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PAIN, MECHANISMS OF FATIGUE AND AUTONOMIC FUNCTION IN RHEUMATOID ARTHRITIS

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Stockholm 2016

Published by Karolinska Institutet.
Printed by Eprint AB 2016

ISBN 978-91-7676-454-1

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THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Reem Altawil

Defense of the thesis will take place on Friday 9th of December, at 9.00 in the CMM lecture Hall, CMM L8:00, Karolinska University Hospital, Solna.

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TO MY FAMILY

ABSTRACT

Rheumatoid arthritis (RA) is a disabling chronic autoimmune disease characterized by joint pain, and potentially leading to a serious decay of life quality. Pain remains the most important problem for patients with RA, and there is an obvious need to increase the knowledge of pain patterns in early disease and relation to anti-rheumatic treatment. The first three papers of this thesis are based on the population-based early RA cohort (EIRA study), linked to the Swedish Rheumatology Register (SRQ). The main focus was to investigate pain patterns in early RA and the relation to other clinical factors. First we studied the frequency of remaining pain after three months' treatment with the first-line agent methotrexate, and found this outcome in a majority of the patients. Moreover, remaining pain was found in almost a third of patients with good clinical response, and predicted by high disability and low inflammatory activity at diagnosis. Further, we found that remaining pain despite satisfactory inflammation control one year after diagnosis, strongly predicts development of widespread pain and fatigue three years after diagnosis. Next, we used a more severe pain outcome, unacceptable pain, and studied the pain course during the first five years after diagnosis. We found that almost a third of the patients still have unacceptable pain after one year of adequate anti-rheumatic treatment, and there is minor further decrease of the proportion with this outcome after five years, suggesting that optimization of immune suppressive treatment can not decrease pain levels further at this stage. Women were more likely than men to develop unacceptable pain and the strongest predictors at diagnosis for this outcome were disability, patients global assessment (PGA) of disease, high number of tender joint count (TJC) and low number of swollen joint count (SJC). At diagnosis, pain correlated to disease activity and SJC/TJC, and this correlation increased after three months to stable levels that remained throughout the first five years of disease. TJC was higher correlated to pain than SJC during the whole early RA disease course. Pain mechanisms are closely linked to the autonomic nervous system (ANS), and dysfunction of autonomic activity is well documented in pain conditions such as fibromyalgia (FM). Our investigation of ANS function in RA and FM revealed different autonomic patterns that could also be coupled to differences in neuroinflammation. Thus, central nervous system (CNS) mechanisms in RA were characterized by an IL-1β dominated intrathecal immune activation, which, unlike in FM, was coupled to reduced parasympathetic activity. These data indicate an earlier unknown interaction between CNS mediators and autonomic activity, which may be of interest to further identify treatment targets in neuro-immune regulation. Conversely in FM, there was an increase of central IL-8, known to associate with pain regulation, and FM also displayed an upregulation of sympathetic activity, which was independent of neuroinflammation.

Altogether, our data imply that remaining pain after anti-rheumatic treatment is not uncommon. The frequency of remaining pain stabilizes during the first years of disease and is a strong risk factor for subsequent generalized pain. Furthermore, we have shown that neuroinflammatory patterns in RA are coupled to autonomic dysfunction, and also clearly differ from FM, indicating different mechanisms behind RA pain and dysfunctional pain. The findings of this thesis have illustrated the pain problem in early RA, and hopefully this knowledge may contribute to early identification and treatment of patients at risk of developing pain conditions in connection to their rheumatic disease.

LIST OF SCIENTIFIC PAPERS

I. **Altawil R**, Saevarsdottir S, Wedrén S, Alfredsson L, Klareskog L, Lampa J. Remaining pain in early rheumatoid arthritis patients treated with methotrexate. *Arthritis Care Res (Hoboken).2016;Aug 68(8):1061–1068.* doi: 10.1002/acr.22790.

II. Schelin MEC, Saevarsdottir S, **Altawil R**, Klareskog L, Alfredsson L and Lampa J. Remaining pain despite inflammation control in rheumatoid arthritis – long-term increased risk for widespread pain and fatigue. *Manuscript.*

III. **Altawil R**, Westerlind H, Schelin M, Klareskog L, Alfredsson L, Lampa J. Repeated pain assessments and the cumulative frequency of unacceptable pain during the first five years after diagnosis in Swedish rheumatoid arthritis patients. *Manuscript*

IV. Kosek E, **Altawil R**, Kadetoff D, Finn A, Westman M, Le Maître E, Andersson M, Jensen-Urstad M and Lampa J.
Evidence of different mediators of central inflammation in dysfunctional and inflammatory pain - interleukin-8 in fibromyalgia and interleukin-1 β in rheumatoid arthritis. *J Neuroimmunol. 2015; Mar 15;280:49-55. doi: 10.1016/j.jneuroim.2015.02.002.*

CONTENTS

LIST OF ABBREVIATIONS

ACPA	Anti Citrullinated Protein Antibody
ANS	Autonomic nervous system
ACR	American college of Rheumatology
CCP	Cyclic citrullinated peptides
CNS	Central Nervous system
CSF	Cerebrospinal fluid,
CRP	C-reactive protein
CTLA4	Cytotoxic T-lymphocyte-associated protein 4
DAS	Disease activity
DMARD	Disease modifying anti-rheumatic drug
ESR	Erythrocyte sedimentation rate
EULAR	European League Against Rheumatism
FM	Fibromyalgia
HRV	Heart rate variability
HAQ	Health Assessment Questionnaire
HLA	human leukocyte antigen
IL	Interleukins
IASP	International Association for the Study of Pain
OR	Odds ratio
PASS	Patient Acceptable Symptom State
PTPN22	Protein tyrosine phosphatase, non-receptor type 22
RA	Rheumatoid arthritis
SJC	Swollen Joint Count
SRQ	Swedish Rheumatology Quality Register
TJC	Tender Joint Count
TNF	Tumor necrosis factor
VAS	Visual analog scale
WSP	Wide spread pain

1 INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease of the joints where pain is a general and important symptom Pain is often the earliest symptom bringing the patient to rheumatology care for the first time and is highly ranked by patients. The recent decade, anti-rheumatic treatment strategies have improved and the aiming for remission has led to better treatment results and less functional impairment in many patients. However, still it is a clinical fact that many patients continue to have pain, sometimes also when the inflammation has been adequately suppressed. The overall aim of this thesis is to investigate the extent of the pain problem in RA, and how this may relate to other clinical factors at diagnosis. Moreover, the mechanisms of pain and fatigue in relation to inflammation and autonomic activity have been studied. A future goal would be to identify patients at risk of developing long-standing pain, and provide efficient preventing strategies in the early phase of the disease.

1.1 RHEUMATOID ARTHRITIS

1.1.1 Clinical course and epidemiology

Rheumatoid arthritis (RA) is a chronic inflammatory joint disease characterized by pain, swelling, and stiffness of the joints, subsequently leading to joint destruction, functional impairment and chronic pain [1] The overall prevalence of RA is 0.5-1% and an incidence of 50 per 100.000 in Sweden [2, 3]. The disease is more common in women, and the female to male ratio is 3:1. RA affects all age groups, with highest incidence between 45 and 65 years [4, 5].

1.1.2 Etiology

The etiology of RA remains unclear, although an interaction between autoimmune mechanisms and environmental exposures have been implicated as important for the pathogenesis [6, 7]. Thus, the concordance of RA is about 15% for homozygotic twins [8, 9] suggesting that environmental factors in addition to the genetic influence may have an important role for development of the disease. Specific HLA alleles (e.g. HLA-DRB1) are among the strongest genetic risk factors for RA, affecting both disease susceptibility and disease severity [10]. Several HLA-DRB1 alleles, for example *01:01 and *04:01 share a specific amino acid sequence (the shared epitope (SE); position 70-74 in the third region of the DRB1 beta chain) and this sequence is located in the peptide-binding groove of the protein. Other important risk genes for RA include PTPN22 and CTLA-4 [11, 12]..

Furthermore, HLA-DRB1*4 strongly associated with presence of anti-CCP antibody positivity patients with RA and may predict more progressive disease [13] while there is no association with RF positivity [14] Among the environmental risk factors, smoking is the most important [15] , and has been found to interact with genetic susceptibility [16]. Other environmental risk factors include breast-feeding, adverse pregnancy outcome, previous blood transfusion and obesity [17].

1.1.3 Autoantibodies in RA

In 1940 rheumatoid factor (RF) was discovered [18, 19] and for many years was used as a marker of disease severity in RA. The later findings of autoantibodies to citrullinated peptides, i.e. anti-citrulline peptide antibodies (ACPA), with a high specificity (96-98%) for RA was a major step forward, both in clinical diagnosis and further understanding of RA pathogenesis [20] . Interestingly, ACPA have also been found in the circulation before disease onset [21], and suggested to play a role in the initiation of the disease [16] as well as osteoclast activation/bone destruction [22, 23] and pain mechanisms [24] .

1.1.4 Treatment considerations

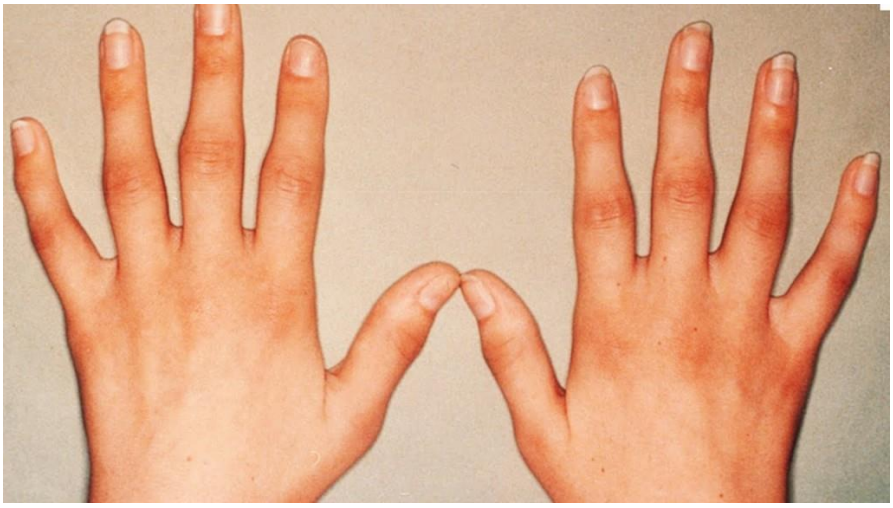
The current pharmacological treatment of RA include nonsteroidal anti-inflammatory drugs (NSAIDs), disease modifying anti-rheumatic drugs (DMARDs), biologics and corticosteroids [25]. Whereas NSAIDs often provide pain relief in the short-term, DMARDs also have the effects to retard or prevent joint destruction. Methotrexate is considered the first-line DMARD agent in RA and has proven efficacious to induce remission in about a third of the patients [26, 27]. During the last decade, the treatment goals in RA have switched from a focus on low disease activity into reaching total inflammatory remission [28]. The use of biologics in disease management has improved disease outcome and may further suppress disease activity and prevent joint destruction in patients with unsufficient clinical response to methotrexate. Biologic agents registered in Sweden include tumor necrosis factor (TNF) antagonists, abatacept (inhibition of the T-cell co-stimulation), tocilizumab (IL-6 receptor inhibition), rituximab (B-cell depletion) and anakinra (IL-1beta receptor antagonist). [29] . Corticosteroids provide important adjunct therapy in the management of arthritis, and addition of low-dose steroids to DMARDs is recommended in moderately active early RA [25] . Moreover, intra-articular injections of steroids may be efficacious for suppressing arthritis in single joints. Further, non-pharmaceutical interventions are important, where physical exercise is a hallmark in the management of RA. Moreover, team work between rheumatologists,

physiotherapists, occupational therapists, social worker and rheumatology nurses provide important management of the newly diagnosed RA patient[30-32]

1.1.5 Clinical features and classification criteria

In the majority of RA patients, the onset of the disease is insidious, with joint pain, stiffness and symmetrical swelling of a number of peripheral joints (figure 1). Initially, pain may be experienced only on movement of joints, but subsequently also pain at rest and prolonged early morning stiffness develops [33]. If left untreated, various degrees of joint destruction, deformity, and a significant decline in functional status which can affect the patient's capacity to perform the activities of daily living [34]. In addition to articular deterioration, constitutional symptoms such as fatigue, malaise, weight loss, and low-grade fever, may be present. Less often, extra articular organ involvement such as the skin, heart, lungs, and eyes can also be significant [35, 36].

Figure 1. Symmetric swelling (arthritis) of small joints in RA.



RA is primarily a clinical diagnosis. No single diagnostic test definitively confirms the diagnosis. However, several tests can provide objective data that increase diagnostic certainty and allow disease progression to be followed [37]. In 1987, the American College of Rheumatology (ACR) established classification criteria for rheumatoid arthritis [38] (Table 1), originally designated for research and clinical trial purposes. Despite that intent, the criteria have been widely used to make the diagnosis of RA.

Table 1. The 1987 revised classification criteria for rheumatoid arthritis [38]

Rheumatoid arthritis is defined by the presence of 4 or more of the following criteria:
1. **Morning stiffness in and around joints lasting at least 1 hour before maximal improvement**
2. Soft tissue swelling (arthritis) of 3 or more joint areas observed by a physician
3. Swelling (arthritis) of the proximal inter phalangeal metacarpophalangeal, or wrist joints
4. Symmetric swelling (arthritis)
***5*. Rheumatoid nodules**
6. The presence of rheumatoid factor
7. Radiographic erosions and/or periarticular osteopenia in hand and/or wrist joints.
Criteria 1-4 must have been present for at least 6 weeks

In order to be able to identify possible rheumatoid arthritis earlier, and prevent delay of diagnosis, new RA classification criteria were formed 2010 by the ACR and the European League Against Rheumatism (EULAR) [39] (Table 2).

Table 2. The 2010 ACR/EULAR classification criteria of rheumatoid arthritis [39]

Target population: patients who (1) have at least 1 joint with clinical synovitis and (2) with synovitis not better explained by other disease.	
Joint involvement (tender/swollen)	
1 large joint	0
2-10 large joints	1
1-3 small joints (± involvement of large joints)	2
4-10 small joints (± involvement of large joints)	3
>10 joints (at least 1 small joint)	5
Serology	
Negative RF & ACPA	0
Low-positive RF/low positive ACPA	2
High-positive RF/high-positive ACPA	3
Acute-phase reactant	
Normal CRP & ESR	0
Abnormal CRP & ESR	1
Duration of symptoms	
< 6 weeks	0
≥ 6 weeks	1
Add score of categories A-D: ≥ 6/10 = define RA	

1.1.6 Disease activity and clinical response to treatment

There has been a dramatic improvement in the treatment of RA in the last 2 decades and disease remission is today considered to be a realistic goal for patients and physicians [40]. Disease activity is measured using a standardized index, the DAS28. DAS28 is a validated index of RA disease activity [41] and calculated using the results of the 28 tender joint count (TJC 28) and the 28 swollen joint count (SJC 28), erythrocyte sedimentation rate (ESR) and patient global assessment (PGA) (Table 3). The level of disease activity and the cut-off points measurement is classified as low (DAS28≤3.2), moderate (3.2<DAS28≤5.1), or high (DAS28>5.1), cut-off point of DAS28 <2.6 is considered as clinical remission (Table 4) [42].

Table 3. Disease activity index

DAS28 index	Scoring scale
TJC	0 - 28
SJC	0 - 28
ESR	0 -140
PGA	0 -100

Table 4. Disease activity cut-off measures

Level of disease activity	DAS28 score
Remission	< 2.6
Low	≤ 3.2
Moderate	> 3.2 and ≤5.1
High	>5.1

For the assessment of the efficacy and response to the anti-rheumatic treatment, changes in the disease activity score 28 (DAS28) is measured by criteria established by the European League Against Rheumatism (EULAR)[43]. According to these criteria, good EULAR response is defined as a decrease in DAS28 of >1.2 and an attained DAS28 of ⩽3.2. No EULAR response is defined as a decrease in DAS28 of ⩽0.6 with an attained DAS28 of >5.1. Patients not fulfilling these criteria are classified as moderate EULAR responders (Table 5) [44].

Table 5. European League Against Rheumatism (EULAR) response criteria in RA.

EULAR Response	Improvement of DAS28 from baseline
Good	>1.2
Moderate	>0.6 and ≤1.2
None	≤0.6

1.2 PAIN PERCEPTION

1.2.1 Definition of pain

There are several descriptions of the pain experience, and one often described is the pain definition from IASP: "Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" . Pain can range from localized, such as joint pain due to synovial inflammation, or it can be more diffuse as in disorders like fibromyalgia. In general, pain is the most common reason that individuals seek medical attention and it has been considered as the "fifth vital sign" [45]

1.2.2 Classifications of pain

Mechanism-based pain diagnosis can be difficult, However, classifying pain could be helpful to the physicians for guiding assessment and treatment. The two most frequently used approaches for classifying pain are based on pain duration i.e. acute vs chronic pain (usually pain more than three months) and underlying pathophysiology i.e. nociceptive vs. neuropathic pain. Moreover, pain with no clear underlying somatic cause as well presence of psychological factors is classified as psychogenic pain. Absence of an identifiable physical or psychologic cause, pain is classified as idiopathic pain [46].

1.2.3 Nociceptive pain

The type of pain that is caused by normal response to noxious insult or injury of tissues, could be somatic such as skin, musculoskeletal and joint pain and is often well localized, or visceral such as organs and smooth muscles, often referral.

Nociceptive pain usually described as a sharp, aching or throbbing pain through activation of nociceptors — specialized sensory neurons that are stimulated by noxious mechanical, thermal or chemical stimuli [47]. Nociceptors transform these stimuli into electrical signals and relay them to the central nervous system. Nociceptive pain tends to be short-lived, but if it persists beyond 12 weeks, it becomes chronic pain.

The link between the nervous and immune systems is important in the mechanism of transition from acute to chronic pain. The pro-nociceptive influence on peripheral nerve fibers is mediated by locally released cytokines by variety of cells such as macrophages, fibroblast and mast cells. However, these peripheral cytokines do not easily cross the blood–brain barrier. Moreover, the central nervous system has its own source of cytokines produced by glia cells that matters in pain processing [48]

The main peripheral mechanism underlying acute nociceptive pain is a change in the activity of the nociceptors located in the tissue which makes them more sensitive to normally painful stimuli (hyperalgesia) or normally non-painful stimuli (allodynia) [49]. However, in chronic pain, central nervous system sensitization and facilitation of nociceptive stimuli, cause a generalized reduction in the pain threshold leading to the appearance of hyperalgesia and allodynia [50],

Involvement of cytokines in nociceptive hypersensitivity has previously been suggested. There is evidence that pro-inflammatory cytokines IL-1β, IL-8 and TNF contribute to pain and hyperalgesia by different mechanisms [51].

1.2.4 Neuropathic pain

Neuropathic pain is caused by a primary lesion or disease in the somatosensory nervous system. Neuropathic pain is often described as sharp, stabbing or shooting. Some possible reasons for neuropathic pain include nerve irritation, nerve damage or the formation of a neuroma.[52]

1.2.5 Psychogenic pain

Type of pain caused by a psychological disorder, such as depression or anxiety and it may have physical complications, such as fatigue and muscle aches and pain. Non-pharmaceutical pain treatments, combined with antidepressants or other psychological medications, are often more effective than traditional painkillers.

1.2.6 Idiopathic pain

Idiopathic pain exists when there is no known physical or psychological cause. Idiopathic pain is more common in people who have a pre-existing pain disorder. These disorders include temporomandibular joint (TMJ) disorders and fibromyalgia. Because its cause is not apparent, idiopathic pain is often difficult to treat.

1.3 PAIN IN RA

Pain is the most common symptom of patients with rheumatic disorders in general, and also a major burden of RA [53]. In spite of effective immune-suppressive therapies, observational studies show that a large number of patients with RA have remaining pain affecting quality of life. This was seen in an international observational study, where the majority of the studied patient cohorts with established RA in Europe (60%) and USA (65%) reported discontent with pain management [54]. the intensity of pain is only weakly correlated with measures of peripheral inflammation [55]

Pain may cause severe suffering in the individual RA patient and also contribute to functional impairment. The peripheral nociceptive mechanisms of arthritis involve direct action of several inflammatory mediators, such as prostaglandins, bradykinins and neuropeptides [56, 57]. In addition, pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin IL-1β, IL-6 and IL-17 have all been shown to sensitize peripheral neurons in experimental arthritis [58]. These mechanisms lead to lower thresholds for mechanical pain in the joint. Moreover, during joint inflammation, afferent neurons in connection to the joint are sensitized. Low threshold nociceptive mechanoreceptors with thick and thin myelinated axons (Aβ and Aδ fibers) show enhanced responses to pressure onto the joint and movements of the joint. Importantly, numerous high-threshold units, defined as nociceptors by their high mechanical threshold, become sensitized and start to respond to light pressure and movements in the working range of the joint. Most of these units are thin myelinated (Aδ fibers) or unmyelinated (C fibers). Finally, initially mechano-insensitive fibers (silent nociceptors) become responsive to mechanical stimulation of the joint and contribute to the afferent inflow into the spinal cord during inflammation. Collectively, these changes provide the afferent sensory basis of joint pain The consequence of these processes is that under inflammatory conditions the nociceptive system is activated by normally innocuous and no painful mechanical stimuli.

On a group level, RA patients have increased pain sensitivity in RA compared to controls [50] and this also increase with disease duration, suggesting dynamics of the pain regulation of the disease. Apart from peripheral effects, earlier data have also depicted central nervous impact on pain modulation in arthritis, and TNF and IL-6 may have important effects in this context [59]. Clinically, this is further underscored by the high co-morbidity between RA and generalised pain syndromes such as fibromyalgia and chronic widespread pain (FM) [60, 61]. In conclusion, RA is associated with an increased pain sensitivity on the basis of both peripheral and central pain sensitization.

1.4 CHRONIC WIDESPREAD PAIN AND FIBROMYALGIA

Fibromyalgia (FM) is a non-inflammatory non-articular rheumatic condition characterized by chronic widespread musculoskeletal pain and generalized tiredness as well as sleep disorders and cognitive dysfunction [62]. According to classification criteria of the American College of Rheumatology 1990, fibromyalgia is defined as the following: History of widespread pain that has been present for at least three months, as well as pain in at least 11 of 18 tender point sites on digital palpation (figure 2 and table 6) [63] Digital palpation should be performed with an approximate force of 4 kg. A tender point has to be painful at palpation, not just "tender."

Figure 2. Tender points in fibromyalgia

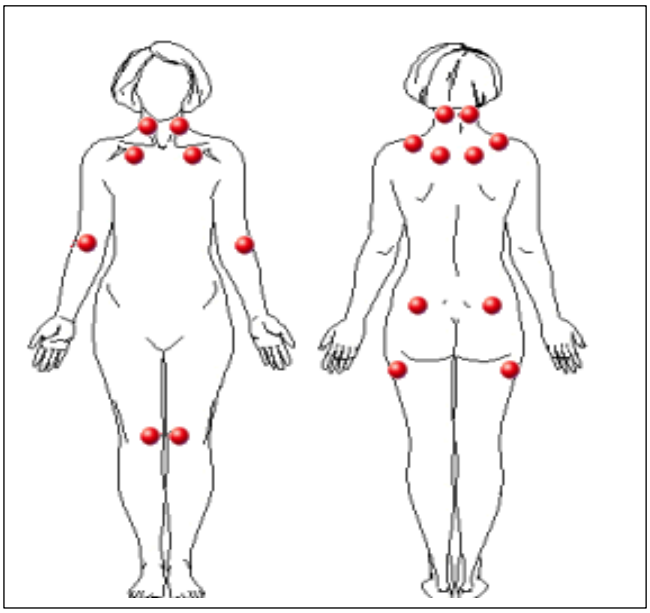


Table 6. ACR 1990 criteria for Fibromyalgia [63]

1- History of widespread pain for at least 3 months Pain is considered widespread when all of the following are present: - Pain in the left side of the body,pain in the right side of the body - Pain above the waist - Pain below the waist - Axial skelital pain (cervical spine or anterior chest or thoracic spine or low back)
2- Pain in 11 of 18 tender points sites on digital palpitation Tender paoint sites: - Bilateral, at the suboccipital muscle insertion - Bilateral, at the anteriar aspects of the intertrasverse space at C5-C7 - Bilateral, at the midpoint of the upper Trapezius border - Bilateral, at Suoraspinatus, above the scapula spine'' - Bilateral, at the second costocondral junctions - Bilateral, 2 cm distal to the lateral epicondyles' - Bilateral, in the upper outer quadrants of the Gluteus muscles - Bilateral, posterior to the greater Trochanter - Bilateral, at the medial fat proximal to the Knee joint

The prevalence of FM in the general global population is 2–7%, and the disease mainly affects women (80%) [62]. FM is often associated with depression and decreased quality of life as well as decreased work capacity [64, 65]. Patients with both FM and depression have a more decreased quality of life and reduced ability to focus attention compared with patients with FM only [66] . The mechanisms of pain in FM has not been fully elucidated, but several studies have reported dysfunction in both the peripheral and central nervous system. Moreover, normal muscle work upregulate sensitivity in pain receptors in the muscle leading to repeated release of nociceptive and inflammatory substances causing long-lasting peripheral and central sensitization. [67, 68].

Recently, there has been focus on activation of glia cells (astrocytes and microglia) as a potential mechanism in dysfunction of pain regulation in the CNS leading to chronic pain [69] [70] Moreover, in patients with fibromyalgia, elevated cerebrospinal fluid and serum concentrations of IL-8 has earlier been reported [71],

Chronic widespread pain is also common in RA, and the prevalence of FM in RA patients may be as high as 10–20% [60]. When a patient has both RA and FM, determining the degree of RA activity may be difficult, because these patients typically have higher scores for pain and disability [55]. RA patient with concomitant FM have significantly higher DAS28 score than patients without FM [72] which is likely due to higher perception of pain in the former group [73]. There are a number of factors influencing pain and pain experience, including age, gender, culture, depression, anxiety and stress [74, 75]. All these factors are of great importance in the overall experience of pain and the interrelationship of these is often referred to as the biopsychosocial model of pain [76] (figure 3)

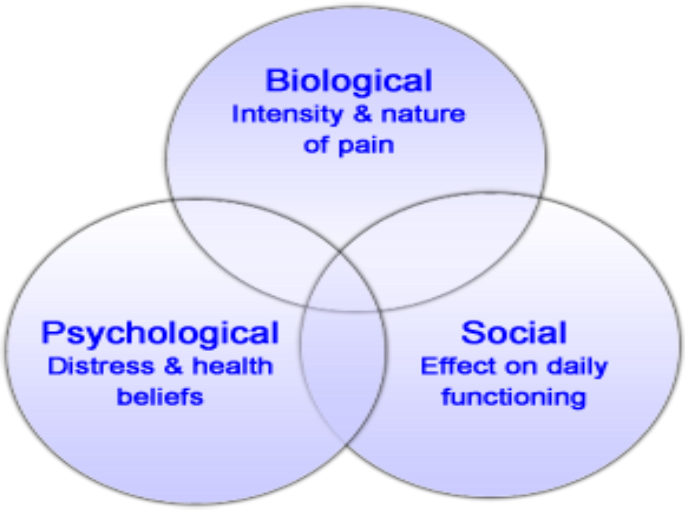


Figure 3. The biopsychosocial model of factors influencing pain

1.5 FATIGUE IN RA

Fatigue can be defined as an overwhelming sense of tiredness, lack of energy and feeling of exhaustion. Women are more likely to feel fatigued than men [77]. Pathological fatigue does not improve with rest and is a complication of several acute and chronic inflammatory diseases, including arthritis [78]. Fatigue can be a severe and distressing phenomenon to the patient and interfere with the patient's life, including work disability and lower quality of life as well fatigue interferes with emotional, physical and social functions.

Fatigue is a common complaint among patients with RA and is regarded as an extra-articular symptom of the disease [79]. More than 80% of RA patients have experienced fatigue [80] and over a half of the patients have reported fatigue as the most problematic symptom [81]. Fatigue in RA is strongly associated with poor sleep, functional disability, pain, depressive symptoms and cause substantial distress and reduced work capacity [81, 82]. Moreover, systemic

inflammation has been shown to associate with activation of immunological mechanisms in the brain, where a role in the processes contributing to fatigue have been suggested. Thus, intrathecal injection of IL-1β in rodents leads to sickness behavior and fatigue, respectively [83, 84]. Moreover, activation of the IL-1 system in RA CNS has been coupled to increased fatigue [85] . Furthermore, in another study, daily administration with IL-1R antagonist significantly reduce fatigue in RA patients [86]. The hypothesis of underlying inflammatory mechanisms behind fatigue is further underscored by fatigue-suppressing effects of TNF-blockade [87].

Related to its multifactorial features, management of fatigue in RA usually involves a combination of non-pharmacological and pharmacological strategies. There is evidence for effects of physiotherapy on fatigue, and in a meta-analysis, non-pharmacological therapies including physical activity and psychosocial intervention provide benefit in management of fatigue in patients with RA [88, 89]. Also pharmacological treatment, both DMARDs and biologic agents, have been shown to reduce fatigue [90]. However, it is important to acknowledge that fatigue is not clearly related to systemic inflammation as measured by CRP and ESR, but instead there is a strong correlation to pain [82]. The treatment effects on fatigue by DMARDs and biologics have thus often been linked to improvement in pain [82, 91, 92].

1.6 AUTONOMIC REGULATION OF INFLAMMATION

The autonomic nervous system (ANS) is an important regulatory system participating in maintenance of homeostasis, in cooperation with other systems, including endocrine and the immune system [93, 94]. The ANS forms important links for neuro-immune regulation, both sympathetic and parasympathetically mediated [94] , The 'cholinergic anti-inflammatory pathway' is a well-documented neuro-regulatory mechanism, known as the inflammatory reflex [95, 96]. Briefly, afferent neurons of the vagus nerve sense inflammation in the periphery and signal to efferent vagal neurons in the nucleus tractus solitarius (NTS) of the brainstem.[97]. The activation of efferent signals in the vagal nerve causes release of norepinephrine from nerve endings in the spleen, and this elicits subsequent acetylcholine production from T-cells, subsequently leading to nicotine acetylcholine receptor subunit a7 (nAChRa7) dependent down regulation of systemic pro-inflammatory cytokine production [98].

1.6.1 Autonomic nervous function in RA

Several previous studies have reported dysfunction in the autonomic activity in patients with RA [99] , Moreover, autonomic dysfunction in RA has been shown to correlate with peripheral inflammation [100] and nACRa7 is expressed in synovium of arthritis patients [101] . The findings of dysfunctional autonomic regulation in patients with RA has led to development of treatment strategies aimed at stimulating or restoring this pathway. For example, selective cholinergic agonists improve outcome in experimental models of arthritis [102]. Furthermore, recent pilot trials have used implantable pacemaker-like vagus nerve stimulating devices for the treatment of RA patients [103].

1.6.2 Autonomic nervous function in FM

The previously confirmed autonomic dysfunction in patients with fibromyalgia has been considered as mainly associated with pain and psychosocial stress [104] . FM autonomic dysfunction is associated with increased sympathetic activity and concomitantly reduced parasympathetic activity [105, 106]. However, autonomic dysfunction has not earlier been linked to neuroinflammatory processes.

2 AIMS OF THE THESIS

2.1 GENERAL AIM

The general aim was to investigate the patterns of pain and fatigue in early RA. Moreover, the relation between pain and inflammation was an objective, as well as an analysis of central nervous mechanisms in RA and FM in relation to autonomic activity.

2.2 SPECIFIC AIMS

1. To investigate pain patterns in newly diagnosed RA after three months treatment with DMARD, with special focus on patients who had achieved good clinical response.

2. To investigate whether pain that remains after adequate antirheumatic treatment leading to inflammation control is a risk factor for later developing widespread pain, and fatigue.

3. To study the association between pain and inflammation at different time points during the course of early RA.

4. To investigate autonomic activity in patients with RA and FM compared to controls in relation to serum and neuroinflammatory mediators.

3 PATIENTS AND METHODS

This thesis includes three epidemiological, observational studies and one clinical case-control study. The epidemiological studies are based on cases reported to a population-based early RA (EIRA) cohort who had follow-up data from the Swedish Rheumatology Quality Register (SRQ). An overview of the different patient cohorts in paper I-IV is presented in table 7.

Table 7. Overview of patients in the studies of the thesis.

	Paper I	Paper II	Paper III	Paper IV
Diagnosis	RA	RA	RA	RA, FM, HC
Total no.	1242	408	2808	14; 15; 15
Description, patient cohorts	EIRA: MTX monotherapy from diagnosis / SRQ	EIRAU3 / SRQ	EIRA/SRQ	RA: Outpatients at the Rheumatology clinic FM: Outpatients at the Department of Rehabilitation Medicine, HC: Recruited by advertising
Age	56 (46-63)	<40 (18%) 40-50 (14%) 51-60 (25%) 61-70 (37%) >70 (5%)	<40 (18%) 40-50 (19%) 51-60 (31%) 61-70 /30%) >70 (2%)	RA: 51 (36-59) FM: 46 (25–60) HC: 44 (25–61)
Female, no (%)	862 (69%)	300 (74%)	1840 (71%)	RA: 14 (100%) FM: 15 (100%) HC: 16 (100%)
VAS pain at baseline	54 (IQR 35-71)	55 (IQR 32-73)	52 (IQR 33-70)	RA: 24 ± 18 (mean±SD) FM: 66 ± 13 (mean±SD) HC: NA

3.1 THE EIRA STUDY AND LINKING TO THE SRQ REGISTER (PAPER I-III)

The population of RA patients was evaluated from the Epidemiologic Investigation of Rheumatoid Arthritis (EIRA) which is a population-based case-controlled study, investigating possible risk factors of rheumatoid arthritis (RA) and increase understanding of which factors contribute to the development of this disease [107, 108]. The EIRA study includes incident RA patients, all fulfilling 1987 ACR criteria for RA [108] and was initiated in 1996. The research project is up and running and EIRA has underwent three phases (referred to as I (1996-2006), II (2007-2016) and soon III will start, including modification of the questionnaires and modernization of the recruitment process). Thus, the study is still enrolling new cases and controls. Starting 2007, all participants also received a follow-up questionnaire 1 and 3 years after inclusion, EIRAU1 and EIRAU3, which include for example assessments of pain, fatigue, sleep problems (see below) and self-reported work capacity. Overall, the EIRA investigation is facilitated by linking to national follow-up system, the

Swedish Rheumatology Quality register (SRQ) for serial clinical data during the course of RA. The patients examined at 0, 3, 6, 9, 12, 18, 24, 36 and 60 months including joint assessment, function, disease activity and DAS28 - EULAR response [44, 109, 110] .

3.2 RA, FM PATIENTS AND HEALTHY CONTROLS IN PAPER IV

RA patients were outpatients at the Unit of Rheumatology, Karolinska University Hospital, Stockholm and fulfilled both the 1987 and 2010 ACR criteria for RA and none fulfilled the ACR criteria for fibromyalgia. For further baseline characteristics see table 7 and paper IV. No NSAIDs were administered within 24 h before CSF sampling and pain and fatigue assessments. No RA patient had any neurological disease.

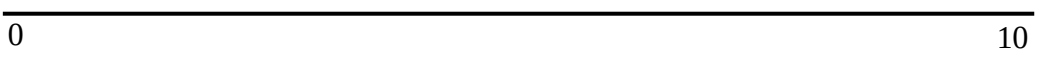
FM patients were outpatients at the Department of Rehabilitation Medicine, Danderyds Hospital, Stockholm and fulfilled the classification criteria of the American College of Rheumatology (ACR) 1990 for fibromyalgia. For further baseline characteristics see table 7 and paper IV. All the patients had normal erythrocyte sedimentation rate, hematology count, liver enzymes, creatinine kinase, thyroid function, rheumatoid factor and antinuclear antibodies. No medications were taken on a regular basis and no analgesics or non-steroidal anti-inflammatory drugs (NSAIDs) had been used on the day of assessment. None of the FM patients had other known painful conditions or neurological diseases.

Fifteen healthy sex- and age-matched subjects participated as controls in the study. They were assessed in the same way as the FM/RA patients except that no lumbar puncture was performed (for ethical reasons). The subjects were recruited by advertising at public places at Danderyds Hospital.

3.3 PAIN ASSESSMENT (PAPER I-IV)

Due to its multi-facetted appearance a fully objective measurement of pain is not possible. There are a number of pain assessment tools in the literature, including multi-item and multidimensional tools [111-118] .The VAS scale has been validated in RA [119-122] and provide a simple easy-to-use tool that is also implemented in the daily work of rheumatology care . Patients included in paper I-IV measured their present pain intensity on a visual analogue scale (VAS) (figure 4), where the responses were on a continuous range from 0 (no pain) to 100 (worst pain). The line was not marked, and patients were unable to see their previous responses.

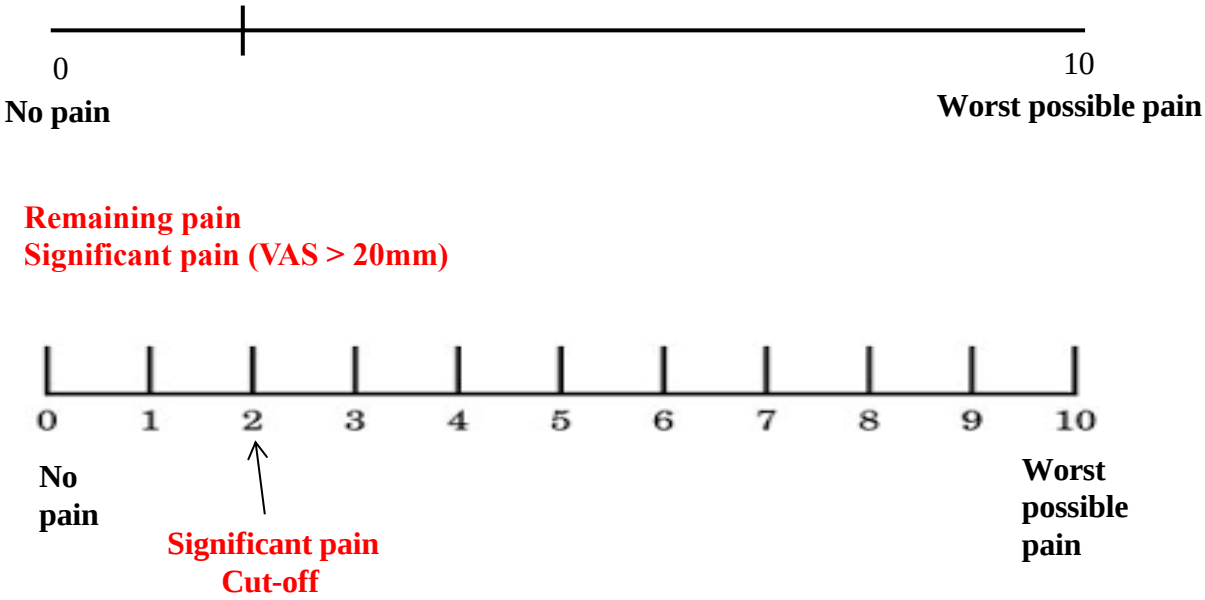
Figure 4. Visual analog scale (VAS) pain



3.4 REMAINING PAIN (PAPER I&II)

Remaining pain (paper I) was defined as VAS pain >20 mm (Figure 5). Above this cut-off was previously described as a significant pain. This cutpoint has ealier been validated by (Wolf F). definings VAS pain < 20mm as an acceptable pain level [119] . The concept remaining pain depicted significant pain after 3 months' treatment with methotrexate in paper I and after one-year treatment with DMARDs in paper II.

Figure 5. Remaining pain

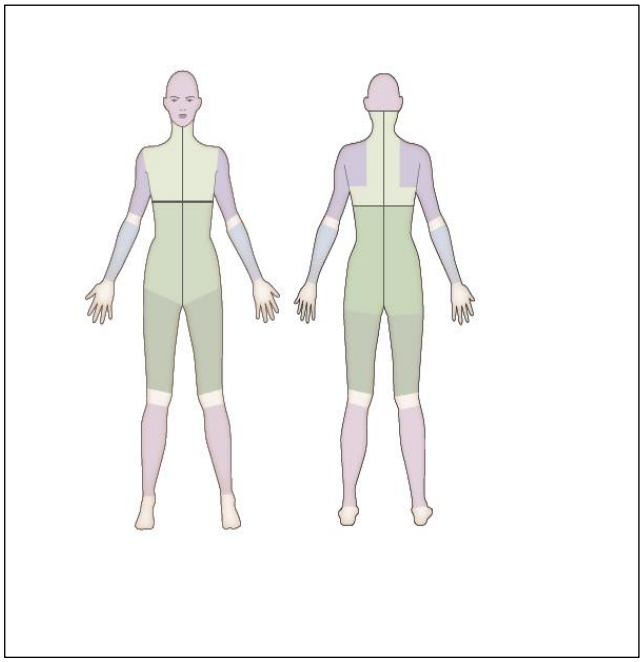


3.5 WIDESPREAD PAIN (PAPER II)

Widespread pain (WSP) was defined based on the EIRAU1 and EIRAU3 follow-up questionnaires of pain outside joints. In the questionnaire, the patients who answered yes to the question on whether they were affected by *pain outside their joints* were asked to indicate all areas affected by pain outside the joints on a schematic drawing of a body (figure 6). The drawing was divided into 24 smaller areas (six per quadrant); the right and left part of the torso, the abdomen, the lower part of the back and the upper part of the back, respectively, and the dorsal and ventral part of each extremity (the lower leg, the upper leg/hip, the forearm, the arm/shoulder). The line dividing the upper and lower part of the body was drawn at the level of the end of the sternum. The question was specifically directed to pain outside joints and the peripheral joint regions (ankle/feet, knee joint, elbow joint and wrist/hands) were not possible to mark on the drawing. Patients were considered to fulfill the requirements for widespread pain if they assessed pain outside joints in one or more areas in all four

quadrants of the body. Patients who had pain outside joints, and marked pain in at least one area were defined has having regional pain. Patients with WSP at one-year follow up (EIRAU1) were excluded in the prediction analysis for WSP and other clinical outcomes in EIRAU3.

Figure 6. Schematic drawing from EIRAU1 and EIRAU3 questionnaire of body areas for assessment of pain outside joints.



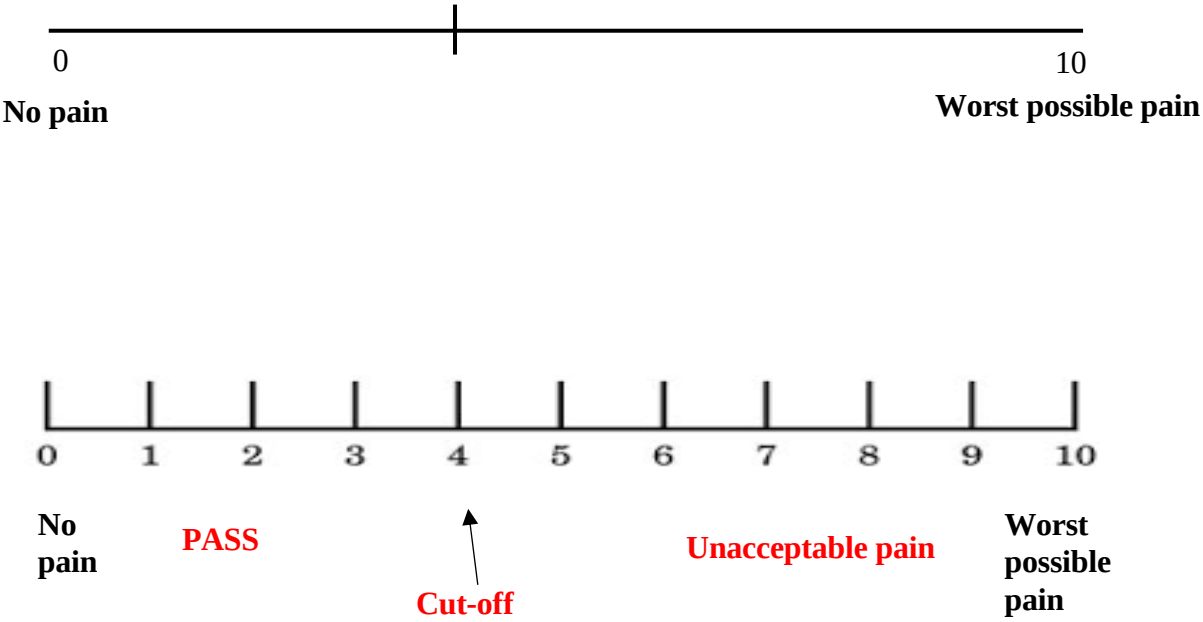
3.6 PATIENT ACCEPTABLE SYMPTOM STATE AND UNACCEPTABLE PAIN (PAPER III)

Patient Acceptable Symptom State (PASS) has been defined as the highest level of symptom beyond which patients consider themselves well [123]. The PASS value is a clinically relevant cutoff from the patient's perspective, as being in "an acceptable state" (with the outcome score < the PASS) or not (with the outcome score > the PASS). In a study on osteoarthritis pain, the PASS was found more relevant than the minimal clinically important improvement outcome (MCII) which recognise a clinically relevant improvement and reflects the concept of improvement ("feeling better") [124]. This study thus concluded that patients experienced an important improvement only if this improvement allowed them to achieve a state they consider satisfactory. The cut-off for PASS concerning pain in

rheumatic diseases has previously been validated as 40 mm on a pain VAS scale (figure 7) [123].

In study III we used the outcome of "unacceptable pain" which was defined as having VAS pain higher than what is defined by PASS, i.e. ≥ 40 mm on a VAS scale. [123, 125, 126].

Figure 7. Unacceptable pain



3.7 FATIGUE AND SLEEP ASSESSMENTS (PAPER I-IV)

Several methods of evaluation have been used to investigate fatigue in RA . Multidirectional scales developed to include the different aspects of fatigue, such as the Short Form 36 (SF-36), the Functional Assessment of Chronic Illness Therapy Fatigue Scale FACIT [127], the RA-specific Multidimensional Assessment of Fatigue (MAF) scale, the Fatigue Severity Scale (FSS) [128] and the Multidimensional Fatigue Inventory 20 item general (MFI-20). MFI-20 covers 5 dimensions of fatigue: general fatigue, physical fatigue, reduced activity, mental fatigue, and reduced motivation (scores within the 5 dimensions range from 4 to 20 , with higher scores indicating higher levels of fatigue) [129, 130]. A simple, but useful method to evaluate fatigue is the visual analog scale (VAS, scores from 0-100, the higher score, the greater the fatigue). This single item scale has earlier been validated and described as more

sensitive than longer scales [131, 132]. In this thesis, fatigue VAS was used in paper II and MFI-20 in paper IV. For sleep assessments in paper II, we used assessment from the EIRAU3 follow-up questionnaires. The following question was asked to the participant; "To what extent is sleep a health problem to you? 1. Very large problem, 2. Quite large problem, 3. Neither large nor small, 4. Small problem, 5. No problem". In the analysis, sleeping problems were assessed as having 1 and 2 (paper II). Sleep disturbance was assessed using (Pittsburg Sleep Quality Inventory) PSQI [133] (paper IV). Physical and mental component of health related quality of life short form (SF-36) [134] was used in paper IV.

3.8 MEASURING CYTOKINES IN PERIPHERAL BLOOD AND CEREBROSPINAL FLUID (PAPER IV)

Venous puncture was performed for collecting blood from both patients and controls and lumbar puncture was performed for collection of cerebrospinal fluid (CSF) from patients only. CSF samples were immediately centrifuged, supernatants frozen and stored in -80°C until use. Cytokine levels in CSF and serum were analyzed with ELISA (R&D, high sensitivity Quantikine). Sensitivity, expressed as the mean of minimum detectable dose (MDD), for the ELISA kits were as follows: IL-1β 0.14 pg/mL; IL-1ra: 6.26 pg/mL; IL-4: 0.11 pg/mL; IL-5 0.29 pg/mL; IL-6 0.039 pg/mL; IL-8 3.5 pg/mL; IL-10 0.09 pg/mL; TNF 0.106 pg/mL. Human CSF was tested for CCL-2 (Cat No L451AYA-1), BDNF (Cat No N45ZA-1), and β-NGF (custom made prototype), in a chemiluminiscence assay based on the MSD technology (Mesoscale Discovery, Gaithersburg, MA, US). Samples were captured on pre-coated MSD plates and detected using a labelled biotinylated antibody directed towards the analyte of interest.

3.9 HEART RATE VARIABILITY (PAPER IV)

Heart rate variability (HRV) is used in clinical research to measure the activity and integrity of the autonomic nervous system [135]. To measure HRV, 24-hour Holter recordings were applied for 24 hours'measurements [136] (figure 8). Recordings were manually read, and readings with a high number of ectopic beats were discarded from analysis.

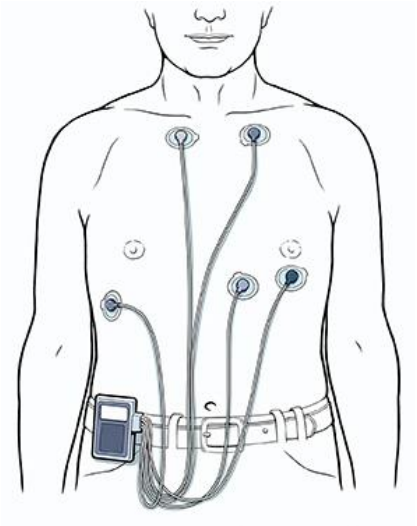
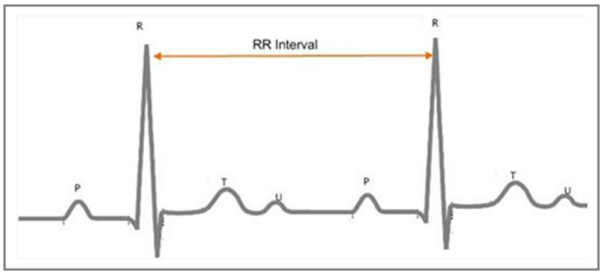


Figure 8. Holter ECG



HRV contains 2 domains: time domain and frequency domain [137]. The component that were included in the study were the major components of the time domain, the root mean square of the standard deviation between normal-to-normal (NN)-intervals (RMSSD) and the standard deviation of intervals between successive QRS complexes (SDNN) are considerd to be indicators of parasympathetic tone [138] .The frequency domain (power spectral) analysis of heart rate variability allows identification of component frequencies of the heart rate spectrum. The components of the frequency domains includes: the high-frequency (HF) component of the heart rate spectrum, considered to reflect parasympathetic influence on the heart rate, while the low-frequency (LF) component has been shown to include contributions from both the sympathetic and parasympathetic nervous system [139] . The HF/LF ratio indicate balance between sympathetic and parasympathetic nervous system [99]. (Table 8).

Table 8. HRV components included in paper IV

RMSSD (ms)	**vagal influence on HRV**
SDNN (ms)	vagal influence on HRV
LF (ms^2)	Sympathetic influence, but also including a parasympathetic component
HF (ms^2)	Only parasympathetic influence
LF/HF	Indicator of autonomic balance

3.10 STATISTICAL METHODS

Paper I: The association between baseline parameters and remaining pain was evaluated by logistic regression and expressed as odds ratios (OR) with 95% confidence intervals (95%CI), adjusted for age at diagnosis (treatment start) and sex. IBM SPSS version 21 was used for all statistical analyses.

Paper II: The correlation between remaining pain at 1 year and patient-reported fatigue (>40 mm on a visual-analog scale), regional pain (pain outside joints in at least one area), wide-spread pain (pain outside joints in at least one area in each of the four quadrants of the body), and sleeping problems (reported by the patient as "quite large" or "very large") at the 3 year follow-up was evaluated by modified Poisson regression [140]. The analysis was performed both crude and adjusted for sex, age and calendar period of RA-diagnosis. All analyses were carried out using SAS Statistical Package 9.3.

Paper III: The correlation between pain, DAS28 and its components was assessed at the time points 0, 3, 6, 12, 48 and 60 months, by using Pearson's correlation and the confidence intervals were assessed using Fisher's transformation. A Kaplan-Meier analysis was run using the survival package [141] to investigate the time to reach stable state of PASS continuous data for the first five years. In this analysis, we defined a stable state of PASS as two consecutive visits with a VAS pain below 40 mm. The time to event was defined as the time to the second of the two visits with VAS pain below 40 mm. The association between baseline parameters and unacceptable pain at one year was assessed using modified Poisson regression and expressed as relative risk ratios with 95% confidence intervals (95%CI), adjusted for sex and age at diagnosis. All statistical analysis in this paper was made using R, except demographics where IBM SPSS version 21 was used.

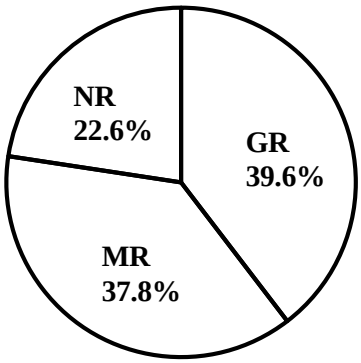
Paper IV: Overall group differences between patients with FM, RA patients and HC were analysed by Kruskal-Wallis Test and post hoc group differences were assessed by Independent Samples Mann-Whitney U-test. Correlations were analyzed by Spearmans' correlation coefficient. $P < 0.05$ was considered as a statistically significant difference.

4 RESULTS

4.1 REMAINING PAIN IS COMMON IN EARLY RA (PAPER I)

The study base consisted of 1241 patients with early RA from the EIRA cohort, who also had follow up data from SRQ. Information about VAS pain and DAS28 were available for 1063 patients at 3 months follow up visit. All patients were initially under treatment with methotrexate as a monotherapy. The frequency of the three response groups according to the EULAR response criteria [44] are displayed in figure 9.

Figure 9. Frequency of EULAR response to treatment at 3 months follow up.



Remaining pain was observed in 58% of all patients and 29% in the good response group (GR), 70% in the moderate (MR) and 83% in the non respose group (NR) at the 3 months' follow-up visit. In the whole group, remaining pain at 3 months follow up visit was significantly associated with the following baseline parameters: higher baseline disability score HAQ (OR 2.17 (1.74-2.71)), higher PGA (OR 1.02 (1.01-1.02)), higher CRP (OR 1.05 (1.02-1.10)), higher TJC (OR 1.05 (1.03-1,07)), higher DAS28 (OR 1.32 (1.19-1.48)) as well as lower age (OR 0.98 (0.97-0.99)), while there were no significant association between sex, ACPA, RF, SJC, smoking and remaining pain.

In the EULAR good response group, remaining pain was significantly and positively associated to high baseline disability HAQ (OR 2.2 (1.4-3.4)) and PGA (OR 1.15 (1.05-1.27)). Furthermore, remaining pain was associated with low baseline ESR (0.81 (0.70-0.93)). Other baseline variables such as CRP, ACPA, rheumatoid factor, swollen and tender joint, smoking and DAS28 were not associated with remaining pain in the good response group.

During the treatment period, increase in VAS pain, defined as a higher VAS pain value at 3-months follow-up compared to baseline, was observed in 19% of the whole cohort, and in the response groups the frequency of increasing in VAS pain, was 9% in the good response group, 15% in the moderate response group and 45% in the non response group.

Increased pain in the whole cohort was associated to lower baseline HAQ (OR 0.57 (0.43-0.75)) , lower ESR (OR 0.90 (0.84-0.97)), lower PGA (OR 0.83 (0.77-0.88)), presence of current smoking (OR 1.54 (1.09-2.18)), lower SJC28 (OR 0.96 (0.93-0.99)) , lower TJC25 (OR 0.97 (0.95-0.99)) and lower DAS28 (OR 0.71 (0.62-0.81)).

In the EULAR good response group, increased pain was associated to lower baseline HAQ (OR 0.25 (0.12-0.54)), lower PGA (OR 0.79 (0.68-0.92)), lower TJC25 (OR 0.90 (0.83-0.99)) and lower DAS28 (OR 0.53 (0.36-0.79)).

4.2 REMAINING PAIN IN SPITE OF INFLAMMATION CONTROL ASSOCIATES WITH LONG-TERM WIDESPREAD PAIN (PAPER II)

The study included patients from the EIRA study that had received a 3-year follow-up questionnaire (EIRAU3), beginning in 2007. At the time of study analysis, information on 785 RA patients was available. Specifically, the questionnaire included questions on pain outside joints, fatigue and sleeping problems. Moreover, a pain drawing was available for patients responding positive to the question of pain outside joints (see methods). 408 patients had data from both EIRA, SRQ and EIRAU3. Of those patients, 6% had wide-spread pain, 33% had regional pain, 19% had significant fatigue and 12% had sleeping problems.

Remaining pain in spite of inflammation control was defined as VAS pain > 20 mm together with a CRP < 10 g/L. The frequency of remaining pain in spite of inflammation control at the one-year follow-up was 35% in the whole cohort. Remaining pain at one-year strongly increased the risk for wide-spread pain, regional pain and fatigue as shown in (table 9). Remaining pain at one-year did not statistically significant increase the risk for sleeping problems.

Table 9. Relative Risk (RR) of remaining pain at one-year for development of wide-spread pain, regional pain, fatigue and sleeping problems after 3 years non-adjusted and * adjusted for sex, age at diagnosis of RA and calendar period of diagnosis of RA.

	N	RR	95% CI	RR*	95% CI
Wide-spread pain	380	**2.42**	**1.07-5.45**	**2.38**	**1.11-5.08**
Regional pain	285	**1.75**	**1.41-2.18**	**1.67**	**1.33-2.09**
Fatigue	319	**1.83**	**1.34-2.50**	**1.68**	**1.22-2.30**
Sleeping problems	357	1.47	0.97-2.24	1.36	0.88-2.10

In absolute terms, the risk to have wide-spread pain and regional pain among the patients with remaining pain at 1 year after diagnosis, was 10% and 52% respectively, while in the patient group without remaining pain at one-year, the risk was 4% and 24% respectively.

Importantly, subsequent analyses have further validated WSP at the 3-year follow-up. Thus, WSP was shown to associate with significantly lower levels of all domains of SF-36 (Lindqvist J et al, Reumadagarna Umeå, 2016).

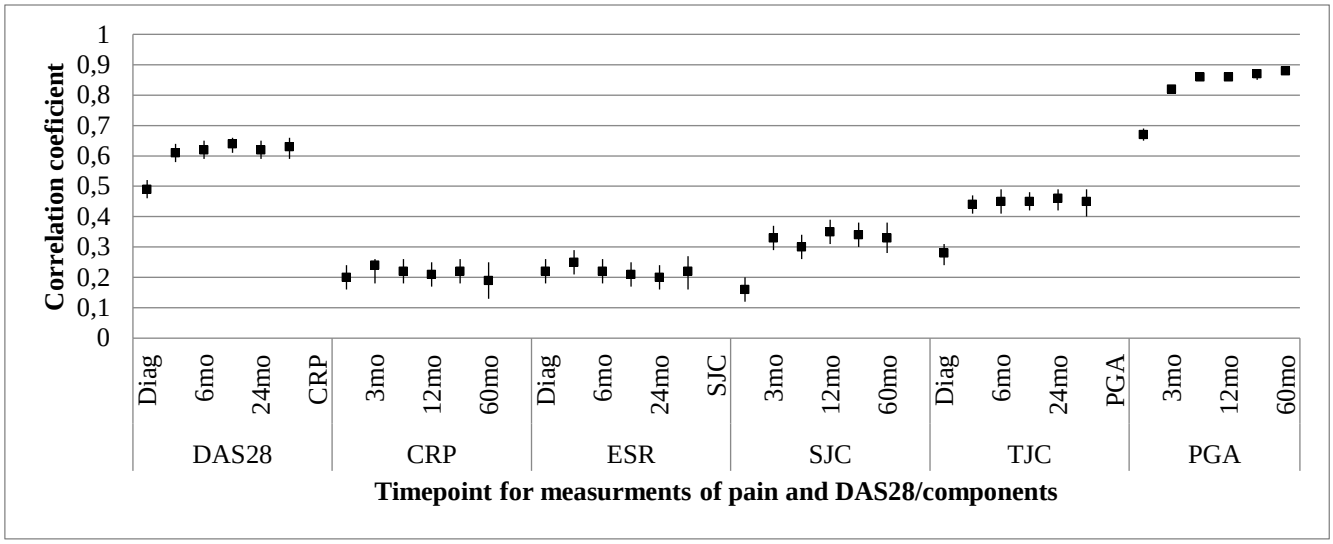
4.3 PAIN PATTERNS IN EARLY RA (PAPER III)

The purpose with paper III was to investigate the association between pain and inflammation during the course of early RA, and also to try to identify predictors of unacceptable pain. 2808 patients from the EIRA study linked to the SRQ were included, 1840 (71%) were females and 1665 (66%) were anti-CCP positive.

At three months' follow-up there was a clear and significant decrease in median VAS-pain from 52 mm (IRQ 37) to 26 mm (IRQ 37) which remains relatively stable in the later on visits up to five years (six months (24 mm; IQR 38); twelve months (22 mm; IQR 41); 24 months (22 mm; IQR 36) and five years (25 mm; IQR 39).

First, we investigated the correlation between pain and DAS28 and its component at different time points (figure 10). There was a significant increase in correlation between pain and DAS28, SJC and TJC from baseline to three months' visit, thereafter the correlation remains stable through the 6 months, 12 months, 24 months and five years visit, while the correlation between pain and PGA significantly increased from diagnosis to three months and continued to increase also at the 6 months' visit. The correlation coefficient between pain and TJC was significantly higher than for SJC throughout the period (figure 10). The correlation of pain with CRP and ESR was relatively constant through-out the follow-up visits.

Figure 10. Correlation between pain and components of DAS28; 6 time-points from diagnosis to 5 years.



Second, we investigated the proportion of patents not reaching stable acceptable symptoms state (stable PASS, for definition, see patients and methods). The proportion of patients reaching stable PASS at six months, one, two and five years is displayed in figure 11A . The proportion of women not reaching stable PASS was significantly higher than men, both after five years (19.5% vs 10%3%, $p<0.0002$), and during the whole disease course from diagnosis to five years later in the Kaplan–Meier analysis (figure 11B).

Figure 11A. Time to achievement of stable PASS. The 95% CI is marked with dashed lines

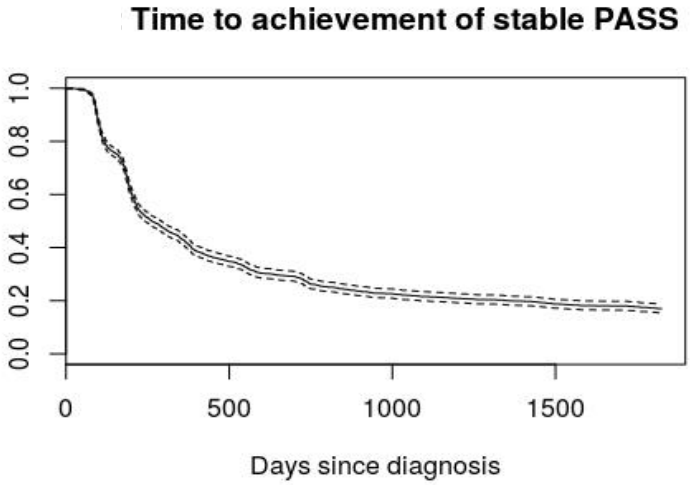
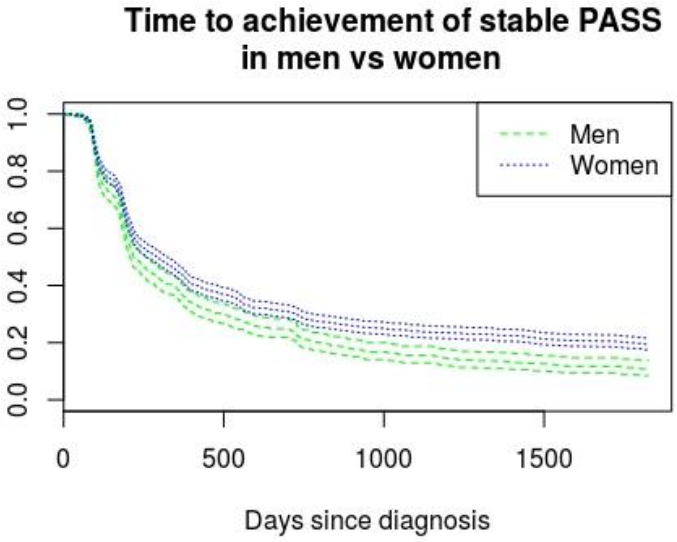


Figure 11B. Time to achievement of stable PASS, men vs women. The 95% CI is marked with dashed lines.



Last, we investigated the association between baseline parameters and unacceptable pain at one year and we found that higher disability, TJC and PGA associated with an increased risk for unacceptable pain, while higher SJC at baseline was associated with a decreased risk (table 10). Moreover, there were significant trends for higher association with higher HAQ,

PGA and TJC. There were no association between anti-CCP (ACPA), nor baseline CRP and ESR and unacceptable pain at the one year follow-up (table 10).

Table 10. Risk factors for unacceptable pain (VAS pain ≥40) at the one-year follow-up visit. Risk ratios (RR) with 95% confidence interval (95%CI), adjusted for age and gender.

		N	RR	95%CI	P	P for trend
Anti-CCP	Negative Positive	753 1401	0.99 1.00	0.86 - 1.13 Ref	0.84	
HAQ at diagnosis (quartiles)	0.0 - 0.5 0.5 - 1.0 1.0 - 1.5 1.5 - 3.0	499 691 515 391	1.00 1.39 1.69 1.97	Ref 1.13-1.71 1.38-2.08 1.60-2.42	 0.0015 5.8x10-7 1.3x10-10	**8.5e-13**
Swollen joint count (quartiles)	≤5 6-9 10-13 >=14	608 593 480 501	1.00 0.93 0.80 0.79	Ref 0.79-1.09 0.66-0.95 0.66-0.95	 0.36 0.014 0.011	**0.0044**
Tender joint count (quartiles)	≤4 5-8 9-12 >13	692 558 453 512	1.00 1.06 1.24 1.40	Ref 0.88-1.27 1.03-1.49 1.18-1.65	 0.56 0.020 9.2x10-5	**2.0e-5**
C-reactive protein (quartiles)	<10 10-19, 20-29 30-39 >=40	815 501 258 144 402	1.00 0.90 0.89 0.86 0.85	Ref 0.76-1.06 0.72-1.10 0.65-1.14 0.70-1.03	 0.21 0.27 0.30 0.094	0.066
ESR (quartiles)	<10 10-20, 20-30 30-40 >=40	286 508 396 291 625	1.00 0.90 0.86 0.86 0.83	Ref 0.73-1.10 0.69-1.08 0.68-1.09 0.68-1.03	 0.31 0.20 0.22 0.085	0.25
Patient global assessment (quartiles)	<20 20-40 40-60 60-80 >80	310 420 594 530 285	1.00 1.34 2.07 2.14 2.63	Ref 0.98-1.82 1.58-2.73 1.62-2.83 1.98-3.49	 0.063 1.9x10-7 7.4x10-8 1.8x10-11	**< 2.2e-16**

In total, 30.8% of the patients had unacceptable pain at one-year visit, this group of patients had significantly higher DAS28, CRP, ESR, SJC, TJC, PGA and HAQ compared to the group of patients reaching PASS. However, there were no differences in ACPA status and current smoking between the groups (table 11).

Table 11. Clinical characteristics for patients with fulfillment of an acceptable symptom state (PASS) vs unacceptable pain.

	PASS	Unacceptable pain	P
Total N	1603	715	
Women N	1022	493	
DAS28 (Mean ± SD)	**2.5 (±1.14)**	**4.1 (±1.3)**	**< 0.001**
CRP (Mean ± SD)	**8.1 (±11,2)**	**13.3 (±18.5)**	**< 0.001**
ESR (Mean ± SD)	**14.4 (±12.4)**	**20.2 (±17.5)**	**< 0.001**
SJC (Mean ± SD)	**1.4 (±2.6)**	**3.5 (±4.1)**	**< 0.001**
TJC (Mean ± SD)	**1.5 (±2.8)**	**5 (±5.3)**	**< 0.001**
PGA (Mean±SD)	**16.9 (±16)**	**55.4 (±19.1)**	**< 0.001**
HAQ (Mean±SD)	**0.32 (±0.4)**	**0.98 (±0.54)**	**< 0.001**
ACPA positive (N (%))	965 (66%)	432 (66%)	NS
Current Smokers (N (%))	458 (31%)	206 (31%)	NS
Biologics (total) (N (%))	**224 (14%)**	**150 (21%)**	**< 0.001**

4.4 PERIPHERAL AND CENTRAL NERVOUS INFLAMMATION – RELATIONS TO AUTONOMIC FUNCTION (PAPER IV)

This clinical study is a comparative investigation of pain, fatigue, peripheral and central nervous inflammation in patients with RA, FM and healthy controls. Compared to RA patients, patients with fibromyalgia rated significantly higher pain intensity. Furthermore, patients with FM had a significantly higher rating of fatigue and sleep problems as well as lower mental and physical quality of life than RA patients. When comparing RA with controls, RA patients had a significantly higher rating of fatigue and sleep disturbance as well as significant lower mental and physical quality of life (Table 12).

Table 12. Differences in clinical characteristics between patients with FM, RA and controls.

Average & SD	FM patients	RA patients	Healthy controls	Group differences
Age	46.2 ± 11.1 n = 15	51.1 ± 7.2 n = 14	44.4 ± 10.7 n = 15	NS
Duration FM/RA (years)	2.9 ± 2.7 n = 15	8.4 ± 8.7 n = 14	NA	P < 0.028
Pain (mm VAS)	65.8 ± 13.2 n = 15	24.0 ± 18.0 n = 14	NA	P < 0.001
Fatigue (MFI-20)	18.1 ± 1.4 n = 15	14.0 ± 4.2 n = 14	5.1 ± 1.0 n = 15	P < 0.001
Sleep (PSQI)	13.2 ± 3.7 n = 15	6.6 ± 3.0 n = 13	1.8 ± 1.7 n = 15	P < 0.001
SF-36 phys	26.4 ± 7.6 n = 15	62.4 ± 18.6 n = 14	97.5 ± 2.7 n = 15	P < 0.001
SF-36 ment	40.3 ± 21.2 n = 15	72.5 ± 21.6 n = 14	90.4 ± 6.3 n = 15	P < 0.001

Analysis of cytokine/chemokine levels in serum and cerebrospinal fluid (CSF) was performed in patients only. We found both patients with RA and FM had significantly lower concentration of TNF and IL-1β in serum compared to healthy controls. Furthermore, RA patients had lower levels of these cytokines in serum compared to patients with FM. Moreover, the concentration of IL-8 in serum was significantly higher in patients with FM compared to RA patients and healthy controls, while RA patients had significant lower levels of IL-8 compared to controls. Further, RA patients had higher concentration in serum IL-6 compared to controls (Table 13).

Table 13. Serum cytokines concentrations in FM patients, RA patients and healthy controls. Overall group differences are shown. Statistically significant differences between FM and RA patients are marked † and significant differences between controls and patients are marked ‡. P < 0.05 is regarded as a statistically significant difference. SD = standard deviation

Serum levels (pg/mL) Means ± SD	FM	RA	Controls	Group differences
IL-1β	0.59±0.08‡ n = 15	0.02±0.06†‡ n = 13	0.83±0.24 n = 15	P < 0.001
IL-8	21.36±5.54‡ n = 15	10.42±6.68†‡n n = 12	16.58±6.20 n = 15	P < 0.001
TNF	2.77±1.61‡ n = 14	1.41±0.96†‡ n = 13	4.42±2.29 n = 15	P < 0.001
IL-6	1.45±0.76 n = 14	7.50±16.07‡ n = 14	1.21±0.70 n = 15	P = 0.054

In the cerebrospinal fluid, RA patients had significantly higher IL-1β and lower IL-1Ra compared to FM patients (table 14). Controversely, FM patients had higher CSF levels of IL-8 and anti-inflammatory cytokines IL-1Ra, IL-4 and IL-10 compared to RA. There was a tendency to higher levels of TNF in CSF in FM patients compared to RA. No significant differences were found in CSF levels of IL-6 and CCL-2 between the two groups. The levels of chemokines BDNF and NGF were undetectable in CSF of both FM and RA patients (Table 14).

Table 14 Concentrations of cytokines and chemokines in cerebrospinal fluid of FM and RA patients. Overall group differences are shown. $P < 0.05$ is regarded as a statistically significant difference. SD = standard deviation.

CSF levels (pg/mL) Means ± SD	FM	RA	Group differences
IL-1β	2.58 ± 1.98 n = 14	8.83 ± 7.21 n = 14	p = 0.002
IL-8	62.35 ± 26.26 n = 14	26.92 ± 14.07 n = 12	p < 0.001
TNF	0.38 ± 0.22 n = 14	0.26 ± 0.09 n = 14	NS (p = 0.056)
IL-6	1.80 ± 0.69 n = 14	1.60 ± 0.73 n = 14	NS
CCL-2	439.03±114.54 n = 12	491.43±134.74 n = 13	NS
IL-1Ra	27.50+4.96 n = 14	17.06+9.82 n = 14	p = 0.002
IL-4	0.25+0.20 n = 14	0.04+0.05 n = 14	p < 0.001
IL-10	0.43+0.19 n = 14	0.13+0.08 n = 14	p < 0.001

Regarding the autonomic activity, both RA and FM patients had significantly higher heart rate compared to controls (table 15). There was a decrease in HF in RA compared to controls in line with the decrease parasympathetic activity in RA. In FM there was increased LF/HF ratio in line with increased sympathetic activity in FM (Table 15).

Table 15. Heart rate variability data in patients with RA and FM as well as HC.

Means ± SD	FM	RA	Healthy Controls	Group differences
Heart rate (bpm)	78+10‡ n = 15	75+6‡ n = 14	68+5 n = 15	p = 0.003
RMSSD (ms)	30.9+12.2‡ n = 15	29.2+8.1‡ n = 14	50.7+24.8 n = 15	p = 0.002
SDNN	124+24.9‡ n = 15	127.8+27.6‡ n = 14	152.9+33.0 n = 15	p = 0.02
LF	836+541 n = 15	530+213‡ n = 13	948+523 n = 15	NS
HF	410+259 n = 15	313+280‡ n = 13	759+657 n = 15	p = 0.018
LF/HF	3.41+1.30‡ n = 15	2.65+0.71 n = 13	2.23+1.0 n = 15	p = 0.036

In RA patients, the time-domain component SDNN correlated negatively with serum IL-6 ($r = -0.868$ $p<0.0001$) and CSF IL-10 ($r = -0.716$ $p=<0.006$). Regarding the frequency-domain, RA CSF IL-1β correlated positively with LF/HF ($r = -0.64$ $p<0.05$), morover, CSF IL-10 displayed an inverse correlation with LF ($r = -0.58$ $p<0.05$). In serum, IL-6 correlated inversely to LF ($r = -0.55$ $p<0.05$).

In FM patients, serum IL-1β correlated inversely to SDNN ($r = -0.646$ $p<0.01$). No significant correlations was found between other serum nor CSF cytokines and HRV.

5 GENERAL DISCUSSION

Musculoskeletal pain is a major symptom affecting quality of life in arthritis patients. The main finding of this thesis is that pain remains despite inflammatory control in early RA. The first three epidemiological studies in this thesis had main focus on pain in early RA patients and the risk for later development of wide spread pain. The fourth clinical study had main focus on central nervous inflammation and autonomic function and found differences in these mechanisms between RA and the dysfunctional pain condition, fibromyalgia. Moreover, CNS inflammation were connected to autonomic function in RA.

5.1 REMAINING PAIN IN RA

Pain VAS scale (0-100mm) has been used widely as a simple clinical measurement of pain intensity and the assessment of VAS pain >20 mm was previously stated as a cut-off for patients with RA reporting significant pain[119]. In order to discriminate between pain associated with inflammation and pain affecting the RA course, we first aimed to define the state of significant pain frequency when patients had received the first-line treatment with MTX during three months, i.e. when a majority of patients are expected to have responded clinically to the drug. In our cohort, we found remaining pain in 58% patients with early RA after 3-month treatment with methotrexate as a standard DMARD (paper I). Notably, previous data from an international observational study that included patients with both early RA and established disease [142] , reported high frequency of dissatisfaction with pain in spite of effective immune-suppressive therapies. Although our study included only patients with early RA, there was a similarity in pain frequency. This is in line with patients suffering from pain in spite of antirheumatic treatment in both early and late RA. Moreover, earlier data has shown discrepancy between decreased inflammation and pain [143] and that pain persists, even among individuals in remission as measured by the DAS28 criteria [144]. In paper I, we therefore focused on investigating pain in a group of RA patients with good clinical response to treatment according to EULAR response criteria [145]. Our data shows that almost one-third of RA patients with good inflammatory response to treatment with methotrexate as a monotherapy at 3-months follow-up reported remaining pain defined as above. In this group, remaining pain was associated with high baseline disability and low systemic inflammation, as measured with ESR. These results are in line with earlier reports of strong association between pain and functional impairment [146] and also that high inflammation or disease activity at baseline is not predictive for development of pain that persist although inflammation is suppressed.

The uncoupling between pain and inflammation is further supported by several other reports. Thus, McWilliams et al showed lack of pain improvement after one year of treatment [147] and a recent publication showed that a majority of the RA patients with high pain rating had minimal signs of inflammation [91] . Moreover, increased pain behavior was detected in experimental arthritis also remaining after the inflammatory phase of the disease [148]. Our results are also in line with the earlier reports of relation between peripheral joint inflammation and development of pain sensitization (see introduction, pain in RA). Pro-inflammatory cytokines like tumor necrosis factor (TNF) and interleukin 6 (IL-6) affect pain thresholds in experimental arthritis as well as long-term sensitization of joint nociceptors. Inflammatory impact on the peripheral nerves may thus lead to long-term sensitization, which may contribute to development of chronic pain. However, the further course of pain seem to be uncoupled from the inflammatory course of the disease. This was also supported by the findings in paper III that inflammation and inflammation markers are less coupled to pain when patients have been adequately treated with antirheumatic agents. In paper I we could also note interesting differences concerning predictive factors between all patients treated with MTX and the EULAR good response group. In both groups, high disability and high PGA was predictive. Baseline high disease activity and inflammation was predictive only in the former group, which is in line with inflammation contributing to remaining pain in some patients at the 3-month follow-up. In the latter group, however, this coupling is lost, which suggest that long-term pain sensitization is not the result of high inflammatory disease from diagnosis, but rather other factors may contribute to development of chronic pain in this context..

5.2 REMAINING PAIN AND WIDESPREAD PAIN

Another objective with the studies of pain was to investigate the long-term consequences of remaining pain and the impact on other patient-reported outcomes (PROs) such as fatigue. The purpose with these studies was also to define a simple, easy-to-use, outcome for pain in spite of inflammation control (after adequate antirheumatic treatment), that could be related to the potential presence of long-term pain and fatigue during the course of early RA. For general purposes, and with the use of different initial treatment strategies (i.e. MTX monotherapy, MTX + prednisolone, combinations with other DMARDS (triple therapy) or biologics in some patients), we found a need to define a new follow-up time - one year after diagnosis - when we considered the antirheumatic treatment to have resulted in optimized inflammatory control of the disease. We therefore modified the definition of remaining pain in paper II with the follow-up time of one year and the addition of CRP<10, as an objective

marker of inflammation control. For note, we had also used this parameter in a sensitivity analysis in paper I, confirming the results of predictive baseline factors in patients with good clinical response. In paper II remaining pain in spite of inflammatory control at one year follow up was observed in more than a third of the early RA patients. These findings are well in line with the frequency of pain related to good clinical response from paper I, and confirm that non-inflammatory factors contribute substantially to pain after antirheumatic treatment.

After defining remaining pain in spite of inflammatory control, the next step was to investigate if this registry-based measurement could be useful for prediction of long-term pain conditions connecting to RA and widespread pain (WSP). In the ERIAU3 follow-up cohort, data on pain outside joints was then combined with data from pain drawing as described (paper II). In order to assess the development of the pain condition, we also excluded patients fulfilling the outcome of WSP at one year. Our finding that remaining pain in spite of inflammatory control strongly predicted development of WSP supports the hypothesis that pain that remains after optimized inflammation is unlikely to improve, and also increase the risk of generalization of pain also outside joints. This stabilization of pain levels is concurrent with our findings in paper III, and depicts a further understanding of pain patterns in early RA. Notably, the frequency of our reported WSP is 6%, which is comparable to the frequency of fibromyalgia earlier reported in early inflammatory arthritis [149] This strengthens the validity of WSP as mirroring generalized pain, however since we do not have serial follow-up data, we can not define this condition as chronic widespread pain. In a later investigation, we have further validated the impact of our WSP-definition, showing that patients with WSP (according to the same definition) reported significantly lower levels in all SF36-domains (Lindqvist J et al, Reumadagarna, Umeå, 2016). Thus, these data confirms the validity of WSP as a measurement of general pain outside joints that is also associated with reduced physical and mental health. The importance to assess chronic and widespread pain in RA should not be underestimated. Thus, patients with RA and concomitant fibromyalgia report worse functional status and quality of life than RA patients without fibromyalgia [150] [151] . Moreover, our studies have shown that also simple, and clinically highly implemented, pain assessement coupled with markers of inflammation may be used to define patients with an increased risk for development of potentially severe pain conditions. Therefore, the definition of remaining pain in spite of inflammatory control could be utilized for early intervention in risk groups, which is an objective for further registry-based and clinically relevant research.

Chronic widespread pain is a component of FM, and the investigation of central nervous

mechanisms in FM compared to RA was also an aim of the thesis. In order to investigate potential differences between RA and FM, known to be associated with dysfunctional pain regulation, paper IV focus on central nervous inflammatory mediators in both these diseases. We then found evidence of different CSF cytokine profiles in these diseases with an upregulation of IL-1β in RA and IL-8 upregulation in FM. Moreover, we found differences in autonomic regulation, further described below. Interestingly, earlier experimental studies have revealed that IL-1β and IL-8, who are both produced by glia cells contribute to pain and hyperalgesia through different pain mechanisms [51, 152] . IL-1β injected intrathecally stimulates COX-2 activity [153] and IL-1β mediated increase in pain sensitivity can be prevented by COX-2 inhibitors [154]. On the contrary, IL-8 induced increase in pain sensitivity is not reversible with COX-2 inhibitors, but instead, beta-adrenergic receptor antagonists or guanathedine [152] can block these effects. These data are also well in line with the inefficacy of COX-2-inhibitors in FM [155]. The exact mechanisms how these cytokines may affect pain regulation are not known, but our data are thus well in line with different profiles in central cytokine release in RA vs FM that may reflect disparate natures of pain regulation in these diseases. Thus, RA was characterized by an IL-1β dominated immune activation that can be associated with prostaglandin-mediated mechanisms, whereas the increase of IL-8 in FM is associated with pain regulatory mechanisms that are independent of prostaglandins, and rather sympathetically mediated. In conclusion, these data indicate that neuroinflammatory processes may be of utmost importance in chronic pain conditions, and support the development of therapies targeted to neuroinflammation and modulation of glia-mediated pain regulation. .

5.3 PAIN PATTERNS IN EARLY RA

In RA, there is a strong correlation between pain and disease activity at diagnosis [156], and this was also confirmed in our studies. Moreover, in paper III this correlation became stronger at the three months' follow-up, and remained stable in the subsequent visits, in line with a stronger impact of pain on disease activity when inflammation is under control [144]. The correlations between pain and the objective inflammation markers were low at diagnosis, and remained low during subsequent visits, in accordance with a minor impact of inflammation on pain after adequate antirheumatic treatment [143, 157]. On the other hand, correlations between pain and swollen joint count / tender joint count significantly increased from diagnosis to three months. Interestingly, there was a significantly higher correlation between pain and tender joints vs swollen joints at all follow-up time points after three months. This is in line with a close connection between joint tenderness and subjective pain,

and important to acknowledge for disease monitoring. After three months, these correlations remained stable through the first year.

The correlation between pain and patient global assessment was as expected very strong at diagnosis, but in contrast to all other measured clinical parameters continued to increase significantly also at the six month follow-up. Furthermore, in paper I these mesures at inclusion predict later remaining pain. These results are in line with earlier reports that pain has a major impact on the individual patient´s well-being [61]. In conclusion, general pain impact becomes more important when the disease has been adequately treated.

5.4 UNACCEPTABLE PAIN AND PAIN COURSE IN RA

The term remaining pain was identified as the minimal level where patients with early RA would have significant pain after adequate anti-rheumatic treatment. Interestingly, also this low cutoff was validated to predict widespread pain (paper II). However, clinically it is vital to also assess a state where patients report a pain that is not acceptable. This level of pain has earlier been validated and defined as patient acceptable symptom state (PASS). We therefore used this higher cutoff (VAS pain ≥ 40, unacceptable pain) and investigated the pain course in early RA. The finding that almost a third of patients had unacceptable pain one year after diagnosis is in line with our earlier observations in paper I & II, and also earlier reports [142, 144, 147]. Next we investigated what baseline factors may predict unacceptable pain. We found strong impact of impaired function with an almost two-fold increased risk of having unacceptable pain after one year. Moreover, high TJC at diagnosis was predictive where high levels of SJC significantly predicted not having unacceptable pain. The latter data is also in line with the earlier described differences concerning tender vs swollen joints in the connection to pain experience.

Patients with unacceptable pain at one year had significantly higher CRP, ESR and DAS28, indicating that there is also a significant remaining inflammation in this context. Interestingly, in the group that reached PASS, both SJC and TJC were at the minimum. Moreover, median DAS28 levels were close to remission levels, thus indicating that pain at this time point of disease is a major contributor to disease activity. On the contrary, in the group with unacceptable pain, the DAS28 median was 4.1, thereby clearly showing moderate disease activity in a majority of these patients. In this group disease activity is driven both by pain, but also apparently by inflammation, since the CRP and ESR levels also were significantly higher in the latter group. To support this, we also found that biologics were more commonly prescribed in the group with unacceptable pain. The differences between these groups likely

represent different phenotypes of RA, with markedly different functional capacity, and potentially large differences in the prognosis. It is thus vital that treatment in early RA should be directed both at adequate immune suppression, but also at decreasing pain, since this is a major symptom affecting functional impairment.

We also followed the pain course during the first five years of disease. Here we could conclude that the achievement of acceptable pain is highest during the first year, correlating with the clinical response to therapy, but after the first year the additional achievement of acceptable pain in remaining patients decrease. There is a stabilization of pain after the first two years of disease, and a significant proportion, almost one fifth, of the patients continue to have stable high pain levels. The proportion of women with unacceptable pain was higher than men at all studied time points, which is in line with the higher pain levels observed in general for women and female RA patients [158, 159]. There was however no other differences in the curve pattern for women vs men, and both showed a clear decrease of unacceptable pain between the three and twelve months follow-up, in line with no effects of sex on immune suppressive treatment effects on pain [160, 161].

5.5 FATIGUE IN RA

Fatigue is common in RA, and confers important impact on quality of life and work capacity. As expected, we could confirm higher fatigue levels in RA compared to controls (paper IV), and also a higher ratings in sleep disturbance and mental and physical health (paper IV). However, both fatigue levels and sleep disturbances as well as mental and physical health were significantly worse in FM than in RA (paper IV), an observation that supports the importance of pain *vs* inflammation on fatigue, although other complex factors, such as disturbed CNS pain regulation are also likely to contribute. In the EIRAU3 follow-up study in paper II, we could report severe fatigue (VAS > 40 mm) in 19% of the early RA patients at the 3-year follow-up, and 12% with sleep problems. The data on fatigue prevalence is in line with earlier reports in RA [80]. We have also investigated the potential connection between remaining pain and fatigue. In paper II, remaining pain one year after diagnosis strongly predict fatigue. In this paper, we use the definition of VAS fatigue, with >40 mm being suggestive of severe fatigue [162, 163]. Due to the registry approach, we were limited to this fatigue assessment only, and could not investigate other potentially important aspects of fatigue. However, also sleep problems were predicted by remaining pain, supporting the general impact of pain on several aspects of fatigue. In conclusion, our data is in line with several earlier studies that report strong relations between pain and fatigue in RA [82, 92, 164],

and underscore the importance of early pain suppression to avoid severe fatigue later in the disease course.

5.6 AUTONOMIC FUNCTION IN RA – RELATED TO CNS MECHANISMS

Endogenous pain mechanisms are closely coupled to the autonomic nervous system, and a reduced vagal tone has been associated with generalized pain syndromes [165] . As mentioned, RA is also associated with a reduced vagal tone[99, 166], but the underlying mechanisms have not been fully elucidated. Furthermore, the reduced autonomic function in RA has not earlier been coupled to intrathecal cytokine levels or any neuromediator in human CNS. In paper IV we could confirm a reduced autonomic function, displayed by a decreased HF value in RA compared to controls. Moreover, the cholinergic anti-inflammatory pathway comprise an important neuroregulatory mechanism, acting through the vagus nerve, and regulating systemic inflammation. In the CNS, the vagus nerve function is regulated by action of muscarinic receptors [167] , and interestingly, earlier studies have shown that IL-1β may cause dysfunction in cholinergic neurotransmission [168] . Notably, our data in paper IV display that RA intrathecal levels of IL-1β was markedly higher than serum levels (figure 12), suggesting local production in the CNS of this cytokine. Actually, IL-1β was the only CSF cytokine having this pattern, and IL-6 and other cytokines instead displayed higher serum than CSF levels (figure 12). These results strengthen the hypothesis that the cell source of the increased IL-1β production may be resident cells, for example glia cells in the CNS. Moreover, CSF IL-1β levels were significantly correlated to reduced parasympathetic activity in the RA patients. Based on our data, we may then propose a possible mechanistic model on the involvement of intrathecal IL-1β in parasympathetic regulation: The systemic inflammation in RA leads to activation of afferent vagus, and signaling to the vagus nuclei of the brain stem. However, high IL-1β levels (as a result of systemic inflammation) leading to an activated state of resident cells of the CNS [70] will hamper the efferent vagus regulation, through negative action on muscarinic receptors in the brain stem. These mechanisms will result in a dysfunctional autonomic regulation through the inflammatory reflex, and further insufficient control of systemic and peripheral inflammation in the disease (figure 13). The coupling between systemic inflammation and autonomic dysfunction is also supported by a recent study by Koopman et al, showing that reduced autonomic activity is found also in arthritis patients at risk of developing RA [103]. The potential action of intrathecal cytokines on autonomic regulation is further supported by the finding that the anti-inflammatory cytokine IL-10 was inversely correlated to LF, and serum IL-6, but not CSF IL-6, was also correlated to reduced autonomic activity. Moreover, in FM patients, we could not find any significant

correlations between intrathecal cytokines and autonomic function, which is in line with the fact that the autonomic balance in FM is not likely to be regulated by central nervous immune activation, but rather by pain regulation, as indicated by the increased sympathetic activity compared to controls and RA (paper IV), which is also in line with earlier reports[105, 169]. In conclusion, reduced vagal function in RA may be related both to the increased systemic inflammation that is a feature of the disease, but also to central nervous immune activation. These findings may be important for further investigations of the inflammatory reflex in the human setting, and also concerning the development of interventions based on cholinergic immune regulation .

Figure 12. Comparison of CSF and serum IL-6 and IL-1beta in RA patients.

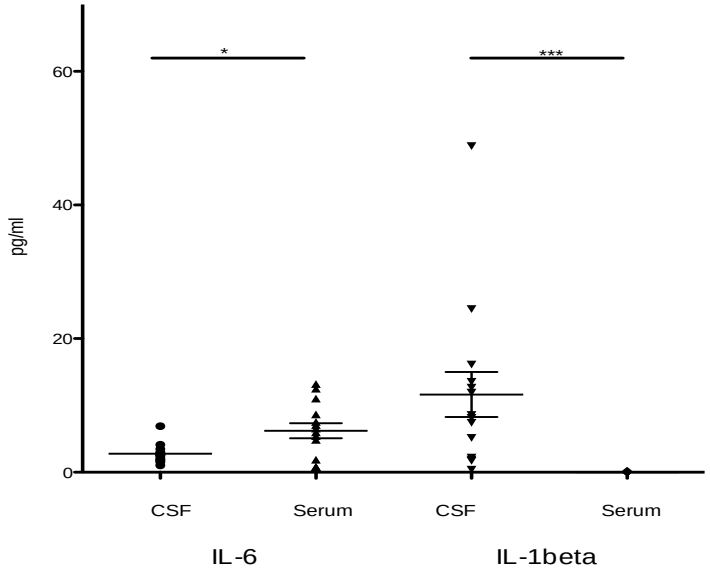
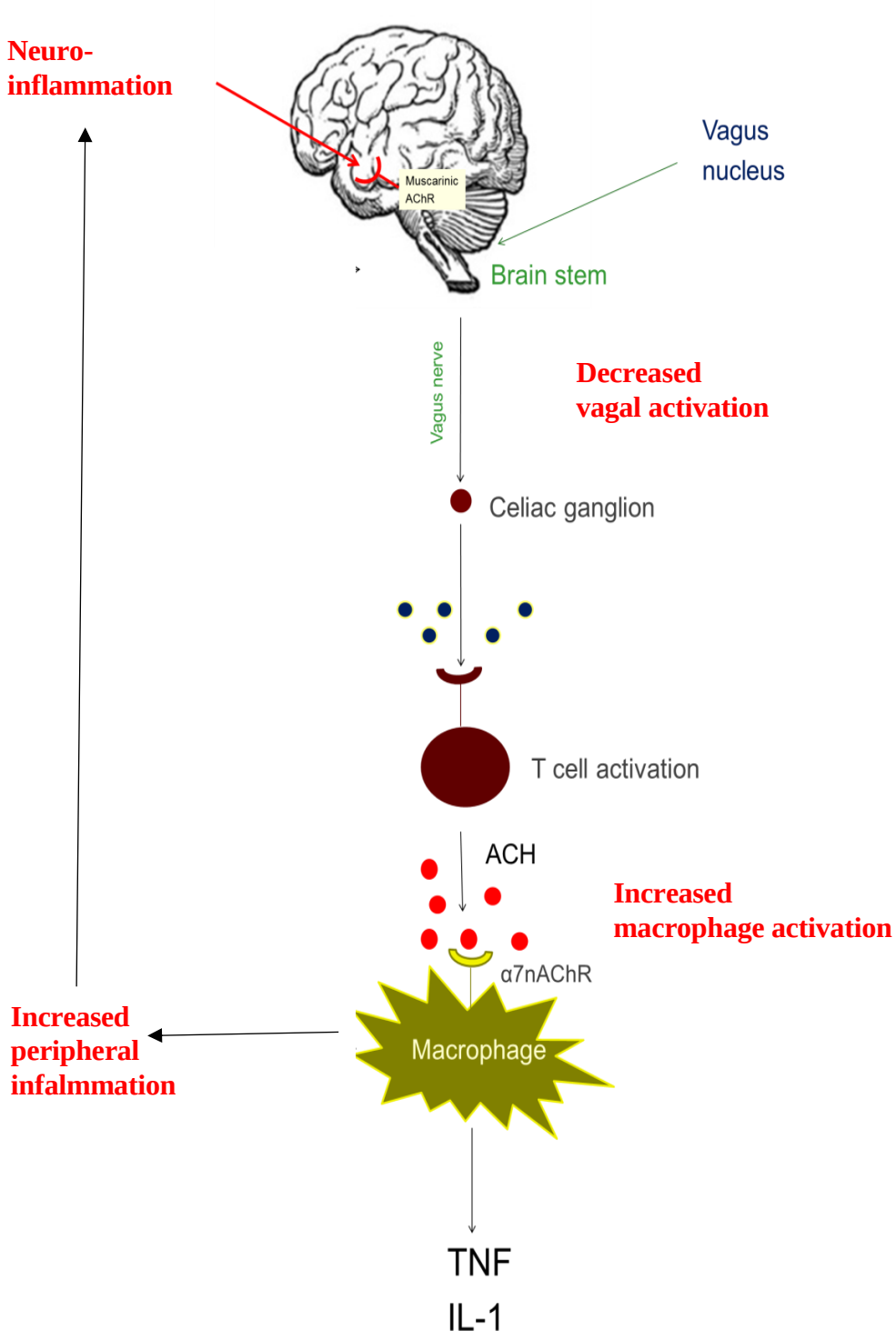


Figure 13. The cholinergic antiinflammatory pathway, with a model for central nervous impact on autonomic regulation in RA (marked in red).



6 CONCLUDING REMARKS

Pain affects all RA patients at diagnosis, and is the major symptom bringing the patient to the rheumatologist for the first time. Several earlier reports have shown that a significant part of patients continue to have pain during the RA course, and that inflammatory remission is not automatically associated with pain relief. Moreover, RA is associated with an increased risk for the development of complicating pain conditions, known as widespread pain, that may further impair function and work ability.

In order to initiate preventive strategies for development of WSP in early RA, it is vital to map the pain problem and the predictive factors for development of WSP. Moreover, there is a need for epidemiological tools, i.e. useful pain outcomes in the defining of pain patterns in early RA. This thesis have aimed to investigate pain patterns in RA with a special focus on the discrepancy between pain in the individual patient on one hand, and objective measures of inflammation on the other. This work has resulted both in the definition of potentially clinically useful pain outcomes as well as the identification of the strongest clinical predictors in this context. Moreover, we have also investigated biological central nervous mechanisms that differ RA pain from dysfunctional pain conditions, such as fibromyalgia.

We show that significant remaining pain after first-line antirheumatic treatment is quite common in early RA, and also affects a third of patients with good clinical response to the drug. This is interesting, and warrants further observational studies with this pain outcome of other antirehumatic treatments both in early and established RA.

We have also defined a registry-based index for WSP, that has later been validated against SF36. This tool may prove important in further observational studies, and subsequent studies on prediction for work ability and sickness leave are ongoing. In addition, our work has also pointed out that development of WSP in RA is not uncommon, and may contribute significantly to decreased quality of life and reduced work capacity.

The aiming for control of inflammation is now a natural hallmark in the treatment strategies for rheumatoid arthritis (RA). However, whereas rheumatologists in general have put more focus into achieve complete inflammation control of the disease, several patient-reported data indicate that although the inflammation is well-treated, other symptoms such as pain and fatigue have a substantial impact on the patient with RA. Data from this thesis also show clearly that the development of stable pain conditions occur mostly the first years after diagnosis of RA. When pain conditions manifest, such as WSP, these are not further

improved with immune suppressive drugs, and there is a lack of efficient pharmacological treatments for WSP. However, if patients at risk for development of pain conditions can be identified early in the disease, this could increase the possibilies to prevent further WSP development.

Hopefully, the next step in rheumatology treatment strategies and guidelines could be aiming also for pain remission in the disease. This could be accomplished through I) early identification of patients at risk of developing pain conditions in connection to RA II) efficient immune suppressive treatment to further suppress inflammation to a minimum, and III) individual non-pharmacological and pharmacological intervention strategies of pain early in the disease.

7 ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to all those who gave me the possibility to complete this thesis. In particular, my thoughts go to:

My main supervisor **Jon Lampa**, brilliant and skillful scientist, for inviting me into the enormously interestingly world of research and for generously sharing scientific and clinical knowledge with me, for giving me patience and continuous support, time and attention so generously, for introducing me to the world of Rheumatology being my excellent former clinical supervisor. I don't know how to thank you enough.

Eva Kosek my co-supervisor and co-author for sharing your expertise in the field of pain research and for your continues encouragement and scientific support.

Saedis Saeversdottir my co-supervisor and co-author for always being such a positive person and for sharing your great knowledge of epidemiology with me.

Sara Wedrén my former co-supervisor and co-author for being such a good-humored person, open and supportive and for all your epidemiological input to my manuscripts

My mentor during these years, **Maryam Dastmalchi** not only for the fantastic lunch meetings but for sincere advice and encouragement in research and clinical life.

Lars Klareskog for creating such a wonderful scientific environment in the clinic and for your valuable contribution to my manuscripts.

Ingrid Lundberg for being such a humble person although your very high scientific knowledge and for promoting research and creating such a positive environment for it.

My co-authors **Mats Jensen-Urstad, Magnus Andersson, Erwan Le Maître, Marie Westman, Anja Finn** and **Diana Kadetoff** for valuable contributions to my manuscript. **Maria Schelin. Helga Westerling** and **Lars Alfredsson** for valuable epidemiological and statistical contributions to my manuscripts.

All members of Jon Lampas research group: **Johanna Estelius**, **Erwan Le Maître, Priya Revathikumar, Helga Westerling, Joakim Lindqvist and Johan Lengqvist** for all the support and good discussions.

Cecilia Carlens, the current Head of Department of Rheumatology, **Joha Bratt**, the former Head of Department of Rheumatology, and **Christina Dorph** my nearest chief as well as my clinical supervisor, thank you for promoting research and providing facilities for it.

My roommate **Louise Ekholm** I always enjoy talking to you about research, work and life.

All amazing **colleagues** and **people** working at the Department of Rheumatology Solna and Huddinge. I'm truly grateful to have been surrounded by so many skillful, nice and helpful

people. **Aikaterini** thanks for all the answers to my questions in the last weeks regarding practical issues, you are so kind.

All the nurses in the in-patient, out-patient and daycare clinics and especial thanks to the nurses in the "purple group" **Lena Pettersson** and **Jenny Isberg** for making sure that my patients are taking care of. **Eva Waldheim**, research nurse, although our work together is not part of this thesis, but talking to such a wise person like you have given me inspiration.

Gunnel Bemerfeldt, Veronica Ebbersten Lindholm, Stina Nordman, Susanne Karlfeldt, Inga Ludin, Christina Ingemarsson, Kristina Nilsson, Pernilla Beckman and **Sirpa Lindberg** for all the help and support from the beginning to the end.

Great thanks to all the **patients and controls**.

All my **friends** and **relatives** in Sweden and all over the world, thank you for the support.

My grandma **Victoria**, soon 95 years old, always calm and wise, I always enjoy talking to you.
My uncle **Walid**, for sharing your amazing statistical knowledge with me.

My mother in -law **Sanaa**, although you are living far far away from us, your love and kindness has given me strength.

Karam and **Ramy** my brothers, for your love and support.

My parents **Fatin** and **Nabil** for always beaning there when I need and for your endless love and support through all times.

My beloved daughters **Victoria** and **Sanna**, I love you so much and I'm looking forward to spend more time with you.

My husband **Khalid** for everything, your love, understanding, endless patience, amazing support and encouragement, practical help, IT support and much more. Without you none of this would have been possible.

8 REFERENCES

1. Firestein, G.S., *Evolving concepts of rheumatoid arthritis.* Nature, 2003. **423**(6937): p. 356-61.
2. Englund, M., et al., *Prevalence and incidence of rheumatoid arthritis in southern Sweden 2008 and their relation to prescribed biologics.* Rheumatology (Oxford), 2010. **49**(8): p. 1563-9.
3. Neovius, M., J.F. Simard, and J. Askling, *Nationwide prevalence of rheumatoid arthritis and penetration of disease-modifying drugs in Sweden.* Ann Rheum Dis, 2011. **70**(4): p. 624-9.
4. Smolen, J.S., D. Aletaha, and I.B. McInnes, *Rheumatoid arthritis.* Lancet, 2016.
5. Silman, A.J. and J.E. Pearson, *Epidemiology and genetics of rheumatoid arthritis.* Arthritis Res, 2002. **4 Suppl 3**: p. S265-72.
6. Pedersen, M., et al., *Environmental risk factors differ between rheumatoid arthritis with and without auto-antibodies against cyclic citrullinated peptides.* Arthritis Res Ther, 2006. **8**(4): p. R133.
7. Yahya, A., et al., *Smoking is associated with an increased risk of developing ACPA-positive but not ACPA-negative rheumatoid arthritis in Asian populations: evidence from the Malaysian MyEIRA case-control study.* Mod Rheumatol, 2012. **22**(4): p. 524-31.
8. Aho, K., et al., *Occurrence of rheumatoid arthritis in a nationwide series of twins.* J Rheumatol, 1986. **13**(5): p. 899-902.
9. Silman, A.J., et al., *Twin concordance rates for rheumatoid arthritis: results from a nationwide study.* Br J Rheumatol, 1993. **32**(10): p. 903-7.
10. Gregersen, P.K., J. Silver, and R.J. Winchester, *The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis.* Arthritis Rheum, 1987. **30**(11): p. 1205-13.
11. Plenge, R.M., et al., *Replication of putative candidate-gene associations with rheumatoid arthritis in >4,000 samples from North America and Sweden: association of susceptibility with PTPN22, CTLA4, and PADI4.* Am J Hum Genet, 2005. **77**(6): p. 1044-60.
12. Stahl, E.A., et al., *Genome-wide association study meta-analysis identifies seven new rheumatoid arthritis risk loci.* Nat Genet, 2010. **42**(6): p. 508-14.
13. Senkpiehl, I., et al., *HLA-DRB1 and anti-cyclic citrullinated peptide antibody production in rheumatoid arthritis.* Int Arch Allergy Immunol, 2005. **137**(4): p. 315-8.
14. Kapitany, A., et al., *Associations between serum anti-CCP antibody, rheumatoid factor levels and HLA-DR4 expression in Hungarian patients with rheumatoid arthritis.* Isr Med Assoc J, 2008. **10**(1): p. 32-6.
15. Sugiyama, D., et al., *Impact of smoking as a risk factor for developing rheumatoid arthritis: a meta-analysis of observational studies.* Ann Rheum Dis, 2010. **69**(1): p. 70-81.

16. Klareskog, L., A.I. Catrina, and S. Paget, *Rheumatoid arthritis.* Lancet, 2009. **373**(9664): p. 659-72.

17. Symmons, D. and B. Harrison, *Early inflammatory polyarthritis: results from the norfolk arthritis register with a review of the literature. I. Risk factors for the development of inflammatory polyarthritis and rheumatoid arthritis.* Rheumatology (Oxford), 2000. **39**(8): p. 835-43.

18. Rose, H.M., C. Ragan, and et al., *Differential agglutination of normal and sensitized sheep erythrocytes by sera of patients with rheumatoid arthritis.* Proc Soc Exp Biol Med, 1948. **68**(1): p. 1-6.

19. Waaler, E., *On the occurrence of a factor in human serum activating the specific agglutintion of sheep blood corpuscles. 1939.* APMIS, 2007. **115**(5): p. 422-38; discussion 439.

20. van Venrooij, W.J., J.J. van Beers, and G.J. Pruijn, *Anti-CCP antibodies: the past, the present and the future.* Nat Rev Rheumatol, 2011. **7**(7): p. 391-8.

21. Rantapaa-Dahlqvist, S., et al., *Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis.* Arthritis Rheum, 2003. **48**(10): p. 2741-9.

22. Harre, U., et al., *Induction of osteoclastogenesis and bone loss by human autoantibodies against citrullinated vimentin.* J Clin Invest, 2012. **122**(5): p. 1791-802.

23. Krishnamurthy, A., et al., *Identification of a novel chemokine-dependent molecular mechanism underlying rheumatoid arthritis-associated autoantibody-mediated bone loss.* Ann Rheum Dis, 2016. **75**(4): p. 721-9.

24. Wigerblad, G., et al., *Autoantibodies to citrullinated proteins induce joint pain independent of inflammation via a chemokine-dependent mechanism.* Ann Rheum Dis, 2016. **75**(4): p. 730-8.

25. Smolen, J.S., et al., *EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2013 update.* Ann Rheum Dis, 2014. **73**(3): p. 492-509.

26. Rau, R. and G. Herborn, *Benefit and risk of methotrexate treatment in rheumatoid arthritis.* Clin Exp Rheumatol, 2004. **22**(5 Suppl 35): p. S83-94.

27. van Vollenhoven, R.F., et al., *Conventional combination treatment versus biological treatment in methotrexate-refractory early rheumatoid arthritis: 2 year follow-up of the randomised, non-blinded, parallel-group Swefot trial.* Lancet, 2012. **379**(9827): p. 1712-20.

28. Smolen, J.S., et al., *Treating rheumatoid arthritis to target: 2014 update of the recommendations of an international task force.* Ann Rheum Dis, 2016. **75**(1): p. 3-15.

29. Avci, A.B., E. Feist, and G.R. Burmester, *Biologicals in rheumatoid arthritis: current and future.* RMD Open, 2015. **1**(1): p. e000127.

30. Combe, B., et al., *EULAR recommendations for the management of early arthritis: report of a task force of the European Standing Committee for International Clinical Studies Including Therapeutics (ESCISIT).* Ann Rheum Dis, 2007. **66**(1): p. 34-45.

31. Hammond, A., A. Young, and R. Kidao, *A randomised controlled trial of occupational therapy for people with early rheumatoid arthritis.* Ann Rheum Dis, 2004. **63**(1): p. 23-30.

32. Withall, J., et al., *Physical activity engagement in early rheumatoid arthritis: a qualitative study to inform intervention development.* Physiotherapy, 2016. **102**(3): p. 264-71.

33. Lee, D.M. and M.E. Weinblatt, *Rheumatoid arthritis.* Lancet, 2001. **358**(9285): p. 903-11.

34. Sokka, T., et al., *Functional disability in rheumatoid arthritis patients compared with a community population in Finland.* Arthritis Rheum, 2003. **48**(1): p. 59-63.

35. De Bandt, M. and O. Meyer, *[Extra-articular manifestations of rheumatoid polyarthritis]*. Rev Prat, 1997. **47**(18): p. 2012-6.

36. Baecklund, E., et al., *Association of chronic inflammation, not its treatment, with increased lymphoma risk in rheumatoid arthritis.* Arthritis Rheum, 2006. **54**(3): p. 692-701.

37. *Guidelines for the management of rheumatoid arthritis: 2002 Update.* Arthritis Rheum, 2002. **46**(2): p. 328-46.

38. Arnett, F.C., et al., *The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis.* Arthritis Rheum, 1988. **31**(3): p. 315-24.

39. Aletaha, D., et al., *2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative.* Arthritis Rheum, 2010. **62**(9): p. 2569-81.

40. Smolen, J.S. and D. Aletaha, *What should be our treatment goal in rheumatoid arthritis today?* Clin Exp Rheumatol, 2006. **24**(6 Suppl 43): p. S-7-13.

41. Prevoo, M.L., et al., *Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis.* Arthritis Rheum, 1995. **38**(1): p. 44-8.

42. Fransen, J., M.C. Creemers, and P.L. Van Riel, *Remission in rheumatoid arthritis: agreement of the disease activity score (DAS28) with the ARA preliminary remission criteria.* Rheumatology (Oxford), 2004. **43**(10): p. 1252-5.

43. van de Putte, L.B., et al., *Efficacy and safety of adalimumab as monotherapy in patients with rheumatoid arthritis for whom previous disease modifying antirheumatic drug treatment has failed.* Ann Rheum Dis, 2004. **63**(5): p. 508-16.

44. van Gestel, A.M., et al., *Development and validation of the European League Against Rheumatism response criteria for rheumatoid arthritis. Comparison with the preliminary American College of Rheumatology and the World Health Organization/International League Against Rheumatism Criteria.* Arthritis Rheum, 1996. **39**(1): p. 34-40.

45. Gallagher, R.M., *Biopsychosocial pain medicine and mind-brain-body science.* Phys Med Rehabil Clin N Am, 2004. **15**(4): p. 855-82, vii.

46. van Hecke, O., et al., *Neuropathic pain in the general population: a systematic review of epidemiological studies.* Pain, 2014. **155**(4): p. 654-62.

47. Cazzola, M., et al., *Physiopathology of pain in rheumatology.* Reumatismo, 2014. **66**(1): p. 4-13.

48. Watkins, L.R., et al., *Norman Cousins Lecture. Glia as the "bad guys": implications for improving clinical pain control and the clinical utility of opioids.* Brain Behav Immun, 2007. **21**(2): p. 131-46.

49. Marchand, F., M. Perretti, and S.B. McMahon, *Role of the immune system in chronic pain.* Nat Rev Neurosci, 2005. **6**(7): p. 521-32.

50. Leffler, A.S., et al., *Somatosensory perception and function of diffuse noxious inhibitory controls (DNIC) in patients suffering from rheumatoid arthritis.* Eur J Pain, 2002. **6**(2): p. 161-76.

51. Sachs, D., et al., *Tumour necrosis factor-alpha, interleukin-1beta and interleukin-8 induce persistent mechanical nociceptor hypersensitivity.* Pain, 2002. **96**(1-2): p. 89-97.

52. Baron, R., *Neuropathic pain: a clinical perspective.* Handb Exp Pharmacol, 2009(194): p. 3-30.

53. *Report of the American College of Rheumatology Pain Management Task Force.* Arthritis Care Res (Hoboken), 2010. **62**(5): p. 590-9.

54. Taylor, P.C., et al., *A structured literature review of the burden of illness and unmet needs in patients with rheumatoid arthritis: a current perspective.* Rheumatol Int, 2016. **36**(5): p. 685-95.

55. Naranjo, A., et al., *Fibromyalgia in patients with rheumatoid arthritis is associated with higher scores of disability.* Ann Rheum Dis, 2002. **61**(7): p. 660-1.

56. Neugebauer, V., H.G. Schaible, and R.F. Schmidt, *Sensitization of articular afferents to mechanical stimuli by bradykinin.* Pflugers Arch, 1989. **415**(3): p. 330-5.

57. McDougall, J.J., *Arthritis and pain. Neurogenic origin of joint pain.* Arthritis Res Ther, 2006. **8**(6): p. 220.

58. Schaible, H.G., *Nociceptive neurons detect cytokines in arthritis.* Arthritis Res Ther, 2014. **16**(5): p. 470.

59. Konig, C., et al., *Involvement of Spinal IL-6 Trans-Signaling in the Induction of Hyperexcitability of Deep Dorsal Horn Neurons by Spinal Tumor Necrosis Factor-Alpha.* J Neurosci, 2016. **36**(38): p. 9782-91.

60. Wolfe, F. and K. Michaud, *Severe rheumatoid arthritis (RA), worse outcomes, comorbid illness, and sociodemographic disadvantage characterize ra patients with fibromyalgia.* J Rheumatol, 2004. **31**(4): p. 695-700.

61. Andersson, M.L., B. Svensson, and S. Bergman, *Chronic widespread pain in patients with rheumatoid arthritis and the relation between pain and disease activity measures over the first 5 years.* J Rheumatol, 2013. **40**(12): p. 1977-85.

62. Wolfe, F., et al., *The prevalence and characteristics of fibromyalgia in the general population.* Arthritis Rheum, 1995. **38**(1): p. 19-28.

63. Wolfe, F., et al., *The American College of Rheumatology 1990 Criteria for the Classification of Fibromyalgia. Report of the Multicenter Criteria Committee.* Arthritis Rheum, 1990. **33**(2): p. 160-72.

64. Gracely, R.H., M. Ceko, and M.C. Bushnell, *Fibromyalgia and depression.* Pain Res Treat, 2012. **2012**: p. 486590.

65. Kivimaki, M., et al., *Increased absence due to sickness among employees with fibromyalgia.* Ann Rheum Dis, 2007. **66**(1): p. 65-9.

66. Jansen, G.B., et al., *Differences in symptoms, functioning, and quality of life between women on long-term sick-leave with musculoskeletal pain with and without concomitant depression.* J Multidiscip Healthc, 2011. **4**: p. 281-92.

67. Kosek, E., J. Ekholm, and P. Hansson, *Sensory dysfunction in fibromyalgia patients with implications for pathogenic mechanisms.* Pain, 1996. **68**(2-3): p. 375-83.

68. Staud, R., *Mechanisms of fibromyalgia pain.* CNS Spectr, 2009. **14**(12 Suppl 16): p. 4-5; discussion 12-4.

69. Milligan, E.D. and L.R. Watkins, *Pathological and protective roles of glia in chronic pain.* Nat Rev Neurosci, 2009. **10**(1): p. 23-36.

70. Watkins, L.R. and S.F. Maier, *Immune regulation of central nervous system functions: from sickness responses to pathological pain.* J Intern Med, 2005. **257**(2): p. 139-55.

71. Kadetoff, D., et al., *Evidence of central inflammation in fibromyalgia-increased cerebrospinal fluid interleukin-8 levels.* J Neuroimmunol, 2012. **242**(1-2): p. 33-8.

72. Haliloglu, S., et al., *Fibromyalgia in patients with other rheumatic diseases: prevalence and relationship with disease activity.* Rheumatol Int, 2014. **34**(9): p. 1275-80.

73. Abbasi, L. and F.R. Haidri, *Fibromyalgia complicating disease management in rheumatoid arthritis.* J Coll Physicians Surg Pak, 2014. **24**(6): p. 424-7.

74. Bair, M.J., et al., *Depression and pain comorbidity: a literature review.* Arch Intern Med, 2003. **163**(20): p. 2433-45.

75. Hall, A.M., et al., *Symptoms of depression and stress mediate the effect of pain on disability.* Pain, 2011. **152**(5): p. 1044-51.

76. Bergman, S., *Management of musculoskeletal pain.* Best Pract Res Clin Rheumatol, 2007. **21**(1): p. 153-66.

77. Pawlikowska, T., et al., *Population based study of fatigue and psychological distress.* BMJ, 1994. **308**(6931): p. 763-6.

78. Sandikci, S.C. and Z. Ozbalkan, *Fatigue in rheumatic diseases.* Eur J Rheumatol, 2015. **2**(3): p. 109-113.

79. Repping-Wuts, H., et al., *Fatigue as experienced by patients with rheumatoid arthritis (RA): a qualitative study.* Int J Nurs Stud, 2008. **45**(7): p. 995-1002.

80. Wolfe, F., D.J. Hawley, and K. Wilson, *The prevalence and meaning of fatigue in rheumatic disease.* J Rheumatol, 1996. **23**(8): p. 1407-17.

81. Hewlett, S., et al., *Patients' perceptions of fatigue in rheumatoid arthritis: overwhelming, uncontrollable, ignored.* Arthritis Rheum, 2005. **53**(5): p. 697-702.

82. Pollard, L.C., et al., *Fatigue in rheumatoid arthritis reflects pain, not disease activity.* Rheumatology (Oxford), 2006. **45**(7): p. 885-9.

83. Kelley, K.W., et al., *Central interleukin-1 receptors as mediators of sickness.* Ann N Y Acad Sci, 1997. **823**: p. 234-46.

84. Rinehart, J., et al., *Phase 1 trial of recombinant human interleukin-1 beta (rhIL-1 beta), carboplatin, and etoposide in patients with solid cancers: Southwest Oncology, Group Study 8940.* Cancer Invest, 1997. **15**(5): p. 403-10.

85. Lampa, J., et al., *Peripheral inflammatory disease associated with centrally activated IL-1 system in humans and mice.* Proc Natl Acad Sci U S A, 2012. **109**(31): p. 12728-33.

86. Omdal, R. and R. Gunnarsson, *The effect of interleukin-1 blockade on fatigue in rheumatoid arthritis--a pilot study.* Rheumatol Int, 2005. **25**(6): p. 481-4.

87. Almeida, C., et al., *Biologic interventions for fatigue in rheumatoid arthritis.* Cochrane Database Syst Rev, 2016(6): p. CD008334.

88. Cramp, F., et al., *Non-pharmacological interventions for fatigue in rheumatoid arthritis.* Cochrane Database Syst Rev, 2013(8): p. CD008322.

89. Neuberger, G.B., et al., *Effects of exercise on fatigue, aerobic fitness, and disease activity measures in persons with rheumatoid arthritis.* Res Nurs Health, 1997. **20**(3): p. 195-204.

90. Louati, K. and F. Berenbaum, *Fatigue in chronic inflammation - a link to pain pathways.* Arthritis Res Ther, 2015. **17**: p. 254.

91. Lee, Y.C., et al., *Subgrouping of patients with rheumatoid arthritis based on pain, fatigue, inflammation, and psychosocial factors.* Arthritis Rheumatol, 2014. **66**(8): p. 2006-14.

92. Bergman, M.J., et al., *Is fatigue an inflammatory variable in rheumatoid arthritis (RA)? Analyses of fatigue in RA, osteoarthritis, and fibromyalgia.* J Rheumatol, 2009. **36**(12): p. 2788-94.

93. Elenkov, I.J., et al., *The sympathetic nerve--an integrative interface between two supersystems: the brain and the immune system.* Pharmacol Rev, 2000. **52**(4): p. 595-638.

94. Thayer, J.F. and E.M. Sternberg, *Neural aspects of immunomodulation: focus on the vagus nerve.* Brain Behav Immun, 2010. **24**(8): p. 1223-8.

95. Tracey, K.J., *The inflammatory reflex.* Nature, 2002. **420**(6917): p. 853-9.

96. Tracey, K.J., *Physiology and immunology of the cholinergic antiinflammatory pathway.* J Clin Invest, 2007. **117**(2): p. 289-96.

97. Pavlov, V.A. and K.J. Tracey, *The cholinergic anti-inflammatory pathway.* Brain Behav Immun, 2005. **19**(6): p. 493-9.

98. Pavlov, V.A., et al., *The cholinergic anti-inflammatory pathway: a missing link in neuroimmunomodulation.* Mol Med, 2003. **9**(5-8): p. 125-34.

99. Janse van Rensburg, D.C., et al., *Autonomic impairment in rheumatoid arthritis.* Int J Rheum Dis, 2012. **15**(4): p. 419-26.

100. Goldstein, R.S., et al., *Cholinergic anti-inflammatory pathway activity and High Mobility Group Box-1 (HMGB1) serum levels in patients with rheumatoid arthritis.* Mol Med, 2007. **13**(3-4): p. 210-5.

101. Westman, M., et al., *Cell specific synovial expression of nicotinic alpha 7 acetylcholine receptor in rheumatoid arthritis and psoriatic arthritis.* Scand J Immunol, 2009. **70**(2): p. 136-40.

102. van Maanen, M.A., M.J. Vervoordeldonk, and P.P. Tak, *The cholinergic anti-inflammatory pathway: towards innovative treatment of rheumatoid arthritis.* Nat Rev Rheumatol, 2009. **5**(4): p. 229-32.

103. Koopman, F.A., et al., *Vagus nerve stimulation inhibits cytokine production and attenuates disease severity in rheumatoid arthritis.* Proc Natl Acad Sci U S A, 2016. **113**(29): p. 8284-9.

104. Figueroa, A., et al., *Resistance exercise training improves heart rate variability in women with fibromyalgia.* Clin Physiol Funct Imaging, 2008. **28**(1): p. 49-54.

105. Martinez-Martinez, L.A., et al., *Sympathetic nervous system dysfunction in fibromyalgia, chronic fatigue syndrome, irritable bowel syndrome, and interstitial cystitis: a review of case-control studies.* J Clin Rheumatol, 2014. **20**(3): p. 146-50.

106. Martinez-Lavin, M., et al., *Circadian studies of autonomic nervous balance in patients with fibromyalgia: a heart rate variability analysis.* Arthritis Rheum, 1998. **41**(11): p. 1966-71.

107. Bengtsson, C., et al., *Non-participation in EIRA: a population-based case-control study of rheumatoid arthritis.* Scand J Rheumatol, 2010. **39**(4): p. 344-6.

108. Stolt, P., et al., *Quantification of the influence of cigarette smoking on rheumatoid arthritis: results from a population based case-control study, using incident cases.* Ann Rheum Dis, 2003. **62**(9): p. 835-41.

109. Jiang, X., et al., *Higher education is associated with a better rheumatoid arthritis outcome concerning for pain and function but not disease activity: results from the EIRA cohort and Swedish rheumatology register.* Arthritis Res Ther, 2015. **17**: p. 317.

110. Pikwer, M., et al., *Parity influences the severity of ACPA-negative early rheumatoid arthritis: a cohort study based on the Swedish EIRA material.* Arthritis Res Ther, 2015. **17**: p. 358.

111. Gloth, F.M., 3rd, et al., *The Functional Pain Scale: reliability, validity, and responsiveness in an elderly population.* J Am Med Dir Assoc, 2001. **2**(3): p. 110-4.

112. Jensen, M.P., et al., *The use of multiple-item scales for pain intensity measurement in chronic pain patients.* Pain, 1996. **67**(1): p. 35-40.

113. Vlaeyen, J.W., et al., *Assessment of the components of observed chronic pain behavior: the Checklist for Interpersonal Pain Behavior (CHIP).* Pain, 1990. **43**(3): p. 337-47.

114. Turk, D.C. and T.E. Rudy, *Towards a comprehensive assessment of chronic pain patients.* Behav Res Ther, 1987. **25**(4): p. 237-49.

115. Kerns, R.D., D.C. Turk, and T.E. Rudy, *The West Haven-Yale Multidimensional Pain Inventory (WHYMPI).* Pain, 1985. **23**(4): p. 345-56.

116. Melzack, R., *The McGill Pain Questionnaire: major properties and scoring methods.* Pain, 1975. **1**(3): p. 277-99.

117. McCracken, L.M., C. Zayfert, and R.T. Gross, *The Pain Anxiety Symptoms Scale: development and validation of a scale to measure fear of pain.* Pain, 1992. **50**(1): p. 67-73.

118. Callahan, L.F., et al., *Quantitative pain assessment for routine care of rheumatoid arthritis patients, using a pain scale based on activities of daily living and a visual analog pain scale.* Arthritis Rheum, 1987. **30**(6): p. 630-6.

119. Wolfe, F. and K. Michaud, *Assessment of pain in rheumatoid arthritis: minimal clinically significant difference, predictors, and the effect of anti-tumor necrosis factor therapy.* J Rheumatol, 2007. **34**(8): p. 1674-83.

120. Huskisson, E.C., *Measurement of pain.* Lancet, 1974. **2**(7889): p. 1127-31.

121. Joos, E., et al., *Reliability and reproducibility of visual analogue scale and numeric rating scale for therapeutic evaluation of pain in rheumatic patients.* J Rheumatol, 1991. **18**(8): p. 1269-70.

122. Carlsson, A.M., *Assessment of chronic pain. I. Aspects of the reliability and validity of the visual analogue scale.* Pain, 1983. **16**(1): p. 87-101.

123. Tubach, F., et al., *Minimum clinically important improvement and patient acceptable symptom state in pain and function in rheumatoid arthritis, ankylosing spondylitis, chronic back pain, hand osteoarthritis, and hip and knee osteoarthritis: Results from a prospective multinational study.* Arthritis Care Res (Hoboken), 2012. **64**(11): p. 1699-707.

124. Tubach, F., et al., *Feeling good rather than feeling better matters more to patients.* Arthritis Rheum, 2006. **55**(4): p. 526-30.

125. Kvien, T.K., T. Heiberg, and K.B. Hagen, *Minimal clinically important improvement/difference (MCII/MCID) and patient acceptable symptom state (PASS): what do these concepts mean?* Ann Rheum Dis, 2007. **66 Suppl 3**: p. iii40-1.

126. Moore, R.A., S. Straube, and D. Aldington, *Pain measures and cut-offs - 'no worse than mild pain' as a simple, universal outcome.* Anaesthesia, 2013. **68**(4): p. 400-12.

127. Cella, D., et al., *Validation of the Functional Assessment of Chronic Illness Therapy Fatigue Scale relative to other instrumentation in patients with rheumatoid arthritis.* J Rheumatol, 2005. **32**(5): p. 811-9.

128. Smets, E.M., et al., *The Multidimensional Fatigue Inventory (MFI) psychometric qualities of an instrument to assess fatigue.* J Psychosom Res, 1995. **39**(3): p. 315-25.

129. Lin, J.M., et al., *Further validation of the Multidimensional Fatigue Inventory in a US adult population sample.* Popul Health Metr, 2009. **7**: p. 18.

130. Rupp, I., et al., *Impact of fatigue on health-related quality of life in rheumatoid arthritis.* Arthritis Rheum, 2004. **51**(4): p. 578-85.

131. Hewlett, S., M. Hehir, and J.R. Kirwan, *Measuring fatigue in rheumatoid arthritis: a systematic review of scales in use.* Arthritis Rheum, 2007. **57**(3): p. 429-39.

132. Wolfe, F., *Fatigue assessments in rheumatoid arthritis: comparative performance of visual analog scales and longer fatigue questionnaires in 7760 patients.* J Rheumatol, 2004. **31**(10): p. 1896-902.

133. Buysse, D.J., et al., *The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research.* Psychiatry Res, 1989. **28**(2): p. 193-213.

134. Contopoulos-Ioannidis, D.G., et al., *Reporting and interpretation of SF-36 outcomes in randomised trials: systematic review.* BMJ, 2009. **338**: p. a3006.

135. Stein, P.K., et al., *Heart rate variability: a measure of cardiac autonomic tone.* Am Heart J, 1994. **127**(5): p. 1376-81.

136. Sandercock, G.R. and D.A. Brodie, *The use of heart rate variability measures to assess autonomic control during exercise.* Scand J Med Sci Sports, 2006. **16**(5): p. 302-13.

137. Adlan, A.M., et al., *Autonomic function and rheumatoid arthritis: a systematic review.* Semin Arthritis Rheum, 2014. **44**(3): p. 283-304.

138. Kleiger, R.E., et al., *Time domain measurements of heart rate variability.* Cardiol Clin, 1992. **10**(3): p. 487-98.

139. Ori, Z., et al., *Heart rate variability. Frequency domain analysis.* Cardiol Clin, 1992. **10**(3): p. 499-537.

140. Zou, G., *A modified poisson regression approach to prospective studies with binary data.* Am J Epidemiol, 2004. **159**(7): p. 702-6.

141. T, T., A Package for Survival Analysis in S. version 2.38. 2015.

142. Taylor, P., et al., *Patient perceptions concerning pain management in the treatment of rheumatoid arthritis.* J Int Med Res, 2010. **38**(4): p. 1213-24.

143. Studenic, P., et al., *Discrepancies between patients and physicians in their perceptions of rheumatoid arthritis disease activity.* Arthritis Rheum, 2012. **64**(9): p. 2814-23.

144. Lee, Y.C., et al., *Pain persists in DAS28 rheumatoid arthritis remission but not in ACR/EULAR remission: a longitudinal observational study.* Arthritis Res Ther, 2011. **13**(3): p. R83.

145. Saevarsdottir, S., et al., *Patients with early rheumatoid arthritis who smoke are less likely to respond to treatment with methotrexate and tumor necrosis factor inhibitors: observations from the Epidemiological Investigation of Rheumatoid Arthritis and the Swedish Rheumatology Register cohorts.* Arthritis Rheum, 2011. **63**(1): p. 26-36.

146. Wolfe, F., *A reappraisal of HAQ disability in rheumatoid arthritis.* Arthritis Rheum, 2000. **43**(12): p. 2751-61.

147. McWilliams, D.F., et al., *Predictors of change in bodily pain in early rheumatoid arthritis: an inception cohort study.* Arthritis Care Res (Hoboken), 2012. **64**(10): p. 1505-13.

148. Christianson, C.A., et al., *Characterization of the acute and persistent pain state present in K/BxN serum transfer arthritis.* Pain, 2010. **151**(2): p. 394-403.

149. Lee, Y.C., et al., *Incidence and predictors of secondary fibromyalgia in an early arthritis cohort.* Ann Rheum Dis, 2013. **72**(6): p. 949-54.

150. Ranzolin, A., et al., *Association of concomitant fibromyalgia with worse disease activity score in 28 joints, health assessment questionnaire, and short form 36 scores in patients with rheumatoid arthritis.* Arthritis Rheum, 2009. **61**(6): p. 794-800.

151. Staud, R., *Evidence of involvement of central neural mechanisms in generating fibromyalgia pain.* Curr Rheumatol Rep, 2002. **4**(4): p. 299-305.

152. Cunha, F.Q., et al., *Interleukin-8 as a mediator of sympathetic pain.* Br J Pharmacol, 1991. **104**(3): p. 765-7.

153. Bartfai, T., *Immunology. Telling the brain about pain.* Nature, 2001. **410**(6827): p. 425, 427.

154. Samad, T.A., et al., *Interleukin-1beta-mediated induction of Cox-2 in the CNS contributes to inflammatory pain hypersensitivity.* Nature, 2001. **410**(6827): p. 471-5.

155. Carville, S.F., et al., *EULAR evidence-based recommendations for the management of fibromyalgia syndrome.* Ann Rheum Dis, 2008. **67**(4): p. 536-41.

156. Leeb, B.F., et al., *Disease activity score-28 values differ considerably depending on patient's pain perception and sex.* J Rheumatol, 2007. **34**(12): p. 2382-7.

157. Sarzi-Puttini, P., et al., *Correlation of the score for subjective pain with physical disability, clinical and radiographic scores in recent onset rheumatoid arthritis.* BMC Musculoskelet Disord, 2002. **3**: p. 18.

158. Hekmat, K., et al., *Changes and sex differences in patient reported outcomes in rheumatoid factor positive RA-results from a community based study.* BMC Musculoskelet Disord, 2014. **15**: p. 44.

159. Barnabe, C., et al., *Sex differences in pain scores and localization in inflammatory arthritis: a systematic review and metaanalysis.* J Rheumatol, 2012. **39**(6): p. 1221-30.

160. Lesuis, N., et al., *Gender and the treatment of immune-mediated chronic inflammatory diseases: rheumatoid arthritis, inflammatory bowel disease and psoriasis: an observational study.* BMC Med, 2012. **10**: p. 82.

161. Arkema, E.V., et al., *Is there a sex bias in prescribing anti-tumour necrosis factor medications to patients with rheumatoid arthritis? A nation-wide cross-sectional study.* Ann Rheum Dis, 2012. **71**(7): p. 1203-6.

162. Repping-Wuts, H., et al., *Persistent severe fatigue in patients with rheumatoid arthritis.* J Clin Nurs, 2007. **16**(11C): p. 377-83.

163. van Steenbergen, H.W., et al., *Fatigue in rheumatoid arthritis; a persistent problem: a large longitudinal study.* RMD Open, 2015. **1**(1): p. e000041.

164. van Hoogmoed, D., et al., *Physical and psychosocial correlates of severe fatigue in rheumatoid arthritis.* Rheumatology (Oxford), 2010. **49**(7): p. 1294-302.

165. Cohen, H., et al., *Autonomic nervous system derangement in fibromyalgia syndrome and related disorders.* Isr Med Assoc J, 2001. **3**(10): p. 755-60.

166. Waldburger, J.M. and G.S. Firestein, *Regulation of peripheral inflammation by the central nervous system.* Curr Rheumatol Rep, 2010. **12**(5): p. 370-8.

167. Pavlov, V.A., et al., *Central muscarinic cholinergic regulation of the systemic inflammatory response during endotoxemia.* Proc Natl Acad Sci U S A, 2006. **103**(13): p. 5219-23.

168. Schliebs, R., et al., *Interaction of interleukin-1beta with muscarinic acetylcholine receptor-mediated signaling cascade in cholinergically differentiated SH-SY5Y cells.* Brain Res, 2006. **1122**(1): p. 78-85.

169. Meeus, M., et al., *Heart rate variability in patients with fibromyalgia and patients with chronic fatigue syndrome: a systematic review.* Semin Arthritis Rheum, 2013. **43**(2): p. 279-87.