed the efficacy of baricitinib versus both placebo and ADA in patients with inadequate disease control despite MTX therapy. Patients were randomised 3:3:2 to receive either placebo, baricitinib 4mg OD or ADA 40mg sc every 2 weeks. At week 12 the baricitinib arm had significantly better rates of ACR20 response than placebo (70% vs 40%) as well as significant advantages in DAS28 and SDAI remission. At 24 weeks the baricitinib group had significantly less radiological progression as measured by modified sharp score. Interestingly at week 12 baricitinib also had a statistically significant advantage in ACR 20 response than ADA (70% vs 61%, p=0.014). This is the first example of a head-to-head trial showing an advantage of one TT over another in the management of RA. Baricitinib has been shown to be efficacious in patients with inadequate responses to prior bDMARD. In RA BEACON (291) patients with inadequate response or side effects to TNFi, non-TNFi biologics or both were randomised to receive either baricitinib 2mg, 4mg or placebo. Statistically significant advantages were seen in the baricitinib 4mg group for ACR 20, 50 and 70, DAS28 and SDAI remission and improvement in HAQ-DI.

Analysis of the clinical trials programme for baricitinib shows infection to be the main adverse event associated with treatment. Like with other JAKi, shingles is more common in patients on baricitinib. Elevation of liver enzymes and serum lipids was also observed (292).

1.8.3.2.1.3 Other Janus Kinase inhibitors.

Recently two further JAKis have been approved for use in RA patients. Upadacitinib and filgotinib, both JAKi that primarily target JAK 1, have shown efficacy in the management of both early RA (293-296) and in patients exposed to previous bDMARDs(297, 298). However, they gained their license after patient recruitment for this study had been completed and as a result, they have not been included in this study.

1.8.4 Choice of targeted therapy in Rheumatoid arthritis

Around 40% of RA patients will gain an inadequate response to csDMARDS and require escalation to a targeted therapy (299). There are, however, limited data available to clinicians regarding the best choice of TT for individual patients. Due to the broadly equivalent efficacy of most of the licensed TTs there is no longer a recommended sequencing of TT in clinical guidelines (163, 300). As a result, treatment decisions are often made based on a combination of factors. Registry data from several countries suggests that TNFi remain the most commonly prescribed 1st line bDMARD (301). This likely reflects the fact that they were the first to be approved. As a result, clinicians are familiar with their use, and they have a wealth of long-term efficacy and safety data to support their use (302).

In clinical practice there are several other factors which may influence choice of 1st line TT. The presence of co-morbidity will influence a clinician's choice of therapy and may exclude the use of certain drugs in some situations. For example, a history of venous thromboembolism is likely to preclude the use of a JAKi where other options exist (303). The

presence of a positive ANA screen may prompt a clinician to try an alternative to a TNFi (304) and a history of diverticulitis may preclude the use of anti-IL-6 as a 1st line therapy (305). Conversely, overlap between RA and other autoimmune conditions may prompt the use of a TT that will treat both conditions, such as RTX in overlap with a connective tissue disease or ILD (306). Monti et al (307) analysed data from the Italian LORHAN registry to find out what factors influenced clinicians first and second line choice of TT. They found that in the presence of comorbidity (particularly hyperlipidaemia, hypertension or pulmonary disease) clinicians favoured ABT, whereas MTX intolerance and high disease activity lead clinicians to favour TCZ. Analysis from the BSRBR of patients who had failed 2 or more classes of TT found that after TNFi, the most common choice for second line therapy amongst British rheumatologists is RTX, followed by TCZ or ABT (308).

Overlap between RA and other connective tissue or autoimmune diseases is relatively common, with some series suggesting as many as 30% of RA patients have a second autoimmune condition (309). This can complicate disease activity assessment and choice of therapy. Other factors associated with choice of TT include patient preference, particularly relating to route of administration and, all other factors being equal, drug cost is an important consideration in some health care systems (310). This is all the more relevant in the age of biosimilars, where the discrepancy between the cost of therapies can be large.

1.9 Predictors of response to targeted therapies

There are very few tools available to Rheumatologists in the clinic at present which can aid in their choice of a first line targeted therapy, and even less to determine which drug to switch to in the event of drug failure. ACPA positivity has been shown to be predictive of a better response to both rituximab (311) and abatacept (312), but has shown inconsistent results for both TNFi and TCZ with some studies finding a link between seropositivity and response (313)

and others showing no association (314). Navares et al have suggested that an elevated CRP and serum IL-6 is predictive of response to tocilizumab (315), but a study by Wang et al did not find this to be the case (316). A G-to-A polymorphism at position -308 in the promoter of the TNFα gene has been shown to be a predicator of response to TNF blockade with etanercept (317) and infliximab (318), although such tests are not routinely available in the clinic. Post hoc analysis of the RA BEACON trial of baricitinib in bDMARD-IR attempted to identify whether any baseline factors including age, weight, disease duration, seropositivity, corticosteroid use, number of prior bDMARDs, TNFi or non-TNFi, or a specific prior TNFi had any effect on baricitinib efficacy. They were unable to identify any factors that influenced response to baricitinib having a consistent effect across all groups (319).

Experimental investigations into both synovial histology and serum biomarkers have provided some interesting possible predictors of response, but these are yet to translate into clinical practice. A meta-analysis of studies looking for predicators of response to therapy in RA patients found 65 examples of serum biomarkers with good predictive value for response in single, small scale studies, but nothing which has been validated on a larger scale (314) and ready to translate into practice.

1.9.1 Synovial biopsy

Synovial biopsy performed either via arthroscopy or under ultrasound guidance has provided useful information in both early and late RA regarding predictors of response to therapy. Humby et al (320) carried out a synovial biopsy study on patients with early RA. Samples were obtained by US guided biopsy in treatment naïve patients and 3 distinct subsets were identified: Lympho-myeloid (B cells and myeloid cells), diffuse-myeloid and pauci-immune. Elevation of lymphoid and myeloid gene expression was associated with higher disease activity, higher inflammatory markers, and increased responsiveness to DMARD therapy. In

addition, lymphoid gene expression was associated with seropositivity and bone erosion. Subsequent analysis by the same study group found that the lympho-myeloid pathotype has a greater risk of progression to biologic therapy within 12 months (321).

A number of biopsy studies have looked into predictors of response to TT and to response to individual bDMARDs. The presence of increased baseline transcription of genes associated with inflammatory processes has been shown to predict responsiveness to TNFi therapy (322). Wijbrandts et al (323) obtained synovial biopsy specimens via arthroscopy prior to commencing patients on infliximab. They found that high synovial expression of TNFα and the presence of TNFα producing inflammatory cells within the synovium was predictive of response to TNFi. However, a similar study failed to reproduce this finding (324). Dennis et al (325) examined synovial tissue from patients' with RA of at least 3 years duration, who had been managed with standard RA therapy, including some patients with prior bDMARD exposure. The aim was to identify different synovial phenotypes and correlate them with serum biomarkers and response to targeted therapy. Four different synovial phenotypes were identified; lymphoid, myeloid, pauci-immune and fibroid, each with distinct gene expression signatures. It is notable that in this cohort that of patients with available serology results, 100% of the patients with myeloid or lymphoid phenotype were seropositive (RF), whereas those with the fibroid type were seronegative. These gene expression signatures were used to interrogate a synovial tissue gene dataset from a previous study aiming to identify synovial genetic markers of response to IFX (326) (This trial did not find any genetic markers predictive of IFX response). It was found that baseline expression of the myeloid gene set was associated with EULAR good response to TNFi, but not the lymphoid or fibroid subtypes.

Subsequent investigation identified genes expressed in the different inflammatory synovial phenotypes (lymphoid and myeloid) that could be reflected as circulating biomarkers. Two candidate biomarkers were identified, ICAM1 in the myeloid type and CXCL13 in the lymphoid type. These biomarkers were tested in serum samples obtained in the ADACTA trail, which

compared ADA therapy to TCZ therapy in MTX intolerant patients (222). They found that patients with high ICAM1 and low CXCL13 (suggestive of a myeloid phenotype) had a higher likelihood of response to ADA, whereas patients with high CXCL13 and low ICAM1 (suggestive of a lymphoid phenotype) had a higher likelihood of responding to TCZ. However, a subsequent study of CXCL13 and ICAM1 in patients treated with TCZ did not find them predictive of response (327). Hogan et al (328) studied arthroscopy specimens of RA patients before and after RTX therapy and were able to identify a baseline gene score that correlated with responsiveness to RTX. The gene score biology suggested that expression of markers of remodelling and interferon-α genes correlated with a poor response, whereas genes associated with T cells and macrophages were associated with a response to therapy. For patients who have failed RTX, Das et al (329) showed that high synovial expression of IL-6 mRNA (as well as elevated serum IL-6), was predictive of response to TCZ.

1.10 Non-response to biologic DMARD

Despite the bDMARD treatment revolution, around 40 % of patients fail to respond to their first line targeted therapy. Most clinical trials of biotherapeutic drugs of differing mechanisms of action across the RA treatment pathway, report an ACR20 response in the region of 60% (330). This figure is consistent with post marketing surveillance data from a number of national registries (331-333). When a patient does fail their first treatment the response to subsequent treatments is reduced when compared to treatment naive patients (334, 335) and decreases sequentially as the number of previous therapies increases(334). Indeed, the best predictor of long term response to GOL therapy has been found to be its use as a first line TT (336). Leon et al (337) investigated the long-term survival of bDMARD agents in patients with RA and found a one-year retention rate of 68% with only 9% of patients remaining on a single biologic drug for 10 years. The most frequent reason for stopping a bDMARD agent was an adverse reaction (45.8%), with inefficacy second (40%) and patient choice or medical reasons

responsible for the remainder. Of those patients who do respond to a bDMARD therapy, around 20% lose their response to treatment within 2 years (338). Extrapolation from RCT evidence and an analysis from the BSRBR suggests that approximately 20% of patients progress to a 3rd line targeted therapy (308, 339).

There are several potential barriers to successful treatment of RA in clinical practice that may make it difficult to achieve remission or low disease activity in some patients. Tymms et al identified several factors which prevented treatment escalation or optimisation in a cohort of patients with high and moderate RA activity (DAS 28>3.2). These barriers included safety concerns (e.g., recurrent infections, abnormal blood results), pre-existing co-morbid conditions (e.g., respiratory disease, renal impairment, malignancy) and patient preference to not escalate treatment (340). Similar results were obtained in an international questionnaire-based study of Rheumatologists, seeking their opinion as to what constitutes difficult to treat RA. Co-morbidity, particularly cardiovascular and lung disease, extra-articular manifestations of RA, pain syndrome, polypharmacy and patient adherence were all felt to be features of difficult to treat RA (341).

Patient compliance is an important issue, as in practice non-compliance is likely to be a reason for suboptimal response in some patients. Indeed, poor adherence has been shown to correlate to reduced treatment response in patients on TNFi (342). Medication adherence rates in patients with RA vary widely between reports, at somewhere between 30-99%. This is a similar rate to other chronic conditions. A belief that the medication is necessary is the main factor in ensuring patient compliance, with sufficient contact with, and the adequate provision of information by the health care provider also being important (343). This underlines the importance of the patient clinician relationship in successful treatment of RA.

Patients may stop or switch a targeted therapy either due to adverse reactions or a lack of clinical response to the treatment. A lack of response after initiation of treatment is described

as primary non-response. In clinical practice response to therapy is usually assessed after three months, with shorter term response potentially being missed or its assessment being confounded by bridging steroid therapy at drug initiation. The exact mechanisms underlying primary non-response are not known, but it has traditionally been thought to be due to incorrect drug targeting (339). An initial response to a biological therapy followed by a subsequent loss of efficacy is known as secondary non-response. There is evidence that this is at least in part mediated by the formation of anti-drug antibodies (ADAbs) that neutralise drug or lead to increased clearance. It should be noted that JAK inhibitors are small, non-biological molecules and therefore are unlikely to elicit an immune response. It remains likely that other mechanisms of secondary drug failure exist, particularly considering the differing relative immunogenicity of the bDMARDs.

The advent of treat-to-target strategies in early RA (121, 344) , with the aim to get patients into remission or a state of low disease activity as early as possible, with subsequent reduction in accumulated damage has been a significant advance. There is limited evidence on how best to proceed when patient's disease proves resistant to this strategy. The available guidelines generally do not address treatment strategies beyond a second targeted therapy (160). Most drug trials of new targeted therapies focus on treatment response after failure of a TNFi. These trials generally show that switching to a drug with an alternative mechanism of action can be effective (226, 237, 251, 253, 256, 291, 345-347). In the ROC trial, Gottenberg et al (348) showed that in patients with an inadequate primary response to a TNFi, switching to a non-TNFi targeted therapy was more likely to be effective. In this study 300 patients with an insufficient response to TNFi were switched to either a second open label TNFi or a non-TNF biologic. 69% of the non-TNFi group obtained a EULAR good or moderate response at 24 weeks versus 52% in the TNFi arm. At week 52 statistically more patients in the non-TNFi arm had low disease activity. Switching between classes has also been shown to increase drug persistence compared to cycling between TNFis (349). Rituximab has been shown to have superior efficacy than a second TNFi, the difference being more marked in seropositive

than seronegative patients (345). Switching to ABT did not show a significant advantage over switching to a second TNFi in one trial (350). However, a meta-analysis of drug trials of RTX, ABT and TCZ in TNFi-IR patients did not show a significant difference between the agents (351). One population based prospective study of 3169 patients in France, did suggest that RTX and TCZ had greater efficacy and drug retention than ABT in TNFi-IR patients (352).

There is even less evidence about choice of subsequent therapy when a non-TNFi TT is started first line. Das et al showed that in patients with inadequate response to RTX, TCZ was more efficacious than ABT therapy (329). Akiyama et al carried out a study looking at whether a TNFi or ABT was more efficacious in patients who failed TCZ. Significantly more patients in the TNFi group obtained low disease activity or remission. However, there was a marked discrepancy between the numbers of patients using concomitant MTX between the arms of this study, which heavily favoured the TNFi group (353). In the recent clinical trials programmes for both tofacitinib and baricitinib, both JAKi were shown to be efficacious in patients who had failed prior bDMARDs, not just a TNFi (283, 354).

Despite the evidence that non-TNFi therapies are more efficacious in previous TNFi-IR there is still RCT evidence suggests that switching to an alternative TNFi in this situation can be effective (201, 208, 355, 356). Those with secondary loss of response are more likely to respond than those with primary inefficacy (357). However, primary inefficacy doesn't preclude an adequate response to a second TNFi (201). There is limited evidence that switching to an alternative TNFi with a different molecular structure (i.e., from a monoclonal antibody to etanercept and vice versa), may be efficacious in some circumstances. Post hoc analysis of the GO-AFTER trial showed lower ACR20 responses in patients receiving GOL after ADA, than after ETN (30.3% vs 46.8%), although there was no similar discrepancy between ETN and IFX (358). When a patient switches to a second TNFi due to an adverse reaction, the chance of discontinuation of the second TNFi due to a further adverse reaction has been reported to be twice as high as those who switch due to inefficacy (359).

There is limited data that directly compares the long-term survival of individual bDMARDs. Most of the available data relates to the first generation TNFis, with cohort study and registry data suggesting that ETN has the best long term drug survival followed by ADA and then IFX (360-362). A number of studies have suggested better retention rates for non-TNFi bDMARDs, particularly TCZ (335, 363, 364) and ABT in elderly patients (365, 366). This is likely a reflection of their relative immunogenicity (which will be discussed in detail in section 1.10.2), and in the case of ABT in elderly patients, it's favourable safety and tolerability profile.

1.10.1 Risk factors for biologic DMARD non-response

RA is a complex and heterogeneous condition, with the interaction of many different environmental and genetic factors involved it is development and evolution. The underlying mechanism of failure to respond to a given treatment is likely to be equally heterogeneous with the reasons for persistence of signs or symptoms varying from patient to patient. The risk factors for bDMARD non-response have conventionally been thought to be the same as those that have been classified as markers of poor prognosis disease. Indeed, seropositivity, high disease activity at baseline (367), female sex and older age at the start of treatment (368) have all been associated with worse response to targeted therapy. However, analysis of prognostic markers for joint damage in the ASPIRE trial found that, although high disease activity and inflammatory markers correlated with progression of joint damage in MTX treated patients, in those treated early with IFX, this correlation was abrogated (169).

A number of studies have attempted to identify risk factors for refractory disease and bDMARD non-response. Becede et al (369) performed a longitudinal study of patients they defined as having refractory RA (RefRA). In this study they defined RefRA as failure of at least 3 courses of DMARD, 1 of which needed to be a bDMARD. Of the 412 patients in their cohort, 70 met this definition of RefRA. They performed logistic regression analysis and identified female sex, high disease activity (defined by CDAI score), younger age and longer delay until starting a

first targeted therapy as being associated with RefRA and using multivariable analysis confirmed these factors as independent predictors of a refractory disease course. Seropositivity and baseline radiographic changes were not predictive of a RefRA in this model. Similarly, Kersley-Fleet et al analysed data from the BSRBR and identified female sex, younger age, shorter disease duration, higher patient global assessment, higher HAQ score, current smoking and obesity as risk factors for RefRA (308). An Italian longitudinal study of patients on TNFi found that male gender, and a negative CRP were predictors of achieving low disease activity. However, in this study younger age (<54) was found to be a predicator of response rather than resistance. The presence of a co-morbidity was a negative predictor of response (370).

Obesity has been established as a risk factor for treatment failure in RA. A meta-analysis by Liu et al, of trials into the effects of obesity of treatment response has shown that an elevated body mass index (BMI) is associated with higher disease activity scores, tender joint counts, inflammatory markers, pain scores, patient global assessments of disease activity and physical function scores (371). Early arthritis patients with an elevated BMI are less likely to achieve EULAR good responses or low disease activity/remission after treatment with csDMARD (372). Obese patients have been shown to be less likely to achieve low disease activity after treatment with TNFis in several studies (373-376). There is less evidence regarding the effects of elevated BMI in patients on non-TNFi therapy. Analysis of the German RABBIT longitudinal cohort suggests that obesity may reduce the likelihood of achieving low disease activity with a JAKi (377). However, evidence seems to suggest that treatment with ABT (378), TCZ (379) and RTX (380) is not effected. A relative reduction in drug exposure due to high body weight in patients on TT administered at a fixed dose had been theorised to be a cause of some TT resistance in obesity. However, the efficacy of IFX, given at a dose calculated on weight, is affected by BMI (375, 376), whereas intravenous ABT and TCZ is not.

An MRI sub-study of the GO-BEFORE trial showed that obese patients are less likely to obtain DAS28 remission than non-obese counterparts despite similarly low levels of synovitis and bone marrow oedema identified on MRI (381). Heimans et al (374) showed that patients with a high BMI reported more pain but didn't have significantly more swollen joints or inflammation. This was also shown in a meta-analysis of trials by Liu (371). This suggests that obesity may not cause refractory disease per se, but instead may bias disease activity measures by, for example, elevating the more subjective components of the DAS28 score. Obesity has also been shown to elevate the inflammatory markers (CRP and ESR) (382) potentially further compounding this propensity to over-estimate disease activity.

Smoking is an environmental risk factor not only for development of RA, but also for higher disease severity and systemic complications such as nodulosis or vasculitis (383-385). This association is true for ACPA positive patients but may be less important for ACPA negative RA (386). Analysis of the BSRBR showed smoking to be a risk factor for lack of response to infliximab treatment (387) and a number of other studies have shown similar effects with TNFis as a class (388, 389), with heavy smokers shown to have the poorest drug survival (390). There is limited evidence regarding the effects of smoking on non-TNFi TTs. There is some evidence that smoking may reduce responsiveness to RTX (391) and ABT (392), but not TCZ (393). The effects of smoking in patients treated with a JAKi are unclear. Smoking has been shown to increase levels of TNFα activity (394) and promote a systemic inflammatory state (395), which likely contributes to its effects on drug responsiveness.

Studies to identify genetic associations with response and non-response to TNFi have identified variants in a number of genes including TNFA, TNFR1A, MED15, PTPRC, FcGR2A and FcGR3A, hinting at a genetic basis for drug resistance in some patients. However, few of these associations have been successfully reproduced in other studies (396). Studies looking into the heritability of DAS28 components themselves (SJC, TJC, VAS and ESR) that may

affect patients disease activity score and thus their relative response to therapy, have also given conflicting results (397, 398).

1.10.2 Primary and secondary targeted therapy failure

As discussed above, a lack of any clinical response after initiation of treatment is described as primary non-response which has traditionally been felt to be due to incorrect drug targeting. Loss of response after a sustained period of efficacy is known as secondary non-response and this has traditionally been attributed to the development of neutralising antibodies.

Bio therapeutic drugs are large exogenous proteins which are foreign to the immune system and are thus capable of provoking an immune response. This leads to the formation of ADAbs that can reduce drug efficacy or lead to complete loss of response. There are two main mechanisms by which an antibody can inhibit the activity of a biotherapeutic drug. ADAb can bind directly to the FAB fragment (the area that binds antigen), competitively inhibiting the binding of endogenous antigen to the therapeutic antibody and thus neutralising it. This is generally the case for the monoclonal antibodies. They can also bind elsewhere, such as the hinge region of fusion proteins or Fc portion of a monoclonal antibody. This is non-neutralising but can lead to immune complex formation and increased clearance of the drug (338). Chimeric monoclonal antibodies with peptide sequences of murine origin are the most immunogenic biologic drugs, although human and humanised mono-clonal antibodies also provoke the formation of ADAb (399). Monoclonal antibodies seem to be more immunogenic than fusion proteins, with Etanercept having the lowest immunogenicity of the TNFis (400).

The presence of ADAb has been associated with reduced serum concentrations of monoclonal TNFis (399). Thomas et al (401) performed a meta-analysis of trials across the RA, PsA and IBD clinical trials programme for TNFi to determine the immunogenicity of the different TNFis

and the effects of ADAb on TNFi efficacy. They determined that IFX was the most immunogenic (25.3%), followed by ADA (14.1%), CTZ (6.9%), GOL (3.8%) and finally ETN (1.2%). The presence of ADAb reduced the chances of clinical response by 67%, although this figure was derived from trials predominantly involving IFX and ADA. Pooled analysis of long-term safety data from the RTX clinical trials programme suggests that 11% of patients had ADAb on at least 1 trial visit (259) with similar analysis of ABT trials suggesting an ADAb rate of 4.8-5.5% with around 50% being neutralising antibodies (262, 402). By contrast, for Tocilizumab, very low levels of ADAb (1.2% for IV, 1.5% for SC) has been described with no clear effect on efficacy (403) .

There are a number of factors which may influence the formation of anti-drug antibodies. There is likely a genetic basis to the formation in ADAb in some patients. For example, polymorphisms in genes encoding IL-10 have been observed in patients with ADAb. IL-10 may regulate immune activation during ADAb formation (404). The co-administration of MTX has been shown to reduce the risk of ADAb formation. MTX has been shown to both increase the trough level and serum half-life of infliximab (405) and adalimumab (406) and reduce the formation of ADAbs (404).The mechanism by which MTX reduces immunogenicity is not clear. It may be by directly supressing ADAb formation directly due to its immunosuppressive effects. It is also postulated that it may reduce ADAb indirectly by suppressing inflammation. Inflammation itself is shown to a risk factor for ADAb formation and reduction in the half-life of TNFis (399). As a result clinical guidelines recommend TTs are prescribed in combination with MTX (300) and the licencing requirement of most of the TTs require their use in combination with MTX (163). Despite these recommendations, registry data suggests up to 33% of TTs are prescribed as a monotherapy due to intolerance and an analysis of healthcare insurance claims data showed that as many as 58% of patients did not collect the MTX prescription regularly (407), increasing the risk of ADAb formation and lowering efficacy. In the case of TCZ, co-administration of MTX has been shown to make no difference in levels of

immunogenicity. It is hypothesised that blocking IL-6 has effects on B-cells and CD4+ T helper cells, which may influence the generation of ADAb (408).

The mechanism of primary non-response is less clear, although the superior efficacy of non-TNFi TT in patients who don't respond to TNFi adds weight to the hypothesis that in many cases the wrong drug target has been selected. A study by Jamnitsky et al (357) recruited a subgroup of patients previously treated with either IFX or ADA and tested them for ADAb prior to switching them to ETN. They then compared their response to TNFi naïve patients starting ETN as a first line TT. Those with prior ADAb positivity responded as well to ETN as treatment naïve patients, whereas those who failed a prior TNFi in the absence of ADAb had a lesser response than either TNFi naïve patients or those with ADAb. This finding supports the theory that primary non-response is more likely to be a result of incorrect drug targeting in a subset of patients.

The results of the EXXELERATE study (201) show that switching TNFi in the event of primary non-response can still be efficacious. In this study 915 patients were randomised to receive either ADA or CTZ in combination with MTX. Both drugs showed comparable efficacy. In the event of non-response at 12 weeks patients were immediately switched to the other TNFi. In both arms around 60% of patients obtained either low disease activity or a good EULAR response. Approximately 40% of switch patients achieved an ACR 20 response to their second TNFi. In clinical trials where patients were switched TNFi after a combination of primary and secondary failure, ACR 20 response has been around 50% (201) and one study which tested CTZ in TNFi-IR patients with only secondary loss of response had an ACR20 rate of 61% (355). This all suggests response in pure primary failure is indeed lower. It should be noted however, that in an analysis of the CORRONA registry from the USA, that in TNFi non-responders who continued TNFi despite non-response at 6 months, around 35% were documented to have obtained a late response by 12 months (409). It is therefore possible that

by switching very early, a small proportion of patients who would have responded later to a first line TNFi were switched.

Response to a second TNFi after primary failure to a first indicates that incorrect therapeutic target is not the whole explanation for primary non-response to therapy. It is possible that this discrepancy is due to pharmacokinetic/pharmacodynamic differences between the drugs within the same class. It raises the possibility that in some patients, either a pre-existing immune mediated response to an individual drug or very early immunogenicity and drug failure akin to secondary non-response may be possible. Pre-existing antibodies to bio therapeutic drugs have been identified in treatment naive individuals (410). In the SERENE study of RTX in MTX-IR patients, 3.6% of patients in the placebo arm had anti-RTX antibodies (249). Anti-mouse antibodies are present in up to 80% of humans, explaining the increased immunogenicity associated with chimeric monoclonal antibodies (411). Even in humanised antibodies, there will still be sequences of amino acids which are foreign to individuals, particularly within the complementarity-determining regions of therapeutic antibodies (412). Antibodies to immunoglobulin fragments and to glycan residues added to bio therapeutic drugs by the cell lines used in their manufacture have been described (412). However, the clinical significance of these pre-existing antibodies in RA patients is unclear.

1.10.3 Management of biologic DMARD non-response

As outlined above, the management of bDMARD non-response is complex and has a limited evidence base. As a result, it is subject to the same pragmatic considerations of drug cost and patient preference as first line bDMARD therapy. Careful consideration of the mode of drug failure (primary non-response, secondary non-response or adverse effects) can help inform the choice of therapy. Switching TT class, when possible, likely gives the best chance of response, but TNFi cycling is a viable alternative, particularly in the case of secondary failure after sustained response to a first line TNFi. Optimising MTX therapy improves efficacy and

reduces ADAb formation. In MTX intolerance, switching to TCZ or a JAKi is likely the best option. Combination bDMARDs are not approved for use and several trials have found this approach increases the risk of adverse events without significantly improving efficacy (255, 413, 414).

1.11 Refractory Rheumatoid Arthritis Disease

The concept of multi-drug refractory RA (RefRA) disease has evolved over the years as the treatment armamentarium has grown. The term, once used to define patients who had an inadequate response to MTX and other synthetic DMARDs, then TNFi, can now be applied to a growing cohort of patients who have either failed to respond to multiple classes of targeted therapy or have stopped therapies due to a combination of non-response, loss of response and adverse effects. Until recently there was no agreed definition of refractory or difficult to treat RA. Over recent years several differing definitions of RefRA and difficult to treat RA, of varying stringency, have been put forward. In their study of risk factors for RefRA, Becede et al defined RefRA as failure of 3 DMARDs, at least one of which is a bDMARD (369). Exposure to ≥3 classes of targeted therapy was used in an analysis of refractory disease in the BSRBR (308).. Failure of csDMARDs followed by at least one anti-cytokine (TNF I, anti-IL-6,) and one cell-targeted (Anti CD-20, anti CTLA4) treatment has also been proposed (339).The recent introduction of the JAKi, introduces a further layer of complexity to this classification. In their survey of Rheumatologist's beliefs in what constituted difficult to treat RA, Roodenrjis et al (341) identified failure of ≥ 2 csDMARDs, ≥ 2 biologic or targeted synthetic DMARD, inability to taper steroids below 10mg equivalent daily, interfering co-morbidity, extra-articular manifestations and polypharmacy as features of difficult to treat RA.

In 2020 the EULAR taskforce on difficult to treat RA produced its consensus definition of difficult to treat RA (9). Under these guidelines a patient can be defined as having difficult to treat RA if they fulfil the following 3 criteria:

1) Treatment according to European League Against Rheumatism recommendation and failure of ≥2 biologic or targeted synthetic DMARDs (with different mechanisms of action) after failing csDMARD therapy (unless contraindicated).
2) Signs suggestive of active/progressive disease, defined as ≥1 of:
    a) At least moderate disease activity (according to validated composite measures including joint counts, for example, DAS28-ESR>3.2 or CDAI>10).
    b) Signs (including acute phase reactants and imaging) and/ or symptoms suggestive of active disease (joint related or other).
    c) Inability to taper glucocorticoid treatment (below 7.5mg/ day prednisone or equivalent).
    d) Rapid radiographic progression (with or without signs of active disease)
    e) Well-controlled disease according to above standards, but still having RA symptoms that are causing a reduction in quality of life.
3) The management of signs and/or symptoms is perceived as problematic by the rheumatologist and/or the patient.

The EULAR criteria provide a definition that allows for standardisation when researching RefRA. However, it is important to make the distinction between difficult to treat RA, where drug intolerance, patient compliance and the interacting effects of co-morbidity can influence the optimisation of therapy and the concept of biologically refractory or ‘true’ refractory RA, where inflammation persists despite the optimisation of therapy.

1.11.1 Burden of refractory Rheumatoid Arthritis

As outlined above, targeted therapy failure is a complex issue; an individual may cycle through different therapies due to a combination of non-response, loss of response and different

adverse effects. Despite recognising the socio- and health-economic impact as well as multi-morbidity of this patient population, robust data are lacking with reported rates of RefRA varying due to differing definitions used to define refractoriness. A recent report from the BSRBR suggested that around 6% of RA patients have refractory disease (415). All patients in this cohort had failed at least 2 classes of TT and had started a TNFi as their 1st line therapy. Becede et al identified a rate of 17% in a tertiary centre cohort, with around 6% of patients meeting their definition in a community hospital validation cohort (369).

1.11.2 Understanding clinical phenotype of refractory Rheumatoid Arthritis

RA is a complex and heterogeneous condition. It is increasingly considered that RA is a syndrome, representing a common clinical phenotype that is underpinned by several biological pathways. Clinical observation also illustrates a spectrum of clinical features contributing to the RA phenotype. Co-existence of RA and other immune-mediated diseases is relatively common, with series suggesting up to 30% of RA patients have a second autoimmune condition (309) and, as previously noted. Although clinical trials of IL-17 blockade failed to meet their primary end points in RA, response was seen in some patients. It is therefore possible that there is a subset of RA patients who are more IL-17 responsive and that in the future, a precision medicine approach which identifies them, may allow these drugs to be used in this group (416). The clinical phenotyping of refractory RA may identify such sub-groups and allow for the interrogation of underlying molecular mechanisms may thus inform on new drug targets for validation.

1.11.3 Limitations of DAS28 in the investigation of bDMARD non-response and refractory disease

In clinical practice, RA disease activity is assessed and monitored using the DAS28 score. This has a number of potential limitations. The exclusion of the feet; done to speed up patient

assessment in clinic, can lead to an underestimate of a patient's disease burden (417). Conversely, it is possible that non-synovial RA pathology may be underestimated by examination for tender or swollen joints missing inflammation in structures distant from the joint. For example, tenosynovitis is common in RA and may occur in the absence of synovitis (418). Enthesitis is also recognised in patients with RA (419) and may be identified by palpation of areas away from those traditionally examined for the DAS28 assessment.

Clinicians tend to give more weight to the swollen joint count as a measure of activity. However, in the DAS 28 calculation the weighting given to the TJC is greater than that given to the SJC (96), despite this being a more subjective measure. There are several confounding factors that may lead to an over-estimate of a patient's disease activity related to the more subjective elements of this scoring system (TJC and VAS). This could potentially lead to a switch in TT in patients without active disease. Acute phase reactant levels may not correlate with disease activity assessed by joint counts and VAS (420), suggesting that other factors may influence the more subjective elements of the DAS 28 score. An estimate of the extent to which these subjective elements contribute to a patient's DAS28 score can be calculated using the DAS28-P score (421). This is calculated by dividing the part of the DAS28 calculation derived from the TJC and VAS scores by the whole DAS28 calculation to determine the proportion of the DAS28 score made up by these more subjective elements. An elevated DAS-P before starting RA treatment has been shown to be associated with less improvement in pain symptoms with treatment than in those with a low DAS-P. Conversely, work by Hensor et al (112) has validated a 2 component DAS28 score using only SJC and CRP, which correlates more accurately with US identified synovitis that the 4 component DAS28 score that also contains TJC and VAS.

VAS has been shown to correlate strongly with depression and cognitive factors in patients with severe RA (422), demonstrating a psychological link with elevated DAS28 independent of inflammation. Ferrier et al have shown in a cross-sectional single centre study that in a

cohort of patients who did not obtain low disease activity or remission, the patient global assessment of disease activity was the sole reason for this and that this was heavily influenced by factors such as fatigue, pain, function, and psychological domains. These patients would be defined as being in remission based on inflammatory response and swollen joint counts (423). It is notable that depression is twice as common in RA patients as the general population (424). Study of the BSRBR has shown that depression at baseline reduces the likelihood of achieving a good treatment response to a TNFi and reduces improvement in disease activity over time (425).

Pain is one of the most important symptoms of RA. It arises from multiple mechanisms, including inflammation, structural damage and central sensitisation and is often associated with psychological distress (426), impacting on patients perception of their general health and thus elevating their VAS score. Pain has shown to be strongly linked to worsening fatigue, a factor that will also influence a patients assessment of their global health (427). Worse pain at baseline has been shown to be an independent predictor of TNFi failure (428). Co-existing fibromyalgia (FM) is common in RA, being reported in 14-17% of patients (429). FM is associated with pain and widespread hyperaesthesia, including joint tenderness, as well as significant fatigue. The risk of developing FM is highest in the first year after diagnosis of IA and doesn't appear to relate to traditional markers of RA severity such as seropositivity or acute phase response (430). It has been shown that both fibromyalgia trigger point tenderness and point tenderness at sites distant from the joint, such as over the sternum and tibia, is associated with an elevation in patient reported DAS28 components (431, 432).

Leeb et al have shown that a cohort of patients with Fibromyalgia had an average DAS28 score of 4.04, not significantly different statistically from a similar cohort of confirmed RA patients, whose average DAS28 was 4.23 (433). Indeed, Fibromyalgia has also been shown to elevate the HAQ and SF-36 scores and can lead to a patient being judged to not be in remission when they would otherwise meet remission criteria (429). An ultrasound study

comparing RA patients with a diagnosis of FM to RA patients without FM found that although the RA FM patients had higher DAS28, CDAI and SDAI scores, their ultrasound synovitis scores were not significantly different to their peers without FM (434).

Osteoarthritis secondary to joint damage is common in RA patients. Around 70% of patients with a disease duration of more than 7 years have been shown to have knee OA (435). The presence of degenerative changes in the joints may lead to chronic joint pain, stiffness and joint tenderness which will influence DAS28 scores. The number and volume of osteophytes in the hand joints of RA patients has been shown to correlate with increasing age, disease duration and the presence of bone erosions (436). Indeed, the correlation in the distribution of osteophytes with bone erosions suggests that osteophyte formation in RA may be a secondary response to bone erosion in some patients. Progression of secondary degenerative changes has been shown to be associated with ongoing RA inflammation (437) and treatment with IFX has been shown to reduce the risk of PIPJ OA in RA patients (438). Refractory patients with sub-optimal disease control, prolonged inflammation and potential progression to bone erosion, are at high risk of secondary OA which may be a confounding factor when assessing their disease activity. Degenerative disease has been suggested as a significant reason why a patient may fail to achieve remission despite treatment (439).

Pain in RA is multifactorial, arising directly as a result of active synovitis, from secondary joint damage and from central sensitisation. Inflammatory cytokines likely play a role in the sensitisation of both peripheral and central nerves to pain, with both TNFα and IL-6 shown to affect pain thresholds in murine models of arthritis (440, 441). It is thus possible that treatment with alternate TT may differentially affect pain control in different individuals. Evidence from the clinical trials programme of baricitinib has suggested that it may improve pain scores to a greater extent than TNFi, even in the presence of similar reduction in swollen joint counts (442). This raises the possibility that there may be a role for JAKi in advanced disease, where

pain secondary to joint damage and central sensitisation may be more prevalent than in early arthritis.

1.12 Phenotyping Rheumatoid Arthritis

As discussed above, RA is a complex and heterogeneous condition, whereas the assessment of patients in the clinic necessarily involves a routine, one size fits all, calculation of disease activity. As a result, for any given DAS28 score there are a number of possible combinations of DAS28 component scores that can contribute to the final score. This is illustrated in **Figure1.1** which shows a number of different combination of DAS28 components which contribute to the same DAS28 score (approximately 5.1). It is possible that patients with different combinations of subjective and objective DAS28 components will respond differently to treatment, for example those patients with high levels of inflammation reflected in elevated SJC and CRP may be more likely to respond to TT than those whose DAS28 score is influenced more by TJC and VAS.

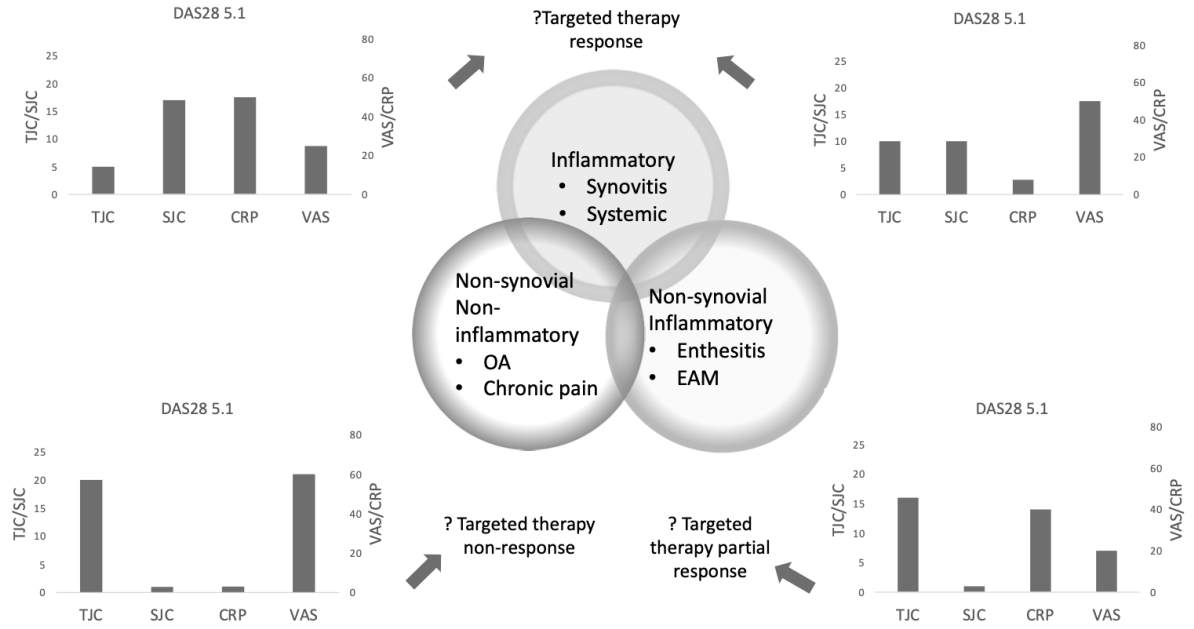

***Figure 1.1**: Illustrative example of potential contributions of differing individual DAS components for a on DAS28 score of approximately 5.1 (severe disease activity) (adapted with permission of M.H Buch).*

TJC – Tender Joint Count, SJC – Swollen Joint Count, CRP – C-Reactive Protein, VAS – Visual Analogue Score, OA – Osteoarthritis, EAM - Extra-Articular Manifestation

With the repertoire of targeted therapies expanding, it is increasingly necessary to identify clinically discernible groups of RA patients and to correlate these subgroup phenotypes with their response to targeted therapies. There are very few published studies in this area. McWilliams et al used latent class analysis using variables related to pain, central sensitisation, and inflammation to analyse groups of patients from the ERAN cohort of early arthritis patients and TNFi starters or those using non-biologic drugs from the BSRBR (10). The principle behind latent class analysis is that you can use statistical modelling to trace back the heterogeneity in a group to a number of latent homogeneous sub-groups (443). Using DAS28 components and results from HAQ and SF-36 scores they identified 4-5 latent classes within these RA cohorts (5 in ERAN cohort, 4 in both BSRBR cohorts). These displayed either expected concordance between the variables (indicative of mild, moderate or severe disease activity); discordantly less severe patient-reported measures despite elevated inflammation or

discordantly worse patient reported measures despite less markedly elevated inflammation. This last grouping is important as it identifies a subgroup of patients in whom elevated subjective measures of disease activity do not correlate with objective markers of active inflammation. Lee et al used self-reported measures and physical examination findings to identify clusters of RA patients who had differing causes for pain. Using a hierarchical agglomerative clustering method, 3 clusters of RA patients were identified; 1 group of individuals with low inflammation, pain, fatigue and psychosocial distress, 1 group with minimal inflammation but high pain, fatigue and psychosocial distress and 1 group of individuals with active inflammatory disease, manifested by high joint counts, high CRP and high pain and fatigue (444).

Several other studies have sought to stratify cohorts of RA patients, using differing statistical methods, to identify different groups of RA patients based on levels of physical limitation and fatigue. Norton et al used latent profile analysis to analyse the long-term trajectories of physical function (HAQ) in patients from the ERAN cohort. 4 classes were identified representing low, moderate, high and severe physical limitation (445). Female sex, older age at onset and high DAS28 at onset were found to be predictors of more severe limitation. Karpouzas et al performed a latent profile analysis on 618 RA patients from a single centre cohort to classify patients based on their patient global assessment of disease activity (PtGA) and physician global assessment (MDGA). They identified 5 clusters (low PtGA/low MDGA, moderate PtGA/moderate MDGA, high PtGA/high MDGA, high PtGA/low MDGA and low PtGA/high MDGA) (446). Basu et al studied the BSRBR register of patients on TNFI and csDMARD to identify clinically relevant clusters of severe fatigue in RA patients. 4 clusters were identified (basic, affective, inflammatory, and global) in the csDMARD cohort, which was validated in the TNFi group (447).

Deeper phenotyping using genetic studies and other biological analytical methods to identify distinct disease phenotypes have also been limited. Using large scale gene expression

profiling, van der Pouw Kraan et al identified two subgroups of RA with differing interferon signatures (IFN high and IFN low) (448). A study using symptom profiles, clinical chemistry and metabolomics measurements identified two subgroups of RA patients (449), but the very small sample size (39 patients) and questionable study design limit its value.

None of the above studies investigated an association between these different phenotypic groups and response to therapy. This will be an important step towards targeting individual therapies at appropriate groups of patients, hopefully avoiding the unnecessary cycling of expensive targeted drugs, and preventing patients from accumulating damage due to poorly controlled disease.

1.13 Musculoskeletal ultrasound

Musculoskeletal ultrasound (MUS) has been used for decades in rheumatology, but recent improvements in image quality and ultrasound availability have increased its utility in both the diagnosis of RA and in its use in monitoring treatment response. MUS is non-invasive, non-irradiating and does not require contrast which means it is safe to use in all patients. As it is deliverable at the point of care, it allows dynamic assessment to be made at the same time as clinical assessment by a trained rheumatologist. Detailed consensus guidance on the indications for MUS from the European Society of Musculoskeletal Radiology (450) and on scanning technique and patient positioning from EULAR (451) make MUS standardised and reproducible. Although it remains dependent on operator skill and experience, good inter-operator reproducibility has been shown between MUS trained rheumatologists (452). Grey scale (GS) ultrasound (B mode ultrasound) allows for the visualisation of synovium, fluid collections, tendons, tendon sheaths, ligaments and bone surfaces and allows for the assessment of bone erosion and cartilage loss (453). Power doppler (PD) ultrasound allows the visualisation of blood flow in vessels and can therefore detect the increased microvascular blood flow present in the actively inflamed rheumatoid synovium (454).

1.13.1 Definitions of ultrasound pathology

In order to standardise the interpretation of MUS, a number of consensus guidelines have been developed, including definitions of ultrasound pathology, scanning technique and scoring systems to define disease activity. OMERACT proposed the first standardised definitions of common ultrasound pathology, the definitions are shown in **table 1.4** (455).

RA Bone Erosion	An intraarticular discontinuity of the bone surface that is visible in 2 perpendicular planes.
Synovial Fluid	Abnormal hypoechoic or anechoic (relative to subdermal fat, but sometimes may be isoechoic or hyperechoic) intraarticular material that is displaceable and compressible but does not exhibit Doppler signal.
Synovial Hypertrophy	Abnormal hypoechoic (relative to subdermal fat, but sometimes may be isoechoic or hyperechoic) intraarticular tissue that is non-displaceable and poorly compressible and which may exhibit Doppler signal.
Tenosynovitis	Hypoechoic or anechoic thickened tissue with or without fluid within the tendon sheath, which is seen in 2 perpendicular planes, and which may exhibit Doppler signal.
Enthesopathy	Abnormally hypoechoic (loss of normal fibrillar architecture) and/or thickened tendon or ligament at its bony attachment (may occasionally contain hyperechoic foci consistent with calcification), seen in 2 perpendicular planes that may exhibit Doppler signal and/or bony changes including enthesophytes, erosions, or irregularity.

***Table 1.4**: OMERACT definitions of musculoskeletal ultrasound pathology.*

1.13.2 Ultrasound scoring systems

The identification of inflammation on ultrasound can help to confirm a clinical suspicion of RA. However, in order to both standardise the use of ultrasound in clinical practise and to be able to measure incremental change in disease activity (i.e. to give information beyond the simple presence or absence of inflammation) to assess response to intervention or loss of response to therapy, it has been necessary to develop scoring systems for ultrasound defined pathology in RA. There are several different scoring systems proposed for each type of ultrasound pathology. Consensus guidelines have been produced by the OMERACT ultrasound task force in an attempt to standardise practice for clinical trials.

1.13.2.1 Synovitis

There are several scoring systems proposed for grading synovitis which have been validated and used in RA research (109, 456-458). The consensus based EULAR-OMERACT scoring system provides a standardised system (459, 460) **(Table 1.5).**

Synovitis	Synovial hypertrophy (SH) (grey scale)	Power doppler (PD)	EULAR-OMERACT combined score
Grade 0 (Normal)	No SH independently of the presence of effusion	No Doppler signal	No SH and no PD signal
Grade 1 (Mild)	Minimal hypoechoic SH up to the level of the horizontal line connecting bone surfaces	Up to three single Doppler spots OR up to one confluent spot and two single spots OR up to two confluent spots	Grade 1 hypoechoic SH and ≤ grade 1 PD signal
Grade 2 (Moderate)	Moderate hypoechoic SH 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 1 but <50% Doppler signals in the total greyscale background	Grade 2 hypoechoic SH and ≤ grade 2 PD signal; or grade 1 SH and a grade 2 PD signal
Grade 3 (Severe)	Severe hypoechoic SH with or without effusion extending beyond the joint line but with the upper surface convex (curved upwards)	>Grade 2 (>50%of the total greyscale background)	Grade 3 hypoechoic SH and ≤ grade 3 PD signal; or grade 1 or 2 SH and a grade 3 PD signal

Table 1.5*: OMERACT EULAR scoring system for synovitis on ultrasound*

SH – Synovial Hypertrophy, PD – Power Doppler

1.13.2.2 Tenosynovitis

Several scoring systems exist for classifying tenosynovitis (461-463). The OMERACT-EULAR consensus scoring system has 4 grades for both grey scale and PD tenosynovitis (463).

	B Mode	Power doppler (PD)
Grade 0	Thin regular hypoechoic halo of the sheath, surrounding normal tendon	No Doppler signal
Grade 1 (mild)	Mild distension of tendon sheath by fluid or tenosynovial hypertrophy	Peritendinous focal Doppler 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 (moderate)	Moderate distension of tendon sheath by fluid or tenosynovial hypertrophy	Peritendinous multifocal Doppler 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 (severe)	Severe distension of tendon sheath by fluid or tenosynovial hypertrophy with tendon body involvement.	Peritendinous diffuse Doppler signal within the widened synovial sheath (i.e., signals filling most of the widened sheath), seen in two perpendicular planes, excluding normal feeding vessels

***Table 1.6**: OMERACT-EULAR scoring system for tenosynovitis on ultrasound*

1.13.2.3 Bone erosion

The presence or absence of bone erosions helps to classify disease severity and prognosis of RA. On US a bone erosion is classified as intra-articular discontinuity of the bone surface that is visible in two perpendicular planes (464). There are several proposed scoring systems for grading of bone erosion (464-467) which grade erosions either by size or the number of erosions in a given area. There is no definitive agreement on the minimum size of the erosion or how progression is shown when the erosion is greater than the maximum size included in the system (usually >4mm) (465).

1.13.2.4 Enthesitis

Enthesitis is a characteristic feature of the spondyloarthropathies, but evidence exists that it is also present and relatively common in RA patients as well (468). Again, there are several scoring systems in the literature used to assess entheses (469-472). The OMERACT definition of enthesitis on ultrasound includes the following features: hypo-echogenicity, increased

thickness, calcifications, enthesophytes, bone irregularity and doppler signal at the enthesis. The enthesis is defined as the 2mm of soft tissue adjacent to the bony cortex (473).

1.13.3 Ultrasound in Rheumatoid Arthritis

MSUS is valuable across the RA continuum from early RA to advanced disease and can assist in both diagnosis and the assessment of treatment response. It is also useful for assessing for differential diagnoses or comorbidity such as crystal arthritis (474). Synovitis is the hallmark of RA and is recognised to be primary driver of joint damage, with bone erosion occurring in its presence and not progressing in its absence (475). The presence of power doppler on ultrasound of RA has been shown to be associated with the presence of synovial inflammation on biopsy (476) and with elevated levels of IL-6 in the serum of RA patients (477). There is evidence that structural damage can continue to accumulate in patients with sub-optimally treated RA who have subclinical synovitis that is not picked up on clinical examination (109, 478). Ultrasound has been shown to be superior to clinical examination in detecting synovitis and joint effusion (479) and more effective than conventional radiology at assessing bone erosion (480).

Ultrasound also has the added benefit of assessing inflammation in extracapsular structures around the joint, as well as identifying tenosynovitis (463) and enthesitis (470). Extracapsular inflammation has been identified as an important finding in patients with palindromic rheumatism which may help distinguish it from early onset RA (481). A potential role of extracapsular inflammation in RefRA is not established but could potentially be a possible source of joint pain in refractory patients in the absence of synovitis.

Several studies have shown the utility of MUS in monitoring treatment response in patients with RA to both csDMARD and TTs. Fillippucci et al performed wrist ultrasound on 24 patients with active RA being treated with ADA (482). This showed a correlation between improvement

in PD and clinical examination as early as week 2 of therapy. Naredo et al carried out a larger study on 278 patients starting on TNFis and found similar results (483) Similar, smaller scale studies have shown similar results with other TNFis (484, 485). D'Agostino et al showed similar results in patients treated with ABT (486).

The features of ultrasound described above show that it can be a valuable tool for determining whether a patient with an elevated DAS28 score has active synovitis, non-synovial inflammation, or joint damage in the absence of active inflammation, as well as identifying patients with normal joints despite an elevated DAS28 score. Ultrasound synovitis scores are not altered by the co-morbid fibromyalgia and are therefore not subject to the same subjective interpretation as the DAS28 score (434). As a result, MUS can be an important tool in establishing whether a patient requires a switch in therapy.

1.14 Rational for thesis and research questions

Refractory RA is a growing problem in the rheumatology clinic and is recognised as a key area for ongoing research in the field. The pathology of RA is heterogeneous, and it is likely that the development of resistant disease is no less complex. The prevalence of RefRA within the general RA population is yet to be fully elucidated. Several recent studies have identified different clusters of RA phenotypes in early RA, highlights the heterogeneity of the RA population. However, no similar studies exist for advanced RA or in patients exposed to many therapies. Identifying whether these groups exist within an established RA population and assessing whether disease phenotype is associated with differential responses to treatment will help to inform on whether some RA phenotypes are predictive of treatment resistance. This will potentially shed light on the different pathways to treatment response, treatment failure and RefRA.

Previous studies in RA phenotyping have focussed on clinical, and patient reported outcome data, showing clusters of patients with both concordant and discordant inflammation when compared to PROs measuring pain, fatigue, and psychological distress. None of the work published to date use MUS to determine differences in the presence of synovitis or non-synovial inflammation between the groups, or whether clusters differ regarding the amount and or distribution of joint damage and how this damage relates to pain and mood. Including MUS would allow us to assess the association of proven synovitis with disease cluster and give an objective measure of response, allowing us to determine whether these different groups respond differently to therapy.

The overarching aim of this thesis is to determine whether differing RA phenotypes exist within a heterogeneous population of established RA patients, both in terms of the composition of the DAS28 component scores, which determine their DAS28 phenotype, and phenotypes of ultrasound defined pathology. Broader disease phenotypes including combinations of both

clinical and MUS derived features and PRO outcomes, also likely exist and may help to give more a more detailed assessment of disease phenotype. Again, if disease clusters can be identified, it is possible that they will differ in their responsiveness to TT. Assembling a cohort of RA patients with established disease, phenotyping them as they start a new TT and tracking their response to therapy over time will allow us to determine if this is the case. It will also allow us to answer the more fundamental question of whether the presence of synovitis at baseline predicts response to treatment, which has yet to be clearly established. If differing groups of established RA patients can be identified and their response to therapy determined to be different this would allow us to tailor different treatments pathways for these different groups. We hope that this will enable us to take a step towards a more personalised approach to managing patients with RefRA.

In the work contained within this thesis I hope to be able to answer the following questions:
In patients with established RA:

1. Is clinical response to targeted therapy mediated by the presence of synovitis i.e. does the presence or absence of synovitis predict clinical response and non-response respectively?
2. Can DAS28 clusters and multi-modality determined phenotypes be identified within a cohort of established RA patients?
3. How do the distinct DAS28 clusters associate with pathophysiological factors i.e. how do they associate with:
    - Clinical, serological and biochemical disease profile?
    - Ultrasound synovial and non-synovial clusters?
    - PROs of pain, quality of life, psychological distress, fatigue and function?
    - How are these factors associated with clinical response and non-response?
4. Do patients with treatment failure defined by DAS28 criteria have persistent synovitis on ultrasound?

5. Do changes with DAS28 correlate with changes in ultrasound score (and does this differ depending on DAS28 cluster)?
6. What are the ultrasound characteristics of patients who demonstrate a lack of DAS28 response at 24 weeks (or 12 weeks in early non-responders) at baseline and at drug fauilure, compared to those who respond?

In a cohort of patients with multi-drug refractory RA:

1. Can different pattens of drug sequencing (multiple non-response, mixed non-response/ loss of response, adverse effects) be identified within a cohort of RefRA patients and what proportion of a RefRA cohort do they comprise?
2. What proportion of multi-TT refractory patients show only primary failure (i.e. no response to treatment)?
3. Are JAK inhibitors efficacious in patients with multiple previous biologic drug failures?

1.15 Hypotheses, Research questions, Aims and Objectives.

1.15.1 Hypotheses

1. Established and active RA comprises distinct groups of patients with disease phenotypes characterised by:
    - Clusters of DAS28 components
    - Clusters of MUS-detected joint pathology
    - Clusters of combined clinical, PRO and imaging traits
2. Ultrasound-determined presence and absence of synovitis is associated with DAS28 response and non-response respectively.
3. Clinical response and non-response correlate with changes in MUS-detected joint pathology.
4. Refractory and active RA captures a heterogeneous patient group of multi-TT failure and/or toxicity but is not necessarily characterised by the presence of synovitis.
5. Recurrent primary failure to successive TT is uncommon.
6. JAK inhibition is effective in refractory RA.

1.15.2 Study aims

1. To accurately phenotype a cohort of patients with established rheumatoid arthritis and to correlate these phenotypes with response to targeted therapy to identify predictors of response and non-response to treatment.
2. To determine whether DAS28 components comprise of distinct clusters

3. To determine the clinical, imaging and patient-reported factors that drive the DAS28 components, and whether all these pathophysiological elements are structured through distinct disease phenotypes
4. To stratify DAS28 according to ultrasound-determined presence or absence of synovitis and evaluate if this associates with clinical response/non-response respectively.
5. To evaluate whether the phenotypes have prognostic significance in themselves with regards to targeted drug response.
6. To explore whether the extent of the clinical response correlates with changes in ultrasound pathology and whether the association differs by DAS28 phenotype.
7. To phenotype a multi-drug refractory cohort and evaluate response to JAK inhibition.

2 Methods

To investigate disease phenotypes in established RA and the prevalence and patterns of RefRA at Leeds teaching hospitals NHS trust, this thesis will include two related clinical investigations. The first part, which forms the basis of most of this thesis, is a prospective study, recruiting patients at the point at which they began their treatment with a new targeted therapy. Patients starting TT across the RA treatment pathway, from first line to multi-refractory were recruited with a view to phenotyping their disease at baseline and tracking their progress over time. This will enable me to determine whether different clusters of patients with established RA exist and, if they do, whether they respond differently to therapy. This will inform on whether baseline disease phenotype plays a role in response to treatment or progress towards RefRA. It will also allow me to establish whether the presence of baseline synovitis on MUS is associated with response to targeted therapy. The second part is a retrospective analysis of patients who have cycled through 3 or more targeted therapies at the trust, aiming to identify the patterns of drug sequencing they went through and thus what proportions of patients had multiple primary failures (“true’ refractory) or mixed response, loss of response and adverse effects, I have also performed a related analysis of the use of JAKi in the LTHT cohort and review of their efficacy in refractory patients.

2.1 Prospective patient recruitment (cohort 1)

The main aim of this thesis is to establish whether different phenotypic clusters of patients exist within a cohort of targeted therapy exposed patients. I therefore sought to establish a cohort of TT exposed patients, phenotype them using clinical assessment, PROs and MUS and then track their progress after initiation of therapy so see how they respond to their treatment.

I established a single centre, prospective longitudinal observational cohort of patients with RA eligible for treatment with targeted therapies. This encompassed patients at all points in the TT treatment spectrum from those starting a first line TT based on NICE guidelines or those switching any line of targeted treatment due to primary or secondary non-response or adverse effects of their current targeted therapy. All patients starting a new TT were thus eligible for recruitment. In addition, it was expected that in practice, some patients meeting the DAS28 criteria required to switch targeted therapy would not have their treatment changed due to a clinical impression that their DAS score is driven by non-synovial pathology. These patients were recognised as being important for our analysis as they potentially represent a proportion of a possible cluster of patients with high PRO responses, but low inflammation and omitting them would be a potential source of bias within the data. To capture these patients in our statistical analysis we aimed to recruit all patients who met DAS28 criteria for TT switch regardless of whether they ultimately changed their therapy.

This is a pragmatic study employing real-life patients managed using standard RA therapy. It is a complex observational study in nature to enable cluster analysis. No specific intervention has been evaluated for its efficacy, but association of drug response to disease activity cluster and pathological phenotypes has been investigated.

As a result, patients starting on any of the TTs licenced for use at the time of initiation of the study (February 2018) or their biosimilar equivalents, were recruited. This included the TNFis (infliximab, etanercept, adalimumab, golimumab, certolizumab pegol), anti-IL-6 monoclonal antibodies (tocilizumab, sarilumab), rituximab, abatacept and two JAKi (tofacitinib and baricitinib).

2.1.1 Target population

All RA patients starting one of the above therapies at Leeds Teaching Hospitals NHS Trust (LTHT).

Patients were recruited predominantly from LTHT rheumatology biologics monitoring clinics, with some first line TT patients referred from the early arthritis clinic. All patients meeting the inclusion criteria were consented to the RADAR (Rheumatoid Arthritis DiseAse Research) main study (version 9) and biological sub study (version 7). Ethical approval for all investigations, imaging and patient reported outcomes was granted under the RADAR protocol (09/H1307/98), which was amended to include all study investigations involved in this work prior to commencing patient recruitment. Ethical approval of this amendment as granted in February 2018.

2.1.2 Inclusion criteria

a) Subject ≥ 18 years of age

b) Is capable of understanding and signing an informed consent form

c) Confirmed diagnosis of RA according to ACR/EULAR 2010 criteria

d) Meets one of the following criteria:

- Ongoing severe disease activity (DAS28 >5.1) despite treatment with 2 synthetic DMARDs as per NICE guidance
- Primary non-response to a targeted therapy (failure to achieve an improvement of DAS28 ≥1.2 or DAS28 <3.2 at 6 months (or 3 months if clear non-response in line with usual practice)
- Secondary failure of targeted treatment determined by the treating rheumatologist.

- DAS28 score of > 5.1, but not deemed suitable to start or switch targeted therapy on clinical grounds.

2.1.3 Exclusion criteria

a) Age < 18 years
b) Lacks capacity to give informed consent or unable to read or understand patient information sheets and consent form
c) Taking ≥ 10mg oral Prednisolone or and equivalent alternative corticosteroid or had an IM depo steroid injection within the last 6 weeks.

Excluding patients currently taking oral prednisolone or those who have recently required a depo steroid injection within the preceding six weeks will potentially introduce a source of bias within the data and may mean that I miss some patients with the most active disease but is unavoidable as steroid treatment will affect clinical and ultrasound findings and therefore results will not be representative of a patient's true disease activity. Where patients required urgent steroid treatment efforts were made to perform same day MUS and if this was not possible patients were counselled and asked to delay start of steroid treatment until after urgent baseline investigation. Those who could not wait were excluded from the study.

2.1.4 Recruitment targets

The aim was to recruit a sufficiently large cohort of advanced RA patients to do a meaningful latent profile analysis. This thesis is aimed to be a pilot to show that disease clusters can be identified within a cohort of advanced RA patients to inform on the potential for a larger piece of work in this area, but it requires sufficient power to allow selection criteria to accurately assess how many clusters are present within the data. In conjunction with the departmental

statistician (LH), it was determined that a minimum of 200 patients should be recruited over a 2-year recruitment period. In one simulation study of the performance of selection criteria for different clustering methods, using different sample sizes and under a variety of conditions (487), the Bayesian Information Criterion (BIC) correctly identified the number of clusters present at n=200 74% of the time, when 10 items were included and the structure was complex (latent profile analysis and selection criteria are discussed in detail in the statistics section of this chapter, section 2.4.3, page 109).The more patients recruited, the greater the accuracy, so we aimed to recruit as many patients as possible during the recruitment period between February 2018 and March 2020. At LTHT around 5 patients per week are seen in the nurse lead biologic counselling clinics to start a new TT, meaning that sufficient numbers of patients would be seen to make these figure achievable.

2.1.5 Study schedule

Table 2.1 Shows the study schedule for patients recruited into the study including the timing of investigations and clinical assessments.

Week	***-1***	***0***	***1***	***12***	***24***	***36***	***52 (3)***
Informed Consent		X					
Demographic data & Unique Identifier (RADAR no)		X					
Diagnosis reviewed		X					
Arthritis treatment review		X					
Vital signs – weight, heart rate, blood pressure		X		X	X		X
Clinical assessments							
Joint count (swollen and tender)		X		X	X		X
DAS28 calculation DAS28 -P calculation		X		X	X		X
Patient Reported Outcomes							
Patient 0-100 Visual analogue score (VAS)		X		X	X		X
HAQ, HADs, FACIT-Fatigue		X		X	X		X
Review or send for blood tests:							
Rheumatoid Factor		X					
ACPA		X					
ANA		X					
FBC, U+E, LFT		X		X	X		X

CRP and ESR		X		X	X		X
Research samples							
Blood for saving		X		X	X		X
Imaging assessment							
Radiographs of hands and feet		X (1)					
Research imaging							
Musculoskeletal ultrasound		X		X (2)	X		

Table 2.1*: Study schedule*

(1) Baseline radiograph done if there is not one available showing previous evidence of erosive changes or a normal x-ray performed within the previous 12 months.

(2) Patients who have failed targeted therapy at 3 months will return to baseline and have a repeat US scan prior to starting a new therapy. They will have a repeat scan at 24 weeks from their initial start date.

(3) Patients will be followed up at 12 months and further data collected to inform future work on this cohort, but for the purpose of this thesis, insufficient 12-month data will be available and won't be included in the analysis presented.

RADAR – Rheumatoid Arthritis DiseAse Research, HAQ – Health Assessment Questionnaire, HADs – Hospital Anxiety and Depression Scale, FACIT – Functional Assessment of Chronic Illness Therapy, ACPA – Anti-Citrullinated Protein Antibody, ANA – Anti-Nuclear Antibody, RF- Rheumatoid Factor, FBC – Full Blood Count, U+E – Urea and Electrolytes, LFT – Liver Function Test, CRP- C Reactive Protein, ESR – Erythrocyte Sedimentation Rate.

2.1.6 Study investigations

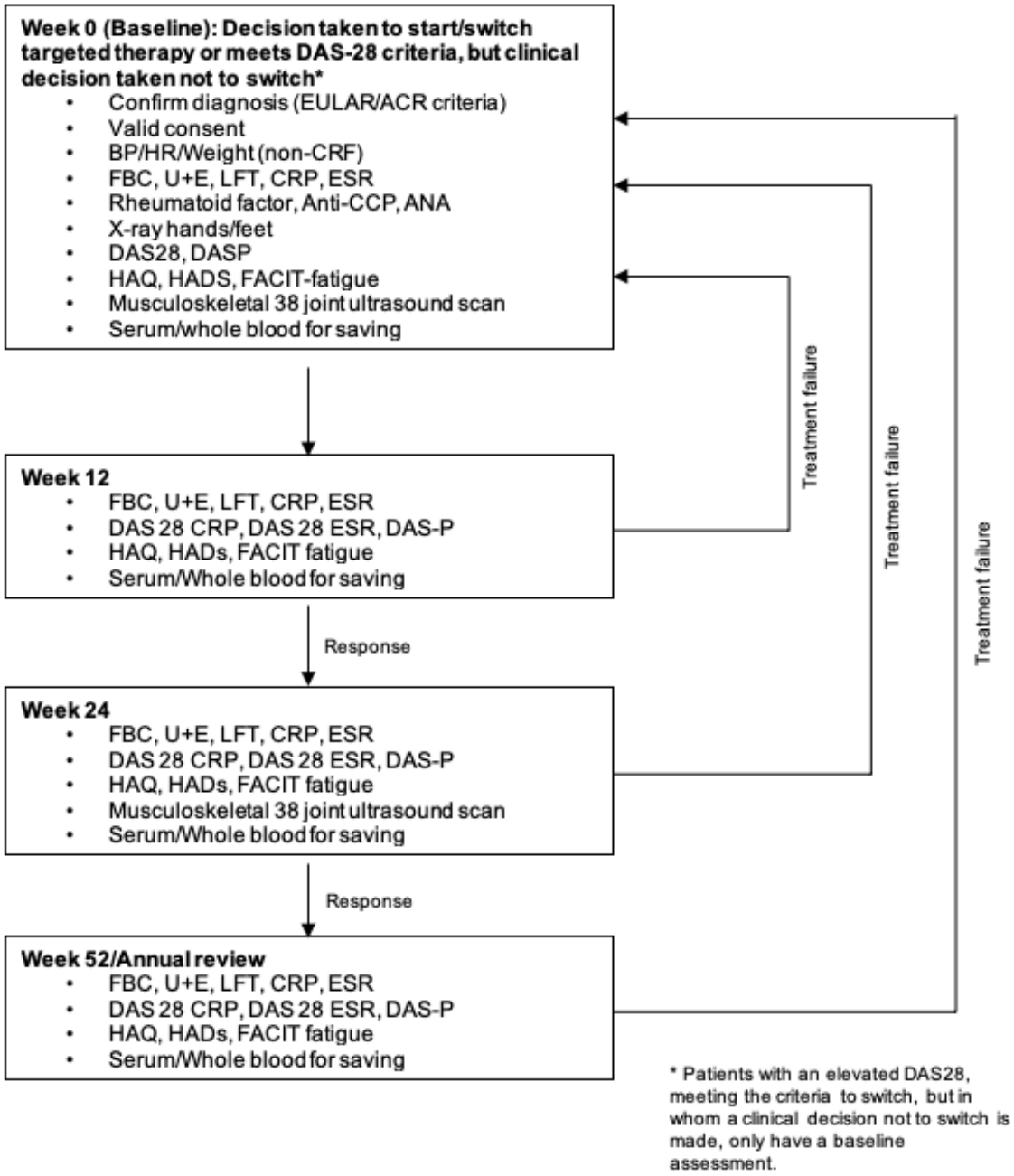

***Figure 2.1**:Study investigations*

RADAR – Rheumatoid Arthritis DiseAse Research, HAQ – Health Assessment Questionnaire, HADs – Hospital Anxiety and Depression Scale, FACIT – Functional Assessment of Chronic Illness Therapy, ACPA – Anti-Citrullinated Protein Antibody, ANA – Anti-Nuclear Antibody, RF- Rheumatoid Factor, FBC – Full Blood Count, U+E – Urea and Electrolytes, LFT – Liver Function Test, CRP- C Reactive Protein, ESR – Erythrocyte Sedimentation Rate.

Figure 2.1 is a flow chart showing clinical assessment and investigations performed at each clinic visit. Demographic information, past medical history and past targeted therapies, and the reason for their withdrawal when available were recorded at baseline.

Patients had clinical assessment at baseline, week 12 and week 24. In addition, a non CRF blood test at week 4 was performed as is standard practice after starting a targeted therapy as part of a safety assessment. Follow up at week 52 was also performed on patients reaching this point on TT. This was to form part of future work on this cohort, as insufficient numbers of patients were expected to reach this time point during the study period for data to be included and analysed in this thesis.

Tender and swollen joint counts, patient general health VAS and inflammatory markers (CRP and ESR) were recorded at each visit in order to calculate DAS28-CRP, DAS28-ESR, DAS-P and DAS28CRP-2C. DAS-P represents the proportion of the DAS28 score which is made up of the subjective elements of the DAS-28 (TJC, VAS) (421). DAS28CRP-2C is a measure of disease activity calculated using only objective elements of the DAS23 score (SJC and CRP). Hensor et al developed and validated this score and have showed that it better correlates with inflammation identifiable on MUS than the standard 4 component DAS28(112). Inclusion of these two scores will thus help to identify individuals whose DAS28 score is being driven primarily by either high subjective component or objective DAS28 component scores.

DAS 28 scores were calculated using the following formulae:

DAS 28-ESR = (0.28 X √SJC) + (0.56 x √TJC) + (0.70 x ln(ESR) + 0.014 X VAS)

DAS 28-CRP = (0.28 X √SJC) + (0.56 x √TJC) + (0.36 x ln(CRP+1) + (0.014 X VAS) + 0.96

DAS 28-CRP-2C = √SJC28+(0.6x ln(CRP+1))

DAS- P is calculated using the following formula:

DASP-ESR = ((0.56 X √ TJC) + (0.014 X GH)) / ((0.28 X √SJC) + (0.56 x √TJC) + (0.70 x ln(ESR) + 0.014 X VAS))

DASP-CRP = ((0.56 X √ TJC) + (0.014 X GH)) / ((0.28 X √SJC) + (0.56 x √TJC) + (0.36 x ln(CRP+1) + (0.014 X VAS) + 0.96)

DAS28-CRP scores at weeks 12 and 24 were be used to classify patients as responders or non-responders to TT. We used the EULAR definitions of good and moderate response (**Table 1.2**) to classify patients as responders or non-responders.

All clinical assessments and the results of blood tests for inflammatory markers were recorded on a biologics clinic proforma, which also formed the CRF for this study. This was copied and stored in a secure site file for this study, which also contained copies of PRO outcomes and ultrasound results for each patient. Information from the CRF was copied onto an excel spreadsheet and stored on a secure university server.

2.1.7 Laboratory investigation

Patients had routine blood tests comprising FBC, U+E, LFTs, CRP and ESR at baseline, 12 weeks, and 24 weeks. Serum samples and whole blood samples were also taken at each visit and have been stored for potential future analysis.

2.1.8 Patient reported outcomes

In order to assess the effects of functional limitation and mood on DAS28 a number of patient reported outcome (PRO) questionnaires were collected. HAQ, FACIT-fatigue and HADs questionnaires were taken at baseline and weeks 12 and 24.

2.1.8.1 Health Assessment Questionnaire-Disability Index (HAQ-DI)

The HAQ score was first developed in 1978 at Stanford university as one of the first measures of self-reported functional status. Modified versions are now widely used in research in a wide variety of rheumatological and non-rheumatological conditions, with improvements in HAQ score primary or secondary outcomes in many drug trials. The HAQ-DI score includes 20 questions across 8 domains relating to physical function (dressing, arising, eating, walking, hygiene, reach, grip, and common activities), and the need for any help or aids to undertake these activities. The extent of disability is scored on a scale from 0 (no disability) to 3 (severe disability) for each item. Patients are then asked to list any aids required to undertake such activities. The use of help or aids increases the category score from 0 or 1 to a 2 if it has been indicated that they are required in that category. If the category score is already a 2 or 3, no adjustment is made. The total score is derived by taking the highest score from all domains, adding together (0-24) and then dividing by 8 to provide an average score (0-3). Higher scores represent greater disability.

2.1.8.2 Functional Assessment of Chronic Illness Therapy (FACIT)-Fatigue

The FACIT-Fatigue scale was originally developed as part of the broader Functional assessment of Cancer therapy (FACT) scale, which was developed to measure functional outcomes of patients following cancer treatment. The short section exploring symptoms of

fatigue has been validated as a stand-alone measure and is now used in a variety of conditions where symptoms of fatigue can predominate, with obvious uses in rheumatology. It is a 13-item symptom-specific questionnaire assessing self-reported severity of fatigue and its impact upon daily activities and function. The score is calculated as the sum of individual item scores (on a 4-point Likert scale), multiplied by 13 and then divided by number of items answered. Scores range from 0 to 52 with higher scores indicating less fatigue.

2.1.8.3 Hospital Anxiety and Depression Scale (HADS)

The HADS was developed in the 1980s as a self-reported measure of anxiety and depression for patients in the general medical outpatient clinic. It consists of 14 questions to assess these symptoms, each of which has 4 responses, ranging from a state of high anxiety / depression to that suggesting a low state of anxiety / depression. It results in two scales, one for each domain, each having a measurement range from 0 to 21. A score of 0-7 is graded as normal, 8-10 as a borderline case and 11-21 as a case of anxiety or depression.

2.1.9 Imaging

2.1.9.1 X-ray

To establish whether patients had radiographic erosions at time of inclusion they underwent x-rays of their hands and feet at baseline if previous x-rays did not show erosive changes and/or if x-ray of the hands and feet had not been undertaken within the previous 12 months. X-rays were checked for the presence or absence of erosions, but no formal scoring of erosive changes was undertaken.

A routine CXR was performed prior to commencing TT as per standard practice.

2.1.9.2 Musculoskeletal ultrasound

Patients underwent blinded musculoskeletal ultrasound of 38 joints (bilateral wrist, MCPs 1-5, PIPs 1-5, elbow, knee, tibiotalar joint, MTPs 1-5), 20 tendons/tendon groups (bilateral wrist extensor compartments 2, 4 and 6, finger flexors 2,3,4,5, tibialis posterior, peroneus longus and brevis) and 10 entheseal sites (bilateral lateral epicondyles, patella ligament proximal insertion, patella ligament distal insertion, Achilles' tendon, plantar fascia) at baseline and 6 months. Those deemed to be failing treatment at week 12 who required a switch in treatment returned to baseline and had a repeat baseline scan, which also serves as a 12-week, treatment failure scan. The treating clinician was not informed of the results of the scan and no treatment decisions were taken based on scan results.

Ultrasound was performed by 2 trained and experienced musculoskeletal sonographers (KS and BS) and 2 trained rheumatologists with specialist interests in MUS and MUS research (RW and AD). Scans were carried out on a General Electric (GE) Logiq E9 machine with linear ML6-15 Megahertz (MHz) transducer and hockey stick linear array 8-18 MHz transducer. Although we haven't formally tested reliability, regular calibration exercises were performed to ensure consistency of image acquisition of scoring. The protocol was shown to take an experienced sonographer around 45 minutes to complete and 15 minutes to score. For pragmatic reasons it was decided to exclude the shoulders and hips from the ultrasound protocol as this would have made scans too long and reduced the number of scans that could be done in a session.

Data were recorded by hand on a paper ultrasound proforma (appendix 1) and transferred to an excel spreadsheet saved on a secure university drive for future analysis. The US protocol is detailed below:

- **Synovitis**: Grey scale (GS) and power Doppler (PD) assessment.

Images of 38 joints were assessed for synovitis. All scans were performed in longitudinal and transverse planes from the dorsal aspect. GS synovitis and PD changes indicating inflammation were defined and scored (0-3) using the OMERACT-EULAR semi-quantitative scoring system (459, 460). We defined the presence of clinically relevant synovitis as PDUS ≥ 2 in at least 1 joint or total PD > 3 (if no joint has PD ≥ 2). This was based on a study by Padavano et al (488) who performed MUS on a large cohort of healthy volunteers and identified common US abnormalities in people without inflammatory arthritis. Our score is based on maximum score across this large study in healthy volunteers. It should be noted that Padavano et al is based on a cohort of relatively young patients, and it was expected that a higher proportion of older patients would be recruited to this study, who may have different prevalence of MUS abnormalities, but to date this is the only paper published on MUS in healthy controls and has therefore been adopted as the standard.

- **Erosions:** Presence or absence of erosions was recorded for each joint scanned.

- **Osteophytes and subluxation;** Structural changes in the form of the presence or absence of osteophytes was recorded at each joint scanned and subluxation was recorded as present or absent for MCPs, Wrists and MTPs.

- **Tenosynovitis:** Grey scale (GS) and power doppler (PD)

Based on the OMERACT-EULAR protocol (463), the following tendons/tendon groups were assessed for GS and PD changes which were scores 0-3 based on the EULAR-OMERACT scoring system:

- Wrist extensor compartment 2 (extensor carpi radialis, brevis and longus)
- Wrist extensor compartment 4 (extensor digitorum communis and extensor indices propius)

- Wrist extensor compartment 6 (extensor carpi ulnaris)
- Finger flexor digitorum superficialis and profundus tendons 2-5 (MCP level)
- Tibialis posterior
- Peroneus longus and brevis.

- **Enthesitis**

Entheseal inflammation is a hallmark of SpA but is also found in RA patients. It remains unclear whether entheseal inflammation is more prevalent in advanced RA compared to early disease and whether it contributes to ongoing joint pains in those who have an improvement in synovitis is response to therapy. It is possible that there is a cohort of advanced patients with more prominent entheseal inflammation. In order to capture this and to give a more complete picture of structures away from the synovium which may contribute to symptoms in advanced I have included assessment of the entheses in the study.

The OMERACT-EULAR scoring system was used for enthesitis (470). The system highlights thickening, hypo-echogenicity and PD as markers of disease activity and calcifications and/or enthesophytes and bone erosions as markers of structural change at the enthesis. We included entheseal sites at the lateral epicondyle, proximal and distal patellar tendon, Achilles' tendon and plantar fascia within our protocol. There are elements of subjectivity in assessing enthesis hypo-echogenicity and tendon thickening. To remove variability in the assessment of tendon thickening between our sonographers we measured tendons against standardised measures of tendon size. For lower limb entheseal sites we used measurements provided in the Glasgow entheseal scoring system (489). No such reference exists for the lateral epicondyle, so another standard was identified from ultrasound literature. Krogh et al studied the ultrasound characteristics of the common extensor tendon insertion of 258 healthy subjects. They identified a mean average measurement of 4.87mm (SD 0.78) in the dominant

arm of patients. We used this measurement plus two standard deviations as our standard of lateral epicondyle tendon thickening. **Table 2.2** shows the measurements used as cut offs for entheseal thickening.

Total scores of all the above ultrasound variables were calculated for individual patients by adding together the scores for PD or GS in each joint, tendon group or entheseal site or adding together the total number of joints with erosions, osteophytes or subluxation or entheses with tendon thickening, calcifications, erosions, hypoechogenicity or enthesiophytes.

Enthesis	Measurement
lateral epicondyle enthesis/tendon	6.4mm
patella ligament proximal insertion	6.1mm
Patella ligament distal insertion	4 mm
Achilles' tendon	5.29 mm
Plantar facia	4.4 mm

Table 2.2*: Standardised measurements for tendon thickening at entheses.*

2.2 Retrospective analysis of Leeds Teaching Hospitals Refractory RA cohort (cohort 2)

LTHT is a tertiary referral centre for Rheumatology and receives patients from all over North Yorkshire and beyond. It has one of the largest cohorts of patients exposed to targeted therapies and has been at the fore front of clinical research into the management of RA with TTs since the early years of the bDMARD revolution. As a result, it has a large cohort of RefRA patients, although this group has not been formally identified and no specific data has been kept on them. I aimed to identify this cohort of patients with a view to performing a descriptive analysis of LTHTs refractory cohort. In the absence of a formalised definition of refractory or resistant RA at the time of inception of this study it was decided to define patients who have tried 3 or more classes of targeted therapy as refractory. This was the definition used in the BSRBRs analysis of RefRA (308) and was more stringent than other definitions available at the time. Unlike the BSRBR analysis, which only included those patients who had started a TNFi as their first line TT, we aimed to include all patients with 3 or more TT exposures, regardless of their first line TT.

2.2.1 Inclusion criteria

All patients with a formal diagnosis of RA consistent with the ACR/EULAR 2010 definition of RA, who had been exposed to 3 consecutive targeted therapies, licenced, and approved by NICE for use in RA in UK at the time that this study took place (August 2017 – March 2020). Patients could have failed at least 2 TTs for any reason. Failure was defined as either primary non-response, where no response to therapy was observed within the first 3-6 months of treatment, secondary non-response, where a response was observed but subsequently lost, or an adverse event, which encompassed drug side effects or the development of disease comorbidity which precluded continuation of the TT.

All patients identified were checked against the RADAR study (09/H1307/98) database, to ensure they had consented to RA research and those who weren't were consented prospectively.

2.2.2 Patient identification

Patients who had been exposed to 3 or more targeted therapies were identified in several ways:

- Through the outpatient clinic. LTHT rheumatology department runs several specialist biologics monitoring clinics for patients with RA at Chapel Allerton Hospital. A dedicated clinical nurse specialist reviewed clinic notes and completed a targeted therapy log for all patients who attend and identified those who had 3 or more TT exposures. This was performed over a period of 18 months from January 2018 to July 2019.
- A dedicated data base of patients involved in several different studies approved under the biologic monitoring clinic arm of the RADAR study is kept, ensuring clinic follow up and sample collection is performed in a timely fashion. Prior drug exposure was recorded on this database, which was used to identify patients who had been exposed to 3 TTs.
- A list of patients who have required an individual funding request for a targeted therapy is maintained by the pharmacy at Chapel Allerton hospital. Patients who had been exposed to 4 targeted therapies require an individual funding request to start subsequent lines of treatment, so patients with 4+ targeted therapy failures were captured on this list.

The following information was collected for each individual patient

- Age

- Sex
- Each individual targeted therapy they have tried, the start date, duration of treatment and reason for stopping (defined as primary failure, secondary failure or adverse effects).
- ACPA and RF status
- The current presence of erosive disease
- Co-morbid diseases
- Smoking status where possible

2.2.3 Analysis of notes for subset of Refractory Rheumatoid Arthritis patients.

It was not possible to collect DAS28 scores or DAS 28 components or disease duration from any of the databases above. Therefore, a further retrospective analysis of the paper clinical notes of a subset of patients was undertaken to identify disease duration and DAS28 scores and components at each switch of therapy was undertaken. The aim was to review the paper notes of all patients identified within the RefRA cohort. However, this was halted after 60 patients due to difficult obtaining some long-term records from storage and the dawn of the COVID-19 pandemic which prevented the collection of paper notes from medical records for non-clinical reasons.

2.3 JAK inhibitor use at Leeds Teaching hospitals NHS trust

We performed an analysis of JAKi use at LTHT to assess their efficacy across the RA treatment pathway, with a particular interest in assessing their efficacy in RefRA patients.

2.3.1 Patient Identification

All patients exposed to a JAKi at LTHT from November 2014 onwards, were recorded on a database at the time of their JAKi prescription by the Rheumatology specialist nursing staff, which allowed identification of all patients with prior JAKi exposure. Retrospective data were collected from clinical notes and electronic health records of patients who started tofacitinib on a compassionate access scheme between November 2014 and November 2017. This scheme was available for patients' refractory to other classes of bDMARD. A prospective database was maintained for all patients with RA at LTHT treated with a JAKi, from time of licensing of both baricitinib and tofacitinib in 2017. This study includes all patients who began a JAKi between November 2014 and November 2019 at LTHT.

Disease duration, serological status, current csDMARD use and history of previous bDMARD exposure were recorded on the database. Disease activity score 28 joint count-C-reactive protein (DAS28-CRP) scores, as well as DAS28-CRP components were recorded at baseline and after 3 and 6 months of therapy, along with reason for drug withdrawal when necessary. Where a patient visual analogue score (VAS) was not recorded a 3-point DAS score of tender joint count (TJC), swollen joint count (SJC) and CRP was calculated, as on a group level there has been shown to be a good correlation between the two measures (490). Where patients discontinued therapy during the study period, discontinuation date and reason for drug withdrawal (lack of effectiveness, adverse event, loss to follow-up) were recorded. Adverse events and serious adverse events of special interest were recorded.

2.4 Statistical analysis

2.4.1 Analysis of a prospective cohort of advanced RA patients.

Patient demographics, clinical characteristics, PRO outcomes, and ultrasound results were summarised for each group using proportions of patients, median with interquartile range or mean with standard deviation as appropriate.

2.4.2 Identifying phenotypic clusters of RA patients: Latent profile analysis.

The main focus of this thesis is to determine whether different phenotypic clusters of patients exist within our cohort of RA patients treated with advanced therapies and, if they exist, to establish whether these differing clusters respond differently to TT. To identify these different clusters, we have used latent profile analysis (LPA).

LPA aims to identify groups or clusters, of individuals within a population that have different configural profiles of personal, environmental or clinical characteristics and thus identify homogeneous clusters from within a heterogeneous population. In the case of this study, I aimed to identify clusters of patients with different profiles of clinical and/or ultrasound variables within the established RA cohort. LPA treats profile membership as an unobserved categorical variable (the latent variable), whose value indicates the profile an individual belongs to with a degree of probability. Individuals are classified into clusters based upon these membership probabilities which are estimated directly from the model (491). Thus, LPA identifies subgroups with similar pattens of variables within a larger population. These variables are known as latent class indicators. The term LPA is traditionally used for data comprised of continuous variables, whereas the term latent class analysis (LCA) is used for categorical variables. However, the model can include continuous or categorical variables or

any combination of these (a mixed model) and the terms are used interchangeably (492). This makes it suitable for use in this study where we have a mixture of categorical and continuous data. In this study we refer to our analysis as a LPA.

In latent Profile/class models, the data are used to estimate the number of classes in the population, the relative size of each class, and the probability of a particular response to each observed variable given class membership. These are estimated using two parameters; the latent class prevalences, the proportion of the population that falls into each latent class, and the item-response probabilities, which represent the probability of a particular response to an indicator variable, conditioned on latent class membership. These are estimated using the expectation maximisation algorithm to gain maximum likelihood estimates of LPA parameters. This is an iterative process which starts with a random allocation of individuals into a prespecified number of classes, with models then run for a number of differing possible classes (491).

We ran three different LPAs using both DAS28 components (SJC, TJC, CRP and VAS) or ultrasound characteristics (total joint B mode, total joint Doppler, total joint erosions, total joint osteophytes, total joint subluxation, total tendon B mode, total tendon Doppler, total tendon thickening, total enthesis hypoechogenicity, total enthesis Doppler, total enthesis thickening, total enthesis erosions, total enthesophytes, total enthesis calcification) as indicator variables. We also ran a combined model with a combination of these clinical and ultrasound variables. LPA was run in Stata MP 16.0 (StataCorp. 2019. Stata Statistical Software: Release 16. College Station, TX: StataCorp LLC). Although LPA models can handle non-normally distributed data, normal distribution is considered optimal. Poisson and negative binominal models were also run for the distributions of the different clinical and ultrasound components to determine the best fit and a trial-and-error approach was taken in which different models were used in trial LPAs to determine the best distributions for model convergence. As a result

of this trial-and error approach, for TJC, VAS and joint grey scale a normal distribution was specified, for enthesis hypo-echogenicity and thickening a Poisson distribution was chosen and for all other variables the negative binomial distribution worked best. Models were run for 2-5 clusters and 10 draws, the random seed 123456 and 20 iterations were specified for each number of classes modelled.

Model fit was compared using the Bayesian information criterion (BIC) and Akaike's information criterion (AIC). Both BIC and AIC attempt to resolve this problem of overfitting by introducing a penalty term for the number of parameters in the model. The BIC is generally considered the best method and favours parsimony, whereas the AIC favours a higher number of clusters. The model with the lowest BIC and AIC is considered the best fit.

2.4.3 Testing for differences between latent profiles.

To compare differences in clinical and ultrasound variables between the latent classes identified we used the Kruskal-Wallis (K-W) test. Kruskal-Wallis is a rank-based nonparametric test that can be used to determine if there are statistically significant differences between two or more groups of an independent variable on a continuous or ordinal dependent variable. To test for differences between categorical variables a Chi^2 test was used. As K-W can only tell us that there is a statistically significant difference between clusters and not which clusters were different from one another, Dunn's test was used on variables with statistically significant K-W results. Dunn's test is a non-parametric pairwise multiple comparisons procedure based on rank sums used post hoc after rejection of a K-W test. These analyses were run in Stata MP 16.0.

2.4.4 Simple rules to approximate latent profiles

Once combined clinical and US latent profiles had been identified an investigation was carried out to see whether there were a limited set of variables that could be used to estimate which latent class an individual would fall into using regression tree analysis. The R recursive partitioning and regression tree package rpart was used to identify this limited set of variables. The data were first split into training (80% of sample) and test (20% of sample) sets. In the training set, 10-fold cross-validation was used to identify the best tuning (complexity) parameter from 20 initial values. The optimal complexity parameter was used to identify variables that could classify patients into the latent classes.

2.4.5 Statistical methods for longitudinal analysis

A variety of approaches were taken to model changes in DAS28 over time in response to TT. Due to the emergence of the COVID-19 pandemic and the shut-down of clinical research in March 2020, a large volume of follow up data were lost from the study. During this time patients could not attend their specified follow up time point and a large proportion of clinical follow up was performed over the phone, making accurate clinical assessment impossible. To model DAS scores and the proportion of EULAR responders at 3 and 6 months, multiple imputation was used to address all missing baseline covariates and missing longitudinal DAS component values, except those resulting from cancelled visits during COVID lockdowns which were assumed to be missing completely at random. Multiple imputation via chained equations was used to create 50 complete datasets. For continuous interval, bounded and ordinal variables predictive mean matching with ten nearest neighbours was used in the imputation model; for binary variables logistic regression was used. Variables imputed were smoker status, presence of synovitis at baseline, disease duration, baseline values for HADS anxiety and depression, FACIT-fatigue score and ESR, and TJC, SJC, CRP, and patient VAS at 3 and 6

months. The imputation models also included age, sex, RF, CCP, ANA, baseline erosions, the number of previous treatments, latent class membership at baseline (clinical and US combined), and baseline values of TJC, SJC, CRP & patient VAS. Following imputation, DAS scores and responder status were re-calculated and results of analyses in the imputed data were combined according to Rubin's rules.

Responder status at 3 and 6 months was compared according to presence of synovitis at baseline, and between the latent classes identified at baseline, using multinomial logistic regression models in which those who responded and those who experienced adverse events were compared to non-responders.

To compare changes over time in DAS scores and their components between latent classes identified at baseline, longitudinal mixed modelling was conducted. Mixed models allow individuals with at least one observation of the outcome to be included, and like multiple imputation, assume missing data are missing at random. Mixed models of changes over 6 months excluded patients whose 6-month visits were cancelled due to COVID lockdowns. Time was treated as a continuous variable, centred at 3 months, and a quadratic term was included to allow for nonlinear changes. Random effects for intercepts and slopes were included. The analyses listed above were conducted in Stata v16.0.

2.4.6 Identifying disease trajectory classes.

To identify whether there were groups of patients sharing similar trajectories of change in DAS over time, growth mixture modelling (GMM) was used, again with quadratic terms for time and random effects for intercepts and slopes. This analysis was conducted in R version 3.5.1 using the package lcmm, which is able to run both latent class mixture models (LCMMs) and growth mixture models (GMMs). The difference between the two is that LCMM fixes the intercepts

(starting values) and slopes (changes over time) to be the same within each class, whereas GMM allows these to vary between patients within a given class. GMM is the recommended approach as it makes fewer restrictions on the model. Minimum Bayesian Information Criterion (BIC) was used to identify the optimum number of classes. The final model was required to meet the following fit criteria for each class: average posterior probability of assignment >0.7, odds of correct classification >5, mismatch close to 0.

2.4.7 Retrospective analysis of Leeds Teaching Hospitals Refractory Rheumatoid Arthritis cohort and JAK inhibitor use.

The retrospective study of RefRA at LTHT is a descriptive analysis of our single centre experience of RefRA. The relatively modest sample size and retrospective nature of the analysis makes it unsuitable for regression analysis to look for causative factors for RefRA. Patient demographics and clinical characteristics were summarised for each group using proportions of patients, median with interquartile range or mean with standard deviation as appropriate and percentages of mild, moderate, and severe RefRA were calculated.

To assess JAKi use at LTHT, patient demographics and clinical characteristics were summarized for the combined JAKi cohort and individual JAKis (tofacitinib and baricitinib) using proportions of patients, median with interquartile range or mean with standard deviation as appropriate. Mean changes in DAS28-CRP score from baseline were calculated at 3 and 6 months and reported for the combined cohort and individual treatment groups. Sub analyses according to number of prior bDMARD failures were performed.

Duration of JAKi treatment was calculated and Kaplan-Meyer survival analysis of JAKi survival in our whole cohort and in sub-groups based on the number of prior TT exposures was carried out. Survival analysis was carried out in IBM SPSS statistics version 26.

3 Latent profile analyses of a cohort of advanced rheumatoid arthritis patients using clinical and ultrasound variables.

3.1 Introduction

I have hypothesised that within a population of established RA patients there will exist sub-groups or clusters of differing clinical phenotypes, both in terms of the configuration of their DAS28 component scores, and broader disease phenotypes comprising clinical and ultrasound features with factors associated with fatigue, general health and psychological wellbeing, reflected by PRO data. As outlined in chapter 1 (page 75) several studies have established the presence of different disease clusters within populations of RA patients (10, 444), although to date this work has been focussed on early RA or in those exposed to 1 or fewer TT and none of these studies have imaging data to confirm the presence of synovitis or inflammation in other joint structures or tendons. To address these hypotheses, I have recruited a cohort of established RA patients beginning TT and performed clinical assessment, 38 joint MUS and collected PRO data. Establishing this cohort allows me to phenotype their disease at baseline (prior to starting their new TT) and then track their response to treatment over time.

In this chapter I will focus on clustering patients according to their clinical characteristics to determine if different DAS28 phenotypes exist. I aimed to determine whether subgroups of patients exist whose DAS28 scores are comprised of varying proportions of subjective and objective DAS28 components as conceptualised in chapter 1, **figure 1.1** (page 75). McWilliam et al (10) established that within the ERAN and BSRBR cohorts there are clusters of patients who have expected concordance between inflammation and PROs, discordantly low PRO outcomes despite elevated disease activity and high PRO outcomes despite low disease activity on clinical assessment. It is therefore also reasonable to hypothesise that similar

clusters will exist within a population of advanced RA patients. In those in whom the more subjective elements of the DAS28 score are predominant, PRO measures in questionnaires focussed on mood, quality of life and fatigue may be higher.

Furthermore, in addition to differences in clinical and PRO outcomes it is possible that a population of patients with longstanding, sub-optimally controlled RA, there will be a proportion of patients in whom pain and stiffness may be attributable to joint damage rather than active RA. The lack of imaging data in previous studies means that it is not established whether this group exists as a discernible cluster. In addition, I have also performed latent profile analysis using MUS identified features to see if clusters of patients with different ultrasound phenotypes also exist.

If clusters of patients with differing clinical phenotypes exist within an established RA population, it is possible that they will respond differently to therapy and that the “one size fits all’ algorithm for establishing eligibility for TT using DAS28 score, may result in some patients getting sub-optimal therapy.

3.2 Chapter 3 aims

- To describe the baseline clinical and ultrasound characteristics of our prospective TT treated cohort.
- To perform a latent class analysis on our cohort using DAS28 component scores as grouping variables to establish if DAS28 clusters exist.
- To compare clinical, PRO and ultrasound variables between the established clusters
- To perform a similar analysis using baseline ultrasound variables as grouping variables and perform a similar comparison between the clusters.

3.3 Chapter 3 Methods

All methods used in the work presented in this thesis are detailed at length in chapter 2, but a brief summary will be provided for work contained in individual chapters. In this chapter I present the results of two separate latent profile analyses on a cohort of patients with established RA (cohort 1). This includes patients who, at a minimum, have failed at least 2 csDMARDs and met the NICE criteria for TT initiation, but more broadly includes patients from across the TT treatment pathway. These patients were recruited directly from LTHT biologics monitoring clinics and, in the case of those starting a first line TT, early arthritis clinics.

All patients recruited to this study, met the EULAR/ACR 2010 RA diagnostic criteria. Once it was established that a patient met the inclusion criteria (chapter 2, page 93) they were recruited onto the RADAR study. They then underwent a clinical assessment including a swollen and tender joint count, laboratory investigations including both CRP levels and ESR an X-ray of the hands and feet to assess for erosions, if no previous evidence of erosive change was documented. Patients were also asked to fill out several PRO questionnaires (FF, HADs, HAQ-DI) and record a VAS GH score. All patients underwent a blinded MUS of 38 joints, 20 tendons and 10 entheseal sites, within a maximum of 2 weeks of their initial assessment. No bridging steroid therapy was given during this time.

Latent profile analyses were performed using either combination of patient DAS28 score components (TJC, SJC, VAS, CRP) as indicator variables or ultrasound variables (total joint B mode, total joint Doppler, total joint erosions, total joint osteophytes, total joint subluxation, total tendon B mode, total tendon Doppler, total tendon thickening, total enthesis hypoechogenicity, total enthesis Doppler, total enthesis thickening, total enthesis erosions, total enthesophytes, total enthesis calcification) as indicator variables. Age, sex and HADs Anxiety were included in the models as covariates. HADs Anxiety was included as a covariate as it was expected that there would be a cluster representing high subjective components and

that anxiety may be a good predictor for this group. Models were run for 2-5 clusters and 10 draws, the random seed 123456 and 20 iterations were specified for each number of classes modelled. Model fit was compared using the Bayesian information criterion (BIC) and Akaike's information criterion (AIC). Clusters were then compared to one another using K-W or Chi^2, where appropriate.

3.4 Chapter 3 Results

3.4.1 Baseline statistics of established Rheumatoid arthritis cohort.

A total of 200 patients were recruited to our prospective cohort between February 2018 and March 2020. 196 patients attended for full baseline investigations, including MUS. The remaining 4 patients did not attend their ultrasound appointment but have all other baseline investigations and have been included in the analysis. Further patient recruitment was halted in March 2020 due to the COVID-19 pandemic which left us 4 patients short of our target of 200 patients with full baseline investigations.

3.4.1.1 Demographics

Of the 200 patients recruited and included in this analysis 150 (75%) were female. The average age of patients was 56.8 years (SD 12.61), the youngest patient was 26.7 and the oldest 88.7. They had a mean disease duration of 11.36 years (SD 8.01) at the time of recruitment.

3.4.1.2 Serology

One hundred and fifty-two (76%) patients were seropositive. **Table 3.1** shows the distribution of autoantibody positivity in the cohort.

Serology		ACPA		
		n	y	total
RF	n	48	33	81
	y	17	102	119
	total	65	135	200

***Table 3.1**: Serology results for established RA cohort*

RF – Rheumatoid Factor, ACPA – Anti-citrullinated Protein Antibodies, n – No, y – Yes.

3.4.1.3 Previous targeted therapy exposure

As outlined in chapter 2, patients were recruited at the point at which they were deemed to require either the initiation of a TT or a switch away from their previous TT due to any reason. Patients recruited to the study had been exposed to an average of 1.8 previous therapies (SD 1.71, median 1, IQR 3) and 1.47 previous classes of TT (SD 1.31, median 1, IQR 2). **Table 3.2** and **3.3** shows the number of patients exposed to each number of previous therapy and each number of classes of therapy respectively. In summary 28.5% of our cohort were starting a first line TT and 44% met our established definition of RefRA (≥2 previous classes of TT).

Number of previous TT	**Number of patients (%) Total n=200**
0	57 (28.5)
1	46 (23)
2	37 (18.5)
3	26 (13)
4	16 (8)
5	11 (5.5)
6	5 (2.5)
7	2 (1)

***Table 3.2**: Number of patients and percentage of cohort exposed to each line of targeted therapy (TT)*

Number of previous classes	Number of patients (%) Total n=200
0	57 (28.5)
1	55 (27.5)
2	40 (20.1)
3	33 (16.58)
4	12 (6.03)
5	3 (1.51)

Table 3.3*: Number of patients and percentage of cohort exposed to different numbers of classes of targeted therapy*

3.4.1.4 Smoking status

Smoking status was recorded for a total of 174 patients. Twenty-seven (15.5%) were current smokers at the time of consent to the study, 56 (32.2%) were former smokers and 91 (52.3%) had never smoked.

3.4.1.5 Clinical and patient reported outcome data

Table 3.4 shows the mean and standard deviation of DAS28 components (TJC, SJC, VAS, CRP, ESR), DAS 28 scores and PRO outcomes in the patients at baseline. **Figure 3.1** and **3.2** show histograms of the distributions of the individual clinical characteristics across the cohort at baseline.

Variable	Mean score (SD)
Tender joint count	13.0 (6.9)
Swollen joint count	5.2 (3.7)
Visual analogue scale (General health)	71.9 (16.4)
CRP (mg/L)	17.7 (23.9)
ESR (mm/hr)	24.3 (23.2)
DAS28-CRP	5.3 (1.0)
DAS28-ESR	5.4 (1.2)
HAQ	1.7 (0.6)
HADs Anxiety	8.8 (4.7)
HADs Depression	8.4 (4.4)
FACIT-fatigue	19.7 (11.7)

***Table 3.4**: Mean (SD) scores of clinical and patient reported outcome data across the cohort.*

SD – Standard Deviation, CRP- C-Reactive Protein, ESR, Erythrocyte Sedimentation Rate, DAS – Disease Activity Score, HAQ – Health Assessment Questionnaire, HADs – Hospital Anxiety and Depression scale, FACIT – Functional Assessment of Chronic Illness Therapy.

Figures 3.1 and **3.2** show histograms of the distributions of DAS28 and DAS28 component scores and PRO outcomes across the cohort respectively.

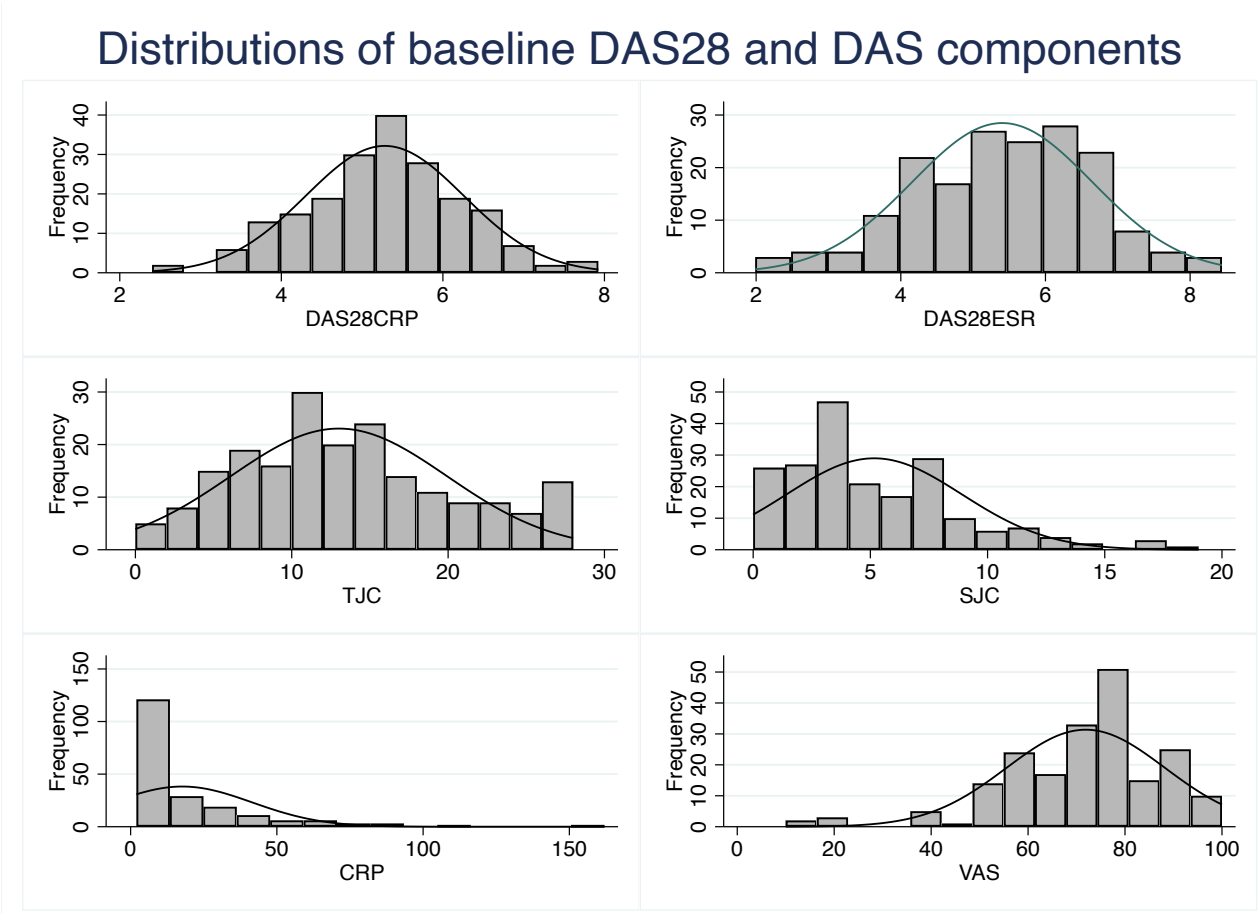

***Figure 3.1**: Distributions of DAS28 scores and DAS28 component scores at baseline across the cohort.*

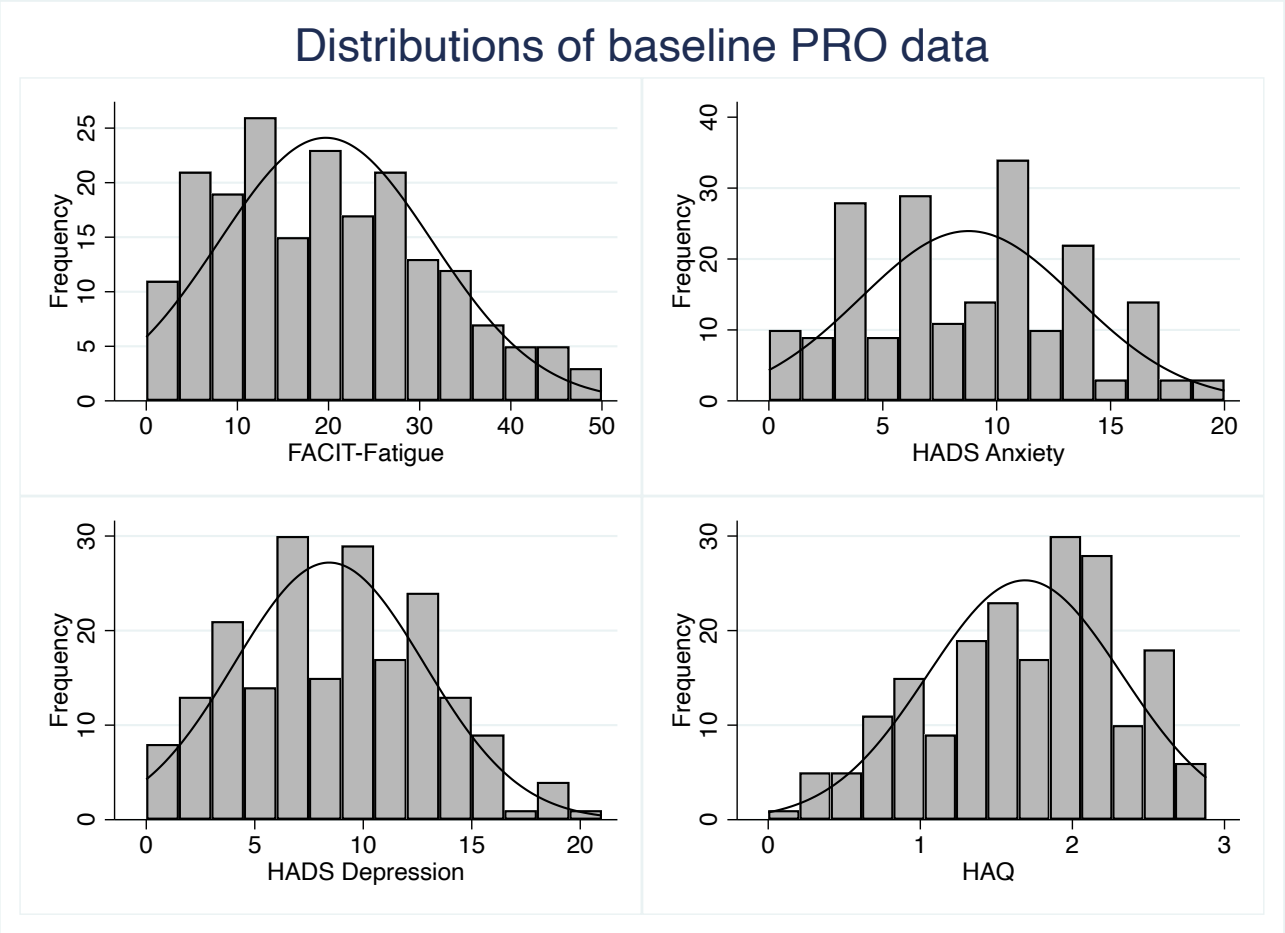

***Figure 3.2**: Distribution of patient reported outcome results at baseline across the cohort.*

3.4.1.6 Baseline ultrasound

Baseline ultrasound was performed on all patients at the time of recruitment to the study and totals of B mode and power Doppler score were calculated for the joints and tendons included. Scores for entheseal pathology were also recorded. **Table 3.5** shows median (IQR) and 90th centile scores for the entire cohort at baseline (n=196).

Ultrasound characteristic (Range)	Median (IQR (90th centile))
Total joint grey scale (0-114)	34.5 (20 (64))
Total joint PD (0-114)	6.5 (15 (32))
Number of joints with erosions (0-38)	2 (3 (7))
Number of joints with osteophytes (0-38)	3 (5 (11))
Number of subluxed joints (0-22)	3 (6 (9))
Total tendon grey scale (0-54)	4 (7 (13))
Total tendon power Doppler (0-54)	1(5 (9))
Number of thickened tendons (0-10)	0 (1 (2))
Total enthesis grey scale (0-30)	3 (3 (6))
Total enthesis power Doppler (0-30)	0 (0.5 (2))
Number of thickened entheses (0-10)	2 (1 (4))
Number of entheses with erosions (0-10)	0 (1 (1))
Number of entheses with enthesophytes (0-10)	1 (2 (4))
Number of entheses with calcifications (0-10)	0 (1 (2))

Table 3.5*: Median (Interquartile range (90th centile)) score for ultrasound variables at baseline*

In this cohort of 200 patients 154 (78.6%) of our cohort met the pre-determined criteria for clinically significant synovitis at baseline (PDUS ≥ 2 in at least 1 joint or total PD > 3 if no joint

has PD ≥ 2). **Figures 3.3 to 3.5** show the distributions of the different ultrasound variables at baseline across the cohort

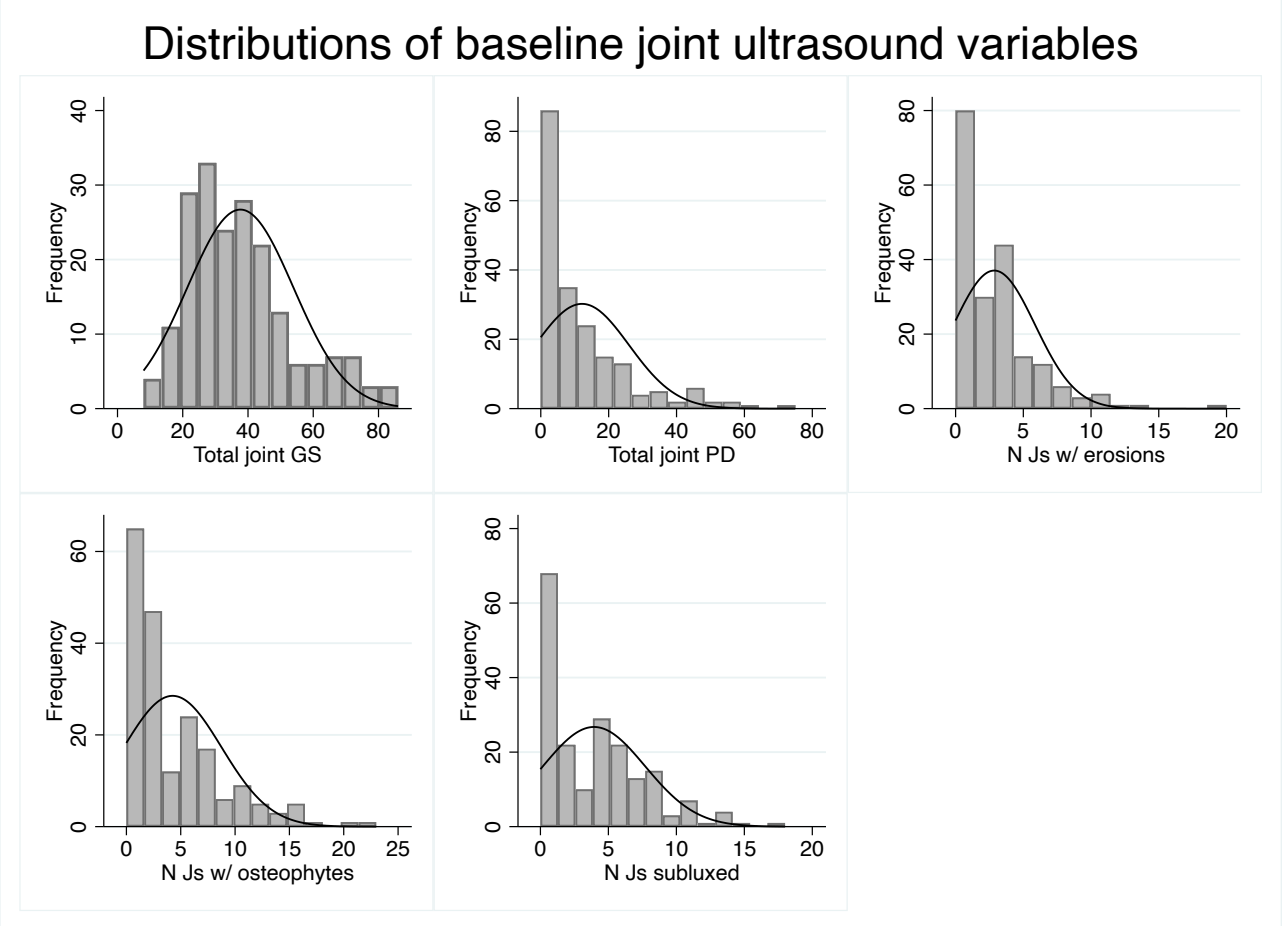

Figure 3.3: *Distributions of joint ultrasound variables across established RA cohort at baseline across the cohort.*

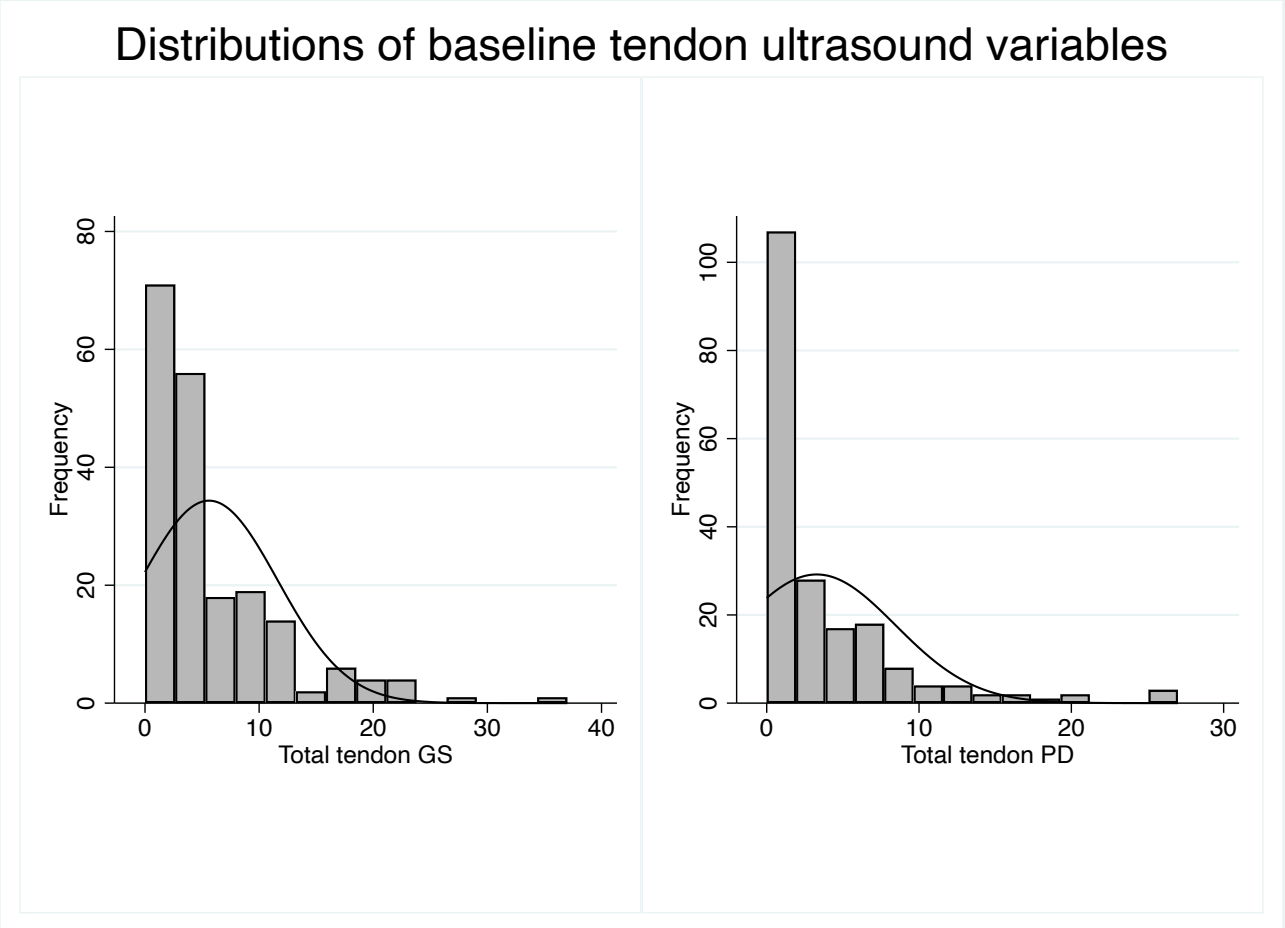

Figure 3.4*: Distributions of tendon ultrasound variables across established RA cohort at baseline across the cohort.*

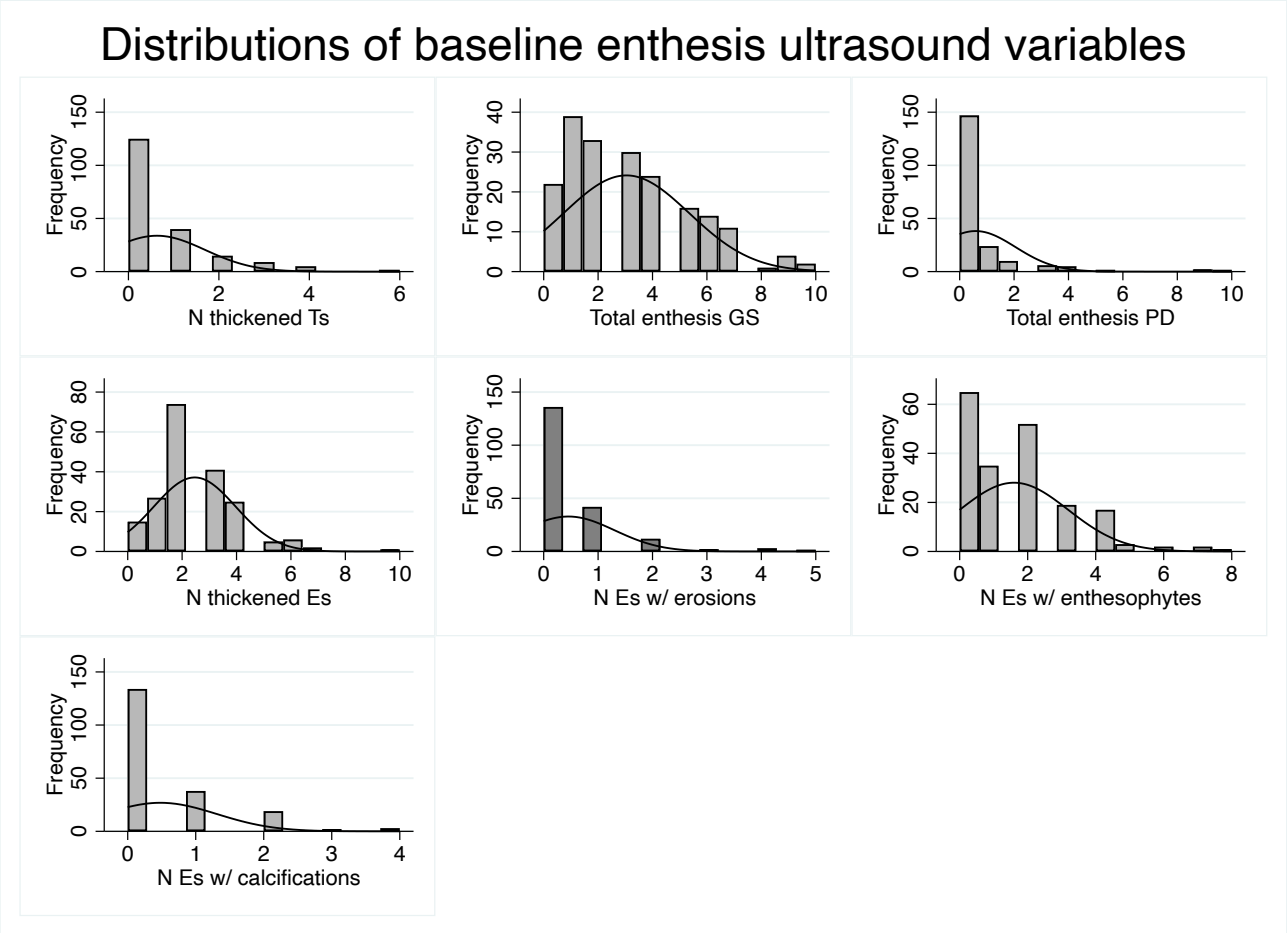

Figure 3.5: *Distributions of enthesis ultrasound variables across established RA cohort at baseline across the cohort.*

3.4.2 Latent profile analysis of established RA cohort using DAS28 components.

In chapter 1, I presented a figure depicting the different combinations of DAS28 components which can potentially make up a DAS28 score of 5.1 to illustrate how patients with different configurations of DAS28 components can be judged to have the same disease activity (**Figure 1.1,** page 75). This potentially encompasses patients with differing levels of inflammation, who may thus respond differently to therapy. I aimed to determine whether such disease phenotypes could be identified within our cohort using latent profile analysis, including DAS28 components as indicator variables as outlined above. **Figure 3.6** shows the fit statistics generated in the analysis.

a

Number of classes	N	df	AIC	BIC
2	200	13	5561.997	5604.875
3	200	18	5536.587	5595.957
4	200	23	5520.17	5596.031
5	200	28	5508.149	5600.501

b

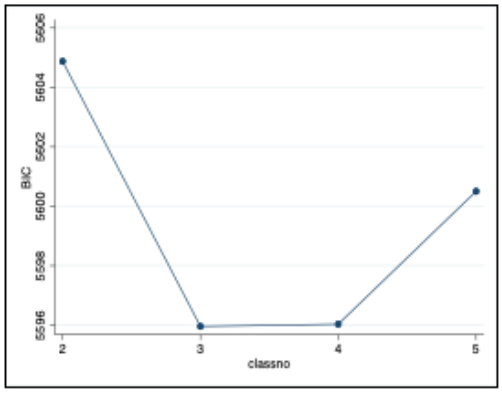

Figure 3.6*: Fit statistics for DAS component latent profile model. a. Table of results for AIC/BIC. b. Graph of BIC results for each number of possible classes.*

Df – degrees of freedom, AIC – Akaike Information Criterion, BIC – Bayesian Information Criterion.

In this analysis AIC doesn't minimise and therefore doesn't give us a clear indication of the best model, but the BIC does minimise at a very similar level for both 3 and 4 classes. This suggests that both the 3 and 4 class solutions are equally valid. Therefore, I will present each model in turn.

3.4.2.1 Clinical model: 3-class solution

Table 3.6 shows the baseline characteristics for patients assigned to each of the clusters in the 3-class solution for the clinical variable model (DAS28 components). Almost half of the patients are in class 1 (98/200), with class 2 being the second most populous (71/200) and class 3 the least common (31/200).

	Clinical predicted class					
	1	2	3	Total		
	N=98	N=71	N=31	N=200	Chi^{2} *	p
Age, years	56.4 (50.0, 61.7), n=98	55.7 (43.1, 63.4), n=71	63.3 (56.4, 73.1), n=31	57.2 (49.8, 65.3), n=200	13.255	**0.0013**
Sex Female	79/98 (80.6%)	50/71 (70.4%)	21/31 (67.7%)	150/200 (75.0%)	3.2203	0.200
Ever smoked	41/87 (47.1%)	28/61 (45.9%)	14/26 (53.8%)	83/174 (47.7%)	0.3742	0.829
Disease duration, years	10.0 (5.0, 16.0), n=84	8.0 (4.0, 16.0), n=63	12.0 (8.0, 18.0), n=26	10.0 (5.0, 17.0), n=173	2.929	0.2312
RF positive	52/98 (53.1%)	45/71 (63.4%)	22/31 (71.0%)	119/200 (59.5%)	4.2203	0.121
ACPA positive	66/98 (67.3%)	45/71 (63.4%)	24/31 (77.4%)	135/200 (67.5%)	1.7193	0.423
ANA positive	12/98 (12.2%)	6/71 (8.5%)	3/31 (9.7%)	21/200 (10.5%)	0.4090	0.815
X-ray erosions present	37/98 (37.8%)	33/71 (46.5%)	18/31 (58.1%)	88/200 (44.0%)	4.7914	0.309
TJC	11.0 (8.0, 15.0), n=98	15.0 (9.0, 19.0), n=71	11.0 (7.0, 19.0), n=31	12.0 (8.0, 17.0), n=200	4.778	0.0917
SJC	4.0 (2.0, 6.0), n=98	5.0 (3.0, 8.0), n=71	8.0 (4.0, 11.0), n=31	4.5 (2.0, 7.5), n=200	26.430	**0.0001**
CRP	2.0 (2.0, 2.0), n=98	16.1 (11.6, 27.0), n=71	55.0 (45.0, 77.0), n=31	8.3 (2.0, 25.0), n=200	175.336	**0.0001**
VAS	70.0 (60.0, 85.0), n=98	75.0 (63.0, 80.0), n=71	80.0 (65.0, 90.0), n=31	75.0 (62.5, 80.5), n=200	3.134	0.2087
ESR	8.0 (3.0, 15.0), n=87	25.0 (17.0, 31.0), n=65	57.0 (36.0, 85.0), n=27	18.0 (7.0, 31.0), n=179	89.396	**0.0001**
HADS Anxiety	9.0 (6.0, 12.0), n=97	8.0 (4.0, 12.0), n=71	9.0 (4.0, 13.0), n=31	9.0 (5.0, 12.0), n=199	0.703	0.7036

	Clinical predicted class					
	1	2	3	Total		
	N=98	N=71	N=31	N=200	Chi^{2} *	p
HADS Depression	8.0 (4.0, 12.0), n=97	8.0 (5.0, 11.0), n=71	11.0 (7.0, 14.0), n=31	8.0 (5.0, 12.0), n=199	5.022	0.0812
FACIT-Fatigue	20.0 (13.0, 28.0), n=97	20.0 (9.0, 28.0), n=71	12.5 (5.0, 19.0), n=30	18.5 (10.0, 28.0), n=198	7.161	**0.0279**
HAQ	1.6 (1.1, 2.0), n=96	1.8 (1.2, 2.2), n=70	2.0 (1.6, 2.5), n=31	1.8 (1.2, 2.1), n=197	11.869	**0.0026**
DAS28CRP	4.8 (4.3, 5.3), n=98	5.7 (5.3, 6.2), n=71	6.1 (5.6, 7.0), n=31	5.3 (4.7, 5.9), n=200	72.006	**0.0001**
DAS28CRP-P	0.6 (0.6, 0.6), n=98	0.5 (0.5, 0.6), n=71	0.5 (0.5, 0.5), n=31	0.6 (0.5, 0.6), n=200	76.124	**0.0001**
DAS28CRP-2C	2.7 (2.1, 3.1), n=98	4.0 (3.3, 4.5), n=71	5.3 (4.6, 5.8), n=31	3.3 (2.4, 4.3), n=200	114.197	**0.0001**
N previous treatments	1.0 (0.0, 3.0), n=98	1.0 (0.0, 3.0), n=71	2.0 (0.0, 3.0), n=31	1.0 (0.0, 3.0), n=200	0.212	0.8996
N previous classes	1.0 (0.0, 2.0), n=97	1.0 (0.0, 3.0), n=71	1.0 (0.0, 3.0), n=31	1.0 (0.0, 2.0), n=200	0.087	0.9576
DAS28CRP>3.2	97/98 (99.0%)	70/71 (98.6%)	31/31 (100.0%)	198/200 (99.0%)		
DAS28ESR>3.2	79/87 (90.8%)	64/65 (98.5%)	27/27 (100.0%)	170/179 (95.0%)		
Clinical synovitis (PD>=2 | total PD>3)	63/95 (66.3%)	60/70 (85.7%)	31/31 (100.0%)	154/196 (78.6%)		
Biologic prescribed						
Baricitinib	32/97 (33.0%)	23/71 (32.4%)	10/31 (32.3%)	65/199 (32.7%)		
Adalimumab	19/97 (19.6%)	15/71 (21.1%)	6/31 (19.4%)	40/199 (20.1%)		
Rituximab	9/97 (9.3%)	9/71 (12.7%)	6/31 (19.4%)	24/199 (12.1%)		
Abatacept	14/97 (14.4%)	6/71 (8.5%)	1/31 (3.2%)	21/199 (10.6%)		
Tocilizumab	9/97 (9.3%)	5/71 (7.0%)	5/31 (16.1%)	19/199 (9.5%)		
Etanercept	6/97 (6.2%)	5/71 (7.0%)	2/31 (6.5%)	13/199 (6.5%)		
Other	8/97 (8.2%)	8/71 (11.3%)	1/31 (3.2%)	17/199 (8.5%)		

	Clinical predicted class					
	1	**2**	**3**	**Total**		
	N=98	**N=71**	**N=31**	**N=200**	**Chi^{2} ***	**p**
Posterior probability >=0.9	83/97 (85.6%)	61/71 (85.9%)	24/30 (80.0%)	168/198 (84.8%)		

***Table 3.6**: Baseline demographics, serology, clinical findings and patient reported outcome measures for each cluster identified in the 3-class clinical model.*

Targeted therapy prescribed for the study also listed. Other – Infliximab, Golimumab, Certolizumab, Sarilumab.

ACPA – Anti-Citrullinated protein Antibody, RF- Rheumatoid Factor, ANA – Anti-Nuclear Antibody, TJC – Tender Joint Count, Swollen Joint Count, VAS – Visual analogue Scale, CRP- C Reactive Protein, ESR – Erythrocyte Sedimentation Rate, HAQ – Health Assessment Questionnaire, HADs – Hospital Anxiety and Depression Scale, FACIT – Functional Assessment of Chronic Illness Therapy, DAS – Disease Activity Score, RF- Rheumatoid Factor.

* Kruscal-Wallis test performed to identify differences between continuous variables, chi^{2} for categorical variables. Significant results highlighted in bold.

Table 3.6 shows the baseline characteristics of each cluster identified within the 3-class model and which TT they were subsequently treated with. It also shows the results for statistical tests of differences between the groups. Simple comparison of the above data suggests that cluster 1 is more female, more seronegative with lower inflammation than the other clusters, whereas cluster 3 is older with a longer disease duration and a higher inflammatory burden. This simple visual analysis allows us to classify class 1 as “low inflammatory”, class 2 as “moderate inflammatory” and class 3 as a “high inflammatory” group. Cluster 3, the most inflammatory cluster, has higher outcomes for all the PROMs recorded (high FF score suggests low fatigue), but a lower DAS-P, suggesting lower proportional TJC and VAS compared to the other clusters. Box and whisper plots of the DAS28 components are shown in **figure 3.7.**

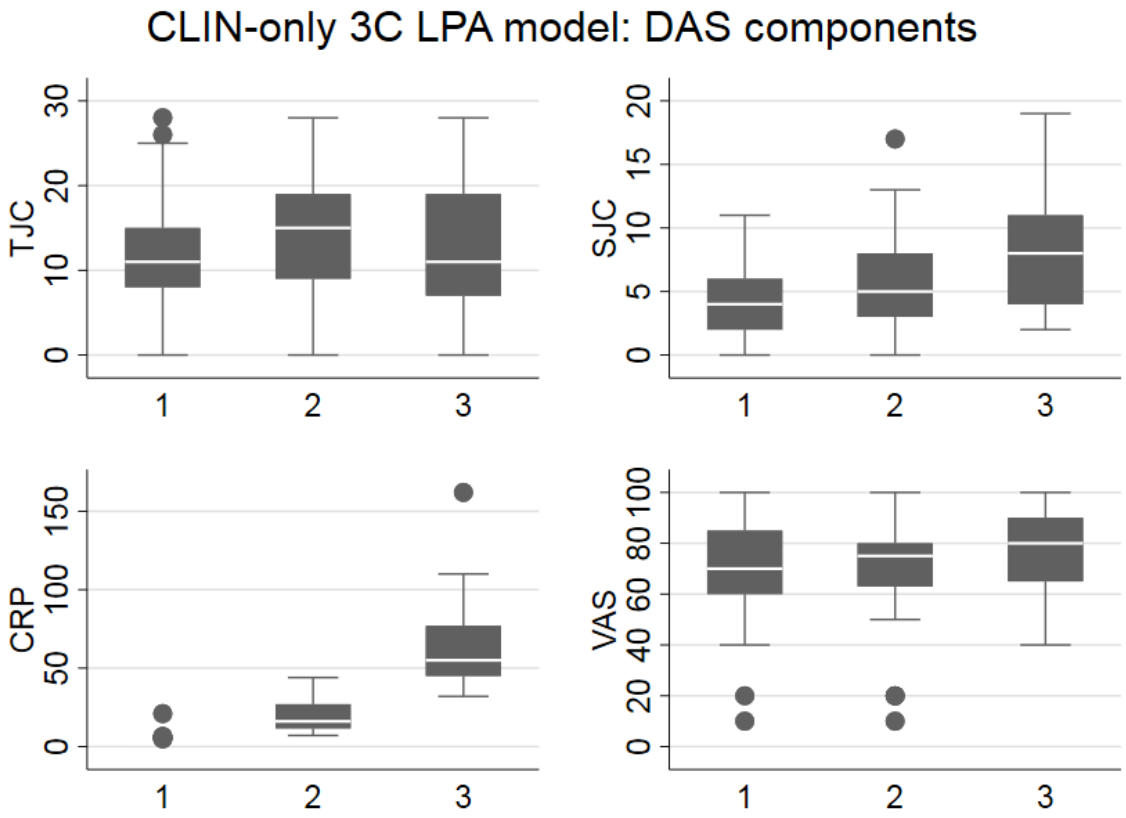

***Figure 3.7**: Box and whisker plots of DAS28 components for each cluster in 3-class clinical model at baseline.*

Figure 3.7 shows box and whisker plots of DAS28 components in each of the 3 clusters identified. It shows the biggest difference between the objective elements of the DAS28 components (SJC and CRP). In this model we are not seeing a clear difference between the subjective elements of the DAS28 (TJC and VAS). Tender joint count is highest in cluster 2, but not significantly so and there is no difference in VAS score between the groups. Therefore, I haven't clearly identified a cluster or clusters with high subjective DAS28 elements, but low levels of inflammation, suggesting that this group is not present within this cohort and if they exist, as is suggested in other studies, may have been screened out of this cohort.

3.4.2.1.1 Statistical differences between clusters

Using the Kruskal-Wallis equality-of-populations rank test for continuous variables or a Chi^2 test for categorical data I aimed to determine whether there are any statistically significant differences between the groups I have identified. Results of these analyses are also shown in **Table 3.6**.

Statistically significant differences exist between the clusters for Age, SJC, CRP, ESR and both DAS28-CRP and 2 component DAS 28 as well as FF and HAQ. A statistically significant difference in DAS-P is also shown, suggesting that for class 1, where it is highest, the subjective DAS 28 components make up a greater proportion of the total DAS28 score than the other clusters. The Kruskal-Wallis tells us that there is a statistically significant difference between the 3 clusters, but not which clusters are significantly different from one another or the direction of that difference. For the continuous variables analysed using K-W testing we could subsequently apply the Dunn test to determine which clusters were different from one another. No suitable test is available for comparison between the categorical variables, although no significant differences were identified between these groups. No differences were identified between disease duration, serology or smoking status between the different clusters. **Table 3.7** shows the p values obtained in the Dunn test analysis, with significant differences highlighted in bold.

Characteristic	Difference between latent profiles (p value)		
	1/2	1/3	2/3
Age	0.3833	**0.0004**	**0.0003**
SJC	**0.0022**	**0.0000**	**0.0036**
CRP	**0.0000**	**0.0000**	**0.0000**
ESR	**0.0000**	**0.0000**	**0.0000**
FF	0.0614	**0.0039**	**0.0164**
HAQ-DI	0.0614	**0.0003**	**0.0161**
DAS-P	**0.0000**	**0.0000**	**0.0007**
DAS28CRP-2C	**0.0000**	**0.0000**	**0.0001**

*T**able 3.7**: Dunn test results for clinical variables with statistically significant differences between clusters in clinical 3-class model. Significant results highlighted in bold.*

SJC – Swollen Joint Count, CRP- C-Reactive protein, ESR- Erythrocyte sedimentation rate, FF – FACIT Fatigue, HAQ-DI – Health Assessment Questionnaire – Disability Index, DAS- Disease activity score.

Using the Dunn test I was able to determine that there were statistically significant differences between all the clusters for SJC with cluster 2 having statistically more swollen joints than cluster 1 and cluster 3 more than clusters 1 and 2, with a similar pattern for markers of inflammation (CRP and ESR). Cluster 3 was significantly different from clusters 1 and 2 for age, with cluster 3 being older than the others. Cluster 3 had significantly more fatigue than clusters 1 and 2, measured by FF and had worse outcomes for effects on physical wellbeing measured by HAQ-DI score. There was no statistically significant difference between clusters 1 and 2 for these variables. There does not appear to be any discordance between PROs and inflammation in this analysis, as where significant differences exist, they are reflective of inflammation. DAS-P is different between all the clusters. With reference to **table 3.6** it appears to be highest in cluster 1 and lowest in 3, with the opposite pattern for DAS28CRP-2C. This reflects higher relative subjective DAS28 components in the lower inflammatory clusters and higher objective components in the more inflammatory groups.

I have extensive ultrasound data on our cohort and as a result I am also able to determine whether there are any differences between features identified on MUS between the clusters. **Figure 3.8** shows a box and whisker graph comparing the ultrasound variables in each cluster.

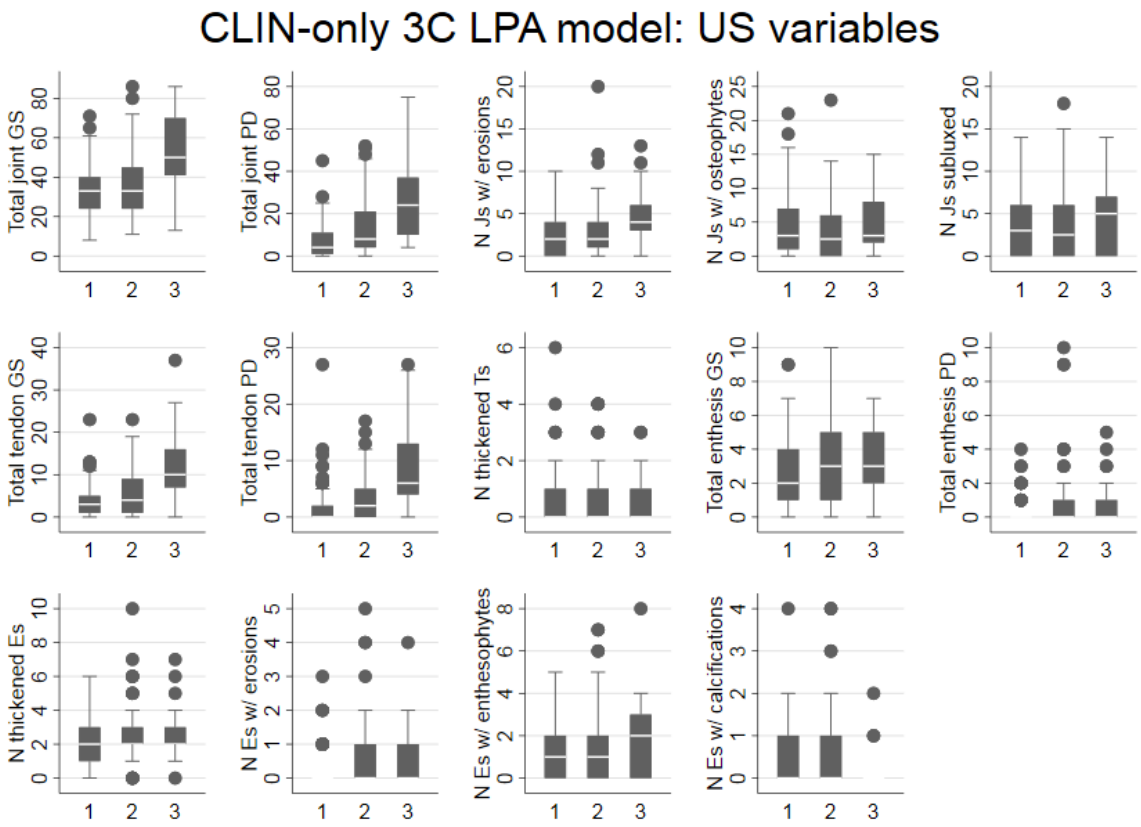

***Figure 3.8**: Box and whisker plots of baseline ultrasound variables for 3-class clinical model*

LPA – Latent profile analysis, US- Ultrasound, GS – Grey Scale, PD – Power Doppler, N – Number, N Js W/ - Number of joints with, N Es w/ - Number of Entheses with, Ts – Tendons.

Figure 3.8 shows box and whisker plots of the ultrasound variables for each cluster in the 3-class clinical model. The more inflammatory nature of cluster 3 is reflected in the ultrasound defined characteristics. Cluster 3 shows more grey scale and PD synovitis and tenosynovitis, and more baseline erosion identified on US. Statistically significant differences between the clusters were identified using K-W testing for joint grey scale, joint power Doppler, tendon grey scale, tendon Doppler and joint erosions. There were also differences in enthesitis scores between the clusters with statistically significant differences in Doppler signal, erosions and calcification (**table 3.8**)

US characteristic	X^2	κ	p
Total joint B mode	27.526	2	**0.0001**
Total joint Doppler	17.456	2	**0.0001**
Total joint erosions	19.520	2	**0.0001**
Total joint osteophytes	2.957	2	0.2279
Total joint subluxation	1.684	2	0.4309
Total tendon B mode	34.506	2	**0.0001**
Total tendon Doppler mode	40.263	2	**0.0001**
Total tendon thickening	0.635	2	0.7281
Total Enthesis hypogenicity	1.712	2	0.4248
Total enthesis Doppler	6.797	2	**0.0334**
Total enthesis thickening	2.173	2	0.3374
Total enthesis erosions	6.683	2	**0.00354**
Total enthesophytes	0.583	2	0.7473
Total enthesis calcification	10.815	2	**0.0045**

***Table 3.8**: Results of Kruskal-Wallis test to determine if differences in ultrasound variables exist at baseline between clusters in 3-class clinical model. Significant results highlighted in bold.*

Results from Dunn tests on the variables with statistically significant differences are shown in **table 3.9**. These revealed statistically significant differences between all 3 groups for tendon Doppler signal and enthesis Doppler with scores rising between each cluster sequentially. Cluster 3 has significantly higher scores than the other clusters for Joint B mode, joint Doppler and enthesis Doppler signal. Cluster 1 has significantly lower scores than the other clusters for enthesis erosion and all clusters differed from each other for enthesis calcifications with cluster 1 having the most, and cluster 2 more than cluster 3. These results highlight the fact that cluster 3 is the most inflammatory group in this analysis.

Ultrasound Characteristic	Difference between latent profile (p value)		
	1/2	1/3	2/3
Total joint B mode	0.1506	**0.0000**	**0.0000**
Total joint Doppler	0.0912	**0.0000**	**0.0002**
Total Tendon B mode	**0.0216**	**0.0000**	**0.0000**
Total Tendon Doppler	**0.0007**	**0.0000**	**0.0001**
Total Enthesis Doppler	0.1297	**0.0048**	**0.0486**
Total Enthesis Erosion	**0.0353**	**0.0098**	0.1799
Total Enthesis Calcification	**0.0483**	**0.0006**	**0.0302**

Table 3.9*: Dunn test results for ultrasound variables with statistically significant differences between clusters in clinical 3-class model. Significant results highlighted in bold.*

3.4.2.2 Clinical model: 4-class solution

The latent profile analysis using DAS28 components also identified an equally valid 4-class solution. Baseline characteristics of the clusters identified in this model are shown in **table 3.10.** In this model most patients fall within clusters 2 (97/200) and 3 (61/200). Class 1 is the smallest group (14/200).

	Clinical predicted class						
	1	2	3	4	Total		
	N=14	N=97	N=61	N=28	N=200	Chi^{2*}	p
Age, years	45.2 (34.9, 56.4), n=14	56.5 (50.7, 62.1), n=97	56.9 (49.1, 63.3), n=61	69.3 (58.9, 74.3), n=28	57.2 (49.8, 65.3), n=200	29.455	**0.0001**
Sex Female	10/14 (71.4%)	78/97 (80.4%)	43/61 (70.5%)	19/28 (67.9%)	150/200 (75.0%)	3.1816	0.364
Ever smoked Yes	3/12 (25.0%)	41/86 (47.7%)	27/53 (50.9%)	12/23 (52.2%)	83/174 (47.7%)	2.2543	0.521
Disease duration, units	6.0 (5.0, 14.0), n=11	10.0 (5.0, 16.0), n=82	8.5 (4.0, 16.0), n=56	13.0 (8.0, 19.0), n=24	10.0 (5.0, 17.0), n=173	4.281	0.2326
RF positive Yes	8/14 (57.1%)	51/97 (52.6%)	40/61 (65.6%)	20/28 (71.4%)	119/200 (59.5%)	4.7340	0.192
ACPA positive Yes	11/14 (78.6%)	65/97 (67.0%)	39/61 (63.9%)	20/28 (71.4%)	135/200 (67.5%)	2.2981	0.513
ANA positive Yes	3/14 (21.4%)	12/97 (12.4%)	3/61 (4.9%)	3/28 (10.7%)	21/200 (10.5%)	4.4494	0.217
X-ray erosions present Yes	8/14 (57.1%)	37/97 (38.1%)	27/61 (44.3%)	16/28 (57.1%)	88/200 (44.0%)	7.9365	0.243
TJC	4.5 (4.0, 8.0), n=14	11.0 (8.0, 15.0), n=97	16.0 (12.0, 21.0), n=61	12.0 (7.0, 18.5), n=28	12.0 (8.0, 17.0), n=200	36.627	**0.0001**
SJC	2.5 (1.0, 3.0), n=14	4.0 (2.0, 5.0), n=97	6.0 (4.0, 8.0), n=61	9.0 (7.5, 12.5), n=28	4.5 (2.0, 7.5), n=200	52.529	**0.0001**
CRP	34.5 (20.0,45.0), n=14	2.0 (2.0, 2.0), n=97	15.6 (11.3, 24.0), n=61	56.0 (40.5, 73.5), n=28	8.3 (2.0, 25.0), n=200	175.770	**0.0001**

	Clinical predicted class						
	1	**2**	**3**	**4**	**Total**		
	N=14	**N=97**	**N=61**	**N=28**	**N=200**	**Chi²***	**p**
VAS	60.0 (50.0, 70.0), n=14	70.0 (60.0, 80.0), n=97	75.0 (66.0, 80.0), n=61	80.0 (75.0, 90.0), n=28	75.0 (62.5, 80.5), n=200	17.201	**0.0006**
ESR	33.5 (26.0, 45.5), n=12	7.5 (3.0, 15.0), n=86	23.0 (16.0, 31.0), n=55	58.5 (38.0, 84.0), n=26	18.0 (7.0, 31.0), n=179	97.012	**0.0001**
HADS Anxiety	9.5 (7.0, 13.0), n=14	9.0 (6.0, 12.0), n=97	8.0 (5.0, 11.5), n=60	8.5 (4.0, 12.5), n=28	9.0 (5.0, 12.0), n=199	1.154	0.7642
HADS Depression	8.0 (7.0, 10.0), n=14	8.0 (4.0, 12.0), n=97	8.0 (5.5, 11.0), n=60	10.5 (6.0, 13.0), n=28	8.0 (5.0, 12.0), n=199	2.497	0.4758
FACIT-Fatigue	18.5 (10.0, 28.0), n=14	19.0 (12.0, 28.0), n=97	20.5 (8.5, 28.0), n=60	11.0 (5.0, 20.0), n=27	18.5 (10.0, 28.0), n=198	5.161	0.1603
HAQ	1.2 (0.9, 2.0), n=14	1.6 (1.1, 2.1), n=96	1.8 (1.4, 2.2), n=59	2.1 (1.8, 2.5), n=28	1.8 (1.2, 2.1), n=197	17.100	**0.0007**
DAS28CRP	4.6 (4.0, 5.4), n=14	4.8 (4.3, 5.3), n=97	5.9 (5.5, 6.3), n=61	6.2 (5.8, 7.0), n=28	5.3 (4.7, 5.9), n=200	107.571	**0.0001**
DAS28CRP-P	0.5 (0.4, 0.5), n=14	0.6 (0.6, 0.6), n=97	0.6 (0.5, 0.6), n=61	0.5 (0.5, 0.5), n=28	0.6 (0.5, 0.6), n=200	91.071	**0.0001**
DAS28CRP-2C	3.7 (2.9, 4.0), n=14	2.7 (2.1, 3.1), n=97	4.0 (3.6, 4.6), n=61	5.4 (4.9, 5.9), n=28	3.3 (2.4, 4.3), n=200	127.369	**0.0001**
N previous treatments	1.0 (0.0, 2.0), n=14	1.0 (0.0, 3.0), n=97	2.0 (0.0, 4.0), n=61	1.5 (0.0, 3.0), n=28	1.0 (0.0, 3.0), n=200	0.908	0.8235
N previous classes	1.0 (0.0, 1.0), n=14	1.0 (0.0, 2.0), n=96	1.0 (0.0, 3.0), n=61	1.0 (0.0, 2.5), n=28	1.0 (0.0, 2.0), n=199	0.970	0.8085
DAS28CRP>3.2 Yes	13/14 (92.9%)	96/97 (99.0%)	61/61 (100.0%)	28/28 (100.0%)	198/200 (99.0%)		

	Clinical predicted class						
	1	**2**	**3**	**4**	**Total**		
	N=14	**N=97**	**N=61**	**N=28**	**N=200**	**Chi²***	**p**
DAS28ESR>3.2 Yes	11/12 (91.7%)	78/86 (90.7%)	55/55 (100.0%)	26/26 (100.0%)	170/179 (95.0%)		
Clinical synovitis (PD>=2 | total PD>3) Yes	12/13 (92.3%)	62/94 (66.0%)	52/61 (85.2%)	28/28 (100.0%)	154/196 (78.6%)		
Biologic prescribed							
Baricitinib	1/14 (7.1%)	32/96 (33.3%)	23/61 (37.7%)	9/28 (32.1%)	65/199 (32.7%)		
Adalimumab	0/14 (0.0%)	19/96 (19.8%)	14/61 (23.0%)	7/28 (25.0%)	40/199 (20.1%)		
Rituximab	6/14 (42.9%)	9/96 (9.4%)	6/61 (9.8%)	3/28 (10.7%)	24/199 (12.1%)		
Abatacept	2/14 (14.3%)	14/96 (14.6%)	4/61 (6.6%)	1/28 (3.6%)	21/199 (10.6%)		
Tocilizumab	0/14 (0.0%)	8/96 (8.3%)	6/61 (9.8%)	5/28 (17.9%)	19/199 (9.5%)		
Etanercept	2/14 (14.3%)	6/96 (6.2%)	3/61 (4.9%)	2/28 (7.1%)	13/199 (6.5%)		
Other	3/14 (21.4%)	8/96 (8.3%)	5/61 (8.2%)	1/28 (3.6%)	17/199 (8.5%)		
Posterior probability >=0.9 Yes	10/14 (71.4%)	82/97 (84.5%)	53/60 (88.3%)	23/27 (85.2%)	168/198 (84.8%)		

***Table 3.10**:Baseline demographics, serology, clinical findings and patient reported outcome measures for each cluster identified in the 4-class clinical model.*

Targeted therapy prescribed for the study also listed. Other – Infliximab, Golimumab, Certolizumab, Sarilumab

ACPA – Anti-Citrullinated protein Antibody, RF- Rheumatoid Factor, ANA – Anti-Nuclear Antibody, TJC – Tender Joint Count, Swollen Joint Count, VAS – Visual analogue Scale, CRP- C Reactive Protein, ESR – Erythrocyte Sedimentation Rate, HAQ – Health Assessment Questionnaire, HADs – Hospital Anxiety and Depression Scale, FACIT – Functional Assessment of Chronic Illness Therapy, DAS – Disease Activity Score, RF- Rheumatoid Factor

* Kruscal-Wallis test performed to determine difference between the clusters for continuous variables, chi^2 for categorical variables. Significant results highlighted in bold.

Table 3.10 shows the clinical and PRO characteristics of the 4-class model identified for DAS28 component clusters. Interestingly, although TJC, SJC, VAS and most PROs progress between the clusters as in the 3-class version, cluster 1 has higher inflammatory markers than cluster 2 and 3, despite lower swollen joints and VAS. Again, the most inflammatory cluster has higher levels of fatigue and higher HAQ scores, but similar scores in the HADs Depression as the other clusters. HADs anxiety is numerically higher in the low inflammatory cluster. DAS28 components are shown graphically in **figure 3.9**.

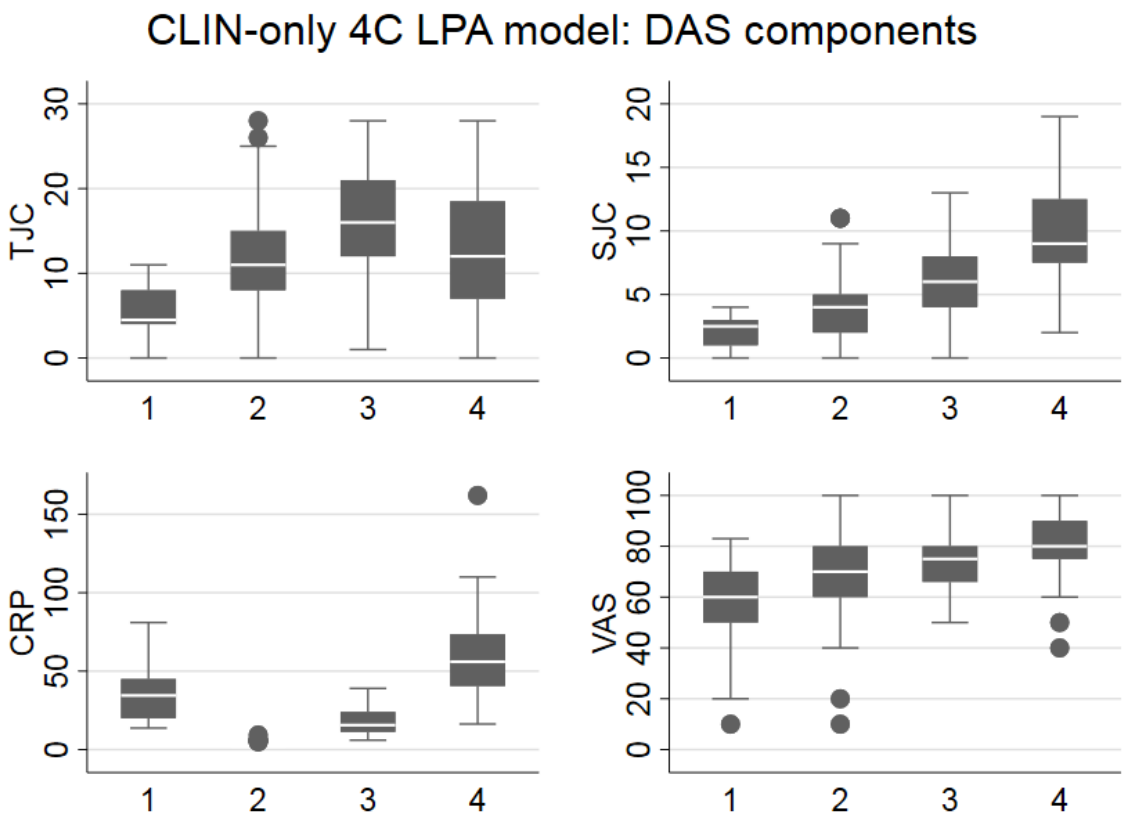

***Figure 3.9**: Box and whisker plots of DAS28 components for each cluster in 4-class clinical model at baseline.*

In the 4-class model, cluster 4 is the most inflammatory with the highest SJC count and CRP, but interestingly it has a lower TJC than cluster 3. Cluster 3 has a high tender joint count and low CRP, but also has a high swollen joint count and VAS, which does not fit with the high pain, low inflammation cluster we expected to see. Cluster 1 has a relatively high inflammatory response, but lower SJCs than the other clusters which may suggest inflammation at sites other than the joint within this group. **Table 3.10** shows the results of the K-W analysis comparing the relationship of these variables between clusters.

Statistically significant difference between the 4 different clusters were identified in all clinical variables (TJC, SJC, VAS, CRP and ESR) as well as HAQ scores. 4 component and 2 component DAS28 and DAS-P were also statistically significantly different. No statistical significance was shown for HADs anxiety between the groups.

Further analysis of these variables using the Dunn test (**Table 3.11**) showed where the differences were for significant results identified using K-W testing. For TJC significant differences existed between all the groups apart from clusters 2 and 4 with cluster 3 having a higher TJC than the others. There was no significant difference in SJC between clusters 1 and 2, but there were differences between all the other clusters, with counts rising through the groups. For CRP and ESR there were no difference between clusters 1 and 4 which showed the most inflammation of the 4 clusters, but there were significant differences between all other clusters. VAS increases from cluster to cluster and showed significant differences between all clusters except 2 and 3. Likewise clusters 2 and 3 did not have significantly differences for age, but differences were shown between all other clusters. For HAQ there was no difference between clusters 1 and 2, but there was for all the other clusters. Again, HAQ rises through the clusters being lowest in cluster 1 and highest in 4.

Characteristic	Difference between latent profiles (p value)					
	1/2	1/3	1/4	2/3	2/4	3/4
Age	**0.0060**	**0.0082**	**0.0000**	0.4765	**0.0000**	**0.0000**
TJC	**0.0003**	**0.0000**	**0.0002**	**0.0000**	0.1772	**0.0175**
SJC	**0.0643**	**0.0002**	**0.0000**	**0.0000**	**0.0000**	**0.0015**
CRP	**0.0000**	**0.0450**	0.1180	**0.0000**	**0.0000**	**0.0000**
VAS	**0.0080**	**0.0029**	**0.0000**	0.2162	**0.0019**	**0.0152**
ESR	**0.0000**	**0.0500**	0.1275	**0.0000**	**0.0000**	**0.0000**
HAQ-DI	0.2251	**0.0495**	**0.0010**	**0.0452**	**0.0001**	**0.0098**
DAS-P	**0.0000**	**0.0000**	0.1434	**0.0000**	**0.0000**	**0.0000**
DAS28CRP-2C	**0.0014**	**0.0567**	**0.0001**	**0.0000**	**0.0000**	**0.0002**

***Table 3.11**: Dunn test results for clinical variables with statistically significant differences between clusters in clinical 4-class model. Significant results highlighted in bold.*

TJC – Tender joint count, SJC- swollen joint count, CRP – C-reactive protein, VAS – Visual analogue score, ESR – Erythrocyte Sedimentation Rate, HAQ-DI – Health assessment questionnaire, DAS – Disease activity score

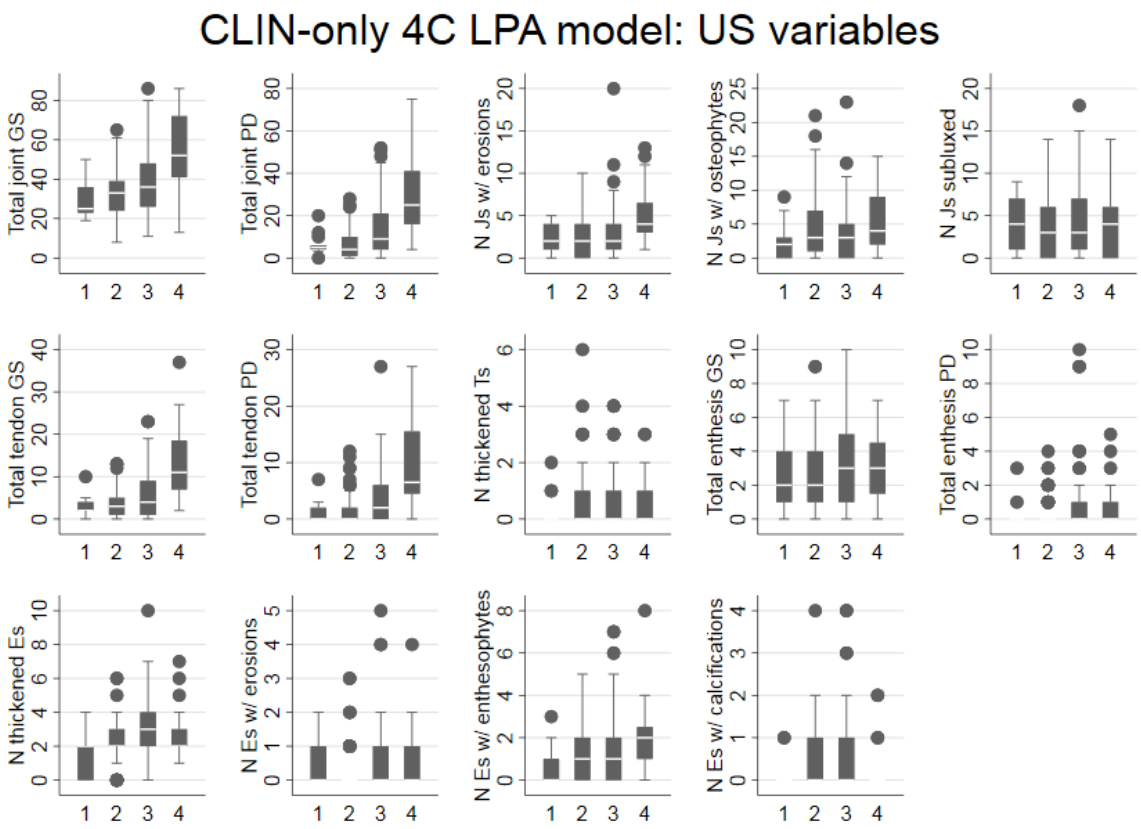

***Figure 3.10**: Box and whisker plots of baseline ultrasound variables for 4-class clinical model.*

LPA-Latent profile analysis, US- ultrasound, GS – Grey Scale, PD – Power Doppler, N – Number, N Js W/ - Number of joints with, N Es w/ - Number of Entheses with, Ts – Tendons.

Ultrasound data shows that inflammation is generally higher as you progress up the clusters and although cluster 1 had higher inflammatory markers than either clusters 2 or 3, it does not appear to have more disease activity on ultrasound than the other groups, with US data more closely mirroring SJC. Differences between the clusters for ultrasound variables identified using K-W analysis are shown in **Table 3.12**. Significant differences were observed between the clusters for joint B mode, Doppler and erosions, tendon B mode and Doppler and enthesis thickening.

US characteristic	X^2	κ	p
Total joint B mode	32.403	3	**0.0001**
Total joint Doppler	53.156	3	**0.0001**
Total joint erosions	24.966	3	**0.0001**
Total joint osteophytes	6.337	3	0.0963
Total joint subluxation	0.024	3	0.9990
Total tendon B mode	46.680	3	**0.0001**
Total tendon Doppler mode	54.564	3	**0.0001**
Total tendon thickening	2.017	3	0.5688
Total Enthesis hypogenicity	4.370	3	0.2242
Total enthesis Doppler	5.782	3	0.1227
Total enthesis thickening	8.8000	3	**0.0321**
Total enthesis erosions	6.133	3	0.1053
Total entheisophytes	6.535	3	0.0883
Total enthesis calcification	6.659	3	0.0836

***Table 3.12**: Results of Kruskal-Wallis test to determine if differences in ultrasound variables exist at baseline between clusters in 4-class clinical model. Significant differences highlighted in bold.*

Dunn tests (**Table 3.13**) showed that there was no significant difference between clusters 1 and 2 for joint B mode and Doppler, whereas significant differences existed between all of the other clusters with scores rising as you move through the clusters. Similar results were obtained for tendon inflammation. Significant differences were shown between the clusters

apart from cluster 1 and 2 and 1 and 3. Cluster 4 had significantly more joint erosions than the other clusters which is in keeping with it being a more inflammatory group with longer disease duration.

Characteristic	Difference between latent profile (p value)					
	1/2	1/3	1/4	2/3	2/4	3/4
Total joint B mode	0.1635	**0.0248**	**0.0000**	**0.0297**	**0.0000**	**0.0000**
Total joint Doppler	0.2263	**0.0603**	**0.0001**	**0.0000**	**0.0000**	**0.0003**
Total joint erosions	0.3907	0.4739	**0.0019**	0.2672	**0.0000**	**0.0000**
Total tendon B mode	0.4074	**0.0663**	**0.0000**	**0.0088**	**0.0000**	**0.0000**
Total tendon Doppler mode	0.3609	0.0606	**0.0000**	**0.0002**	**0.0000**	**0.0000**
Total enthesis thickening	0.0596	**0.0040**	**0.0428**	**0.0170**	0.2951	0.1542

Table 3.13*: Dunn test results for ultrasound variables with statistically significant differences between clusters in clinical 4-class model. Significant results highlighted in bold.*

3.4.3 Latent profile analysis of established RA cohort using ultrasound features.

Having established that clusters of patients exist within the cohort determined by their DAS28 component scores, I sought to determine whether similar clusters could be established using baseline ultrasound features as grouping variables and using the same methodology as with the clinical phenotypes described above.

Figure 3.11 shows the fit statistics for the US based phenotyping of our cohort. Once again, the AIC does not minimise, but the BIC favours the 3-class solution.

a

Number of classes	N	df	AIC	BIC
2	196	41	11309.38	11443.78
3	196	56	11153.25	11336.83
4	196	71	11129.63	11362.38
5	196	86	11099.83	11381.75

b

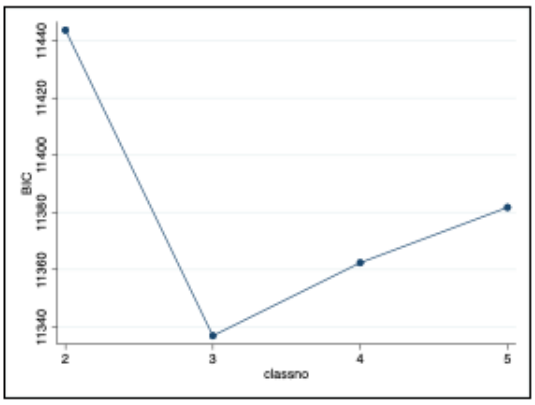

Figure 3.11*: Fit statistics for Ultrasound characteristic latent profile model. a. table of results for AIC/BIC. b. Graph of BIC results for each number of classes.*

Df – degrees of freedom, AIC – Akaike Information Criterion, BIC – Bayesian Information Criterion.

	Ultrasound predicted class					
	1	2	3	Total		
	N=76	N=84	N=40	N=200	Chi^{2*}	p
Age, years	54.0 (47.7, 61.1), n=76	57.6 (49.8, 65.4), n=84	60.2 (54.2, 70.8), n=40	57.2 (49.8, 65.3), n=200	9.367	**0.0092**
Sex Female	66/76 (86.8%)	63/84 (75.0%)	21/40 (52.5%)	150/200 (75.0%)	15.3494	**0.000**
Ever smoked Yes	26/63 (41.3%)	37/76 (48.7%)	20/35 (57.1%)	83/174 (47.7%)	4.4945	0.106
Disease duration, units	10.0 (5.0, 16.0), n=64	8.0 (3.5, 16.5), n=72	13.0 (6.0, 20.0), n=37	10.0 (5.0, 17.0), n=173	4.524	0.1042
RF positive Yes	35/76 (46.1%)	51/84 (60.7%)	33/40 (82.5%)	119/200 (59.5%)	16.3561	**0.000**
ACPA positive Yes	45/76 (59.2%)	58/84 (69.0%)	32/40 (80.0%)	135/200 (67.5%)	6.6129	**0.037**
ANA positive Yes	5/76 (6.6%)	12/84 (14.3%)	4/40 (10.0%)	21/200 (10.5%)	2.7548	0.252
X-ray erosions present Yes	30/76 (39.5%)	38/84 (45.2%)	20/40 (50.0%)	88/200 (44.0%)	4.8997	0.298
TJC	12.0 (6.0, 16.0), n=76	11.0 (8.5, 16.0), n=84	14.0 (9.0, 20.0), n=40	12.0 (8.0, 17.0), n=200	5.240	0.0728
SJC	3.0 (1.0, 5.0), n=76	5.0 (3.0, 7.5), n=84	8.0 (5.5, 11.5), n=40	4.5 (2.0, 7.5), n=200	58.045	**0.0001**
CRP	2.0 (2.0, 12.1), n=76	6.4 (2.0, 19.6), n=84	31.5 (14.5, 48.0), n=40	8.3 (2.0, 25.0), n=200	42.503	**0.0001**
VAS	70.0 (60.0, 80.0), n=76	78.0 (65.0, 85.0), n=84	74.0 (62.5, 80.0), n=40	75.0 (62.5, 80.5), n=200	2.577	0.2757
ESR	11.0 (4.0, 25.0), n=68	19.5 (8.0, 31.0), n=74	30.0 (15.0, 57.0), n=37	18.0 (7.0, 31.0), n=179	26.468	**0.0001**
HADS Anxiety	9.0 (6.0, 13.5), n=76	9.0 (5.0, 11.0), n=84	7.0 (4.0, 13.0), n=39	9.0 (5.0, 12.0), n=199	1.425	0.4905
HADS Depression	8.0 (5.0, 12.0), n=76	9.0 (5.0, 11.0), n=84	9.0 (4.0, 12.0), n=39	8.0 (5.0, 12.0), n=199	0.396	0.8204
FACIT-Fatigue	18.0 (10.0, 26.0), n=76	20.5 (11.5, 28.5), n=84	15.5 (9.0, 30.0), n=38	18.5 (10.0, 28.0), n=198	1.999	0.3681
HAQ	1.8 (1.2, 2.1), n=76	1.7 (1.1, 2.1), n=82	1.9 (1.5, 2.5), n=39	1.8 (1.2, 2.1), n=197	3.974	0.1371
DAS28CRP	5.1 (4.3, 5.5), n=76	5.3 (4.8, 5.9), n=84	6.0 (5.3, 6.6), n=40	5.3 (4.7, 5.9), n=200	28.170	**0.0001**
DAS28CRP-P	0.6 (0.5, 0.6), n=76	0.6 (0.5, 0.6), n=84	0.5 (0.5, 0.6), n=40	0.6 (0.5, 0.6), n=200	23.649	**0.0001**
DAS28CRP-2C	2.7 (2.1, 3.5), n=76	3.4 (2.9, 4.1), n=84	4.8 (3.9, 5.5), n=40	3.3 (2.4, 4.3), n=200	59.320	**0.0001**

	Ultrasound predicted class					
	1	**2**	**3**	**Total**		
	N=76	**N=84**	**N=40**	**N=200**	**Chi²***	**p**
N previous treatments	2.0 (1.0, 3.0), n=76	1.0 (0.0, 2.5), n=84	1.0 (0.0, 3.5), n=40	1.0 (0.0, 3.0), n=200	4.605	0.1000
N previous classes	2.0 (1.0, 2.0), n=75	1.0 (0.0, 2.0), n=84	1.0 (0.0, 3.0), n=40	1.0 (0.0, 2.0), n=199	3.661	0.1603
DAS28CRP>3.2 Yes	76/76 (100.0%)	83/84 (98.8%)	39/40 (97.5%)	198/200 (99.0%)		
DAS28ESR>3.2 Yes	62/68 (91.2%)	72/74 (97.3%)	36/37 (97.3%)	170/179 (95.0%)		
Clinical synovitis (PD>=2 | total PD>3) Yes	37/76 (48.7%)	81/84 (96.4%)	36/36 (100.0%)	154/196 (78.6%)		
Biologic prescribed						
Baricitinib	28/76 (36.8%)	24/83 (28.9%)	13/40 (32.5%)	65/199 (32.7%)		
Adalimumab	9/76 (11.8%)	24/83 (28.9%)	7/40 (17.5%)	40/199 (20.1%)		
Rituximab	9/76 (11.8%)	11/83 (13.3%)	4/40 (10.0%)	24/199 (12.1%)		
Abatacept	11/76 (14.5%)	8/83 (9.6%)	2/40 (5.0%)	21/199 (10.6%)		
Tocilizumab	5/76 (6.6%)	9/83 (10.8%)	5/40 (12.5%)	19/199 (9.5%)		
Etanercept	4/76 (5.3%)	4/83 (4.8%)	5/40 (12.5%)	13/199 (6.5%)		
Other	10/76 (13.2%)	3/83 (3.6%)	4/40 (10.0%)	17/199 (8.5%)		
Posterior probability >=0.9 Yes	63/76 (82.9%)	72/84 (85.7%)	33/38 (86.8%)	168/198 (84.8%)		

***Table 3.14**: Baseline demographics, serology, clinical findings and patient reported outcome measures for each cluster identified in the ultrasound 3-class model.*

Targeted therapy prescribed for the study also listed. Other – Infliximab, Golimumab, Certolizumab, Sarilumab.

ACPA – Anti-Citrullinated Cyclo-protein Antibody, RF- Rheumatoid Factor, ANA – Anti-Nuclear Antibody, TJC – Tender Joint Count, Swollen Joint Count, VAS – Visual analogue Scale, CRP- C Reactive Protein, ESR – Erythrocyte Sedimentation Rate, HAQ – Health Assessment Questionnaire, HADs – Hospital Anxiety and Depression Scale, FACIT – Functional Assessment of Chronic Illness Therapy, DAS – Disease Activity Score, RF-Rheumatoid Factor, USpred class – ultrasound predicted class.

* Kruscal-Wallis test performed to identify differences between the clusters for continuous variables, chi^2 for categorical variables. Significant differences highlighted in bold.

Table 3.14 shows clinical and PRO characteristics for our ultrasound defined clusters. Similar to the 3-class solution determined using only DAS28 components, inflammation in the form of SJC and inflammatory markers appear to progress through the groups whereas there doesn't appear to be any real difference between the TJCs and VAS. The proportion of patients with seropositive disease also rises through the clusters. Again, there are more males in the most inflammatory cluster. In this analysis HADs anxiety is higher in the low inflammatory cluster 1 and intermediate cluster 2, than in the most inflammatory group. The other PRO outcomes are consistent with relative levels of inflammation within the groups. Interestingly, the more inflammatory cluster has the highest age. Traditionally age is associated with low inflammatory state, "burned out RA", but it is increasingly recognised that high inflammatory disease is more common than thought in elderly patients and the burden of co-morbidity makes this condition more difficult to treat in older patients.

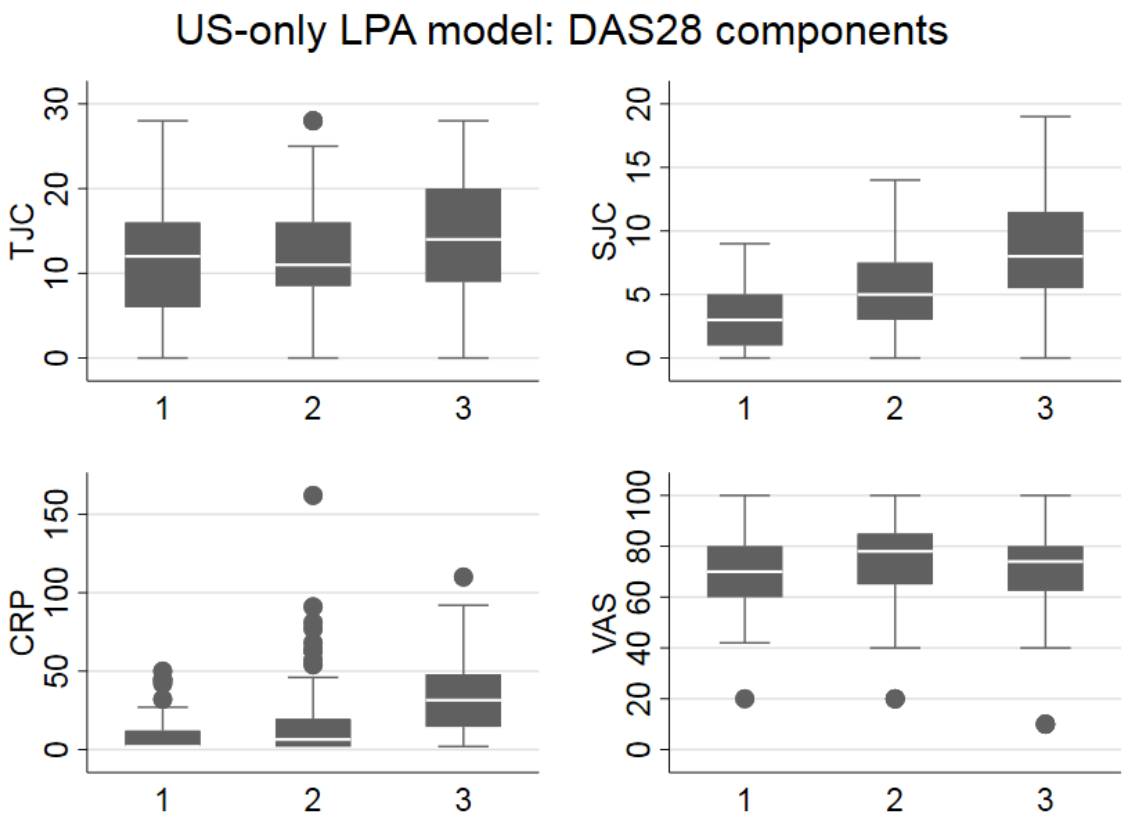

***Figure 3.12**: Box and whisker plots of DAS28 components for each cluster in the ultrasound model at baseline.*

Figure 3.12 shows box and whisker plots of the DAS28-CRP component scores for the ultrasound defined clusters. Again, the SJC and CRP rise as the clusters progress, but there is no clear difference between the VAS scores. TJC is slightly higher in cluster 3 than the other clusters which is in keeping with the higher SJC in this group.

Kruskal-Wallis or Chi^2 testing of the individual clinical components (**table 3.14**) shows statistically significant differences between age, sex, seropositivity (both RF and ACPA), SJC and inflammatory markers (CRP and ESR) and resulting statistically significant differences between DAS28 scores, including DAS-P and DAS28CRP-2. The results of the Dunn tests for these variables (**table 3.15**) shows statistically significant differences between all the clusters for SJC, CRP and ESR, which increase as you go up through the clusters. Age also rises as you progress up the clusters.

Characteristic	**Difference between latent profile (p value)**		
	1/2	1/3	2/3
Age	**0.0408**	**0.0013**	**0.0471**
SJC	**0.0000**	**0.0000**	**0.0000**
CRP	**0.0024**	**0.0000**	**0.0000**
ESR	**0.0098**	**0.0000**	**0.0004**
DAS-P	**0.0176**	**0.0000**	**0.0007**
DAS28CRP-2C	**0.0000**	**0.0000**	**0.0000**

Table 3.15*: Dunn test results for clinical variables with statistically significant differences between clusters in ultrasound model. Significant results highlighted in bold.*

SJC – Swollen Joint Count, CRP – C-Reactive Protein, Erythrocyte sedimentation rate, DAS – Disease activity score.

As categorical variables, neither sex nor seropositivity are not suitable for K-W analysis and have been analysed using Chi^2 for which there is no post hoc test to determine the where the differences lie between the clusters. Visual analysis of **table 3.14** shows clearly that both seropositivity and male sex are more common in cluster 2 than cluster 1 and cluster 3 than cluster 2.

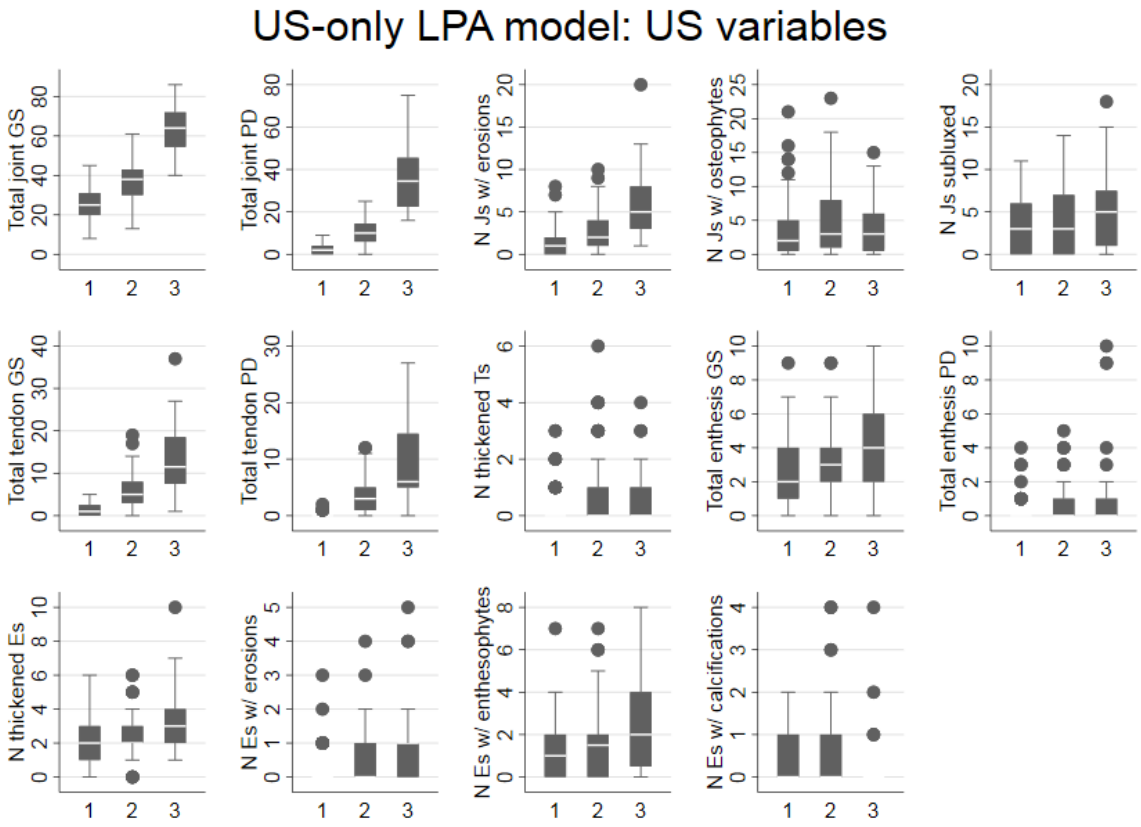

Figure 3.13: Box and whisker plots of baseline ultrasound variables for ultrasound model.

US- ultrasound, LPA – Latent profile analysis, GS – Grey Scale, PD – Power Doppler, N – Number, N Js W/ - Number of joints with, N Es w/ - Number of Entheses with, Ts – Tendons.

Figure 3.13 shows similar results for the ultrasound features of the clusters. Cluster 3 is the most inflammatory with GS and PD synovitis and tenosynovitis most prominent in this cluster. There also looks to be more erosive change and enthesophytes in the cluster than the others. **Table 3.16** shows the results of statistical comparisons between the groups with significant differences between multiple ultrasound characteristics including Joint B mode, Doppler mode, erosions, tendon B and Doppler modes and enthesis Doppler, hypo-echogenicity, thickening and erosions.

US characteristic	X^2	κ	p
Total joint B mode	113.116	2	**0.0001**
Total joint Doppler	140.645	2	**0.0001**
Total joint erosions	47.162	2	**0.0001**
Total joint osteophytes	3.696	2	0.1575
Total joint subluxation	2.968	2	0.2267
Total tendon B mode	119.385	2	**0.0001**
Total tendon Doppler mode	112.360	2	**0.0001**
Total tendon thickening	10.385	2	**0.0056**
Total Enthesis hypogenicity	9.526	2	**0.0077**
Total enthesis Doppler	7.069	2	**0.0292**
Total enthesis thickening	12.114	2	**0.0023**
Total enthesis erosions	18.425	2	**0.0001**
Total entheisophytes	3.442	2	0.1789
Total enthesis calcification	8.003	2	0.0679

Table 3.16*: Results of Kruskal-Wallis test to determine if differences in ultrasound variables exist at baseline between clusters in ultrasound model.*

Dunn testing of these variables (**table 3.17)** shows that statistically significant differences between all the clusters for joint B mode, Doppler mode, joint erosions, tendon B mode and tendon Doppler mode, all of which are higher in sequential clusters. Statistically significant differences also exist between all the clusters for enthesis hypoechogenicity, enthesis

thickening and enthesis erosions. Significant differences between clusters 1 and 3 for enthesis Doppler have also been shown, with cluster 3 having significantly more than 1. Differences in tendon thickening at the enthesis was identified between clusters 1 and 2 and 1 and 3, but not 2 and 3 and differences were observed between clusters 1 and 3 and 2 and 3 for enthesis calcification, but not clusters 1 and 2. In this case cluster 3 had less calcification than the other groups. These results highlight that cluster 3 is more inflammatory than the other groups with inflammation rising through the clusters in joints, tendons and entheseal sites. Although entheseal inflammation is uncommon in all the groups, entheseal calcification is more common in the less inflammatory clusters.

US characteristic	**Difference between latent profiles (p value)**		
	1/2	1/3	2/3
Total joint B mode	**0.0000**	**0.0000**	**0.0000**
Total joint Doppler	**0.0000**	**0.0000**	**0.0000**
Total joint erosions	**0.0002**	**0.0000**	**0.0000**
Total tendon Doppler mode	**0.0000**	**0.0000**	**0.0000**
Total tendon thickening	**0.0008**	**0.0313**	0.2646
Total Enthesis hypogenicity	**0.0495**	**0.0010**	**0.0337**
Total enthesis Doppler	0.0657	**0.0045**	0.0724
Total enthesis thickening	**0.0313**	**0.0003**	**0.0217**
Total enthesis erosions	**0.0052**	**0.0000**	**0.0132**

Table 3.17*: Dunn test results for ultrasound variables with statistically significant differences between clusters in ultrasound model. Significant results highlighted in bold.*

3.4.4 Comparing clusters defined by clinical features and ultrasound

It is not possible to perform a valid statistical test comparing 2 different cluster analyses on the same cohort of patients, but it is possible to look at which patients were assigned to which cluster based on their posterior probabilities. These results are shown in **table 3.18** and **3.19** below.

Ultrasound predicted class	**Clinical predicted class (3 class solution)**			**Total**
	1	2	3	
1	49	25	2	76
2	42	31	11	84
3	7	15	18	40
total	98	71	31	200

Table 3.18*: Comparing in the 3-class clinical latent profile model to the ultrasound model.*

Ultrasound predicted class	**Clinical predicted class (4 class solution)**				**Total**
	1	2	3	4	
1	7	49	20	0	76
2	6	42	26	10	84
3	1	6	15	18	40
Total	14	97	61	28	200

Table 3.19*: Comparing in the 4-class clinical latent profile model to the ultrasound model*

For the 3-class solutions there is considerable overlap between the clinical and ultrasound derived clusters with most patients in the ultrasound derived cluster 1 also in clinical cluster 1 and the greatest proportion of ultrasound cluster 3 also in clinical cluster 3. There is a less clear association between the ultrasound and clinical clusters in the 4-class model. Most patients from clusters 1 and 2 in the US model fall within clusters 2 and 3 of the 4-class clinical model, with small numbers falling into the smaller class 1 which has potentially been separated

from the other low inflammatory clusters due to the relatively high levels of CRP in this small group.

3.5 Chapter 3 summary and conclusions

In this chapter I have used latent profile analysis (mixed models) to identify different clusters of patients from the baseline characteristics identified in our prospective cohort of targeted therapy treated established RA patients. I have been able to identify equally valid 3 and 4-cluster models based on DAS28 component scores and a 3-cluster model when using ultrasound characteristics as grouping variables. This confirms the hypothesis that homogeneous clusters of patients can be identified with in a heterogeneous established RA cohort using these variables and that different DAS28 and US disease phenotypes exist within this cohort. In all the models, particularly the 3-class solutions, the clusters are most clearly differentiated by the levels of joint and tendon inflammation on ultrasound and corresponding higher SJC and inflammatory markers which progress through the clusters. This is reflected in differences between the DAS28CRP-2C scores in each group, which is shown to reflect MUS findings more closely than the conventional DAS28 score (112).

In all 3 models, the most inflammatory cluster is more male, older, has a longer disease duration and has a higher proportion of seropositive patients, although not all these differences reach statistical significance. Seropositivity is known to be associated with more active disease (56), but in many analyses female sex is associated with worse prognosis disease (21), whereas in this analysis there are more males in the most inflammatory cluster.

Ultrasound results tend to corroborate clinical findings within all these analyses, with the groups with higher SJC and inflammatory markers having highest levels of joint GS and PD synovitis, tenosynovitis, and entheseal inflammation. The exception to this is found in cluster

1 in the 4-class DAS-28 component models, which has higher levels of CRP despite low levels of MUS identified inflammation, potentially suggesting sources inflammation distant from the joint. Interestingly in all models, enthesis calcification is more common in the lower inflammatory cluster 1. These groups are more seronegative than the other clusters and it is possible that this group contains more patients on the SpA spectrum of disease who meet RA diagnositic criteria.

Using either DAS components or Ultrasound variables to perform LPA does not clearly identify a cohort of patients with discordantly high PROs or high subjective DAS 28 components. When significant differences exist between the clusters, it tends to correlate with inflammation with no clear evidence of discordance. However, in all the analyses DAS-P is different between the groups, suggesting proportionally higher contributions of the subjective DAS elements to the overall DAS28 score within clusters 1 and 2. No significant differences between these individual subjective DAS component variables are identified. Likewise, in all cases PRO data was either the same between the clusters or rose in parallel with inflammation, apart from anxiety in the ultrasound derived 3-class model, which was higher in the low inflammation cluster 1, than in the intermediate cluster 2. However, this result did not meet statistical significance. I have not identified a cluster of patients which have more damage and less active inflammation on their ultrasound in either analysis. This would represent a cluster of patients with more osteoarthritis than active disease, which does not appear to be present within this cohort based on these LPAs.

4 Phenotyping of the advanced Rheumatoid arthritis cohort: Combined clinical and ultrasound latent profile analysis

4.1 Introduction

In chapter 3, I confirmed that clusters of patients could be identified within our targeted therapy treated cohort by using latent profile analysis. Using DAS28 component scores I identified equally valid 3 and 4-class models and using ultrasound variables as indicators I identified a 3-class model. In all these analyses the clearest differences between the clusters were in the relative amount of inflammation at baseline. In this chapter I aimed to combine both ultrasound and clinical variables into a single model to give a more comprehensive phenotyping of our cohort and to see whether this combination of variables allows me to identify more or different types of clusters to those outlined in the previous chapter. If these more comprehensively phenotypes clusters exist, I aimed to identify whether it was possible to estimate group membership using a smaller number of variables to provide a more manageable set of investigations that could more readily be used in clinical practice. These more comprehensive phenotypic clusters will be used for further work assessing treatment response and disease trajectory which will be covered in subsequent chapters of this thesis.

4.2 Chapter aims

- To perform a latent profile analysis on our cohort using DAS28 component scores and ultrasound scores as grouping variables in one analysis to establish if more deeply phenotyped clusters of patients can be identified.
- To compare clinical, PRO and ultrasound variables between the established clusters.
- To use regression tree analysis to identify whether a limited number of variables can be used to estimate which latent class a patient would fall into.

4.3 Chapter 4 methods

The analysis in this chapter uses the same methodology as chapter 3 using the baseline characteristics of the prospectively recruited established RA cohort to perform LPA to identify different phenotypic clusters. I have performed a latent profile analysis similar to those in chapter 3, using the same methodology but on this occasion including both DAS28 component scores and baseline ultrasound characteristics as latent class indicators in a single model, once again with age, sex and HADs anxiety added to the model as covariates. I aimed to identify clusters of patients, this time defined by both clinical and ultrasound variables, and then compare the clinical and PRO outcomes between the groups identified.

Clusters have been compared using K-W with a Dunn extension for significantly different continuous variables or Chi^2 for categorical variables. A regression tree analysis has also been performed to identify a small number of variables that can be used to predict latent class in this model.

4.4 Chapter 4 Results

4.4.1 Latent profile analysis of established RA cohort using DAS28 components and ultrasound variables

The latent profile model was run using DAS28 component scores and ultrasound scores as grouping variables using the same methodology as in chapter 3. Fit statistics for the models are shown in **figure 4.1**. Once again, the AIC does not minimise, but the BIC minimises at 3 and thus favours a 3-class solution. Demographic and clinical variables are shown in **table 4.1**.

a

Number of classes	N	df	AIC	BIC
2	200	53	16845.05	17019.86
3	200	72	16677.08	16914.56
4	200	91	16639.25	16939.4
5	200	110	16616.33	16979.15

b

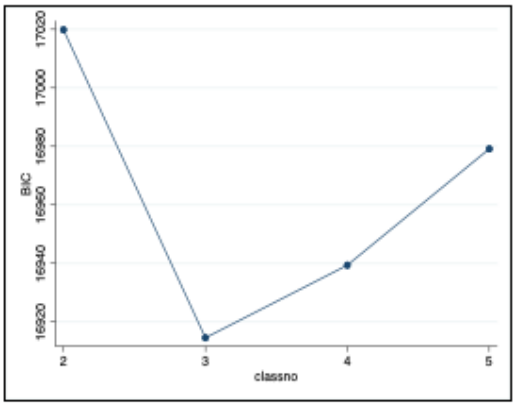

Figure 4.1*: Fit statistics for combined clinical and ultrasound variable latent profile model. a. table of results for AIC/BIC. b. Graph of BIC results for each number of classes.*

Df – degrees of freedom, AIC – Akaike Information Criterion, BIC – Bayesian Information Criterion.

	Combined clinical/ultrasound latent profile					
	1	**2**	**3**	**Total**		
	N=76	**N=82**	**N=42**	**N=200**	**Chi²***	**p**
Age, years	54.7 (48.8, 61.4), n=76	57.1 (47.9, 63.1), n=82	62.2 (54.5, 72.2), n=42	57.2 (49.8, 65.3), n=200	8.971	**0.0113**
Sex Female	64/76 (84.2%)	61/82 (74.4%)	25/42 (59.5%)	150/200 (75.0%)	8.8199	**0.012**
Ever smoked Yes	25/63 (39.7%)	34/74 (45.9%)	24/37 (64.9%)	83/174 (47.7%)	6.0843	**0.048**
Disease duration, years	10.0 (5.0, 15.5), n=64	8.0 (4.0, 16.0), n=69	13.0 (6.5, 20.0), n=40	10.0 (5.0, 17.0), n=173	6.799	**0.0334**
RF positive Yes	33/76 (43.4%)	51/82 (62.2%)	35/42 (83.3%)	119/200 (59.5%)	18.3012	**0.000**
ACPA positive Yes	42/76 (55.3%)	58/82 (70.7%)	35/42 (83.3%)	135/200 (67.5%)	10.3776	**0.006**
ANA positive Yes	6/76 (7.9%)	11/82 (13.4%)	4/42 (9.5%)	21/200 (10.5%)	1.3328	0.514
X-ray erosions present Yes	26/76 (34.2%)	40/82 (48.8%)	22/42 (52.4%)	88/200 (44.0%)	5.6476	0.227
TJC	12.0 (6.5, 16.5), n=76	11.0 (8.0, 16.0), n=82	15.0 (12.0, 21.0), n=42	12.0 (8.0, 17.0), n=200	8.963	**0.0113**
SJC	3.0 (1.0, 5.0), n=76	5.0 (3.0, 7.0), n=82	9.0 (6.0, 12.0), n=42	4.5 (2.0, 7.5), n=200	69.785	**0.0001**

	Combined clinical/ultrasound latent profile					
	1	2	3	Total		
	N=76	N=82	N=42	N=200	Chi^{2*}	p
CRP	2.0 (2.0, 9.2), n=76	6.7 (2.0, 20.0), n=82	33.0 (16.3, 55.0), n=42	8.3 (2.0, 25.0), n=200	58.270	**0.0001**
VAS	70.0 (60.0, 80.0), n=76	75.0 (65.0, 85.0), n=82	79.5 (65.0, 80.0), n=42	75.0 (62.5, 80.5), n=200	4.878	0.0872
ESR	10.0 (3.0, 21.0), n=69	19.5 (8.0, 30.0), n=72	35.0 (23.0, 66.0), n=38	18.0 (7.0, 31.0), n=179	39.838	**0.0001**
HADS Anxiety	9.0 (6.0, 14.0), n=76	9.0 (5.0, 11.0), n=82	7.0 (4.0, 13.0), n=41	9.0 (5.0, 12.0), n=199	1.460	0.4818
HADS Depression	8.0 (5.0, 12.0), n=76	8.0 (5.0, 11.0), n=82	10.0 (5.0, 12.0), n=41	8.0 (5.0, 12.0), n=199	1.204	0.5477
FACIT-Fatigue	17.5 (10.0, 25.5), n=76	21.5 (12.0, 29.0), n=82	15.5 (8.5, 25.5), n=40	18.5 (10.0, 28.0), n=198	4.043	0.1325
HAQ	1.8 (1.2, 2.1), n=76	1.6 (1.1, 2.1), n=80	2.0 (1.8, 2.5), n=41	1.8 (1.2, 2.1), n=197	7.954	**0.0187**
DAS28CRP	4.9 (4.2, 5.5), n=76	5.3 (4.8, 5.7), n=82	6.2 (5.8, 6.7), n=42	5.3 (4.7, 5.9), n=200	50.854	**0.0001**
DAS28CRP-P	0.6 (0.5, 0.6), n=76	0.6 (0.5, 0.6), n=82	0.5 (0.5, 0.6), n=42	0.6 (0.5, 0.6), n=200	31.008	**0.0001**
DAS28CRP-2C	2.4 (2.1, 3.2), n=76	3.5 (2.9, 4.1), n=82	5.1 (4.4, 5.7), n=42	3.3 (2.4, 4.3), n=200	91.192	**0.0001**
N previous treatments	2.0 (1.0, 3.0), n=76	1.0 (0.0, 2.0), n=82	2.0 (0.0, 4.0), n=42	1.0 (0.0, 3.0), n=200	5.350	0.0689
N prev classes	2.0 (1.0, 2.0), n=75	1.0 (0.0, 2.0), n=82	1.5 (0.0, 3.0), n=42	1.0 (0.0, 2.0), n=199	5.088	0.0785
DAS28CRP>3.2 Yes	75/76 (98.7%)	81/82 (98.8%)	42/42 (100.0%)	198/200 (99.0%)		
DAS28ESR>3.2 Yes	61/69 (88.4%)	71/72 (98.6%)	38/38 (100.0%)	170/179 (95.0%)		
Clinical synovitis (PD>=2 | totalPD>3) Yes	34/72 (47.2%)	78/82 (95.1%)	42/42 (100.0%)	154/196 (78.6%)		
Biologic prescribed						
Baricitinib	29/76 (38.2%)	19/81 (23.5%)	17/42 (40.5%)	65/199 (32.7%)		
Adalimumab	8/76 (10.5%)	24/81 (29.6%)	8/42 (19.0%)	40/199 (20.1%)		
Rituximab	7/76 (9.2%)	13/81 (16.0%)	4/42 (9.5%)	24/199 (12.1%)		

	Combined clinical/ultrasound latent profile					
	1	**2**	**3**	**Total**		
	N=76	**N=82**	**N=42**	**N=200**	**Chi²***	**p**
Abatacept	11/76 (14.5%)	9/81 (11.1%)	1/42 (2.4%)	21/199 (10.6%)		
Tocilizumab	5/76 (6.6%)	8/81 (9.9%)	6/42 (14.3%)	19/199 (9.5%)		
Etanercept	6/76 (7.9%)	4/81 (4.9%)	3/42 (7.1%)	13/199 (6.5%)		
Other	10/76 (13.2%)	4/81 (4.9%)	3/42 (7.1%)	17/199 (8.5%)		
Posterior probability >=0.9 Yes	63/76 (82.9%)	71/82 (86.6%)	34/40 (85.0%)	168/198 (84.8%)		

***Table 4.1**: Baseline demographics, serology, clinical findings and patient reported outcome measures for each cluster identified in the combined clinical and ultrasound 3-class model.*

Targeted therapy prescribed for the study also listed. Other – Infliximab, Golimumab, Certolizumab, Sarilumab.

ACPA – Anti-Citrullinated Cyclo-protein Antibody, RF- Rheumatoid Factor, ANA – Anti-Nuclear Antibody, TJC – Tender Joint Count, Swollen Joint Count, VAS – Visual analogue Scale, CRP- C Reactive Protein, ESR – Erythrocyte Sedimentation Rate, HAQ – Health Assessment Questionnaire, HADs – Hospital Anxiety and Depression Scale, FACIT – Functional Assessment of Chronic Illness Therapy, DAS – Disease Activity Score, RF-Rheumatoid Factor.

* Kruscal-Wallis test performed to identify differences between clusters for continuous variables, chi^2 for categorical variables. Significant differences highlighted in bold

Table 4.1 shows the demographic, clinical and PRO outcomes for the latent profile analysis for the 3 classes identified in the combined model. It also shows numbers of previous therapies and next TTs prescribed. Visual investigation shows similar trends to both the DAS28 component model (**Table 3.6**, Page 131) and US characteristic model (**Table 3.14**, page 152) identified in chapter 3. Once again, we see progression in the objective components of the DAS28 score as you rise through the clusters with subsequent higher baseline DAS28 scores. Cluster 1 is notable for being more female and more seronegative than the other clusters. PRO data suggests that physical limitation, defined by HAQ-DI score rises in parallel with inflammation as you ascend the clusters, but interestingly anxiety scores are numerically higher in clusters 1 and 2 than in cluster 3 and fatigue levels are worse in cluster 1 than the

more inflammatory cluster 2. **Table 4.1** also shows the results of the Kruskal-Wallis and chi squared tests for the above variables.

Significant differences have been shown between the clusters in several clinical characteristics including DAS28 components and age, but there are also differences in sex, disease duration, smoking status and seropositivity between the clusters. **Table 4.2** shows the p values for Dunn tests performed on the significantly different continuous variables shown in **table 4.1**. For the categorical variables no suitable post hoc test is available, but by revieing the data in **table 4.1** we can see that cluster 1 is the most female cluster and cluster 3 has markedly more smokers than the other clusters.

Characteristic	**Difference between latent profile (p value)**		
	1/2	1/3	2/3
Age	0.2240	**0.0016**	**0.0095**
Disease duration	0.1915	**0.0353**	**0.0047**
TJC	0.2569	**0.0092**	**0.0017**
SJC	**0.0000**	**0.0000**	**0.0000**
CRP	**0.0000**	**0.0000**	**0.0000**
ESR	**0.0025**	**0.0000**	**0.0000**
HAQ	0.3894	**0.0077**	**0.0037**
DAS-P	**0.0014**	**0.0000**	**0.0010**
DAS28CRP-2C	**0.0000**	**0.0000**	**0.0000**

Table 4.2*: Dunn test results for clinical variables with statistically significant differences between clusters in the combined clinical and ultrasound model. Significant results highlighted in bold.*

TJC – Tender joint count, SJC – Swollen Joint Count, CRP – C-Reactive Protein, ESR – Erythrocyte sedimentation rate, HAQ – Health Assessment Questionnaire, DAS – Disease Activity Score.

Dunn test results show where the significant differences lie between the clusters. Cluster 3 is significantly older than the other clusters and has a significantly longer disease duration. There is no significant difference between clusters 1 and 2 in these variables. With regards to DAS28 component scores, there is no significant difference between clusters 1 and 2 in the TJC, but cluster 3 had significantly more tender joints than either of clusters 1 or 2. All the clusters were significantly different from one another for SJC, CRP and ESR. Visualisation of the data shows that these scores increase in each successive cluster (**figure 4.2**). For VAS there was no significant difference between clusters 2 and 3, but both clusters have significantly higher VAS scores than cluster 1.

The only PRO to show any significant difference between the clusters was the HAQ score with cluster 3 having a significantly higher HAQ than either of clusters 1 or 2. The numerically higher scores for fatigue and anxiety PROs did not reach statistical significance. DAS28CRP-2C reflects the higher inflammatory activity ascending through the clusters and once again DAS-P is significantly higher in the less inflammatory clusters.

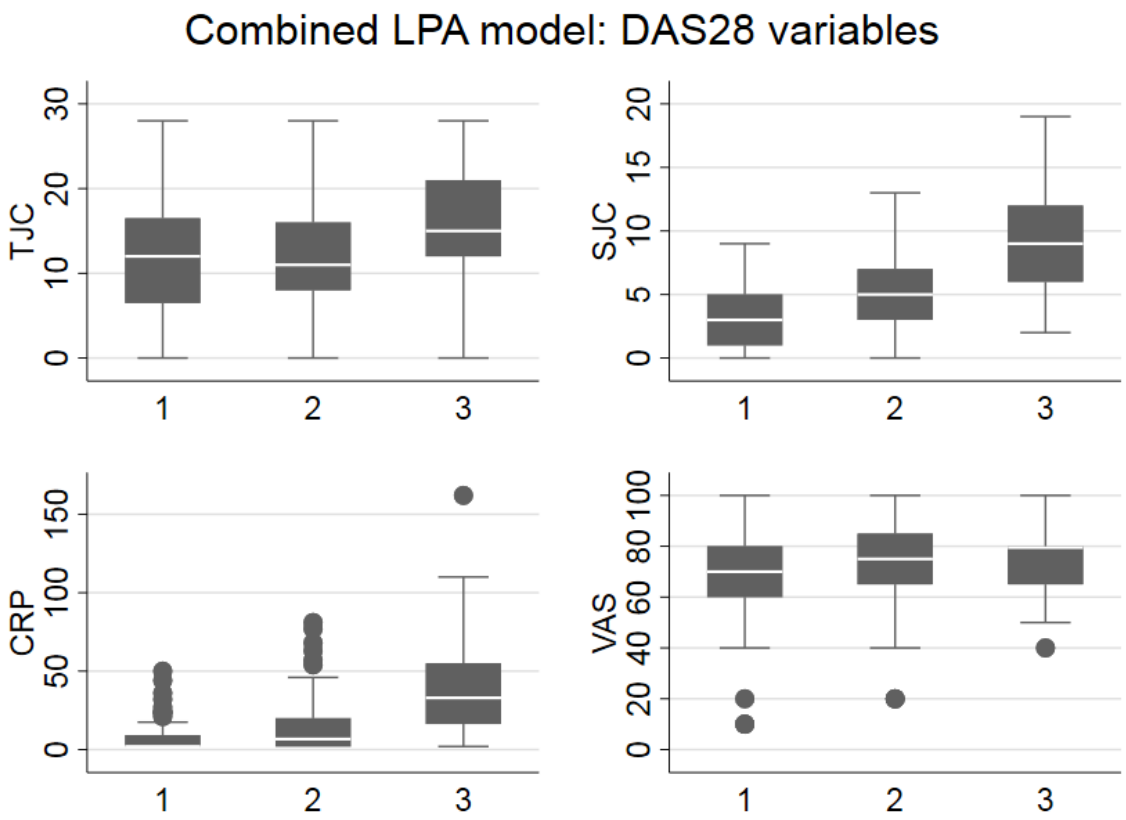

***Figure 4.2**: Box and whisker plots of DAS28 components for each cluster in the combined clinical and ultrasound model at baseline.*

Figure 4.2 shows box and whisker plots for DAS28-CRP components of the 3 clusters identified in this combined clinical and ultrasound latent profile analysis. Like the previous analyses, the combined clinical and ultrasound clusters show a progression in CRP and SJC, with less clear differences between the subjective elements of the DAS28 (TJC and VAS).

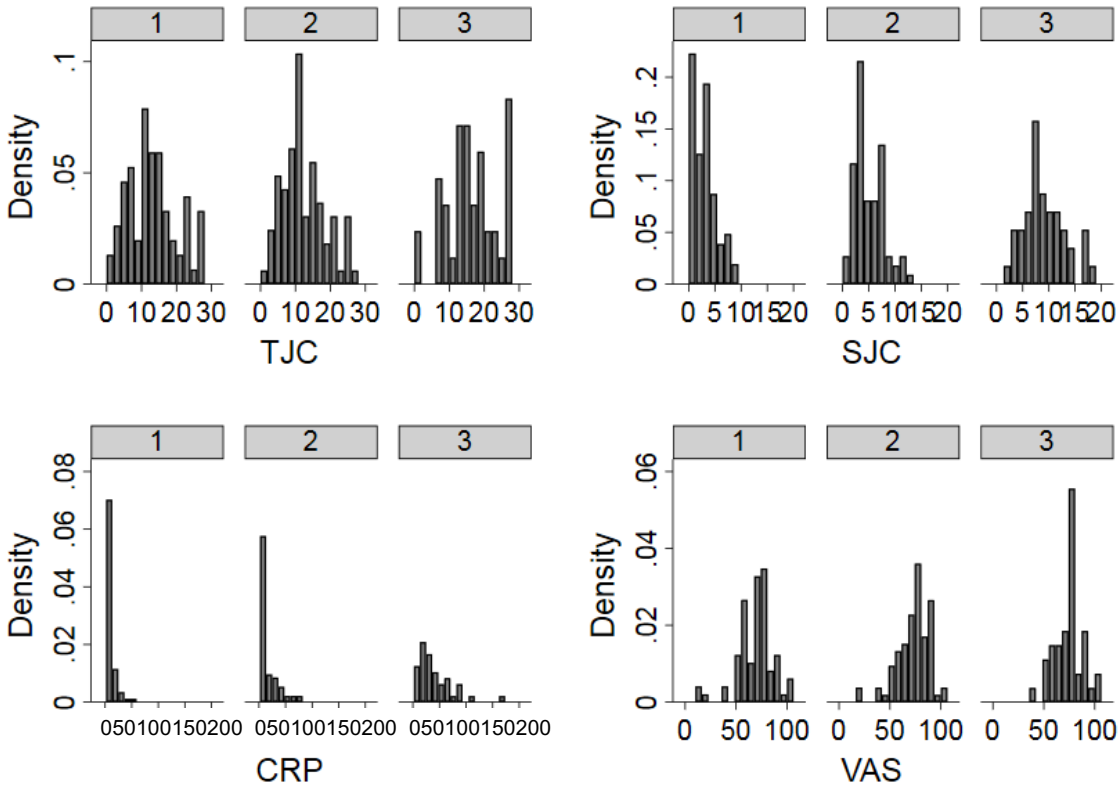

***Figure 4.3**: Histograms of DAS28 components by latent profile in the combined clinical and ultrasound model*

Figure 4.3 shows histograms of the distributions of the DAS28-CRP components within the different clusters. Although there is no discernible difference in the distributions of TJC and VAS in our clusters it is notable that in cluster 1 there is a greater proportion of patients with no or very low levels of SJC and CRP, whereas in cluster 3 there are more patients with high levels of these variables. Cluster 2 shows an intermediate distribution, between that of clusters 1 and 3.

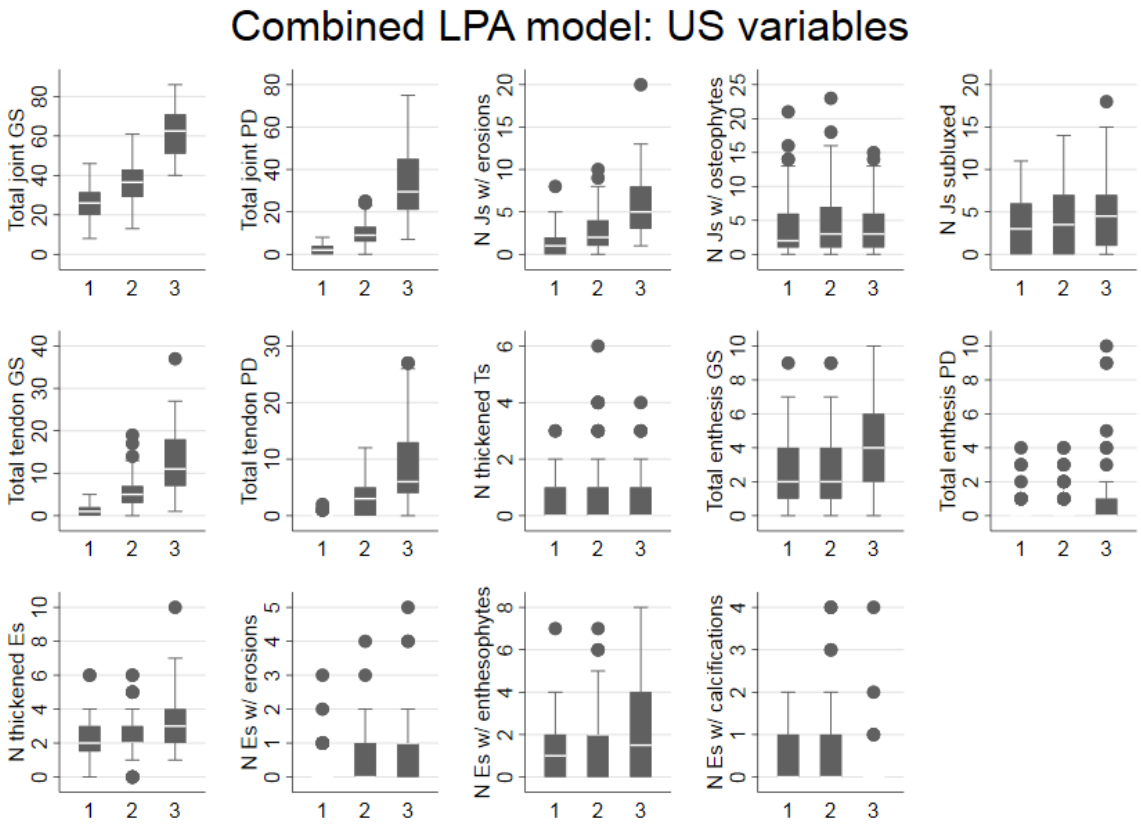

***Figure 4.4**: Box and whisker plots of baseline ultrasound variables for the combined clinical and ultrasound model.*

GS – Grey Scale, PD – Power Doppler, N – Number, N Js W/ - Number of joints with, N Es w/ - Number of Entheses with, Ts – Tendons.

Figure 4.4 shows box and whister plots of the ultrasound variables of each of our clusters. Once again the clusters show a progression of inflammation defined by joint and tendon B mode and power Doppler. Statistical analysis of the differences between the clusters using the K-W test is shown in **table 4.1**. It shows significant differences for joint grey scale, Doppler and erosions. There was no statistical difference in the presence of erosion at baseline on xray, highlighting the advantage of MUS over xray in identifying erosive changes. Tendon grey scale and Doppler were also significantly different as were a number of changes at the entheses (enthesis thickeneing, tendon thickeneing, enthesis Doppler, hyopechogenicity, erosions and calcification).

US characteristic	X^2	κ	p
Total joint B mode	108.125	2	**0.0001**
Total joint Doppler	143.370	2	**0.0001**
Total joint erosions	60.323	2	**0.0001**
Total joint osteophytes	0.623	2	0.7325
Total joint subluxation	2.352	2	0.3085
Total tendon B mode	120.783	2	**0.0001**
Total tendon Doppler mode	106.638	2	**0.0001**
Total tendon thickening	6.047	2	**0.0486**
Total Enthesis hypogenicity	10.444	2	**0.0054**
Total enthesis Doppler	9.419	2	**0.0090**
Total enthesis thickening	9.632	2	**0.0081**
Total enthesis erosions	18.250	2	**0.0001**
Total entheisophytes	1.443	2	0.4860
Total enthesis calcification	12.879	2	**0.0016**

Table 4.3*: Results of Kruskal-Wallis test to determine if differences in ultrasound variables exist at baseline between clusters in combined clinical and ultrasound model.*

Table 4.4 shows the p values obtained for the post hoc Dunn test analysis of the significantly different continous variables.

Characteristic	Differences between latent profiles (p value)		
	1/2	1/3	2/3
Total joint B mode	**0.0000**	**0.0000**	**0.0000**
Total joint Doppler	**0.0000**	**0.0000**	**0.0000**
Total joint erosions	**0.0001**	**0.0000**	**0.0000**
Total tendon B mode	**0.0000**	**0.0000**	**0.0000**
Total tendon Doppler mode	**0.0000**	**0.0000**	**0.0000**
Total tendon thickening	**0.0199**	**0.0168**	0.3364
Total Enthesis hypogenicity	0.2336	**0.0008**	**0.0047**
Total enthesis Doppler	0.1205	**0.0011**	**0.0164**
Total enthesis thickening	0.1378	**0.0010**	**0.0128**
Total enthesis erosions	**0.0237**	**0.0000**	**0.0037**
Total enthesis calcification	0.0879	**0.0002**	**0.0060**

***Table 4.4**: Dunn test results for ultrasound variables with statistically significant differences between clusters in the combined clinical and ultrasound model. Significant results highlighted in bold.*

Dunn tests showed that there were significant differences between all of the clusters for joint B mode, Doppler and the presence of joint erosion, with scores rising through the clusters. Similar results are also seen for tendon B mode and Doppler.

For enthesitis scores, significant differences were identified between clusters 1 and 2 and 1 and 3 for tendon thickening, but no significant difference between 2 and 3. For Enthesis Doppler mode, hypoechogenicity, thickenening and calcification cluster 3 differed significantly from the other clusters, but clusters 1 and 2 showed no significant difference

between these variables. Interestingly, for enthesis calcification, cluster 3 has significantly less than the others, but the the other variables cluster 3 has higher scores. There were significant differences between all of the clusters for enthesis erosion, which again appears to increase up the clusters.

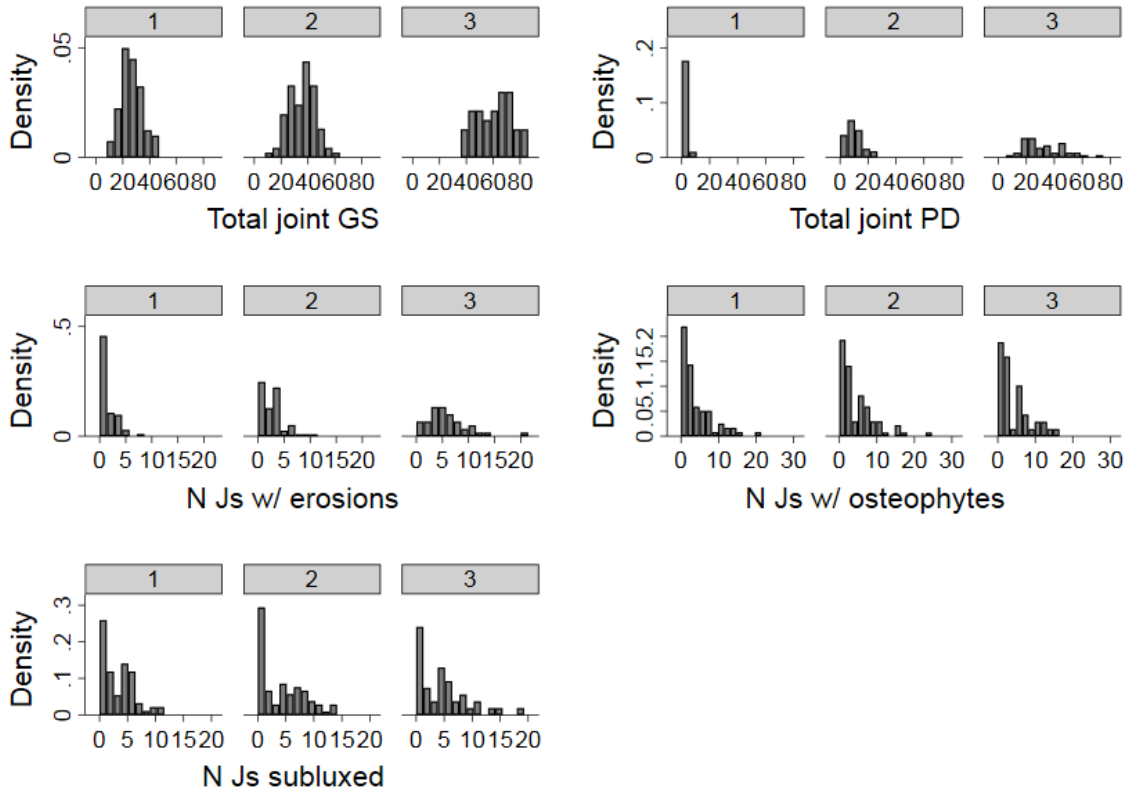

***Figure 4.5**: Histograms of US joint variables by latent profile in the combined clinical and ultrasound model.*

GS – Grey scale, PD – Power Doppler, N Js w/ - Number of joints with. N Js – Number of joints.

The above figure shows the distributions of ultrasound variables reflecting joint synovitis and damage. The higher scores as we ascend the clusters, for joint B mode and doppler, representing synovial inflammation are clear with cluster 1 having lower grey scale and comparatively little doppler activity. As we know that active synovitis, demonstrated by Doppler activity on ultrasound is associated with progressive erosion, we would expect that this inflammation will be associated with erosive change and this is reflected in our distributions of joint erosion, with cluster 3 having more patients with erosive changes in more joints. The distributions of osteophytes and joint subluxation, which may be expected to also be indicators of joint damage, do not show any obvious difference, even though cluster 3 has been shown to have longer disease duration than the other clusters and could reasonably have been expected to have accumulated more joint damage. There are no formal guidelines for the

assessment of subluxation on MUS and this metric was based on sonographer opinion. A formal, validated measure of subluxation would be useful to differentiate these differences more clearly.

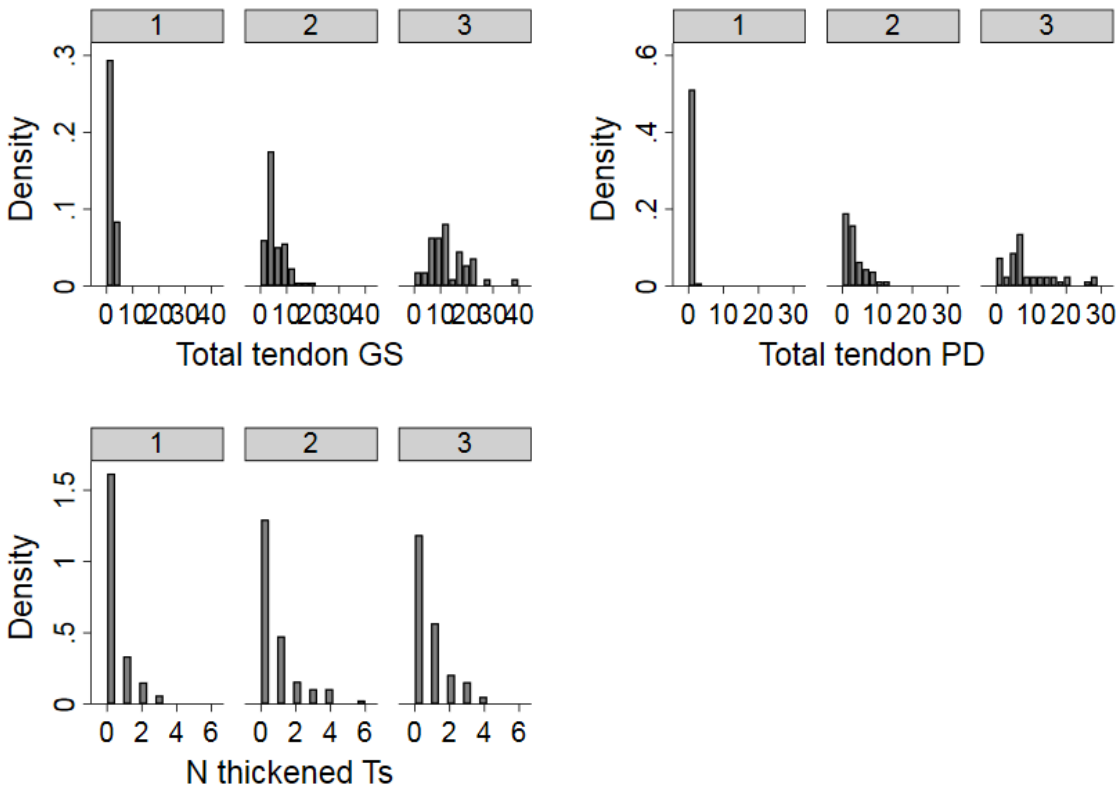

***Figure 4.6**: Histograms of US tendon variables by latent profile in the combined clinical and ultrasound model.*

GS – Grey scale, PD – Power Doppler. N – Number, Ts – Tendons.

Figure 4.6 shows the distributions and densities of tendon variables identified by ultrasound. It is most notable for showing that in cluster 1 there is almost no active tendon inflammation. Tendon inflammation progresses as we ascend the clusters in the same way that it does with joint inflammation.

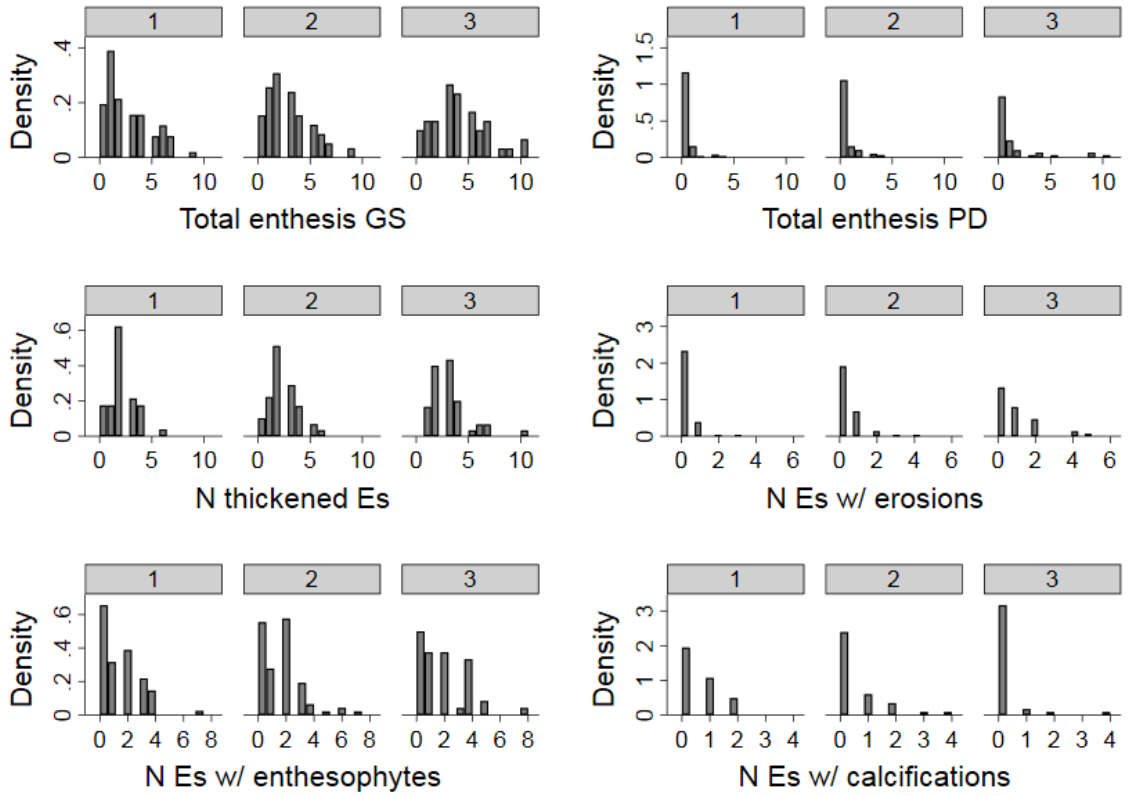

***Figure 4.7**: Histograms of US enthesis variables by latent profile in the combined clinical and ultrasound model.*

GS – Grey Scale, PD – Power Doppler, N – Number, Es – Entheses, N Es w/ - Number of entheses with.

Figure 4.7 shows the distributions of the entheseal variables identified on ultrasound. The densities of entheseal changes are low, but again there are more patients with evidence of inflammation at the enthesis in the more inflammatory cluster 3 than the other clusters. There are more entheses with calcifications in cluster 1 and 2, but the numbers are low in all of the clusters.

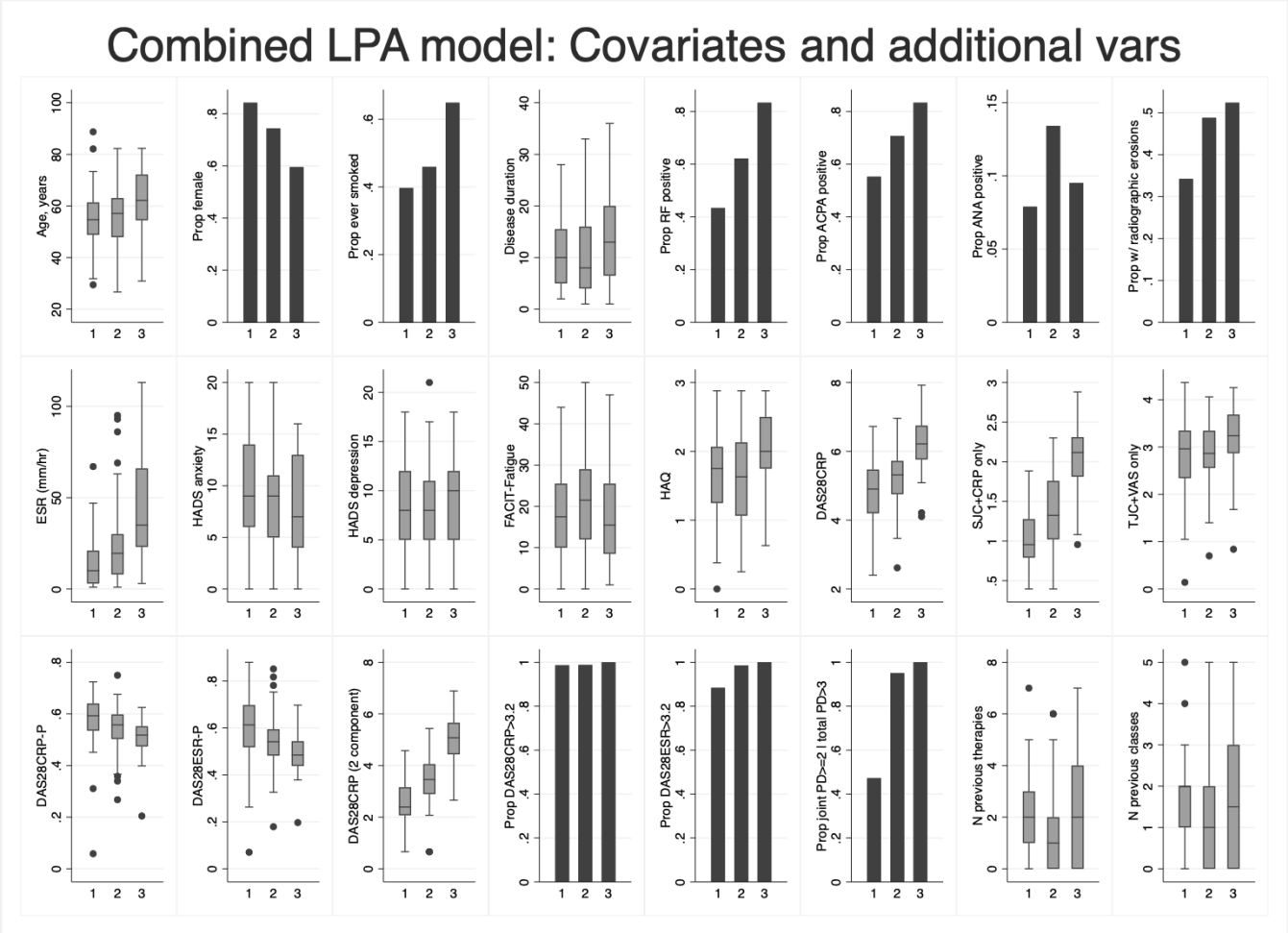

Figure 4.8*: Graphs of covariates and additional variables associated with the clusters identified in the combined clinical and ultrasound model.*

LPA – Latent profile analysis, Vars – Variables, ACPA – Anti-Citrullinated protein Antibody, RF- Rheumatoid Factor, ANA – Anti-Nuclear Antibody, TJC – Tender Joint Count, Swollen Joint Count, VAS – Visual analogue Scale, CRP- C Reactive Protein, ESR – Erythrocyte Sedimentation Rate, HAQ – Health Assessment Questionnaire, HADs – Hospital Anxiety and Depression Scale, FACIT – Functional Assessment of Chronic Illness Therapy, DAS – Disease Activity Score, RF- Rheumatoid Factor.

Figure 4.8 shows a mixture of box and whisker plots and bar charts for the covariates and additional variables associated with each of the clusters identified, graphically representing information in **table 4.1.** This again shows that age, proportion of male patients, proportion of smokers and seropositivity progresses from cluster 1 to cluster 3. These graphs also show higher exposure of cluster 3 to previous TTs and classes of TT, but these results have not met statistical significance.

4.4.2 Simple rules to approximate latent profiles

I next looked at whether it was possible to use a limited number of variables to approximate which latent class a patient would fall into using regression tree analysis. The R recursive partitioning and regression tree package rpart was used to identify a limited set of variables that could approximate the latent classes. If latent class membership is shown to be significantly related to treatment response, then identifying a limited set of variables that can identify latent class will be useful in the clinical setting. The final tree identified Total Joint PD (split <6, >=6 to <19, >=19), Total tendon GS (split <4, >=4) and CRP (split <10 mg/L, >=10mg/L) as variables that could classify patients into the latent classes. **Table 4.5** shows the results of the analysis.

		Total Joint PD				
		<6	>=6 & <19	>=19		
Total Tendon GS	<4	LC1	LC2	LC2	<10	CRP (mg/L)
	<4	LC1	LC2	LC3	>=10	
	>=4	LC2	LC2	LC2	<10	
	>=4	LC2	LC2	LC3	>=10	

***Table 4.5**: Results of regression tree analysis of limited variables predicting latent class (LC) for the combined clinical and ultrasound model.*

GS – Grey scale, PD – Power Doppler, LC – Latent Class, CRP – C-Reactive Protein.

4.5 Chapter 4 summary and conclusions

Using latent profile analysis and including both clinical and US variables in the analysis I have identified 3 clusters of patients within the established RA cohort. As with the previous LPAs this confirms the hypothesis that broader phenotypic clusters can be identified within a group of established RA patients. Once again, in this analysis the clusters vary mainly by the amount of joint and tendon inflammation visible on ultrasound and the corresponding objective components of the DAS28 score. I have not clearly identified any clusters with discordant

inflammation and PRO results or clusters that are determined by the amount of joint damage. However, we do once again see higher DAS-P scores for clusters 1 and 2 than in cluster 3, suggesting the subjective elements of the DAS28 score make up a higher proportion of the DAS28 in these clusters. Anxiety, measured by the HADs, was also numerically higher in these clusters but did not meet statistical significance, but again hints at a degree of discordance in the lower inflammatory disease clusters, but I cannot clearly state that clusters with discordant inflammation and PRO results exist within this analysis.

A number of similarities with the previous LPAs exist with male sex, smoking status and age rising through the groups. There also more patients within cluster 3 with higher exposure to TT suggesting that high inflammation could be associated with a more refractory disease course in this cohort as has been suggested in a number of analyses of RefRA (369) but differentials in the number of previous TTs did not meet statistical significance in this analysis.

I haven't identified any additional disease clusters by combining more indicator variables into the analysis than were identified in either the DAS28 component or US variable LPAs and indeed, there remains significant overlap between all of the 3-class solutions that I have identified, both in terms of the baseline characteristics in the groups and in the patients assigned to the groups based on their posterior probability of class membership. This consistency across multiple analyses, with different numbers of indicator variables used in LPA gives support to the fact that 3 distinct and discreet phenotypic clusters are present in this cohort. I have determined that patients class membership can be estimated by using total Joint PD, total tendon GS and CRP, which will be clinically useful if these latent classes can be shown to respond differently to TT.

5 Response to targeted therapy based on the presence of baseline synovitis and baseline combined clinical and ultrasound phenotypic cluster.

5.1 Introduction

In chapters 3 and 4, I have shown that different clusters of patients, with different clinical and imaging phenotypes can be identified within our cohort of advanced RA patients. In latent profile analyses using either DAS28 component scores or US characteristics as grouping variables, and in a broader analysis combining these elements, clusters emerge with varying degrees of inflammation in joints, tendons and at entheses. This inflammation is best shown by differences in SJC and inflammatory markers for DAS 28 scores and PD and grey scale synovitis, tenosynovitis and, to a lesser extent, inflammation at the entheses identified by ultrasound.

Synovitis is the defining lesion in RA and its suppression can be said to be the primary goal of rheumatoid arthritis directed therapy. It is therefore reasonable to hypothesise that those patients with confirmed active synovitis at baseline will stand a better chance of making a moderate to good EULAR response to TT than those with no evidence of significant synovial inflammation. It does however remain possible, particularly in advanced RA patients, that non-response to therapy may be driven by non-synovial inflammation or structural damage, rather than persistent active synovitis. MUS has been shown to be more accurate than clinical examination at confirming the presence of synovitis but there is little published work that shows whether the presence of baseline synovitis, confirmed using imaging, can be used to predict a response to treatment.

In this chapter I aim to determine whether, in a cohort of advanced RA patients, the presence of ultrasound determined synovitis at baseline, before a new TT is started, is associated with

a response to targeted therapy and if the absence of baseline synovitis predicts treatment failure. Furthermore, I aim to determine whether the clusters identified in chapter 4, derived from the combined clinical and ultrasound model, with their differences in levels of inflammation, also associate with a different profile of response to therapy. I will also look at patterns of joint involvement in responders and non-responders after 6 months of therapy to see if clear differences in inflammation between patients with differential responses to treatment exist. Finally, I will explore whether there is a differential in disease distribution, both in terms of joint involvement and whether differences in entheseal or tendon inflammation exist between responders and non-responders.

5.2 Chapter 5 aims

- To determine whether the presence of clinically relevant MUS identified synovitis at baseline associates with response to therapy.
- To determine whether a combined clinical/ultrasound cluster associates with response to therapy.
- To evaluate for association between individual ultrasound characteristics at baseline and response to targeted therapy.

5.3 Chapter 5 methods

I have used ultrasound to confirm the presence or absence of synovitis at baseline for patients in the prospective established RA cohort. For the purpose of this thesis, I have defined clinically significant synovitis as the presence of power doppler synovitis of grade 2 or above in a single joint or total PD synovitis of greater than 3 if no single joint has grade 2 PD. Response to treatment has been defined using DAS28-CRP, which is standard practice within our department. The EULAR response criteria for good or moderate response were used i.e. of a change in DAS28-CRP of ≥0.6 or a change of ≥1.2 in a patient with a DAS28 >5.1 at

endpoint. Any patient with a moderate or good response was classified as a responder. Where analysis of response by latent profile has been performed the broader phenotypic clusters identified using combined clinical and US findings in chapter 4 have been used.

I followed up our cohort of TT exposed patients at 3 and 6 months to determine whether they responded to therapy. The ongoing COVID-19 pandemic, which started in early 2020, disrupted my ability to follow up this cohort of patients as planned as all non-COVID face-to-face clinical research was paused in March 2020. Unfortunately, this meant that a proportion of our cohort were not seen at three and six months as these time points fell during the research lockdown. Of those 200 patients included in our cluster analyses 134 patients had 3 month follow up and 111 were followed up at 6 months, although only 97 of these were able to have their final MUS, before research activity was halted. The majority of this follow up data were lost due to COVID-19, with smaller numbers of patients with missing data due to other factors such as patient loss to follow up or adverse events precluding continuation of TT.

In order to model DAS scores and the proportion of EULAR responders at 3 and 6 months, multiple imputation was used to address all missing baseline covariates and missing longitudinal DAS component values, except those resulting from cancelled visits during COVID lockdowns which were assumed to be missing completely at random. Multinomial logistic regression models have been used to compare response in those with synovitis at baseline and those without and to compare responses between cluster 1 and clusters 2 and 3. Longitudinal mixed modelling (LMM) has been used to determine if there are differences in response to TT over the 6 month follow up period between the clusters. LMM was performed in unimputed data as it handles missing data directly using the same assumptions as multiple imputation i.e. that the data are missing at random, but instead of filling in multiple complete datasets it directly models the outcome as a function of the observed data. Sample size was matched to be the same as the multinomial logistics modelling

5.4 Chapter 5 Results

5.4.1 Does the presence of baseline synovitis or baseline latent profile affect response to therapy?

Responder status at 3 and 6 months was compared according to presence of synovitis at baseline, and between the latent classes identified at baseline, using multinomial logistic regression models in which those who responded and those who experienced adverse events were compared to non-responders. In total 12 patients within the cohort were determined to be failing treatment at 3 months to require an immediate switch in therapy at that point. **Table 5.1** shows the relative risk ratios for both response and adverse effects of these groups at 3 and 6 months.

	Responded %	Rel. risk ratio (95% CI)	AE %	Rel. risk ratio (95% CI)
Response at 3 months (n=164)				
Clinical Synovitis at BL				
No (estimated 21%)	65%	Reference	6%	Reference
Yes (estimated 79%)	70%	1.18 (0.47, 2.97)	4%	0.74 (0.12, 4.51)
Latent class				
1 (estimated 39%)	64%	Reference	5%	Reference
2 (estimated 38%)	72%	1.38 (0.61, 3.15)	3%	0.84 (0.12, 5.71)
3 (estimated 23%)	70%	1.34 (0.51, 3.57)	5%	1.43 (0.20, 10.25)
Response at 6 months (n=152)				
Clinical Synovitis at BL				
No (estimated 23%)	66%	Reference	6%	Reference
Yes (estimated 77%)	65%	1.05 (0.38, 2.93)	9%	1.75 (0.31, 9.76)
Latent class				
1 (estimated 40%)	61%	Reference	8%	Reference
2 (estimated 38%)	72%	1.50 (0.61, 3.70)	3%	0.53 (0.09, 3.22)
3 (estimated 22%)	58%	1.24 (0.43, 3.60)	18%	2.88 (0.66, 12.60)

Table 5.1*: Proportions of responders at 3 and 6 months by US determined clinical synovitis and by combined clinical and ultrasound latent class at baseline*

BL – Baseline, AE – Adverse Event.

The results show a relative risk ratio (RRR) of response for patients with clinical synovitis versus those without, of 1.18 at 3 months and 1.05 at 6 months. They also show an increased RRR for response of clusters 2 and 3 over cluster 1, which has been used as the reference cluster, at both 3 and 6 months. However, despite these RRRs showing an increased chance of response in patients with baseline synovitis and an increased rate of response in our more inflammatory disease clusters, the confidence intervals of all the above analyses suggest that we do not have statistical significance. Therefore, within our cohort, we cannot see any significant difference in response to TT either between those with synovitis at baseline and those without, or between the individual latent profiles in response to TT, despite the differential in inflammation between our clusters.

5.4.2 Changes in DAS28 components at 3 and 6 months

Figure 5.1 shows the changes in the individual DAS28 components in clusters 1-3 for both the observed and imputed data at 3 and 6 months. It is most notable for the improvement in CRP in patients from cluster 3. Cluster 3 also shows a greater improvement in the SJC over the first 3 months of treatment than the other clusters which is consistent with this cluster having the hight SJC at baseline. All the clusters have similar patterns of improvement in the TJC, regardless of the relative differences in inflammation at baseline and regardless of the differential in improvement in the SJC over time. The greatest variation between the clusters is in the SJC and VAS. In both the observed and imputed data, cluster 2 shows the biggest improvement in VAS over the first 3 months.

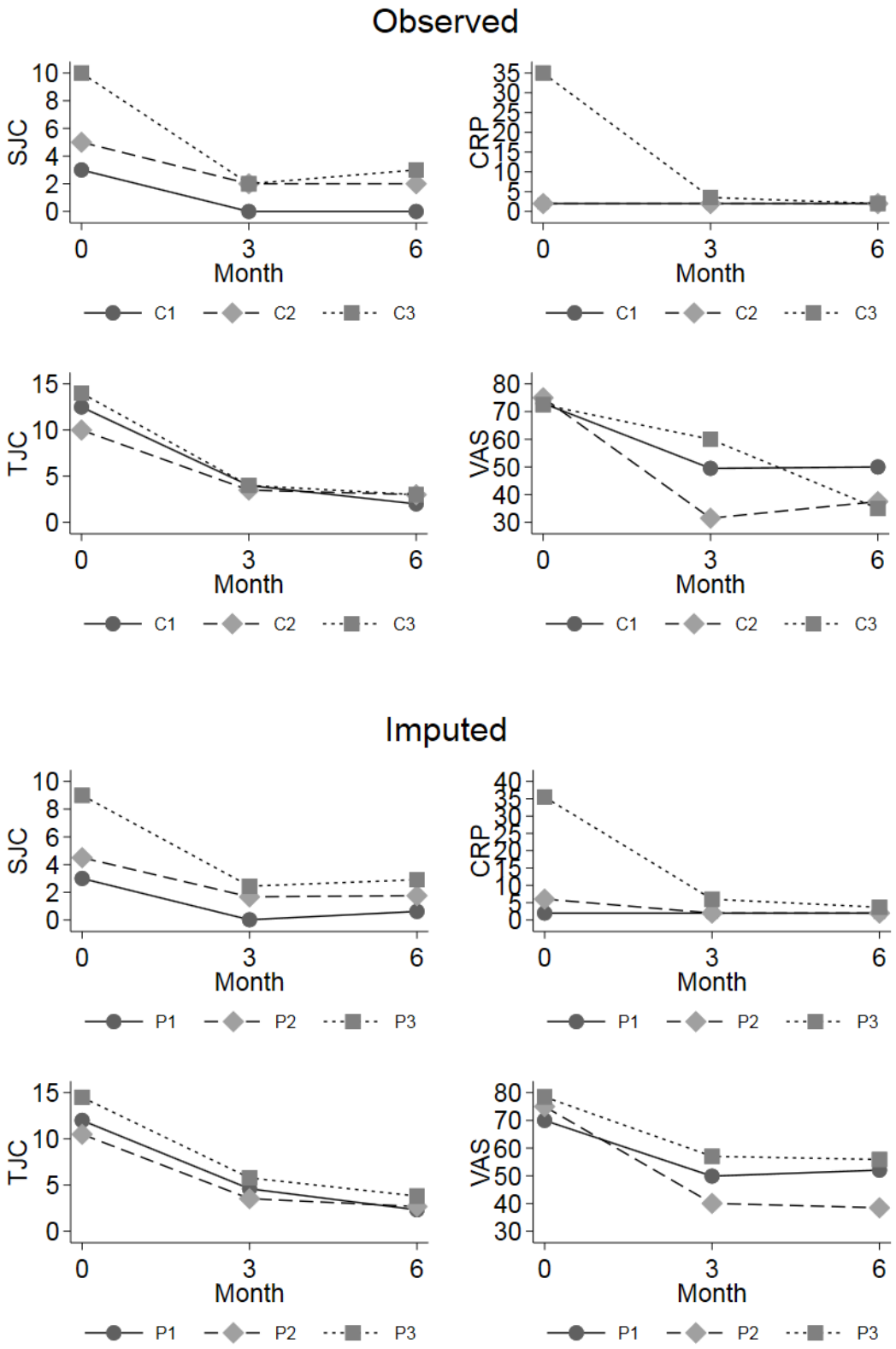

***Figure 5.1**: Median DAS28CRP components over time in observed & imputed data*

C1 – cluster 1 in observed data, C2 – cluster 2 in observed data, C3 – cluster 3 in observed data
P1 – cluster 1 in imputed data, P2 – cluster 2 in imputed data, P3 – cluster 3 in imputed data.

5.4.3 Differences in change in DAS-28 CRP over time in response to therapy between the latent classes

I next sought to model the changes in DAS28 over time in response to TT in each of our combined clinical and US clusters and to see whether there were any significant differences in response to TT over time between the individual disease clusters, to determine whether the clusters had differing trajectories of response to TT over the 6-month study period. To compare changes over time in DAS28-CRP scores between the combined clinical and US latent classes identified at baseline, I have performed longitudinal mixed modelling.

Table 5.2 shows the results of the model for DAS28 -CRP scores where changes for clusters 2 and 3 are shown relative to cluster 1 which is once again used as the reference (constant). The tables give the results for both class by month and class by month squared to account for non-linear change associated with the 3 time points that disease activity was assessed (i.e. it allows for the line to bend at 3 months). The results in **Table 5.2** allow us to work out the non-linear change in DAS28 over time in each cluster and then perform Chi^2 analysis to determine if there are significant differences between these clusters in their change in DAS28 in response to TT over the study period (**table 5.3**).

das28crp	Coef.	Std. Err.	z	P>|z|	[95% Conf. Interval]	
Clinical/US class						
2	0.17	0.24	0.70	0.483	-0.31	0.65
3	0.80	0.28	2.87	0.004	0.25	1.35
c month	-0.23	0.04	-6.35	<0.001	-0.30	-0.16
Clinical/US class x cmonth						
2	-0.06	0.05	-1.14	0.256	-0.16	0.04
3	-0.14	0.06	-2.42	0.016	-0.26	-0.03
$Cmonth^2$	0.07	0.02	3.79	<0.001	0.03	0.10
Clinical/US class x $cmonth^2$						
2	0.01	0.03	0.25	0.801	-0.04	0.06
3	0.01	0.03	0.47	0.635	-0.04	0.07
cons	3.48	0.17	20.97	<0.001	3.15	3.80

***Table 5.2**: Linear mixed regression of DAS28CRP (fixed effects)*

cmonth – Centred month, Cons – Constant. Constant = latent class 1.

The results in **table 5.2** are not directly interpretable but allow us to determine the coefficient for each cluster and the non-linear change in DAS28. The results for the coefficient are all relative to cluster 1 which is used as the constant. At baseline (time point 0) the coefficient for cluster 1 is 3.48 and for cluster 2 is 3.65 (3.48+0.17) and cluster 3 is 4.28 (3.48+ 0.80). c month represents the change over time in cluster 1 and cmonth x cmonth the change in time over month squared. We can calculate the non-linear change in cluster 1 with the formula cmonth x month + $cmonth^2$ x $month^2$. Therefore, for cluster 1 non-linear change will be

calculated as 0.23xmonth+0.07xmonth$^{2.}$ and in cluster 2 (0.23+0.06) x month+(0.07+0.01) x month^2 and so on. The results of this model are shown graphically in **Figure 5.2**.

	df	chi2	P>chi2
das28crp			
Clinical/US class x cmonth	2	5.86	0.053
Clinical/US class x cmonth^2	2	0.23	0.892
Overall	4	6.55	0.162

Table 5.3:*Tests of interaction from linear mixed regression of DAS28-CRP: latent class by month and latent class by month squared*

cmonth – Centred month

Table 5.3 shows the overall interactions from the above analysis tested using Chi^2. It does not show a significant difference in the overall changes in DAS-28 CRP over time between the clusters in response to targeted therapy (p=0.162). Once again this can be seen in **Figure 5.2** where the disease trajectories and differences between the groups at each time point shows minimal change relative to each other.

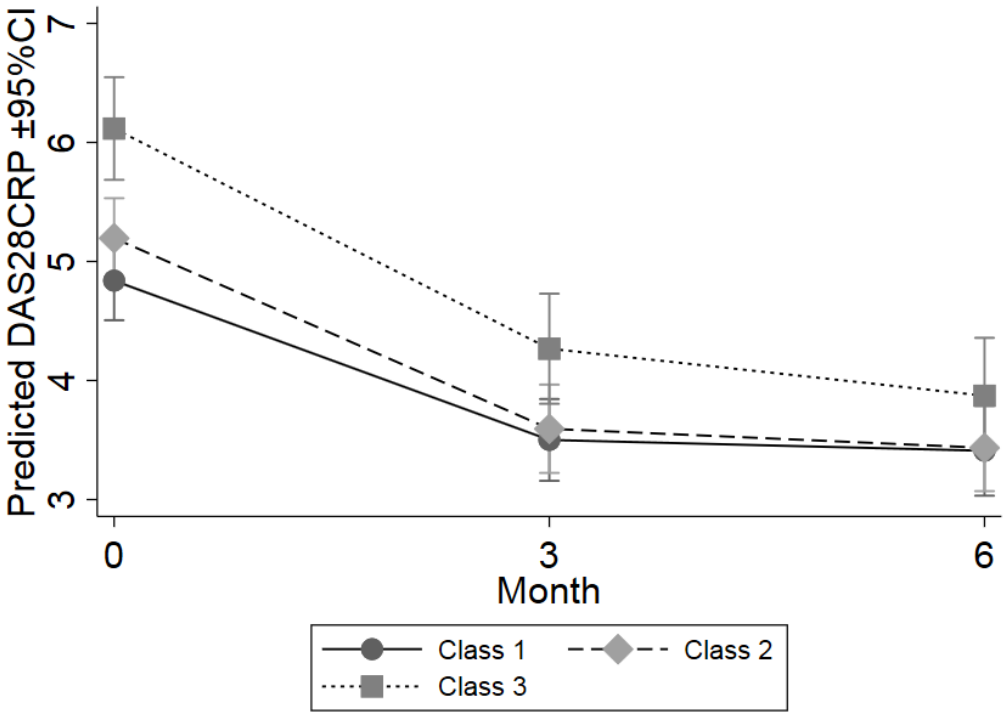

***Figure 5.2**: DAS28CRP over time by latent class (fixed effects from mixed model)*

The above figure shows the predicted disease trajectories or changes in DAS28-CRP over the study period. There is minimal difference either in the shapes of the disease trajectory, which all follow a similar pattern, or the relative differences between each disease cluster at each assessment timepoint.

5.4.4 Differences in change in DAS28CRP-2C over time in response to therapy between the latent classes.

In chapter 1 I discussed the 2 component DAS28 score calculated using just the SJC and CRP, which was modelled and validated to be better at representing active synovitis than the traditional 4 component scores. I have also shown for all the LPAs performed in this study, including the combined clinical and ultrasound model used in the analysis in this chapter, that there is a significant difference in baseline DAS28CRP-2C, between all of the clusters. As a result, I tested whether there were any differences in the changes in DAS28CRP-2C over time

between our clusters using the same methods outlined above. The results are shown in **table 5.4.**

DAS28CRP_2C	Coef.	Std. Err.	z	P>|z|	[95% Conf.	Interval]
Clinical/US class						
2	0.79	0.22	3.55	<0.001	0.35	1.22
3	1.34	0.26	5.21	<0.001	0.84	1.84
cmonth	-0.13	0.04	-3.80	<0.001	-0.20	-0.07
Clinical/US class x cmonth						
2	-0.08	0.05	-1.54	0.123	-0.17	0.02
3	-0.21	0.06	-3.57	<0.001	-0.33	-0.09
$cmonth^2$	0.06	0.02	3.79	<0.001	0.03	0.10
Clinical/S class x $cmonth^2$						
2	-0.01	0.02	-0.37	0.711	-0.06	0.04
3	0.05	0.03	1.89	0.059	-0.00	0.11
cons	1.49	0.15	9.83	<0.001	1.19	1.79

Table 5.4*:Linear mixed regression of DAS28CRP-2C (random effects model)*

cmonth – Centred month

Once again **table 5.4** is not directly interpretable but allows us to identify the coefficient for each class and the non-linear change in DAS28CRP-2C over time using the same method described for **table 5.2** (page 188). Tests of the main interactions from this analysis are shown in **table 5.5.**

	df	chi2	P>chi2
DAS28CRP-2C			
Clinical/US class x cmonth	2	12.74	0.002
Clinical/US class x cmonth^2	2	5.10	0.078
Overall	4	19.95	0.001

***Table 5.5**:Tests of interaction from linear mixed regression of DAS28CRP-2C: latent class by month and latent class by month squared*

cmonth – Centred month

Table 5.5 shows the overall interactions. In the case the overall interaction shows that there is a significant difference in the change in the DAS28-CRP2C between the clusters over time (p=0.001). This is shown graphically in **figure 5.3** where there is a clear separation in the trajectories at each time point.

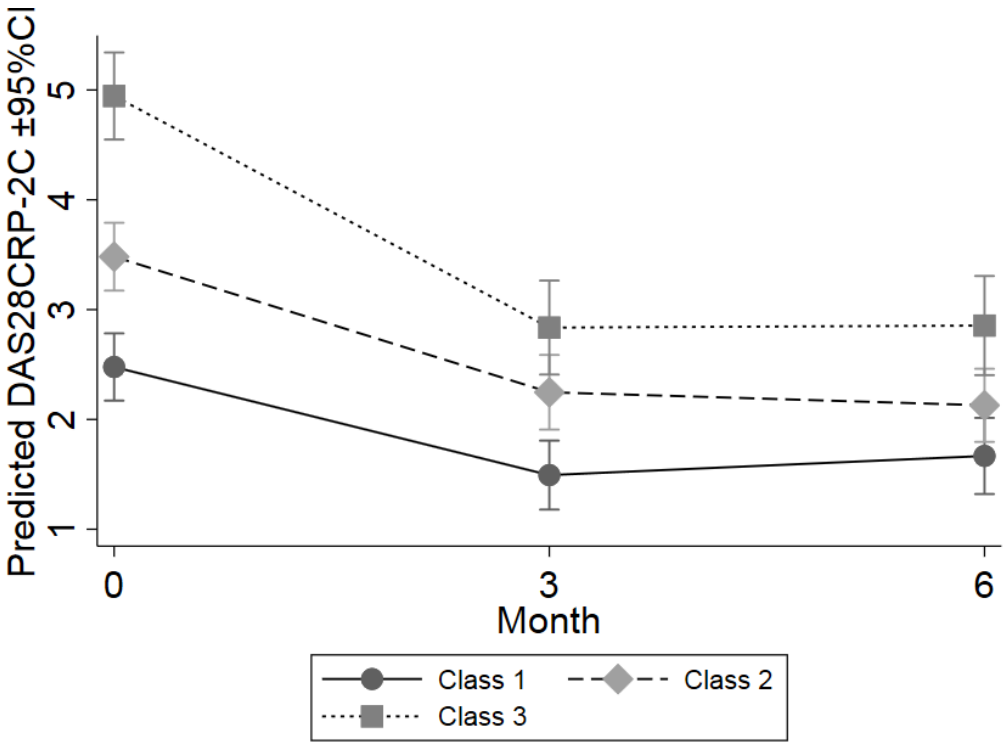

***Figure 5.3**: DAS28CRP-2C over time by latent class (fixed effects from mixed model)*

Figure 5.3 shows predicted DAS28CRP-2C over time by latent class. There are visible differences in the trajectories shown with class 3 showing a rapid improvement before a stabilisation in DAS28CRP-2C, cluster 2 showing a rapid improvement followed by a further slower improvement and cluster 1 initially improving until 3 months, before a worsening between 3 and 6 months. There is also clear differenced in the amount of separation of the clusters at the 3 time points.

5.4.5 Differences in change in VAS over time in response to therapy between the latent classes.

In the analyses above I have not been able to show a difference in the overall DAS28 score between the clusters over time, but that there is a difference in the changes in the DAS28-2C over time in response to TT. DAS28-2C is designed to better reflect MUS measured markers of inflammation (PD), but the composite DAS28 score was developed to reflect all aspects of a patient's disease, including their feeling of well-being. DAS28-2C does not include measure of how a patient feels about disease and quality of life and as a result I have decided to run a similar analysis using just the VAS score.

VAS	Coef.	Std. Err.	z	P>|z|	[95% Conf.	Interval]
Clinical/US class						
2	-5.74	4.83	-1.19	0.235	-15.20	3.73
3	2.32	5.55	0.42	0.676	-8.56	13.20
cmonth	-3.25	0.72	-4.52	0.000	-4.66	-1.84
Clinical/US class x cmonth						
2	-2.07	1.00	-2.07	0.039	-4.04	-0.11
3	-1.41	1.17	-1.20	0.228	-3.71	0.89
$Cmonth^2$	1.10	0.39	2.85	0.004	0.34	1.86
Clinical/USx $Cmonth^2$						
2	0.44	0.56	0.78	0.434	-0.66	1.55
3	-0.07	0.65	-0.11	0.913	-1.35	1.20
_cons	48.66	3.27	14.87	0.000	42.25	55.08

***Table 5.6**: Linear mixed regression of VAS (random effects model)*

As above, table 5.6 is not directly interpretable but allows us to identify the coefficient for each class and the non-linear change in VAS over time using the same method described for **table 5.2** (page 190). Tests of the main interactions from this analysis are shown in **table 5.7.**

	df	chi2	P>chi2
VAS			
Clinical/US class x cmonth	2	4.39	0.112
Clinical/US class x $cmonth^2$	2	0.83	0.660
Overall	4	5.65	0.227

***Table 5.7**: Tests of interaction from linear mixed regression of DAS28CRP-2C: latent class by month and latent class by month squared*

Table 5.7 shows the overall interactions from the above analysis tested using Chi^2. It does not show a significant difference in the overall changes in VAS over time between the clusters in response to targeted therapy (p=0.227). **Figure 5.4** shows a graphical representation of the trajectories of VAS scores in each cluster in response to TT highlighting a lack of separation between the trajectories for each cluster.

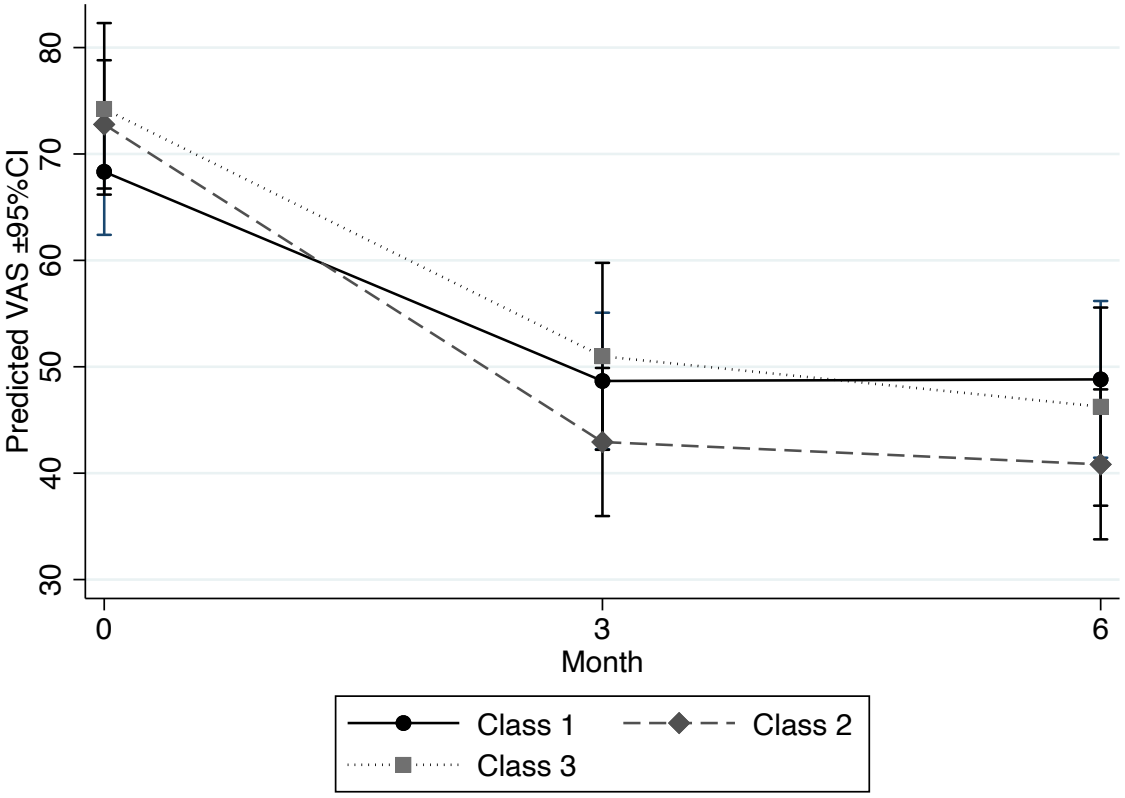

Figure 5.4: *VAS over time by latent class (fixed effects from mixed model)*

5.4.6 Differences in ultrasound between responders and non-responders.

Tables 5.8 shows the baseline scores for ultrasound characteristics for each of our combined clusters expressed as average score for the cluster (median) and percentages of patients within each cluster with scores > 0 for each characteristic. **Table 5.9** shows the same results for the follow up ultrasound scans at 6 months, to allow comparison. Only 97 of the total cohort had a 6- month ultrasound scan, predominantly due to loss of data due to COVID-19, so the observed data is shown.

	Latent Profile			
	1	**2**	**3**	**Total**
	N=76	**N=82**	**N=42**	**N=200**
Total joint GS	26.0 (20.0, 31.5), n=72	36.5 (29.0, 43.0), n=82	62.5 (51.0, 71.0), n=42	34.5 (25.0, 45.0), n=196
Total joint GS>0 Yes	72/72 (100.0%)	82/82 (100.0%)	42/42 (100.0%)	196/196 (100.0%)
Total joint PD	2.0 (0.0, 4.0), n=72	9.0 (6.0, 13.0), n=82	29.5 (21.0, 45.0), n=42	6.5 (3.0, 18.0), n=196
Total joint PD>0 Yes	51/72 (70.8%)	80/82 (97.6%)	42/42 (100.0%)	173/196 (88.3%)
N Js w/ erosions	1.0 (0.0, 2.0), n=72	2.0 (1.0, 4.0), n=82	5.0 (3.0, 8.0), n=42	2.0 (1.0, 4.0), n=196
N Js w/ erosions>0 Yes	39/72 (54.2%)	67/82 (81.7%)	42/42 (100.0%)	148/196 (75.5%)
N Js w/ osteophytes	2.0 (1.0, 6.0), n=72	3.0 (1.0, 7.0), n=82	3.0 (1.0, 6.0), n=42	3.0 (1.0, 6.0), n=196
N Js w/ osteophytes>0 Yes	56/72 (77.8%)	63/82 (76.8%)	33/42 (78.6%)	152/196 (77.6%)
N Js subluxed	3.0 (0.0, 6.0), n=72	3.5 (0.0, 7.0), n=82	4.5 (1.0, 7.0), n=42	3.0 (0.0, 6.0), n=196
N Js subluxed>0 Yes	53/72 (73.6%)	60/82 (73.2%)	32/42 (76.2%)	145/196 (74.0%)
Total tendon GS	1.0 (0.0, 2.0), n=72	5.0 (3.0, 7.0), n=82	11.0 (7.0, 18.0), n=42	4.0 (1.0, 8.0), n=196
Total tendon GS>0 Yes	42/72 (58.3%)	80/82 (97.6%)	42/42 (100.0%)	164/196 (83.7%)
Total tendon PD	0.0 (0.0, 0.0), n=72	3.0 (0.0, 5.0), n=82	6.0 (4.0, 13.0), n=42	1.0 (0.0, 5.0), n=196
Total tendon PD>0 Yes	6/72 (8.3%)	60/82 (73.2%)	39/42 (92.9%)	105/196 (53.6%)
N thickened Ts	0.0 (0.0, 1.0), n=71	0.0 (0.0, 1.0), n=82	0.0 (0.0, 1.0), n=42	0.0 (0.0, 1.0), n=195
N thickened Ts>0 Yes	18/71 (25.4%)	33/82 (40.2%)	19/42 (45.2%)	70/195 (35.9%)
Total enthesis GS	2.0 (1.0, 4.0), n=72	2.0 (1.0, 4.0), n=82	4.0 (2.0, 6.0), n=42	3.0 (1.0, 4.0), n=196

	Latent Profile			
	1 N=76	2 N=82	3 N=42	Total N=200
Total enthesis GS>0 Yes	62/72 (86.1%)	73/82 (89.0%)	39/42 (92.9%)	174/196 (88.8%)
Total enthesis PD	0.0 (0.0, 0.0), n=72	0.0 (0.0, 0.0), n=82	0.0 (0.0, 1.0), n=42	0.0 (0.0, 0.5), n=196
Total enthesis PD>0 Yes	12/72 (16.7%)	20/82 (24.4%)	17/42 (40.5%)	49/196 (25.0%)
N thickened Es	2.0 (1.5, 3.0), n=72	2.0 (2.0, 3.0), n=82	3.0 (2.0, 4.0), n=42	2.0 (2.0, 3.0), n=196
N thickened Es>0 Yes	63/72 (87.5%)	76/82 (92.7%)	42/42 (100.0%)	181/196 (92.3%)
N Es w/ erosions	0.0 (0.0, 0.0), n=72	0.0 (0.0, 1.0), n=82	1.0 (0.0, 1.0), n=42	0.0 (0.0, 1.0), n=196
N Es w/ erosions>0 Yes	12/72 (16.7%)	26/82 (31.7%)	22/42 (52.4%)	60/196 (30.6%)
N Es w/ erosions	0.0 (0.0, 0.0), n=72	0.0 (0.0, 1.0), n=82	1.0 (0.0, 1.0), n=42	0.0 (0.0, 1.0), n=196
N Es w/ erosions>0 Yes	12/72 (16.7%)	26/82 (31.7%)	22/42 (52.4%)	60/196 (30.6%)
N Es w/ enthesophytes	1.0 (0.0, 2.0), n=72	2.0 (0.0, 2.0), n=82	1.5 (0.0, 4.0), n=42	1.0 (0.0, 2.0), n=196
N Es w/ enthesophytes>0 Yes	45/72 (62.5%)	56/82 (68.3%)	30/42 (71.4%)	131/196 (66.8%)
N Es w/ calcifications	0.0 (0.0, 1.0), n=72	0.0 (0.0, 1.0), n=82	0.0 (0.0, 0.0), n=42	0.0 (0.0, 1.0), n=196
N Es w/ calcifications>0 Yes	32/72 (44.4%)	26/82 (31.7%)	4/42 (9.5%)	62/196 (31.6%)

***Table 5.8**: Baseline Ultrasound findings by latent profile.*

GS – Grey Scale, PD – Power Doppler, N Js w/ - Number of Joints with. N – Number, Ts – Tendons, N Es w/- Number of Entheses with.

Baseline ultrasound findings for the combined clinical and ultrasound clusters have been discussed in chapter 4 (Comparison between the baseline ultrasound variables using K-W is shown in chapter 4, **figure 4.4**, page 172). They show progression between the clusters in joint GS and PD and tendon GS an PD and more inflammation at the entheses in the more inflammatory cluster 3 (more enthesis PD and enthesis erosion), but greater numbers of enthesis calcification in cluster 1 and 2 than cluster 3, with cluster 1 having the most calcification. These results are shown again above. Other notable results to highlight above are the lack of difference in the clusters in osteophytosis or joint subluxation. There is more joint erosion in cluster 3, the most inflammatory cluster.

	Latent Profile			
	C1 N=38	C2 N=38	C3 N=23	Total N=97
Total joint GS	26.5 (21.0, 34.5), n=36	30.5 (25.0, 41.0), n=38	49.0 (36.0, 60.0), n=23	32.0 (24.0, 45.0), n=97
Total joint GS>0 Yes	36/36 (100.0%)	38/38 (100.0%)	23/23 (100.0%)	97/97 (100.0%)
Total joint PD	2.0 (0.0, 5.5), n=36	4.0 (1.0, 11.0), n=38	12.0 (8.0, 35.0), n=23	5.0 (1.0, 11.0), n=97
Total joint PD>0 Yes	24/36 (66.7%)	31/38 (81.6%)	23/23 (100.0%)	78/97 (80.4%)
N Js w/ erosions	1.0 (0.0, 2.0), n=36	2.0 (1.0, 4.0), n=38	5.0 (3.0, 6.0), n=23	2.0 (1.0, 4.0), n=97
N Js w/ erosions>0 Yes	22/36 (61.1%)	33/38 (86.8%)	21/23 (91.3%)	76/97 (78.4%)
N Js w/ osteophytes	2.0 (1.0, 4.0), n=36	3.5 (0.0, 5.0), n=38	4.0 (2.0, 7.0), n=23	3.0 (1.0, 5.0), n=97
N Js w/ osteophytes>0 Yes	28/36 (77.8%)	28/38 (73.7%)	20/23 (87.0%)	76/97 (78.4%)
N Js subluxed	3.0 (1.0, 5.0), n=36	3.0 (0.0, 7.0), n=38	4.0 (1.0, 10.0), n=23	3.0 (1.0, 6.0), n=97
N Js subluxed>0 Yes	28/36 (77.8%)	28/38 (73.7%)	19/23 (82.6%)	75/97 (77.3%)
Total tendon GS	1.0 (0.0, 3.0), n=36	4.0 (1.0, 8.0), n=38	5.0 (3.0, 12.0), n=23	3.0 (1.0, 6.0), n=97
Total tendon GS>0 Yes	22/36 (61.1%)	31/38 (81.6%)	23/23 (100.0%)	76/97 (78.4%)
Total tendon PD	0.0 (0.0, 1.0), n=36	0.0 (0.0, 4.0), n=38	4.0 (1.0, 10.0), n=23	0.0 (0.0, 3.0), n=97
Total tendon PD>0 Yes	11/36 (30.6%)	17/38 (44.7%)	19/23 (82.6%)	47/97 (48.5%)
N thickened Ts	0.0 (0.0, 1.0), n=36	0.0 (0.0, 2.0), n=38	0.0 (0.0, 1.0), n=23	0.0 (0.0, 1.0), n=97
N thickened Ts>0 Yes	10/36 (27.8%)	15/38 (39.5%)	9/23 (39.1%)	34/97 (35.1%)
Total enthesis GS	3.0 (1.0, 4.0), n=36	2.0 (1.0, 5.0), n=38	3.0 (0.0, 6.0), n=23	3.0 (1.0, 4.0), n=97
Total enthesis GS>0 Yes	34/36 (94.4%)	33/38 (86.8%)	17/23 (73.9%)	84/97 (86.6%)
Total enthesis PD	0.0 (0.0, 0.0), n=36	0.0 (0.0, 0.0), n=38	0.0 (0.0, 1.0), n=23	0.0 (0.0, 0.0), n=97
Total enthesis PD>0 Yes	6/36 (16.7%)	9/38 (23.7%)	9/23 (39.1%)	24/97 (24.7%)
N thickened Es	2.0 (1.0, 2.5), n=36	2.0 (1.0, 3.0), n=38	3.0 (2.0, 4.0), n=23	2.0 (1.0, 3.0), n=97
N thickened Es>0 Yes	35/36 (97.2%)	35/38 (92.1%)	23/23 (100.0%)	93/97 (95.9%)
N Es w/ erosions	0.0 (0.0, 0.0), n=36	0.0 (0.0, 0.0), n=38	0.0 (0.0, 1.0), n=23	0.0 (0.0, 0.0), n=97
N Es w/ erosions>0 Yes	4/36 (11.1%)	9/38 (23.7%)	6/23 (26.1%)	19/97 (19.6%)
N Es w/ erosions	0.0 (0.0, 0.0), n=36	0.0 (0.0, 0.0), n=38	0.0 (0.0, 1.0), n=23	0.0 (0.0, 0.0), n=97
N Es w/ erosions>0 Yes	4/36 (11.1%)	9/38 (23.7%)	6/23 (26.1%)	19/97 (19.6%)

	Latent Profile			
	C1	**C2**	**C3**	**Total**
	N=38	**N=38**	**N=23**	**N=97**
N Es w/ enthesophytes	2.0 (0.0, 3.0), n=36	1.0 (0.0, 3.0), n=38	1.0 (0.0, 3.0), n=23	1.0 (0.0, 3.0), n=97
N Es w/ enthesophytes>0 Yes	23/36 (63.9%)	25/38 (65.8%)	17/23 (73.9%)	65/97 (67.0%)
N Es w/ calcifications	0.0 (0.0, 1.0), n=36	0.0 (0.0, 1.0), n=38	0.0 (0.0, 0.0), n=23	0.0 (0.0, 1.0), n=97
N Es w/ calcifications>0 Yes	17/36 (47.2%)	12/38 (31.6%)	4/23 (17.4%)	33/97 (34.0%)

Table 5.9*: US scores at 6 months summarised by latent class (observed data)*

GS – Grey Scale, PD – Power Doppler, N Js w/ - Number of Joints with. N – Number, Ts – Tendons, N Es w/- Number of Entheses with.

Table 5.9 Shows ultrasound results of the 97 patients who were able to attend for follow up before the COVID-19 pandemic. It is notable that, although there is no difference change in DAS28-CRP over time between the clusters, there are numerically greater changes in median joint and tendon GS and PD scores as you ascend the clusters, with minimal improvement in cluster 1, and marked improvement in cluster 3, with an intermediate result for cluster 2. Cluster 3 also has the greatest improvement in tendon PD (there was little tendon PD at baseline in cluster 1). **Figures 5.5-5.7** shows comparisons of US variables at baseline and 6 months between responders and non-responders.

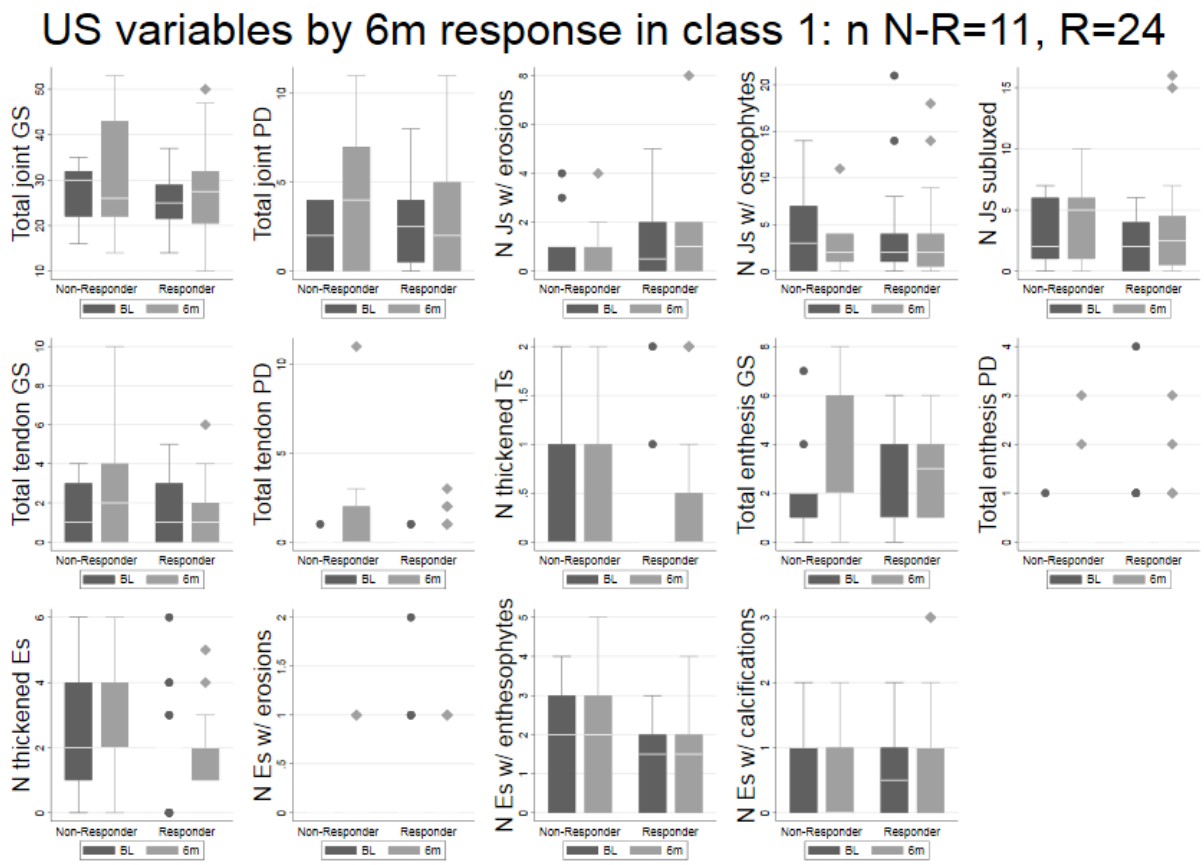

Figure 5.5*: US scores at baseline and 6 months by response status at 6 months in combined clinical and ultrasound class 1.*

N-R – Non-response, R – Response, GS – Grey Scale, PD – Power Doppler, N Js w/ - Number of Joints with. N – Number, Ts – Tendons, N Es w/- Number of Entheses with. Es – Entheses.

Figure 5.5 shows box and whisker plots of US variables at baseline and 6 months for cluster 1, separated into responders and non-responders. Response in this study is defined by improvements of DAS28-CRP according to the EULAR response criteria. Cluster 1 is the least inflammatory of the clusters identified, but 24 of the 35 patients with full US data at 6 months were deemed to have responded according to DAS28 criteria. However, there is little difference between the responders and non-responders on US in most variables.

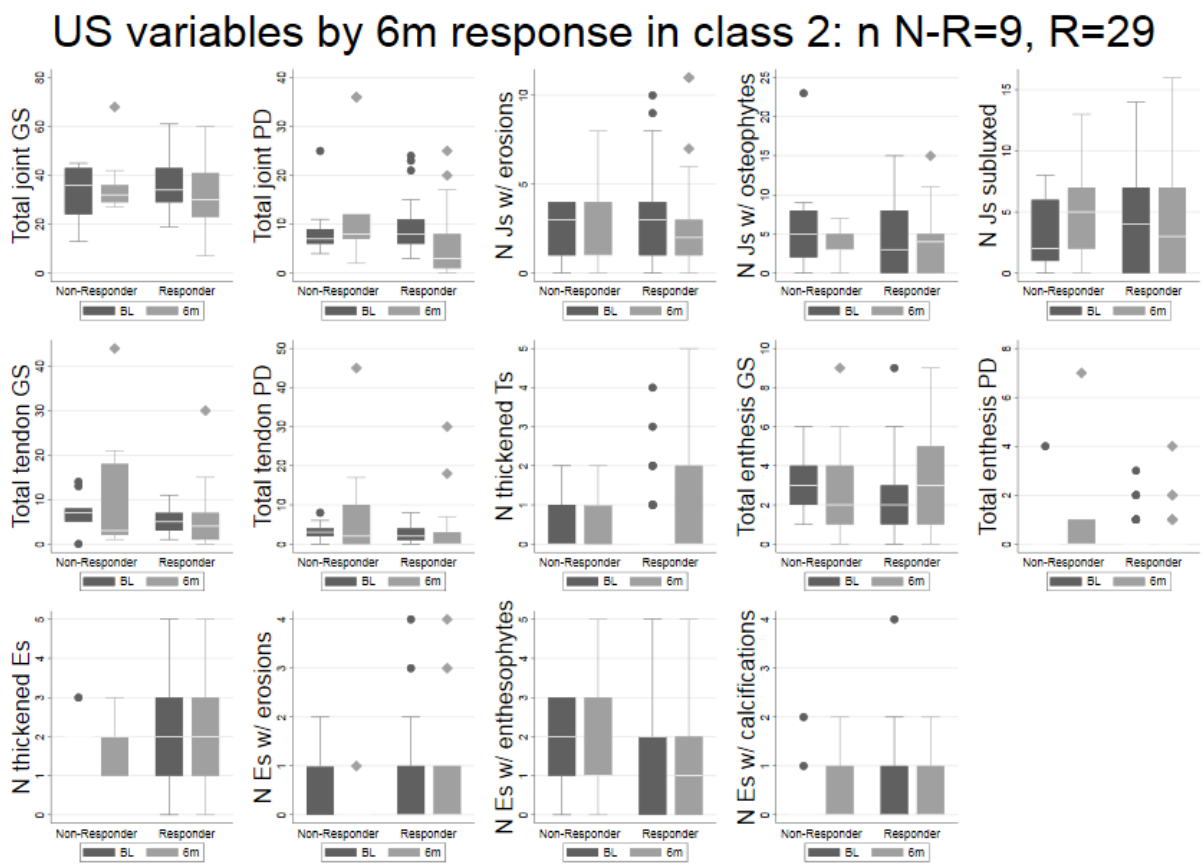

***Figure 5.6**: US scores at baseline and 6 months by response status at 6 months in combined clinical and ultrasound class 2.*

N-R – Non-response, R – Response, GS – Grey Scale, PD – Power Doppler, N Js w/ - Number of Joints with. N – Number, Ts – Tendons, N Es w/- Number of Entheses with. Es – Entheses.

Figure 5.6 shows box and whisker plots of US variables at baseline and 6 months for cluster 2, separated into responders and non-responders. There is a small improvement in joint PD in responders relative to non-responders and a slight improvement in tendon Doppler as well, but for most variables there is minimal difference between responders and non-responders.

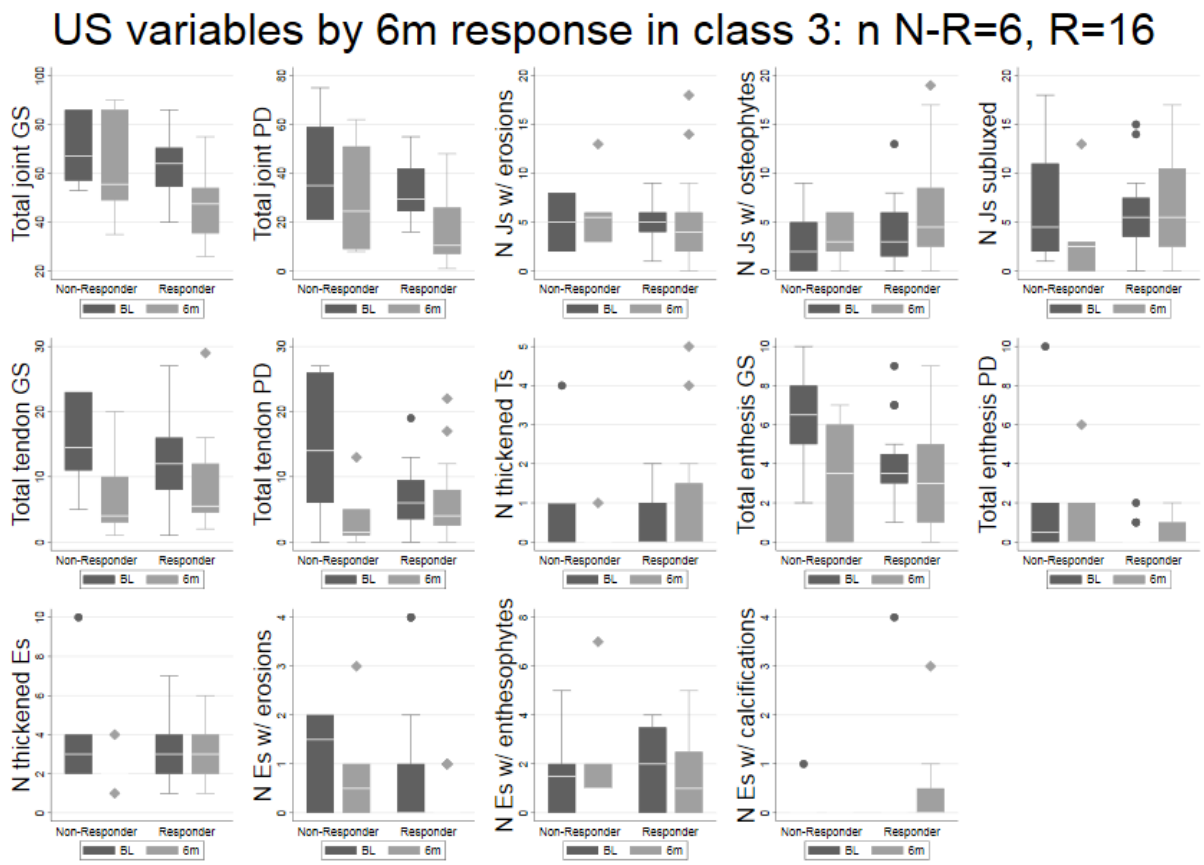

***Figure 5.7**: US scores at baseline and 6 months by response status at 6 months in combined clinical and ultrasound class 3.*

N-R – Non-response, R – Response, GS – Grey Scale, PD – Power Doppler, N Js w/ - Number of Joints with. N – Number, Ts – Tendons, N Es w/- Number of Entheses with. Es – Entheses.

Figure 5.7 shows box and whisker plots of US variables at baseline and 6 months for cluster 3, separated into responders and non-responders. Cluster 3 is the most inflammatory of the clusters and the results are notable for improvement in a number of ultrasound variables in both responders and non-responders. In cluster 3 there are more clear improvements in joint GS and PD in responders than in non-responders. Improvements in tendon inflammation (GS and PD) are present in both groups but appear more marked in non-responders than responders.

5.4.7 Differences in the distribution of joint, tendon and enthesis inflammation between responders and non-responders.

Figures 5.8-5.19 show the distributions of ultrasound identified synovitis, tenosynovitis and entheseal changes in both non-responders and responders to determine whether there are any visible differences in the distribution of these variables between these groups.

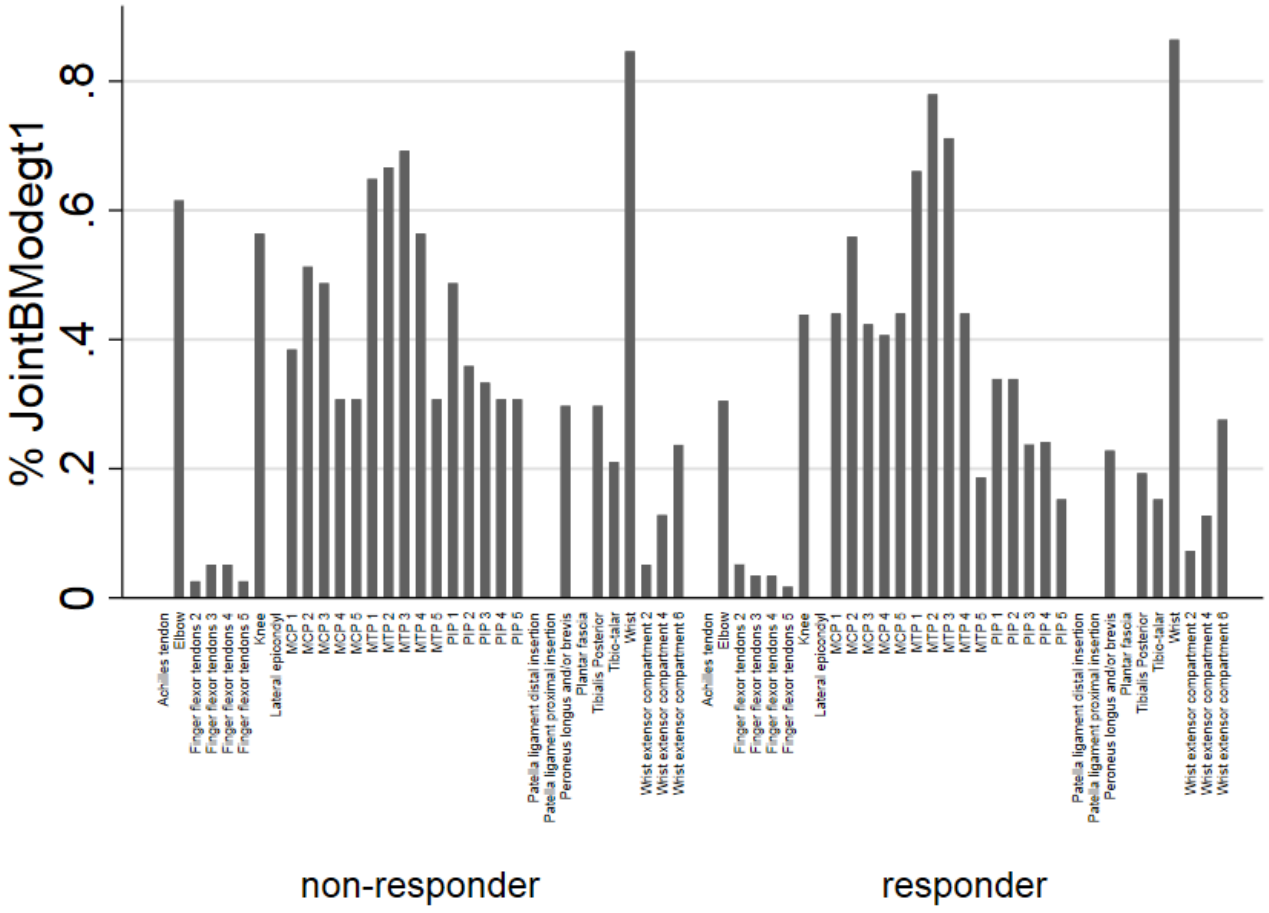

Figure 5.8: *Proportion with Joint B Mode greater than 1 at 6 months by response status (observed data only)*

Figure 5.8 shows the proportion of patients with GS (B mode) synovitis of greater than grade 1 in each joint at 6 months in responders and non-responders. There is no clear difference in the distribution of GS synovitis between responders and non-responders.

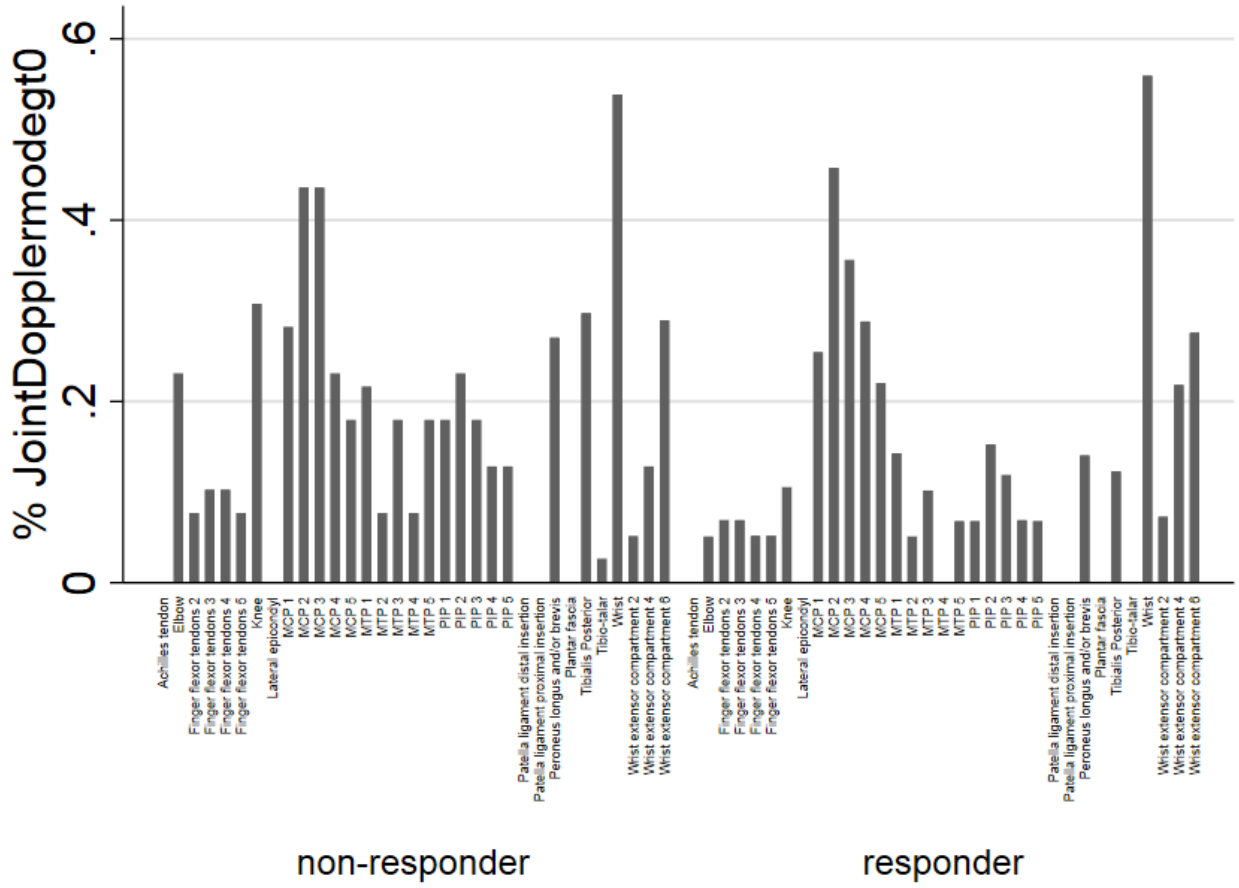

Figure 5.9*:Proportion with Joint Doppler mode greater than 0 at 6 months by response status (observed data only)*

Figure 5.9 shows the proportion of patients with PD synovitis of greater than grade 0 in each joint at 6 months in responders and non-responders. Responders show proportionally lower levels of PD across the PIPJs in particular, but there isn't a clear difference in the patten of joint involvement.

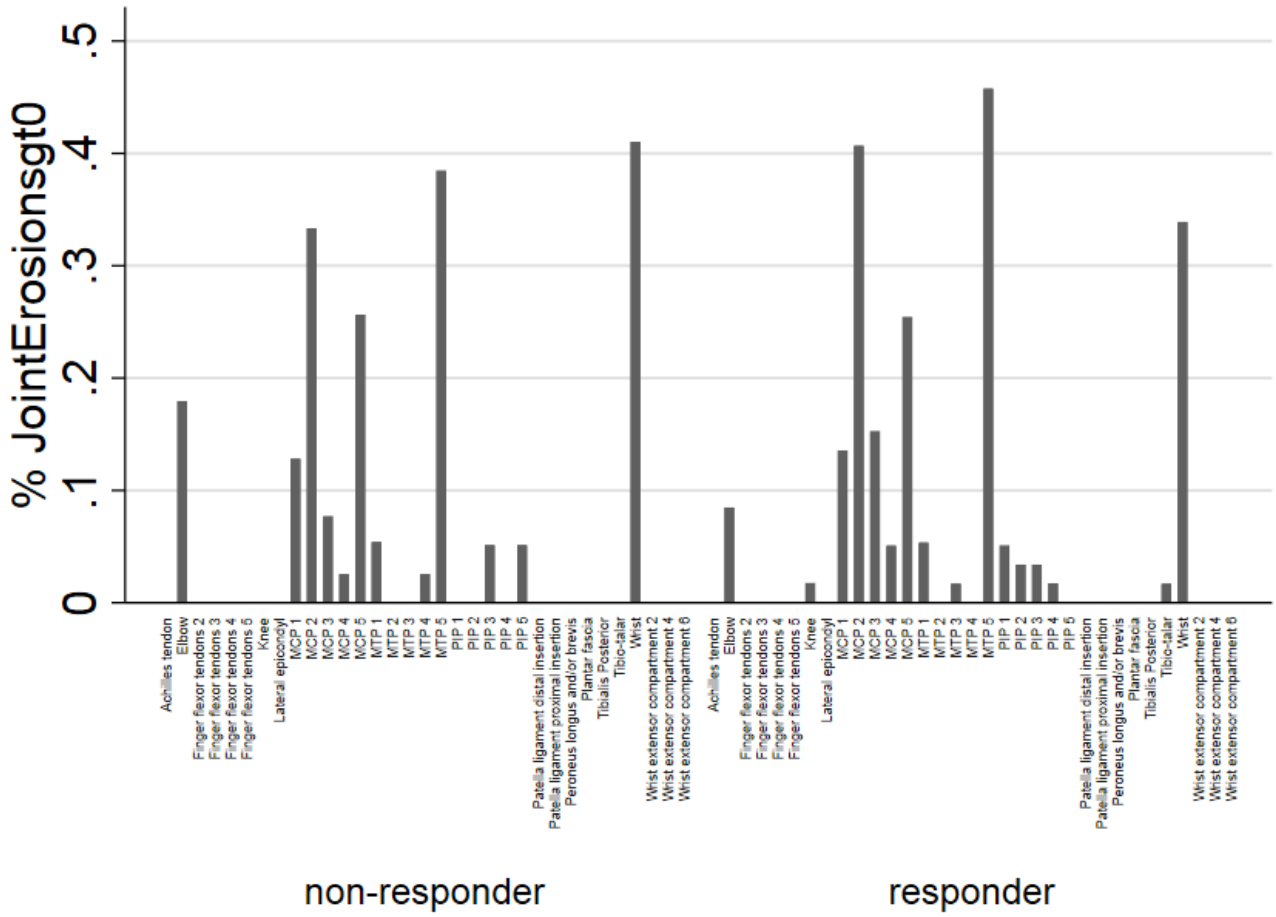

***Figure 5.10**: Proportion with Joint Erosions greater than 0 at 6 months by response status (observed data only)*

Figure 5.10 shows the proportion of patients with more than 0 erosions in each joint at 6 months in responders and non-responders. Once again there is no clear difference, with a similar pattern of erosive change in each group.

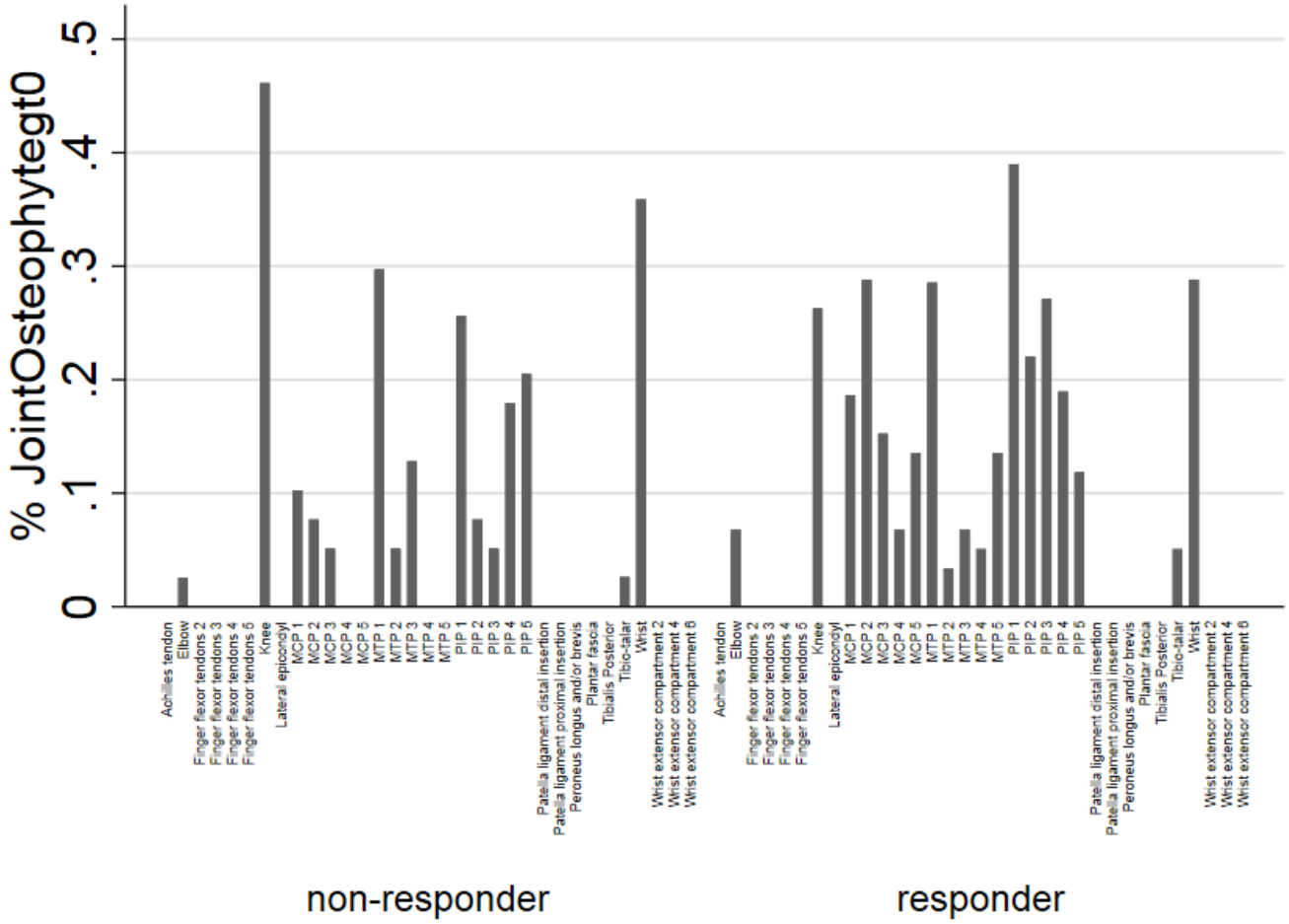

***Figure 5.11**: Proportion with Joint Osteophytes greater than 0 at 6 months by response status (observed data only)*

Figure 5.11 shows the proportion of patients with osteophytes greater than 0 in each joint at 6 months in responders and non-responders. Responders have osteophytes in more joints in this analysis.

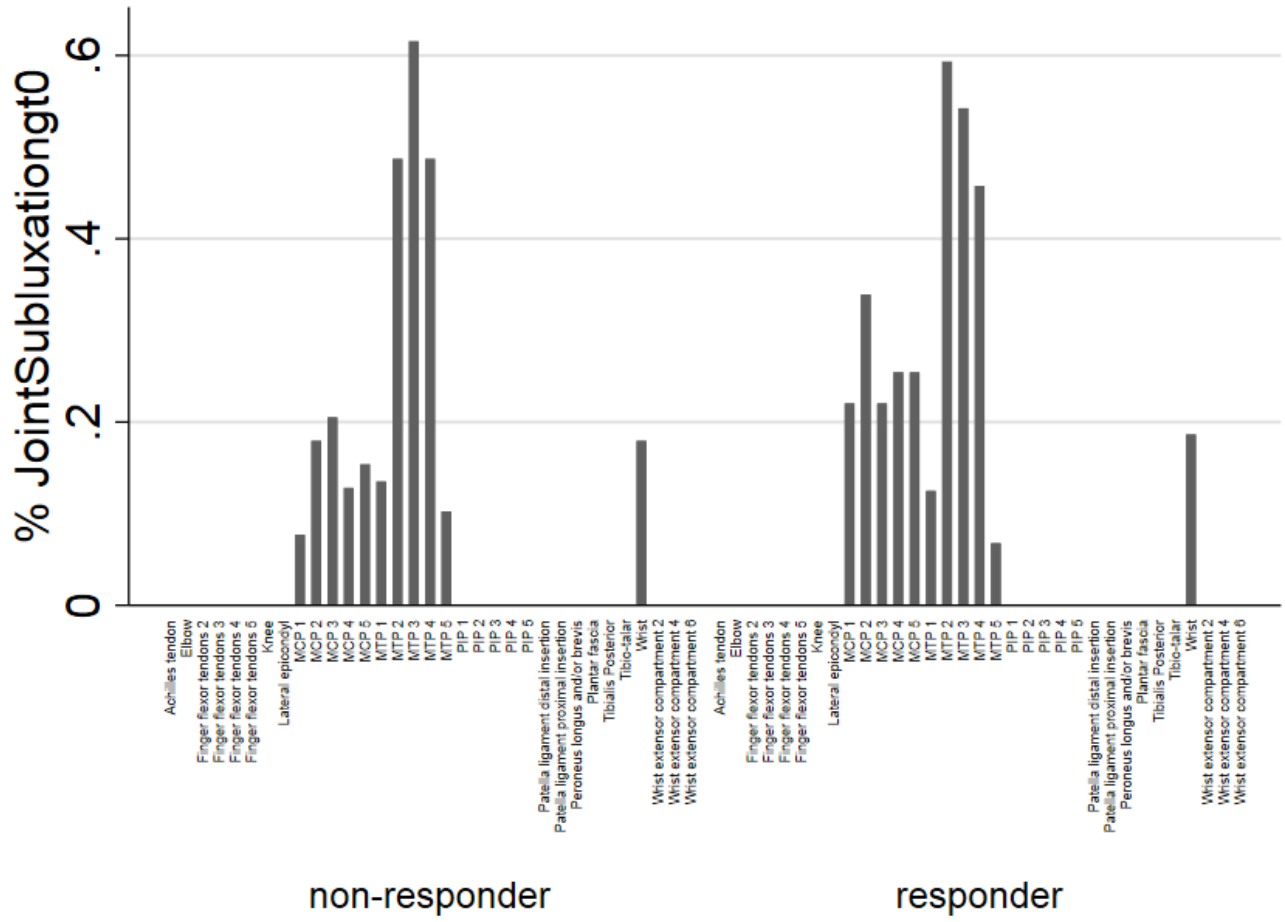

***Figure 5.12**: Proportion with Joint Subluxation greater than 0 at 6 months by response status (observed data only)*

Figure 5.12 shows the proportion of patients with joint subluxation greater than 0 in each joint at 6 months in responders and non-responders. There is no clear difference in the distribution of subluxation, with responders once again having proportionally more subluxation.

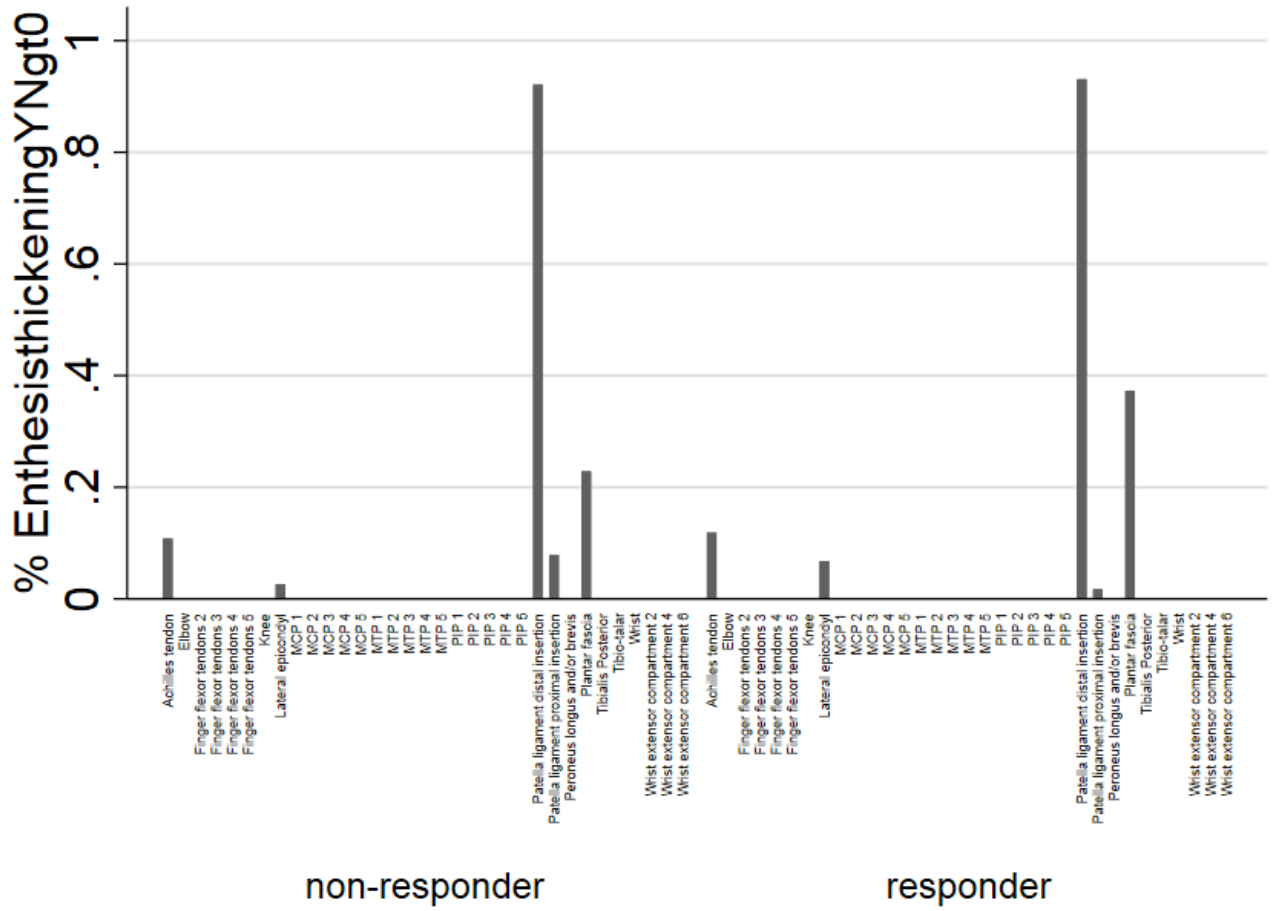

***Figure 5.13**: Proportion with Enthesis thickening at 6 months by response status (observed data only)*

Figure 5.13 shows the proportion of patients with enthesis thickening at 6 months in responders and non-responders. There is no clear difference in the distribution between the groups.

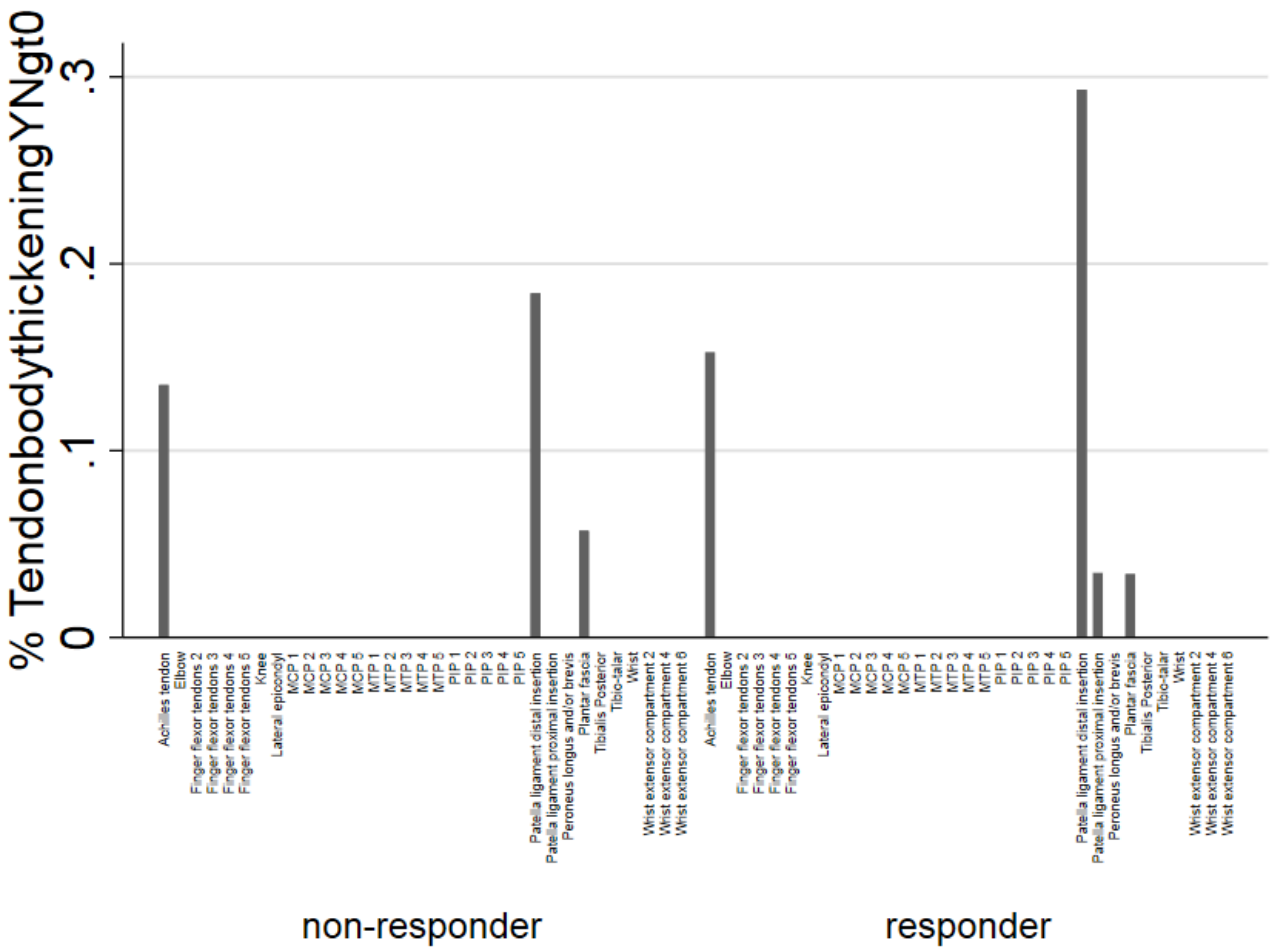

***Figure 5.14**: Proportion with Tendon body thickening at 6 months by response status (observed data only)*

Figure 5.14 shows the proportion of patients with tendon body thickening at the enthesis at 6 months in responders and non-responders. There is little tendon body thickening within the cohort and no clear difference in the distribution between the groups.

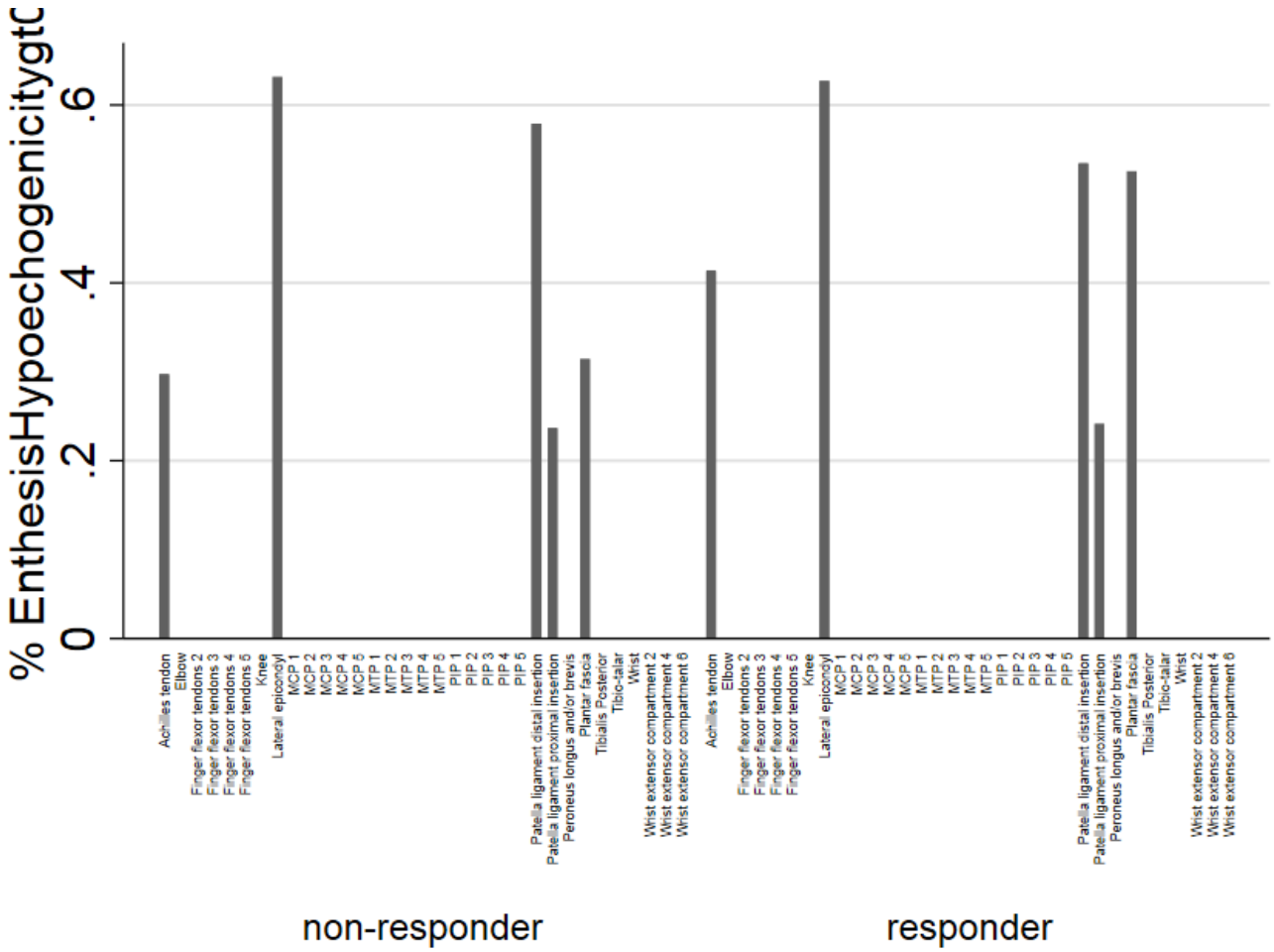

***Figure 5.15**:Proportion with Enthesis Hypoechogenicity greater than 0 at 6 months by response status (observed data only)*

Figure 5.15 shows the proportion of patients with enthesis hypoechogenicity at 6 months in responders and non-responders. There is no clear difference in the distribution between the groups.

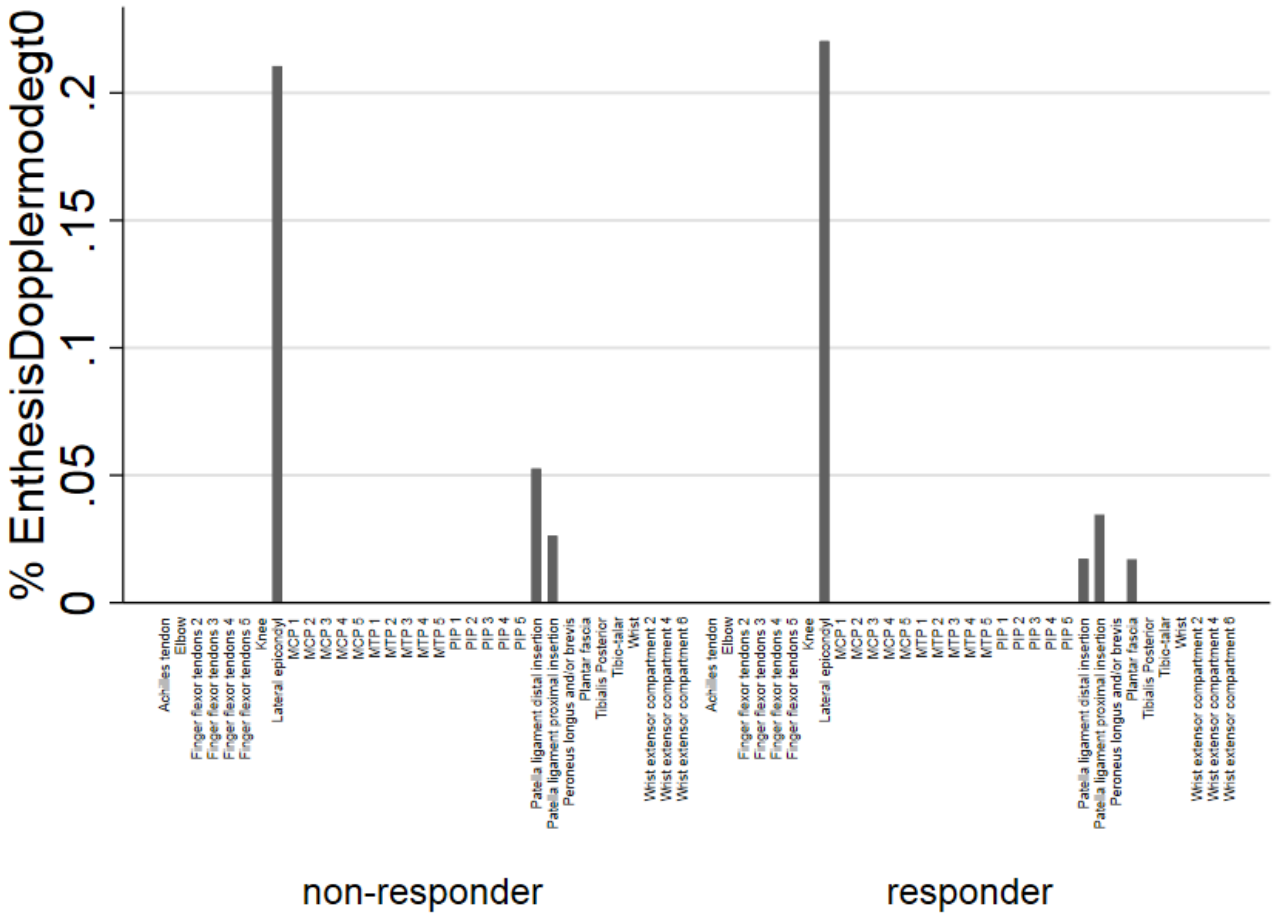

***Figure 5.16**: Proportion with Enthesis Doppler mode greater than 0 at 6 months by response status (observed data only)*

Figure 5.16 shows the proportion of patients with PD at the entheses at 6 months in responders and non-responders. A small number of responders were shown to have PD at the plantar fascia, but the small number is unlikely to be significant and the distribution otherwise appears the same between the groups.

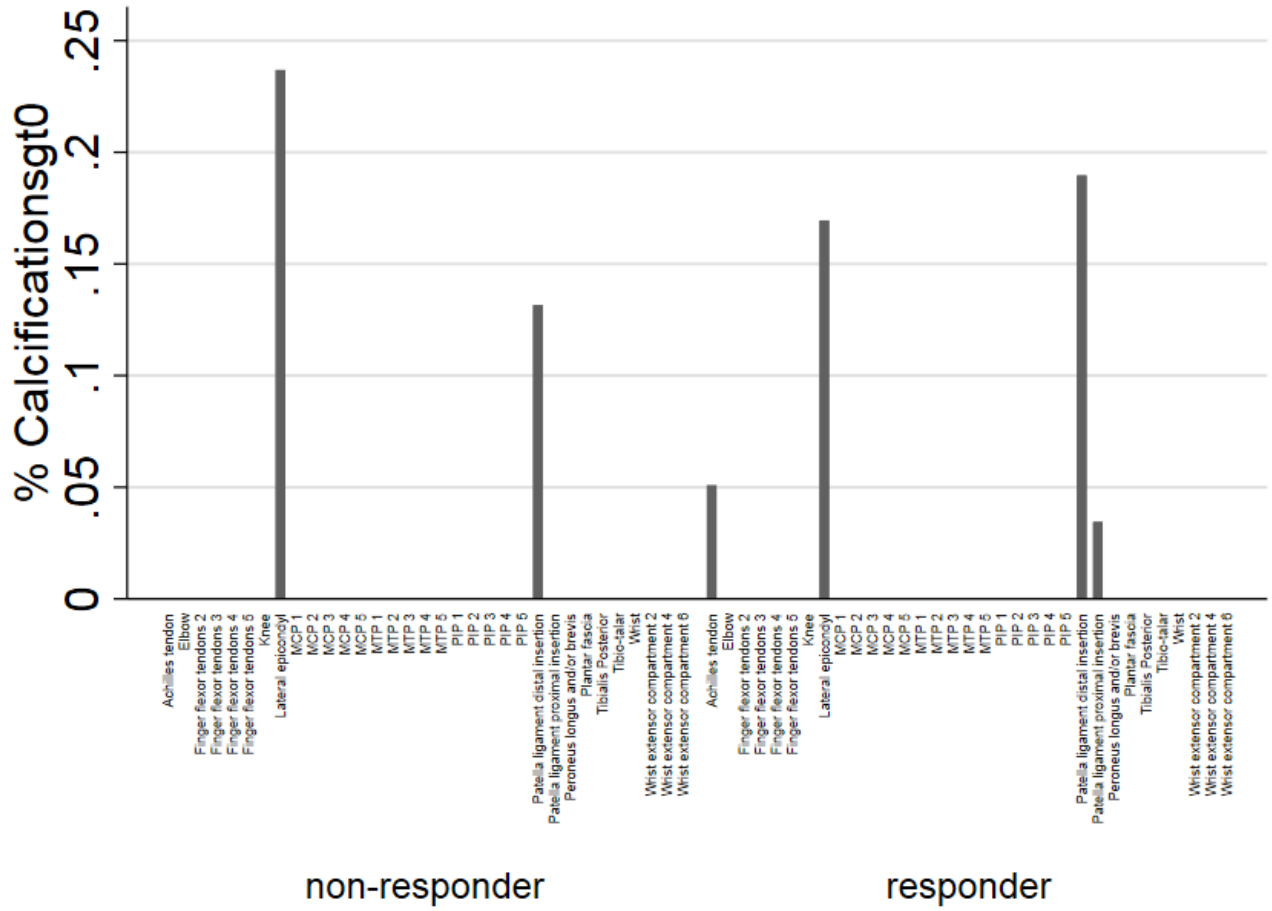

Figure 5.17:*Proportion with Calcifications at 6 months by response status (observed data only)*

Figure 5.17 shows the proportion of patients enthesis calcification at each site at 6 months in responders and non-responders. A small proportion of responders have calcification at the Achilles tendon and proximal insertion of the patella tendon which was interestingly not seen in non-responders, but again the numbers of patients within the cohort with changes at the enthesis is small.

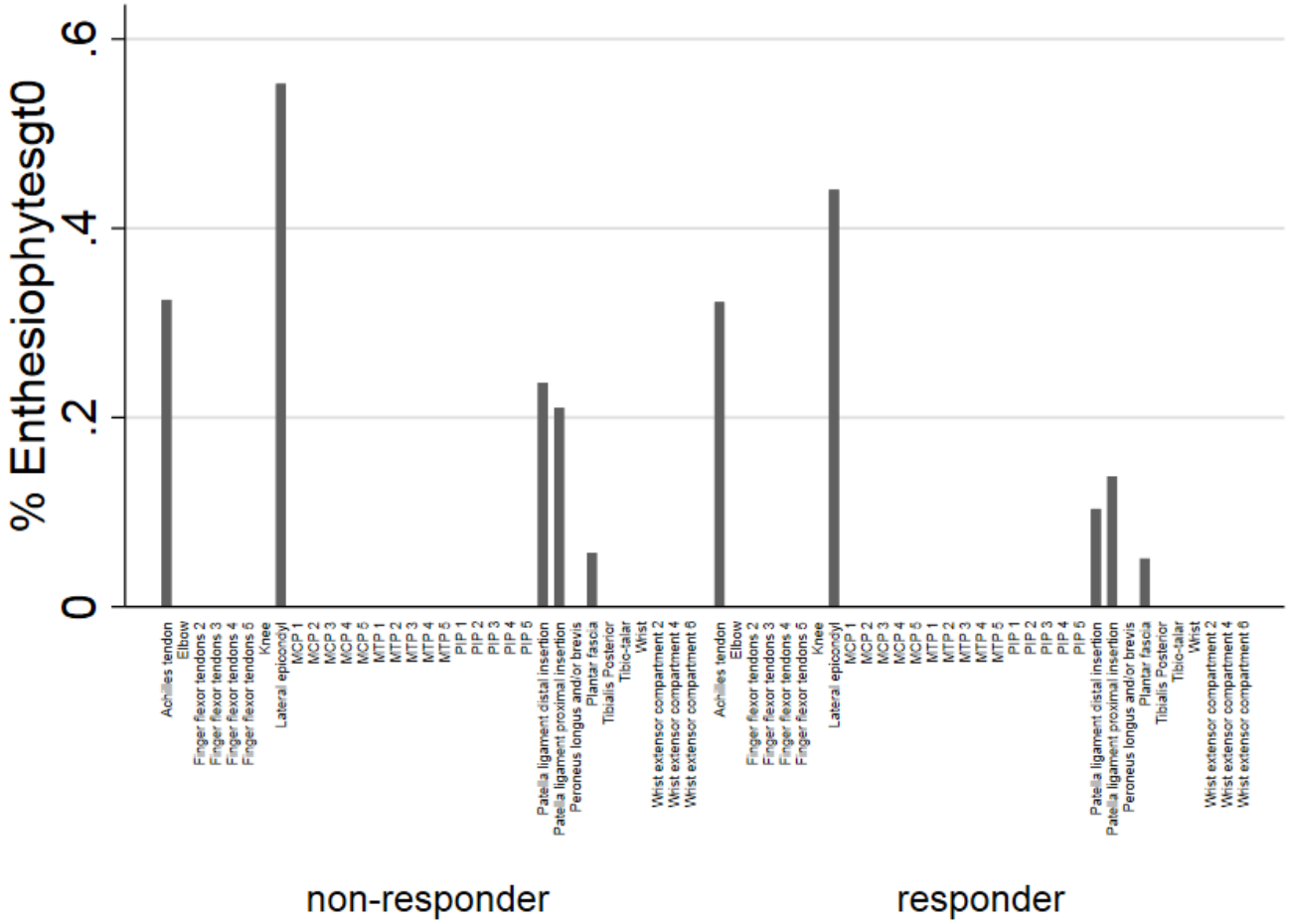

***Figure 5.18**:Proportion with Enthesiophytes at 6 months by response status (observed data only)*

Figure 5.18 shows the proportion pf patients with enthesiophytes at each enthesis at 6 months in responders and non-responders. The distribution is the same with a slightly greater proportion of non-responders having enthesiophytes.

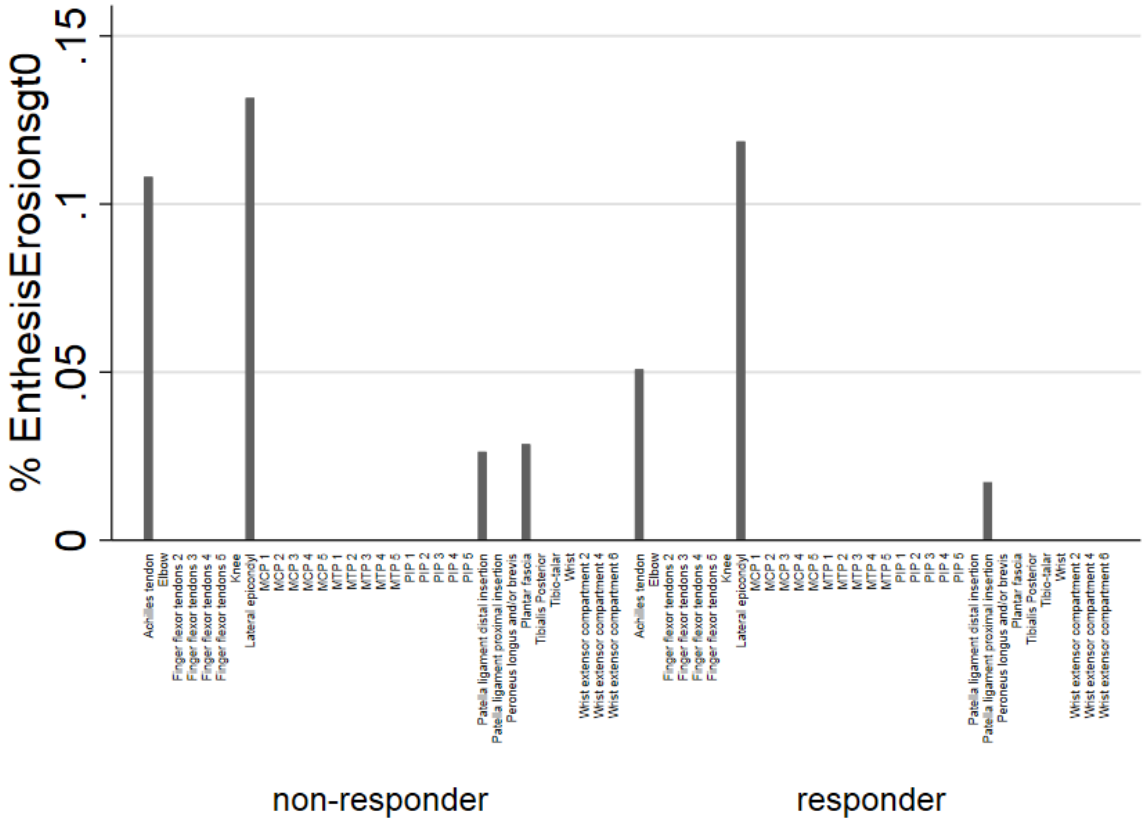

***Figure 5.19**: Proportion with Enthesis Erosions at 6 months by response status (observed data only)*

Figure 5.19 shows the proportion pf patients with enthesis erosions joint at 6 months in responders and non-responders. There is no clear difference in the distribution of erosions between the groups.

Figures 5.8 to 5.19 show proportions of patients with ultrasound characteristics at individual joints split into responders and non-responders to see if there are any clear differences in the distributions of joint inflammation between the two groups. Although the proportions of patients with GS and doppler synovitis are lower in responders at 6 months there are no clear differences in the patterns of joint or enthesis inflammation between responders and non-responders after 6 months of therapy. Interestingly the proportion of patients with osteophytes and subluxation, markers of joint damage, were higher in responders than non-responders. This may be indicative of responders having higher baseline activity and therefore accumulating more damage.

5.5 Chapter 5 summary and conclusions

Within this cohort I have not been able to show a statistically significant association with baseline synovitis or latent profile and response to therapy. I have therefore been able to refute the hypothesis that the presence of synovitis at baseline is associated with a response to TT. As a high proportion of patients in cluster 1, with limited inflammation, also respond to therapy it also does not appear that an absence of synovitis is associated with non-response. This highlights the complexity of treatment response in RA patients treated with TT as groups of patients with minimal disease activity on MUS still appear to respond well to treatment. For ethical reasons this study does not contain a placebo arm, which may account for clinical response in some patients with limited inflammation within this cohort, but it is also possible that differential effects of TT on non-synovial pathology or effects on pain independent of effects on inflammation may account for this phenomenon. JAKi has been suggested to improve pain independently of its effects on inflammation (442).

I have also not been able to show that the separate combined clinical and ultrasound derived clusters of established RA patients have a difference in changes in DAS-28 CRP over time in response to TT. However, I have shown a difference in the change in DAS28-CRP2C over time between the classes. DAS28CRP-2C was developed as a score which more accurately reflects PDUS than the conventional DAS28-CRP score (112) . These results do correlate with the proportionally greater improvements in both PD and GS synovitis and tenosynovitis shown in responders in cluster 3, the most inflammatory cluster, over the less significant improvements shown in the less inflammatory clusters. There was little difference in the ultrasound appearance between responders and non-responders after 6 months of therapy in the least inflammatory cluster 1, despite that fact that the majority had a EULAR response to TT. There was no statistically significant difference in chage in VAS score over time in response to TT between the clusters.

Review of the distributions of joint, tendon and entheseal MUS results in responders and non-responders at 6 months of therapy do not show any clear differences. This suggests that there is neither a clear US phenotype that is associated with non-response nor are there groups of non-responders whose ultrasound phenotype changes in response to therapy, but which is not associated with an improvement in DAS28 score.

6 Do different response trajectories exist within our cohort and how does this relate to baseline latent class?

6.1 Introduction

In Chapter 5, I investigated whether either the presence of baseline synovitis or membership of latent class was associated with a response to targeted therapy. I was not able to show a significant association with baseline synovitis or baseline disease cluster and subsequent DAS28-CRP response within our cohort. There was also no difference between the baseline latent classes in improvement in the 4 component DAS28-CRP over time. However, for the 2 component DAS28-CRP, there was a difference in change in DAS28 over time between the clinical and ultrasound variable derived clusters, which is reflected by changes identified on MUS. I looked further at ultrasound data which suggested that there was numerically greater improvement in both grey scale and PD synovitis and tenosynovitis in class 3, the most inflammatory cluster, than the other clusters. This likely accounts for the differential in DAS28-CRP2C, which has been shown to be more reflective of the presence of MUS defined synovitis, than the 4 factor DAS28 score.

Having established in chapter 5 that there was no difference in DAS28 response profile between the combined clinical and ultrasound clusters, I aimed to investigate whether treatment with targeted therapy causes a subset of patients to transition from one phenotypic cluster to another and how this transition is related to response and non-response. The initial plan to investigate this was to use latent transition analysis (LTA). This is a longitudinal extension of latent profile analysis which would allow us to determine whether patients change disease cluster over time. Non-response to therapy is likely to be a heterogeneous process. In some patients disease may not change at all after the initiation of TT, but in others a response may occur which changes their disease phenotype, but which is not reflected by a EULAR response on clinical assessment. For example, a patient may transition from a high inflammatory cluster to another cluster with less inflammation, but higher subjective DAS28

components, maintaining an elevated DAS28 score despite an identifiable response to treatment. Using LTA may allow us to identify this kind of transition. Unfortunately, the loss of follow up data due to the COVID-19 pandemic has meant that this approach is not viable. I have therefore performed an alternative analysis to identify whether different disease response trajectories exist within our data.

Growth mixture modelling (GMM) is another method for identifying unobserved sub-populations within data and describing longitudinal change within these sub-groups. GMM identifies subgroups who have similar patterns of change over time. It is therefore different from LPA/LTA which identifies clusters from baseline characteristics. GMM is an extension of the longitudinal mixed modelling performed in chapter 5. I have used GMM to identify sub-groups with differing disease trajectories and then compared these differing trajectories to the pre-existing combined clinical and ultrasound latent profiles identified in chapter 4 (Table 4.1, page164).

6.2 Chapter 6 aims

- To use growth mixture modelling to identify subgroups within the data associated with different treatment response trajectories.
- To explore the baseline characteristics of patients assigned to each trajectory and to look for differences between them.
- To compare the combined clinical and ultrasound latent classes identified in chapter 5 to these disease trajectories.

6.3 Chapter 6 methods

GMM was performed to identify different trajectories of change within the prospective cohort of established RA patients. One hundred and eleven patients had complete clinical follow up data. Analysis was performed in unimputed data as the GMM handles missing data directly in

the same way as LMM and sample sizes were matched to the previous analyses. GMM was first attempted using original 4-component DAS28CRP but according to the BIC values there were no solutions that improved over the single class model. In chapter 6 I identified differential treatment responses, or changes in DAS28CRP-2C over time, between the latent profiles. Therefore, I have also tested for different disease response trajectories using DAS28CRP-2C using GMM. Using DAS28CRP-2C, minimum BIC was achieved for four classes. Patients were assigned to the class for which the posterior probability of class membership was highest. The average posterior probability of assignment for classes 1-4 was 92%, 88%, 85% and 91% respectively (it is recommended that this is at least 70% in all classes). The odds of correct classification were 133.4, 5.8, 17.5 and 85.3 respectively (recommended to be >5). Mismatch scores were 0.001, 0.044, -0.033, -0.012 respectively (recommended to be close to zero). As a result, this four-class model was considered to be an acceptable fit. The 4-trajectory classes are shown graphically in **figure 6.1**

6.4 Chapter 6 results

6.4.1 Do groups of patients with differing disease trajectories exist within our cohort?

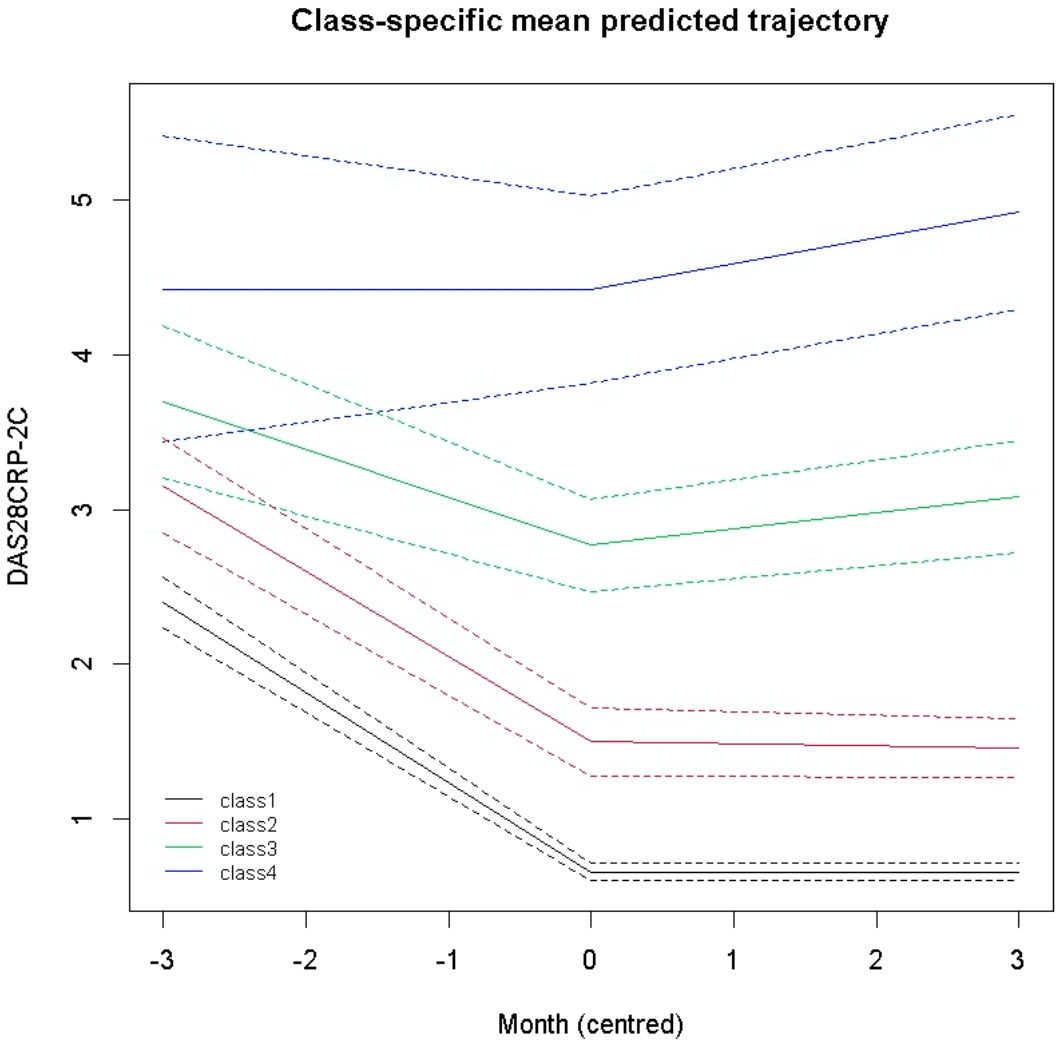

***Figure 6.1**: Predicted mean trajectories of change in DAS28CRP-2C within each of four classes identified via growth mixture modelling.*

Figure 6.1 shows a plot of the mean DAS28-CRP2C for the 4 different classes identified using GMM. Class 1 has the lowest disease activity at baseline and shows a rapid improvement at 3 months with a maintenance of response up to 6 months. Class 2 shows a rapid improvement at 3 months with a much slower further improvement up to 6 months. In class 3 there is an improvement up to 3 months with an increase in DAS28-CRP2C after 3 months, although not

back up to their baseline DAS28-CRP. In class 4, the class with the highest average baseline DAS28-CRP2C, on average there is no improvement up to 3 months and then DAS28CRP-2C gets worse up to 6 months.

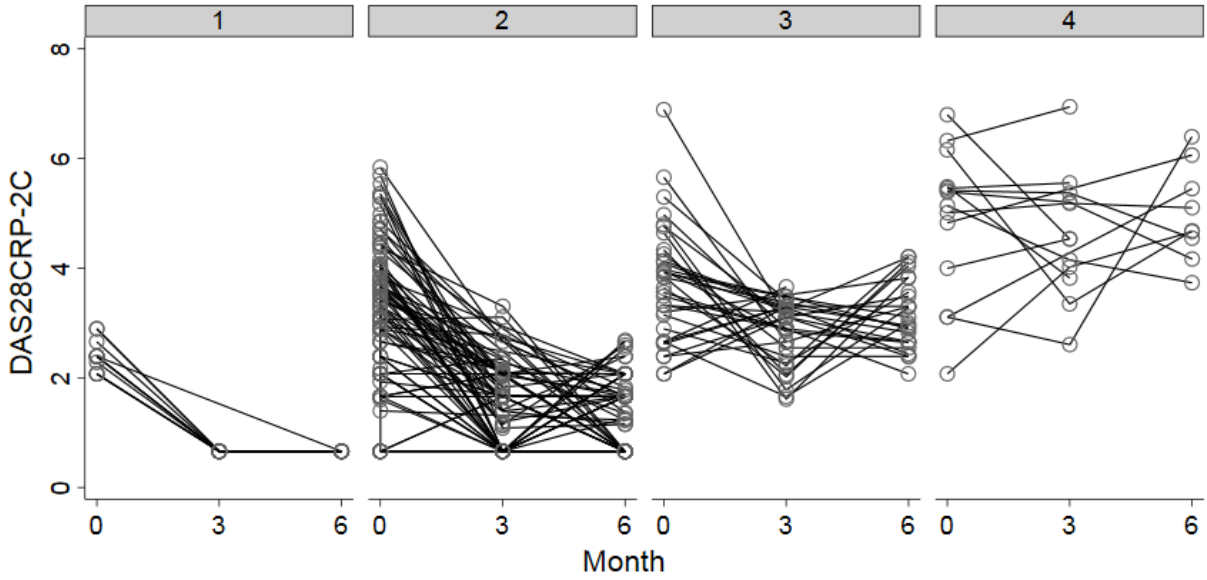

***Figure 6.2**: Patient-level trajectories of change in DAS28CRP-2C within each of four classes identified via growth mixture modelling.*

Figure 6.2 shows patient level plots within each class. Most patients fall within classes 2 (92/153) or 3 (33/153) for their disease trajectory. Trajectory 1 is the smallest group (12/153) and the individual trajectories show significant consistency between the patients within the profile. There is more heterogeneity in the other trajectory classes. Not all patients within the cohort will fit neatly into one of the identified trajectory classes, although the pattens shown in figure 7.1 are clearly discernible from the graphs of individuals patient trajectory.

Table 6.1 shows the baseline demographics, clinical characteristics, and PRO outcomes for patients in each response trajectory class expressed as median (IQR) scores for each class for continuous variables and numbers of patients and percentages of the total cohort for categorical outcomes.

	DAS28CRP-2C Trajectory						
	1 N=12	2 N=93	3 N=33	4 N=15	Total N=153	Chi^{2*}	p
Age, years	54.1 (46.0, 61.0), n=12	57.3 (50.0, 63.3), n=93	53.6 (45.3, 60.1), n=33	59.5 (52.0, 68.6), n=15	56.5 (49.6, 63.3), n=153	3.435	0.3293
Sex Female	9/12 (75.0%)	70/93 (75.3%)	25/33 (75.8%)	8/15 (53.3%)	112/153 (73.2%)	3.3508	0.341
Ever smoked Yes	2/10 (20.0%)	33/77 (42.9%)	16/30 (53.3%)	9/15 (60.0%)	60/132 (45.5%)	4.8540	0.183
Disease duration, years	8.5 (2.5, 18.5), n=8	8.0 (5.0, 15.0), n=75	10.5 (4.5, 19.0), n=32	13.0 (10.0, 19.0), n=12	10.0 (5.0, 17.0), n=127	5.549	0.1358
RF positive Yes	7/12 (58.3%)	55/93 (59.1%)	18/33 (54.5%)	11/15 (73.3%)	91/153 (59.5%)	1.5388	0.673
ACPA positive Yes	10/12 (83.3%)	58/93 (62.4%)	22/33 (66.7%)	12/15 (80.0%)	102/153 (66.7%)	3.4742	0.324
ANA positive Yes	1/12 (8.3%)	9/93 (9.7%)	4/33 (12.1%)	3/15 (20.0%)	17/153 (11.1%)	1.5214	0.677
X-ray erosions present Yes	7/12 (58.3%)	38/93 (40.9%)	13/33 (39.4%)	11/15 (73.3%)	69/153 (45.1%)	10.1303	0.119
TJC	10.0 (6.0, 15.0), n=12	12.0 (6.0, 16.0), n=93	14.0 (8.0, 18.0), n=33	16.0 (9.0, 19.0), n=15	12.0 (7.0, 17.0), n=153	4.152	0.2455
SJC	3.0 (2.0, 3.5), n=12	4.0 (2.0, 7.0), n=93	4.0 (3.0, 6.0), n=33	8.0 (6.0, 12.0), n=15	4.0 (2.0, 7.0), n=153	14.703	**0.0021**
CRP	2.0 (2.0, 2.0), n=12	6.0 (2.0, 23.0), n=93	13.3 (2.0, 32.0), n=33	37.0 (2.0, 79.0), n=15	8.2 (2.0, 27.0), n=153	18.981	**0.0003**
VAS	74.0 (69.0, 80.0), n=12	75.0 (60.0, 80.0), n=93	70.0 (60.0, 80.0), n=33	75.0 (65.0, 90.0), n=15	75.0 (60.0, 80.0), n=153	0.703	0.8725
ESR	8.5 (1.0, 23.0), n=8	15.5 (8.0, 30.0), n=86	22.5 (10.0, 32.5), n=32	29.0 (11.5, 64.0), n=12	18.0 (8.0, 31.0), n=138	6.831	0.0775
HADS Anxiety	9.5 (5.0, 12.5), n=12	8.0 (4.5, 11.0), n=92	10.0 (5.0, 13.0), n=33	11.0 (7.0, 13.0), n=15	9.0 (5.0, 12.0), n=152	3.129	0.3722
HADS Depression	9.0 (5.5, 12.0), n=12	7.5 (4.5, 11.0), n=92	9.0 (7.0, 14.0), n=33	12.0 (11.0, 13.0), n=15	9.0 (5.0, 12.0), n=152	13.170	**0.0043**

	DAS28CRP-2C Trajectory						
	1	2	3	4	Total		
	N=12	N=93	N=33	N=15	N=153	Chi^{2*}	p
FACIT-Fatigue	19.0 (11.0, 25.5), n=12	19.0 (10.0, 30.0), n=92	14.0 (8.0, 23.0), n=33	15.5 (13.0, 25.0), n=14	18.0 (10.0, 28.0), n=151	2.359	0.5013
HAQ	1.8 (1.0, 2.2), n=12	1.6 (1.1, 2.1), n=92	1.9 (1.5, 2.5), n=33	2.1 (2.0, 2.4), n=15	1.8 (1.2, 2.2), n=152	10.359	**0.0157**
DAS28CRP	4.8 (4.1, 5.0), n=12	5.2 (4.5, 5.7), n=93	5.4 (5.1, 6.1), n=33	6.1 (5.4, 6.7), n=15	5.3 (4.5, 5.9), n=153	17.939	**0.0005**
DAS28CRP-P	0.6 (0.6, 0.6), n=12	0.6 (0.5, 0.6), n=93	0.6 (0.5, 0.6), n=33	0.5 (0.5, 0.6), n=15	0.6 (0.5, 0.6), n=153	7.125	0.0680
DAS28CRP-2C	2.4 (2.2, 2.5), n=12	3.3 (2.2, 4.0), n=93	3.8 (2.9, 4.3), n=33	5.1 (3.1, 5.5), n=15	3.3 (2.4, 4.1), n=153	23.395	**0.0001**
N previous treatments	1.5 (0.0, 3.5), n=12	1.0 (0.0, 2.0), n=93	2.0 (1.0, 3.0), n=33	3.0 (2.0, 5.0), n=15	1.0 (0.0, 3.0), n=153	11.189	**0.0107**
N previous classes	1.5 (0.0, 3.0), n=12	1.0 (0.0, 2.0), n=93	1.0 (1.0, 3.0), n=33	3.0 (1.0, 4.0), n=15	1.0 (0.0, 3.0), n=153	11.028	**0.0116**
DAS28CRP>3.2 Yes	12/12 (100.0%)	92/93 (98.9%)	33/33 (100.0%)	15/15 (100.0%)	152/153 (99.3%)		
DAS28ESR>3.2 Yes	8/8 (100.0%)	80/86 (93.0%)	29/32 (90.6%)	12/12 (100.0%)	129/138 (93.5%)		
Clinical synovitis (PD>=2 | totalPD>3) Yes	8/11 (72.7%)	63/90 (70.0%)	31/33 (93.9%)	14/15 (93.3%)	116/149 (77.9%)		
N previous treatments	1.5 (0.0, 3.5), n=12	1.0 (0.0, 2.0), n=93	2.0 (1.0, 3.0), n=33	3.0 (2.0, 5.0), n=15	1.0 (0.0, 3.0), n=153		
N previous classes	1.5 (0.0, 3.0), n=12	1.0 (0.0, 2.0), n=93	1.0 (1.0, 3.0), n=33	3.0 (1.0, 4.0), n=15	1.0 (0.0, 3.0), n=153		

***Table 6.1**: Baseline clinical characteristics by response trajectory*

ACPA – Anti-Citrullinated protein Antibody, RF- Rheumatoid Factor, ANA – Anti-Nuclear Antibody, TJC – Tender Joint Count, Swollen Joint Count, VAS – Visual analogue Scale, CRP- C Reactive Protein, ESR – Erythrocyte Sedimentation Rate, HAQ – Health Assessment Questionnaire, HADs – Hospital Anxiety and Depression Scale, FACIT – Functional Assessment of Chronic Illness Therapy, DAS – Disease Activity Score, RF- Rheumatoid Factor.

* Kruscal-Wallis test performed to identify differences between clusters for continuous variables, chi^2 for categorical variables. Significant differences highlighted in bold

Table 6.1 shows the baseline clinical data and PRO outcomes for patients assigned to each different trajectory class. Interestingly there are similar outcomes shown in this analysis as we saw with the LPA despite the different methodology, with clear differences in baseline inflammation, which increases as we ascend the trajectories. The baseline characteristics are notable for showing that trajectory 4, which shows no response to therapy, not only has higher baseline disease activity, longer disease duration and more erosive change, but has also been exposed to more previous TTs and classes of TT, suggesting that this represents a group of refractory patients. This trajectory was also more male and contains more current or former smokers than the other groups. Smoking is associated with worse prognosis RA. PRO outcomes at baseline in these trajectory classes follow inflammation and are universally highest in the high inflammatory class (trajectory 4). Further analysis using K-W testing with a post hoc Dunn test for continuous variables, and Chi^2 testing for categorical outcomes has been performed to determine which classes are different from one another for variables where statistical significance has been shown. The results are shown above in **table 6.1**.

Statistically significant differences are shown for SJC and CRP, HADs depression and HAQ as well as DAS28-CRP, DAS28CRP-2C and numbers of previous TTs and classes of TT. **Table 6.2** shows the p values generated using the Dunn test for characteristics with significant differences identified in K-W test, significant differences are highlighted in bold.

Characteristic	Comparison between response trajectories (p value)					
	1/2	1/3	1/4	2/3	2/4	3/4
SJC	0.0648	**0.0128**	**0.0003**	0.0778	**0.0009**	**0.0317**
CRP	**0.0117**	**0.0016**	**0.0000**	0.0685	**0.0008**	**0.0316**
HADS D	0.1712	0.3373	**0.0545**	**0.0164**	**0.0005**	0.0620
HAQ	0.4504	0.1162	**0.0327**	**0.0149**	**0.0035**	0.1591
Previous TTs	0.2554	0.3693	**0.0375**	0.0605	**0.0007**	**0.0319**
Previous classes of TT.	0.3029	0.3694	**0.0274**	0.0910	**0.0006**	**0.0213**

***Table 6.2**: Dunn test results for clinical and PRO variables with statistically significant differences between disease response trajectories. Significant results highlighted in bold.*

SJC – Swollen Joint Count, CRP – C-Reactive Protein, HADs D – HADs Depression, HAQ – Health assessment questionnaire, TT – Targeted Therapy.

Table 6.2 shows that the swollen joint count is significantly higher in trajectory 4 than in all the other trajectories. It is also significantly higher in trajectory 3 than 1, but otherwise there is no difference between the other groups. Trajectory 4 also has a significantly higher CRP than all the other trajectories, and trajectories 2 and 3 are higher than 1. Trajectory 4 also has significantly higher levels of baseline depression score (HADSs D) and HAQ-DI than trajectories 1 and 2, but not 3. Trajectory 4 has been exposed to significantly more TTs and classes of TT than the other trajectories.

Table 6.3 shows baseline ultrasound results for the response trajectories. 149 of the 153 patients had full baseline ultrasound results.

	DAS28CRP-2C Trajectory				
	1	2	3	4	Total
	N=11	N=90	N=33	N=15	N=149
Total joint GS	30.0 (25.0, 37.0), n=11	32.0 (24.0, 45.0), n=90	36.0 (29.0, 47.0), n=33	56.0 (37.0, 70.0), n=15	34.0 (25.0, 45.0), n=149
Total joint GS>0 Yes	11/11 (100.0%)	90/90 (100.0%)	33/33 (100.0%)	15/15 (100.0%)	149/149 (100.0%)
Total joint PD	5.0 (2.0, 9.0), n=11	5.0 (2.0, 14.0), n=90	10.0 (4.0, 20.0), n=33	22.0 (7.0, 45.0), n=15	6.0 (3.0, 16.0), n=149
Total joint PD>0 Yes	10/11 (90.9%)	75/90 (83.3%)	32/33 (97.0%)	14/15 (93.3%)	131/149 (87.9%)
N Js w/ erosions	2.0 (0.0, 4.0), n=11	2.0 (0.0, 4.0), n=90	2.0 (1.0, 4.0), n=33	5.0 (3.0, 8.0), n=15	2.0 (1.0, 4.0), n=149
N Js w/ erosions>0 Yes	7/11 (63.6%)	63/90 (70.0%)	28/33 (84.8%)	15/15 (100.0%)	113/149 (75.8%)
N Js w/ osteophytes	2.0 (0.0, 5.0), n=11	2.0 (1.0, 6.0), n=90	4.0 (2.0, 7.0), n=33	3.0 (0.0, 8.0), n=15	3.0 (1.0, 6.0), n=149
N Js w/ osteophytes>0 Yes	7/11 (63.6%)	71/90 (78.9%)	27/33 (81.8%)	11/15 (73.3%)	116/149 (77.9%)
N Js subluxed	4.0 (0.0, 6.0), n=11	3.0 (1.0, 6.0), n=90	6.0 (2.0, 7.0), n=33	5.0 (2.0, 8.0), n=15	4.0 (1.0, 6.0), n=149
N Js subluxed>0 Yes	7/11 (63.6%)	70/90 (77.8%)	28/33 (84.8%)	14/15 (93.3%)	119/149 (79.9%)
Total tendon GS	4.0 (0.0, 5.0), n=11	3.0 (1.0, 7.0), n=90	5.0 (2.0, 10.0), n=33	9.0 (6.0, 16.0), n=15	4.0 (1.0, 8.0), n=149
Total tendon GS>0 Yes	8/11 (72.7%)	75/90 (83.3%)	26/33 (78.8%)	15/15 (100.0%)	124/149 (83.2%)
Total tendon PD	0.0 (0.0, 3.0), n=11	0.0 (0.0, 4.0), n=90	2.0 (0.0, 4.0), n=33	5.0 (1.0, 13.0), n=15	1.0 (0.0, 4.0), n=149
Total tendon PD>0 Yes	5/11 (45.5%)	37/90 (41.1%)	22/33 (66.7%)	12/15 (80.0%)	76/149 (51.0%)
N thickened Ts	0.0 (0.0, 1.0), n=11	0.0 (0.0, 1.0), n=89	0.0 (0.0, 1.0), n=33	0.0 (0.0, 1.0), n=15	0.0 (0.0, 1.0), n=148

	DAS28CRP-2C Trajectory				
	1	**2**	**3**	**4**	**Total**
	N=11	**N=90**	**N=33**	**N=15**	**N=149**
N thickened Ts>0	4/11	27/89	12/33	5/15	48/148
Yes	(36.4%)	(30.3%)	(36.4%)	(33.3%)	(32.4%)
Total enthesis GS	4.0 (1.0, 6.0), n=11	2.0 (1.0, 4.0), n=90	3.0 (2.0, 5.0), n=33	2.0 (2.0, 4.0), n=15	3.0 (1.0, 4.0), n=149
Total enthesis GS>0 Yes	10/11 (90.9%)	77/90 (85.6%)	30/33 (90.9%)	15/15 (100.0%)	132/149 (88.6%)
Total enthesis PD	0.0 (0.0, 1.0), n=11	0.0 (0.0, 0.0), n=90	0.0 (0.0, 1.0), n=33	0.0 (0.0, 1.0), n=15	0.0 (0.0, 0.0), n=149
Total enthesis PD>0 Yes	3/11 (27.3%)	14/90 (15.6%)	9/33 (27.3%)	4/15 (26.7%)	30/149 (20.1%)
N thickened Es	2.0 (1.0, 4.0), n=11	2.0 (1.0, 3.0), n=90	2.0 (2.0, 3.0), n=33	2.0 (2.0, 3.0), n=15	2.0 (2.0, 3.0), n=149
N thickened Es>0 Yes	10/11 (90.9%)	80/90 (88.9%)	30/33 (90.9%)	15/15 (100.0%)	135/149 (90.6%)
N Es w/ erosions	0.0 (0.0, 0.0), n=11	0.0 (0.0, 1.0), n=90	0.0 (0.0, 1.0), n=33	1.0 (0.0, 2.0), n=15	0.0 (0.0, 1.0), n=149
N Es w/ erosions>0 Yes	2/11 (18.2%)	27/90 (30.0%)	11/33 (33.3%)	9/15 (60.0%)	49/149 (32.9%)
N Es w/ enthesophytes	1.0 (0.0, 4.0), n=11	1.5 (0.0, 2.0), n=90	1.0 (0.0, 2.0), n=33	2.0 (1.0, 3.0), n=15	1.0 (0.0, 2.0), n=149
N Es w/ enthesophytes>0 Yes	7/11 (63.6%)	61/90 (67.8%)	19/33 (57.6%)	13/15 (86.7%)	100/149 (67.1%)
N Es w/ calcifications	0.0 (0.0, 2.0), n=11	0.0 (0.0, 1.0), n=90	0.0 (0.0, 1.0), n=33	0.0 (0.0, 0.0), n=15	0.0 (0.0, 1.0), n=149
N Es w/ calcifications>0 Yes	5/11 (45.5%)	28/90 (31.1%)	10/33 (30.3%)	1/15 (6.7%)	44/149 (29.5%)

***Table 6.3**: Ultrasound findings by response trajectory*

GS – Grey scale, PD – Power Doppler, N Js w/ - Number of joints with. N – Number, Ts – Tendons, N Es w/ - Number of entheses with.

Table 6.3 shows the baseline ultrasound scores expressed as median scores and number and percentages of joints with each US variable. Both GS and PD synovitis and tenosynovitis are highest in trajectory 4 corresponding with the clinical findings. Trajectory 3, which is characterised by an initial improvement and then a worsening after 3 months, has the highest levels of osteophytes and joint subluxation of the groups, suggestive of numerically more joints with osteoarthritis in this trajectory.

US characteristic	X^2	κ	p
Total joint B mode	12.423	3	**0.0061**
Total joint Doppler	15.315	3	**0.0016**
Total joint erosions	15.474	3	**0.0015**
Total joint osteophytes	2.860	3	0.4138
Total joint subluxation	4.810	3	0.1863
Total tendon B mode	18.148	3	**0.0004**
Total tendon Doppler mode	11.822	3	**0.0080**
Total tendon thickening	0.532	3	0.9118
Total Enthesis hypogenicity	7.228	3	0.0650
Total enthesis Doppler	2.714	3	0.4379
Total enthesis thickening	1.003	3	0.8005
Total enthesis erosions	7.322	3	0.0623
Total entheisophytes	3.269	3	0.3520
Total enthesis calcification	6.352	3	0.0957

***Table 6.4**: Results of the K-W tests comparing ultrasound scores between the different response trajectory classes.*

Statistically significant differences between the different disease trajectories were found in joint GS and PD, joint erosions and tendon GS and PD. No statistically significant difference was found in osteophytes or subluxation and no differences were identified for enthesis scores.

US characteristic	**Comparison between trajectory classes (p value)**					
	1/2	1/3	1/4	2/3	2/4	3/4
Total joint B mode	0.3020	0.0967	**0.0033**	0.0792	**0.0005**	**0.0225**
Total joint Doppler	0.3616	0.0598	**0.0031**	**0.0176**	**0.002**	**0.0404**
Total joint erosions	0.4866	0.3124	**0.0033**	0.1869	**0.0000**	**0.0018**
Total tendon B mode	0.4207	0.1591	**0.0011**	0.0817	**0.0000**	**0.0026**
Total tendon Doppler mode	0.4667	0.1049	**0.0138**	**0.0220**	**0.0012**	0.0796

Table 6.5*: Dunn test results for ultrasound variables with statistically significant differences between disease response trajectories. Significant results highlighted in bold.*

Table 6.5 shows the p values generated by performing Dunn's test for the statistically significant results from the K-W analysis. Trajectory 4 has significantly more Joint GS and PD, joint erosion and tendon GS than all of the other trajectory classes. Trajectory 4 also has significantly more tendon Doppler signal than trajectories 1 and 2, but not trajectory 3.

I have now identified 4 different classes of disease trajectory within our cohort, but how do these classes associate with the 3 latent clusters identified in chapter 5. Our LPA clusters were identified using all 4 components of the DAS28-CRP as indicator variables, as well as

US variables, but were best defined by differences in their SJC and CRP (page170). **Table 6.6** shows the numbers and percentages of patients in each DAS28-CRP2C trajectory class who have been assigned to each of the combined clinical and ultrasound latent classes. As shown above, most patients fall within disease trajectory class 2 and this is the most common trajectory for each of our 3 latent classes. However, it is notable that 75.41% of latent class 1 follow trajectory 2, which is associated with a sustained response to therapy, with none in trajectory class 4 which is characterised by a lack of response. This is despite the fact that latent class 1 has low levels of inflammation and minimal changes on ultrasound in response to therapy or differences between responders and non-responders. Latent class 2 has 55.17% of patients within trajectory class 2, with 29.31% in class 2. More patients from clinical and US class 3 are found in trajectory class 4 than the other classes (27.27%), possibly reflecting their more severe active disease at baseline.

US&Clin Class	**DAS28CRP-2C Trajectory**				
	1	**2**	**3**	**4**	**Total**
1 (Frequency**)**	7	46	8	0	61
(%)	11.48	75.41	13.11	0.00	100.00
2	4	32	17	5	58
	6.90	55.17	29.31	8.62	100.00
3	1	15	8	9	33
	3.03	45.45	24.24	27.27	100.00
Total	12	93	33	14	152
	7.89	61.18	21.71	9.21	100.00

Table 6.6*: Proportions of patients following each DAS28CRP-2C trajectory according to their baseline US & clinical variable latent class membership (total N=152).*

6.4.2 How does DAS28-CRP EULAR response associate with disease trajectory?

Next, I have looked at how response to TT, defined using EULAR response criteria relates to disease trajectory class. There is no validated definition of response that relates to DAS28CRP-2C, so response was determined according to DAS28CRP. In this study responders are those with a EULAR moderate or good response. Trajectories were defined using DAS28CRP-2C. **Table 6.7** shows the percentages of patients within each trajectory class with a EULAR response.

	DAS28CRP-2C Trajectory			
	1	**2**	**3**	**4**
N (152)	12	93	33	14
Resp%	93	74	48	18
AE%	0	11	3	14

Table 6.7*: Proportions of DAS28CRP EULAR responders at 6 months by DAS28CRP-2C trajectory (estimated in imputed data; total N=152)*

The majority of patients in trajectory 1 are EULAR responders as expected. The proportions of responders decreases as we ascend the trajectories. Trajectory 4, the most resistant, group also has the highest proportion of patients who suffered an adverse effect from their therapy, explaining why some of this groups did not respond well to treatment.

Figure 6.3 shows individual patient disease trajectories divided by EULAR responders and non-responders.

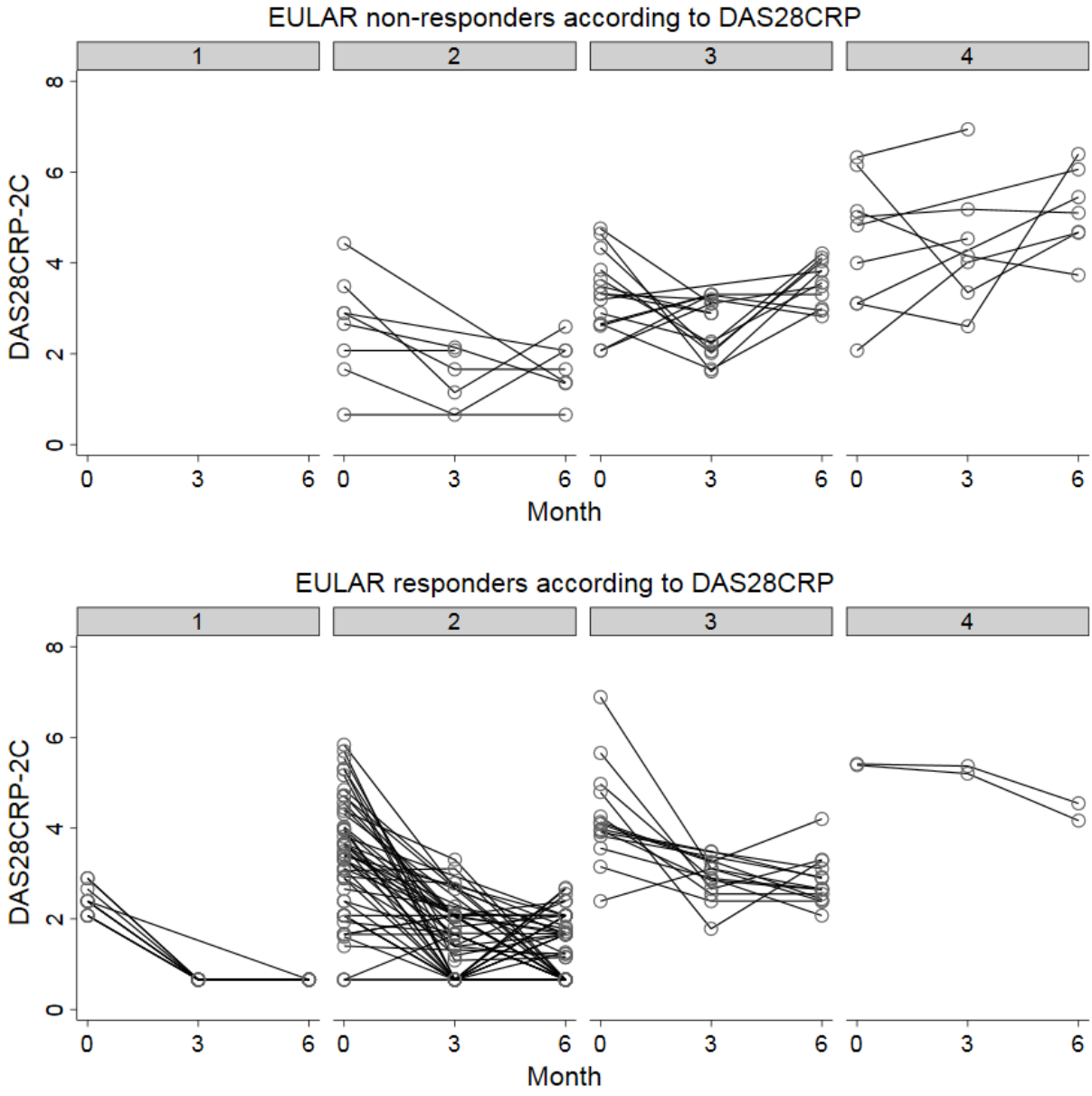

***Figure 6.3**:Patient-level trajectories of change in DAS28CRP-2C within each of four classes identified via growth mixture modelling, split by EULAR response status at 6 months*

Figure 6.3 shows patient level disease trajectories divided by responders and non-responders using observed data. All patients in trajectory class 1 are EULAR responders, as are the majority of class 2. Trajectory class 3, which has a mixture of responders and non-responders. Two patients in trajectory class 4 responded to therapy, but the majority in this group are non-responders.

6.5 Chapter 6 summary and conclusions

Using growth mixture modelling we have been able to identify 4 disease trajectories in response to targeted therapy. Most patients within our cohort are responders and most follow trajectory 2, which is characterised by an initial rapid response to treatment up to 3 months and a slower, but ongoing response up to 6 months. A small number of patients follow trajectory 1 which is characterised by a good response followed by sustained low disease activity. Trajectory 4, which is characterised by a lack of response to treatment, appears to represent a group of refractory patients who have previously been exposed to a significantly greater number of TTs than the other groups. This is a relatively small group of patients (15), but interestingly it is characterised by having the most significant levels of both clinical and ultrasound identified synovitis, suggesting persistent inflammation despite exposure to multiple TTs. Although they show more erosive disease, there is no significant difference in osteophytosis or joint subluxation and there is no statistically significant difference in PRO outcomes, suggesting this may represent a trajectory of “true RefRA’’ patients.

Comparing our disease trajectories to the combined clinical and ultrasound LPA model identified in chapter 5, in the most inflammatory cluster identified (cluster 3), a greater proportion of patients follow trajectory 4, which is associated with non-response. However, most still respond to therapy. Interestingly more patients in cluster 1, which is characterised by low levels of baseline inflammation, follow disease trajectories characterised by a good response to therapy. This once again highlights a lack of association between baseline synovitis and TT response within this cohort.

7 Refractory rheumatoid arthritis and JAK inhibitor use at Leeds Teaching Hospitals

7.1 Introduction

Over the previous 4 chapters I have discussed the analysis performed on our prospectively recruited cohort of established RA patients starting TT (cohort 1). I have identified clusters of patients within our cohort using DAS28 components, ultrasound characteristics and a combination of these factors as clustering variables. I have shown that neither the presence of baseline synovitis nor baseline disease cluster predicts a EULAR response to therapy. I have also identified a number of differing disease response trajectories within our cohort, including a RefRA disease trajectory. In this chapter I will discuss the retrospective analysis of RefRA patients at LTHT (cohort 2).

In chapter one I covered the various definitions of RefRA currently available in the literature, including the recently published EULAR definition of difficult to treat RA (9). The EULAR guidance gives a broad classification encompassing a wide variety of patients with differing reasons for a lack of response to treatment and helps to provide a framework for research into RefRA. However, a distinction can be drawn between the concept of difficult to treat RA, where inadequate disease control is a multifactorial process combining non-response to therapy with co-morbidity and poor adherence amongst other factors, and the concept of “true” biological refractory disease, where synovitis persists despite the optimisation of multiple sequential therapeutic strategies. I have also highlighted the discrepancy between the reported prevalence of RefRA due to the differing definitions of RefRA used in the literature. These different definitions make it difficult to determine the exact extent of refractory disease in the RA population and thus, what proportion of patients can be expected to develop RefRA.

Around 40% of RA patients require escalation to TT and current estimates suggest somewhere between 6% and 20% of these patients cycle through at least two TTs (339).

LTHT has more than 1500 RA patients who have been exposed to 1 or more TT and has been at the forefront of research into the treatment of RA for more than 20 years. As a result, many patients have been exposed to bDMARDs since they first became available in the late 1990s, and many have cycled through several drugs in that time. I performed a retrospective analysis of our cohort of patients in order to explore the prevalence of Ref RA within our population and to see if a subpopulation of patients exists within our cohort who have exhibited multiple primary drug failures, representing a cohort of patients with “true” RefRA.

On a pragmatic level, the state of refractory RA has evolved based on when all the potentially useful therapeutic options available at the time, have been exhausted. A term that, in practice, once meant failure to respond to MTX now includes patients who have failed all the available classes of bDMARD. Over recent years the introduction of the JAK inhibitors, small molecules which inhibit the intracellular signalling cascade common to multiple cytokines, has added a further layer of complexity. In theory JAK inhibition may be an important therapeutic option in advanced RA. The ability to inhibit multiple inflammatory cytokines in tandem may help to avoid the issue of cytokine redundancy, where multiple cytokines can perform the same biological function, which could potentially be one reason for lack of efficacy with single cytokine inhibitors. Emerging evidence from JAKi clinical trials also suggests that JAKi may show greater efficacy in the improvement of pain than other bDMARDs (442). This could be beneficial in a subset of patients with RefRA, whose pain is related to the accumulation of joint damage due to prolonged periods sub-optimal disease control. However, clearly not all patients respond to JAKi and thus there is now a growing cohort who have not responded to one, or even both of the available JAKis.

I used these concepts to stratify our cohort into 3 subclasses; class 1 who have failed 2 classes of bDMARD and are on the milder end of the spectrum of refractory disease, class 2 who have failed 3-4 classes of bDMARD and are thus more refractory and class 3, who have failed at least 2 bDMARD and a JAKi. This group represents patients in whom the broader spectrum of cytokine blockade by JAKi has not been successful and I have highlighted this cohort as it has been suggested that JAKi may have a particular role in RefRA as outline above. This classification is represented in **Figure 7.1. I** report the proportions of each subclassification within our cohort.

I also report initial experience with the JAKi tofacitinib and baricitinib at LTHT with the objective of highlighting the real-world efficacy of JAK inhibition across the treatment pathway from first-line targeted therapy to their use in those who have tried and failed multiple targeted therapies. As JAK selectivity differs between the available JAKi I will also report on a small number of cases of patients who have switched directly between the two available JAKi and assess JAKi efficacy in patients previously exposed to an IL-6 blocking TT.

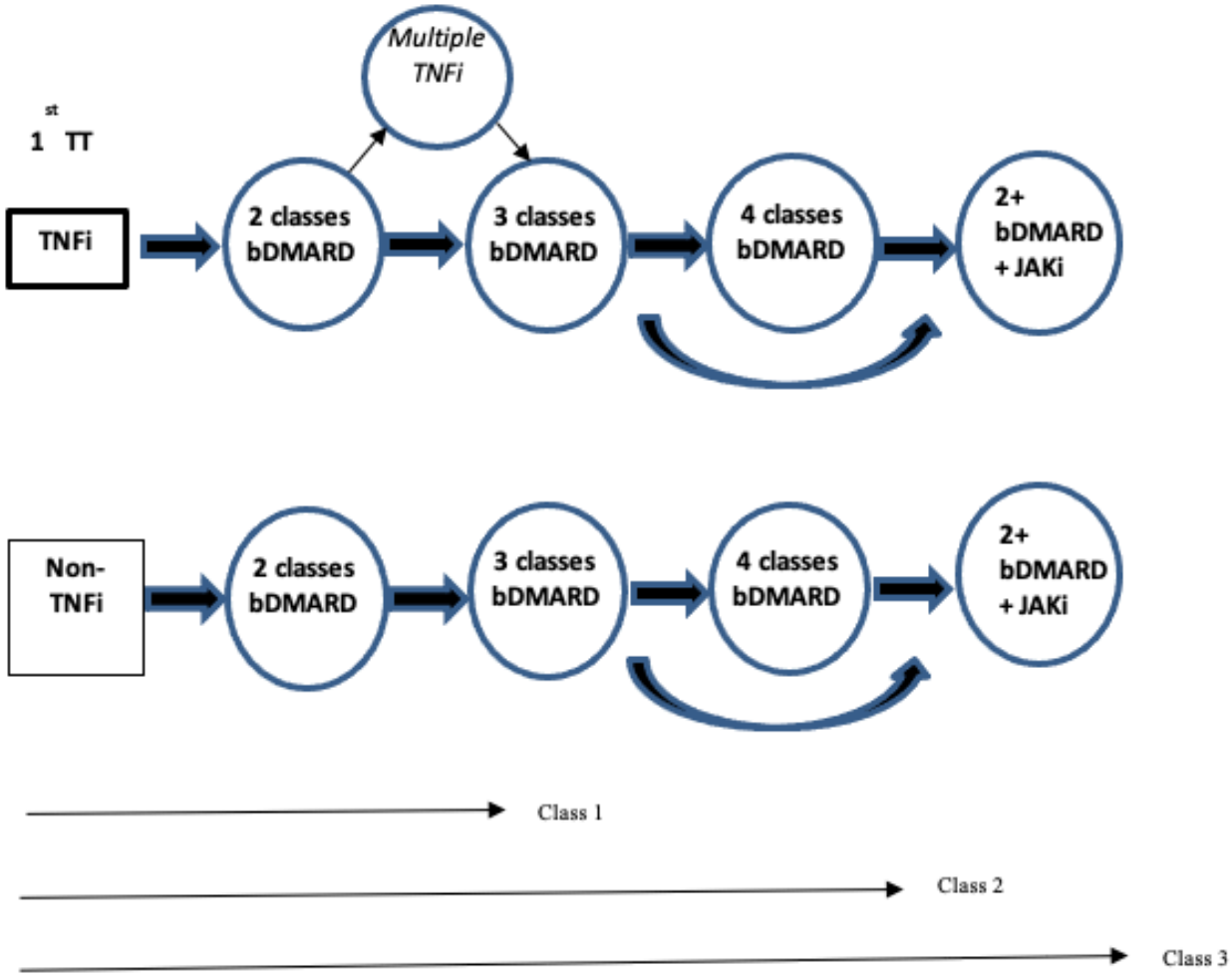

Figure 7.1: Pragmatic classification of LTHT refractory RA (RefRA) cohorts.

7.2 Chapter 7 Aims

- To determine the extent of RefRA within the LTHT RA patient population using a pragmatic classification system
- To explore the patterns of drug sequencing associated with development of RefRA.
- To identify a group of patients with RefRA who had demonstrated only lack of efficacy to all available classes of TT, a group we have defined as “true refractory RA”, and to determine what proportion of the total they represent.
- To assess the efficacy of JAKi in the RA cohort at LTHT and to determine whether JAKi shows efficacy in patients with more refractory disease.

7.3 Chapter 7 methods

7.3.1 Refractory Rheumatoid Arthritis at Leeds Teaching Hospitals Trust

Patients with confirmed RA, meeting the 2010 ACR-EULAR criteria, who had failed at least 2 TT (i.e. exposed to 3 or more TT) were identified as they attended LTHT biologic monitoring clinics at Chapel Allerton Hospital, over an 18-month period. This definition was chosen based on the BSRBR paper on RefRA by Kearsley-Fleet et al (308) but is also consistent with the recently published EULAR guidelines. Further patients were identified from a hospital pharmacy database of patients who had required an individual funding request (IFR) to start one or more of their treatments. At the time of the study an IFR was required to initiate a new therapy for any patient who had failed four previous targeted therapies and thus represents the most refractory patients at LTHT.

Data on the number of TT a patient had been exposed to, the individual patient drug sequence (i.e which targeted therapies they had been prescribed and when) and, where available, the reason for each individual drug failure have been recorded in a database and clinical records. Reason for drug failure was classified as either primary non-response, secondary non-response or toxicity. Patient notes were obtained for a subset of 60 patients to confirm disease duration, duration from diagnosis until the initiation of TT and DAS-28 scores prior to starting therapies. This number was limited as some long-term records could not be obtained and the process was halted by the COVID 19 pandemic as research activity and the requesting of notes for non-clinical reasons were halted by the trust.

Patient demographics and clinical characteristics were summarised for each group using proportions of patients, median with interquartile range or mean with standard deviation as appropriate and percentages of mild, moderate and severe RefRA were calculated.

7.3.2 JAK inhibitor use at Leeds Teaching Hospitals Trust

A separate database was collated of all patients at LTHT who had been exposed to a JAKi. A prospective database was maintained for all patients with RA at LTHT treated with a JAKi, from time of licensing of both baricitinib and tofacitinib in 2017. In addition, retrospective data were collected from clinical notes and electronic health records, where indicated, for patients starting tofacitinib on a compassionate access scheme between November 2014 and November 2017. This scheme was available for patients' refractory to other classes of bDMARD. This study includes all patients who began a JAKi between November 2014 and November 2019.

All patients had previously tried and failed two conventional synthetic (cs)DMARDs, including methotrexate as per NICE guidelines. Most had previously been treated with one or more bDMARDs. Disease duration, serological status, current csDMARD use and history of previous bDMARD exposure were recorded on the database. DAS28-CRP scores, as well as DAS28-CRP components were recorded at baseline and after 3 and 6 months of therapy, along with reason for drug withdrawal when necessary. Where a patient visual analogue score (VAS) was not recorded, a 3-point DAS28 score of tender joint count (TJC), swollen joint count (SJC) and CRP was calculated (490). Where patients discontinued therapy during the study period, discontinuation date and reason for drug withdrawal (lack of effectiveness, adverse event, loss to follow-up) were recorded. Adverse events and serious adverse events of special interest were recorded.

Patient demographics and clinical characteristics are summarized for each group using proportions of patients, median with interquartile range or mean with standard deviation as appropriate. Mean changes in DAS28-CRP score from baseline were calculated at 3 and 6 months and reported for the combined cohort and individual treatment groups. Sub analyses

according to number of prior bDMARD failures were performed. All patients had baseline DAS 28 scores and components recorded. Ten patients had data missing at 3 months but had results available at 6 months and were included for 6 month analysis. Twenty patients had data missing at 6 months, partly due to the interruption in follow-up with the onset of the COVID-19 pandemic.

7.4 Chapter 7 Results

7.4.1 Refractory Rheumatoid Arthritis at Leeds Teaching Hospitals NHS trust.

In our analysis of RefRA at LTHT we identified one hundred and seventy-two patients who had failed 2 or more targeted therapies (median (IQR) 4 (2)). 81.3% were female, 80.1% seropositive with a mean (SD) age of 63 (12.3) years. Reasons for drug discontinuation were identified in 166 cases. One hundred and fifty-two of these patients had failed both an anti-cytokine and a cell-targeted therapy, a definition of RefRA previously proposed in the literature (339) and included in our analysis to determine the extent to which this definition compared with other definitions of RefRA. An additional 50 patients had received multiple (≥2) TNFi and at least 1 other class of targeted therapy (80% female, 76% seropositive, Mean (SD) age 63.5 (14.3) years). **Table 7.1** shows the numbers in each category of refractory RA. The 60 patients who had notes available for more detailed analysis had a median (IQR) disease duration of 22 (10.75) years, with a median (IQR) duration from diagnosis to initiation of first TT of 5 (10) years. Mean (SD) baseline DAS28CRP prior to starting a 1st line TT was 5.91 (0.84).

	Whole group (n=166)	Refractory RA category		
		1 (2 class bDMARD)	2 (3/all 4 classes bDMARD)	3 (Multi bDMARD (min. 2 class) + JAKi)
Number of patients	166	64*	76	26
Age (Mean (SD))	63 (12.3)	62 (12.5)	64 (11.6)	61 (14.3)
Gender (Female)	135 (81.3)%	51 (79.7%)	64 (84.2%)	20 (76.9%)
RF and/or CCP+	134 (80.1%)	48 (75%)	66 (86.8%)	20 (76.9%)
1st Targeted Therapy				
TNFi	139 (83.7%)	54 (84.4%)	61 (80.3%)	24 (92.3%)
Rituximab	15 (9%)	5 (7.8%)	9 (11.8%)	1 (3.8%)
Tocilizumab	4 (2.4%)	3 (4.7%)	1 (1.3%)	0 (0%)
Abatacept	8 (4.8%)	2 (3.1%)	5 (6.6%)	1 (3.8%)
Reason for drug discontinuation				
Mixed NR	65 (39.2%)	31 (48.4%)	28 (36.8%)	6 (23.1%)
Primary NR only	10 (6%)	5 (7.8%)	3 (3.9%)	2 (7.7%)
Secondary NR only	13 (7.8%)	7 (10.9%)	6 (7.9%)	0 (0%)
Mixed NR and toxicity	72 (43.4%)	19 (29.7%)	36 (47.4%)	17 (65.4%)
Multiple toxicity	6 (3.6%)	2 (3.1%)	3 (3.9%)	1 (3.8%)
***49 patients received one anti-cytokine and one cell-targeted bDMARD, 12 received two anti-cytokine bDMARD (TNFi and tocilizumab) and 3 received two cell targeted treatments (rituximab and abatacept).**				

Table 7.1: *Demographics, first line targeted therapy and reasons for drug switching in whole cohort and by classification of severity of refractory disease.*

bDMARD – biologic disease modifying anti rheumatoid drug, JAKi – Janus kinase inhibitor, TNFi – Tumour Necrosis Factor inhibitor, RF – Rheumatoid factor, NR – non-response

Eighty-eight patients in total have demonstrated only non-response to targeted therapies with no history of significant adverse effects leading to a cessation of therapy. Eight of these cases had failed all 4 classes of bDMARD and a JAKi, although 1 of these patients subsequently derived a response to a second JAKi. Sixty-five patients have demonstrated a combination of primary non-efficacy and secondary loss of response, including 28/76 exposed to 3 or more bDMARD classes and 6/26 patients exposed to 2 or more bDMARD and a JAKi. 13 patients responded to all their treatments, but subsequently lost their response (sequential secondary failure). Ten patients showed successive primary non-response to two or more bDMARD classes, 5 patients to 3 or more drugs. Two had severe refractory disease with multiple primary drug failures. **Figure 7.2** shows reasons for drug discontinuation by group.

Fifty patients failed multiple TNFi, which is predominantly reflective of a time when TNFis were the only class of TT available. Of this group, 39 were switched to a cell-targeted therapy (31 Rituximab, 7 Abatacept). Thirty-six of these patients have maintained their response to their cell-targeted therapy. Eleven of the 50 were switched from a TNFi to tocilizumab and all were still on it at the time of cessation of the study.

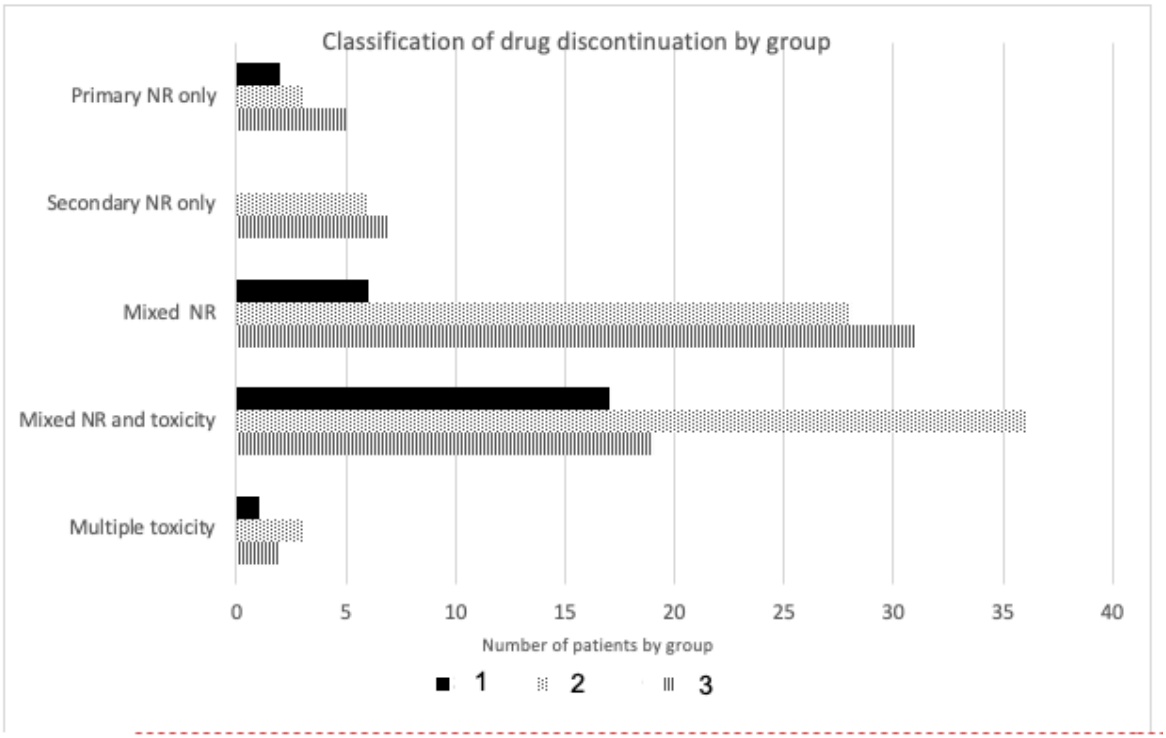

***Figure 7.2**: Reasons for drug discontinuation for each severity group*

NR – Non-response

7.4.2 JAK inhibitors at LTHT

7.4.2.1 Baseline characteristics

Between November 2014 and November 2019, 115 patients with RA were treated with a JAKi (76.4% female; mean (SD) age 57.3 (14.3) years). Eight patients were treated with both JAKi sequentially (7 switching from tofacitinib to baricitinib and 1 from baricitinib to tofacitinib). **Table 7.2** shows the baseline characteristics of the combined JAKi cohort and those treated with tofacitinib and baricitinib, including the number of classes of targeted therapy that patients had been exposed to prior to starting JAKi.

Characteristic	Combined JAK inhibitor group (n=123)	Tofacitinib (n=54)	Baricitinib (n=69)
Demographics			
Age (mean) (SD)	57.2 (14.3)	59.1 (14.0)	55.8 (14.3)
Female, n (%)	94 (76.4)	40 (74.1)	54 (78.2)
RA profile			
Disease duration, years; median (IQR)	16 (7)	17 (5)	14 (8)
Seropositivity (ACPA and/or RF), n (%)	90 (73.2)	41 (75.9)	49 (71)
Treatment history			
Concomitant csDMARD (%) Any (%) MTX other	 73 (59.3) 54 (43.9) 19 (15.4)	 34 (62.9) 27 (50) 7 (13)	 39 (56.5) 27 (39.1) 12 (17.4)
Targeted therapy naïve (%)	11(9)	1 (1.9)	10 (14.5)
Number of previous TT(median, range)	3 (0-9)	4 (0-8)	3 (0-9)
Number of previous classes of TTs (%)			
TNFi Only	13 (10.6)	4 (7.4)	9 (13)
1 Non-TNFi TT only	8 (6.5)	3 (5.6)	5 (7.2)
2 classes	20 (16.3)	7 (13)	13 (18.8)
3 classes	39 (31.7)	21 (38.9)	18 (26.1)
4 classes	26 (21.1)	18 (33.3)	8 (11.6)
5 classes	6 (4.9)	0 (0)	6 (8.7)
Previous anti-IL-6 therapy	76 (61.8)	39 (72.2)	37 (53.6)
Reason for failure of previous TTs			
Primary non-response only	16 (13)	8 (15.1)	8 (11.6)
Secondary loss of response only	20 (16.3)	6 (11.3)	14 (20.3)
Mixed primary non-response/secondary loss of response	22 (17.9)	15 (28.3)	6 (8.7)
Adverse effects	8 (6.5)	2 (3.8)	6 (8.7)
Mixed primary/secondary/adverse events	48 (39)	23 (40.4)	25 (36.2)

***Table 7.2**: Baseline characteristics of the combined JAKi cohort and those treated with tofacitinib and baricitinib*

JAK – Janus Kinase inhibitor, ACPA – Anti-Citrullinated protein antibody, RF – Rheumatoid factor, TNFi – Tumour necrosis factor inhibitor, TT – Targeted therapy.

As should be expected, given the fact that tofacitinib was available at LTHT for a subset of patients who were deemed to have exhausted their treatment options as early as 2014 on a compassionate access scheme, those treated with tofacitinib were more refractory. They had a longer disease duration and had been previously exposed to a greater number of targeted therapies, than those on baricitinib. Thirty-three patients in total were treated under this scheme. The majority of the patients had been exposed to between 2 and 4 previous targeted therapies and had failed them due to a combination of non-response and adverse effects.

Table 7.3 shows the demographics of compassionate access patients.

Characteristic	Compassionate access tofacitinib (n=33)
Demographics	
Age (mean) (SD)	61.1 (12.2)
Female, n (%)	23 (69.7)
RA profile	
Disease duration, years; median (IQR)	18 (4)
Seropositivity (ACPA and/or RF), n (%)	25 (75.8)
Treatment history	
Concomitant csDMARD (%) Any (%) MTX other	 17 (51.1) 12 (33.4) 4 (12.1)
Number of previous bDMARDs (Median ,Range)	5 (3-7)
Number of previous classes of bDMARD (median, Range)	3 (2-4)

Table 7.3*: Demographics and disease profile of patients commence on Tofacitinib on compassionate access scheme.*

SD – standard deviation, IQR – inter quartile range, ACPA – Anti-citrullinated protein antibody, RF – Rheumatoid factor, csDMARD – conventional synthetic disease modifying anti-rheumatoid drug, bDMARD – biologic disease modifying anti-rheumatoid drug.

7.4.2.2 Efficacy

Table 7.4 shows baseline DAS28-CRP score, DAS28-CRP components, and the mean change in these measures at 3 and 6 months for the combined JAKi cohort and individual tofacitinib and baricitinib cohorts. Fourteen of 54 patients treated with tofacitinib stopped treatment due to lack of efficacy during the study period. Twenty-six patients remain on tofacitinib with a median treatment duration of 23.5 months (Interquartile range, IQR 23) to date. Two patients have been lost to follow up. Fifteen of 69 patients have stopped baricitinib due to lack of efficacy. Forty-five patients remain on baricitinib with a median treatment duration of 13 months (IQR 6). In the combined JAK inhibitor group 84.6% of patients remained on their JAKi at 3 months and 73.2% of patients were still on drug at 6 months after the combined effects of lack of efficacy and toxicity (see below)

	Baseline	3 months	6 months
Combined JAKi group	n=123	n=94	n=68
DAS28	5.62 (1.14)	-1.49 (1.44)	-1.67 (1.61)
TJC	14.30 (7.84)	-6.73 (9.24)	-7.31 (9.71)
SJC	6.50 (5.13)	-3.31 (5.54)	-3.49 (5.39)
VAS GH	77.01 (17.97)	-26.34 (26.47)	-23.32 (26.92)
CRP	26.33 (39.81)	-10.93 (37.06)	-16.74 (47.69)
Tofacitinib	n=54	n=36	n=27
DAS28	5.85 (1.23)	-1.71 (1.78)	-1.81 (1.77)
TJC	15.13 (8.46)	-8.0 (10.50)	-8.56 (8.33)
SJC	7.45 (5.26)	-4.35 (5.94)	-5.11(5.49)
VAS GH	79.63 (18.87)	-27.55 (26.55)	-23.86 (26.50)
CRP	31.58 (47.12)	-18.98 (53.08)	-32.62 (69.11)
Baricitinib	n=69	n=58	n=41
DAS28	5.45 (1.04)	-1.35 (1.19)	-1.57 (1.50)
TJC	13.65 (7.32)	-5.96 (8.39)	-6.44 (10.58)
SJC	5.77 (4.95)	-2.68 (5.25)	-2.28 (5.05)
VAS GH	74.89 (17.06)	-25.86 (26.71)	-22.97 (27.59)
CRP	22.3 (33.05)	-6.81 (21.64)	-5.7 (17.85)

Table 7.4*: Mean (SD) DAS28-CRP score and change in DAS28-CRP components at baseline, 3 and 6 months.*

DAS – Disease activity score, TJC – tender joint count, SJC – swollen joint count, VAS GH – Visual analogue score general health, CRP – C-reactive protein.

On average, in our cohort, treatment with both JAK inhibitors improved DAS28-CRP and all DAS28 components at both 3 and 6 months compared to baseline.

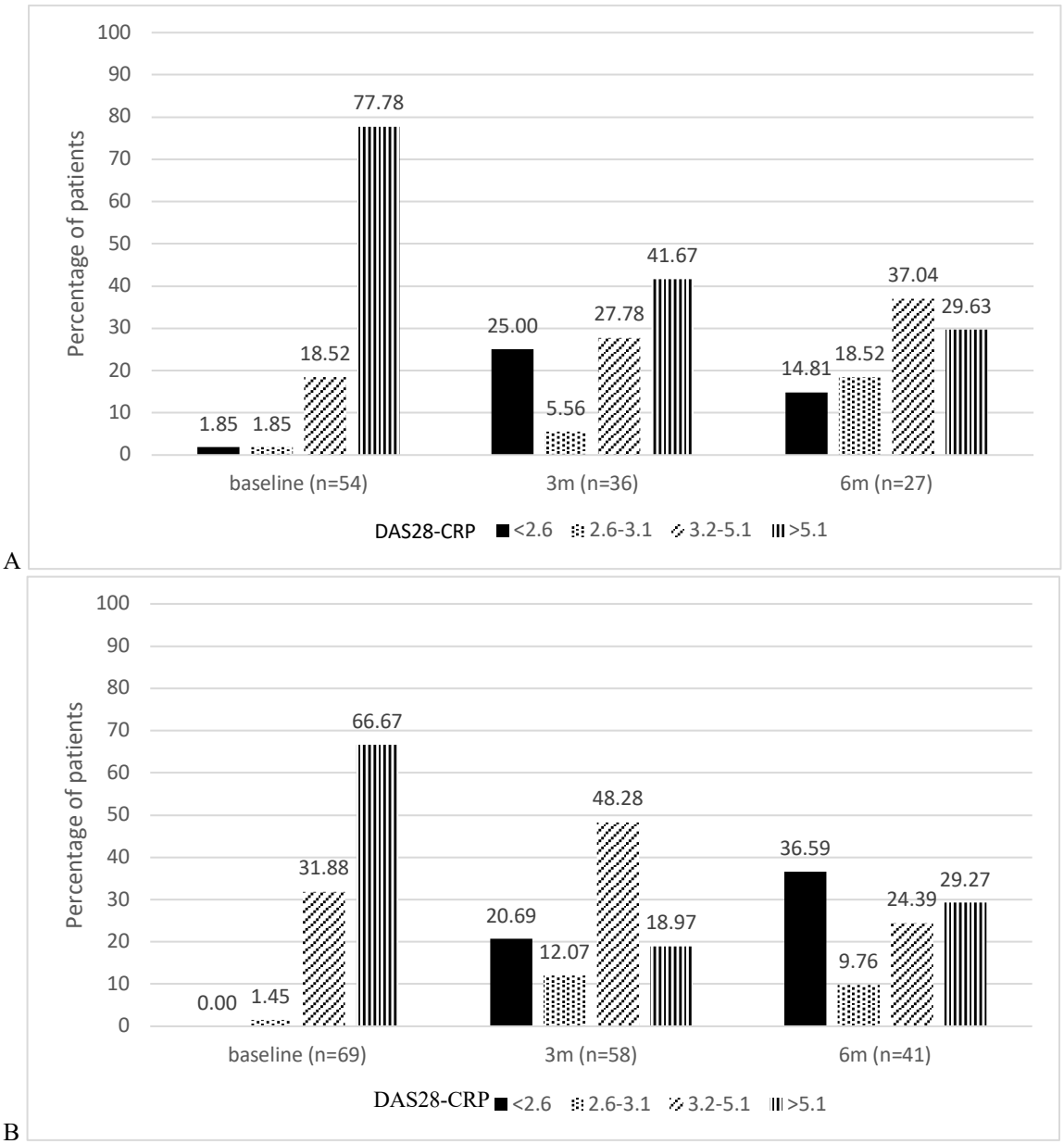

***Figure 7.3**: DAS28-CRP categories at baseline, 3 and 6 months of (A) Tofacitinib and (B) Baricitinib*

Percentage of patients in each DAS28-CRP cut-off category at baseline, 3 and 6 months treated with A. Tofacitinib; B. Baricitinib.

DAS28-CRP: Disease activity score 28-joint C-reactive protein

Figure 7.3 shows the percentages of patients in each DAS28-CRP cut-off category >5.1, >3.2-≤5.1, ≥2.6-≤3.2 and <2.6 at baseline, 3 and 6 months for both tofacitinib and baricitinib. This reflects the EULAR response criteria, although these are defined by the DAS28-ESR, rather than CRP, but ESR is used less frequently in normal clinical practice at LTHT. Both JAKi

reduced the proportion of patients with severe disease activity and increased the numbers in remission by the end of 6 months follow up.

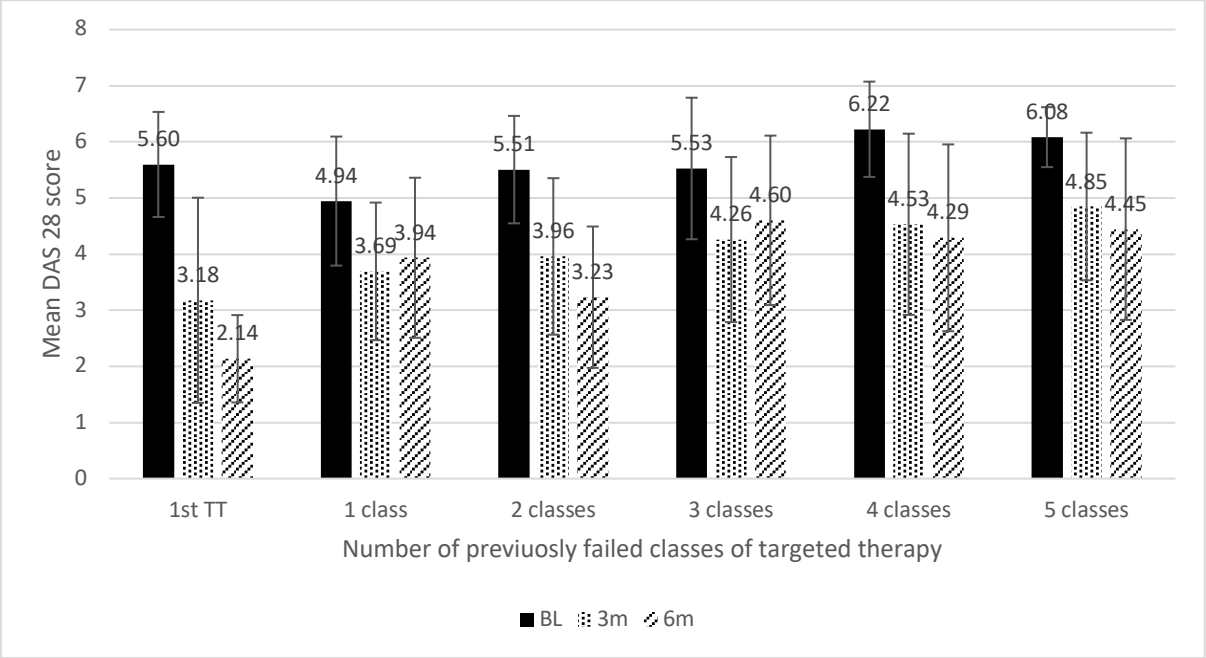

***Figure 7.4**: Response to JAKi based on prior number of classes of targeted therapy (combined group tofacitinib and baricitinib).*

DAS28-CRP: Disease activity score 28-joint C-reactive protein; JAKi: Janus Kinase inhibitor

Figure 7.4 shows the combined results for both JAK inhibitors based on the number of previous classes of targeted therapies the patient had been treated with prior to the introduction of a JAKi. JAKi lead to an improvement in disease activity across the treatment pathway, with even the most refractory patients on average responding to therapy.

7.4.2.3 JAKi survival

I undertook a survival analysis of the whole JAKi cohort, and an analysis based on the number of lines of TT a patient had been exposed to determine whether prior drug exposure resulted in worse JAKi survival. A total of 40.7% of patients stopped their JAKi during the entire study

period. Kaplan Meier survival analysis for the cohort over the whole study period, according to prior bDMARD exposure, suggests lower cumulative survival of JAK inhibitors in patients who have previously been exposed to 2 -4 previous classes of targeted therapy (**Figure 7.5**), suggesting that more refractory patients have worse JAKi survival. However, it should be noted that the most refractory patients started their JAKi drug earlier, through a compassionate access scheme, meaning longer follow-up compared to patients with less refractory RA that have been censored at the end of the study period. The more refractory cohort would also have had more limited (or no) further treatment options. This may have influenced the decision to continue therapy for longer than would have been done in patients with multiple treatment options available, which may introduce a bias to the data.

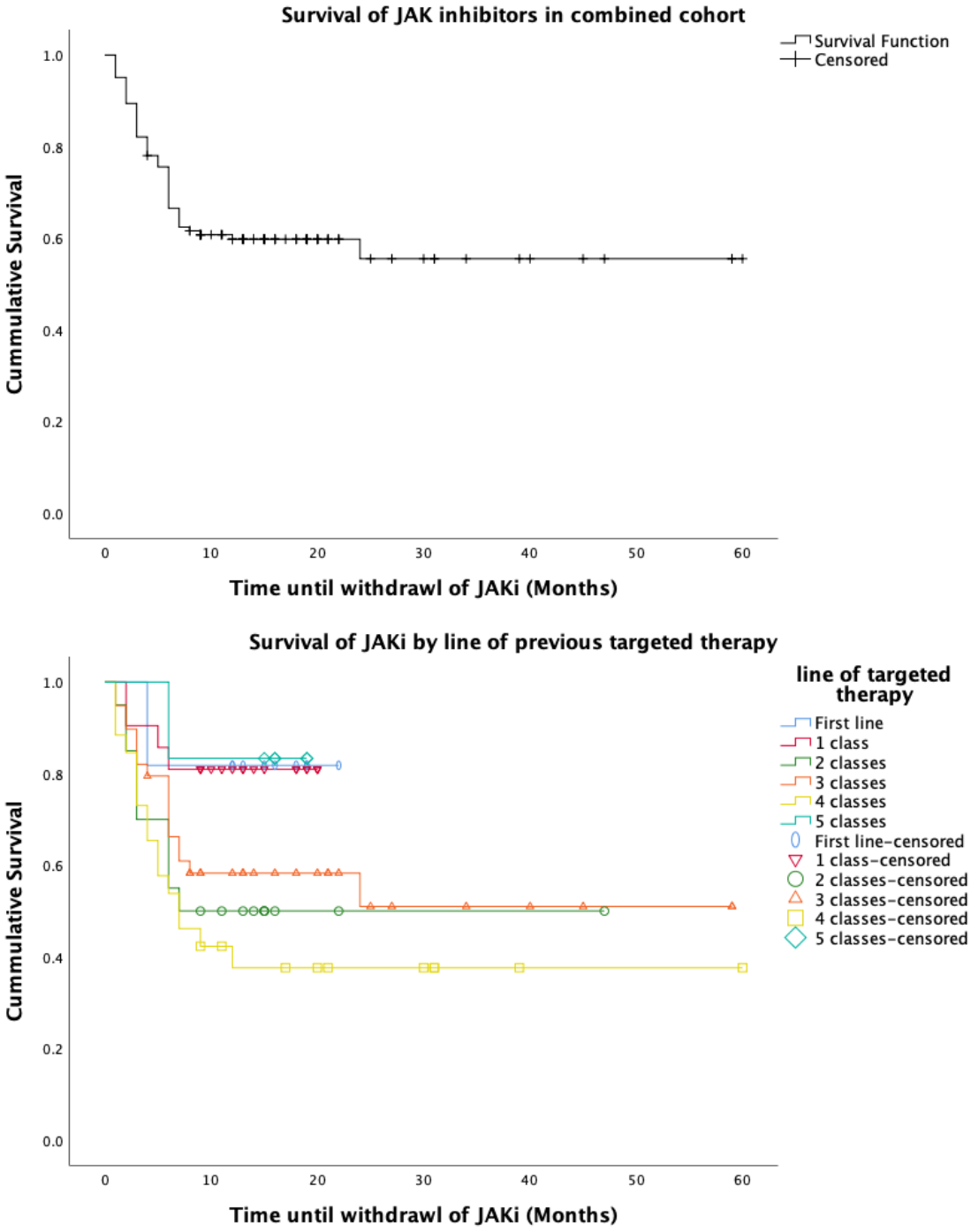

Figure 7.5*: survival of JAKi in our combined JAKi cohort over the treatment period (A) and survival of JAKi according to number of previously failed targeted therapies (B).*

7.4.2.4 Response in patients previously treated with IL-6 inhibition

As previously outlined, IL-6 is a pivotal cytokine in the initiation and maintenance of the inflammatory response in RA, which has been underlined by the efficacy of tocilizumab and sarilumab in its management. The IL-6 receptor signals via JAK1 and JAK2, thus both tofacitinib and baricitinib will have effects on IL-6 signalling. I looked at the previous responsiveness of our cohort to IL-6 blockade with TCZ to see whether patients who failed a previous IL-6 blocker showed efficacy with JAKi.

Seventy-six patients had previously been treated with the anti-IL-6 receptor monoclonal antibody tocilizumab. Twenty-eight patients in our cohort had a documented primary non-response to tocilizumab, 25 had secondary loss of response and 23 had stopped due to adverse effects. Thirty-six of these patients responded to a JAKi with a mean DAS28-CRP improvement of 2.29 (SD1.19), Twenty-six did not respond and 14 stopped due to adverse effects. Twelve of 28 of those with a primary non-response to tocilizumab responded to JAKi (4 tofacitinib, 8 baricitinib), with a mean improvement in DAS28-CRP score of 2.18 (SD 1.08) at 6 months (mean DAS28 score improving from 5.54 to 3.36).

7.4.2.5 JAKi cycling

Seven patients switched from tofacitinib to baricitinib due to either lack of response (n=3) or intolerance (n=4). Five patients responded to baricitinib after 6 months of treatment and mean (SD) DAS28-CRP improvement of 1.42 (SD 2.03). One patient switched from baricitinib to tofacitinib due to lack of response but did not have improvement in DAS28-CRP. The patient continued tofacitinib however due to an improvement in a co-existing skin condition.

7.4.2.6 Toxicity

Twelve patients treated with tofacitinib stopped treatment due to adverse effects (3 infection, 1 rash, 2 deranged liver function tests, 2 headache, 1 dizziness, 2 diarrhoea and 1 malignancy). Nine patients treated with baricitinib stopped treatment due to adverse events (2 headache, 2 deranged LFTs, 5 due to infections and 1 angioedema). However, a further 4 patients down-titrated from 4mg daily to 2 mg daily due to nausea and/or other gastrointestinal side effects and continued to respond at this lower dose. Two patients out of the 69 treated with baricitinib developed a deep vein thrombosis, although in both cases other provoking factors were present (long haul travel and lower limb trauma, and obesity). Due to lack of alternative treatment options (after multiple bDMARD failure in one case and severe needle phobia in the other) and after careful consultation with the patients involved and haematology colleagues, both patients elected to remain on baricitinib with anticoagulation.

7.5 Chapter 7 conclusions

More than 10% of the total LTHT TT exposed RA cohort have RefRA according to either the EULAR definition of difficult to treat RA or a definition of RefRA that includes failure of both anti-cytokine and anti-cell targeted therapies. This is consistent with the proportions of patients with RefRA observed in other studies published at the time this work was carried out (308, 369). This cohort also includes a group of patients who have failed one or both the available JAKis, which was not the case in previous studies. I have stratified the cohort into 3 groups, mild, moderate and severe and found that 15.7% of the total meet the definition of severe disease, having failed at least 2 classes of bDMARD and a JAKi. Most patients failed TT due to a combination of primary inefficacy, secondary loss of response and adverse effects, which confirms my initial hypothesis that this would be the case. Likewise, a small identifiable group of around 6% of our RefRA cohort have what I have termed “true RefRA” and have multiple

primary non-responses to therapy. Two of these patients were classed as severe refractory according to the pragmatic classification system.

JAKi have shown to be efficacious across the treatment pathway at LTHT but were most beneficial as a first line TT. However, they have also shown to be effective in patients with multiple previous TT failures. This efficacy across the treatment pathway aligns with what we have seen within the clinical trials programmes for tofacitinib (276, 280, 347) and baricitinib (288, 289). I have shown that JAKi is effective in patients with previous failure of IL-6 blockade and that JAKi cycling can be efficacious in the event of failure of a first JAKi.

8 Discussion

Rheumatoid arthritis is a common, chronic autoimmune inflammatory arthropathy. If not successfully suppressed with medication it can cause significant morbidity and increased mortality. Over the last two decades its treatment has been revolutionised by the arrival of the TTs and the treat to target era meaning that, in a large proportion of patients, RA can be controlled sufficiently to maintain a high quality of life. However, over recent years it has become increasingly recognised that a cohort of patients who have tried and, for a variety of reasons, failed multiple TTs has begun to develop. Thus, RefRA is has begun to re-emerge, despite these treatment advances and increasingly represents an area of unmet need in the rheumatology clinic.

RefRA is an increasing focus of RA research, with several studies published over recent years on its characteristics and risk factors (308, 359, 369, 493, 494). However, given the long time periods involved from diagnosis to multiple drug failure, much of the work in this area to date has focused on patients in established cohorts and registries. Prospective studies which include detailed imaging to confirm the presence of synovitis at baseline are lacking. MUS has been shown to be more efficacious than clinical examination at picking up synovitis (109) and has a role in differentiating damage from active disease (495). Including MUS variables relating to joint, tendon and enthesis inflammation in the analysis of established RA patients is valuable. Incorporating these variables into statistical models that will allow different disease clusters to be identified will give greater depth to the phenotypic analysis of RA patients and hasn't been done previously. This has the potential to enable us to clearly define whether groups of patients exist with different proportions of synovial and non-synovial inflammation, or whether clusters of patients with no synovial inflammation or predominant damage are present within the established RA pathway. Studies that include MUS will also facilitate a less subjective assessment of therapy response, establishing the presence of baseline synovitis,

and determining if it persists in patients who fail to respond. This will help to differentiate persistent synovitis from other causes of difficult to treat RA. Simultaneously, it will help to determine whether the presence of synovitis prior to therapy initiation can help to predict treatment response which is a fundamental question not yet clearly answered in the literature.

Treatment failure is a multifactorial process, not only encompassing persistent synovitis, but also joint damage and the overlapping effects of medical and psychological co-morbidity. The use of PRO measures in research highlights the effects of chronic pain and psychological co-morbidity on elevating disease activity scores, particularly by increasing the more subjective components of the DAS28, providing an addition dimension to the interpretation of disease activity (429, 433, 496). Thus, the use of PRO results in addition to clinical examination, laboratory tests and MUS, can help to give a more complete picture of disease phenotype. Assessing changes in these metrics over time and in response to therapy can help to give a better understanding of the differing pathways taken by patients with established RA towards either response to treatment or RefRA.

The overarching aim of this thesis was to establish whether homogeneous clusters of patients within a heterogeneous population of advanced RA patients treated with TT can be identified and investigated. I aimed to evaluate DAS28, and broader pathophysiological and imaging phenotypes in established RA, identify differences between these phenotypes at baseline, and by including longitudinal analysis, evaluate whether an association between baseline phenotypic cluster and drug response can be identified. If these different phenotypic clusters can be identified and shown to respond differentially to TT, this will raise the possibility that we could tailor different treatment pathways for these different groups, allowing us to take a step towards a more personalised approach to the management of RA. Furthermore, I hoped to establish whether the presence of baseline synovitis on MUS predicts a response to therapy in established RA patients. Finally, I aimed to classify refractory rheumatoid arthritis, establish

the extent of RefRA within our local population and determine whether the most recently introduced TTs, the JAKis, were effective in patients' refractory to bDMARDs in our real-world population.

As detailed in chapter 1, RA is a heterogeneous condition, which develops over many years as a result of the complex interaction between myriad genetic and environmental risk factors. RefRA is no less complex with patients failing therapies due to multiple factors including incorrect drug targeting, the development of immunogenicity, drug induced side effects or the emergence of comorbid conditions that limit the use of some treatments. To add further layers to this complexity, the accrual of damage due to periods of prolonged suboptimal disease control can cause pain and reduce quality of life, which may affect perceived treatment response. It also remains possible that the nature of drug resistance changes over time as the biology of disease evolves in response to prolonged inflammation or exposure to treatment.

For example, epigenetic changes in lymphocytes and FLS have been suggested to play a role in the development of RA and it has also been suggested that changes such as methylation or histone modification occur in established RA, both due to advancing age and potentially due to exposure to treatment (497). Evidence suggests that epigenetic changes in FLS can lead to the loss of capacity to repress inflammatory genes induced by TNFα (498) and can lead to these stomal cells playing a key role in the persistence of synovitis independently of cytokines secreted by other inflammatory cells. Changes such as these may lead to shifts in dominant cytokine networks and the inflammatory cell types driving inflammation. This may have the effect of moving drug targets, making the treatment of RefRA complex. These processes again take years, and it may take a long period before an individual meets any definition of RefRA.

Because of this complexity and the long time periods involved in the development of RefRA, I have used two parallel investigations to answer my research questions. A prospective longitudinal cohort study that allows us to use LPA to phenotype patients with established disease at baseline, both in terms of their DAS28 profiles and more broadly with MUS and PRO data, and then track their response to treatment. Using baseline MUS, we will also be able to establish whether the presence of synovitis predicts response to therapy within our cohort. A second retrospective cohort has allowed us to establish the extent of RefRA within our local population and to assess the responses of RefRA patients to the most recently licensed TTs; the JAK inhibitors.

8.1 Phenotyping DAS28 and broader phenotyping of established RA patients

I hypothesised that a number of homogeneous clusters of patients, with differing clinical and ultrasound phenotypes, exist within a larger heterogenous cohort of advanced RA patients. If this is the case, it is possible that there will be differences in their response to TT and that some of these differences may be important predictors of the development of RefRA. Identifying them may allow for a more tailored approach to managing these different groups. To investigate whether these clusters could be identified, I established a cohort of 200 patients being treated with TT (196 with full baseline ultrasound results) for latent profile analysis. The cohort includes patients from across the RA targeted therapy treatment spectrum from a 1st line TT up to more refractory patients, with 1 patient starting their eighth line TT. Eighty-eight (44%) of our cohort meet our definition of RefRA, but all have established disease and have failed multiple csDMARDs. I have included number of targeted therapies and number of previous classes of therapy in the analysis to allow me to assess whether patients with earlier disease fall into different clusters compared to patients with multi-drug RefRA. The cohort was predominantly female (75%) and seropositive (76%), both of which have been linked to higher disease activity and a refractory course of RA (369). The patients within the cohort had a mean

baseline DAS28-CRP of 5.3 and DAS-28-ESR of 5.4 and had on average been exposed to 1.47 previous classes of TT (SD 1.31, median 1, IQR 2).

I have performed several different latent profile analyses on the cohort using the same methodology but using different combinations of clinical and MUS characteristics as indicator variables. I have used these different models to address my hypotheses that clusters of patients comprising differing combinations of DAS28 variables, different ultrasound phenotypes and broader combined clinical and ultrasound phenotypes could be identified. In chapter 3 I investigated whether clusters of patients could be identified by using DAS28 component scores as indicator variables to assess whether discrete groups of patients can be identified with different DAS28 phenotypes. This model identified essentially equally valid 3 and 4 class solutions. I also investigated whether MUS variables can be used to cluster patients, producing a 3-class solution as the best fit. Finally, in chapter 4, I used a combination of these metrics to identify broader phenotypic clusters, again this favoured a 3-class solution. The results of these separate analyses confirm that distinct and discrete clusters of patients can be identified within our broader cohort of established RA patients.

8.1.1 Phenotyping DAS28

Using DAS28 component scores I identified 2 models with essentially equal validity, although the absolute BIC score was lowest for the 3-class version the difference in BIC between the 3 and 4-class models was negligible. The 3-class solution could be easily classified into a low inflammatory group (cluster 1) and intermediate inflammatory group (cluster 2), and a high inflammatory group (cluster 3) based on statistically significant differences between all these clusters in the objective markers of inflammation from the DAS28 component scores; the SJC and inflammatory markers (CRP and ESR). These differences lead to significant differences in DAS28 scores. Cluster 1 was the most common cluster with just under half of the patients

(98 patients) falling within this group, followed by cluster 2 (71 patients). Cluster 3, the most inflammatory cluster was the least common (31 patients).

Cluster 1 is more female, more seronegative with lower inflammation than the other clusters. Traditionally seronegative disease has been considered to be less inflammatory than seropositive disease, which could explain lower levels of inflammation within this cluster, although these differential levels of inflammation between the subtypes of RA is controversial and severe resistant inflammation in seronegative patients is well recognised (499). It should also be noted that despite having a greater proportion seronegative patients', 67% of patients in cluster 1 are still seropositive versus 77% in cluster 3. Cluster 3 is older with a longer disease duration and a higher inflammatory burden than the other clusters. The older age of the more inflammatory cluster is notable as traditionally, RA was felt to be less inflammatory in older age groups, but this paradigm is increasingly recognised to be incorrect, with severe inflammatory disease recognised in elderly patients. This is complicated by increasing co-morbidity creating more barriers to successful treatment in elderly patients (500). It should be noted that the age differential between the clusters is relatively small and the longer disease duration in cluster 3 is indicative of the fact that cluster 3 is not made up of patients with significantly later onset disease than the other groups.

In this analysis I did not see any clusters of patients emerge with clearly discordant PRO data across all the questionnaires collected, relative to levels of inflammation. There was no difference in either depression or anxiety measured in HADs. Significant differences in HAQ-DI score were noted and were highest in cluster 3, the most inflammatory cluster. In this case the higher level of physical limitation is associated with higher disease activity, as should be expected. FACIT fatigue levels were significantly lower in cluster 3 than the other clusters which again is expected as higher FF scores suggest lower fatigue and are associated with better quality of life. The DAS28-P was significantly higher in the low inflammatory cluster 1

than in the other clusters (and higher in cluster 2 than cluster 3), suggesting that the subjective DAS components (TJC and VAS) made up a greater proportion of the DAS-28 score in patients in this cluster. However, it should be noted that patients in cluster 1 had a lower starting DAS-28 score on average than the others, and thus the higher DAS-P was not reflected in numerically higher TJC or VAS scores within this cluster when compared to the more inflammatory groups. Thus, the predominance of the subjective DAS-28 elements may reflect the relatively lower objective markers of inflammation in this group. These higher proportional levels of subjective DAS28 components were not reflected in differences in levels of anxiety, depression, physical limitation or fatigue.

I was not able to show a cluster of patients with elevated PRO outcomes across the measures we included in this study in this model. Previously published work by both Lee et al (444) and Mcwilliams et al (10), using differing clustering methods, had previously clearly identified groups of patients with discordant PRO data in patients with low levels of inflammation. Both studies used different combinations of PROs to this study. Lee et al used the Brief Pain Inventory-short form (BPI-sf), Hospital Anxiety and Depression scale, Multi-dimensional Health Assessments Questionnaire (MDHAQ), Medical Outcomes Study (MOS), sleep questionnaire 2 and Pain Catastrophising scale (PCS) along-side clinical examination to cluster patients with K means clustering. They identified 3 clusters, one of which had low inflammation, but high fatigue, pain and psychological distress. McWilliams et al used HAQ and Short-Form 36 in their analysis, identifying a group with discordance between PRO outcomes and inflammation. Roodenrijs et al (501) have also recently published a report using K means clustering on a cohort of 52 difficult to treat RA patients using a large number of differing PROs related to pain, fatigue and fibromyalgia. This identified a small cluster of patients with pain syndromes. In my study I have used HAQ, HADs and FF and this different use of PROs may possibly account for some of the difference in the types of disease cluster identified. We did not include specific measures related to fibromyalgia and chronic pain or

include the SF-36 questionnaire, which includes a domain on pain symptoms. All PROs used in this study are validated measures used in RA research, but inclusion of a PRO which included a pain metric may help to characterise a fibromyalgic cluster more clearly and would be included in future work.

My study is unique in that it also contains comprehensive 38 joint ultrasound data to identify the extent and pattern of joint, tendon and enthesis involvement and as a result we can compare ultrasound outcomes between these clusters. In our 3-class DAS28 component solution, US characteristics again reflect the relative differences in inflammation between the groups. There is good concordance between the clinical clusters and findings on ultrasound with higher SJC and inflammatory markers corresponding to relatively higher levels of active inflammation confirmed on ultrasound. Cluster 3 has significantly more power doppler and grey scale synovitis than the other 2 clusters. Every patient within cluster 3 had clinically relevant synovitis identified on MUS, whereas approximately one third of cluster 1 patients did not meet our threshold for clinically relevant synovitis, highlighting the relatively low levels of inflammation in this group. The 3 clusters also had significantly different levels of tenosynovitis which progressed from cluster 1 to cluster 2 and from cluster 2 to cluster 3, again reflecting the differing levels of inflammation between the clusters.

There are only small differences in disease activity at the entheses in between the clusters, which supports the notion that enthesis involvement in RA is a less common finding than it is in SpA (502). Although cluster 3 has evidence of an increase in active inflammation at the enthesis, with greater power Doppler signal in keeping with its more inflammatory phenotype, cluster 1 has greater number of enthesis calcifications. The number of patients with changes at the entheses is low in the entire cohort, so this represents a small difference between the clusters. Enthesis calcification is a more common feature in spondyloarthropathy than RA. Given that cluster 1 is more seronegative than the other clusters, it is possible that the higher

level of enthesis calcification may be due to cluster 1 including patients that fall across the seronegative spondyloarthropathy spectrum of disease who meet the ACR criteria for RA. Disease patterns in keeping with a more SpA like phenotype have been described in seronegative RA patients (503).

Using DAS28 components as clustering variables I also identified 4-class solution with equal validity to the 3-class solution discussed above. This showed a similar pattern to the 3-class solution, with SJC and VAS progressing as we ascend the clusters, but in this analysis class 1 had higher average inflammatory markers than either of class 2 or class 3. Class 4 was the most inflammatory cluster. Given the small number of patients within cluster 1, it was possible that this elevated inflammation may have been due to the data being skewed by a small number of individuals with a very high inflammatory response, but closer review of the data shows that this is a true elevation in CRP across individuals assigned to cluster 1 based on their posterior probability. The corresponding ultrasound data for this group is interesting in that overall, this cluster does not show evidence of significant disease activity with total GS and PD synovitis and tenosynovitis scores lowest in this cluster. However, a high proportion (92%) of this group met the threshold for clinically relevant synovitis (PDUS ≥ 2 in at least 1 joint or total PD > 3). Therefore, this group appears to represent a cluster with active disease, but lower levels of clinical synovitis and raises the possibility that the source of some inflammation within this group could be from other unrecognised extra-articular manifestations of their RA.

It should be noted that a number of factors such as diet, obesity and female sex hormones have been linked to CRP levels in RA patients (504) which may also play a role in discordantly high CRP in patients with limited US evidence of disease activity. We do not have data on BMI or dietary factors in this cohort. Bradford et al (505) have described a group of RA patients who do not mount a significant CRP response when having a flare of RA with MUS identifiable

synovitis. These patients have different immunological profiles to patients with high CRP during flares with higher levels of T regulatory cells and a loss of synergy between CRP levels and serum inflammatory cytokines. These patients have a worse prognosis than those who do mount a CRP response. This phenomenon could contribute to clusters 2 and 3 having higher levels of synovitis but lower CRP. Once again within the 4-cluster solution there was no group identified with clearly discordant inflammation and PRO outcomes.

8.1.2 Can phenotypic clusters be identified using ultrasound?

An LPA using ultrasound derived variables as grouping variables was also carried out. This also produced a 3-class solution as the best fit for our model. In this analysis, once again SJC and CRP ascended through the clusters giving us low, intermediate, and high inflammatory groups. In this analysis this increase in inflammation is also mirrored by a corresponding rise in seropositivity in each cluster. Seropositivity is known to be associated with worse prognosis disease (56). Once again, in our ultrasound derived clusters, US variables matched clinical findings with higher GS and PD synovitis, tenosynovitis and enthesis Doppler in the most inflammatory groups and higher enthesis calcification in the lower inflammatory groups (in this case both clusters 1 and 2 have significantly more enthesis calcifications than cluster 3). As cluster 3 has the longest disease duration this increase in calcification is not due to a more prolonged inflammatory process and as seropositivity is lowest in these clusters, it once again raises the possibility that there are SpA phenotype individuals contained within our seronegative RA patients as described above for our 3-class DAS28 component model solution (page 261). There are no statistically significant differences in PRO outcomes between these clusters, although some subtle differences are present which were similarly identified in the combined clinical and MUS model discussed below.

8.1.3 Can broader phenotypic clusters be identified combining clinical and ultrasound variables

In chapter 5 I undertook a broader LPA including both clinical and ultrasound variables within our analysis to give a deeper phenotyping of our cohort. Once again, the best fit for our model was the 3-class solution. This analysis again produced similar results to both of our previous LPAs, with inflammation defined by SJC and inflammatory markers rising through the clusters. Cluster 1 is more female and more seronegative than the other clusters, with the proportion of seropositive patients going up in each successive cluster. In this analysis, cluster 3, the most inflammatory cluster, also contained a significantly higher proportion of smokers than the other clusters. Smoking is a known risk factor for both severe and refractory disease (33, 386, 390). Cluster 2 had the most patients (82 patients) followed by cluster 1 (76 patients) and then cluster 3 (42 patients). Ultrasound data once again corroborated the clinical findings with higher amounts of joint, tendon and enthesis inflammation in the most inflammatory cluster, but numerically more calcification within the lower inflammatory, more seronegative cluster, as with my previous models.

Interestingly in this analysis there is some evidence of discordant PRO data when compared to the relative inflammation between the clusters. Physical limitation, defined by HAQ score rises in parallel with inflammation as you ascend the clusters as expected. The results for other PROs within this analysis are more nuanced. Anxiety scores, measured by HADs are numerically higher in clusters 1 and 2 than in cluster 3 (there were no differences in depression scores) and interestingly, fatigue scores were highest (indicating lowest levels of fatigue) in cluster 2, suggesting that the least inflammatory cluster (cluster 1), had greater fatigue than the intermediate group. However, these differences did not meet statistical significance.

Fatigue is a frequent symptom in RA and has a negative impact on quality of life. It is associated with active inflammation, but patients with high levels of fatigue and low

inflammatory burden are increasingly recognised. As discussed in chapter 1, fibromyalgia, a condition in which fatigue is a significant symptom, is found in higher proportions in RA patients and is associated with an elevated DAS28 score (429, 433). A recent analysis of early RA patients from the ARCTIC trial, a study looking at the inclusion of MUS in treat to target strategies, showed that patients with fewer swollen joints, lower PD ultrasound scores and high patient global assessment at baseline, but who met the inclusion criteria of DAS28 >3.2, were more likely to have persistent fatigue after 24 months of treatment (506). Cluster 1 in my analysis shares some of these features, having both lower SJC and lower Doppler scores than the other groups. Hammer et al (507) have recently published on a cohort of RA patients starting on TT, who were assessed with MUS and PROs related to fatigue, global disease activity, catastrophising and mental wellbeing. They found that fatigue levels correlated with PRO results, but not objective measures of inflammation. However, significant fatigue was associated with lower remission rates on composite disease activity indices.

Likewise, anxiety has been shown to be more common in RA patients (508) and associated with relatively higher disease activity and worse functional status (509). In my 3-class combined clinical and MUS model, there are subtle differences between the clusters in these metrics, particularly in cluster 1 the lowest inflammatory cluster which has higher rates of anxiety and relatively higher fatigue, hinting at a degree of discordance between PROs and inflammation within this group, but there isn't a statistically significant difference on K-W testing, meaning I haven't clearly identified the cluster with discordant PRO data that I had expected based on the previously published literature. DAS-P is significantly higher in cluster 1 than cluster 3, suggesting once again that the subjective elements of the DAS28 score make up a relatively greater proportion of the overall DAS28 in this group.

I have described above the results of 3 different LPA performed on our advanced RA cohort, one using DAS-28 component scores as indicator variables, one using ultrasound variables

and a more extensive one using both outcomes to perform LPA. All 3 analyses have provided similar results with 3 (or 4) clusters best defined as low, intermediate or high inflammatory based on the presence of elevated SJC and inflammatory markers and US GS and PD synovitis/tenosynovitis. Although it is not possible to directly compare the results of differing LPAs using statistical methods, review of the patients assigned to each of the 3 class solutions in our data shows a significant overlap between the analyses with high proportions of the same patients assigned to the same class in each analysis. This shows high consistency between the analyses and suggests that 3 distinct and discreet clusters exist within my established RA cohort.

At the outset of this project, it was expected that there would be clearly definable clusters with high disease activity represented by high levels of all the DAS-28 components, clusters of patients with high objective measures of inflammation (CRP and SJC) and clusters of patients with high subjective markers of disease activity (TJC and VAS). These expected groups were demonstrated in **figure 1.1**, chapter 1, page 75. In the high subjective cluster/clusters it was further expected that there would be an association with discordantly high PRO data in patients with low inflammation. This expectation was based on evidence that chronic pain and fibromyalgia are associated with elevated disease activity. It was also based on several studies using clustering methods on cohorts of RA patients. These included an analysis of the ERAN and BSRBR cohorts performed by Mcwilliams et al (10) and a report by Lee et al (444) on 169 patients from the Brigham Rheumatoid Arthritis Sequential Study. McWilliams et al identified 5 clusters of patients which displayed either expected concordance between clinical and PRO variables (indicative of mild, moderate or severe disease activity); discordantly less severe patient-reported measures despite elevated inflammation or discordantly worse patient reported measures despite less markedly elevated inflammation. Lee et al identified 3 clusters of patients, 1 with high inflammation and high pain and fatigue, 1 with low inflammation and

low pain, fatigue and psychological distress and one cluster with low inflammation, but high pain, fatigue and psychological distress. More recent evidence also supports these analyses.

Identifying clusters of patients within populations of RA patients to inform precision medicine is an increasing focus of research. Several recent studies involving a form of cluster analysis of RA patients have been performed. A number of these studies support the existence of clusters of patients with higher pain and psychological co-morbidity, suggesting that this group of patients exists within a population of RA patients. A recent study by Curtis et al (510) used a different clustering method, K-means clustering, to identify phenotypic clusters within the Brigham and Women's Rheumatoid Arthritis Sequential Study (BRASS). Using a large number of variables, including extensive data on comorbidities, they identified 5 complex clusters, one of which they defined as having less RA disease activity/multimorbidity, longer RA duration, more infections, psychiatric comorbidities and high health care utilization. The presence of a cluster with lower disease activity and high psychiatric co-morbidity supports the hypothesis that a low inflammation, high psychological burden cluster may exist within a heterogeneous group of RA patients.

A recent study by Roodenrijs et al (501) using clustering in difficult to treat RA patients also identified a sub-group of patients with higher psychological co-morbidity. They recruited a cohort 52 patients meeting the EULAR criteria for difficult to treat RA and collected multiple PRO outcomes and data on previous therapies alongside clinical findings. They compared them to 100 RA controls, not meeting the EULAR definition. Using logistic regression, they identified lower socioeconomic status as an independent risk factor for difficult to treat RA and several factors contributing to its development including co-morbidity, fibromyalgia and poor coping strategies. The also performed K means clustering of their cohort and identified a 3-cluster model that they deemed to be a good fit. The 3 clusters identified which they termed as 'non-adherent dissatisfied patients'; patients with 'pain syndromes and obesity'; and

'patients closest to the concept of 'true' refractory RA'. The pain syndrome and obesity group represent a subset of patients with high psychological comorbidity, although the numbers in this study were small it is supportive of the existence of a group with pain syndrome, rather than active inflammation. In this study the 3rd cluster comprised patients in whom treatment non-adherence, anxiety, depression and poor coping strategies were uncommon, which they have described as being closer to true refractory RA but it must be noted that, unlike my study, there is no imaging to confirm the persistence of synovitis in this group.

Mars et al (511) performed agglomerative hierarchical clustering on a cohort of 939 RA patients from a single centre in Finland. This cohort included RA patients from across the treatment spectrum, not just those treated with TT. They used HAQ score, DAS28, pain VAS and total annual health service-related direct costs as clustering variables. They were able to identify 4 clusters; Cluster 1 contained 550 patients and was characterized by relatively young patients with low health service-related costs, low disease activity, and minimal disability. Cluster 2 contained 269 patients and included those with the highest pain and fatigue levels. Disability was common within this cluster. Cluster 3 contained 97 patients had rather high mean costs and the highest average disease activity, but lower average levels of pain and less disability than Cluster 2. Cluster 4 only had 23 patients and was a heterogeneous group characterized by exceptionally high costs incurred by comorbidities rather than by high RA activity. Cluster 2, in this analysis once again represents a group of patients with discordant pain and fatigue relative to disease activity, which I did not definitively find within out cohort.

The recent evidence outlined above does suggest that clusters of discordant PRO and inflammation patients exist, and that these groups can be identified. However, I haven't clearly identified this group within any of my analyses. There are several reasons why this may be the case, in addition to the different PROs used in different studies as mentioned above (page 259). At the outset I had aimed to recruit all patients meeting DAS28 criteria to switch TT

regardless of whether they were switched. I was conscious of the possibility that clinicians may screen out patients who in their clinical judgment, had chronic pain or fibromyalgia and that this screening could not be assumed to be uniform across all rheumatologists. In total I only identified and recruited 3 patients who met DAS28 criteria but did not go on to be prescribed a new therapy. One of these patients rapidly went on to develop clear synovitis requiring targeted therapy within weeks and baseline ultrasound was suggestive of active disease. It therefore remains possible, that despite our best efforts, these patients were not reliably referred onto the study by the treating rheumatologist and that a degree of screening did take place producing a selection bias within the data. The reasons for this are unclear. It is possible that in many cases where chronic pain is suspected and no plan to initiate a new TT is made, formal DAS-28 scoring is not performed reliably and therefore eligibility for referral on to this study was not established. DAS28 scoring requires a laboratory measure of inflammation, which is not immediately available to the treating clinician and thus scores may not have been calculated retrospectively when there was no intention to change treatment.

Furthermore, it must be noted that my analysis was based on a significantly smaller cohort of patients (200) than analysis of the ERAN (828) and BSRBR (12,486) cohorts. However, my cohort was recruited prospectively to include detailed baseline MUS and was designed as a pilot/proof of concept to determine that phenotypic clusters with different clinical and ultrasound phenotypes could be identified within a group of advanced RA patients. It is possible that my study lacked the power to identify all clusters identified in a much larger analysis. In both the clinical and combined clinical and ultrasound analysis, cluster 1 contains a significant proportion of patients with no clinically significant ultrasound defined synovitis, and there is evidence of higher anxiety in this group relatively high fatigue relative to the intermediate cluster (cluster 2), as well as elevations in DAS-P. However, differenced in PRO results did not meet statistical significance, but hint at the possibility that these patients are represented within our dataset.

In addition to clusters of patients with discordant PRO data we had expected that we may also find groups of patients whose symptoms and DAS28 score were driven by joint damage rather than active disease. Once again, this cluster does not appear to be evident in our analyses. There are no significant differences in either joint subluxation or osteophytosis between the clusters that we have identified. The cluster with the most joint erosion is the cluster which simultaneously has the most inflammation and this is in keeping with well-established evidence that active MUS determined synovitis is associated with progression of erosions (478). Patients with long disease duration and particularly patients with poor disease control for prolonged periods (i.e. RefRA patients) may have been expected to have accumulated the most damage. Only in our combined analysis was there a significant difference in disease duration between the high inflammatory cluster and the others, but this hasn't translated into greater numbers of osteophytes or subluxation suggesting more OA in this group. In none of our analyses have we found any statistically significant differences between the number of TT or the number of classes of TT that our groups have been exposed to, so our LPA doesn't suggest that disease clusters are determined by the degree of refractoriness of the patients assigned to them.

Our LPA analyses all favour either 3 or 4 class solutions that identify clusters that a broadly definable by the levels of inflammation. The results give the impression that our data set contains a continuum of patients on an inflammatory spectrum. The appearance of dividing up a continuum into clusters is a common outcome in several studies using LPA (512, 513). It should however be noted that latent profile analysis identifies clusters which are both exhaustive and exclusive and that the output of LPA is categorical (492) and thus these represent real clusters of patients within our cohort.

8.2 Does the presence of baseline synovitis predict a response to targeted therapy?

Synovitis is the pathological hallmark of RA, and its suppression can be said to be the primary aim of RA treatment. Despite this there is little evidence that imaging confirmed synovitis at baseline is predictive of a response to RA treatment. Synovial biopsy studies have shown that a more inflammatory pathotype in synovial tissue has a better response to therapy. Dennis et al (325) showed that myeloid and lymphoid synovitis is more likely to respond to TT than pauci-immune or fibroid types, although it is unclear how this correlates with imaging phenotype. I have already hypothesised that within our cohort there would be clusters of patients with low inflammatory burden, but high TJC, VAS and PRO outcomes and clusters of patients with high inflammation manifesting as high levels of GS and PD synovitis on MUS. Although I did not identify a cluster with discordant PRO outcomes, cluster 1 in the combined clinical and MUS clusters does represent a group of patients with moderate to high DAS-28 EULAR disease activity, but with low levels of inflammation on MUS. I aimed to explore whether these different levels of inflammation led to differential responses to TT and hypothesised that ultrasound-determined presence and absence of synovitis would be associated with DAS28 response and non-response respectively.

I have compared EULAR responder status at 3 and 6 months according to presence of clinically relevant US defined synovitis at baseline using a multinomial logistic regression model in which those who responded and those who experienced adverse events were compared to non-responders. Although I showed numerically higher relative risk ratios of response and adverse events for patients with MUS defined clinically relevant synovitis, this difference did not reach statistical significance. Similar results were obtained when performing the same analysis to determine whether there was a differential response between the combined clinical and ultrasound phenotypic clusters. Therefore, within this cohort, I can say that there is no significant difference in DAS-28 CRP response between patients with

significant MUS defined synovitis at baseline and those that did not have significant synovitis at baseline. In all clusters a majority of patients responded to therapy regardless of the relative amount of inflammation associated with that cluster.

This is the first study to clearly show that baseline synovitis identified on MUS is not associated with response to therapy in advanced RA. Several studies have used MUS and MRI in early RA to determine whether using the presence of imaging defined synovitis, alongside clinical examination to inform an aggressive treat to targeted strategy improved outcomes. These have universally shown that escalating treatment based on the presence of synovitis has no additional benefit (514, 515) when used alongside a treat to target strategy. It should be noted that a criticism of these studies is that the use of very aggressive therapy strategies meant that both groups had very tight disease control and as a result there was a limited difference between the conventional management and imaging groups as synovitis was comprehensively suppressed in both treatment arms. Therefore, the power to detect any difference was limited. One extension study of the ARCTIC trial has suggested that the presence of synovitis on MUS or MRI is not predictive of a lack of response to MTX (516), but there are no published data to show the opposite, that the presence of synovitis predicts response. In my study there are clear differences in inflammatory activity between the clusters, but no statistically significant difference in DAS28 responses between the groups.

It should be noted that when comparing the US results of responders and non-responders (chapter 5, **figures 5.5-5.7**, pages 203-205) within the clusters that there is a clear improvement in GS and PD synovitis in patients who responded to therapy in clusters 2 and 3, with the most marked improvement in cluster 3, the most inflammatory cluster. In cluster 1, the least inflammatory cluster, there is minimal difference in the ultrasound characteristics of responders and non-responders. Based on this data one would assume that the absence of baseline synovitis would predict a failure to respond to therapy as there was no clear difference

in the average US scores between responders and non-responders. However, 69% of patients within cluster 1 met EULAR criteria for a moderate or good response to therapy. In cluster 3 even non-responders showed some improvement, particularly in scores for tenosynovitis, which showed marked improvements in both responders and non-responders. In the VEDERA trial (184), which compared early use of combination ETN and MTX with treat to target MTX, the absence of MUS determined PD signals in the hands of around one-third of participants, despite lower than expected rates of clinical remission, suggested that in real-world clinical practice, a substantial proportion of even symptomatic individuals with early RA might not have local inflammation to modify and that there is a disconnect between MUS results and clinical findings, with similar results found in other studies of early RA (517) . My results suggest a similar finding in established RA, that in this cohort, baseline synovitis does not necessarily predict clinical response, even when imaging is suggestive of an improvement in levels of GS and PD synovitis/tenosynovitis. It is also notable that in this cohort there were no clear differences in the distribution of synovitis, tenosynovitis or enthesitis between those who responded to TT and those who were classified as non-responders (**Figures 5.8-5.19**) after 6 months of therapy. This highlights the complexity of measuring and defining treatment response in RA.

There are several possible explanations for why patients with no clear improvement in disease activity on US may have an improvement in DAS-28 score and vice versa. Firstly, my study does not contain a placebo arm as ethically it would not be acceptable to withhold active treatment in patients with severe RA. It is well established that untreated disease leads to joint damage and an increase in morbidity and potentially mortality. It is therefore possible that a placebo effect exists in patients with no clear improvement in the MUS which has improved their perception of general health and thus improved DAS28. **Figure 5.1** (page 188) shows that in cluster 3 there is a more marked improvement in SJC and CRP, whereas TJC and VAS improve in all groups, with more marked improvements in VAS in clusters 1 and 2 in the

observed data. This improvement in subjective disease components will be more influenced by placebo than objective measures of inflammation. Secondly, it is likely that treatment with targeted therapy has effects beyond improvement in synovitis. For example, blockade of IL-6 has been shown to improve anaemia of chronic disease, improve sleep and has been associated with both weight gain and an improvement in muscle mass (518), all of which will serve to improve general health, with similar effects likely for other TTs. The use of JAKi has been suggested to produce an improvement in pain that is independent of their effects on inflammation (442, 519) which may account for an improvement in DAS28 in the absence of an improvement in MUS defined synovitis in a subset of patients. This study does not have the power to compare different TTs with different mechanisms of action, particularly because of the loss of follow up data caused by COVID-19.

In addition to exploring whether there was a difference in responder status between the combined clinical and ultrasound clusters we have used longitudinal mixed modelling to determine whether there were differences between the clusters in their response to TT over time (**table 5.2-5.3**, pages 190-191). I aimed to determine whether the individual clusters differed in their trajectory of response to TT over our 6 month follow up period, even when these differences did not manifest as significant differences in DAS28 responder status. An analysis of the traditional 4 component DAS28 score did not show any statistically significant differences between the overall DAS-28 response between groups. **Figure 5.2** (page 192) shows each of the clusters had similar pattern of response, with a rapid improvement at 3 months, with a slower further improvement up to 6 months. This treatment response over time is in keeping with my previous results showing no difference in the likelihood of a EULAR response to TT at 3 and 6 months between the clusters as all our clusters are following approximately the same disease trajectory.

Hensor et al (112) have previously modelled and validated a 2 component DAS 28 score using the SJC and CRP, which they have determined has a better association with MUS determined synovitis and radio- graphic progression than the original DAS28. As a result of this observation, and the fact that there were significant differences between the clusters in terms of inflammation, manifested by statistically significant differences in SJC and CRP, I also investigated whether there were differences in response to TT over time in the 2C DAS score. In this case there were significant differences in DAS28 score identified between the clusters over time. **Figure 5.3** (page 194) shows the fixed effects from the mixed model of DAS28CRP-2C over time by latent class. Unlike the 4 component DAS28 model, different pattens of disease trajectory can be observed, with cluster 3 showing a rapid improvement at 3 months and then a plateau up to 6 months, cluster 2 showing a slower improvement to 3 months, but ongoing improvement up to 6 months and cluster 1 improving to 6 months and then a deterioration at 6 months. Once again this illustrates, that although the clusters identified in the combined clinical and ultrasound model do not show a clear difference in EULAR response to TT, like the observable difference in MUS measured inflammation, there is a differential response in DAS28CRP-2C overtime. This supports the findings of the Hensor et al paper that suggests DAS28CRP-2C is a better measure of MUS defined inflammation than DAS28CRP-4C. This analysis would benefit from more data from further time points to track these trends further, as given the shape of the trajectories identified in this analysis it is possible that the differential response is at baseline with each group trending towards the mean over time. There was no difference between the clusters in the improvement of their VAS score over time in response to TT which suggests that despite improvement in the objective markers of disease activity there was no difference in the way patients felt about their disease activity with explains the lack of difference change in in DAS28 scores over time.

8.3 Do clusters of patients with differing disease trajectories exist within our cohort?

Further to the above analysis of DAS28 response over time, in chapter 7 I aimed to determine whether I could identify different trajectories of response to TT within our cohort and compare these trajectories to the combined clinical and ultrasound clusters. I performed growth mixture modelling to determine whether we could identify any different disease response trajectories within our cohort. The initial analysis plan had been to use latent transition analysis, a longitudinal extension of LPA, to determine whether patients could transition between clusters in response to therapy, but the loss of follow up data caused by the COVID-19 pandemic has meant that this analysis was not possible.

Instead, I have used growth mixture modelling, a different method for identifying multiple unobserved sub-populations within a larger population, describing longitudinal change within each unobserved sub-population, and examining differences in change between them. Initial analysis used the DAS28CRP-4C, but the BIC did not minimise, suggesting that no trajectories could be identified within the cohort that improved on a single class, possibly because of a limited sample size due to follow up data loss. However, using DAS28CRP-2C we identified 4 different classes of response trajectory within the cohort.

Of these classes, Class 1 had the lowest disease activity at baseline and showed a rapid improvement at 3 months with a maintenance of response up to 6 months. This class achieved very low levels of DAS28CRP-2C by 3 months and may have achieved disease remission and thus had little room for further improvement, explaining the plateau in disease activity at a very low level, but they maintained this low level of disease activity over the study period. Class 2 showed a rapid improvement at 3 months with a much slower further improvement up to 6 months. In this study patients were not allowed to have steroid treatment in the 6 weeks prior

to baseline clinical assessment, but bridging steroid treatment was allowed post ultrasound, whilst awaiting commencement of TT, which may account for some of the initial rapid response to therapy. In class 3 there was an improvement up to 3 months with an increase in DAS28-CRP2C after 3 months, although not back up to their baseline DAS28-CRP. In class 4, the class with the highest average baseline DAS28-CRP2C, on average shows no improvement up to 3 months and then interestingly their disease activity gets worse up to 6 months. Most patients (93, 61.2%) fall within trajectory 2. The trajectory classes were associated with a EULAR response in 93% of patients in trajectory 1, 74% in trajectory 2, 48% in trajectory 3 and only 18% in trajectory 4, highlighting the refractoriness of patients in disease trajectory 4.

Analysis of the baseline characteristics of patients within the different trajectory classes confirms a number of similarities with the baseline characteristics of our previous LPA analyses. The level of inflammation, reflected by the SJC and CRP is higher in each of the trajectories than the preceding one and this is reflected clearly in the differing baseline DAS28CRP-2C. Trajectory 4 has statistically significantly more inflammation than the other groups. This trajectory class, which shows no response to therapy, not only has higher baseline disease activity but also longer disease duration and more erosive change. Interestingly patients within this group have also been exposed to more previous TTs and classes of TT (Median 3 previous classes of TT) suggesting that this trajectory represents a group of refractory patients. Baseline ultrasound scores in this group are also higher suggesting that these refractory patients have persistent inflammatory disease, rather than elevated disease activity due to damage associated with their more prolonged disease course.

Comparing our trajectory classes to the combined clinical and ultrasound latent clusters identified in chapter 5 shows that most patients fall within disease trajectory class 2 and this is the most common trajectory for each of our 3 latent classes. 75.41% of latent class 1 follow trajectory 2, which is associated with a sustained response to therapy, with none in trajectory

class 4 which is characterised by a lack of response. This is interesting as it once again shows a positive response to therapy in our least inflammatory cluster of patients. Latent class 2 has 55.17% of patients within trajectory class 2, with 29.31% in class 3. More patients from Clinical and ultrasound class 3 are found in trajectory class 4 than the other classes (27.27%). This likely reflects the more severe disease activity at baseline in this cluster.

Several studies have been published on trajectories of response in clusters of patients with RA treated with TT. Courvoisier et al performed GMM on a total of 3898 RA patients stating abatacept, pooled from 9 different national registries, followed up over 2 years (520). They identified 3 response trajectories, although most patients fell into a single cluster. This cluster was labelled as gradual responders which included 91·7% of the total cohort. These patients improved gradually over time. A second cluster of rapid responders (5·6%) started with a high DAS28 at baseline and improved quickly. A final small cluster of inadequate responders (2·6%), who had a stable and relatively high disease activity over the first two years was also identified. This corresponds well with the results of my analysis, with the majority of patients falling within 1 major cluster characterized by a more gradual response to therapy. In my analysis the most populous cluster (cluster 2) has an initial quick response followed by an ongoing gradual improvement. Similarly, trajectory 1 could be described as rapid responders, who rapidly obtain and then maintain very low disease activity.

Dagliati et al performed latent class mixture modelling on 2,991 rheumatoid arthritis (RA) patients starting treatment with a biologic disease-modifying antirheumatic drug within the Biologics in Rheumatoid Arthritis Genetics and Genomics Study Syndicate cohort (521). They modelled both DAS28CRP-4C and DAS28CRP-2C response trajectories. For the 4 component DAS28 they identified 3 clusters including the following groups: a rapid responder group (67%) with quick improvement in the first observation period followed by stabilisation or slight increases in disease activity, a gradual responder group (30.73%) with slower but

consistent decrease in disease activity, and a poor responder group (2.27%). These are similar clusters to those identified by Courvoisier. They also identified 7 trajectory clusters using DAS28CRP-2C. These trajectory groups included 2 groups of good responders, 2 groups of gradual responders, 2 groups of secondary non-responders, and 1 group of low disease activity. The DAS28CRP-2C clusters were associated with different baseline characteristics in time from diagnosis, HAQ scores, and number of comorbidities and also ADA levels and drug adherence, meaning that these clusters were clinically significant. The baseline data in this study differs from what I have in my analysis, but in my data set, not only was trajectory 4 significantly more inflammatory at baseline, with higher HAQ scores, it also had exposure to a statistically significantly higher number of previous classes of TT, suggesting that this trajectory also represents a group of refractory patients.

Recent analyses have also been published relating to trajectories of change in both radiographic progression and pain in RA patients. Platzer et al (522) published a recent study identifying differing trajectories of radiographic progression in 1887 patients treated with TNFi for RA. They identified 4 trajectories of radiographic progression one with a stable Sharp-van der Heijde score (SHS) over the whole 2-year study period (86%); one with relentless progression (5.8%); one with decreasing SHS (6.9%); one trajectory of the SHS going up and down (1.4%). Although not directly comparable with my analysis, trajectories with relentless progression and stable or even improving SHS could be comparable with our high inflammatory/worsening inflammation (Trajectory 4), stable (Trajectory 2) and low activity trajectories (trajectory 1).

Finally, Mcwilliams et al (523) performed GMM on the ERAN and BSRBR cohorts to investigate differing trajectories of pain in RA patient based on SF36-Bodily Pain scores. Discrete persistent pain (59% to 79% of cohort participants) and resolving pain (19% to 27%) trajectories were identified in each cohort. In ERAN, a third trajectory displaying persistently

Low Pain (23%) was also identified. In people with normal levels of inflammatory markers after 3 years, 65% of the were found to follow a persistent pain trajectory. When trajectories were compared, greater disability and smoking history were risk factors for persistent pain trajectories in each cohort. This once again highlights the importance of discordant pain in treatment response in RA patients. I haven't found comparable results in my cohort as I was unable to identify distinct trajectories in 4 component DAS28 model. However, as highlighted above the results correspond well to a number of other recent analyses plotting disease trajectories in RA patients and importantly highlights the presence of a refractory disease trajectory within my cohort. Patients in trajectory class 4 appear to represent a small group of patients with RefRA, which warrants further investigation in future work on this cohort.

8.4 Refractory rheumatoid arthritis at Leeds Teaching hospitals NHS trust.

In chapter 7 I looked at the prevalence of RefRA in the LTHT cohort of RA patients treated with TT. We used a definition of RefRA of exposure to 3 classes of TT (i.e. failure of 2 or more TT classes), based on work by Kersley-Fleet et al on BSRBR data (308), which was the most recently published work in this area at the time I began this investigation. This definition is however, also in line with the more recently published EULAR guideline on difficult to treat RA (9). The term difficult to treat RA is a broad definition which encapsulates sub-optimal disease control due to a combination of factors including drug resistance and loss of response alongside inability to optimise therapy due to poor adherence, adverse drug effects and limitation of treatment options due to comorbidity. This can be differentiated from the concept of "true" RefRA, where synovitis persists despite to optimisation of sequential therapies. I have also compared my data to a second definition of RefRA published by Buch (339), which defined RefRA as the failure of 1 or more anti-cytokine therapy and 1 directly cell targeting therapy, to see how the extent of RefRA at LTHT changes when using the different definitions of refractoriness available at the time this piece of work was begun. According to both

definitions, more than 10% of our TT exposed cohort have RefRA (172 failed ≥2 classes of TT, 152 failed at least 1 anti-cytokine and 1 cell targeted therapy).

A figure of 10% for RefRA is similar to the proportions of RefRA patients in previous studies described in chapter 1 which estimated the extent of RefRA to be between 6% and 20% of the RA population. Since I began this work several studies that have been published that also investigate the prevalence of RefRA. These have put the proportion of RefRA in their respective populations of between 7.9% (524) and 10% (493, 525). I have presented a single centre experience of RefRA from a tertiary referral centre. Therefore, this review must come with the usual caveats that it may not be generalisable to other institutions with different patient populations and differing approaches to management of RA with TT. However, when comparing these results to the literature available at the inception of this work and subsequently published data, my results are in line with what has been found elsewhere.

The definitions of RefRA used within this study were published when the first JAKi had only recently been licensed for use in the UK (November 2017) and their inclusion in this study of RefRA patients added a further layer of complexity to the definition of RefRA. A number of the patients within LTHTs cohort had prior JAKi exposure due to a compassionate access scheme available in the trust from 2014. There are several reasons why JAK inhibition may be beneficial in RefRA patients. Due to their unique mechanism of action, inhibiting the common intracellular signalling cascade of multiple cytokines, it has been postulated that JAKi could bypass the issue of cytokine redundancy, where multiple cytokines can perform the same biological function, which could give them an advantage over single cytokine inhibitors in complex, advanced disease. Secondly, JAKi are synthetic, small molecules which act intracellularly and therefore are less likely to elicit an immune response than large protein bDMARDs. Many patients cycle through TTs due to the development of neutralising ADA, which should not be an issue with JAKi. Because of this added complexity, I created a

pragmatic classification system for our RefRA cohort (**Figure 7.1,** page 240), which included failure of 2 or more classes of TT and a JAKi as a more severe RefRA (class 3). 15.7 % of our refractory patients met this definition of refractory disease according to this pragmatic classification system. All 26 patients who met the most stringent definition of RefRA had tried and failed at least one anti-cytokine therapy, one anti-cell targeted therapy and a JAKi.

This cohort includes a larger, albeit still modest, proportion of patients with RefRA than that identified in a BSRBR report (6%) (308). The BSRBR report only included patients whose first line TT was a TNFi. My cohort includes patients who started any TT first line which may account for some of this difference. However, the majority (80%) used a TNFi first line which reflects common practice in the UK, with similar practice in several other European countries suggested by registry data (301). In many cases, the choice of first line bDMARD and the subsequent sequencing of therapies reflects the order in which the different bDMARDs came on to the market. This is reflected in the fact that 77 of the LTHT refractory cohort cycled through 2 or more TNFi, before receiving a non-TNFi bDMARD, predominantly because TNFi was the only option available when they first started TT. TNFi cycling is shown to be effective, even in the case of primary non-response to a first line TNFi (201, 208), but on a group level, switching class of biologic is likely to be more effective (345, 348).

The lack of alternative targets for therapy in those who started bDMARDs in the early 2000s may have contributed to the refractory nature of their disease. Treating RA to target (i.e., rapidly obtaining low disease activity) has helped to revolutionise RA management and studies have suggested that delay to starting a TT is associated with development of RefRA (369). It is possible that failing to gain disease control due to failure to “hit the correct target” with TT early in disease may carry a similar risk.

The absence of predictive biomarkers of drug response has partly ensured TNFi as the predominant first-line TT to be prescribed, due to the factors described above. However, in

clinical practice, several other factors contribute to the decision on which drug to start first line including patient characteristics, disease phenotype, physician and patient preference and cost. In many cases the presence of co-morbidity (e.g., malignancy, lung disease, recurrent infection), is an important consideration when starting a bDMARD. There is evidence which suggests that clinicians may favour the use of non-TNFi bDMARD in patients with co-morbidity due to the perception that they have a more favourable safety profile (307). Multimorbidity has been shown to reduce the chances of obtaining low disease activity (526) and has been identified as a risk factor for difficult to treat RA in an international survey of rheumatologists (341). Takanashi et al have recently shown that elderly patients with higher co-morbidities are more likely to meet the EULAR definition of difficult to treat RA (521). A recent study of the KUMARA cohort in Japan has shown pulmonary disease to be a risk factor for the development of RefRA, likely due to concerns both about the use of concomitant MTX and the risk of infections with bDMARD in those with underlying respiratory conditions (525). Whilst I do not have the necessary data in the LTHT cohort to describe wider difficult to treat characteristics, the inclusion of patients starting non-TNFi bDMARD first line is likely to be another contributary factor as to why this cohort appears more refractory than that evaluated by the BSRBR.

It is notable that the proportion of the LTHT cohort that is female (81.5%) is higher than a general RA population. Female gender is a recognised risk factor for a refractory disease course (369). Other risk factors identified include delay to starting targeted therapy, higher disease activity at baseline and younger age when starting a first line TT (369, 493, 525). My analysis of a subset of 60 patients shows an average delay to starting a biologic of 5 years. This is, in large part, because many patients were diagnosed with RA years before bDMARDs first became available. Exposure to an increasing number of conventional synthetic DMARDs before initiation of adalimumab has been shown to be a risk factor for a worse response to therapy (527). In the case of patients with longstanding disease prior to the introduction of

bDMARDs, csDMARD cycling will have been the only treatment option. Again, it is possible to postulate that in this situation, it is the failure to obtain early disease control which contributes to the development of RefRA, although the need to csDMARD cycle may also indicate an inherent refractoriness in these patients.

Several recent studies have published on risk factors for RefRA with conflicting results. Novella-Novaro et al (493) followed up a cohort of 41 patients who had failed 2 or more classes of TT and compared them to 71 patients who obtained remission with their first bDMARD. Using multivariate analysis, they found younger patients with erosive disease and the early absence of clinical response to the first bDMARDs were predictors of multi-refractoriness to consecutive biologics. Watanabe et al (524) identified high RF and co-existing pulmonary disease were risk factors for meeting the EULAR definition of difficult to treat RA. Takanashi et al (525) identified female sex, low body weight, older age, disease duration, and seropositivity as being associated with RefRA.

It is well established that early intervention and tight disease control prevent disease progression and structural damage in RA, leading to better outcomes for RA patients (103). Structural damage, in the form of bone erosion and secondary osteoarthritis can cause pain and stiffness in joints. In RA patients the number and volume of osteophytes in the hand joints is shown to correlate with increasing age, disease duration and the presence of bone erosions (436). Buch et al have recently introduced the concept of non-inflammatory refractory RA (NIRRA); apparent refractory patients whose symptoms are predominantly driven, not by ongoing synovial inflammation, but by structural damage and pain sensitisation (528). In a study looking for risk factors for RefRA, Becede et al (369) compared refractory patients to a non-refractory RA control and showed no difference inflammatory markers and no significant difference in SJC between the cohorts, suggesting that NIRRA may make up a substantial proportion of the RefRA population. The risk of structural damage is likely to increase with

disease duration, this may be the case for some patients within the LTHT cohort. The window of opportunity hypothesis states that early intervention can have the effect of attenuating the disease process (529), and thus reducing the risk of biologically refractory or persistent inflammatory refractory RA (PIRA). Interestingly in the prospective patient cohort (which we discuss in detail above), I did not find a group of patients with significantly more degenerative disease. The most inflammatory cluster of patients had a longer disease duration, but no significant increase in joint subluxation or osteophytosis. In all of the LPA analyses, cluster 1 represents a cohort of patients with relatively low disease activity on MUS and may best approximate a NIRRA group, but there isn't clear evidence of more damage or discordant PRO data within the cluster. In the trajectory analysis I did find a disease trajectory of patients who had failed multiple TTs and had persistent MUS defined synovitis suggesting this group may represent PIRA patients. We do not have imaging results for the LTHT retrospective cohort to explore this further, which is a limitation in this arm of our study.

I hypothesised that patients would predominantly fail sequential targeted therapies for a combination of reasons and that "true" RefRA would be rare. This has been borne out in this investigation. As expected, this cohort shows that in a large proportion of cases (over 40%), patients fail multiple targeted therapies due to a mixture of inadequate response and adverse effects. Only 6 patients in total had multiple drug intolerances as their sole reason for cycling therapies. 53% patients however failed multiple drugs due to a lack of efficacy which highlights the challenges in delivering precision medicine. I have further described non-response as mixed primary and secondary non-response although the value of this in providing a possible biological context is not clear.

Primary drug failure is characterised by absence of response to therapy. This is established at the earliest time of clinical assessment (and often varies between 12-24 weeks) which may miss a very early response with subsequent rapid loss of efficacy. Primary non-response has

traditionally been hypothesised to be due to mismatch between drug target and disease; whereas secondary failure, which develops following an initial response to therapy may be due to underlying drug immunogenicity (339). However, these definitions can be imprecise, and determination of response status falls within a spectrum. In addition, evidence that drug cycling within class is efficacious even in the event of a primary non-response (201) challenges this paradigm. Interestingly, 10 patients (6% of the refractory cohort) had shown no response to any targeted therapy to date including 1 patient who is in the severe category and had failed a total of 8 targeted therapies. These patients represent a rare group which I have defined as "true refractory". This small group warrants further biological investigation, which is beyond the scope of this study, but this represents important future work on this cohort.

The main limitation of this retrospective analysis of RefRA at LTHT is that it is a modest sized cohort with no control group to compare to. This makes it unsuitable for regression analysis to look for risk factors for refractory disease. This section of my study is designed to be descriptive and aims to provide a better understanding of the extent of RefRA using a historical cohort. As such, it lacks the wider outcome data to be able to determine the consequences of such a refractory cohort in detail but provides important insights into the prevalence of RefRA and the patterns of treatment failure within this cohort.

8.5 Janus Kinase inhibitor use at Leeds Teaching Hospitals NHS trust.

In the final part of this thesis, I have reported on one of the largest single centre UK real-life experiences of RA patients treated with JAKi at the point that these data were collected and published. There were over 120 RA patient exposures at the time the study terminated, a significant proportion of patients were refractory to several previous targeted therapies. These results confirm the efficacy of JAK inhibition in both bDMARD naïve and experienced patients, including those who meet the definition of refractory disease outlined in the previous section.

As discussed above, JAK inhibition offers an alternative approach in the treatment of RA, through blockade of the signalling of multiple cytokines, including a number implicated in disease pathogenesis. This report in a real-life population further consolidates previous data which demonstrates meaningful clinical improvement in RA patients treated with JAKi. The patient population in this thesis represents a typical mixed population, three quarters of which were seropositive, with wide-ranging disease duration (from 1 year to over 57 years) and a spread of previous exposure to both csDMARDs and bDMARDs. A quarter of patients had previous exposure to one or fewer bDMARDs. In contrast, three quarters (73.4%) of patients met the criteria for RefRA (2 or more classes of TT failure). A subset of patients within this cohort have received tofacitinib on a compassionate access scheme (33 out of a total of 54 patients treated with tofacitinib). This was for those who had exhausted previous treatment options, either through multiple failures or contraindication to individual bDMARDs prior to NICE approval of JAKi in 2017. This means that within the overall JAKi cohort, patients treated with tofacitinib had on average a longer disease duration, had been exposed to a greater number of bDMARDs and had higher baseline disease activity. Nevertheless, meaningful clinical improvements were still observed in patients in this subgroup.

Within this cohort meaningful DAS28-CRP improvements were observed across the TT treatment pathway, even in the most refractory groups, with 41.2% patients achieving DAS28-CRP <3.2 (70.8% starting in DAS28-CRP >5.1). DAS28-CRP treatment responses were recorded in 14 patients who received tofacitinib on the compassionate access scheme. This includes a good EULAR response to baricitinib in combination with LFL in one patient who had documented primary non-response to seven previous TTs (including compassionate access tofacitinib). This patient met DAS28-CRP <2.6 criteria after 6 months of treatment (DAS28-CRP improved from 6.40 at baseline to 2.02). Nevertheless, the most notable

improvement in DAS28-CRP scores was still observed in patients who received a JAKi as their first line targeted therapy (**figure 7.4,** page 252).

The success of tocilizumab and sarilumab has demonstrated that IL-6 is an important therapeutic target in RA. JAK is involved in signal transduction of type I and II cytokine receptors, including IL-6 receptor which signals via JAK1, 2 and TK2. JAK 1 is thought to be most important for IL-6 function as genetic ablation of JAK1 severely inhibits gp130 signalling, whereas knock out of the other JAK molecules has little effect (530). All the available JAKi currently licensed for use in RA inhibit JAK1, with varying avidity. This suggests that IL-6 blockade may be an important factor in the clinical efficacy of JAKi and has been postulated to be the primary mechanism by which JAKi derive their efficacy in RA (531). As a result, I explored whether previous failure of targeted IL-6 blockade was related to JAKi failure. A meaningful DAS28 response was noted in 47.3% of patients who had previously failed an IL-6 targeted therapy within this cohort (36/76). This included responses in 12 patients who had documented primary non-response to tocilizumab therapy. This implies that the clinical efficacy of JAK inhibition may be due to effects beyond the interruption of IL-6 signalling and JAKi remains a good therapeutic option on those who have failed IL-6 blockade. Dua et al (532) have recently published data from a study of patients in the CORRONA registry in which 122 patients were switched from JAKi to IL-6 blocker and 144 patients switched the other way. Response was shown in both arms of this study, confirming our findings and also showing that switching from a JAKi to an IL-6 blocker can be efficacious.

The role of switching between JAKi, particularly in those with limited therapeutic options, is also of interest as no data on the efficacy of this approach was published at the time of this study. Switching within class for other bDMARDs has shown efficacy. TNFi cycling has been shown to be effective, even in the event of apparent primary non-response to the first drug (201). There is more limited data on the efficacy of switching between IL-6 blockers, with a

long term extension of the ASCERTAIN study showing ongoing efficacy when switching from IV TCZ to sc SAR (533), but this was in patients with ongoing efficacy, rather than those with failure of a first IL-6 blocker. Differences in drug molecule, binding affinity, target, and pharmacokinetics all likely play a role in response to drug therapy (534). The differing selectivity of JAKi, individual drug specific bioavailability and tissue penetrance may provide a rationale for why switching between them may be successful in patients who failed to respond to their first JAKi.

This preliminary data shows that this approach can be successful with five of seven patients switching from tofacitinib to baricitinib responding to treatment. Since publication of this data several other case series have produced similar results, suggesting JAKi cycling is efficacious in a subset of patients (535, 536). Diminishing response of DMARDs including bDMARDs is generally recognised following successive treatment failure (68, 334). Clinical trial data suggest this may not be the case with JAKi, likely attributable to the broader targeting of JAK inhibition. Comparable response profiles have been reported in MTX-IR and the more refractory bDMARD-IR (291, 298, 354) in JAKi trial cohorts, with impressive efficacy in head-to-head trials against TNFi (primarily ADA), the most established bDMARDs.

I performed a Kaplan Meier survival analysis for the cohort over the whole study period, according to prior bDMARD exposure to investigate whether exposure to multiple previous bDMARD was associated with lower cumulative survival of JAK inhibitors. Lower survival was identified in patients who have previously been exposed to 2-4 previous classes of targeted therapy. This does suggest that even with JAKi, shorter drug survival may be associated with more refractory disease. However, in this cohort this result needs to be interpreted with some caution. The longer follow-up of patients that started their JAKi through the compassionate access scheme is compared to patients who started treatment after NICE approval, potentially with less refractory RA and who have been censored at the end of the study period. This

introduces a source of bias. The more refractory cohort with more limited (or no) further treatment options may also have influenced the decision to continue therapy for longer than would have been done in patients with still treatment options available.

Toxicity with the two JAK inhibitors was as expected and similar to the adverse events highlighted in the clinical trials programs. These mostly comprised infections and deranged liver function. Two patients sustained a DVTs, but both had known risk factors. Although there are a number of warnings regarding VTE in JAKi patients and early trial data suggested an excess of VTE over controls, more recent analysis casts doubt on this (537) and the risk of VTE in JAKi exposed patients remains controversial. Nevertheless, the decision to continue both patients on JAKi highlights the challenging decisions that occur in clinical practice compared to clinical trials. Here, a multi-disciplinary discussion with colleagues in haematology and a shared decision-making approach with fully informed patients were central components to the management plan.

Again, this report has its obvious limitations. It is a modest-sized, observational cohort combining both prospective and retrospective data, with all the associated caveats including absence of a ‘control’ cohort, channelling bias, and descriptive outcomes. Again, these factors make the study unsuitable for regression analysis to identify predictive factors of good response, which would have been valuable. Longer-term outcomes for evidence of attrition on JAKi will also be clearly important to evaluate. Whilst the focus is of clinical responses of the entire cohort, I have also presented data for tofacitinib and baricitinib separately – but would caution that this unmatched, observational study does not permit direct comparison of outcomes between the two drugs.

In summary in a markedly heterogeneous cohort, I clearly observed clinical improvement in patients treated with JAK inhibition following MTX-inadequate response and in the most

bDMARD-refractory of patients. These data underscore the potential for JAKi to avoid some of the effects of cytokine redundancy, which could give them a unique role in the broad management of RA as well as more complex, advanced disease.

8.6 Limitations and future work

I have shown in this study that heterogeneous phenotypic clusters of patients exist within a larger homogeneous group of advanced RA patients, both in terms of their baseline characteristics and their subsequent disease trajectories. However, this study has several limitations that would be addressed in future work in this area. This was always aimed to be a proof-of-concept study, to show that LPA could be used in a cohort of established RA patients, not only using PRO and clinical data, but also extensive ultrasound data as clustering variables, to identify differing phenotypic clusters. It was aimed to serve as a pilot to inform a larger piece of work with greater power to detect differences. The two main limitations of this study are the sample size and the subsequent loss of follow up data caused by the COVID-19 pandemic. Many studies using LPA, or other methods of identifying disease clusters, use existing registry data, with large sample sizes that allow greater statistical power to identify disease clusters. My study is unique in that it includes detailed ultrasound data containing imaging of multiple joints, tendons and entheses in the analysis, but this meant that this cohort had to be collected prospectively and this limited the number of patients that could be included. This study was performed in a single centre and the recruitment time-period was constrained to fit in with a PhD study period and then ended prematurely by a pandemic.

At the outset I had aimed to recruit as many patients as possible during a 2-year recruitment period with a minimum target of 200. It was observed that around 4 patients were started on a new TT every week at LTHT giving approximately 400 patients starting a TT during the recruitment period and thus the potential to have recruited more. The rate of recruitment was

primarily limited by ultrasound capacity as each scan took 45 minutes. It was therefore not possible to arrange scans for every possible recruit due to resource limitations. For future work it would be desirable to run the study over multiple sites to improve capacity and allow greater recruitment of patients. Greater MUS capacity, including same day MUS would also allow the recruitement of patients with significant disease activity who were unable to wait for baseline investigation as they required immediate steroid treatment. Some patients, including a number with significant disease activity were lost from this study for this reason and it would be beneficial to include them in the study as they likely represent some of the patients with the most active disease.

It would also be beneficial to extend the recruitment period to allow for a larger cohort of patients to be recruited. For LPA sample size needs to be sufficiently large for the selection criteria to accurately assess how many clusters are present in the data. In one simulation study of the performance of selection criteria at sample sizes of n=200, n=500 and n=1000 under a variety of conditions, the BIC correctly identified the number of clusters present at n=200 74% of the time, when 10 items were included and the structure was complex (487). This increased to close to 100% of the time at n=500. Thus, a target of 500 patients would give statistical power to detect clusters with greater accuracy and could possibly lead to the detection of more disease clusters than I have identified in this smaller cohort.

Although I have been able to perform a growth mixture modelling on my data set to determine that different disease trajectories exist within the cohort, I was not able to carry out a latent trajectory analysis due to data loss caused by the COVID-19 pandemic. Latent trajectory analysis is a longitudinal extension of LPA and it would have allowed me to determine whether patients change their latent profile over time and how that change in profile was related to treatment with targeted therapy. It is probable that individuals change disease cluster in response to TT treatment, most obviously from high inflammatory to low inflammatory in the

case of response. It would be interesting to determine whether some patients who do not have a clear DAS28 response to a targeted therapy also change cluster, for example from a high inflammatory cluster to a lower inflammatory cluster with discordant PRO outcomes and higher subjective DAS28 components. This would suggest a biological response to TT but ongoing pain sensitisation or psychological co-morbidity leading to a clinical non-response (my ultrasound data shows an improvement in synovitis in some non-responders from the high inflammatory cluster). This would potentially allow me to identify clusters representing NIRRA and PIRA as proposed by Buch et al (528) and to tailor further treatment in these clusters accordingly. Repeating the study, not only with a larger population of patients to increase its statistical power, but also with complete follow up data would allow me to perform this analysis.

Having a larger cohort of patients for a latent transition analysis would also allow me to directly compare whether differential effects of different targeted therapies had an influence on disease trajectory or transition between clusters. As has been highlighted above, evidence from the clinical trials programme of baricitinib have suggested that it may have effects on pain that are independent of its ability to supress inflammation. It would thus be interesting to determine whether patients treated with JAKi transitioned to different clusters than patients treated with bDMARDs. It is also shown in the clinical trials programmes of multiple JAKis that they have a rapid onset of action when compared to bDMARD. This may give patients treated with JAKi a different disease trajectory, with a more rapid initial response which could be identified clearly. These analyses would require randomisation of patients onto differing TTs, as baseline disease phenotype may influence a clinician's decision as to which therapy to prescribe, introducing a channelling bias into the data.

This study was pragmatic in design in order to represent standard UK clinical practice. This introduces several limitations which could be corrected in future work. We do not know the rate of drug compliance within the cohort and how that influenced response and disease

trajectory. Dagliati et al (521) used both patient reported compliance and drug and antibody levels to determine adherence to treatment and were able to identify a DAS28CRP-2C disease trajectory that was associated with low adherence. Drug and antibody levels could be included in a future study, not only to clarify drug adherence, but also to help classify reasons for TT failure within patients within the cohort by confirming ADA formation in non-response or loss of response. I have collected large amounts of serum and whole blood samples as part of this study which has been stored for future analysis and this could also be screened for drug levels and ADA. Secondary failure of TT is thought primarily to be driven by ADA development, but it remains possible that a proportion of primary non-response is driven by very early immunogenicity. Including a drug and anti-body level at the 4-week safety blood test would help to identify early ADA production and could help to inform whether ADA has any role in primary non-response in the research cohort.

In this study, patients with non-response to targeted therapy switched treatment to a second drug at 3 months and started back at baseline. To accurately track all possible treatment response trajectories, it would be beneficial for all patients to complete at least 6 months of therapy, including those with no response at 3 months. Late responders to TT therapy have been identified in registry data, suggesting that there may be a cluster of patients that have an initial non-response followed by an improvement after the 3-month follow up period. I have not identified this disease trajectory as clear non-responders stopped their treatment at this point and switched therapy. However, it should be noted that for this study, it is felt that it would not be appropriate, from an ethical point of view, to continue with a treatment that wasn't working due to the risks of ongoing morbidity. On a pragmatic level, in normal clinical practice and particularly in patients with limited further treatment options available, patients may stay on a treatment for longer than 3 months in order to assess for later response, so it may be possible to identify this potential disease trajectory. A greater number of follow up time points and a longer follow up period, may also identify more treatment response trajectories.

Furthermore, a control group of patients not starting TT, but maintained on standard therapy, including the use of steroid treatment following baseline investigation would be valuable to track disease trajectory and response. However, again this comes with the same ethical considerations of not optimising therapy.

Finally, this established RA cohort has been phenotyped using clinical, PRO and ultrasound data. It would be beneficial to also include biological investigations to more deeply phenotype the cohort. Blood samples could be analysed for proteomic and genetic markers that could be both compared to our existing clusters to determine if identifiable differences exist between them and used for further cluster analyses to identify more deeply phenotyped cohorts of patients. It is recognised that there are some genetic differences between seropositive and seronegative disease, and it would be interesting to determine whether there were any differences that associated with different disease cluster. It would also be informative to perform synovial biopsy on a subset of patients to determine whether previously identified synovial pathotypes, Lymphoid, myeloid, pauci-immune and fibroid synovitis, correlate with our differing phenotypic clusters.

8.7 Conclusions

At the outset of this work I hypothesised that RefRA would be a heterogeneous condition, with patients failing TT for a variety of reasons, with the most common pattern of drug sequencing being due to a mixture of non-response loss of response and adverse effects. Furthermore, we hypothesised that “true” refractory disease, patients who make no significant or sustained response to TT, would be a rare but real and identifiable subgroup. I have shown both hypotheses to be correct. More than 10% of our RA cohort were refractory and most cycled therapies due to a combination of non-response and adverse events. A small percentage (6%) of our RefRA cohort had multiple sequential TT failures. This small subgroup warrants further

biological investigation to further inform of the nature of TT resistance. I have also shown that in a real-world population of RA patients that JAKi are an effective treatment for RefRA patients and published some of the first results showing that both JAK sequencing and use after IL-6 blockade are effective treatment options.

Using a prospectively recruited cohort of RA patients starting on TT, I have been able to confirm the existence of distinct established RA disease clusters, best characterised by varying degrees of inflammation, measured both by clinical assessment and ultrasound. This is the first study to use comprehensive ultrasound data as clustering variables, as well as PRO and clinical data. I have shown that this is a viable method to identify RA disease clusters to inform future work in this area. Interestingly, I was unable to show that either baseline synovitis or disease cluster was associated with response to TT. Patients with minimal inflammation on US responded well to therapy and patients without EULAR clinical response were shown to have had improvements in MUS measured inflammation, highlighting the complexity of measuring treatment response in RA. I haven't confirmed either clusters of patients with discordant inflammation and PRO data or groups with greater amounts of joint degeneration driving their high disease activity, emblematic of non-inflammatory disease activity driven by chronic pain or osteoarthritis, but a more nuanced picture with clusters with differing levels of inflammation, but with similar levels of response to treatment, suggesting that factors beyond improvement in synovitis are associated with treatment response in RA.

Finally, I have shown that within a cohort of advanced RA patients, a number of different treatment response trajectories exist. Most patients followed a trajectory of gradual response to treatment, and this is consistent with other examples in the recent literature, but more refractory trajectories exist, including a trajectory of patients with persistent ongoing inflammation despite TT treatment. This small group of patients (trajectory 4) have failed multiple previous TTs and represent a true refractory disease trajectory classified by marked

ongoing inflammatory activity on ultrasound, rather than chronic pain or damage. The significant loss of follow up data, caused by the advent of the COVID-19 pandemic has limited my ability to determine the differential effects of different classes of targeted therapy, but this study has shown that these trajectories exist and I have highlighted how future work in this area may help to answer these questions.

I have used two parallel studies to attempt to answer a series of questions regarding the nature, extent and pattern of refractory rheumatoid arthritis and to phenotype a cohort of advanced RA patients in order to inform on the heterogeneity of the condition. The ability to identify differing subgroups of patients within the broader RA population will allow us to begin to tailor treatments towards patients with differing disease phenotypes and this study helps to start this process and informs on the feasibility of ongoing work in this area. I hope this helps to take a step towards precision medicine for difficult to treat RA patients.

9 References

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10 Appendix 1: Ultrasound Proforma

	Right					Left				
	B Mode	Doppler mode	Erosions	Osteophyte	Subluxation	B Mode	Doppler mode	Erosions	Osteophyte	Subluxation
	0-3	0-3	Yes/no	Yes/No	Yes/No	0-3	0-3	Yes/no	Yes/No	Yes/No
Wrist										
MCP 1										
MCP 2										
MCP 3										
MCP 4										
MCP 5										
PIP 1										
PIP 2										
PIP 3										
PIP 4										
PIP 5										
Elbow										
Knee										
Tibio-talar										
MTP 1										
MTP 2										
MTP 3										
MTP 4										
MTP 5										
Other (please specify)										

Tenosynovitis

	Right		Left	
	Tenosynovitis SQ grading 0-3		Tenosynovitis SQ grading 0-3	
	GS	PD	GS	PD
Wrist extensor compartment 2				
Wrist extensor compartment 4				
Wrist extensor compartment 6				
Finger flexor tendons 2*				
Finger flexor tendons 3*				
Finger flexor tendons 4*				
Finger flexor tendons 5*				
Tibialis Posterior				
Peroneus longus and/or brevis				

*MCP level

Entheses

	Right							Left						
	Enthesis thickening	Tendon body thickening	Hypoechogenicity	Doppler mode	Calcifications	Enthesiophytes	Erosions	Enthesis thickening	Tendon body thickening	Hypoechogenicity	Doppler mode	Calcifications	Enthesiophytes	Erosions
	Yes/No	Yes/No	Yes/No	0-3	Yes/No	Yes/No	Yes/No	Yes/No	Yes/No	Yes/No	0-3	Yes/No	Yes/No	Yes/No
	mm	mm						mm	mm					
Lateral epicondyle														
Patella ligament proximal insertion														
Patella ligament distal insertion														
Achilles tendon														
Plantar fascia														

Report/Clinical impression.

Synovitis secondary to active RA

Tenosynovitis ☐ ☐

OA related joint damage ☐

Mixed OA and RA related synovitis ☐

Erosions ☐

Normal ☐

Other (*please include free text*):