

From THE DEPARTMENT OF MEDICINE, SOLNA  
Karolinska Institutet, Stockholm, Sweden

**PREDICTING AND MONITORING DISEASE  
COURSE IN RHEUMATOID ARTHRITIS:  
IMAGING, BIOMARKERS, RISK FACTORS,  
AND INTEGRATIVE MEDICINE**

Adrian Levitsky



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# Predicting and monitoring disease course in rheumatoid arthritis: Imaging, biomarkers, risk factors, and integrative medicine

## THESIS FOR DOCTORAL DEGREE (Ph.D.)

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*The Road goes ever on and on*  
Down from the door where it began  
Now far ahead the Road has gone,  
And I must follow, if I can,  
Pursuing it with eager feet,  
Until it joins some larger way  
Where many paths and errand meet.  
And whither then? I cannot say.

❖ *The Old Walking Song*

*All that is gold does not glitter,*  
Not all those who wander are lost;  
The old that is strong does not wither,  
Deep roots are not reached by the frost.

❖ *The Riddle of Strider*

*The Lord of the Rings*  
By *J.R.R. Tolkien*

*To my dear wife, Rongrong,*  
*To my parents, Margareta and Alexander,*  
*To my siblings, Anna and Alex*

Your vision will become clear only when you look into your heart. Who looks outside, dreams.  
Who looks inside, awakens.

- Carl Gustav Jung



## ABSTRACT

Rheumatoid arthritis (RA), the most common inflammatory arthritis, is a chronic, potentially debilitating autoimmune disease that can lead to functional disability, bone erosion, and chronic pain. The modern era of treatment has led to major advancements in treating this condition, especially if patients are treated early within a ‘window of opportunity’ with potent disease-modifying antirheumatic drugs (DMARDs) and a ‘treat-to-target’ approach aiming towards low disease activity or remission. Personalized integrative medicine may lead to further advancements in the care of individuals suffering from autoimmune conditions such as RA through the application of imaging, biomarker and risk factor identification, and integrative manual therapy.

After simulating a true radiographic progression control group in several randomized clinical trials, early application of intensive or biological DMARDs was demonstrated to be superior to conventional monotherapy in early RA, and that rheumatoid factor-positive patients on an intensive strategy may benefit more with a half-year induction of anti-tumor necrosis factor (TNF) therapy (**Papers I-II**).

For the first time, it was revealed that the proto-oncogene *survivin*, expressed in one third of patients with early RA, prevents a sustained clinical response to gold-standard methotrexate. Additionally, further allocation to combination DMARDs may be favorable to the allocation of anti-TNF therapy among *survivin*-positive patients (**Paper III**).

Lifestyle risk factors were shown to play an important role in early RA disease outcome, and obesity in particular was found to be a strong independent predictor of long-term non-remission, in addition to smoking. Obesity was associated with worse clinical outcomes over time, measured by disease activity, pain, and functional disability (**Paper IV**).

A novel approach was explored with integrative manual mobilization therapy and its potential to further enhance patient care in RA. This was demonstrated through systemic subjective and objective hand improvements – including pain, synovial fluid, and joint space (**Paper V**).

Together with the goal of aiming for early, tight RA disease control, when utilizing imaging tools; identifying biomarkers and lifestyle risk factors; and applying integrative medicine, allopathic practice can move towards even better proactive patient care in RA. Altogether, these findings support the value of incorporating personalized integrative medicine into clinical practice for patients with RA.

# LIST OF SCIENTIFIC PAPERS

This thesis is founded upon five scientific papers, which will be referred to as **Papers I-V** throughout this book.

- I. Adrian Levitsky**, Kristina Forslind, Ronald F. van Vollenhoven;  
SWEFOT trial group. *Predicted vs. observed radiographic progression in early rheumatoid arthritis (POPeRA): results from a randomized trial.*  
Scandinavian Journal of Rheumatology. 2015;44(5):348-53.  
Epub 2015 May 20.
  
- II. Adrian Levitsky**, Marius Wick, Timo Möttönen, Marjatta Leirisalo-Repo, Leena Laasonen, Markku Korpela, Ronald F. van Vollenhoven, Vappu Rantalaiho. *Early treatment intensification induces favourable radiographic outcomes according to predicted versus observed radiographic progression in early rheumatoid arthritis: a subanalysis of the randomized FIN-RACo and NEO-RACo trials.*  
Clinical and Experimental Rheumatology. 2016 Nov-Dec;34(6):1065-1071.  
Epub 2016 Aug 31.
  
- III. Adrian Levitsky**, Malin C. Erlandsson, Ronald F. van Vollenhoven\*, Maria I. Bokarewa\*. *Serum survivin predicts responses to treatment in active rheumatoid arthritis: a post hoc analysis from the SWEFOT trial.*  
BMC Medicine. 2015 Sep 30;13:247. \* Contributed equally as senior authors
  
- IV. Adrian Levitsky**, Kerstin Brismar, Ingiäld Hafström, Karen Hambarzumyan, Cecilia Lourdudoss, Ronald F. van Vollenhoven, Saedis Saevarsdottir; SWEFOT trial group.  
*Obesity is a strong predictor of worse clinical outcomes and treatment responses in early rheumatoid arthritis: results from the SWEFOT trial.*  
RMD Open (Minor revision submitted, 2017 Apr).
  
- V. Adrian Levitsky**, Yogan Kisten, Sara Lind, Patric Nordström, Helene Hultholm, Jessica Lyander, Viveka Hammelin, Cidem Gentline, Ioanna Giannakou, Francesca Faustini, Eva Skillgate, Ronald F. van Vollenhoven, Tobias Sundberg.  
*Joint mobilization of the hands in rheumatoid arthritis: Results from an assessor-blinded, randomized crossover study.*  
Manuscript (Submitted 2017 Apr).

## RELATED PUBLICATIONS

- I. **Adrian Levitsky**, Yogan Kisten, Tobias Sundberg, Ronald F. van Vollenhoven. *Clinical roundup: selected treatment options for rheumatoid arthritis. Kaltenborn manual mobilization.* Alternative and Complementary Therapies. 2016 Aug;22(4):175.
  
- II. Yogan Kisten, Noemi Györi, Erik af Klint, Hamed Rezaei, **Adrian Levitsky**, Anna Karlsson, Ronald F. van Vollenhoven. *Detection of clinically manifest and silent synovitis in the hands and wrists by fluorescence optical imaging.* RMD Open. 2015 Jun 19;1(1):e000106.

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## LIST OF ABBREVIATIONS

RA	Rheumatoid arthritis
DMARDs	Disease-modifying anti-rheumatic drugs
NSAIDs	Non-steroidal anti-inflammatory drugs
EULAR	European League Against Rheumatism
ACR	American College of Rheumatology
PCs	Plasma cells
ACPA	Anti-citrullinated protein antibodies
RF	Rheumatoid factor
Ig	Immunoglobulin
SE	Shared epitope
TNF	Tumor necrosis factor
IL	Interleukin
DCs	Dendritic cells
FDCs	Follicular dendritic cells
Th2	T helper 2 (cell)
TCR	T cell receptor
BCR	B cell receptor
MHC II	Multihistocompatibility complex class II
CD	Cluster of differentiation
M $\phi$	Macrophages
FLS	Fibroblast-like synoviocytes
MMPs	Matrix metalloproteinases
OCs	Osteoclasts
NETs	Neutrophil extracellular traps
ARA	American Rheumatism Association
DIP	Distal interphalangeal (joints)
PIP	Proximal interphalangeal (joints)
MCP	Metacarpophalangeal (joints)
MTP	Metatarsophalangeal (joints)

MTX	Methotrexate
CRP	C-reactive protein
ESR	Erythrocyte sedimentation rate
ULN	Upper limit of normal
TT	Triple therapy
SSZ	Sulfasalazine
HCQ	Hydroxychloroquine
JAK	Janus kinase
STAT	Signal transducer and activator of transcription
FDA	Food and Drug Administration
EMA	European Medicines Agency
T2T	Treat-to-target
DAS28	28-joint count disease activity score
SHS	Sharp-van der Heijde Score
SWEFOT	The Swedish pharmacotherapy trial
POPeRA	Predicted vs. Observed Progression in early RA
FIN-RACo	Finnish RA Combination therapy trial
NEO-RACo	New Finnish RA Combination therapy trial
MBDA	Multi-biomarker disease activity score
BMI	Body mass index
HAQ	Health assessment questionnaire
CAM	Complementary and alternative medicine
TM	Traditional medicine
T&CM	Traditional and complementary medicine
NCCIH	National Center for Complementary and Integrative Health
WHO	The World Health Organization
TwHF	<i>Tripterygium wilfordii</i> Hook F
OA	Osteoarthritis
VAS-pain	Visual analog scale for pain
VAS-global	Visual analog scale for global health
IPR	Inferred progression rate

PRP	Predicted radiographic progression
ELISA	Enzyme-linked immunosorbent assay
BL	Baseline
W	Week
PROMs	Patient-reported outcome measures
H-rand	Randomized hand
H-control	Initial control hand
PBO	Placebo
IQR	Interquartile range
OR	Odds ratio
CI	Confidence interval
R	Right hand (proportion)
Y	Years
Q-Doppler	Quantitative Doppler activity (%)
Tx	Treatment (mobilization)
Foll-up	Follow-up
CMC	Carpometacarpal (joints)

# 1 INTRODUCTION

## 1.1 PRELUDE

With the impact of innovations through western allopathic medicine, public health, and industrialization to abrogate infectious diseases and significantly prolong life, modern humanity has been able to convert itself in a stepwise fashion from a state of – paraphrasing the words of Professor Hans Rosling – ‘dying in ecological balance’ to that of ‘living in ecological balance.’

Now in the modern era with advanced nations taking small steps towards science fiction becoming the reality, our globe – in addition to the cumbersome weight of political, ethical, and environmental concerns – is faced with new challenges: the ever-advancing, looming shadow and inescapable burden of chronic illnesses. To tackle these challenges, the novel scientific minds of our age are advocating personalized medicine, where – through knowledge and identification of biomarkers and genetic expression – the design and allocation of therapies can be targeted to individuals instead of populations.

Through the studies included in this thesis book that centralize around rheumatoid arthritis, the intention was to investigate the advances of allopathic medicine upon tackling challenges with personalized care: through proper identification and treatment of individuals by imaging techniques, biomarkers, and environmental & lifestyle risk factors; yet, to also potentially help fill a void in the care paradigm. Perhaps by taking a more inclusive medical approach, chronic illnesses and the surging problem of disarray, stress, and psychosocial issues could be further eased through a combined approach; namely, personalized integrative medicine.

## 1.2 RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a chronic autoimmune disease and the most prevalent inflammatory arthritis – affecting around 0.5-1% of adults in high-income countries, and is two to three times as common among women (1-4). RA has an estimated global prevalence of 0.24% due to regional fluctuations: being highest in Oceania, North America, and Western Europe (0.45%); while being lowest in East/Southeast Asia, North Africa, and the Middle East (0.16%) (5). About two-thirds of patients with RA have autoantibodies, and the condition is characterized by elevated acute-phase reactants, swollen and tender joints due to synovial inflammation, functional disability, work productivity losses, bone erosion, and chronic pain; and is also associated with an enhanced risk of cardiovascular morbidities (1-10).

Disease-modifying anti-rheumatic drugs (DMARDs) in particular, whether synthetic or biological – in addition to non-steroidal anti-inflammatory drugs (NSAIDs) – are utilized in the management of patients with RA. These treatments are today capable of inducing increasingly-achievable states of remission (11), however, it has been identified that even some of these patients, despite clinical response, lose health-related functional capability over time (12). Efforts have been made by the European League Against Rheumatism (EULAR) and the American College of Rheumatology (ACR) – the two largest international research organizations within rheumatology – in creating the 2010 classification criteria for RA, which may help patients to get treated in time by a ‘window of opportunity’ with early, aggressive therapy (13, 14). This stratagem has been shown to be more efficacious than standard of care in the clinical setting (15, 16).

There exist, however, several avenues which have not been carefully addressed prior to the work demonstrated in this thesis. Namely, exactly how early, intensive therapy in a large randomized trial in early RA might benefit patients by radiographic imaging in relation to true control projections through linear modelling; the clinical value of predictive biomarkers and risk factors in early RA; and the potential importance of monitoring patients with early-to established RA through integrative medicine with manual mobilization therapy.

### 1.3 OUTLINE

In this thesis, the following background topics will be covered:

- ❖ A background of RA, including its preclinical stage and pathogenesis, diagnosis, as well as allopathic treatment strategies.
- ❖ Imaging tools utilized in RA, including conventional radiographic analysis, musculoskeletal ultrasound, and a brief mentioning of novel imaging instruments.
- ❖ Predictive and associative biomarkers, including how they may play a role in RA, as well as how they may potentially predict disease course.
- ❖ Lifestyle and environmental risk factors as potentially influential determinants of pathogenesis and predictors of disease activity in RA.
- ❖ Integrative medicine, where complementary treatment modalities are included together with allopathic medicine. Here, a description of various integrative modalities will be laid out, in addition to a summary of the literature pertaining to integrative medicine tested in autoimmune rheumatic conditions thus far.

The thesis then includes the original research addressed through **Papers I-V**, including:

- ❖ An overarching aim and study-specific aims for each paper.
- ❖ Materials and methods for each paper.
- ❖ Study-specific results, points for discussion, and conclusions.



## 2 BACKGROUND

### 2.1 RHEUMATOID ARTHRITIS IN DEPTH

#### 2.1.1 Pathogenesis and promulgation

There is increasing evidence that the body reveals important pathogenic signs prior to disease onset in autoimmune conditions such as RA (17-25). Monitoring these markers, and thereafter responding with therapy, may provide a ‘window of opportunity’ for action in early RA – within three months to maximum two years after symptom onset (26, 27) – to prevent outcomes which could later become potentially irreversible.

Autoreactive B cells that differentiate into plasma cells (PCs) are capable of producing anti-citrullinated protein antibodies (ACPA) and rheumatoid factor (RF), which have been found to be present in individuals who would later develop RA several years afterward (21-23). Although RF is traditionally associated with RA, it does not specifically identify RA as it is present in several inflammatory conditions, and appears to be involved in T cell-related immunity to immunoglobulin G (IgG) immune complexes (28-30). ACPA, on the other hand, is more RA-specific as these antibodies occur in <2% of healthy individuals and are only marginally present in other inflammatory conditions (28). ACPA specificities in RA include citrullinated epitopes as antigens on vimentin, fibrinogen,  $\alpha$ -enolase, histones, type II collagen, and tenascin C (28, 31-36). Somatic hyper-mutation and affinity maturation in the follicles/germinal centers of lymph nodes allows for highly specialized B cells and their antibody progeny to effectively respond to antigens. However, this specialization may decrease affinity and thus increase autoreactivity – creating antibodies like ACPA or RF. This, together with genetic and environmental risk factors, may lead to the initiation and prolongation of inflammation/bone erosion in RA (2, 28, 37, 38).

RA may be initiated and prolonged through the formation of several potential stakeholders. The most widely-accepted, evidence-based environmental trigger is smoking (39-45) – especially upon combination with the HLA-DRB1 shared epitope (SE) and PTPN22; as these genes are especially susceptible RA risk candidates of protein citrullination caused by smoking and triggering the immune system by ACPA as a result (28, 32, 37-39, 46). Alternative triggers include exposure to silica (47, 48), textile dust (49), and possibly some microorganisms (50-52). The cascade then begins with (citrullinated) antigen presentation and co-stimulation, where dendritic cells (DCs) and autoreactive B cells activate the effector functions of T cells at mucosal sites: the lungs, gums, or gut (28, 38); leading to pro-inflammatory cytokines such as

tumor necrosis factor (TNF), interleukin (IL)-6, interferon- $\gamma$ , and lymphotoxin- $\alpha$  (37); as well as ACPA/RF autoantibodies and the resulting formation of immune complexes. Finally, ectopic lymphoid structures are present in ~40% of patients with RA and can form in the sublining of the synovial tissue surrounding the joints (53), as well as extra-articular sites such as the lungs and bone marrow (54, 55). They are induced by cytokines such as lymphotoxin- $\alpha_1$ - $\beta_2$ , chemokines such as B lymphocyte chemoattractant CXCL13, and vascular adhesion molecules such as VCAM1; and display functional germinal center features with autoreactive B cells, T cells, follicular dendritic cells (FDCs), and PCs that are capable of releasing localized cytokines, chemokines, and autoantibodies (37, 53, 56-58). These elements, in their own way, shape, or form, play an individualized role in the creation and sustainability of adaptive autoimmunity in RA (**Figure 1**).

Once the pathogenesis and promulgation of RA has been set in motion, synovial cells and the joint microenvironment are activated and infiltrated by peripheral immune cells – the result being synovitis or inflammation of the synovium and the formation of the pannus, which degrades cartilage and erodes bone (2, 59). RA, however, can manifest through several different pathways; subdivided for example into at least three possible microstructural synovial phenotypes: *lymphoid*: follicular synovitis with B- and T cells that form ectopic lymphoid structures; *myeloid*: diffuse infiltration pattern of monocytes and macrophages (M $\phi$ ); and *fibroid*: minimal synovitis with limited to no immune cell infiltration (53, 60-64). An example of a metacarpophalangeal joint of the hand affected by RA synovitis is shown in **Figure 2A-B**.

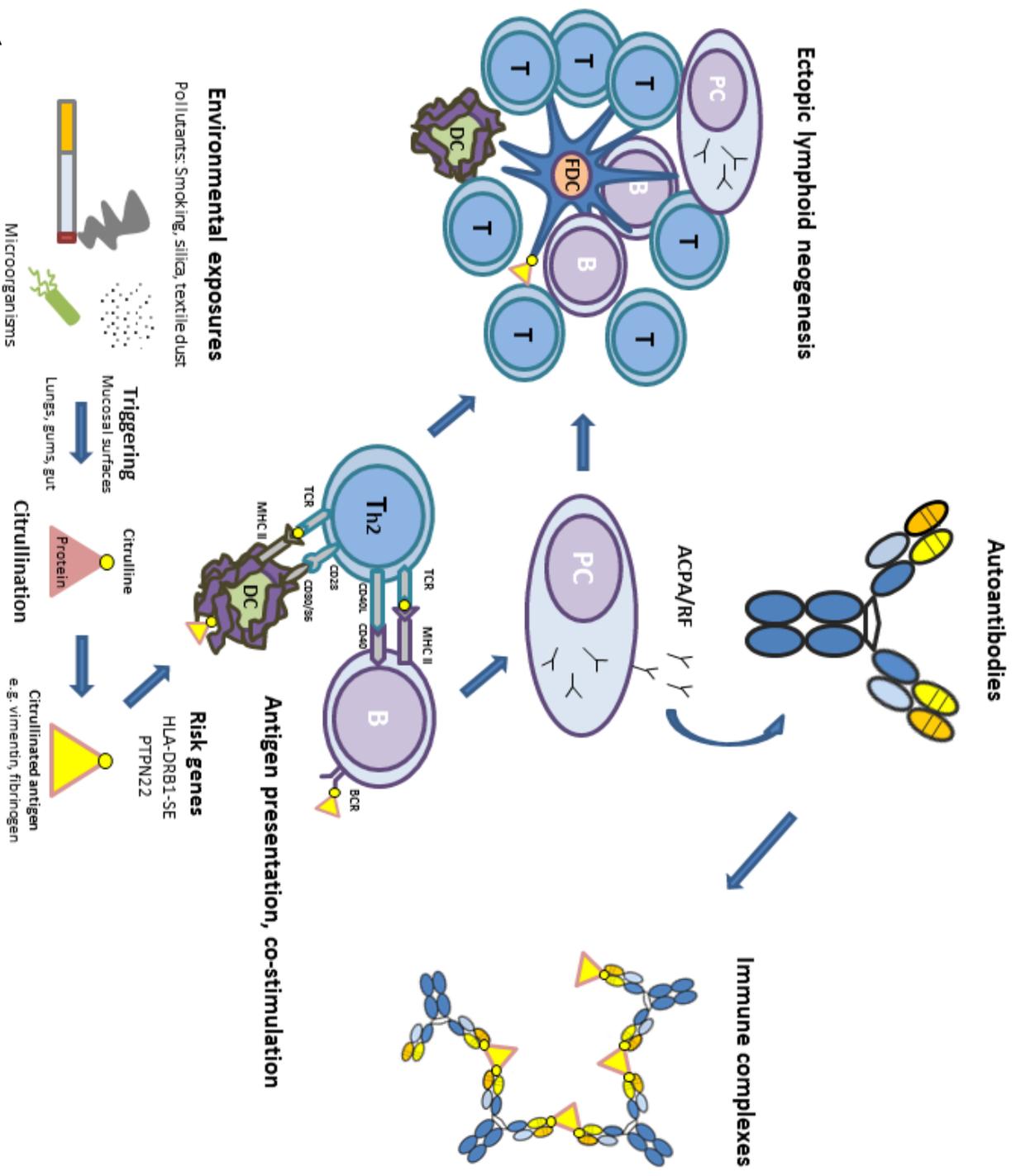


Figure 1.

### **Figure 1. Pathogenesis and promulgation of autoimmunity in rheumatoid arthritis**

In the pre-arthritis phase of rheumatoid arthritis (RA), environmental exposures in the form of pollutants (smoking) or microorganisms (bacteria), mucosal surfaces are triggered and post-translational modification can ensue with citrullination of vimentin, fibrinogen,  $\alpha$ -enolase, histones, type II collagen, and tenascin C. Risk genes associated with anti-citrullinated protein antibodies (ACPA), such as HLA-DRB1 shared epitope (SE) and PTPN22, can trigger pollutant-based exposures – set forth first through antigen presentation by professional antigen-presenting cells (dendritic cells (DC) and B cells) and co-stimulation of T cells in lymph nodes. T helper 2 (Th2) cells are then able to activate the differentiation of autoreactive B cells to produce autoantibodies such as ACPA or rheumatoid factor (RF) which can gather as immune complexes. Ectopic lymphoid structure neogenesis through resulting cytokines, chemokines, and immune cell infiltration can also be triggered and form in the sublining of synovial tissue – with follicular dendritic cells (FDC) and B cells in the core of its apparent germinal center, surrounded by T cells and later by autoantibody-secreting plasma cells (PCs). Autoimmunity in RA is then promulgated from proinflammatory cytokines such as tumor necrosis factor (TNF) and interleukin 6 (IL-6) (1, 2, 28, 37, 38, 53).

T/BCR, T/B cell receptor; MHC II, multihistocompatibility complex class II; CD, cluster of differentiation.

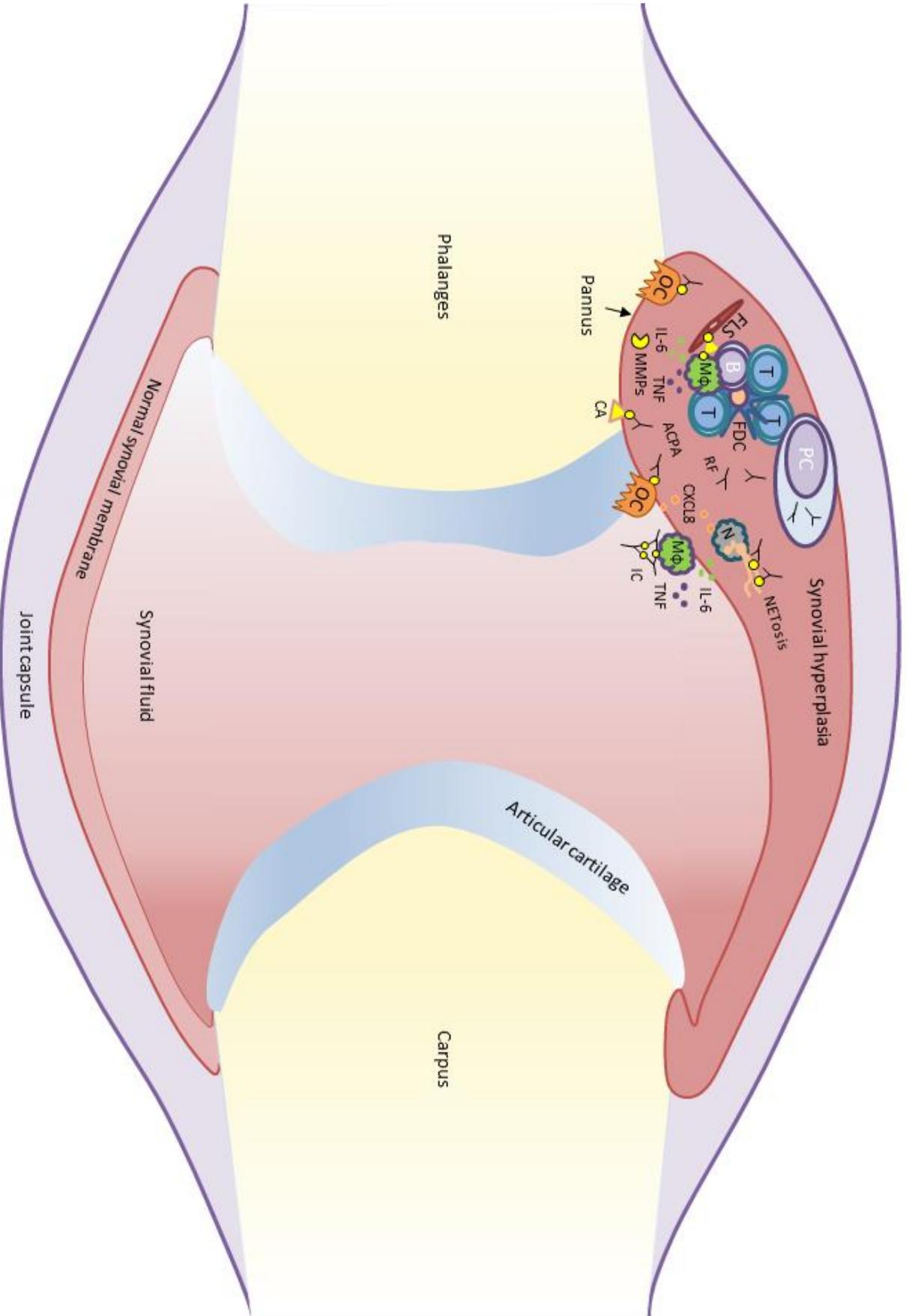
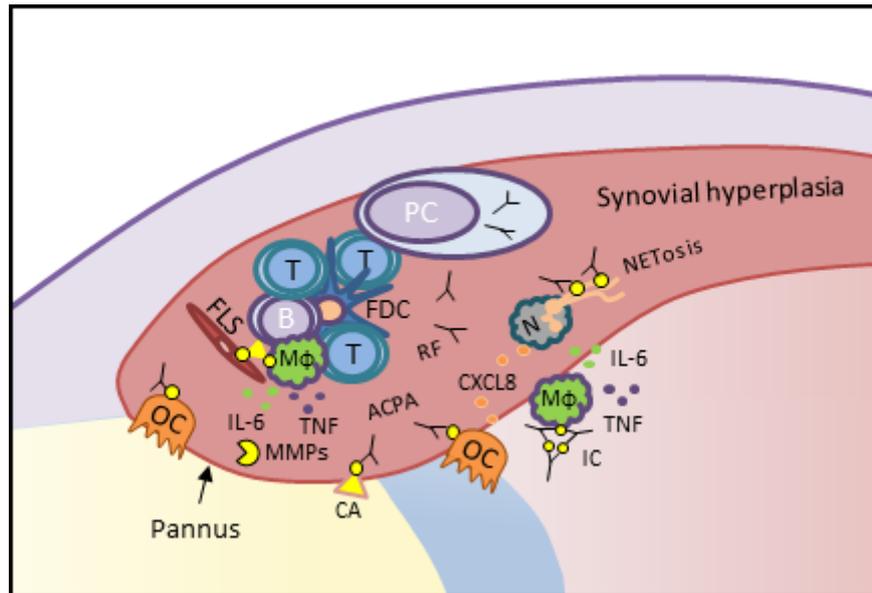


Figure 2A.



**Figure 2B. Synovitis caused by rheumatoid arthritis in the metacarpophalangeal joint**

An example of a metacarpophalangeal hand joint affected by rheumatoid arthritis (RA). The unaffected portion on the lower end of the joint (bottom of **Figure 2A**) shows normal synovial tissue and healthy bone and cartilage. The affected portion on the top of the joint (zoomed in here in **2B**) highlights ectopic lymphoid structures of B- and T cells, follicular dendritic cells (FDC), antigen-presenting cells such as macrophages (Mφ), fibroblast-like synoviocytes (FLS), and plasma cells (PCs): an environment of autoimmune inflammation trapped in a chronic loop. PC-secreted autoantibodies such as anti-citrullinated protein antibodies (ACPA) and rheumatoid factor (RF) contribute to joint inflammation by targeting antigens (ACPAs target citrullinated antigens (CA) such as vimentin and fibrinogen) and form immune complexes (IC) which can engage Mφ to secrete pro-inflammatory cytokines classic to RA: tumor necrosis factor (TNF) and interleukin 6 (IL-6). Upon activation by the auto-antigenic milieu, FLS, like Mφ, secrete IL-6 and matrix metalloproteinases (MMPs), leading to biomechanical dysfunction. FLS inflammatory activation leads to a rapid increase in synovial cell number (synovial hyperplasia), and they also introduce chemokine CXCL8, which, together with ACPA binding, induce osteoclasts (OCs) to proliferate and erode bone and cartilage, releasing additional CXCL8. In addition to triggering pain through nociceptive nerves, CXCL8 can also draw in neutrophils to eventually release neutrophil extracellular traps (NETs), with citrullinated histones captured by ACPA to promote further NETosis and resulting inflammation. At the vanguard of the invasive synovial hyperplasia is the pannus, eating away at cartilage and bone (1, 2, 28, 53, 59).

## 2.1.2 Classification criteria

### 2.1.2.1 Older rheumatoid arthritis classification criteria

Prior to the joint venture of ACR and EULAR to develop the new RA classification criteria at the turn of the current decade in 2010 (13, 14), the older 1987 revised RA criteria (**Table 1**) by the ACR (formerly known as the American Rheumatism Association, ARA) had been utilized for over 20 years in diagnosing and classifying RA – with a sensitivity from 91-94% to a specificity of 89% for correct classification in comparison to controls with other rheumatic diseases (65). Prior to this, the 1958 revised criteria of the ARA were the most widely used for a long period of time – but were eventually challenged and changed to the 1987 criteria due to the risk of inaccuracy, extensive criterion, and unnecessary invasive procedures (65, 66).

Despite having good classification accuracy for established RA, the 1987 ACR criteria were challenged by their inaccuracy in identifying individuals with RA at an early stage (67). This is due to the fact that these criteria were formed to discriminate patients with established RA from those with other rheumatic diagnoses; thus, they weren't designed to identify patients who could benefit from early intervention – which became one of the most important modern paradigms: treating patients within the aforementioned 'window of opportunity' could prevent the chronic, erosive disease state highlighted by the 1987 criteria (13, 14, 26, 27).

**Table 1 – Summary of the 1987 ACR classification criteria for RA by traditional format**

Criterion	Description
1. Morning stiffness	Morning stiffness in/around joints $\geq$ 1 hour before maximal improvement
2. Arthritis of $\geq$ 3 joint areas*	Simultaneous soft tissue swelling/fluid: PIP/MCP/wrist/elbow/knee/ankle/MTP
3. Arthritis of hand joints*	$\geq$ 1 swollen area (as defined above) in a wrist/MCP/PIP joint
4. Symmetric arthritis*	Simultaneous bilateral joint involvement of the same joint areas
5. Rheumatoid nodules	Subcutaneous nodules over bony prominences/extensor/juxtaarticular regions
6. Serum rheumatoid factor	Abnormal serum rheumatoid factor: <5% of normal control subjects
7. Radiographic changes	Posteroanterior hand/wrist radiographic changes typical of RA, which must include erosions/unequivocal bony decalcification localized in or adjacent to involved joints (osteoarthritis changes alone do not qualify)

1987 American College of Rheumatology (ACR) criteria for rheumatoid arthritis (RA), adapted from ref (65).

In order for a patient to be classified with RA, four out of the seven above criterion must be met. Criteria 1-4 must be present  $\geq$ 6 weeks. Patients with two clinical diagnoses are not excluded, and designation by classic, definite, or probable RA as in the 1958 revised criterion is not to be made.

\*14 possible joint areas (right/left): PIP, proximal interphalangeal joints; MCP, metacarpophalangeal joints, wrists, elbows, knees, ankles, and MTP, metatarsophalangeal joints. Bilateral involvement without absolute symmetry is acceptable for PIP/MCP/MTP.

### 2.1.2.2 *New rheumatoid arthritis classification criteria*

The new 2010 classification criteria (**Table 2**) were prepared by a joint ACR/EULAR working group with three phases of development, the first two being: 1) identifying variables to predict the decision to give methotrexate (MTX) in an early undifferentiated arthritis population (68), and 2) using real-life patient cases to address rheumatologist-based decisions on the contribution of each variable in influencing the probability of developing RA (69). The resulting four criteria formed the basis of the final criteria set (phase three), which were published both in the EULAR (13) and ACR (14) flagship journals. Here, definite RA according to these new criteria (score  $\geq 6$ ) had proportions of 97%, 91%, and 87% in three different cohorts of patients treated with MTX within a year from onset of symptoms.

Studies have since confirmed that the new 2010 ACR/EULAR criteria have acceptable discriminative ability in classifying more patients with early RA, who may have otherwise been classified as having undifferentiated disease (70-74), although there are also limitations as the new criteria may identify less autoantibody positive patients and more with monoarthritis (71), and they could possibly overdiagnose very early RA (75). Nonetheless, it does appear that the new 2010 criteria are superior in discriminative capacity when compared to the older 1987 criteria (76). They have a good overall sensitivity performance (0.82), although the overall lower specificity (0.61) needs to be taken into consideration for potential improvements in the future (77). A EULAR task force has thus far added an erosive disease definition for use in the 2010 ACR/EULAR criteria for patients who had radiographic erosions in at least three separate joints but did not otherwise meet the 2010 criteria (score of  $< 6$  points) (78).

**Table 2 – Summary of the 2010 ACR/EULAR classification criteria for RA**

Target population, who should be tested?	
<ol style="list-style-type: none"> <li>1. Patients who have <math>\geq 1</math> joint with definite clinical synovitis (swelling)*</li> <li>2. Patients with synovitis not better explained by another disease*</li> </ol>	
Classification criteria for RA: a score-based algorithm of the sum of categories A-D, where a score $\geq 6/10$ is needed for classification of a patient as having definite RA**	
A. Joint involvement ***	Score
1 large joint ***	0
2-10 large joints	1
1-3 small joints (with/without involvement of large joints) †	2
4-10 small joints (with/without involvement of large joints)	3
>10 joints (at least one small joint) ††	5
B. Serology ( $\geq 1$ test result needed for classification) ‡	
Negative rheumatoid factor (RF) <i>and</i> anti-citrullinated protein antibodies (ACPA)	0
Low-positive RF <i>or</i> ACPA	2
High-positive RF <i>or</i> ACPA	3
C. Acute-phase reactants ( $\geq 1$ test needed for classification)	
Normal C-reactive protein (CRP) <i>and</i> erythrocyte sedimentation rate (ESR)	0
Abnormal CRP <i>and</i> ESR	1
D. Duration of symptoms ‡‡	
<6 weeks	0
$\geq 6$ weeks	1

2010 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) criteria for rheumatoid arthritis (RA), adapted from ref. (13, 14).

\* Aimed at classifying newly-onset patients. Patients with RA-typical erosive disease, or long-standing disease with a history compatible with prior 2010 criteria fulfillment should be classified with RA. Other diseases: expert rheumatologist should be consulted if unclear. \*\* Status reassessment possible over time if not fulfilling 6/10 criteria. \*\*\* Any swollen/tender joint which may be confirmed by synovitis imaging evidence (distal interphalangeal (DIP), first carpometacarpal-, and first metatarsophalangeal (MTP) joints are excluded). Joint distribution categories classified by location and number of involved joints; highest category placement based on joint involvement pattern. Large joints: shoulders/elbows/hips/knees/ankles. † Small joints: Metacarpophalangeal-, proximal interphalangeal (PIP), MTP II-V, and thumb interphalangeal joints and wrists. †† Any combination of large- and additional small joints. ‡ Negative:  $\leq$  upper limit of normal (ULN) for lab/assay; low-positive:  $>ULN$  but  $\leq 3xULN$ ; high-positive:  $>3xULN$ . If only RF is available, a positive should be scored as low-positive for RF. ‡‡ Patient-reported symptom duration of signs/symptoms of synovitis.

## 2.1.3 Treatment

### 2.1.3.1 *Synthetic, biological, and novel disease-modifying antirheumatic agents*

Traditionally, the treatment for RA involves the combination of conventional synthetic DMARDs – MTX being the gold standard – with glucocorticoids and NSAIDs. In cases of non-response, more intensive treatment with conventional triple therapy (TT: MTX + sulfasalazine (SSZ) + hydroxychloroquine (HCQ)) can be administered. Otherwise, expensive biologics that typically come in the form of humanized or chimeric monoclonal antibodies may be necessary. Biologics are indicated in particular for patients who do not respond through the conventional approach, and they include anti-TNF agents (infliximab, etanercept, certolizumab-pegol, golimumab, and adalimumab) and agents with other modes of action (IL-1 receptor antagonist, anakinra; T cell costimulation inhibitor, abatacept; anti-CD20 (B cell) agent, rituximab; and IL-6 receptor inhibitor, tocilizumab) (79-82).

More recently, the first targeted synthetic DMARD for RA (small-molecule inhibitor) blocking the intracellular Janus kinase/signal transducer and activator of transcription (JAK-STAT) pathway via JAK1 and 3, tofacitinib, was approved by the United States Food and Drug Administration (FDA) for moderate to severe RA as of November, 2012, and has been utilized first in the United States, Russia, and Japan (83). Due to the demonstrated efficacy, tofacitinib and the newer JAK1-2 inhibitor, baricitinib, are included as possible treatment options in the latest EULAR treatment guidelines (84). The European Medicines Agency (EMA) had initially rejected tofacitinib's approval due to safety concerns, but has now in January 2017 forwarded its recommendation to the European Commission; two months after recommending baricitinib.

Additionally, it is now recognized that biosimilars – less expensive, near-identical copies of the original biologic product upon patent expiration – are as effective as their originators; and that targeting the IL-6 pathway or ligand through novel biologics sarilumab, clazakizumab, sirukumab; or granulocyte-monocyte colony stimulating factor receptor  $\alpha$  inhibition with mavrilimumab, can provide potential benefits in RA (82, 84).

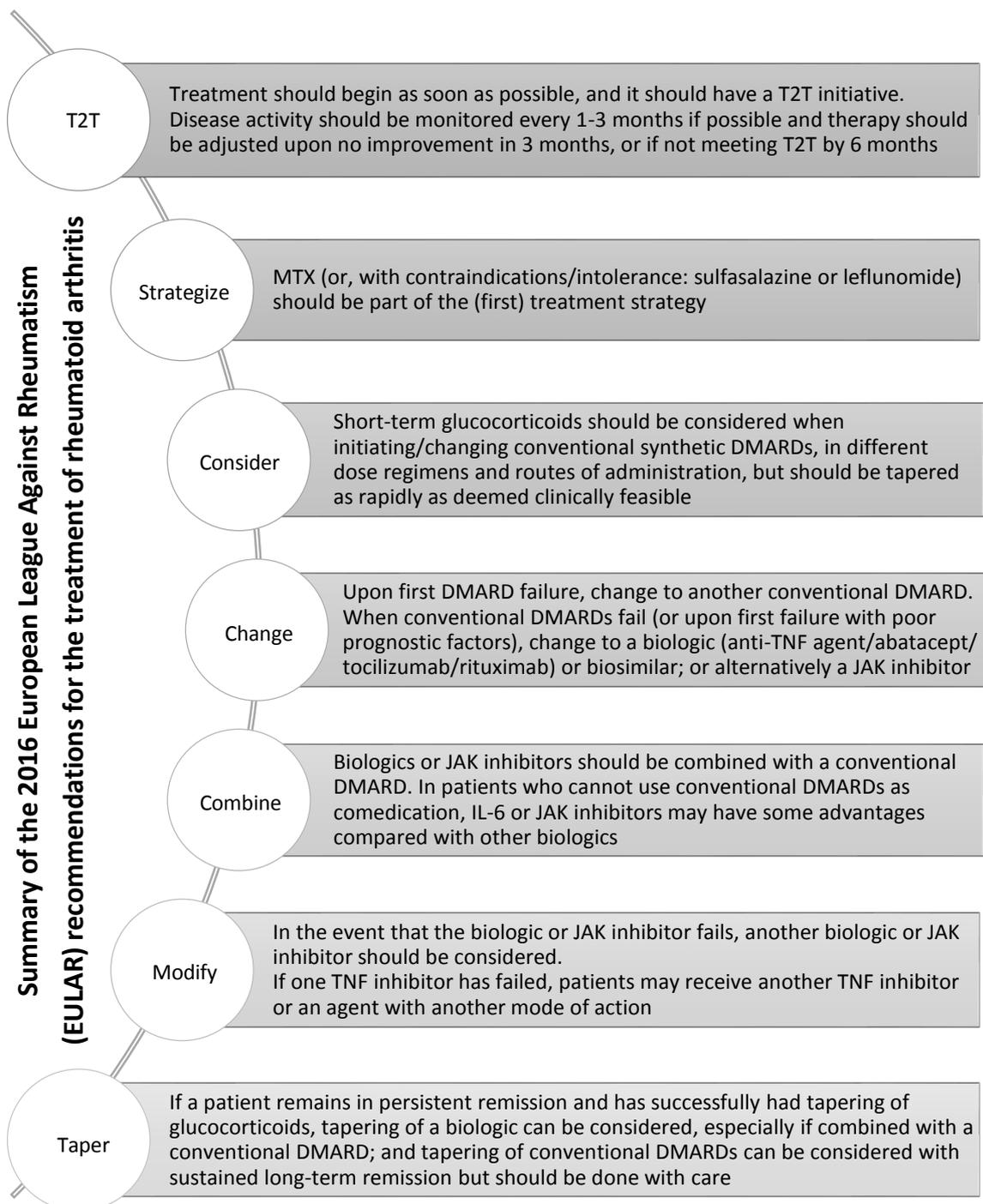
### 2.1.3.2 Rheumatoid arthritis treatment paradigms and recommendations

It has been demonstrated that, just as by treating within a ‘window of opportunity,’ a more effective strategy than routine care is to plan treatment goals with patients by aiming to achieve remission or low disease activity – a concept called ‘treating to target’ or ‘treat-to-target’ (T2T) (85, 86), which has been gaining ample systematic evidence pointing to its superiority to routine care (87). The initial 2010 T2T strategy formed by a panel of rheumatology experts resulted in 10 recommendations, with the ultimate goal of remission (or low disease activity in patients with long-standing disease); to be achieved by following-up with patients with active disease every one to three months, and followed by appropriate therapeutic management to reach the ultimate goal within three- to a maximum of six months (85). It has since been updated in 2014, with the same amount of recommendations but with the order changed in addition to partial adaptations as deemed necessary (88).

The four overarching principles in 2014 T2T for RA (88) are as follows:

- ❖ **A.** Treatment must be based on shared patient and rheumatologist decision-making.
- ❖ **B.** The primary goal should be to maximize long-term health-related quality of life through symptom control, structural damage prevention, and normalization of function and participation in social and work-related activities.
- ❖ **C.** Abrogation of inflammation is the most important way to achieve these goals.
- ❖ **D.** T2T by measuring disease activity and adjusting therapy optimizes outcomes.

For the management of RA, a merged summary of the 2016 EULAR treatment recommendations (84) can be found in **Figure 3**. These were an update of the 2013 recommendations (81, 83), further informed by the efficacy of older and new biologics, including biosimilars; more data on novel synthetic JAK inhibitors (tofacitinib, baricitinib); and information on switching, spacing, and dose reduction strategies (82).



**Figure 3.** Concise summary of the EULAR 2016 updated recommendations for the management of rheumatoid arthritis (RA) with synthetic or biological disease-modifying antirheumatic agents (DMARDs), adapted from ref. (84)

T2T, Treat-to-target (aim: low disease activity/remission); MTX, methotrexate; TNF, tumor necrosis factor; JAK, Janus kinase; IL-6, interleukin 6.

## **2.2 IMAGING**

As patients who may be in clinical remission by the 28-joint count disease activity score (DAS28) (89) are still able to develop health-related functional disability (12), so too can these patients also develop bone erosions (termed radiographic progression) according to the Sharp-van der Heijde (SHS) scoring method, as was shown in the group of patients who responded to (and remained on) MTX monotherapy in the randomized care-based Swedish pharmacotherapy (SWEFOT) trial (90). This stresses the importance of imaging as a necessary gold standard for verifying whether or not a patient is indeed in remission.

### **2.2.1 Conventional radiography**

X-ray analysis has for a long time served as the conventional method of imaging-based diagnosis and prognosis in rheumatology. Although X-rays do not show signs of ongoing inflammation and have limitations in detecting early disease, they reveal erosions and joint-space narrowing; and also correlate with physical function – a highly important indicator of long-term outcomes (91-93). The most widely used conventional radiographic scoring method in rheumatology is SHS, which has an erosive component where an analyst scores 32 joints in the hands and wrists and 12 in the feet; as well as a joint-space narrowing component, which scores 30 joints in the hands and wrists and 12 in the feet. The total score is the summed score of both components, the maximum being 448 (94, 95).

### **2.2.2 Musculoskeletal ultrasound and other novel instruments**

Although conventional radiography is still recommended over clinical criteria alone as one of the imaging modalities to be used when there is an uncertainty of diagnosis, musculoskeletal ultrasound and magnetic resonance imaging have instead rather recently been accepted as new gold standards of imaging in rheumatology by EULAR – in the sense that they are superior to conventional radiography in detecting inflammation and disease progression (96). Ultrasound, for example, has been shown to improve diagnostic certainty of an auto-inflammatory condition through the testing of a probabilistic Bayesian analysis (97). Finally, even more novel instruments such as fluorescence optical imaging – which utilizes an intravenous fluorescent dye that emits visible light after excitation by light at short wavelengths – was shown through one of

our studies to have good agreement with ultrasound and is capable of detecting clinically non-apparent synovitis (98). Beyond the scope of this thesis, we are further evaluating this method in how it may help with diagnostic certainty through a probabilistic Bayesian analysis.

### 2.2.3 A novel approach with conventional radiography

If novel and expensive instruments would not be available, however, there is a method by which radiographic progression may be predicted by using conventional radiography and symptom duration before diagnosis, whereby the simulation of bone erosion or joint-space narrowing over time (as if patients were not on treatment) is created. Although not an exact representation of a true control group, as radiographic progression is not entirely linear, the prediction offered by this method (Predicted vs. Observed Progression in early RA, POPeRA) has first been shown by Wick et al. as being most similar to the outcomes of non-responders to treatment (99). This was later demonstrated in **Paper I**, where the POPeRA method was applied to the randomized SWEFOT trial (100). Here, the original SWEFOT findings – that radiographic efficacy of anti-TNF treatment over TT were significantly apparent after two years (101, 102) – were also tested to determine if potential radiographic progression could be *prevented* more among anti-TNF than TT (100). **Paper II** utilized POPeRA to potentially confirm or add new insights into the original findings from the randomized Finnish RA Combination therapy (FIN-RACo) and New Finnish RA combination therapy (NEO-RACo) trials (103).

The first publication that utilized the POPeRA method by Wick et al. compared MTX with SSZ and with auranofin, a now out-of-phase DMARD also known as oral gold. Here, after one year, patients on either MTX or SSZ had significant reductions from predicted; however, the auranofin group had similar progression to predicted – as did another group deemed a control due to a lack of response to several medications (99).

Naturally, patients with RA are to be treated immediately upon diagnosis. Therefore, the simulation that POPeRA provides – which is an approximation of how patients would progress as if not on treatment – is a vital method for validating the relative radiographic efficacy of various DMARDs.

## 2.3 BIOMARKERS

For those who have RA, despite sharing the same diagnosis, every patient represents a case study due to their unique profile of genetic background and environmental exposures. Biomarkers are unique indicators (typically proteins) which have associative or predictive roles in inflammation, aspects of disease activity, or prognosis. The biomarkers RF and ACPA (e.g. against fibrinogen, vimentin, and  $\alpha$ -enolase), together with acute-phase reactants C-reactive protein (CRP) and/or erythrocyte sedimentation rate (ESR), are the only major associative biomarkers to inflammation and disease activity utilized as part of standard clinical practice in rheumatology and RA. There exist, however, a multitude of other biomarkers that have entered the knowledgebase in the field, such as anti-collagen II, anti-binding Ig protein, anti-peptidylarginine deiminases, and anti-histones (28, 46).

Novel, standalone biomarkers such as cellular signaling protein 14-3-3 $\eta$ , or the inhibitor of apoptosis, *survivin*, have been shown to be associated with radiographic progression and worse outcome (104-107). As elevated *survivin* can also signal the onset of RA (108), **Paper III** was conducted to assess the clinical value of elevated *survivin* levels and how it may potentially predict therapy responses in early RA (109).

An intriguing concept is the combination of several pro-inflammatory markers, acute-phase reactants, and matrix metalloproteinases (MMPs) combined together to form a multi-biomarker disease activity (MBDA) score, which – with a high score at baseline – has been able to predict worse radiographic progression over one year (110, 111); and, when combined with ACPA, can predict relapses in over 80% of patients (112). Finally, it is worth mentioning that randomized controlled trials in RA often exclude patients with low CRP ( $\leq 10$  mg/L). Patients with a high MBDA score despite low CRP were found to have outcomes comparable to patients with a high CRP; thus, recruitment in clinical trials can be substantially enhanced with the inclusion of this metric (113).

Despite a bold undertaking, the results thus far indicate that the investigation of biomarkers may point to successful prospects for healthcare, where personalized medicine could one day become a reality to achieve.

## **2.4 RISK FACTORS: ENVIRONMENT AND LIFESTYLE**

### **2.4.1 Known environmental risk factors in rheumatoid arthritis**

As introduced earlier, smoking is a strong trigger for RA onset (39-45), particularly when combined with genetic factors (HLA-DRB1-SE, PTPN22) (28, 32, 37-39, 46). Smoking has also been shown to be a strong independent predictor of radiographic progression in early RA in the SWEFOT trial (114), in addition to being a risk factor for MTX failure (115). Silica (47, 48) and textile dust (49) as potentially hazardous occupational exposures for construction- and textile workers – and, possibly, invasive microorganisms (50-52) – are additional risk factors according to most studies for the development of RA that can trigger autoimmunity at mucosal sites (28, 38).

### **2.4.2 Lifestyle risk factors in rheumatoid arthritis**

Poor diet/nutrition such as elevated salt intake (116) may be a potential RA risk factor, particularly at a younger age (117, 118). Fortunately, a relatively recent popular field of investigation in RA is diet; the consumption of fish-derived omega-3 fatty acids in particular. A large meta-analysis in 2014 found a 20-24% reduced risk of RA with one to three servings of fish per week when compared to never-consumption, although it wasn't statistically significant (119). Nonetheless, a large prospective cohort study of women the same year found a significant 35% risk reduction of developing RA if consuming fish >0.21 g/day; and by an even greater amount (52% reduced risk) if this amount of consumption was maintained for 10 years (120). Omega-3 also has documented modest effects on reducing pain and inflammation in RA (121). More recently, it has been shown to be associated with refractory pain suppression (122); and high-dose fish oil supplements might even increase remission rates (123). Interestingly, it has been recently implicated that omega-3 may be of particular benefit for preventing ACPA-positive RA and protecting RA-susceptible individuals with the risk gene, HLA-DRB1-SE (124, 125).

Poor diet – together with the increased prevalence of sedentary behavior in RA (126) – brings forth the question of one of its consequences: an elevated body mass index (BMI). BMI, obtained by dividing weight by the squared height, is divided into four categories: obese (BMI $\geq$ 30 kg/m<sup>2</sup>), overweight (25-29.9), normal (18.5-24.9), and underweight (<18.5), respectively. Obesity and overweight have been recognized as an epidemic in modernized and

urbanizing countries alike (127), and their link to metabolic syndrome and diabetes are concerns in and of themselves. An elevated BMI (overweight, and obesity in particular) has been identified in a meta-analysis of eleven studies as a potential risk factors for developing RA (128), and the risk may be higher among seropositive smokers (129). A large population-based study – corrected for potential confounders including smoking – indicated a reduced risk of developing RA among overweight or obese men, but not among women (130). Prior to this, an even larger prospective observational cohort of female registered nurses found that being overweight or obese contributed to the risk of developing RA, either seropositive or seronegative (131).

An elevated BMI has previously been associated with persistent disease activity/non-remission, functional impairment, and pain; as well as a lower odds of a good treatment response in early RA (majority on MTX) (132). Similar findings, with obesity in particular, have been observed in other observational studies, mostly in established RA (reviewed in: 133, 134). On the other hand, an elevated BMI has also been shown to be associated with less radiographic damage (133, 135). **Paper IV** was thus conducted with data from the SWEFOT trial to determine the potential clinical and predictive role of BMI (obesity) in the randomized setting of early RA.

## 2.5 INTEGRATIVE AND COMPLEMENTARY MEDICINE

### 2.5.1 What is it?

Due to the global popularity of non-allopathic complementary and alternative medicine (CAM) and traditional medicine (TM), the National Institutes of Health of the United States were pushed to set up a tailored division of research: the National Center for Complementary and Integrative Health (NCCIH) (formerly NCCAM), which today funds CAM- or TM-specific studies. Despite large investments in North America, Asia, and Australia for CAM or TM research – and despite the European Commission taking interest in the matter – the majority of European countries (including Sweden) are lagging behind in research of complementary medicine primarily due to scarce funding (136). In research, the term CAM is a catchy acronym that is still used but could be considered somewhat out of phase in the sense that the ‘alternative’ aspect refers to treatments taken instead of conventional allopathic medicine (137), which is unusual in the West and would not be recommendable, especially pertaining to autoimmune conditions where tight disease control is required. Many modern approaches outside of allopathic medicine with a sound scientific basis such as manual therapy – despite having roots at least as far back as the established Father of Medicine, Hippocrates (138) – could be regarded by some as falling into the ‘complementary’ category, but it is perhaps even less fitting for other practices intricately tied to culture that originate thousands of years earlier than allopathic medicine. According to the World Health Organization (WHO), TM ranges from ancient Chinese or Indian health-related practices such as tai chi, qigong, acupuncture, meditation, or yoga; to medication-based approaches such as herbal Ayurveda, Arabic unani, and other indigenous medicines (139).

Complementary medicine according to NCCIH can be divided into ‘natural products’ (vitamins and herbal supplements) and ‘mind and body practices’ (manual mobilization or manipulation, massage, therapeutic touch, and relaxation therapy; but also includes TM practices such as acupuncture and yoga). Importantly, NCCIH stresses the importance of ‘integrative medicine,’ which is the practice of merging allopathic with non-allopathic medicine cooperatively (137). In a unified approach, WHO has decided upon the acronym, traditional and complementary medicine (T&CM), to refer to the very broad spectrum of non-allopathic methods (140). In the context of this thesis, it will generally be referred to as integrative and complementary medicine, or simply, integrative medicine.

## 2.5.2 Prevalence of integrative and complementary medicine

Results from a nationally representative survey in the United States performed in 1990 and 1997 found that there was a substantial increase in the use of at least one integrative and complementary medicine within the previous year, from 33.8% to 42.1%; that seeking out a complementary practitioner also increased substantially (36.3% to 46.3%); and that, despite the increase in complementary medicine use, the low disclosure rate to doctors and large proportion of patients paying entirely out of pocket for complementary care remained similar over time (39.8% vs. 38.5%; 64.0% vs. 58.3%, respectively) (141). The national survey also revealed that expenditures on complementary care alone equated to \$30 billion per year – which, at that time, even exceeded the country's out-of-pocket payments for allopathic treatments prescribed by primary care physicians (141, 142). The prevalence of complementary medicine use and out-of-pocket payments has remained stable since (143).

Results from WHO's 2002-2005 TM strategy estimate the European national use of integrative and T&CM at least once per lifetime as being 75% (France), 70% (Canada), 48% (Australia), and 38% (Belgium) (139). China and India have a very prevalent TM integration in health care. Chinese TM, unlike in the West, is integrated in hospitals and exclusive TM-based pharmacies exist. No less than 40% of all healthcare services delivered in China is through Chinese TM alone (139). In India, the use of Indian TM such as Ayurveda or yoga for primary health care equated to as much as 70% (139). Largely as a result of the 2002-2005 initiative, the number of WHO Member States that have implemented either T&CM policies, or regulated herbal medicine, have greatly increased from 25 and 65 in 1999, respectively; to 69 and 119 in 2012, respectively (140). Despite the large prevalence of integrative and complementary medicine globally and its noticeable popularity, finding funding for well-controlled studies is extremely challenging. Cochrane systematic reviews in turn report the difficulties of appropriate methodology for its research and find only a tendency towards a possibility of positive effects for several non-allopathic medicines, including acupuncture, tai chi, yoga, manual therapy, massage, and others (144).

### 2.5.3 Integrative care of autoimmune conditions

Studies powered and funded adequately that may be able to give us insight on the prevalence of the use of integrative and complementary medicine and its effectiveness in autoimmune diseases are scarce; and are, at best, only a relatively new phenomenon. Cochrane systematic reviews have thus far been able to summarize that, for RA, balneotherapy (spa therapy) does not have enough evidence to show effectiveness (145); physical activity and psychosocial interactions have a beneficial effect on self-reported fatigue (146); electroacupuncture is capable of reducing knee pain but more evidence is warranted due to methodological flaws (147); tai chi has significantly beneficial effects on the lower extremities of motion (148); and oils containing gamma linolenic acid have moderate evidence of symptom relief, while *Tripterygium wilfordii* Hook F (TwHF) (thunder god vine) – a Chinese TM herbal therapy which has already been integrated in Chinese rheumatology practice for decades – provides symptom relief but could lead to mild to moderate adverse events if administered orally (149). Well after these systematic reviews were conducted, a carefully-constructed randomized controlled trial was published in the *New England Journal of Medicine* and demonstrated that tai chi had major benefits for subjects with fibromyalgia (150), a complex pain syndrome that is more prevalent among patients with RA and other rheumatic conditions. Additionally, a relatively recent, open-label randomized controlled trial demonstrated that TwHF has comparable efficacy to MTX against disease activity in active RA, and is also statistically superior to MTX monotherapy when both are combined; with a relatively good safety profile (151).

In Sweden, one study has thus far mapped out the use of integrative and complementary medicine among outpatients with inflammatory rheumatic conditions (152). The prevalence was 65%; and patients who sought out complementary care had more often poorer health, indicative of non-response to conventional therapy. Stress has been shown to predict fatigue and pain (153); which may influence patients to seek out complementary care for qualitative purposes that allopathic medicine alone might otherwise not be able to address.

The effects of integrative or complementary medicine can also be measured quantitatively. We first performed a pilot study involving patients with RA who were non-responders to antirheumatic therapy, to determine if a manual therapy treatment method that is normally used for pain relief in osteoarthritis (OA) – manual mobilization of the extremities, founded upon conventional medical principles of anatomy, physiology, physiotherapy, and manual therapy; and developed by the Norwegian physiotherapist Freddy Kaltenborn (154-156) – could also be effective in RA. In three treatment sessions within a week, 20 metacarpophalangeal (MCP) hand

joints (MCP II-V of one hand) were treated with repeated Kaltenborn within-the-slack Grade I-II manual mobilizations – which sufficiently provides movements of traction to counteract compressive joint forces but avoids soft tissue stretching (154, 156) – both for safety and feasibility, as well as monitoring a potential dose-based response over time. After one week, pain and tenderness decreased significantly, and inflammation as assessed via musculoskeletal Doppler ultrasound decreased by a mean of 21% from baseline to post-final treatment (157, 158). These results motivated the creation of a larger randomized crossover pilot study (**Paper V**), which also included a hand OA comparator group and a longer follow-up period, in order to investigate the potential effectiveness of integrative medicine in more novel manners, not only qualitatively, but quantitatively.

### 3 OVERARCHING AIM

The overall aim of this thesis was to evaluate personalized integrative medicine, by:

- ❖ Reviewing the state of established knowledge pertaining to RA, including its pathogenesis, clinical course, classification, and treatment paradigms.
- ❖ Identifying imaging modalities, biomarkers, environmental and lifestyle risk factors, and integrative medicine of potential importance for RA.
- ❖ Investigating the disease course of RA through *predicting* – with the simulative capacity of POPeRA and radiography (*imaging*); the theranostic capability of serum survivin (*biomarkers*); and the clinical importance of BMI and lifestyle factors (*risk factors*) in disease outcome – as well as *monitoring* RA outcome with the applied approach of integrating manual therapy with the standard of care (*integrative medicine*).

## 3.1 STUDY-SPECIFIC AIMS

### 3.1.1 Paper I

The first paper sought to apply the POPeRA method, previously called ‘Estimated prediagnosis radiological progression’ (99), to a large randomized clinical trial in early RA (SWEFOT) to verify its original findings – that anti-TNF infliximab therapy was radiographically superior to TT over two years (101, 102). The primary outcome of POPeRA, however, was to specifically determine if anti-TNF could *prevent* more potential radiographic progression than TT, as the radiographic progression in this case is a simulation of how patients with early RA would progress without DMARDs. Thereby, the comparison to actual radiographic findings can be done, and % reduction from predicted can be compared across treatments.

### 3.1.2 Paper II

The second paper sought to apply the POPeRA method and verify or add insight to the findings of two large randomized clinical trials in early RA (FIN-RACo and NEO-RACo) by determining how various treatments can prevent radiographic progression as the primary outcome. FIN-RACo originally found that intensive DMARD combination therapy (TT with glucocorticoids) was radiographically superior to monotherapy (primarily SSZ or MTX) over two and five years (159, 160), and NEO-RACo originally found that intensive combination therapy + six-month induction of anti-TNF infliximab was only superior to intensive combination therapy + placebo at two years, but not at five years (161, 162).

### 3.1.3 Paper III

The proto-oncogene *survivin* has previously been shown to be elevated in pre-RA individuals (108), and has in RA been associated with erosive disease, RF/ACPA, and smoking (105-107). Due to the lack of literature on how *survivin* might affect therapeutic clinical choices, the aim of the third paper was to determine the clinically-predictive role of *survivin* as a potential theranostic (selective targeted therapy) biomarker. Primary outcomes included assessment of the clinical ‘core set’ (DAS28, HAQ, and the 0-100 mm visual analog scale for pain (VAS-pain) and global health (VAS-global)) over 2 years.

### **3.1.4 Paper IV**

Due to the previously observed discordance between worse clinical outcomes, pain, and function, but better radiographic outcomes related to elevated BMI (obesity and/or overweight), particularly in established RA (132-135, 163, 164), we sought to investigate the potentially predictive role of obesity with clinical and radiographic outcomes in the randomized SWEFOT trial in the early RA setting (101, 102). Outcomes were the clinical ‘core set’; EULAR non-remission ( $DAS \geq 2.6$ ), EULAR good response, and radiographic progression ( $SHS \geq 1$ ;  $SHS \geq 5$ ) over two years.

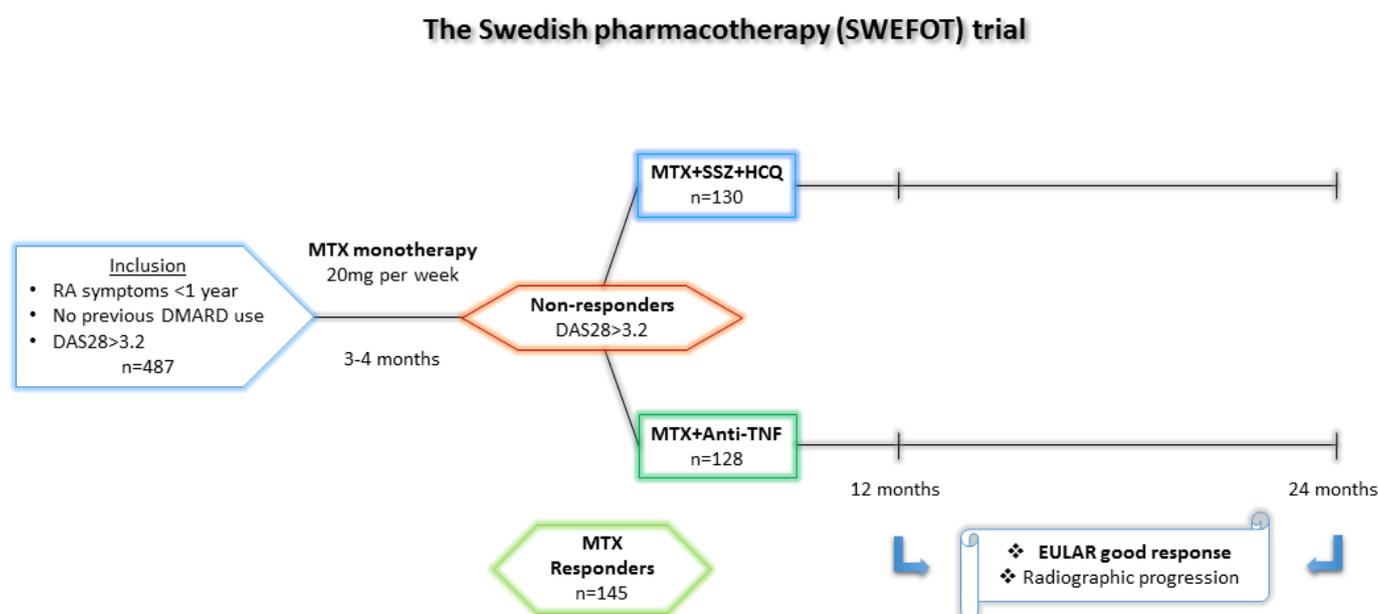
### **3.1.5 Paper V**

Despite major advancements in the care of patients with RA (165), pain remains a persistent concern – even when patients respond well to DMARDs (166). Thus, the initiative to find a complementary method of care to potentially integrate into the practice of rheumatology has been in need. After the promising results of an initial pilot study that achieved successful short-term control of pain and even inflammation in RA (157, 158), the aim of the final paper was to take a longer-term prospective experimental approach in the care of patients with RA in a larger randomized crossover pilot study to assess the clinical feasibility, safety and effectiveness of Kaltenborn manual mobilization in RA compared to a clinical comparator group with hand OA. The primary outcomes of the study was assessment of the hands with VAS-pain, physician’s tender/swollen joint count; as well as quantitative (%) Doppler signal, synovial fluid, and MCP joint space by musculoskeletal ultrasound.

## 4 MATERIALS AND METHODS

### 4.1 THE SWEDISH PHARMACOTHERAPY TRIAL

**Papers I, III, and IV** are based on the patient populations with early RA from the SWEFOT trial. SWEFOT was a two-year multicenter open-label randomized clinical trial in early RA conducted in Sweden from 2002-2005 and was one of the first to apply the modern T2T approach (85, 88) in advance, aiming for low disease activity or remission (101, 102). All patients were first allocated to MTX monotherapy for three to four months, and, of the remaining 403 patients, 258 did not respond to MTX ( $\text{DAS28} \leq 3.2$ ) and were randomized either to treatment intensification with TT or with add-on of anti-TNF infliximab. The primary endpoint was a EULAR good response (present  $\text{DAS28} \leq 3.2$  and a  $\Delta\text{DAS28}$  improvement  $>1.2$ ) at 12 and 24 months of follow-up, where radiographic damage by SHS was also assessed (**Figure 4**).



**Figure 4. Schematic of the randomized clinical SWEFOT trial**

RA, rheumatoid arthritis; DMARD, disease-modifying antirheumatic drug; DAS28, disease activity score; MTX, methotrexate; SSZ, sulfasalazine; HCQ, hydroxychloroquine; anti-TNF, anti-tumor necrosis factor; EULAR, European League Against Rheumatism. See ref. (101).

## 4.2 THE FIN-RACO AND NEO-RACO TRIALS

**Paper II** included patients with early RA from the FIN-RACo (159, 160) and NEO-RACo (161, 162) trials, including data from the primary two-year endpoints as well as the more recent five-year follow-ups. Like SWEFOT, both FIN-RACo and NEO-RACo were already built on a T2T approach and were an inspiration for T2T when it became official as of 2010 (85).

FIN-RACo was a two-year multicenter Finnish randomized early RA clinical trial conducted from 1993-1995 (159). Of 195 patients, 97 were randomized to intensive combination TT (MTX+SSZ+HCQ with glucocorticoids) and 98 to DMARD monotherapy (primarily SSZ or MTX, with or without glucocorticoids). A five-year follow-up was also carried out (160), and the primary outcomes were preliminary ACR strict remission (167) and radiographic damage by the Larsen score (168). NEO-RACo was another two-year multicenter Finnish randomized early RA clinical trial, conducted from 2003-2005 (161). Of 99 patients, 50 were randomized to receive intensive combination TT with a six-month induction of anti-TNF infliximab, versus 49 who were randomized to intensive combination TT with a six-month induction of placebo. A five-year follow-up was also carried out (162) and the primary outcomes were the same as in FIN-RACo, except that SHS was also available for radiographic damage scoring.

## 4.3 THE POPERA METHOD IN EARLY RHEUMATOID ARTHRITIS

The POPeRA method (99) was applied in **Papers I-II**, where 343 patients from SWEFOT; 144 from FIN-RACO; and 90 from NEO-RACO had available radiographic scores at all time points. The radiographic score upon early RA diagnosis at baseline (SHS for SWEFOT and NEO-RACO, and Larsen for FIN-RACO) was first divided by the patient-reported symptom duration in months before baseline to obtain an inferred progression rate (IPR) (see below). The radiographic score at symptom onset is assumed to be zero (thus the x-intercept). Following this calculation, each time point in months was multiplied by the IPR, and then added to the baseline radiographic score. Altogether, this formula simulates linear progression as if the patient were not treated – thus generating a true control reference value that can be compared to observed progression. A detailed figure of the POPeRA technique can be found in **Paper I** (100). To include as many patients as possible into the model (some started with a score of zero that was maintained throughout the follow-ups), all radiographic scores were imputed with an increase of one unit.

Formula for calculating the IPR, required for calculating the predicted progression score:

$$IPR = \frac{\text{Radiographic score [baseline SHS or Larsen]}}{\text{Symptom duration [months before baseline]}}$$

Formula for calculating predicted radiographic progression (PRP) in SWEFOT (**Paper I**):

$$\begin{aligned} \text{PRP score [12; 24 months]} \\ = IPR \times 12 + \text{baseline SHS}; IPR \times 24 + \text{baseline SHS} \end{aligned}$$

Formula for calculating PRP in FIN-RACo (Larsen) and NEO-RACo (SHS) (**Paper II**):

$$\begin{aligned} \text{PRP score [24; 60 months]} \\ = IPR \times 24 + \text{baseline score}; IPR \times 60 + \text{baseline score} \end{aligned}$$

#### 4.4 SERUM SAMPLES, ENZYME-LINKED IMMUNOSORBENT ASSAY

For **Paper III**, available stored frozen serum samples at -80 °C from baseline – and from 3, 12, and 24 months – from 302 early RA SWEFOT patients were organized and sent for analysis so that a sandwich enzyme-linked immunosorbent assay (ELISA) (DYC647, R&D Systems, Minneapolis, MN, USA; detection limit 0.1 ng/mL) (105, 169) could identify *survivin* positivity by a matched-antibody pair (rabbit anti-human *survivin*), with a threshold >0.45 ng/mL indicating positivity – being present in <5% of healthy controls (105, 170).

#### 4.5 BODY MASS INDEX

For **Paper IV**, BMI was calculated from available baseline data of height (meters, m) and weight (kg) from 260 patients with early RA in the SWEFOT trial. BMI was calculated by kg/m<sup>2</sup> into its respective scale, and was also converted into BMI categories: obese (≥30 kg/m<sup>2</sup>; n=43), overweight (25-29.9; n=74), and normal (18.5-24.9, n=143). One patient was underweight (<18.5) and was included under normal due to a BMI close to the normal threshold (17.9).

## 4.6 THE INTEGRATIVE KALTENBORN MANUAL MOBILIZATION STUDY

**Paper V** is a novel randomized blinded crossover pilot study that integrates established manual therapy into conventional rheumatology practice, and was carried out in the Rheumatology Clinic of the Karolinska University Hospital in Solna. Here, 12 research participants with RA were recruited who were also patients receiving standard of care with background medication, including either conventional or biological DMARDs. A clinical comparator group was also included, consisting of eight research participants with hand OA, which is a more common manual therapy target group. For primary measures, 320 hand joints were assessed.

All participants were recruited from February 2015 to December 2016 through the Rheumatology Clinic and the premises of the Karolinska University Hospital in Solna, as well as through the Swedish Rheumatism Association (*Reumatikerförbundet*). The study schematic is shown in **Figure 5**.

Within-the-slack Grade I-II Kaltenborn mobilization (154, 156) was carried out for 28 minutes per participant once/week for four weeks. Four MCP joints (MCP II-V) were each treated for three minutes + one minute rest + three minutes. The study included five licensed therapists (four naprapaths; one physiotherapist) blinded to diagnosis and ultrasound; six physicians for joint assessments blinded to the diagnosis, ultrasound, and treated hand; and an ultrasonographer blinded to the diagnosis and treated hand. Hands/wrists were assessed by musculoskeletal ultrasound (General Electric LOGIQ EQ; Wauwatosa, WI, USA) with previously-reported instrument presets (97, 98, 171).



## 4.7 ETHICAL CONSIDERATIONS

All studies included in this thesis were conducted in accordance to the Declaration of Helsinki, where written informed consent was obtained from all participants. The SWEFOT, FIN-RACo, and NEO-RACo trials were approved by the regional ethical boards of all participating sites (101, 159, 161). Additionally, **Papers I-V** were individually approved by the regional ethics board of Stockholm (EPN, *Etikprövningsnämnden*).

For the prospective clinical study, **Paper V**, careful thought was taken into consideration regarding the treatment of the patients with RA, as, to our knowledge, there have been no published studies with manual mobilization in RA prior. Within-the-slack Kaltenborn Grade I-II mobilization was chosen due to being a safe manual therapy method, while at the same time having the ability to reduce pain and relax tense joints without applying the stretching forces of Grade III mobilization (154-156). All treatments were carried out by registered and licensed providers of physiotherapy or naprapathy in the Rheumatology Clinic of the Karolinska University Hospital in Solna, where research physicians were readily available in case of any adverse event. Specific exclusion criteria were developed so that the treatment would not end up being a risk for the patient: chronic bone damage or soft tissue injuries in the hands; acute inflammation within the latest week in any finger joints; any surgery on the shoulder, arm, or hand within the latest 3 months; or pregnancy within the latest 3 months.

## 4.8 STATISTICAL ANALYSES

Non-parametric Mann-Whitney U tests and Kruskal-Wallis tests with Dunn-Bonferroni correction for continuous variables – and Pearson's  $\chi^2$  and Fisher's exact test for proportions – were carried out for **Papers I-V**. Additionally, the Wilcoxon signed rank test and Friedman test with Dunn-Bonferroni correction were carried out for **Papers III** and **V**; and univariate and multivariate binary logistic regression was applied in **Paper IV**. Two-tailed p values <0.050 were considered significant.

## 5 RESULTS

### 5.1 PAPERS I-II: POPERA IN EARLY RHEUMATOID ARTHRITIS

#### 5.1.1 Patient characteristics and standard radiographic follow-up

In **Paper I**, MTX responders at three months who thus continued on monotherapy were generally older than the other SWEFOT patients – significant when compared to patients randomized to anti-TNF (**Table 3**). It was more common for a MTX non-response among females; thus, fewer females remained on MTX. The TT arm had a non-significantly lower proportion of ACPA/RF-positivity than the anti-TNF arm. There were no differences in baseline radiographic scores (all scores are one SHS unit higher, which was required to include all patients in the model).

**Table 3. Characteristics of eligible patients from the SWEFOT trial for POPeRA**

<b>Outcomes</b>	<b>MTX</b> (n=117)	<b>TT</b> (n=114)	<b>Anti-TNF</b> (n=112)	<b>P value</b>
Age (years)	60 (49, 67)*	56 (43.8, 62.3)	55 (43, 61)*	<b>0.005</b>
Sex (female)	69 (59%)†	89 (78.1%)†	86 (76.8%)†	<b>0.002</b>
Duration	5 (3, 7)	5.5 (4, 8)	6 (4, 8)	0.127
ACPA (+)	67/112 (59.8%)	64/111 (57.7%)	71/102 (69.6%)	0.163
RF (+)	80/116 (69.0%)	71/113 (62.8%)	78/111 (70.3%)	0.455
SHS BL	4 (2, 7)	4 (2, 8.3)	4 (2, 8)	0.173
SHS 12m	5 (2, 10)††	8 (2.3, 16.8)††	7 (2, 12.8)	<b>0.043</b>
SHS 24m	6 (3, 12.5)‡	11 (2.5, 18)‡	6.5 (2, 14)‡	<b>&lt;0.050</b>

**MTX**: methotrexate responders, **TT**: triple therapy arm, **Anti-TNF**: anti-tumor necrosis factor arm. Outcomes in medians (interquartile range in parentheses): age in years, patient-reported symptom duration in months before baseline, and the Sharp-van der Heijde Score (SHS) at baseline, 12 months (m), and 24m, respectively. Anti-citrullinated protein antibody positivity (ACPA) (+) or rheumatoid factor (RF) antibody positivity (+) were reported as proportions. Kruskal-Wallis tests were performed with Dunn-Bonferroni correction. Post hoc analyses: \* MTX > anti-TNF, p=0.005; † MTX < TT and anti-TNF: p<0.010; †† MTX (n=107) < TT (n=104): p=0.037; ‡ MTX (n=101) vs. TT (n=109) vs. anti-TNF (n=106), post hoc not significant: p=0.070. However, Mann-Whitney U-tests comparing MTX vs. TT or anti-TNF vs. TT both were statistically significant: p<0.050.

Among absolute radiographic scores, in **Paper I**, MTX responders had superior radiographic outcomes over one and two years to MTX non-responders randomized to TT, and MTX non-responders randomized to anti-TNF attained superior two-year outcomes to the TT arm (**Table 3**). In **Paper II**, there were no baseline differences across the treatment arms in FIN-RACo or NEO-RACo, and intensive TT with glucocorticoids in FIN-RACo (Combo) was superior to monotherapy over two and five years (**Table 4**).

**Table 4. Characteristics of FIN-RACO and NEO-RACO patients eligible for POPeRA**

Outcomes	Combo (n=72)	Single (n=72)	P value <sup>1</sup>	Combo + aTNF (n=44)	Combo + PBO (n=46)	P value <sup>1</sup>
Age (Y)*	48.0 (39.3, 52.8)	50.0 (40.3, 56.8)	0.184	51.0 (44.5, 54.0)	47.5 (36.0, 55.0)	0.472
Sex (F)†	43 (59.7%)	49 (68.1%)	0.298 <sup>2</sup>	29 (63.0%)	32 (72.7%)	0.326 <sup>2</sup>
Duration (Months)*	6.0 (4.0, 9.8)	7.0 (4.0, 10.0)	0.582	4.0 (2.0, 5.8)	4.0 (3.0, 6.0)	0.516
RF Positive†	53 (73.6%)	49 (68.1%)	0.463 <sup>2</sup>	33 (71.7%)	34 (77.3%)	0.547 <sup>2</sup>
Radiograph Baseline*	1.0 (1.0, 5.8)	3.0 (1.0, 8.5)	0.349	1.0 (1.0, 3.0)	1.0 (1.0, 3.3)	0.593
Radiograph 2 years*	5.0 (1.0, 16.5)	14.5 (5.0, 23.0)	<b>0.001</b>	1.0 (1.0, 4.0)	2.5 (1.0, 5.3)	0.226
Predicted 2 years*	9.0 (7.0, 22.5)	10.0 (6.25, 35.5)	0.435	13.5 (7.25, 26.5)	9.5 (7.0, 25.75)	0.194
Radiograph 5 years*‡	12.0 (3.3, 27.3)	25.0 (11.5, 34.5)	<b>0.001</b>	2.0 (1.0, 5.8)	3.0 (1.0, 9.0)	0.302
Predicted 5 years*‡	21.0 (13.75, 48.0)	22.0 (13.25, 70.5)	0.445	31.5 (16.25, 62.5)	21.5 (16.0, 61.75)	0.176

**Combo:** triple therapy (TT) combination arm (FIN-RACo), **Single:** monotherapy arm (FIN-RACo); **Combo + aTNF:** TT combination + anti-TNF arm (NEO-RACo); **Combo + PBO:** TT combination + placebo arm (NEO-RACo).

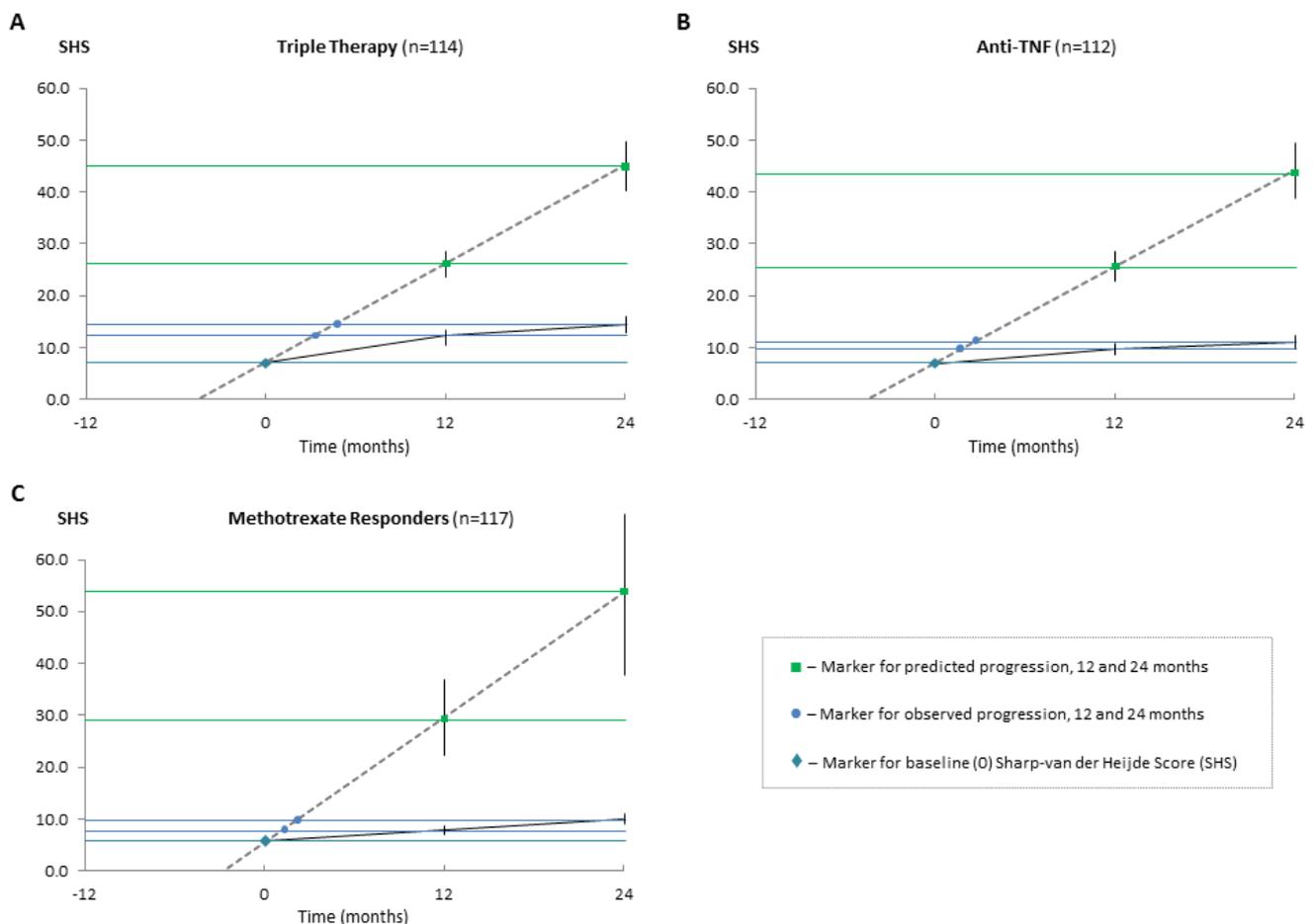
1. Mann-Whitney U test, unless otherwise stated, 2. Pearson's  $\chi^2$ . All the following outcomes were reported as the median, followed by the interquartile range in parentheses:

\* Age in years, patient-reported symptom duration in months before baseline (BL), and the observed or predicted Larsen (FIN-RACo) or Sharp-van der Heijde (NEO-RACo) radiographic score at BL, two-, and five years, respectively. The following were reported as proportions: † Sex (% female) and % rheumatoid factor (RF) antibody positive.

‡ Treatments in all groups became unrestricted after two years.

### 5.1.2 Results from the POPeRA method

In **Paper I**, observed radiographic progression was reduced from predicted by 50.1% in TT, by 72.3% in anti-TNF, and by 73.9% among MTX responders at year one; and by 87.2% (TT), 89.8% (anti-TNF) and 87.8% (MTX) at year two (**Figure 6A-C**, respectively). There was a significantly greater reduction of radiographic progression from predicted at year two in the anti-TNF arm when compared either to the MTX or TT arms ( $n=316$ , 89.8% vs. 87.8%,  $p=0.013$ ; 89.8% vs. 87.2%,  $p=0.021$ , respectively; **Table 5**). Among completers who remained on their assigned therapy throughout the entire study, reductions of 56.7% (TT) and 76.5% (anti-TNF) from predicted at year one and of 91.0% (TT) and 96.0% (anti-TNF) from predicted at year two were observed (**Table 5**).



**Figure 6. Predicted versus observed radiographic progression in the SWEFOT trial**

Predicted progression (broken line) versus observed progression (solid). Scores are plotted as means with standard error for graphical purposes. For more information, see **Paper I** (100).

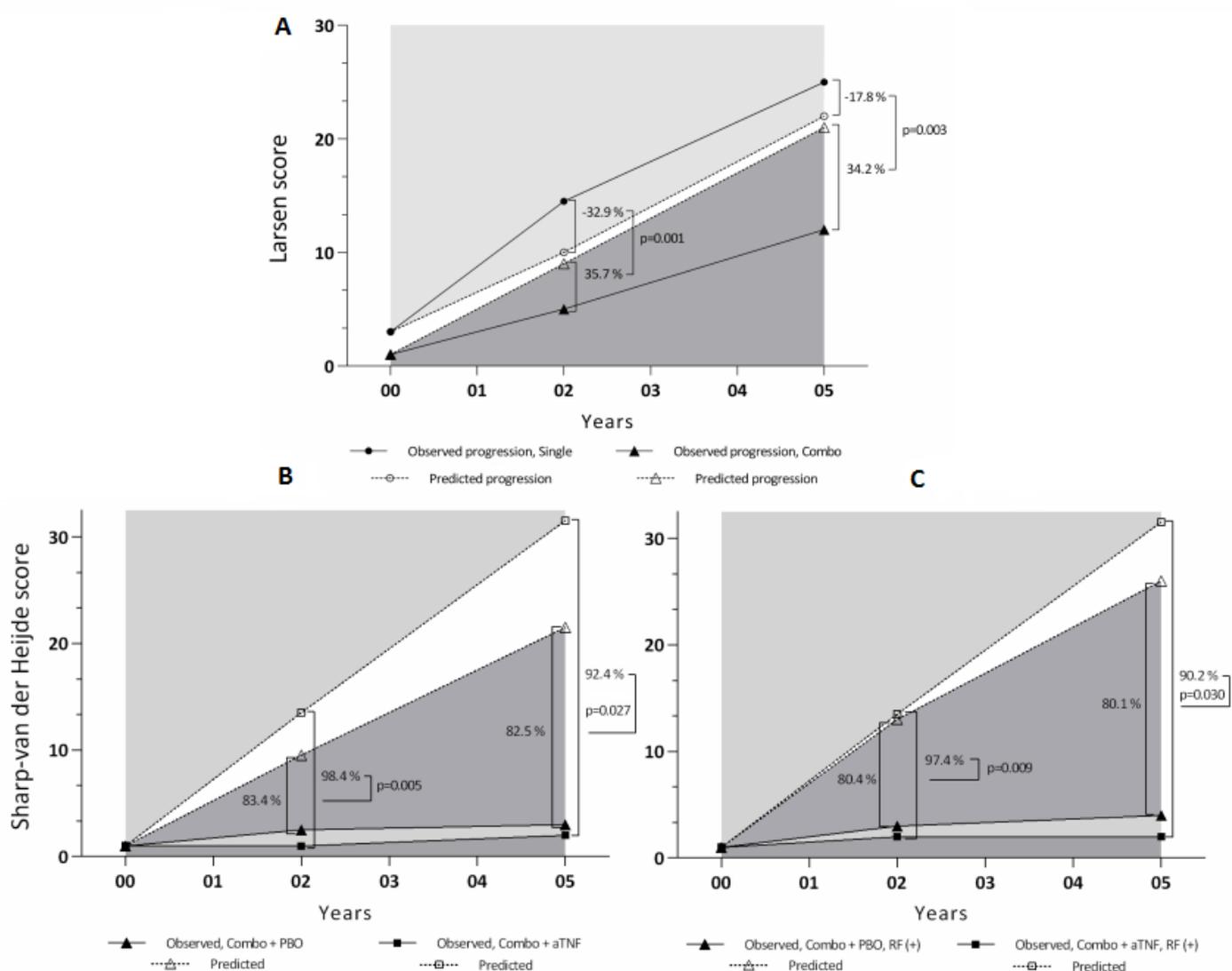
**Table 5. Percent reduction of predicted radiographic progression in SWEFOT**

Time point	MTX	TT	Anti-TNF	TT (C)	Anti-TNF (C)
<b>12m</b>	73.9 ( $\pm$ 56.7)	50.1 ( $\pm$ 103)	72.3 ( $\pm$ 56.4)	56.7 ( $\pm$ 110.8)	76.5 ( $\pm$ 41.2)
Median (IQR)	100 (71, 100)	92 (61.8, 100)	100 (59.3, 100)	94 (67, 100)	100 (56.8, 100)
<b>24m</b>	<b>87.8*</b> ( $\pm$ 27.8)	<b>87.2<sup>†</sup></b> ( $\pm$ 32.2)	<b>89.8<sup>†*</sup></b> ( $\pm$ 32.0)	91.0 ( $\pm$ 3.4)	96.0 ( $\pm$ 1.8)
Median (IQR)	100 (88, 100)	100 (87.5, 100)	100 (96, 100)	100 (92, 100)	100 (96, 100)

**12/24m:** 12 or 24 months; **MTX:** methotrexate responders (12m: n=107, 24m: n=101); **TT:** triple therapy arm (12m: n=104, 24m: n=109); **Anti-TNF:** anti-tumor necrosis factor arm (12m: n=100, 24m: n=106); **TT (C):** completers, 12m: n=61, 24m: n=64; **Anti-TNF (C):** completers, 12m: n=74, 24m: n=78. Results are reported as the mean reduction in % with standard deviation in parentheses, or the median reduction in percent with the interquartile range (IQR) in parentheses; \* Anti-TNF vs. MTX: p=0.013, <sup>†</sup> Anti-TNF vs. TT: p=0.021; Mann-Whitney U test.

In **Paper II**; in FIN-RACo, intensive TT with glucocorticoids vs. monotherapy resulted in superior outcomes in the change from predicted progression over two and five years (mean 35.7% reduction vs. -32.9%, a worsening from predicted, p=0.001; 34.2% vs. -17.8%, p=0.003, n=72, respectively; **Figure 7A, Table 6A**), and was superior regardless of RF positivity (**Table 6B**).

In NEO-RACo, intensive TT with glucocorticoids + a six-month induction of anti-TNF therapy (n=44) led to significantly greater reductions from predicted progression than with the addition of placebo (n=46), both at two and five years of follow-up (98.5% vs. 83.4%, p=0.005; 92.4% vs. 82.5%, p=0.027, respectively; **Figure 7B, Table 6A**). However, initial anti-TNF add-on treatment was superior only among RF-positive patients when stratifying for serostatus (n=34, 33, respectively): two years: 97.4% vs. 80.4%, p=0.009; five years: 90.2% vs. 80.1%, p=0.030 (**Figure 7C, Table 6B**). ACPA serostatus was unavailable.



**Figure 7. Predicted versus observed radiographic progression in the FIN-RACo and NEO-RACo trials**

The differences, as a result of therapy, in the percent change of the predicted scores of all patients were compared. The mean percent change for all patients within each respective therapy is shown; **A.** (FIN-RACo): Combo: triple therapy (TT) combination arm; Single: monotherapy arm; **B-C.** (NEO-RACo): Combo + aTNF: TT combination + anti-TNF arm; Combo + PBO: TT combination + placebo arm; **C.** Rheumatoid factor (RF)-positive patients. The median predicted and observed Larsen (**A**) or Sharp-van der Heijde (**B-C**) scores are plotted at baseline, two, and five (00, 02, and 05) years. Median scores are plotted (interquartile range not in the figure for graphical purposes). Treatment in all groups became unrestricted after two years. Predicted slopes might not appear linear all throughout due to medians being plotted. For additional information, see **Paper II** (103).

**Table 6. Percent reduction of predicted radiographic progression in the FIN-RACO and NEO-RACO trials**

<b>A</b>	<b>Combo</b> (n=72)	<b>Single</b> (n=72)	P value <sup>1</sup>	<b>Combo + aTNF</b> (n=44)	<b>Combo + PBO</b> (n=46)	P value <sup>1</sup>
<b>2 years</b>	35.7 (±127.4)	-32.9 (±211.6)	<b>0.001</b>	98.4 (±7.6)	83.4 (±40.6)	<b>0.005</b>
Median	94.2	47.8		100.0	100.0	
(IQR)	(30.8, 100.0)	(-83.0, 98.4)		(100.0, 100.0)	(82.6, 100.0)	
<b>5 years*</b>	34.2 (±127.7)	-17.8 (±175.4)	<b>0.003</b>	92.4 (±19.8)	82.5 (±41.3)	<b>0.027</b>
Median	80.0	47.2		100.0	98.2	
(IQR)	(21.7, 100.0)	(-62.5, 86.0)		(96.3, 100.0)	(75.0, 100.0)	
<b>Rheumatoid factor (+)</b>				<b>Rheumatoid factor (-)</b>		
<b>B</b>	<b>Combo</b> (n=53)	<b>Single</b> (n=49)	P value <sup>1</sup>	<b>Combo</b> (n=19)	<b>Single</b> (n=23)	P value <sup>1</sup>
<b>2 years</b>	21.1 (±143.1)	-51.2 (±225.7)	<b>0.003</b>	76.7 (±49.4)	6.2 (±176.1)	0.114
Median	88.9	0.0		100.0	91.7	
(IQR)	(-14.3, 100.0)	(-93.8, 77.5)		(75.0, 100.0)	(-25.0, 100.0)	
<b>5 years*</b>	15.9 (±143.3)	-31.1 (±176.9)	<b>0.047</b>	85.2 (±35.0)	10.4 (±172.7)	<b>0.002</b>
Median	60.0	37.8		100.0	66.7	
(IQR)	(-2.2, 94.8)	(-86.7, 76.1)		(80.0, 100.0)	(10.0, 90.3)	
	<b>Combo + aTNF</b> (n=34)	<b>Combo + PBO</b> (n=33)	P value <sup>1</sup>	<b>Combo + aTNF</b> (n=10)	<b>Combo + PBO</b> (n=13)	P value <sup>1</sup>
<b>2 years</b>	97.4 (±8.0)	80.4 (±46.2)	<b>0.009</b>	101.6 (±5.4)	91.0 (±20.2)	0.343
Median	100.0	97.5		100.0	100.0	
(IQR)	(92.6, 100.0)	(82.0, 100.0)		(100.0, 100.4)	(75.0, 100.0)	
<b>5 years*</b>	90.2 (±22.0)	80.1 (±46.7)	<b>0.030</b>	99.9 (±2.8)	88.7 (±22.7)	0.648
Median	100.0	96.7		100.0	100.0	
(IQR)	(92.9, 100.0)	(73.4, 100.0)		(98.3, 100.0)	(80.0, 100.0)	

**A.** All patients in the FIN-RACo and NEO-RACo trials. **B.** Patients distinguished as rheumatoid factor positive (+) or negative (-).

**Combo:** triple therapy (TT) combination arm (FIN-RACo); **Single:** monotherapy arm (FIN-RACo); **Combo + aTNF:** TT combination + anti-TNF arm (NEO-RACo); **Combo + PBO:** TT combination + placebo arm, intention-to-treat (NEO-RACo).

1. Mann-Whitney U test. Results are reported as the mean reduction in percent of the predicted Larsen (FIN-RACo) or Sharp-van der Heijde score (NEO-RACo), respectively, at two and five years with standard deviation in parentheses, or the median reduction in percent with the interquartile range (IQR) in parentheses. Negative values indicate a worsening from predicted. \* Treatments in all groups became unrestricted after two years.

## 5.2 PAPER III: SERUM *SURVIVIN* IN EARLY RHEUMATOID ARTHRITIS

### 5.2.1 Patient characteristics

The baseline characteristics of patients with early RA from the SWEFOT trial with available serum samples for **Paper III** are shown in **Table 7**. Here, no baseline differences were observed across *survivin* status, except that RF positivity was significantly more prevalent among the *survivin*-positive patients, and ACPA positivity was marginally more prevalent.

**Table 7. Percent reduction of predicted radiographic progression in the FIN-RACO and NEO-RACO trials**

Variables	<i>Survivin</i> -positive (n=114)	<i>Survivin</i> -negative (n=188)	P-value
Age (years)	56.0 (43.0, 62.25)	57.0 (44.0, 67.0)	0.332
Sex (F)	77 (68%)	143 (76%)	0.107
Duration	5.0 (4.0, 8.0)	5.0 (4.0, 8.75)	0.905
RF (+)	91/113 (81%)	108/187 (58%)	<b>&lt;0.001</b>
ACPA (+)	71/107 (66%)	103/183 (56%)	0.091
VAS-pain	60.0 (45.75, 72.0)	54.0 (39.0, 71.0)	0.269
VAS-global	60.0 (39.0, 77.0)	58.0 (35.25, 74.0)	0.452
HAQ	1.25 (0.85, 1.75)	1.0 (0.75, 1.5)	0.079
TJC	8.0 (5.0, 13.0)	9.5 (6.0, 14.0)	0.134
SJC	11.0 (6.0, 14.0)	10.0 (7.0, 14.0)	0.692
ESR	35.0 (21.5, 63.0)**	34.0 (19.25, 50.0)	0.260
CRP	17.0 (9.0, 54.5)**	18.0 (9.0, 37.0)	0.628
DAS28	5.78 (5.06, 6.35)**	5.72 (5.02, 6.43)	0.751

Serum levels of *survivin* >0.45 ng/mL indicated *survivin* positivity. F, females; RF, rheumatoid factor (positive (+)); ACPA, anti-citrullinated protein antibody (+); VAS-pain, visual analog scale for pain; VAS-global, patient's global health; HAQ, health assessment questionnaire; TJC/SJC, tender/swollen joint count; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; DAS28, 28 joint-count disease activity score. \*\* n=113. Medians are shown (interquartile range in parentheses). Group comparisons were done by Mann-Whitney U tests for continuous variables, and by Pearson's  $\chi^2$  tests for frequencies.

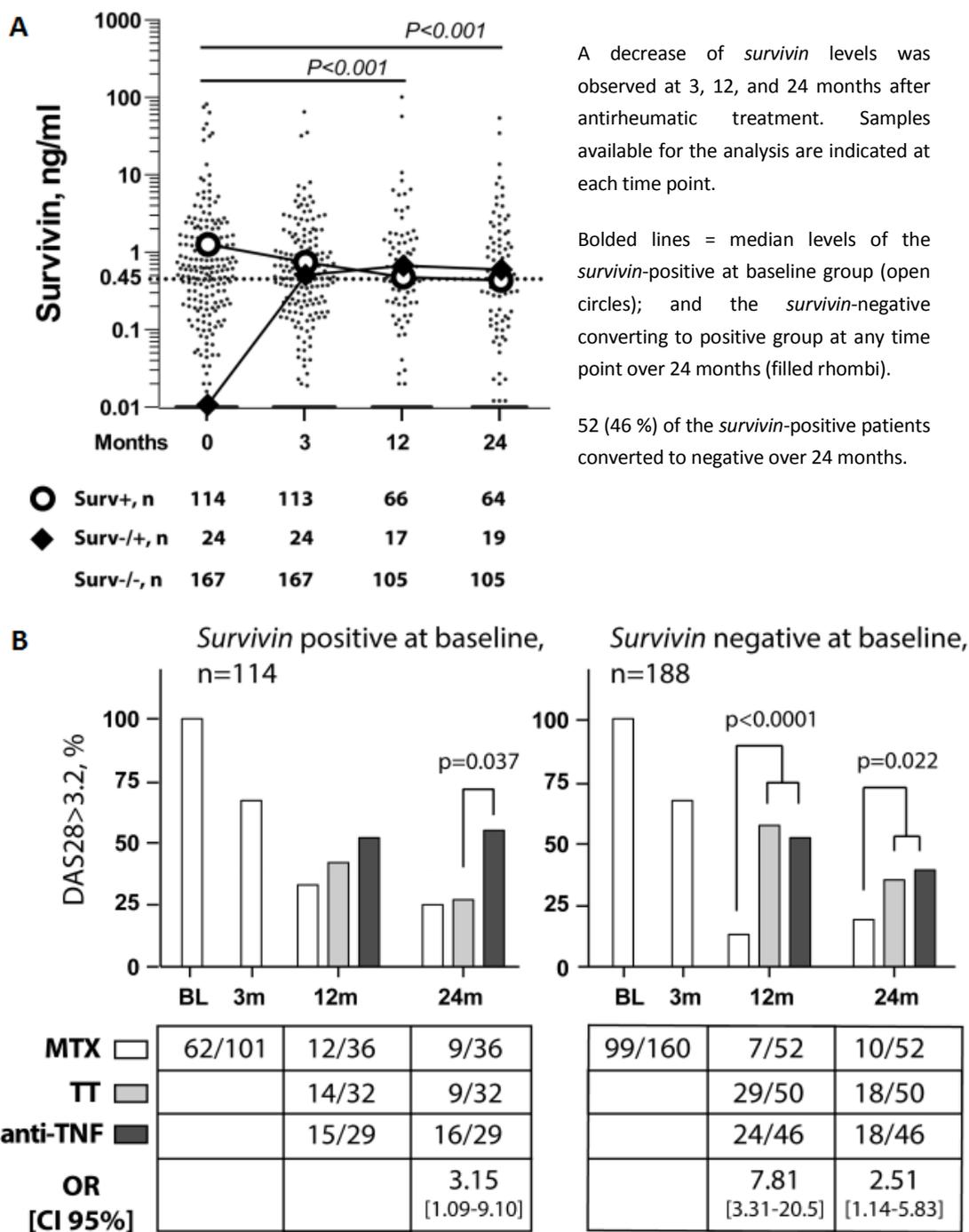
### 5.2.2 *Survivin* as a theranostic, predictive biomarker

Regardless of treatment modality, *survivin* levels decreased significantly at every time point over two years (**Figure 8A**).

*Survivin*-positive patients at baseline who responded to MTX monotherapy at month three and continued on that treatment had a higher odds of disease re-activation (DAS28 >3.2) at year one than if they were *survivin*-negative (odds ratio (OR) 3.21 (95% confidence interval (CI) 1.12-9.24),  $p=0.032$ ); in addition to failing to improve in DAS28, HAQ, and VAS-global health over two years if they still remained positive by three months (see **Paper III**). *Survivin*-negative MTX responders retained superior low disease activity rates over one year (OR 7.81 (95% CI 3.31-20.5,  $p<0.001$ ) and two years OR 2.51 (95% CI 1.14-5.83,  $p=0.022$ ) than to the MTX non-responders who were randomized to treatment intensification, but not if the MTX responders were *survivin*-positive (**Figure 8B**). Additionally, *survivin*-positive patients who ever smoked were less likely to have an initial three-month MTX response than those who were *survivin*-negative (OR 1.91 (95% CI 1.01-3.62),  $p=0.045$ ).

In *survivin*-positive patients, randomization to TT led to better improvements in disease activity than randomization to MTX + anti-TNF infliximab. *Survivin*-positive patients on anti-TNF had a higher risk of active disease at year two than if they were on TT (OR 3.15 (95% CI 1.09-9.10),  $p=0.037$ ) (**Figure 8B**).

For additional information on how *survivin* fluctuations over time affected the clinical ‘core set’ measures such as DAS28 and HAQ, see **Paper III** (109) for more information.



**Figure 8. Serum *survivin* in the SWEFOT trial**

**A.** Changes of serum *survivin* levels during the SWEFOT trial. Serum levels of *survivin* were measured in 302 patients with available serum samples at baseline, where 114 patients were *survivin*-positive (*survivin* >0.45 ng/mL, dashed line), and the remaining 188 were negative.

**B.** Prevalence of active disease (DAS28 >3.2) among *survivin*-positive or negative patients in SWEFOT, with odds ratios (OR) and 95% confidence intervals (CI) indicated. MTX, methotrexate responders; TT, triple therapy arm; anti-TNF, anti-tumor necrosis factor arm.

## 5.3 PAPER IV: OBESITY IN EARLY RHEUMATOID ARTHRITIS

### 5.3.1 Patient characteristics

For all patients from the SWEFOT trial with available BMI (n=260) for **Paper IV**, the characteristics of the patients at baseline who had available follow-up data for the univariate and multivariate binary logistic regression analyses did not differ from the original SWEFOT dataset (**Table 8**). Variables at baseline also did not differ across BMI categories, except that obese patients were marginally older (Supplementary Material, **Paper IV** Manuscript).

**Table 8. Baseline characteristics of early RA SWEFOT trial patients with available body mass index for univariate analyses or the multivariate model did not differ from the original SWEFOT trial population**

	SWEFOT* (n=403)	Univariate** (n=215)	Multivariate† (n=154)
<i>Proportions, n (%)</i>			
Obese (BMI $\geq$ 30 kg/m <sup>2</sup> )	43 (17)	39 (18)	26 (17)
Overweight (BMI=25-29.9)	74 (28)	62 (29)	39 (25)
Normal weight (BMI<25)	143 (55)	114 (53)	89 (58)
Female sex	285 (71)	152 (71)	111 (72)
Current smokers	41 (23)	34 (22)	34 (22)
RF positive	274 (69)	141 (66)	95 (62)
ACPA positive	237 (63)	127 (64)	92 (63)
Concurrent prednisolone	58 (14)	28 (13)	19 (13)
<i>Medians (IQR)</i>			
Age, years	56 (45-64)	56 (44-63)	56 (44-63)
Symptom duration, months	5 (4-8)	6 (4-8)	6 (4-9)
DAS28	5.7 (4.9-6.3)	5.9 (5.1-6.5)	5.9 (5.2-6.6)
HAQ score	1.1 (0.8-1.5)	1.3 (0.9-1.8)	1.2 (0.8-1.8)

\* Missing data were as follows in number, n: body mass index (BMI), 143; smoking status, 221; rheumatoid factor (RF) status, 4; anti-citrullinated protein antibody (ACPA) status, 25; concurrent prednisolone use, 3; and health assessment questionnaire (HAQ), 6.

\*\* Univariate model, where the 28-joint count disease activity score (DAS28) at 24 months and baseline BMI were required. Missing data (n) were as follows: smoking status, 59; RF status, 1; ACPA status, 15; concurrent prednisolone use, 3; and HAQ, 4.

† Multivariate model shown in Table 2 and Figure 3A. Missing data points (n) were as follows: RF status, 1; ACPA status, 7; concurrent prednisolone use, 2.

Abbreviations: RA, rheumatoid arthritis; IQR, interquartile range. Proportions, n (%), were compared with Pearson's  $\chi^2$ . Continuous data, medians (IQR), were compared with independent samples Mann-Whitney U tests.

**Table 9. Baseline predictors of two-year non-remission in the SWEFOT trial population with available body mass index, odds ratios and 95% confidence intervals**

Parameters	Univariate (n=215)*	Univariate (n=154)†	Multivariate (n=154)†
Obesity	<b>4.1 (1.8 - 9.1)</b>	<b>5.4 (1.9 - 15.2)</b>	<b>5.2 (1.8 - 15.2)</b>
Female sex	<b>2.5 (1.4 - 4.6)</b>	<b>2.4 (1.1 - 4.9)</b>	<b>2.6 (1.1 - 5.8)</b>
Current smokers	1.8 (0.9 - 4.0)	1.9 (0.9 - 4.0)	<b>2.6 (1.1 - 6.3)</b>
HAQ	<b>2.0 (1.2 - 3.2)</b>	<b>1.9 (1.1 - 3.2)</b>	<b>1.9 (1.1 - 3.4)</b>
Age, years	1.0 (1.0 - 1.0)	1.0 (1.0 - 1.0)	1.0 (1.0 - 1.0)
DAS28	<b>1.7 (1.3 - 2.3)</b>	<b>1.9 (1.3 - 2.7)</b>	<b>1.9 (1.3 - 2.9)‡</b>
Tender joints	<b>1.1 (1.0 - 1.1)</b>	<b>1.1 (1.0 - 1.2)</b>	<b>1.1 (1.0 - 1.2)‡</b>

Risk of not achieving clinical remission (disease activity score, DAS28 $\geq$ 2.6) after two years was calculated using uni- and multivariate binary logistic regression (significant findings are bolded). Obese- (body mass index (BMI)  $\geq$ 30 kg/m<sup>2</sup>) (\* n=39; † n=26) were compared with non-obese patients (BMI<30, \* n=176; † n=128). Additional outcomes assessed: female sex; current vs. non-current smokers (\* n=156); per-unit increase in health assessment questionnaire (HAQ) (\* n=211); per-year increase in age; disease activity score (DAS28); and tender joints. Non-significant univariate/multivariate predictors included concurrent use of prednisolone; patient-reported symptom duration in months before baseline; presence of erosions & Sharp-van der Heijde Score; swollen joints; visual analog scale for global assessment or pain; erythrocyte sedimentation rate; C-reactive protein; and anti-citrullinated protein antibody or rheumatoid factor positivity.

† Among patients who had available data for all parameters included in the final multivariate model (highlighted in grey: obesity, sex, smoking status, DAS28, HAQ, and age) (n=154), each predictor was also tested by univariate analysis individually.

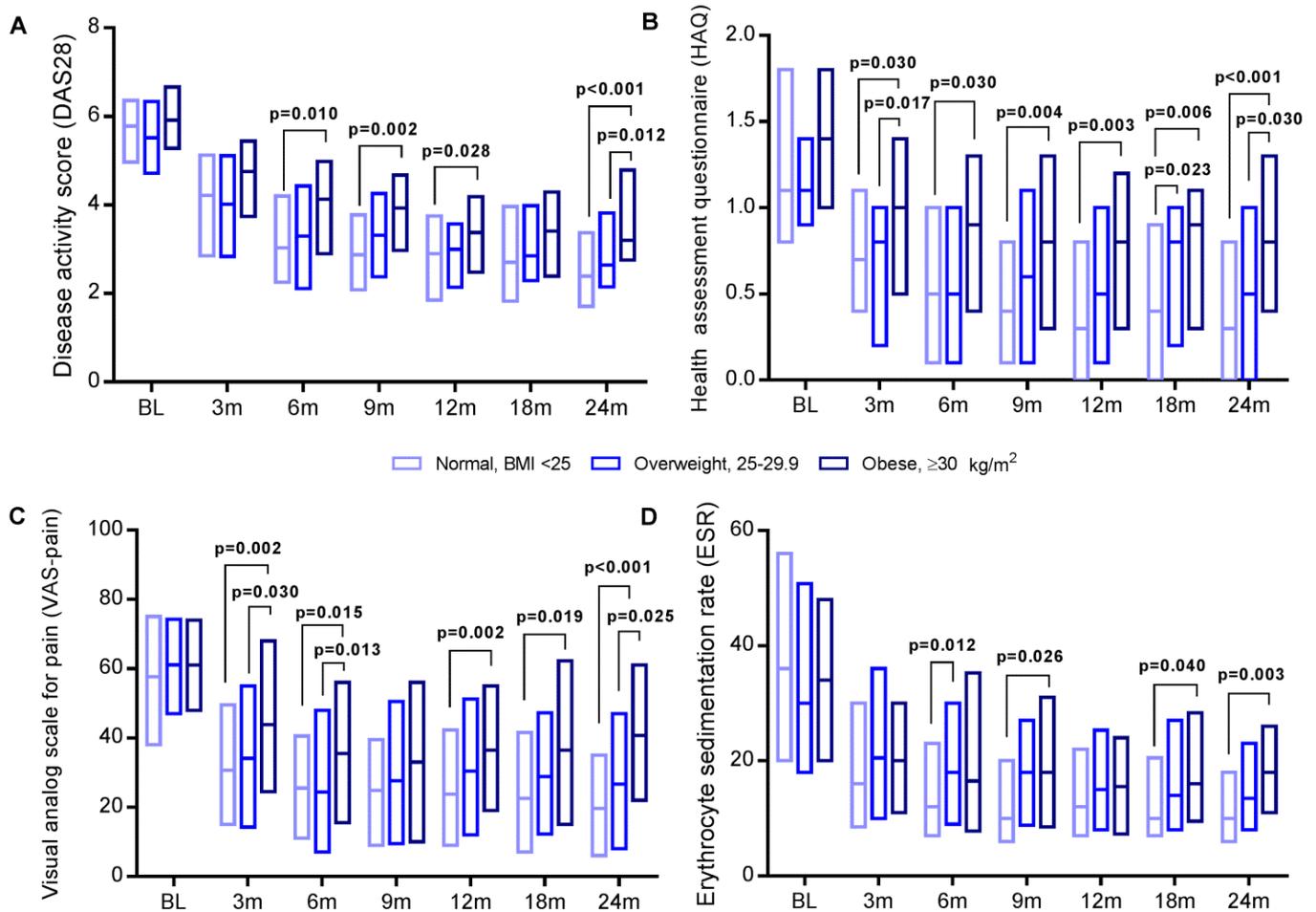
‡ DAS28 and tender joints were not chosen as predictors in the final model due to collinearity. These variables were tested and weighed against their counterparts in a multivariate model (DAS28 or tender joints instead of HAQ). The final model provided the greatest predictive capacity; predicting non-remission with a classification accuracy rate of 67.5% versus a null proportional-by-chance accuracy rate of 50%.

### **5.3.2 Obesity as a predictor of worse clinical outcome measures**

Upon diagnosis of early RA, despite no baseline differences, obesity was associated with worse 'core set' clinical outcomes over two years (DAS28, HAQ, and VAS-pain; year two: obese vs. normal:  $p < 0.001$ ; obese vs. overweight:  $p < 0.050$ ) (**Figure 9**).

Obese- compared to non-obese patients had independently greater odds of non-remission at year two (adjusted OR 5.2, 95% CI 1.8-15.2). Other independent predictors were female sex (adjusted OR 2.6, 95% CI 1.1-5.8), current smoking (adjusted OR 2.6, 95% CI 1.1-6.3), and HAQ (per-unit increase, adjusted OR 1.9, 95% CI 1.1-3.4) (**Figure 10**).

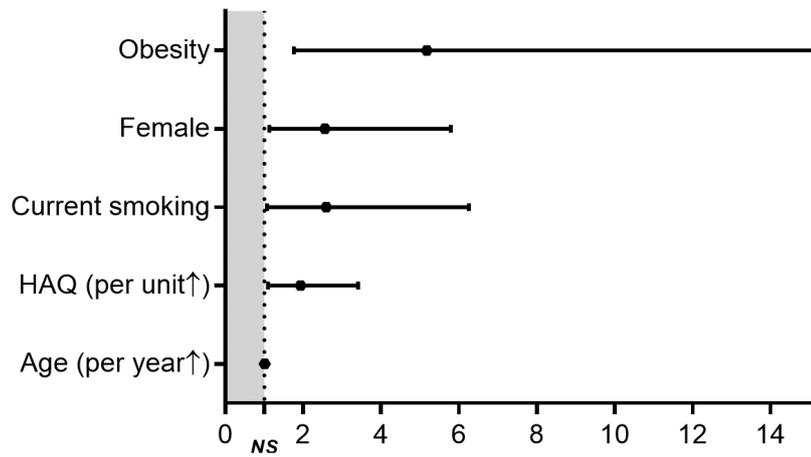
The pattern was similar among MTX responders, and among MTX non-responders randomized at three months to TT, although significance was not found among obese patients randomized to anti-TNF infliximab (**Figure 11**). ACPA/RF positivity did not distinguish differences for these findings, and obesity had no independent association to radiographic progression (**Paper IV Manuscript**).



**Figure 9. Changes in clinical outcome measures over two years in the SWEFOT trial by baseline body mass index (BMI) categories**

Mann-Whitney U tests were performed for all calculations. Medians and interquartile range are plotted for each BMI category. **A-D**: DAS28, HAQ, VAS-pain, and ESR, respectively, are plotted at baseline (BL); and at 3, 6, 9, 12, 18, and 24 months (m).

A



B

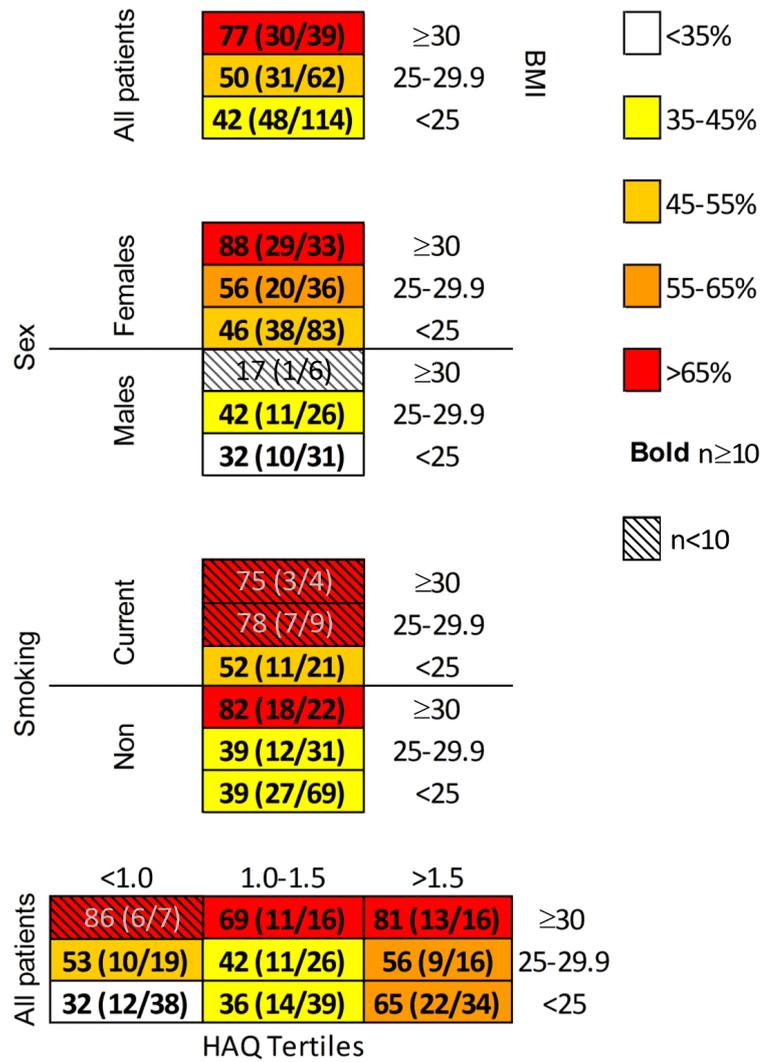
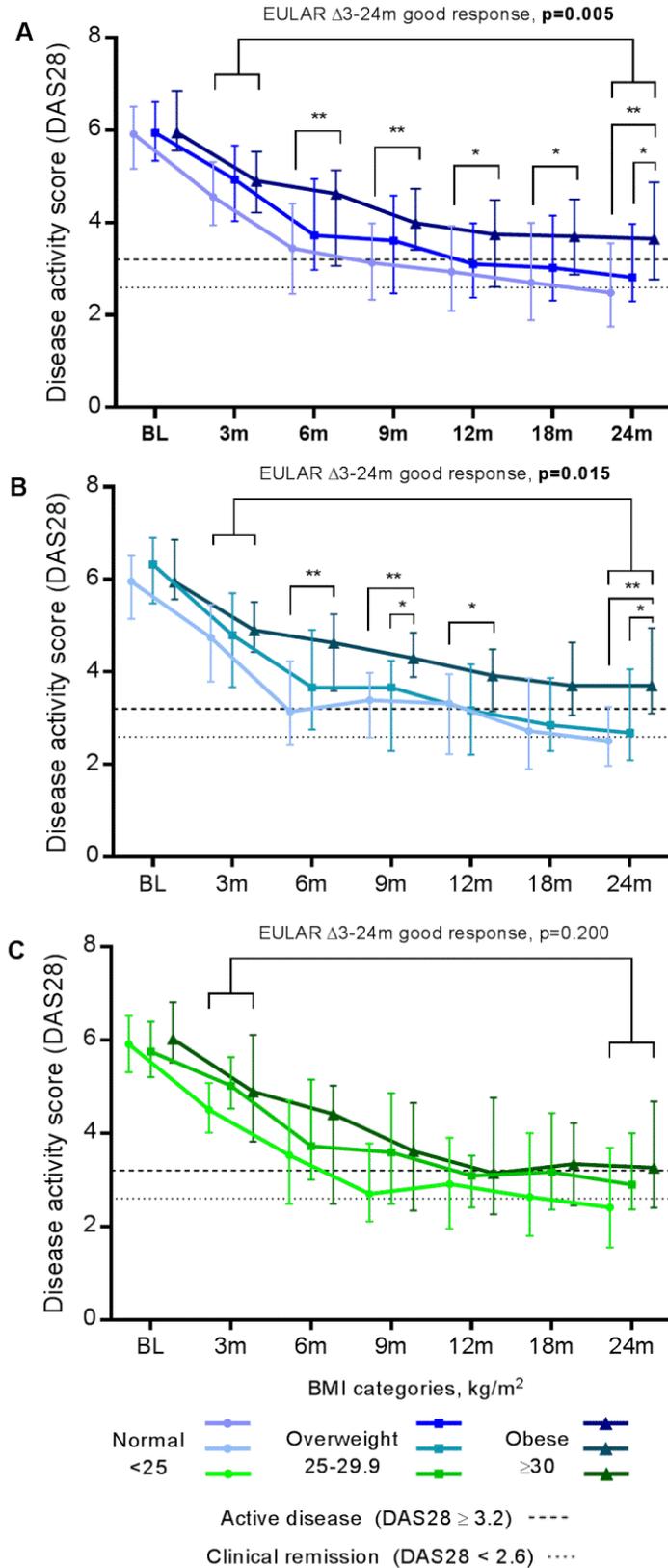


Figure 10. Independent baseline predictors of two-year non-remission in the SWEFOT trial



**Figure 11. Changes in clinical disease activity over two years in SWEFOT trial participants randomized to triple therapy or anti-TNF with available baseline body mass index (BMI) categories**

### Figure 10. Legend

**A:** Adjusted odds ratios (OR) with 95% confidence intervals (CI) for significant predictors in a binary logistic multivariate analysis of non-remission. NS: not significant. Additional information can be found in **Table 9**.

**B:** Risk matrices showing the likelihood (%) of non-remission with different combinations of predictors presented in **A**.

Body mass index (BMI) and two-year disease activity was available for 215 out of originally 403 Swedish pharmacotherapy (SWEFOT) trial participants. Of these, the health assessment questionnaire (HAQ) was available for 211 patients; and smoking habits for 156 patients. Of those with HAQ and smoking habits (n=154), 26 were obese; 34, current smokers; 22, methotrexate (MTX) responders; 65, randomized to triple therapy (TT); 67, randomized to MTX+tumor necrosis factor inhibitor (anti-TNF) infliximab.

### Figure 11. Legend

Patients not achieving low disease activity (DAS28<3.2) at the 3-month (m) follow-up visit were randomized, and the European League Against Rheumatism (EULAR) good response at the two-year follow-up was calculated using randomization at 3m as the baseline value (obese versus normal weight). Individual timepoints with DAS28 over two years are plotted with medians and interquartile range. Included among all patients (**A**) is the combination of the two randomized groups, triple therapy (TT) (**B**, n=94) or anti-tumor necrosis factor (TNF) (**C**, n=91). Responders to methotrexate (MTX) (DAS28≤3.2) continued on monotherapy and are not included due to non-randomization. Sample size (n) for normal weight, overweight, and obese in **A**: 103, 48, 32; **B**: 52, 22, 20; and **C**: 53, 26, 12, respectively.

Obese versus normal- or overweight: \* p<0.050; \*\* p≤0.002.

## 5.4 PAPER V: INTEGRATIVE MEDICINE IN RHEUMATOID ARTHRITIS

### 5.4.1 Participant characteristics

Among baseline characteristics and primary outcome measures, the research participants with RA did not differ from the hand OA participants, except that those with hand OA were older and started with numerically more overall VAS-pain (**Table 10**).

**Table 10. Baseline parameters of research participants with rheumatoid arthritis or osteoarthritis in the randomized Kaltborn manual mobilization crossover study**

	RA (n=12)		OA (n=8)	
<i>Proportions, n (%)</i>				
Female sex	8 (67)		7 (88)	
Randomized hand (R)	7 (58)		3 (38)	
Dominant hand (R)	10 (83)		6 (75)	
Ever-smokers	7 (58)		5 (63)	
ACPA/RF positive	4/8 (50)		-	
<i>Medians (IQR)</i>				
Age	61 (49.5 - 63.8)*		69.5 (66.5 - 73)*	
Disease duration (Y)	6 (1.1 - 6)		7 (4.3 - 19.3)	
<b>Outcomes by hand</b>	<b>H-rand</b>	<b>H-control</b>	<b>H-rand</b>	<b>H-control</b>
Hand pain MCP	1.5 (0 - 43.9)	7.3 (0 - 55.1)	10 (0 - 37.9)	15 (6.3 - 50.4)
Hand pain region	3.3 (0 - 49.1)	1.9 (0 - 40.8)	12.5 (0 - 46.9)	25 (6.3 - 50)
Swollen joints‡	1.5 (0 - 3)	1 (0 - 3.8)	1.5 (1 - 2)	1 (0 - 2)
Tender joints‡	4.5 (2.3 - 7)	4 (2.3 - 5)	4 (1.5 - 13)	5 (2.5 - 14.8)
Q-Doppler, MCP†	0 (0 - 2)	0 (0 - 22.2)	-	-
Q-Doppler, region†	0 (0 - 15.9)	0 (0 - 2.2)	-	-
Synovial fluid, MCP†	0 (0 - 7.2)	0 (0 - 2.5)	-	-
Synovial fluid, region†	0 (0 - 3.4)	0 (0 - 2.1)	-	-
Joint space, MCP	1.1 (1 - 1.3)	1.1 (1 - 1.3)	1 (0.9 - 1.1)	1 (0.9 - 1.1)
Joint space, region	0.9 (0.7 - 1.1)	0.9 (0.7 - 1.1)	0.8 (0.6 - 1)	0.8 (0.7 - 1)
VAS-pain	33.7 (15.1 - 71.3)		63.8 (42.3 - 85.9)	

RA: Twelve participants with rheumatoid arthritis. OA: Eight participants with hand osteoarthritis (serostatus and acute-phase reactants unavailable).

Abbreviations and parameter explanations: randomization: proportion of right hand, R, vs. left hand randomized; dominant hand: proportion right, R vs. left; ever-smokers: current + past-smokers vs. never-smokers; ACPA/RF, anti-citrullinated protein antibody and/or rheumatoid factor positive; IQR, interquartile range; Y, years; H-rand, randomized hand; H-control, control hand; Hand pain, participant-reported pain intensity by visual analog scale (VAS) in the metacarpophalangeal, MCP joints II-V before treatment, followed by regional pain intensity among hand joints, Region: MCP II-V + proximal- and distal interphalangeal, PIP & DIP, joints II-III, respectively; Quantitative (Q) color Doppler musculoskeletal ultrasound (MSUS) activity indicates the absolute score (%) of hyperemia/blood flow activity within an inflamed joint; synovial fluid (area of synovial hypertrophy and fluid effusion by MSUS, mm<sup>2</sup>); joint space (radiographic distance between the MCP and/or interphalangeal bone space, mm, measured by MSUS); VAS-pain (overall). Exploratory measures can be found in the **Paper V** Manuscript.

† Due to a limited amount of participants expressing color Doppler signal or synovial hypertrophy and/or fluid effusion by MSUS, medians are shown with 5<sup>th</sup> - 95<sup>th</sup> percentiles in parentheses. The participants with hand OA had negligible signals or fluid.

‡ Swollen/tender joints were instead based on a modified physician's evaluation of the hands and wrists of the participants, including all DIPs/PIPs but excluding the shoulders, elbows, and knees. Between-group differences (RA versus OA): \* Statistical significance: age, p=0.002

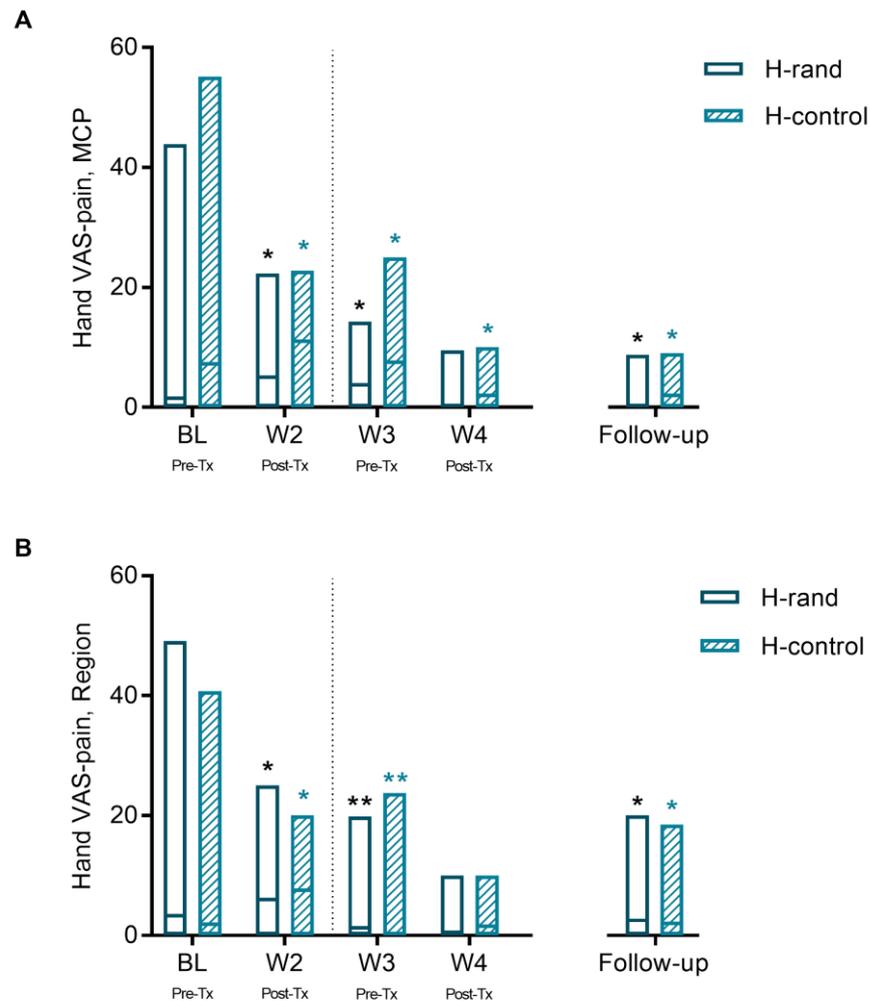
#### 5.4.2 Subjective and objective improvements from mobilization therapy

Among the participants with RA, systemic subjective and objective improvements were observed. Both the initially-randomized- and contralateral hand improved significantly from baseline-crossover-follow-up at two months (pain outcomes/Doppler signal,  $p < 0.050$ ; synovial fluid/MCP joint space,  $p \leq 0.001$ ) (**Figures 12-13**). The proportion of MCP joints that were synovitis-positive (exhibiting Doppler activity and synovial hypertrophy/effusion) decreased significantly from baseline to follow-up (randomized hand MCP joints: 20.8% (10/48) vs. 8.3% (4/48); initial control hand MCP joints: 18.8% (9/48) vs. 10.4% (5/48);  $p \leq 0.025$ , respectively). The change from effusion presence to null effusion from baseline to follow-up for the hand region was also significant (randomized hand joints: 20.8% (20/96) vs. 7.3% (7/96); initial control hand joints: 18.8% (18/96) vs. 8.3% (8/96);  $p \leq 0.004$ , respectively). The participants in the hand OA arm had negligible Doppler activity or effusion.

From baseline-crossover-follow-up, highly significant increases in MCP joint space were observed ( $p \leq 0.001$ ); namely, joint space from BL-Follow-up increased from 1.1 to 1.4 mm (median 21.2% increase [IQR 10.3-32.8%]) (**Figure 13**). **Figure 14** shows improvements in MCP joint space and synovial fluid (A-B), and Doppler activity (C-D) over two months.

In the participants with hand OA, they started out with less MCP joint space and a large increase was observed from baseline-crossover-follow-up, from 1.0 to 1.4 mm (26.7% [23.1-35.2%]). Pain in addition to MCP joint space improved in hand OA, and the specific results from these participants can be found in the **Paper V** Manuscript (Supplementary Material).

There were no dropouts or reported adverse events in either RA or hand OA.

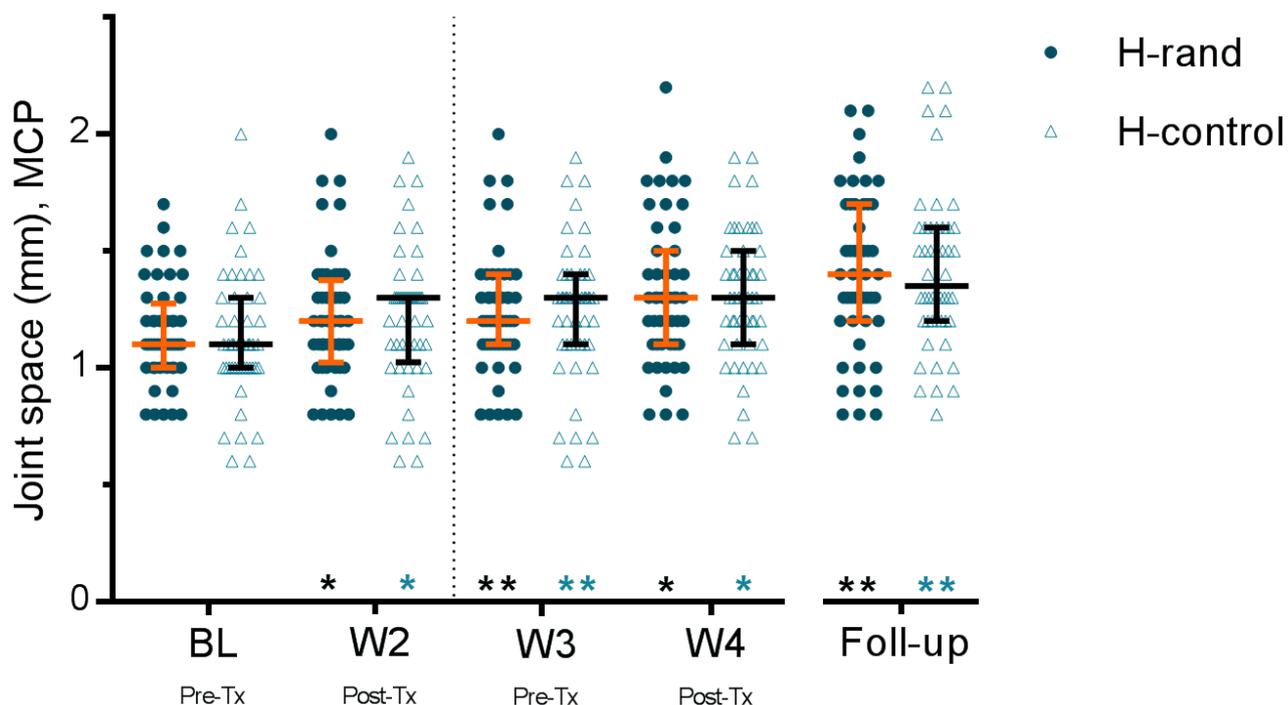


**Figure 12. Hand visual analog scale for pain among participants with rheumatoid arthritis treated with Kaltenborn manual mobilization**

**A.** Hand-pain composite score by visual analog scale (VAS), medians and interquartile range, among metacarpophalangeal (MCP) joints II-V that were treated with Kaltenborn mobilization either from baseline to week two (BL-W2) (randomized hand, H-rand) or from W3-W4 (initial control hand, H-control), n=48 vs. n=48, respectively; **B.** Regional hand-pain composite score among MCPs II-V in addition to the pain scores from the untreated proximal- and distal interphalangeal (PIP/DIP) joints II-III, n=96, H-rand vs. n=96, H-control, respectively. Dashed line indicates crossover at W3 (H-rand becomes control; H-control is now treated directly after the W3 assessment).

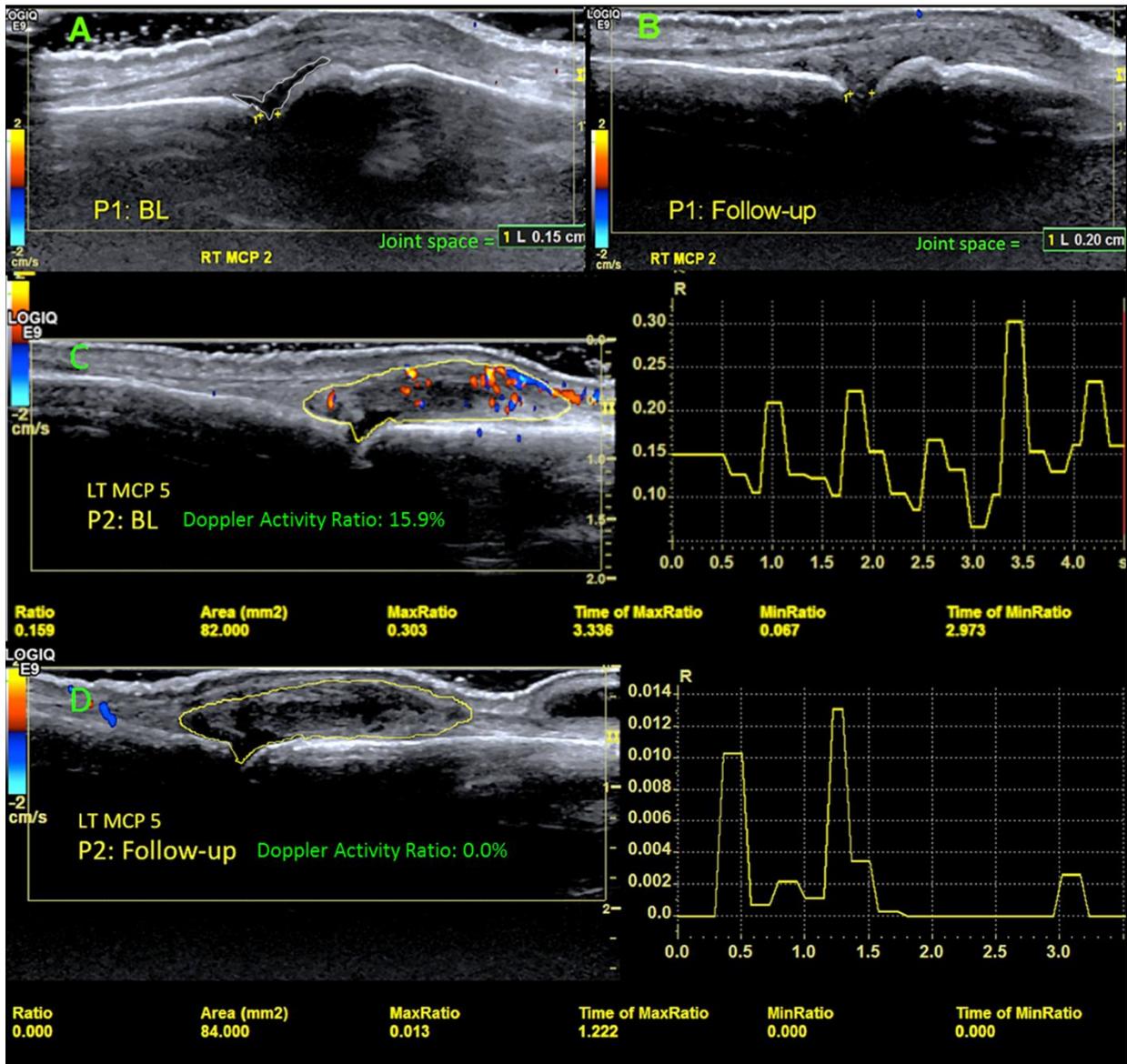
Follow-up: one month after the last treatment at W4; Pre-Tx (BL and W3), VAS-pain directly before treatment with mobilization; Post-Tx (W2 and W4), VAS-pain directly after treatment with mobilization. Medians and interquartile range are plotted. H-rand vs. H-control: No significant between-group differences. \* Statistical significance ( $p < 0.050$ ), \*\*  $p \leq 0.001$ .

BL vs. W2: **A.** H-rand,  $p=0.046$ , H-control,  $p=0.032$ ; **B.**  $p=0.002$ , respectively. BL vs. W3: **A.** H-rand,  $p=0.018$ , H-control,  $p=0.009$ ; **B.**  $p<0.001$ , respectively. Crossover: W3 vs. W4: **A.** H-control (treated),  $p=0.018$ . BL vs. W4 (not plotted in figure): **A.** H-rand,  $p=0.007$ , H-control,  $p=0.002$ ; **B.** H-rand/control,  $p<0.001$ , respectively. BL vs. Follow-up: **A.** H-rand,  $p=0.029$ , H-control,  $p=0.010$ , **B.** H-rand,  $p=0.009$ , H-control,  $p=0.025$ .



**Figure 13. Metacarpophalangeal joint space among participants with rheumatoid arthritis treated with Kaltenborn manual mobilization**

Metacarpophalangeal (MCP) joint space (between tip of phalanges to tip of metacarpus) among MCP joints II-V that were treated with Kaltenborn mobilization either from baseline to week two (BL-W2) (randomized hand, H-rand) or from W3-W4 (initial control hand, H-control),  $n=48$  vs.  $n=48$ , respectively. Follow-up: one month follow-up after the last treatment at W4; Pre-Tx (BL and W3), joint space directly before treatment with mobilization; Post-Tx (W2 and W4), joint space directly after mobilization treatment. Medians and interquartile range are plotted in addition to each individual value. H-rand vs. H-control: No significant between-group differences. \* Statistical significance ( $p<0.050$ ), \*\*  $p\leq 0.001$ . BL vs. W2: H-rand,  $p=0.018$ , H-control,  $p=0.003$ ; BL vs. W3: H-rand,  $p=0.001$ , H-control,  $p<0.001$ . Crossover: W3 vs. W4: H-rand (untreated),  $p=0.002$ , H-control (treated),  $p<0.001$ . BL vs. W4 (not plotted in figure) or BL vs. Follow-up: H-rand/control,  $p<0.001$ , respectively.



**Figure 14. Changes in metacarpophalangeal joint space, synovial fluid, and Doppler activity in rheumatoid arthritis over 2 months with Kaltenborn manual mobilization**

The following images are from two participants (P1, A-B; and P2, C-D, respectively) with rheumatoid arthritis included in this study who gave consent to publication of their musculoskeletal ultrasound images. **A-B.** Metacarpophalangeal joint space of P1 at baseline (BL, 1.5 mm) and follow-up one month after the final treatment at W4 (2.0 mm), respectively; including presence of synovial fluid at BL (0.8 mm<sup>2</sup>), which is gone by follow-up. **C-D.** Presence of active Doppler quantification of P2 at BL (Ratio, 15.9%; Max: 30.3%), which is virtually gone by follow-up (Ratio: 0%; Max: 1.3%), respectively.

## 6 DISCUSSION

The overall aim of this thesis was to evaluate an approach of personalized integrative medicine. First, the state of established knowledge pertaining to RA was reviewed, including its pathogenesis, clinical course, classification, and treatment paradigms. Next, imaging modalities, biomarkers, environmental and lifestyle risk factors, and integrative medicine in RA were identified. An investigation was then carried out for *predicting* the disease course of RA – with the simulation provided through POPeRA and radiography (*imaging*) (**Papers I-II**); the theranostic capability of serum survivin (*biomarkers*) (**Paper III**); and the clinical relevance of BMI and lifestyle factors (*risk factors*) for disease outcome (**Paper IV**) – as well as *monitoring* RA outcome with the applied approach of integrating established manual therapy with the standard of care (*integrative medicine*) (**Paper V**).

### 6.1 SUMMARY AND INTERPRETATION OF MAIN RESULTS

#### 6.1.1 Papers I-II: The POPeRA method

In **Papers I-II**, the simulation of predicted vs. observed radiographic progression through POPeRA identified the value of creating a true control reference point – which is otherwise impossible to observe in RA due to the good standard of care in allopathic medicine and rheumatology to treat a newly-diagnosed patient with early RA immediately. With POPeRA, we can now emulate how patients diagnosed with RA would progress if they had not received DMARDs; and can thus compare the relative radiographic efficacy of various treatments with more sensitivity than by comparing radiographic scores – especially today since radiographic differences are more difficult to observe across treatments due to the successful standards of the modern T2T approach (85, 88) and treating within the ‘window of opportunity’ (13-16).

In **Paper I**, significant reductions were observed from predicted radiographic progression regardless of the treatment modality. However, the original findings from the randomized SWEFOT trial with POPeRA were confirmed – where it was found that after a three-month MTX non-response, randomization to anti-TNF+MTX was radiographically superior to TT at year two (and clinically superior by a EULAR good response at year one) (101, 102). We demonstrated through POPeRA that anti-TNF therapy was not only superior by the radiographic SHS score but was also able to *prevent* more radiographic progression than TT.

In **Paper II**, significant reductions from predicted radiographic progression were also observed with POPeRA regardless of the DMARD being utilized. The original results of the randomized FIN-RACo trial were confirmed: that intensive TT with glucocorticoids was radiographically superior to monotherapy with SSZ or MTX over two and five years, regardless of RF status (159, 160). Upon application of POPeRA in the randomized NEO-RACo trial, additional treatment intensification to the same TT modality with an induction of six months of anti-TNF infliximab therapy provided additional radiographic benefits, not only over two years (161), but also over five years compared to intensive TT + placebo – which was not seen in the original five-year follow-up publication by traditional radiographic score analysis (162). However, this beneficial effect was observed exclusively among RF-positive patients upon stratification by serostatus. POPeRA was therefore able to further inform the NEO-RACo results by identifying a subgroup of patients with early RA who could particularly benefit from just receiving a short-term induction of anti-TNF therapy.

### **6.1.2 Paper III: Serum *survivin* in early rheumatoid arthritis**

The importance of serum *survivin* in early RA was investigated and established with an in-depth post hoc analysis carried out in the SWEFOT trial. Considering the rarity of elevated *survivin* presence in the serum of healthy individuals (<5%) (105, 170), *survivin* positivity was a rather prevalent subset in early RA – accounting for one third of all SWEFOT patients. The results confirm that *survivin* positivity at baseline generally predicts worse one- or two-year clinical outcomes, particularly among initial MTX responders who continue on monotherapy and have later disease reactivation; and among patients randomized to the addition of anti-TNF therapy. The risks of active disease (DAS28 >3.2) that *survivin*-positivity was associated with, however, appeared to at least be partly abrogated upon randomization to TT – so much so that clinical outcomes were more favorable among TT vs. anti-TNF at year two. It was also found that *survivin*-positive ever-smokers had a higher chance of initial MTX non-response at three months – thus backing up the established knowledge of smoking as a risk factor of worse outcome in RA (115, 172).

Complementing the predictive baseline findings, in addition to disease reactivation by DAS28, continued *survivin* positivity up to three months was associated with long-term functional deterioration by HAQ and worse overall health by VAS-global over two years despite initial response to MTX. These findings inform T2T-steered treatment and observational studies that had previously identified the importance of the initial MTX

response (172-176) that *survivin* is a clinically-useful biomarker that can be included to predict a maintained long-term MTX response. These results also provide a potential alternative treatment pathway through TT upon *survivin*-associated MTX failure. If additional studies do not confirm our results with TT being an effective approach to *survivin* positivity, perhaps anti-*survivin* treatments now being tested in cancer (as *survivin* is expressed in a plethora of cancer types) could one day be incorporated into RA practice (177-181). Synovial hyperplastic growth in RA – likely sustained by *survivin* – is not unlike cancer, and the success of TT in SWEFOT for *survivin* positivity could be due to the synergistic effects of HCQ, which has been demonstrated (in addition to its originator, chloroquine) to improve cancer patient responsiveness to both conventional anti-cancer therapies like MTX as well as novel agents by directly inhibiting autophagy (182, 183) – which is a key component in sustaining synovial hyperplasia in RA (184).

### **6.1.3 Paper IV: Obesity in early rheumatoid arthritis**

In RA, obesity has been associated with worse clinical-, but not radiographic outcomes (133, 134). However, there are only limited data from early disease onset and from randomized clinical trials in the context of specific treatments. Furthermore, it is not clear whether known predictors of outcome might explain this association.

In this early RA SWEFOT trial reflecting today's standard treatment (MTX with add-on of anti-TNF or triple therapy in non-responders), a clear dose-response relationship with BMI categories and clinical disease activity was observed over two years. A consistent dose-response in all individual components of the DAS28 was observed, except for significance not being reached with the swollen joint count. Obesity strongly lowered the chance of attaining good clinical outcomes; including remission. Namely, obese patients at baseline – despite no differences in disease activity – had over a five-times higher independent odds of not reaching remission at year two compared to non-obese patients. Other independent predictors of non-remission included female sex, current smoking, and functional impairment. When stratifying by individual treatments, the same pattern was observed for MTX responders and the TT arm; however, obese vs. non-obese patients assigned to anti-TNF did not have significantly worse disease activity over two years. The results were independent of ACPA/RF, and obese patients had numerically but non-independently less radiographic progression.

These findings demonstrate that the likelihood of remission can be predicted using measures that are easily-available in the clinic at diagnosis, and highlight the importance of considering lifestyle modifications as a new treatment paradigm in the care of patients with RA.

Potential mechanisms for fat mass to be involved with inflammation in RA naturally include the chronic state of inflammation caused by adiposity, with increased M $\phi$  activation/infiltration in white adipose tissue from free fatty acids (185, 186) – thus leading to increased TNF and IL-6 secretion. Two potential biomarkers that could explain the discrepant findings between better clinical outcomes but more progression among lean patients with RA, and more inflammation and less erosions among obese patients with RA, are adiponectin and leptin, respectively. Adiponectin is under normal conditions an anti-inflammatory biomarker, yet it appears to play an erosive role in autoimmunity. It is elevated with normal BMI and has been shown to be associated with erosive disease in RA (163, 187, 188). Leptin, on the other hand, is directly proportional to BMI, and may explain the clinically apparent inflammatory-associated phenotype of RA (163, 189, 190). More investigations on these two biomarkers are warranted.

#### **6.1.4 Paper V: Integrative medicine in rheumatoid arthritis**

The integrative randomized Kaltenborn mobilization crossover pilot study – carried out over a total time period of two years from start to finish – was the first of its kind, where, to our knowledge, no prior studies had ever been performed pertaining to mobilization in RA. The purpose was to test the clinical feasibility, safety, and effectiveness of Kaltenborn manual mobilization in RA. The aims were met by the results, in that we observed significant reductions in overall pain, hand pain (MCPs or the hand region); hand joint Doppler activity and synovial fluid; as well as highly-significant improvements in MCP joint space over two months. Equivalent improvements in pain and joint space were observed in the clinical comparator group with hand OA. Importantly, there were no study dropouts or reported adverse events in either RA or hand OA. While all the improvements were systemic regardless of which hand was treated, a placebo effect or regression to the mean cannot be disregarded. However, with the objective joint improvements in either hand as observed through ultrasound – regardless of which hand was treated – it cannot be ruled out that mobilization may affect both hands through a potential systemic stimulatory effect on the central nervous system. Thus, Kaltenborn within-the-slack Grade I-II mobilization (154, 156)

was found to be a feasible, safe, and effective approach for treating RA patients with refractory pain.

Despite Kaltenborn mobilization being a worldwide modality used by multiple manual therapy professions for treating joint pain and improving mobility, the scientific literature of its use beyond mobilization of the shoulder and elbow joints is limited (155). Therefore, we had first conducted an initial pilot study of five women with RA to preliminary determine its safety and effectiveness with three extended mobilization sessions within one week, and observed significant reductions in pain and Doppler quantification (157, 158). In light of these results, the inception was made for the current longer-term integrative mobilization study. The initial pilot study had included participants with active Doppler (mean 52% activity) and a 21% mean reduction in hyperemia was observed. However, the current study had recruited patients with minimal Doppler activity despite experiencing daily bilateral pain in the hands (median 0%; maximum 32.1% activity). Despite this, we observed a significant reduction in the proportion of hyperemic MCP joints over two months (8.4-12%, depending on the hand).

Though subjective or objective improvements were indistinguishable, regardless of which hand was treated, there is evidence pointing to a potential systemic effect of mobilization. For example, mobilizing one hand in carpometacarpal (CMC) OA has been shown to have systemic improvements in the untreated contralateral hand, including pain and grip strength (191-193). Strength or range of motion in exercise has also been shown to affect the untreated contralateral side of the body (194-199); as well as pain analgesia with transcutaneous electrical nerve stimulation (200-202). Additionally, a vacuum phenomenon followed by an increase in MCP joint space has previously been reported as a result of mobilization in healthy individuals (203), which could be an underlying mechanism to the displacement or clearance of excessive synovial fluid observed in our study. Additional mechanisms of action may also include reduction of TNF and IL-6, as has been suggested through massage therapy research (204).

Altogether, Kaltenborn manual mobilization of the hands appears both feasible and safe as a clinically integrative method to potentially bring into the practice of rheumatology, and larger randomized controlled studies to confirm or refute these results are warranted.

## 6.2 METHODOLOGICAL CONSIDERATIONS

### 6.2.1 Strengths and limitations

#### 6.2.1.1 *Papers I-II*

The strengths of the POPeRA method are that it is a simple technique to adapt and can be utilized practically by any rheumatology clinic. The only requirements are: 1) the symptom duration reported by the patient before diagnosis, which is date of first symptom onset in months; 2) a baseline radiograph and radiographic score such as SHS or Larsen; and 3) an additional radiograph/score at least at one point of follow-up. The first application of the technique by Wick et al. was verified with the results of subsequent non-responders to DMARD therapy (99), who had minimal reductions from predicted progression; also verified as a sensitivity analysis in **Paper I** (100). Through the confirmation that POPeRA provided of all results in several randomized clinical trials (SWEFOT, FIN-RACo, NEO-RACo), in addition to providing new insight in identifying responders to targeted therapy (**Paper II**, RF-positive patients with anti-TNF induction), its future use in other datasets is encouraged – as conventional radiographic analysis cannot simulate how patients may progress if untreated.

Before POPeRA, a similar ‘benchmark’ method was published in a brief report where the authors argued against comparing progression rates of patients with early RA (205), however, their approach did not take into consideration the important ingredient of patient-reported symptom duration before diagnosis. Instead, disease duration from diagnosis until inclusion in a trial was utilized. We have confirmed that POPeRA is a valid approach in early RA when based on the patient’s self-reported symptom duration before diagnosis (diagnosis was the same as baseline inclusion in our context). Potential methodological weaknesses include disease duration errors (recall bias from the patient), which requires a reliable patient-physician relationship to prevent. Additionally, POPeRA may overestimate progression as it is not entirely linear among individuals (99, 206, 207), yet relatively accurate in the context of groups (208). To further improve the POPeRA simulation, an equation could be incorporated which would be sensitive to the trend that the rate of radiographic damage lessens after one year. Though there were fewer women among the MTX responders in **Paper I** as well as a higher age compared to the randomized arms, the radiographic damage at baseline was the same; additionally, it has previously been reported that neither sex nor age played significant roles concerning progression in early RA (209), despite older age having

the potential chance to be associated with marginally higher SHS due to concurrent hand osteoarthritis (210).

While the POPeRA findings from **Paper II** demonstrated considerable numerical mean differences (e.g. 10% favorable difference over five years in prevention of radiographic progression with anti-TNF induction, **Figure 7**), the results of **Paper I** at two years might not appear so numerically different for anti-TNF arm vs. TT (**Table 5**), especially when taking into consideration the costs of anti-TNF over TT (211-213), in addition to no greater prevention of sick leave or disability pension over several years (214). It could thus be argued that anti-TNF should only be considered upon TT failure as a treatment option. However, in addition to the findings reaching statistical significance, first quartile comparisons revealed a percentage reduction difference of 8.5% in favor of anti-TNF. It is important to stress that each physician should decide the clinical value of each DMARD and to take a good amount of thought into the correct treatment option for their patients.

Finally, in **Paper II**, radiographic progression was more similar to the predicted score in the FIN-RACo dataset. This could be due to wider inclusion criterion: maximum-allowed symptom duration of 24 months, instead of 12 for NEO-RACo. Utilizing the Larsen score may also have limitations in generating a lower slope. As a result, it appeared as if patients on monotherapy had worsened mean changes than predicted. The median change, however, was a 47% improvement at two and five years in these patients. For future POPeRA analyses, inclusion criteria of a maximum of 12 months of symptom duration should thus be used, and most ideally with SHS.

### 6.2.1.2 Paper III

The strengths of this study were that a large proportion of samples were available, particularly at baseline and 3 months; and they were all utilized to create an in-depth analysis of *survivin* in the clinical setting of early RA. We had robust statistical results and per protocol sensitivity analyses that confirmed our main findings. Additionally, our predictive baseline findings were confirmed by creating *survivin* follow-up groups among all individual therapies.

Factors that could have influenced the results include possible drug cytotoxicity, which could have influenced *survivin* level release; however, the small proportion of patients who had fluctuating *survivin* status over several time points were excluded from analysis. A combined ACPA/RF and *survivin* analysis could have further strengthened the findings due to their coexistence in severe RA (107, 215); however, autoantibody status is often associated with a strong anti-TNF response (216) – which was the opposite for *survivin*-positive patients in SWEFOT. Though autoantibody status in the SWEFOT trial was not a predictor of ‘core set’ clinical outcomes, which were the outcome measures utilized for this post hoc study, future studies directed in testing *survivin* clinically as a theranostic marker should nonetheless consider testing *survivin*’s independent predictive value in relation to ACPA/RF. Thus far, no interfering cross-reactions have been found when testing *survivin* against ACPA/RF (107).

Finally, a somewhat limited final number of patients within TT and anti-TNF for *survivin* follow-up stresses the importance of additional studies to be carried out to investigate TT as an option for treating *survivin*-positive patients and to determine the potential mechanism behind its success – such as the potential impact of HCQ and/or SSZ, and whether the effectiveness may be unique to these drugs alone and/or if it is their combination that enhances the efficacy of MTX against inflammation made resistant through *survivin*.

### 6.2.1.3 Paper IV

The strengths of this study were that, in the randomized clinical setting of SWEFOT, despite no baseline differences, BMI categories were consistently shown to have a significant dose-response relationship with clinical disease activity in the majority of follow-up occasions. Obese patients had the poorest clinical outcomes, including disease activity, pain, and functional disability. Advanced multivariate binary logistic regression was carried out to rule out potential confounding of the results, and obesity was found to be a strong independent predictor of non-remission over two years.

A limitation in this study was that SWEFOT wasn't specifically powered to test for post hoc analyses including BMI or smoking, particularly among individual therapies. Thus, several patients did not have such data, particularly in follow-up visits. Secondly, the size of the individual treatment groups does not allow a definite conclusion on our results, particularly pertaining to obese patients having better anti-TNF responses than with TT. It could be that the infliximab responses were attributable to the fact that obese patients in fact received more of the drug due to their weight; however, this was not seen in other studies in RA involving elevated BMI and infliximab in different contexts (163, 217, 218).

Additional studies investigating conventional vs. biological agent response with BMI in early RA (obesity in particular) are needed, including weight-adjusted infliximab vs. the other non-adjusted anti-TNF agents, as well as dose escalation strategies with conventional therapy. In SWEFOT, for example, only SSZ could be elevated from 1000 to 1500mg twice daily in TT. It could be possible that the obese patients treated with MTX and TT required even higher doses to have effective responses, as it has been shown that MTX polyglutamates have a strong inverse association to BMI (219), which ought to be taken into consideration for future study designs.

#### 6.2.1.4 Paper V

The strengths of this study of integrative manual therapy are that subjective improvements of pain relief have been quantified by objective improvements in the joints. Additionally, due to the strict design of the study, several biases have been controlled for with the following setup: at least two different manual therapists per participant, and two per hand, who were blinded to diagnosis, outcomes, and physician/ultrasound evaluation; at least two different physicians per participant, who were blinded to diagnosis, the treated hand, outcomes, and ultrasound; and an ultrasonographer (blinded as physicians) who randomly selected participant ultrasound images for analysis together with the study coordinator (blinded to the random selection and thus the treated hand) – who both analyzed the ultrasound images together with the ultrasonographic reference atlas by Hammer et al. (220), where disputes were resolved by consensus. Additionally, the ultrasonographer conservatively analyzed joint space for all follow-up visits with slight undermeasurement. An image-measurement repeat pilot exercise was also performed for the first participant's images and was concluded with minimal measurement error (<5%). Lastly, the study yielded clinically relevant results that could be incorporated as an integrative medicine in practice; with a limited amount of sessions over four weeks, carried out once a week for 28 minutes each. Importantly, the observed improvements in RA were sustainable and did not have a one-month wash-out effect.

Limitations of this study include the monocentric design and small patient sample size, although a considerable amount of individual joints were analyzed for primary outcomes (n=320). Participants could have also attempted to mobilize their own hands; however, they were specifically instructed not to do so. Background medication could also have interfered with the results; however, participants were instructed to not take their medication during the day before- or the day of mobilization (if possible). Methodology that could be taken into consideration for future studies include considering a larger patient sample size; a longer follow-up time and duration before crossover; and to include a standard of care RA reference control group receiving non-therapeutic ultrasound, as was done in CMC OA (191-193, 221), to further test the possibility of a placebo vs. systemic mobilization-induced therapeutic effect. Additionally, a larger proportion of participants with active joint inflammation should be included. Finally, the synergistic treatment effect that was observed ought to be evaluated physiologically with more mechanistic studies; and qualitatively by studies involving interviews or focus group discussions to investigate perceptions and experiences of treatment effects and their relations to the intervention itself and/or the totality of care including the interaction with the providers of care.

## 6.2.2 Statistical analyses

Conservative non-parametric statistical analyses as indicated under **Materials and Methods** were applied so that a consistent approach could be demonstrated across **Papers I-V**; and so as to prevent type I errors – as much of the data did not have parametric distributions, and several subgroup analyses ended up having small sample sizes.

### 6.2.2.1 Sensitivity analyses

Several sensitivity analyses were applied so as to confirm the main findings of **Papers I-V**.

In **Papers I-II**, various sensitivity analyses were carried out, e.g. to test for different subsets of patients. For example, the final sensitivity analysis did not utilize imputation and included patients who only had a positive radiographic score at baseline. All sensitivity analyses confirmed the main findings.

Upon conducting **Paper II**, an important factor that was decided to control for was serostatus in the POPeRA model as a sensitivity analysis, which was not included in the methodology of **Paper I** as its purpose was only to confirm the original SWEFOT results. Upon carrying out this analysis, we discovered that the association that was driving the more favorable reductions from predicted progression with six-month anti-TNF induction was among the RF-positive patients in particular. ACPA – which is an established potential predictor of radiographic progression (222-226) – was not available in the FIN-RACo and NEO-RACo datasets. In light of these results with RF, additional studies involving POPeRA ought to include both RF and ACPA as sensitivity analyses.

In **Papers I, III, and IV**, per protocol (completers who remained true to their assigned drug) and last observation carried forward sensitivity analyses were carried out to test the main analyses, all of which confirmed the original results.

In **Paper V**, sensitivity analyses that were carried out were all analyses for the clinical comparator group with hand OA, who had equivalent improvements in pain and joint space as to the participants with RA. Doppler activity and synovial fluid was negligible in the OA participants.

## 7 CONCLUSIONS

In **Papers I-II**, it was demonstrated how the novel POPeRA approach may be utilized in conventional radiography, which can be adapted by virtually any rheumatology clinic to simulate a true control and thereby compare the relative radiographic efficacy of various treatments. POPeRA has now both confirmed and informed the results of three large randomized clinical trials in early RA: SWEFOT, FIN-RACo, and NEO-RACo.

In **Paper III**, *survivin* was demonstrated in a randomized clinical trial to be a prevalent and relevant biomarker for clinical and theranostic response in early RA, signaling a risk for worse long-term disease activity upon positivity at diagnosis. *Survivin*-positive patients are at a higher risk for MTX non-response, but appear to benefit more from TT than anti-TNF upon MTX failure.

In **Paper IV**, being obese upon diagnosis of early RA was shown to be a strong independent predictor of non-remission over two years; in addition to female sex, current smoking status, and functional disability. These results stress the need to consider lifestyle in randomized controlled trials, and to work proactively with patients in changing modifiable risk habits.

In **Paper V**, a new approach for integrating Kaltenborn manual mobilization was developed and tested in an experimental research group of participants with RA and a clinical comparator group with hand OA in a blinded randomized crossover pilot study. Mobilization of the MCP II-V joints was found to be a clinically feasible, safe, and potentially effective approach of integrative medicine in RA, which merits further testing in larger randomized controlled trials.

To conclude, we established an updated, evidence-based proactive research approach investigating if personalized integrative medicine for patients with RA – through the application of imaging methods; identification of biomarkers and risk factors; and integration of manual therapy – can lead to enhanced, individualized care. The resulting emerging evidence suggests that personalized integrative medicine is a strategy that may benefit patients with RA. Future studies of individualized and integrative therapeutic approaches in RA and other similar autoimmune conditions are warranted.



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