Effect of antirheumatic treatment on endothelial function and levels of pentraxin 3 and selenium in patients with inflammatory arthritis

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Bærum, June 2019

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Sammendrag

For å forbedre behandlingen av aterosklerose og dens akselererte form ved inflammatorisk artritt (IA), og av IA selv, er det viktig å forbedre innsikten i patofysiologien av disse tilstandene, og finne optimale biomarkører for overvåking av IA-aktivitet og kardiovaskulær (KV) risiko. Derfor fokuserte vi i dette PhD arbeidet på tre parametere som har vært mistenkt for å være involvert i patofysiologi av KV sykdommer (KVD) og / eller inflammatoriske sykdommer: Endotelfunksjon (EF), pentraxin 3 (PTX3) og selen. Svekket EF er et av de første trinnene ved ateroskleroseutvikling, og er en verdifull biomarkør av KV risiko. PTX3, et viktig molekyl i immunsystemet har vært foreslått som en potensiell nyttig biomarkør for både betennelse og KV risiko. Den produseres blant annet direkte i det betente vevet og dens nivåer responderer raskt på endringer i sykelige tilstander i kroppen. Underskudd på selen ser ut til å øke KV risiko og betennelse. Vi undersøkte pasienter fra PSARA, en prospektiv observasjons-studie som inkluderte 140 pasienter med revmatoid artritt, psoriasis artritt eller ankyloserende spondylitt som var i ferd med å starte metotrexat og / eller anti-TNF behandling på grunn av aktiv sykdom. Vi vurderte pasientene ved oppstart og etter 6 ukers og 6 måneders behandling. PTX3 verdien hos IA pasientene var høy under hele oppfølgingen (lå ovenfor referanseområdet). Selv om andre inflammatoriske biomarkører ble redusert av antirevmatisk behandling, forble PTX3 nivået uendret. Derfor kan de høye PTX3-nivåene i IA gjenspeile en pågående subklinisk immunreaksjon som ikke responderer på anti-revmatisk behandling. Hos IA-pasienter med endoteldysfunksjon forbedret EF seg raskt med anti-revmatisk behandling, uavhengig av endring i sykdomsaktivitet. Derfor kan også andre virkemåter av DMARDs, i tillegg til de anti-inflammatoriske effektene, bidra til deres aterobeskyttende effekter. Selen-nivået (72 µg/L) var innenfor referanseområdet, men under grensen på 80-85 µg/L som anses som optimal for KV helse. Dermed er det nødvendig med ytterligere forskning for å avklare om selen-mangel bidrar til økt KV risiko ved IA, eller om den tidligere påviste assosiasjon mellom lavt selen nivå og KV risiko skyldes underliggende inflammasjon. Selen-nivået økte med behandling, muligens på grunn av hemning av selens forbruksfremmende proinflammatoriske prosesser. Våre resultater kan bidra til bedre innsikt i patogenesen av IA/ akselerert KV-risiko og i virkemåten av to av de mest vanlige anti-revmatiske behandlingene, noe som kan muliggjøre utvikling av bedre strategier for behandling av disse tilstandene.

Summary

In order to improve treatment of atherosclerosis and its accelerated form in inflammatory arthritis (IA), as well as of IA themselves, it is essential to improve insights into pathophysiology of these conditions, and to find optimal biomarkers for monitoring IA activity and cardiovascular (CV) risk.

Therefore, we focused on three parameters suspected to be involved in pathophysiology of CV disease (CVD) and/or inflammation: endothelial function (EF), pentraxin 3 (PTX3) and selenium levels. Impaired EF, one of the first steps of atherosclerosis, is a valuable biomarker of CV risk. PTX3, an important molecule of the innate immune system, has been proposed to be a useful biomarker of both inflammation and CV risk due to its production in the inflamed tissue and fast response. Selenium deficit appears to enhance CVD risk and inflammation. We examined patients from PSARA, a prospective longitudinal observational study comprising 140 patients with rheumatoid arthritis, psoriasis arthritis or ankylosing spondylitis starting with methotrexate and/or anti-TNF therapy due to active disease. We assessed the patients at baseline and after 6 weeks and 6 months of treatment. In IA patients with endothelial dysfunction, EF rapidly improved with antirheumatic treatment, independent of change in inflammatory status. Hence, besides the anti-inflammatory effects, also other modes of actions of disease modifying antirheumatic drugs may contribute to their atheroprotective effects. The IA patients had increased PTX3 levels that, in contrast to other inflammatory factors, did not change with treatment. Hence, the high PTX3 levels in IA might reflect an ongoing immune process not modifiable by the given antirheumatic treatment. Selenium levels (72µg/L) were within the reference range but below the limit of 80-85 µg/L that is considered optimal for CVD protection. Thus, further research is necessary to clarify if selenium insufficiency contributes to increased CV risk in IA, or if the previously observed link between selenium levels and CV risk is caused by underlying inflammation. Intriguingly, selenium levels increased with treatment, potentially due to inhibition of selenium consuming proinflammatory processes.

Taken together, our results can contribute to better insights into the pathogenesis in IA and the associated accelerated CVD, and in pharmacological actions of two of the most common antirheumatic regimens, and consequently facilitate development of better management strategies for these conditions.

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List of Papers

Paper I

Deyab G, Hokstad I, Whist JE, Smastuen MC, Agewall S, Lyberg T, Bottazzi B, Meroni PL, Leone R, Hjeltnes G and Hollan I. *Anti-rheumatic treatment is not associated with reduction of pentraxin 3 in rheumatoid arthritis, psoriatic arthritis and ankylosing spondylitis*. PloS one. 2017;12: e0169830. Retrieved from http://dx.doi.org/10.1371/journal.pone.0169830

Paper II

Deyab G, Hokstad I, Whist JE, Smastuen MC, Agewall S, Lyberg T, Ronda N, Mikkelsen K, Hjeltnes G and Hollan I. *Methotrexate and anti-tumor necrosis factor treatment improves endothelial function in patients with inflammatory arthritis*. Arthritis research & therapy. 2017; 19:232. Retrieved from http://dx.doi.org/10.1186/s13075-017-1439-1

Paper III

Deyab G, Hokstad I, Aaseth J, Småstuen MC, Whist JE, Agewall S, Lyberg T, Tveiten D, Hjeltnes G, Zibara K and Hollan I. *Effect of anti-rheumatic treatment on selenium levels in inflammatory arthritis.* Journal of Trace Elements in Medicine and Biology. 2018; 49:91-97. Retrieved from http://dx.doi.org/10.1016/j.jtemb.2018.05.001

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Abbreviations

ACPA	Anti-citrullinated peptide antibodies
ACR	American college of Rheumatology
Anti-TNF	Anti- tumor necrosis factor
Anti-TNF±MTX	Anti-TNF with or without Methotrexate
AS	Ankylosing spondylitis
BMI	Body mass index
CRP	C- reactive protein
CV	Cardiovascular
CVD	Cardiovascular disease
DMARDs	Disease modifying anti rheumatic drugs
ECs	Epithelial cells
ED	Endothelial dysfunction
EF	Endothelial function
ELISA	Enzyme linked immunosorbent assay
ESR	Erythrocyte sedimentation rate
FMD	Flow mediated dilation
IA	Inflammatory arthritis
ICAM-1	Intercellular adhesion molecule-1
IRD	Inflammatory rheumatic diseases
GCA	Giant cell arthritis
HbA1C	Glycated hemoglobin
HDL	High density lipoprotein
IL1	Inter leucin 1
LDL	Low density lipoprotein
MTX	Methotrexate
NO	Nitric-oxide
NSAIDs	Non-steroidal anti-inflammatory drugs
PGA	Physicians' Global Assessment Score of Disease Activity
PsA	Psoriatic arthritis
PtGA	Patients' Global Assessment Score of Disease Activity
PTX3	Pentraxin 3
PWA	Pulse wave amplitude

RA	Rheumatoid arthritis
RF	Rheumatoid factor
RHI	Reactive hyperemia index
RH-PAT	Reactive Hyperemia Peripheral Arterial Tonometry
SC	Subcutanus
SCMs	Smooth cell muscles
SpA	Spondyloarthritis
s-PTX3	Serum Pentraxin 3
s-selenium	Serum Selenium
TG	Triglycerides
TNFR	Tumor necrosis factor receptor
VCAM-1	Vascular cell adhesion molecule-1
WBC	White blood cell

1. Introduction

1.1 Chronic inflammatory arthritis

Chronic Inflammatory arthritis (IA) is characterized by marked inflammation in joints (peripheral joints and/or joints of the spine) that is commonly accompanied by systemic inflammatory state. Moreover, these diseases can also affect other body structures including inner organs (Figure 1).

Among typical symptoms of IA belong joint swelling, pain, stiffness, reduced physical function and systemic symptoms such as fatigue.

Diagnosis of IA usually depends on a combination of symptoms, physical findings, and imaging and laboratory analyses [1].

The main cause of chronic IA is autoimmunity, i.e. overreaction of the immune system to natural components of the body, resulting in damage of healthy tissues [2].

The exact cause of autoimmune IA is still unknown, and causal curative treatment is therefore not available. Hence, immunosuppressive drugs including anti-tumor necrosis factor (anti-TNF) and methotrexate (MTX), which inhibit disease activity and slow down disease progression, play a pivotal role in management of IA [3].

In this thesis, we will focus on three of the most common forms of IA, rheumatoid arthritis (RA) psoriatic arthritis (PsA) and ankylosing spondylitis (AS) [2, 4].

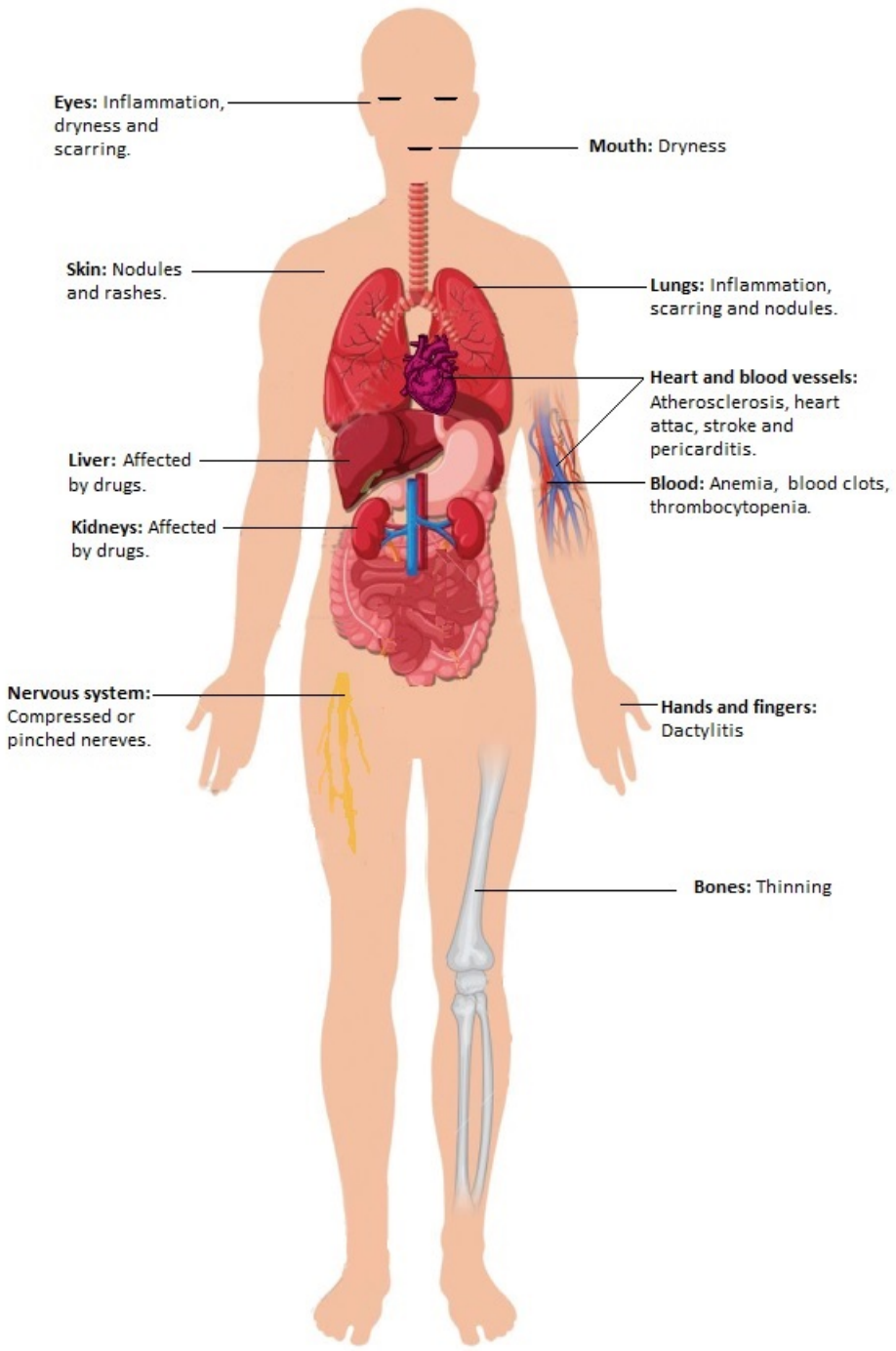

Figure 1: Overview of main extra articular manifestations in IA.

Figure constructed by author.

1.1.1 Rheumatoid arthritis

RA typically causes symmetric chronic inflammation of peripheral joints that may lead to their destruction (Figure 2) [5]. RA can also affect spine and extra-articular tissues, such as vessels and heart, skin, kidneys, lungs and nervous system (Figure 1) [6].

RA occurs in 0.3- 1% of the population, and is more common in women than in men [7]. Almost 40 per 100 000 people develop this condition each year [8].

Majority of RA patients have antibodies to rheumatoid factor (RF) and/or anti-citrullinated peptide antibodies (ACPA), which facilitate RA diagnostics. RA associated with these antibodies, i.e. seropositive RA, is characterized by more severe disease course and higher cardiovascular (CV) risk compared to patients with seronegative RA.

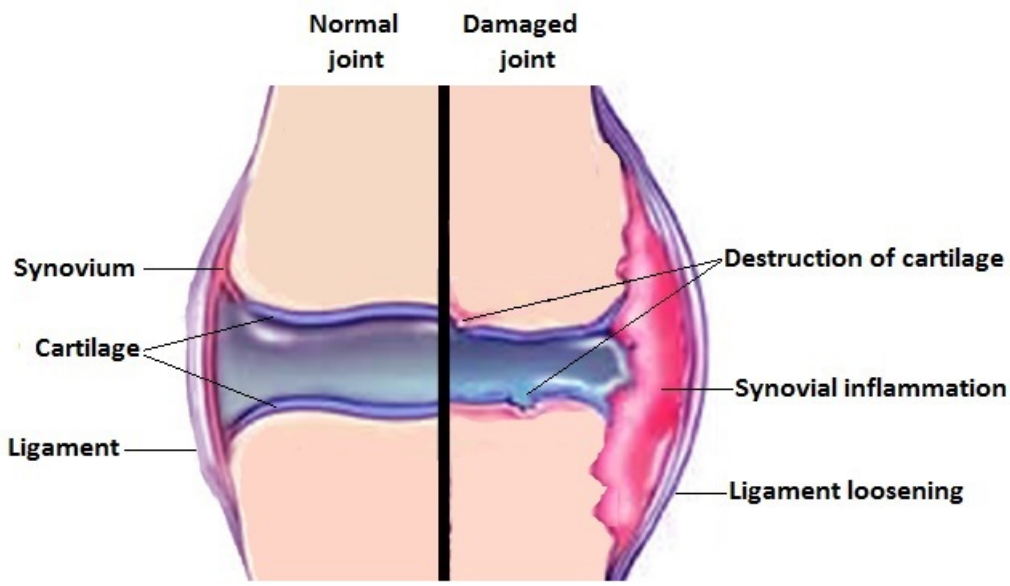

Figure 2: Normal joint and joint affected by rheumatoid arthritis.

Joints affected by RA are characterized by synovial inflammation, ligament loosening and cartilage destruction. Figure constructed by author.

1.1.2 Spondyloarthritis

Spondyloarthritis (SpA) is an umbrella term referring to a group of inflammatory rheumatic diseases (IRD) characterized with axial arthritis, including spondylitis and sacroiliitis (Figure 3).

SpA can also be associated with peripheral arthritis (usually in an asymmetric form), dactylitis, enthesitis and inflammation outside of the musculoskeletal system, such as in the aorta and heart (Figure 1).

SpA comprises several diseases including AS and PsA [1].

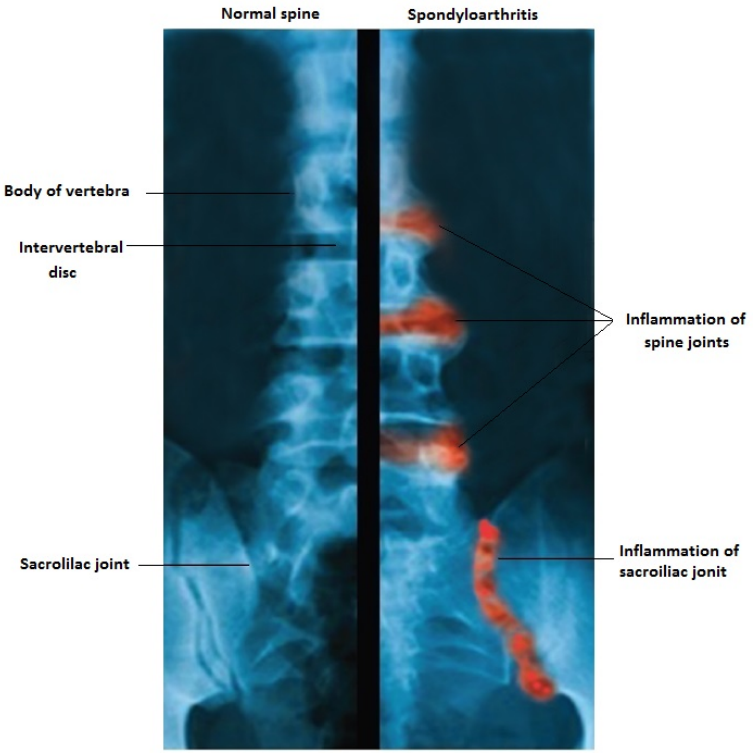

Figure 3: X-ray image of normal spine and spine in patient with SpA.

In patients with SpA, inflammation is located in the spine joints and in sacroiliac joints. If untreated, the spine joints may eventually fuse together. Figure constructed by author.

1.1.2.1 Ankylosing spondylitis

AS is the prototype of SpA. It usually starts in second or third decade of life and occurs predominantly in males. The prevalence of AS varies between 0.1-1.4% globally [9]. Majority of AS patients have human leukocyte antigen B27 (HLA-B27), and HLA-b27 positivity is taken into consideration in diagnostics of AS [10, 11].

1.1.2.2 Psoriatic arthritis

PsA occurs in approximately 30% of people with psoriasis, with similar frequencies in males and females [12]. In some patients, PsA evolves already before the onset of psoriasis.

The prevalence of PsA varies up to 0.42% globally [13].

There are still no known PsA specific circulating biomarkers suitable for PsA diagnostics [12].

1.2 Inflammation

Inflammation is a complex defense mechanism of the body that aims to eliminate harmful stimuli (such as pathogens and irritants), clear out damaged tissues and promote healing [14].

Inflammation may be localized to the affected tissue only, but it may also trigger a systemic reaction, associated with increased levels of circulating inflammatory factors, such as acute phase proteins e.g. C-reactive protein (CRP).

Acute inflammation is characterized by increased blood flow and vascular permeability, along with accumulation of fluid, leukocytes and inflammatory mediators in the affected tissue. While the innate immune system plays a crucial role in an acute inflammatory phase, chronic inflammation includes specific humoral and cellular immune responses [15].

Mediators involved in inflammatory responses include for example cellular adhesion molecules and chemokines that facilitate leukocyte recruitment to the infected area, and a

variety of cytokines that coordinate the inflammatory response, and regulate activation, proliferation and migration of immune cells [15].

Pro-inflammatory cytokines include e.g. interleukin 1 (IL-1) and tumor necrosis factor (TNF). TNF is produced by fibroblasts, mast cells, endothelial cells and, in particular, by activated macrophages and T lymphocytes [16]. TNF binds to one of the two structurally distinct receptors: TNF receptor type I and II (TNFR I and II), which both are present in most cell types except erythrocytes [17]. The binding of TNF to TNFR triggers a series of intracellular events that induce expression of genes important for diverse biological processes, such as cell growth and death and immune and inflammatory responses [18]. Thus, TNF exerts pleiotropic biological activities, including induction of fever and expression of acute phase reactants [15].
TNF is involved in pathogenesis of various IA including RA and SpA, and TNF inhibition is therefore used therapeutically in order to reduce disease activity and slow-down disease progression in these diseases [17, 19-21].

Introduction of anti-TNF treatment and other biologics targeting certain components of the immune system into clinical practice has dramatically improved the course of IA. However, as the cause of these diseases is still unknown, curative treatment is not available, and even with the most modern therapeutic regimens most patients still do not achieve complete sustained remission.

1.3 Accelerated cardiovascular disease in IA

Cardiovascular disease (CVD) is the number one cause of death worldwide. In 2015, CVD accounted for 31% of all global deaths [22].
Patients with IRD, including IA, have increased CVD morbidity and mortality compared to the general population. Even though IA patients are predisposed to a wide variety of CV pathologies, the main reason to their CVD excess is accelerated atherosclerosis [23-26]. The cause of premature atherosclerosis in IA has not been fully clarified, but immune dysregulation and inflammation appear to play important roles [24, 27-32].

Recent studies have brought promising results indicating that mortality and CV morbidity may be decreasing in RA, which is thought to be due to improvements in antirheumatic treatment and/or CV prevention. However, there are some indications that the relative CV risk remains increased, at least in some geographic areas, as the absolute CV risk declines both in the RA and general population [33-36]. Therefore, it is important to identify reasons for increased CV risk in IA patients, and why the risk remains increased in spite of introduction of new highly efficient treatment regimens into the therapy of IA.

1.4 Endothelial dysfunction

The endothelium consists of a simple sheet of endothelial cells (ECs), that lines the inner surface of the entire vascular system and exhibits multiple important functions [37, 38].

It plays a crucial role in maintenance of vascular homeostasis and provides an anticoagulant barrier between the vessel wall and blood [39]. ECs synthesize soluble nitric oxide (NO), which modulates vascular tone, protects the vessel from injuries caused by circulating platelets and cells, and regulates local cell growth [40, 41]. Measurement of NO is used as a surrogate marker for assessment of endothelial function (EF). Endothelium also regulates immune and metabolic responses.

Semipermeability of the endothelium secures selective transport of cells and proteins between blood and the vessel wall [39, 42].

Various factors, including smoking, diabetes, hypertension, obesity, hyperlipidaemia and inflammation, may lead to activation of the endothelium and impairment of its functions [43-46]. The endothelial dysfunction (ED) is characterized by decrease in bioavailability of NO in the vessel, increased expression of pro-inflammatory cytokines (including TNF and IL-1) and adhesion molecules, including vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and E-selectin [39, 47]. Adhesion molecules are designed to interact with leukocytes and platelets, allowing them to roll along the endothelium and eventually move into the vessel wall [42, 48-50].

1.5 Atherosclerosis

ED is an early and reversible step in the development of atherosclerosis. Therefore, its early diagnostics and treatment may be of great importance for CV risk reduction [46].

Formation of atherosclerotic lesions involves accumulation of macrophages, smooth muscle cells (SMCs) and low density lipoprotein (LDL) in the intima, i.e., the innermost layer of a vessel [51]. The retained LDL undergoes oxidization and enzymatic modification, and is taken up by macrophages that therefore evolve into foam cells [44]. Atherosclerotic plaques can also contain other immune cells (in particular lymphocytes) and calcifications. The intimal SMCs, that migrated to the intima from the media, digest modified lipoproteins and synthesize collagen and proteoglycans that contribute to the formation of a fibrous cap overlying the plaque. Further plaque growth is controlled, among other factors, by pro-inflammatory cytokines secreted by various cells including activated ECs, macrophages, platelets and SMCs [52, 53].

Atherosclerotic lesions can lead to narrowing of the vessels, and therefore to impaired flow of nourishment and oxygen-rich blood to the respective organs.

Inflammation and changes in lipid content and type of calcifications in the plaque, as well as thinning of the fibrous cap, can lead to destabilization of the plaque and its rupture, with consequent adjacent thrombogenesis, leading to acute coronary syndromes. Obstructive thrombogenesis may also be initiated by erosions of plaques, characterized by disruption of the endothelial layer [54].

1.6 Effect of antirheumatic treatment on CV risk in IA

IA is treated by disease modifying antirheumatic drugs (DMARDs) and glucocorticoids in order to control disease activity, and by non-steroidal anti-inflammatory drugs (NSAIDs) in order to reduce subjective symptoms.

The mechanisms of action of many current DMARDs, including MTX and anti-TNF, are only partly understood. Therefore, more insights into the modes of action of these drugs are needed, in order to fully utilize therapeutic potential of these drugs, as well as to develop new therapeutic approaches.

MTX and anti-TNF inhibit inflammatory activity through different mechanisms [55, 56]. As TNF is one of the key pro-inflammatory cytokines involved in the pathogenesis of IA, its inhibition represents one of the most efficient and common types of current antirheumatic treatment [17, 56]. MTX, a folate-antagonist, is given as the drug of choice to most patients with newly detected chronic peripheral arthritis. Both these drugs appear to reduce disease activity by multiple actions, such as by inhibition of secretion of TNF and other pro-inflammatory molecules, and by down-regulating expression of adhesion molecules on endothelial cells [55, 57-59].

Importantly, while NSAIDs and high-dose glucocorticoid treatment may increase CV risk, DMARDs such as MTX and anti-TNF treatment can reduce CVD morbidity and mortality [60-67]. Interestingly, the cardioprotective effects of MTX and anti-TNF can be partly independent of their anti-inflammatory effects [68].

1.6.1 Need for improved understanding of pathogenesis of IA and CVD

The etiology of IA and CVD is multifactorial and involves genetic and environmental factors. Increased understanding of the underlying pathogenic pathways (including determination of the involved molecules and cells) may help to detect new therapeutic targets and bring clues regarding potential causes of these diseases.
Improved insight into the molecular and cellular mechanisms may also help to identify new biomarkers for diagnosing these conditions as well as for monitoring their activity and severity, and for prediction of prognosis of IA and CVD (which may facilitate decision-making regarding individual choice of treatment strategies).

Among immune factors that have been proposed to possibly play a role in the pathogenesis of inflammation as well as atherosclerosis belongs the acute phase protein pentraxin 3 (PTX-

3). Interestingly, there are also theories that selenium deficiency and excess might predispose to these two conditions [69-72]. We have therefore focused on these two factors, alongside with ED, in this PhD work.

1.7 Evolution of clinical laboratory

Medical signs and symptoms have been used in clinical practice as long as medicine has existed. Already Hippocrates, known as the "Father of Modern Medicine", introduced diagnostic tools such as listening to the lungs, observing skin color and even tasting the patient`s urine, into management of patients [73, 74]. From that time, the clinical laboratory practice became a pivotal source of medical decisions [74].

1.7.1 Biomarkers

National Institutes of Health Biomarkers Definitions Working Group defines a biomarker as: “A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention” [75].

1.7.2 Blood biomarkers in IA

Different blood biomarkers have been found to be associated with diagnosis of IA, but their usability is limited. For example, inflammatory markers, such as CRP and erythrocyte sedimentation rate (ESR), are widely used in diagnostics and monitoring of IA activity, but they do have flaws in terms of relatively low specificity and sensitivity [76]. Although majority of patients with active IA have elevated levels of CRP and/or ESR, they also may have normal levels[77-80]. For example, 58% of patients with active RA had neither elevated ESR nor CRP [79].

In recent years, a multi-biomarker disease activity blood test has been developed as an objective tool for assessment of RA patients [81]. It is based on a score of 12-biomarkers on

a scale between 1-100, which is consistently associated with established clinical disease activity measures. The 12 biomarkers comprise VCAM-1, epidermal growth factor, vascular endothelial growth factor A, IL-6,TNF-alpha receptor 1A, leptin, resistin, matrix metalloproteinases 1 and 3, human cartilage glycoprotein 39 , serum amyloid A and CRP [82]. This score has been shown to reflect disease activity in patients with early and established RA, and to associate with risk of its radiographic progression in RA, and to be related to disease activity score in AS [82, 83].

However, there is still need for new feasible and reliable clinical biomarkers suitable for detection and monitoring of both IA and CVD risk factors, and for prediction of their prognosis (that might be efficient either on their own or as part of a multi-biomarker panel).

1.8 Pentraxin 3

Pentraxin 3 (PTX3) is an acute phase inflammatory glycoprotein from the same pentraxin superfamily as the well-established inflammatory biomarker CRP [70]. PTX3 is a pattern recognition molecule of the innate immune system, and its production is induced by proinflammatory mediators including IL-1 and TNF [84]. While CRP is produced in the liver, PTX3 is produced directly at inflammatory sites by various cell types including vascular ECs, SMCs and macrophages. Moreover, it is stored in granules of neutrophils, ready to be rapidly released upon stimulation [85]. The PTX3 response is faster than the CRP response; thus, PTX3 is believed to provide a more accurate picture of the current inflammatory reaction (in particular of local inflammatory processes). PTX3 has multiple important functions including anti-microbial effects, participation in clearance of apoptotic cells, induction of immunologic tolerance, and regulation of inflammation.

Serum PTX3 (s-PTX3) levels may play a role both in IA and atherosclerotic CVD [86-94]. In support of this notion, Hollan et al. revealed that coronary artery disease patients with IA had higher mean s-PTX3 levels than patients without IA and healthy controls [92]. Further, Jenny et al. reported that PTX3 was associated with CVD risk factors, subclinical CVD, coronary artery calcification, and clinical coronary heart disease event [89]. Savchenko et al. demonstrated expression of PTX3 in advanced atherosclerotic lesions. PTX3 was expressed

in neutrophils infiltrating the atherosclerotic plaques, suggesting that neutrophils releasing PTX3 might play important role in the atherosclerotic process [95, 96]. Further, Baldini et al. indicated that local expression of PTX3 is a feature of vascular inflammation in giant cell arteritis (GCA). Circulating levels of PTX3 were significantly higher in patients with recent GCA diagnosis (less than 6 months ago) compared to patients diagnosed with GCA for more than 6 months ago, probably reflecting the strong vascular inflammation in the early phases of the disease [97].

In spite of the direct association between PTX3 and CVD, PTX3 itself does not necessarily increase the CV risk. On the contrary, there are indications that PTX3 may have a cardioprotective effect [98, 99]. During inflammation, PTX3 plays an immunoregulatory role through interactions with P-selectin, thereby modulating neutrophil recruitment as well as complement activation, and can inhibit exaggerated inflammation [100-102]. It has been suggested that PTX3 may act as a molecule at the crossway between proinflammatory and anti-inflammatory stimuli, perhaps balancing the overactivation of a proinflammatory, proatherogenic cascade [103].

This modulation is thought to contribute to the cardioprotective function of PTX3, as dysregulation of inflammation plays a major role in IA and CVD [101, 103].

In theory, PTX3 might be a useful biomarker for vascular inflammation and CVD, which might reflect other aspects of inflammation than CRP [88, 89, 96]. In fact, The Japanese Atherosclerosis Society suggested that PTX3 should be considered as a marker for atherosclerotic CVD [104].

1.9 Selenium

Intriguingly, there is evidence that deficit in selenium might enhance CVD risk [105, 106]. Selenium is a trace element that exerts its biologic effects mainly through incorporation into various selenoproteins. Selenium is involved in a wide-range of biologic processes in the human body, including those essential for proper function of brain and thyroid gland and the

circulatory, immune and reproductive systems. For example, selenium has antioxidative, anti-inflammatory and antiplatelets properties, regulates protein folding, cellular transport of Ca^{2+} and intracellular signaling, and influences differentiation, activation and proliferation of immune cells [107-109]. Furthermore, selenium might influence glucose and lipid metabolism [110, 111].

Selenium occurs in various common foods, including grains, seeds, and meats, and its content is particularly high in tuna, eggs, chicken, spinach and Brazilian nuts [112]. Hence, optimal selenium levels may be reached by natural diet. However, because concentrations of selenium in foods are highly dependent on the quality of soil where they are produced, the amount of selenium available in diets varies with geographic area. Of note, the content of selenium in soil in various European countries, including Norway, is known to be low [113-115].

The optimal level of serum selenium (s-selenium) is not known. Although the reference range in Norway is 50-120 µg/L, research indicates that levels below 80-85 µg/L might be insufficient for optimal protection against CVD [116]. Further, there is evidence that associations between selenium levels and risk of CVD, diabetes, dyslipidemia, hypertension, and metabolic syndrome have U-shaped forms: their risk increases not only with low, but also with high selenium levels [106, 110, 117, 118].
Moreover, s-selenium deficiency may occur also due to other reasons, such as poor diet or compromised nutrient absorption from the gastrointestinal tract.

A decade ago, it was shown that circulating s-selenium levels were lower in patients with RA than controls, possibly due to the chronic inflammatory state [119]. Thus, one might speculate that low selenium levels might contribute to the premature CVD in RA [120]. However, although selenium deficit might theoretically play a role in development of premature CVD in IA, this field has not been studied yet [105, 120]. Therefore, it may be of importance to evaluate selenium levels in IA (in particular in relationship to disease activity), and its potential suitability as biomarker to evaluate the CVD risk in these patients.

2. Aims

The main purpose of this thesis was to improve insights into the pathogenesis of accelerated CVD in IA (RA, PsA and AS), and to search for potential biomarkers of IA activity and CV risk. Further, we aimed to examine effects of MTX and/or anti-TNF treatment (antirheumatic treatment) on markers of CV risk (including EF) and other variables that might be involved in pathogenesis of CVD in these IA.

This thesis consists of three papers with the following specific aims:

Paper I

- To compare s-PTX3 levels in patients with RA, AS and PsA.
- To examine the effects of antirheumatic treatment on s-PTX3 levels in the three diagnostic groups.
- To evaluate if s-PTX3 is related to IA characteristics including inflammatory activity and to selected CV risk factors.

Paper II

- To compare EF in patients with RA, AS and PsA.
- To examine the effect of antirheumatic treatment on EF in the three diagnostic groups.
- To evaluate if EF is related to IA characteristics including inflammatory activity and to selected CV risk factors.

Paper III

- To compare s-selenium levels in patients with RA, AS and PsA.
- To examine the effect of antirheumatic treatment on s-selenium levels in the three diagnostic groups.
- To evaluate if s-selenium levels and clinical and laboratory parameters, including markers of disease activity and CVD risks factors.

3. Methods

The present study is based on the bio- and databank from PSoriatic arthritis, Ankylosing spondylitis, Rheumatoid Arthritis (PSARA) study, which is a prospective, open label, observational study of patients with RA, PsA or AS starting with MTX or anti-TNF with or without MTX co-medication (anti-TNF±MTX) due to active IA. The patients were examined at baseline and 6 weeks and 6 months after the initiation of antirheumatic treatment.

3.1 Patients

In brief, the inclusion criteria included age 18-80 years, RA according to the American College of Rheumatology (ACR) 1987 criteria, PsA according to Moll and Wright 1973 criteria or AS according to the modified New York diagnostic criteria for AS, clinical indication for starting with either MTX monotherapy or anti-TNF± MTX (anti-TNF regimens). Exclusion criteria included lack of co-operability, any contraindication for MTX and anti-TNF, any significant infection (including subclinical tuberculosis), immunodeficiency, pregnancy or breastfeeding, congestive heart failure, uncontrolled diabetes mellitus, recent stroke (within 3 months), demyelinating disease, use of systemic glucocorticoid > 10 mg/day during the last 2 weeks or anti-TNF during the last 4 weeks before the inclusion, malignancy and any chronic inflammatory disease other than RA, AS or PsA.

In all patients, oral glucocorticoids were kept at a steady dose, corresponding to prednisolone ≤10 mg day, throughout the study.

All patients gave oral and written informed consent, were included at Lillehammer Hospital for Rheumatic Diseases between October 2008 and May 2010 and all were Caucasians [121].

In this PhD work, we examined only PSARA patients who had completed the whole 6 month follow-up (n=114). See Figure 4 for more detailed information.

Paper I and III evaluated data from all 114 patients. In Paper II, we evaluated only 113 of these patients as reliable EF measurements were not possible in one patient: the patient

smoked before the measurement and did not sit still during the procedure.

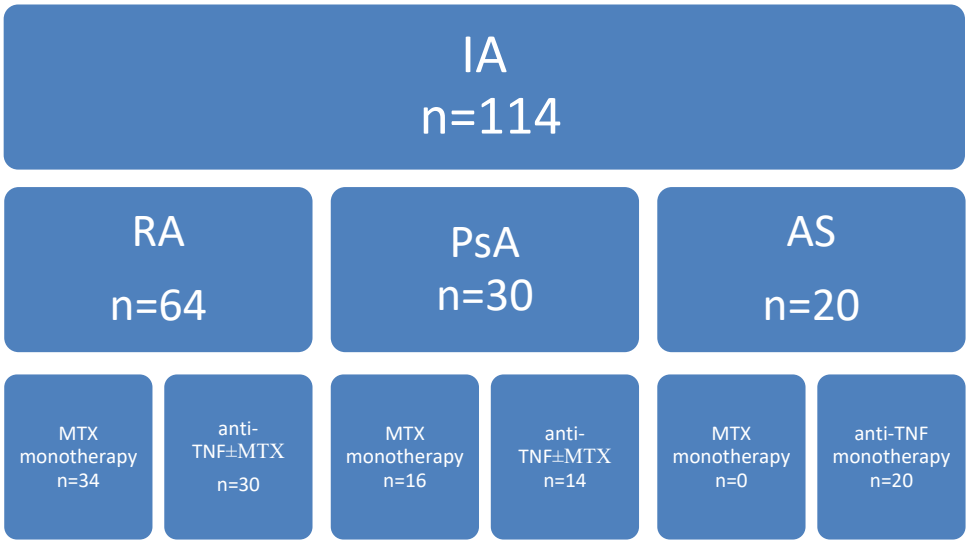

Figure 4: Overview of patients who completed 6 months follow-up in the PSARA study.

3.2 Antirheumatic treatment

The type and doses of antirheumatic treatment were decided by clinical rheumatologists not involved in the study, upon clinical judgment, and in accordance with the Norwegian guidelines. Patients prescribed anti-TNF used either adalimumab (n=37), infliximab (n=7) or etanercept (n=20) with or without MTX co-medication. Doses were as follows: etanercept 50 mg subcutaneous (SC) injection once a week, adalimumab 40 mg SC injection every other week, infliximab 3–5 mg/kg intravenous injection at baseline, then following standard dosing regimen. MTX doses were 15-25 mg orally once a week.

Norwegian guidelines consider MTX as first-line antirheumatic treatment in patients with peripheral IA [122]. Therefore, anti-TNF is usually prescribed to these patients only if they did not have satisfactory response to MTX or if they did not tolerate it. In PSARA study, all patients with peripheral IA starting with anti-TNF were MTX non-responders.

Due to limited effect of conventional DMARDs on SpA (AS and PsA patients), the axial manifestations are treated by TNF inhibition if they do not sufficiently respond to NSAIDs

[123]. Anti-TNF is commonly used in combination with MTX (also in MTX non-responders) in patients with peripheral arthritis as MTX can limit antidrug autoimmune reaction against anti-TNF preparations. However, in patients with axial arthritis anti-TNF drugs have been frequently used as monotherapy.

3.3 Data collection

The data registry in PSARA includes demographic data, lifestyle factors, medical history, CV risk factors and manifestations, medications and measures of IA activity/severity and physical function (Table 1) [124, 125]. At all visits, findings from medical history, physical examination, self-reported questionnaires, routine laboratory analysis and EF assessment were registered.

Table 1: Clinical measures for evaluation of physical function and activity in RA and SpA used in PSARA study.

Test	*Abbreviation*	*Patient group*	*Measures*
Physicians' Global Assessment Score of Disease Activity	(PGA)	All	Disease Activity
Patients' Global Assessment Score of Disease Activity	(PtGA)	All	Disease Activity
Bath Ankylosing Spondylitis Disease Activity Index	(BASDAI)	PsA and AS	Disease Activity
Bath Ankylosing Spondylitis Metrology Index	(BASMI)	PsA and AS	Joint Mobility
Bath Ankylosing Spondylitis Functional Index	(BASFI)	PsA and AS	Physical Function
Bath Ankylosing Spondylitis Patients Global Score	(BAS-G)	PsA and AS	Well-Being
Disease Activity Score for 28 Joints	(DAS28)	RA	Disease Activity
Modified Health Assessment Questionnaire	(MHAQ)	All	Physical Function

3.4 Laboratory methods

At each visit, venous blood samples were drawn after fasting for minimum of 8 hours and absence of any form of tobacco use for 12 hours.
Routine test standards of the hospital laboratory were used to analyze hematological and biochemical routine tests, including ESR, white blood cells counts (WBC), hemoglobin, thrombocytes, CRP, triglycerides (TG), cholesterol, high density lipoprotein (HDL), LDL, glucose, glycated hemoglobin (HbA1C), RF, ACPA and lipoprotein (a) [59, 126].

Small aliquots of serum and plasma were stored at -80°C for later analyses.

These were used for analyses of s-PTX3 and s-selenium. The frozen samples were sent on dry ice to the respective laboratories, and analyzed in batches, by assessors blinded for clinical data, in random order.

PTX3 analyses were performed at Humanitas Research Hospital in Milan, Italy, by an in-house sandwich enzyme-linked immunosorbent assay (ELISA) based on the rat monoclonal antibody as capturing and a rabbit antiserum raised against human PTX3, affinity purified and biotinylated, as detection antibody. Streptavidin-horse radish peroxidase was used and absorbance at 450 nm (Abs 450) was measured with an automatic ELISA reader. For each biological sample, 2 dilutions in duplicate wells were evaluated and mean PTX3 content was calculated converting Abs 450 values to protein concentration by means of a standard curve with recombinant purified human PTX3 (range from 75 pg/ml to2.4 ng/ml). Detection limit for this assay is 100 pg/ml and the inter-assay variability ranges from 8 to 10% (Paper I) [88].

Lab1, Sandvika, Norway (SYNLAB group; SYNLAB International GmbH) examined s-selenium by atomic absorption spectrometry (Varian AA 240Z Zeeman-GFAAS). A hollow cathode lamp was used to measure the absorption at 196 nm. The intra-assay variability coefficient of variation at 53.1 µg/L is 5.1%. The inter-assay variability coefficient of variation and the assays accuracy at 118 µg/L is 5.2% and 2% respectively (Paper III) [116].

3.5 Endothelial function

The EF was measured by the Reactive Hyperemia Peripheral Arterial Tonometry (RH-PAT) technique using EndoPAT2000 (Itamar) device, which evaluates EF by measuring finger arterial pulsatile volume changes [127]. This non-invasive technique has shown a significant and linear relationship with the commonly used non-invasive method, flow-mediated-dilation (FMD) [128, 129]. The EndoPATs technique is described in detail by Bonetti et al. and Rozanski et al. [127, 130]. The EndoPAT quantifies endothelium-mediated changes in vascular tone. Briefly, a cuff is placed on the upper arm of the experimental arm, and a detector is set on the distal finger phalanx of both experimental and control arms. The change in the pulsatile arterial volume is measured before, during and after 5 minutes occlusion of the upper arm. When the cuff is released, the surge of blood flow causes endothelium-dependent FMD. The measurement in the control arm continues during the whole procedure (Figure 5). The reactive hyperemia index (RHI) is calculated as the ratio between the magnitude of the average post-obstructive pulse wave amplitude (PWA) and the average of baseline PWA (pre-occlusion). To compensate for potential systemic changes, RH-PAT values from the experimental arm are normalized for findings from the control arm [127].

ED was defined as RHI≤1.67, as recommended by the manufacturer, and in accordance with findings from populations at risk for ischemic heart disease [127].

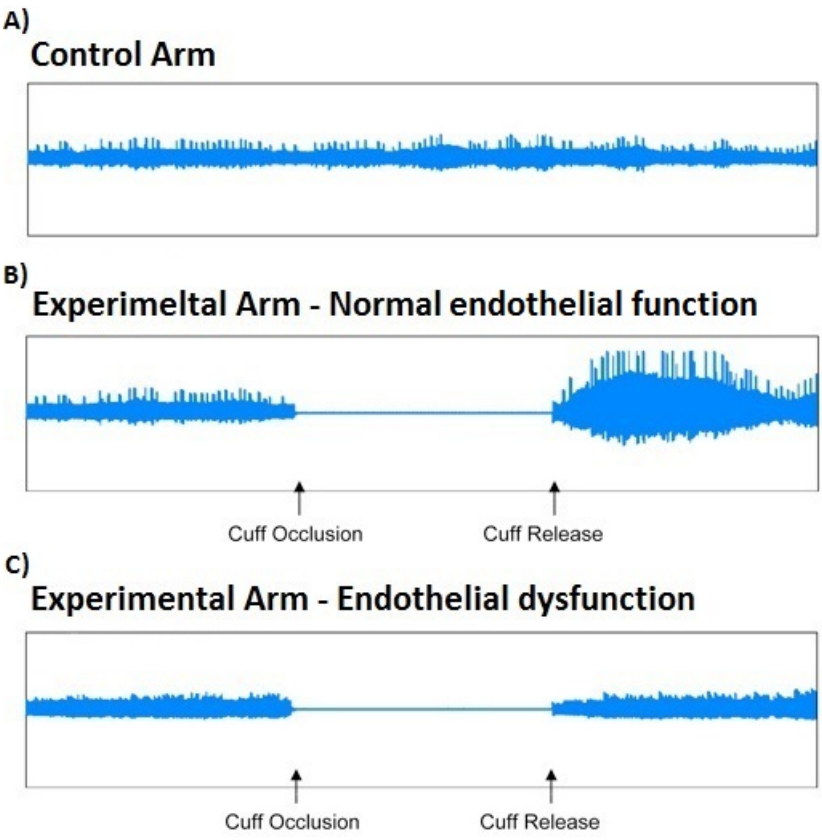

Figure 5: RHI measurement using finger plethysmograph

A) Control arm with no cuff. B) Normal endothelial function is characterized by an increase in the pulse wave amplitude after cuff release in the experimental arm. C) Endothelial dysfunction is characterized by a small or no increase in the pulse wave amplitude after cuff release in the experimental arm.

3.6 Statistical analysis

Continuous data are presented as medians and ranges and categorical data as proportions and percentages. Because the vast majority of all variables, including s-PTX3, EF and s-selenium levels, were non-normally distributed, and due to the small sample size of our study, we universally used non-parametric tests because they do not make any assumptions on the shape of distribution [131].

Mann-Whitney U test and Wilcoxon sign test were applied for comparisons of continuous variables between and within the examined groups, respectively. Pearson Chi-square test was used to assess possible associations between pairs of categorical variables.

Linear regression analyses were used to assess associations between the main variables (PTX3, EF and selenium) modeled as the dependent variable (baseline, 6 weeks and 6 months) and selected laboratory and clinical variables such as medications, characteristics of IA (disease severity, activity and duration and physical function) and CV risk variables (presence of CVD, CVD co-morbidity and EF (when EF is not the dependent variable)). In a similar manner we looked for associations between changes in the main variables and changes in the aforementioned variables (if it was relevant to expect their change) during 6 weeks and 6 months treatment. Age, gender, rheumatic diagnosis, and variables that showed a significant association with the dependent variable in simple regression analyses were included in multiple linear regression models.

In all papers, several multiple adjusted analyses were performed, to check if the presented data were consistent across multiple models, and therefore robust with respect to small changes of the independent variables. However, due to multiple testing in Paper I, p-values ≤ 0.01 were considered statistically significant. For both Paper II and Paper III, p-values ≤ 0.05 were considered statistically significant.

All analyses were considered exploratory, all tests were two-sided and performed using IBM SPSS statistics software.

4. Main Results

Most patient characteristics were similar between both the diagnostic and treatment groups. Of all the IA diagnostic groups, RA patients were the oldest, had the lowest proportion of men and the highest levels of Physicians' global assessment score of disease activity (PGA) and number of swollen joints. The MTX group had significantly shorter rheumatic disease duration (p= 0.043), higher PGA score (p=0.002) and had used fewer DMARDs (p<0.001) than the anti-TNF group.

4.1 Paper I

In the total IA group, median baseline level of s-PTX3 was 3.9 ng/mL, and there were no significant differences in the baseline levels between the three diagnostic groups. We examined the effect of antirheumatic treatment on PTX3 and other disease activity measures at baseline and both follow-up visits. In the total IA group and in the RA group, CRP, ESR, WBC, PGA and Patients' global assessment score of disease activity (PtGA) levels decreased significantly after 6 weeks of therapy (p-value for all <0.001). Of these variables, only PGA and PtGA decreased further from 6 weeks to 6 months, and these decreases were apparent only in the total IA and RA groups (p-values for both p<0.003).
In the PsA group, there was a significant improvement only in PtGA, and only after 6 weeks of treatment. In the AS group, levels of ESR, WBC, PGA and PtGA, decreased significantly only from baseline to 6 weeks (Figure 6).

There were no statistically significant changes in s-PTX3 levels between patients treated with MTX and anti-TNF regimens. Further, no associations were found between s-PTX3 and any of the examined inflammatory markers and CV risk factors such as smoking, BMI, EF, hypertension, hyperlipidemia and levels of HDL, LDL and total cholesterol in both simple and multiple linear regression analyses.

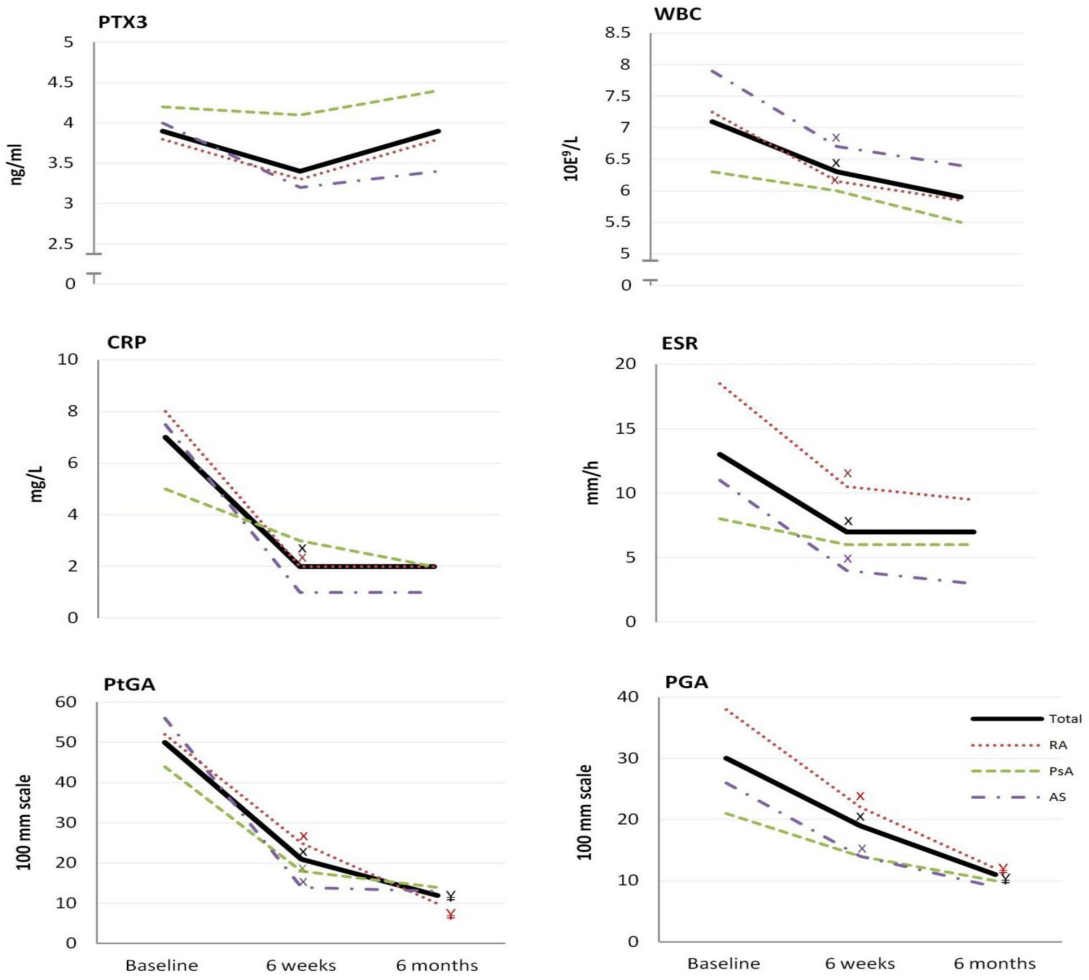

Figure 6: Changes in s-PTX3 and established markers of disease activity during antirheumatic treatment in RA, PsA and AS patients.

Values are given in median. RA, rheumatoid arthritis; PsA, psoriatic arthritis; AS, ankylosing spondylitis; PTX3, pentraxin 3; WBC, white blood cells; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; PGA, Physicians Global Assessment Score of disease activity; PtGA, Patients' Global Assessment Score of disease activity.

X $p<0.01$ for difference between the evaluation at baseline and at 6 weeks.

¥ $p<0.01$ for difference between the evaluation at 6 weeks and 6 months.

4.2 Paper II

Among the three diagnostic groups, the AS patients were the most likely to have ED and CV co-morbidity although they were the youngest, had the worst median RHI value, which was significantly different from PsA group (p=0.040), had a similar disease duration as RA and PsA groups, were more likely to use statins and had lower proportion of patients using systemic glucocorticoids than the RA group. ED at baseline was observed in 40 (35%) of the total 114 IA patients. Among these patients, a significant improvement in EF from baseline to 6 weeks and 6 months visit was revealed only in the total IA group and RA group. There was a trend towards EF improvement also in the PsA group at both points of time, and in the AS group after 6 months, however these differences were not statistically significant (Figure 7).

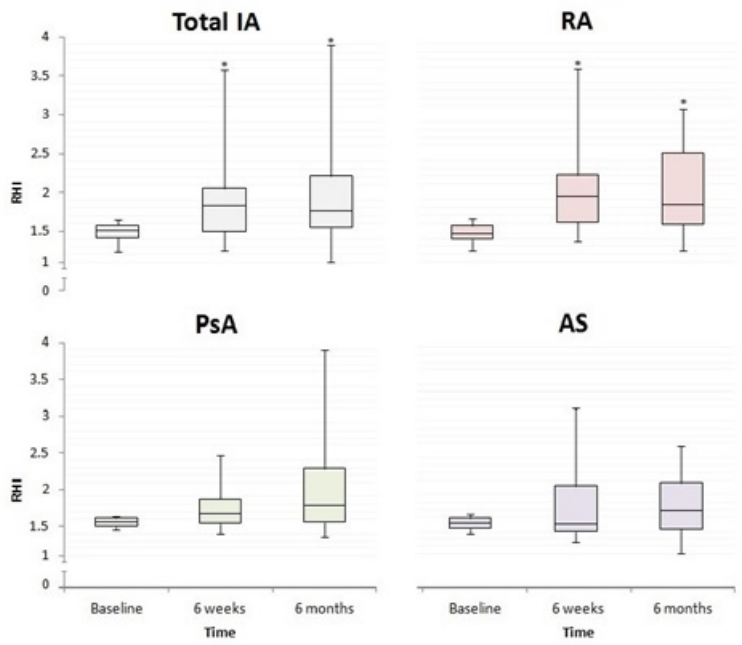

Figure 7: RHI values in RA, PsA, and AS patients with ED at all visits.

*p < 0.05 versus baseline. Midline represents median values. Bottom and top of the box represent 25 and 75 percentile and whiskers represent minimum and maximum values. IA, inflammatory arthritis; RA, rheumatoid arthritis; PsA, psoriatic arthritis; AS, ankylosing spondylitis; RHI, Reactive Hyperemic Index.

Significant EF improvement was observed at both follow-up visits compared to baseline in both treatment regimens (MTX: $p_{baseline\text{-}6weeks} = 0.002$, $p_{baseline\text{-}6months} = 0.001$, anti-TNF±MTX: $p_{baseline\text{-}6weeks} = 0.004$, $p_{baseline\text{-}6months} = 0.024$).

After 6 weeks of treatment, EF continued to improve in the MTX group but decreased in the anti-TNF± MTX group, resulting in a statistically significant difference in EF (RHI values) between the two treatment groups at 6 months (Figure 8). This difference remained statistically significant after adjustments for age, gender, rheumatic disease duration and diagnostic IA group.

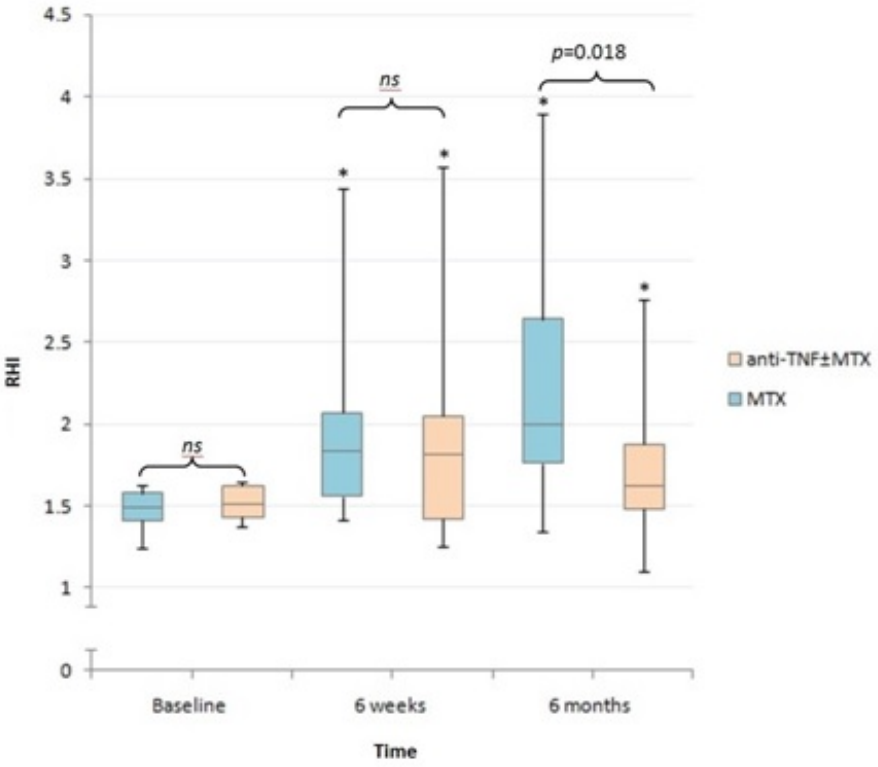

Figure 8: RHI values in patients with ED treated with MTX monotherapy or anti-TNF regimens.
*p<0.05 compared to baseline value. The lines inside of the boxes show the median; the whiskers of the boxes show maximum and minimum values. MTX, methotrexate; anti-TNF, anti-tumor necrosis factor; ns, not statistically significant; RHI, Reactive Hyperemic Index.

EF in forms of RHI and ED were not associated with any inflammatory marker, including CRP and ESR and any CVD characteristics, neither in simple nor multiple regression analysis.

Female gender was related to a greater improvement in EF between baseline and 6 months than male gender, independently of age, rheumatic disease duration and type of IA

diagnosis. Further, rheumatic disease duration was negatively related to RHI change$_{baseline-6months}$ and remained statistically significant in several multiple regression models including those adjusted for age, gender and diagnostic IA group and/or type of antirheumatic treatment.

4.3 Paper III

In the total IA group, median baseline level of s-selenium was 72 µg/L, and there were no significant differences in the baseline levels between the three diagnostic groups.

We examined the effect of antirheumatic treatment on s-selenium at baseline and both follow-up visits. In the total group, levels of s-selenium increased significantly from baseline to 6 weeks and from baseline to 6 months. Between 6 weeks to 6 months the selenium levels remained unchanged.

In the RA group, the increase from baseline was statistically significant after 6 weeks whereas in PsA after 6 months of treatment. The improvement in AS group did not reach the level of statistical significance at any point of time. Levels of s-selenium increased from baseline to both follow-up visits in both treatment regimens, but the improvements were statistically significant only in the MTX group (Figure 10).

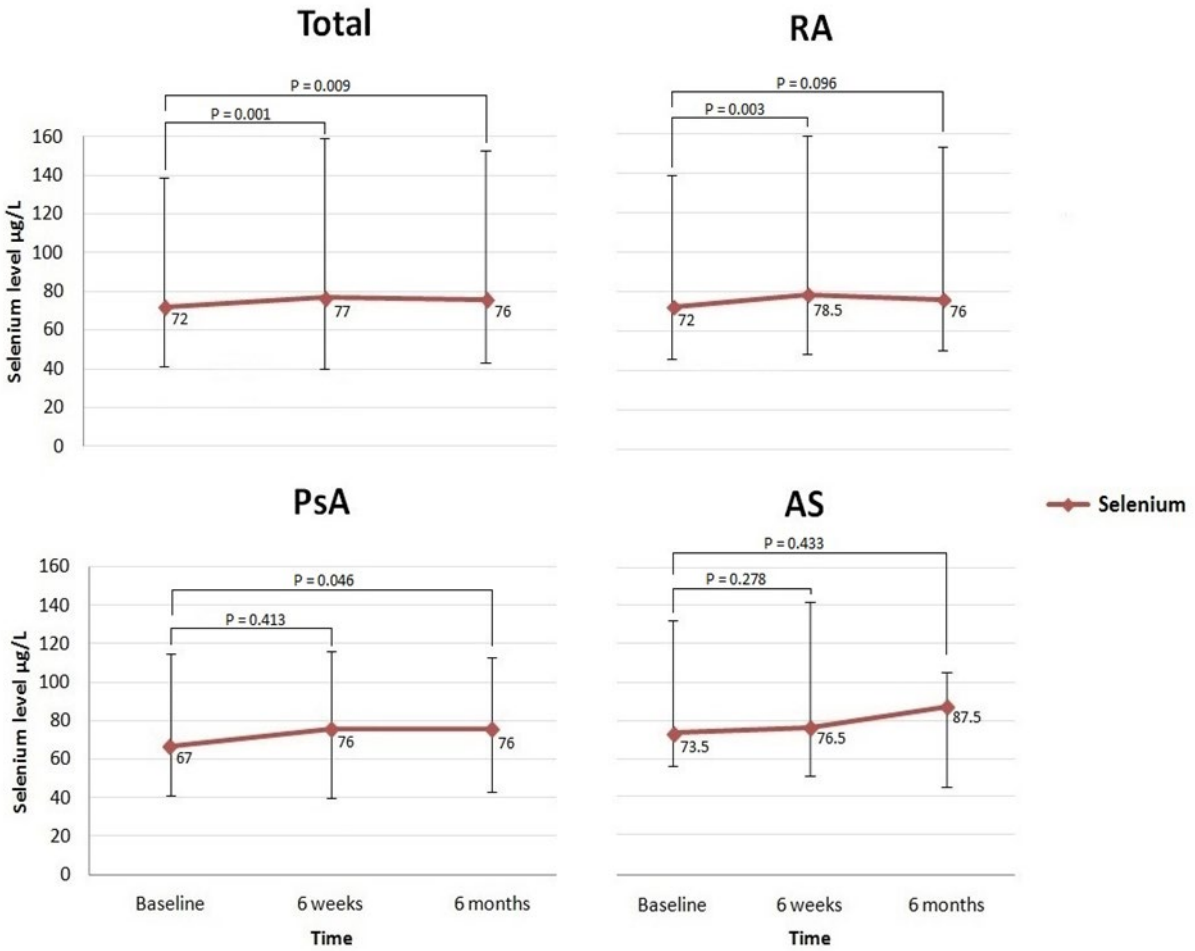

Figure 9: Changes in s-selenium levels in patients with RA, PsA and AS during treatment with MTX monotherapy or anti-TNF regimens.

Values are given as median and range (minimum-maximum).

IA, Inflammatory arthritis; RA, Rheumatoid arthritis; PsA, Psoriatic arthritis; AS, Ankylosing spondylitis.

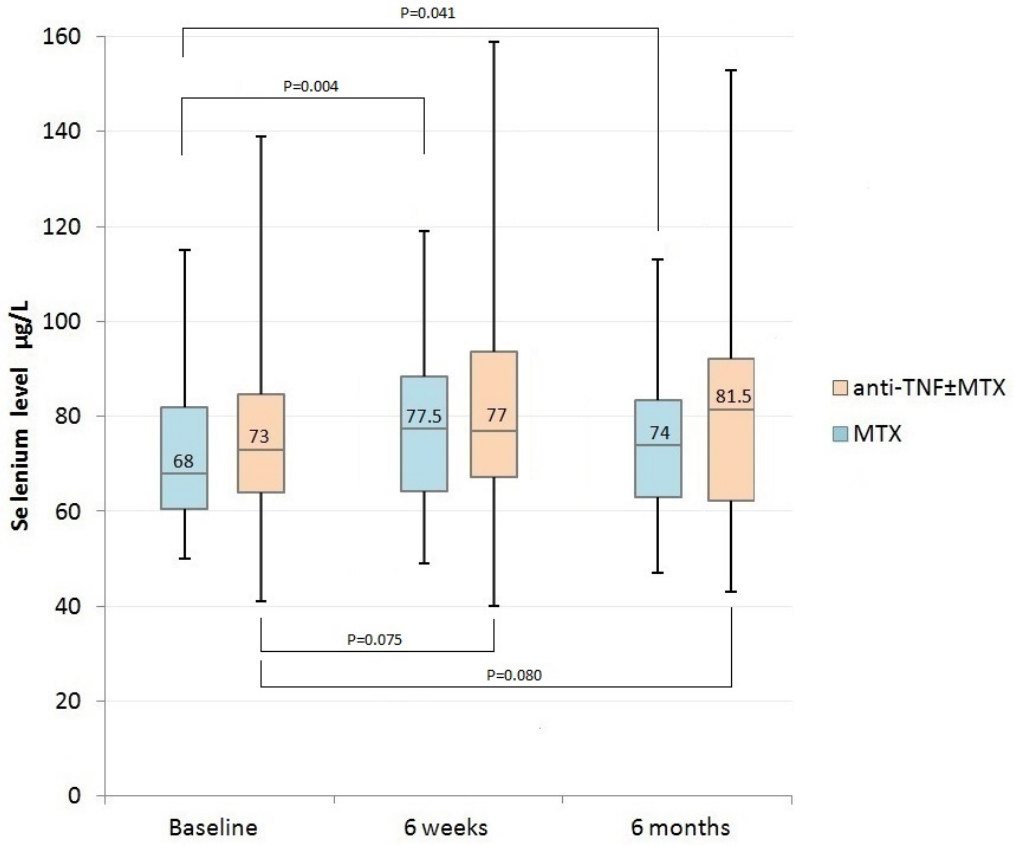

Figure 10: Changes in s-selenium levels in patients with RA, PsA and AS during treatment with MTX monotherapy or anti-TNF regimens.

Midline represents median values. Bottom and top of the box represent 25 and 75 percentile and whiskers represent minimum and maximum values. Anti-TNF, anti-tumor necrosis factor; MTX, methotrexate.

The change in s-selenium after 6 months of treatment was negatively associated with change in CRP and ESR in simple regression analyses. These associations remained statistically significant after adjustments for age and gender. Baseline s-selenium level were not statistically significantly related to CV risk parameters including EF, proportion of patients with ED, markers of disease activity, severity and duration or to medications. Similarly changes in s-selenium during therapy were not related to changes in any of the relevant variables at any visit.

5. Discussion

5.1 Methodological considerations

5.1.1 Study design

Due to ethical considerations, PSARA was designed as a longitudinal prospective observational study.

It was deemed unethical to perform randomized control trial (RCT) as randomization could lead to prescription of MTX monotherapy to patients non-responsive or intolerant to MTX, who were therefore in need of other DMARDs, such as anti-TNF drugs. On the other hand, randomization could also lead to overtreatment of patients who could be sufficiently treated with MTX monotherapy, and who would thus be unnecessarily exposed to potential anti-TNF related severe side-effects [132].

Consequently, we could not secure the same level of similarity between study groups at baseline as in an RCT. Nonetheless, observational studies have some advantages to RCTs and have therefore been increasingly called for during the last years. For example, they are easier to perform, and they can more accurately reflect the real life, and have greater reproducibility (in contrast to RCTs that often examine highly selected populations) [133-137]. Observational studies enable external validation of the data generated from RCTs. Moreover, they can help to plan future RCTs by generating hypotheses, detecting outcomes and providing information for sample-size calculation [138]. RCTs and observational studies complement each other, and both should be taken into consideration when evaluating treatment effects.

Longitudinal observational studies enable studying a group of individuals over an extended period of time, by collecting new data on the same variables for each time point. Thus, we were able to evaluate effects of two main antirheumatic regimens on several selected outcome variables in three common IA diseases over a 6 month period.

Prospective studies have clear advantages to retrospective studies. For example, results from prospective studies are less exposed to biases and confounding factors, in particular recall error. In retrospective designs the data collection might not have been designed to

answer the chosen research questions/hypotheses, and some variables influencing the outcomes might not have been collected, which can prevent appropriate adjustments for confounding factors [139].

Besides the longitudinal evaluation, our study design enabled comparison of the chosen groups in a cross-sectional manner. Consequently, we could search for differences between patients with active RA, AS and PsA at baseline. This cross-sectional evaluation is of importance as limited data exist on this area.

One of the main advantages of our study is the detailed clinical and laboratory characterizations of the patients that allows for a comprehensive evaluation of the study population.

5.1.2 Treatment groups

There are essential differences between IA patients starting with MTX monotherapy and those starting with anti-TNF in the clinical practice. For example, as chronic peripheral arthritis is usually treated by anti-TNF treatment only in patients where sufficient disease control cannot be achieved by synthetic DMARDs (in particular MTX), patients receiving anti-TNF are likely to have longer disease duration and a more aggressive and therapy-resistant arthritis [140, 141]. Thus, given the observational design of PSARA, the MTX and anti-TNF regimens were not directly comparable. Indeed, patients using anti-TNF in our study had longer disease duration and had used several DMARD regimens previously than those using MTX monotherapy. However, there were no other significant differences indicating higher IA severity and activity in the anti-TNF regimens. Conversely, patients using MTX monotherapy had higher PGA (reflecting higher self-perceived IA activity) than patients using anti-TNF. This could be explained by overrepresentation of AS patients in the anti-TNF group, but adjustments for diagnosis did not change the results.

To reduce confounding effects when comparing the two treatment regimens, we adjusted for baseline differences as well as other potential confounders using statistical methods.

Our main goal was to evaluate effects of antirheumatic treatment on selected parameters within IA. Therefore, we did not examine any control group without IA, and our study does not elucidate any differences between IA and non-IA individuals.

We were not able to evaluate differences in monotherapies with MTX or anti-TNF as most of the patients using anti-TNF also used MTX. All patients with peripheral arthritis starting with anti-TNF had used MTX previously without sufficient effect (MTX was given to these patients due to a standard procedure to avoid development of antibodies to anti-TNF). Thus, as all patients with peripheral arthritis treated with anti-TNF were MTX non-responders, it is likely that the effect of MTX on disease activity (and possibly other pathophysiologic pathways) in this group was relatively poor.

Reflecting the current guidelines, and in contrast to patients with RA and PsA, all AS patients in our study used anti-TNF in form of monotherapy. Three patients with AS had previously used MTX due to peripheral arthritis (MTX was later discontinued due to lack of effect, and anti-TNF treatment was given instead). The rest of the AS patients received anti-TNF as the initial treatment.

To reduce potential bias in assignment of patients to the respective treatment regimens, the treatment was decided by a physician not involved in the study.

5.1.3 Laboratory analysis

5.1.3.1 Endothelial function

EF was assessed using finger pletysmograph (EndoPAT2000), by calculating RHI (see chapter 3.5).

RHI has been shown to significantly correlate with findings from FMD of the brachial artery, which is commonly used for measuring EF in clinical studies [129]. Both FMD and EndoPAT are non-invasive methods that reflect bioavailability of NO, which is an essential molecule in vascular hemostasis and regulation of EF [142] . The advantage of EndoPAT is its relative simplicity, accessibility and reliability [143]. EndoPAT is user-friendly, and less operator-dependent than FMD. Further, in contrast to FMD, EndoPAT controls for concurrent non-

endothelial changes in the vascular tone (chapter 3.5), and is less time consuming [144]. Because EndoPAT recordings are sensitive to mental stress, anxiety, smoking, high blood glucose and other factors, our patients were examined under standard condition, including rest, fasting and non-smoking during pre-defined periods before the measurements [145-147].

5.1.4 Blood samples

Blood samples were taken and treated in a standardized manner. To minimize bias response, the samples were analyzed under standard conditions, in random order and by assessors blinded to clinical data. To reduce influence of food and stress on the blood markers, the patients fasted for 12 hours and had a rest period of 15 minutes before the blood draw. To avoid errors in measurements caused by thawing processes, no thawing was allowed until the desired analysis was performed. The frozen samples were analyzed in batches.

5.1.4.1 PTX3

Serum PTX3 levels were determined by the standard detection method, i.e. ELISA, at Humanitas Research Hospital, which has high expertise and long experience in detecting PTX3.
There was no cross-reaction with human CRP and serum amyloid P component protein and the used antibodies.

5.1.4.2 Selenium

In PSARA biobank, the blood samples were not collected on specific tubes for trace elements examination that have caps free of metals (to avoid contamination of the sample by metals). Thus, ideally, we should have included some blank samples, to ensure that there was no contamination of the examined samples. However, the blood samples were taken on regular serum tubes which do not contain any selenium contamination, and that are widely used for

selenium analyses. Atomic absorption spectrometry, which we used, is state-of-the-art method for selenium measurement [148].

5.1.5 Statistical considerations

When choosing the correct and most clinically meaningful approach to statistical analyses, many issues have to be considered. Although statistics as a science is based on exact calculations and formulas, medical expertise and logical thinking is necessary for both the choice of statistical methods and interpretations of their results. Hence, a close collaboration between statisticians and the responsible clinicians/scientists is warranted.
Our main variables of interest (PTX3, selenium and RHI) had skewed distributions, there were several outliers, and the sample size was limited, especially when analyses were stratified by diagnostic groups. Thus, after recommendations from the involved statistician, we chose to conduct all statistical analyses using non-parametric methods.
Moreover, decision-making about "normality" of variables is into a certain degree open for subjective interpretation. However, the non-parametric methods are more robust, i.e. statistically significant results from these tests are less likely to be achieved by chance and are likely to be confirmed with parametric methods. This is not true for parametric methods which rely heavily on the normality assumption and might lead to wrong results, especially in small samples [149].

In medical research, the level of statistical significance is usually set to 5% (with corresponding $p<0.05$ for determination of statistically significant results). In general, the significance level should be adjusted for each additional statistical test performed on the same dataset, to reduce the chance of type-I error and therefore of false positive conclusions. The significance level should be stricter if several hypotheses are tested simultaneously, to reduce the probability that the positive results are accidental [150]. However, this rule has not been universally applied [151]. Well-defined approaches and traditions exist for some situations, such as for comparisons of continuous variables in multiple groups using analysis of variance (e.g., Bonferroni correction) [152]. Also, in confirmatory RCTs with several primary outcomes or several treatments, and in exploratory

studies with large datasets (e.g., in genomics), it is generally required to use correction for multiple testing.
However, for some other tests (e.g., multiple crosstabs analyses) corrections for multiple testing have not been extensively applied, and there are no clear cutoffs for the total number of tests implying change of the level of statistical significance per article or study (e.g. in case of biobank research) [150].

In our exploratory study, we tested the effects of the chosen treatment regimens on the main variables and examined their relationships to other variables. Moreover, we performed several comparisons of baseline characteristics between the different patient groups. Therefore, in order to reduce the risk of type-I error, the significance level was set to 1% in *Paper I*. This approach on the other side naturally increased the chance of type-II error, i.e. false negative conclusions [153]. In analyses performed in *Paper I*, the conclusion was very similar when we used 1% significance level instead of 5%: only a few variables that were significant at 5% level did not reach the level of statistical significance at 1% level (in particular change in CRP in the AS group and changes in WBC, PGA, CRP and ESR in the PsA group). Nevertheless, we cannot be entirely sure which results correctly reflect the truth. It is possible that the stricter approach, i.e. 1% significance level, captured true lack of some differences, but also that it, due to type-II error, underestimated differences that were correctly apparent at 5% significance level. Of note type-II error is also more likely to occur in small samples.

In *Paper II* and *Paper III*, we chose to set the level of statistical significance at 5% as we slightly reduced the number of tests, and as we, for readability, wanted to adhere to the most commonly applied strategy in medical research. Nonetheless, independent of the level of statistical significance, it is generally important that the readers do not overinterpret the results, but keep in mind the aforementioned potential pitfalls of research analyses.

In summary, it is important to interpret statistically significant as well as non-significant results with caution. As in any study, our findings may be accidental or influenced by confounding factors and biases and limited power of the study. Therefore, further studies, are needed for their validation.

To partly compensate for the observational design of our study, we used multiple linear regression analyses to reduce effects of potential confounders. However, given the limited sample size of our study, we were able to adjust for only a relatively low number of confounders. Otherwise, our regression models would contain more independent variables than what could be justified by the sample size of the data set, resulting in "overfitted models" describing random error rather than relationships between the variables [154].

Moreover, it is important to realize that a complete exclusion of every potential confounder is impossible as some may not be detectable in a study while others may be unknown.

5.2 Discussion of results

5.2.1 PTX3

5.2.1.1 PTX3 levels in patients with IA

The median s-PTX3 baseline value in the total IA group was 3.9 ng/mL, and it stayed above the upper limit of the reference range (1-3 ng/mL) during the whole 6 month follow-up period [155]. These findings are in accordance with other studies reporting increased PTX3 levels in IRD, such as RA, PsA, AS, polymyalgia rheumatica, giant cell arteritis, systemic lupus erythematosus and small vessel vasculitis compared to individuals without these diseases [92, 156-161]. A systematic review also confirmed that both serum and plasma levels of PTX3 are increased in patients with autoimmune diseases compared to others [162].
The s-PTX3 values in this study differed from some of the previous observations. Our patients had higher median PTX3 levels than patients with IRD in a previous study by Hollan et al. (where median s-PTX3 levels was 1.85 ng/mL in RA, 1.55ng/mL in PsA and 2.72 in AS group), even though the s-PTX3 analyses were performed in the same laboratory, using the same method [92]. This might possibly be attributed to a higher disease activity in our patient group, as all PSARA patients were recruited at a rheumatology clinic, while they were in need of initiation of antirheumatic treatment due to active IA, (whereas the IA patients in the previous study by Hollan et al. were recruited in connection to their coronary artery bypass surgery, during a period of a relatively low disease activity).

On the other hand, although in our study the PsA group had the highest s-PTX3 levels at all visits (median PTX3baseline =4.2 ng/mL), their levels were far lower than those observed in a study by Okan et al. (median PTX3=11.21 ng/mL) [156]. These differences might potentially be caused by different laboratory techniques or geographic factors (e.g. diet, genetics or toxins), because also the control group in Okan`s study had higher PTX3 values (mean= 7.79 ng/mL) than the reference range in our laboratory.
Interestingly, the s-PTX3 level in our patient group were comparable to PTX3 levels in patients with active small vessel vasculitis in an Italian study (whereas patients with quiescent vasculitis had mean s-PTX3 levels =1.42 ng/mL while those with active disease had mean=4.97 ng/mL) [159].

There were no statistically significant differences in s-PTX3 at baseline between RA, PsA and AS patients, despite the differences in their demographic characteristics and several measures of IA disease activity, including ESR and PGA (which were the highest in RA).

Although the discrepancies in traditional disease activity markers between the three diagnostic groups might be accidental, they may also indicate that the respective diseases may be considered as active and at need of initiation of new antirheumatic therapy at different levels of some of the inflammatory measures, such as ESR (possibly due to differences in clinical picture and pathophysiology, including the involved immune pathways).

5.2.1.2 PTX3 and inflammation

Several markers of disease activity (CRP, ESR, WBC, PGA and PtGA) decreased already after 6 weeks of therapy and remained decreased for the remainder of the 6 month treatment period (Figure 6). However, in the PsA group, CRP, ESR, WBC and PGA levels did not decrease significantly at 6 weeks. Thus, MTX and/or anti-TNF appears to have less pronounced effect on inflammation, in terms of the aforementioned parameters, in PsA than in RA and AS [163].

Importantly, in spite of the apparent reduction of inflammation, there were no statistically significant changes in s-PTX3 levels in the total IA group and in the different diagnostic groups, neither after 6 weeks nor 6 months of antirheumatic treatment.

There are contradicting data on PTX3 role in IA and inflammation. Although some studies have shown that PTX3 might be a potential biomarker of disease activity in IA, others failed to confirm such relationship.

In our study we did not find any association between PTX3 and disease activity (including CRP and ESR), neither at baseline nor for changes during treatment. Hollan et al. and Sharma et al. found a correlation between serum and synovial PTX3 and CRP, respectively, in patients with RA [92, 160]. On the other hand, others did not find any positive correlation between PTX3 and inflammatory markers including CRP, ESR, WBC and neutrophil/leucocyte ratio in patients with AS, PsA, RA, small vessel vasculitis, Takayasu's arteritis and acute myocardial infarction [87, 157, 159, 161, 164-166]. Of note, even though PTX3 does not seem to be related to CRP in psoriasis, it is reportedly positively related to the extent and severity of skin psoriasis [167].

In a study by Ramonda et al. examining PsA patients, the PTX3 baseline levels did not differ from healthy controls and were not associated with disease activity. Surprisingly, after 24 months of anti-TNF treatment, the PTX3 values significantly increased compared to the baseline [168]. Ramonda et al. do not give a clear explanation for this phenomenon. Similar to Ramonda´s study, after a transient decrease in PTX3 levels during the first 6 weeks, the levels increased between 6 weeks and 6 months visits in all our diagnostic groups, and it even exceeded the baseline level in the PsA group (although these changes were not statistically significant). Thus, there is need for further research to explain the reason for the high and possibly even increasing levels in PsA patients.

MTX and anti-TNF inhibit inflammatory activity through different modes of action [17, 55]. TNF is one of the key pro-inflammatory cytokines involved in the pathogenesis of RA and other IA diseases, and TNF inhibition represents one of the most efficient and common types of current antirheumatic therapy [17, 56]. MTX, a folate-antagonist, is an anchor antirheumatic drug given as the drug of choice to most patients with newly detected chronic

peripheral arthritis. It appears to reduce disease activity by multiple actions, such as by inhibition of secretion of IL-1β and other pro-inflammatory molecules important in IA pathogenesis [55].

As proinflammatory cytokines can induce PTX3 expression, one might speculate that the high baseline s-PTX3 levels in our IA patients could be at least partly secondary to their high levels during an active disease phase. However, as neither TNF-inhibition nor MTX treatment seemed to reduce s-PTX3 levels, in spite of their anti-inflammatory effects, other factors than changes in levels of these cytokines are likely to substantially contribute to the excess PTX3 formation and secretion in our patient group [169]. In hypothesis, s-PTX3 might reflect an active inflammatory pathway that sustains PTX3 production even in patients apparently responding to the traditional antirheumatic therapy. Indeed, antirheumatic treatment does not eliminate the cause of these IA diseases, and usually does not lead to a total and permanent remission of the disease. Hence, further studies are needed to explore if PTX3 might be a marker of the residual inflammatory activity in patients with apparently satisfactory therapeutic response.

Taken together, our results indicate that s-PTX3 does not reflect the actual systemic disease activity in RA, PsA and AS patients, and disease activity amelioration after antirheumatic treatment, as defined by the traditional clinical and biochemical disease activity measures.

It is not clear what are the principal sources and triggers of the increased PTX3 levels in IA. PTX3 can originate from various cells involved in local inflammatory processes, e.g. from circulating neutrophils or from cells in the local tissue, e.g. in joints and in the cardiovascular system (see 1.8). One might speculate that the local PTX3 production may be sustained, e.g. due to persistent stimulation by certain triggers (e.g. ongoing localized inflammation), or by reduced inhibition of the PTX3 response, even in patients with low levels of systemic inflammation (not sufficient to induce high production of CRP in the liver and marked inflammatory symptomatology). Theoretically, the persisting production of PTX3 in IA could go on for example in vessels, as there are some indications that inhibition of inflammatory disease activity does not prevent progression of vascular damage in IA [170]. Hence, PTX3 might more reliably reflect subtle subclinical pathologic, and even pathogenetic, changes in IA compared to CRP. However, the origin of PTX3 excess in IA and the potential value of PTX3 as biomarker remain to be determined in other studies. Further, there is need to clarify the

roles of different possible triggers for the increased PTX3 production, such as autoimmune inflammation, infections (including subclinical infections due to IA-related dysregulation or immunosuppressive therapy, or infection agents playing a pathogenic role in IA), and atherogenic lipoproteins, and how PTX3 levels influence long-term outcomes in IA [91, 94, 96, 171, 172].

5.2.1.3 PTX and CV risk

In general, PTX3 is thought to be a strong predictor of CVD risk [173]. A recent study concluded that increased PTX3 levels may be associated with cardiovascular involvement in PsA patients independently of disease activity [174]. Consequently, one might expect a positive association between s-PTX3 and EF, as EF is related to CV risk [92, 155, 175]. However, this hypothesis is not supported by our data, e.g. due to possibility of type-2 error. One of the potential explanations for the lack of association between s-PTX3 and EF in our study could be that damaged endothelial cells may increase their PTX3 and maintain it for a long time. According to Bjorklund et al., endothelial cells from irradiated human artery express PTX3 even years after the irradiation [176]. In theory, this could suggest that damage to endothelial cells may result in prolonged PTX3 induction. However, as mentioned earlier, PTX3 might also stem from other cells and tissues and might be induced by various mechanisms (see 1.8).

It is important to keep in mind that the high CV risk associated with high PTX3 levels might be secondary to the trigger of PTX3 production and release, rather than to PTX3 itself (i.e., PTX3 might be just a bystander of this relationship). It is possible that the trigger of PTX3 expression might be involved in the pathogenesis of CVD and/or IA [88, 91].
In fact, in animal models, PTX3 administration has been observed to protect from CVD, probably via modulation of the complement cascade and immuno-inflammatory balance and other mechanisms [98, 103, 177]. If PTX3 have protective functions, then it is possible that the sustained and increased s-PTX3 production in IA might be beneficial, counteracting negative effects of the traditional and non-traditional CV risk factors [98, 100, 103].

Indeed, these hypotheses could not be answered by our study as it was not designed to examine the predictive role of PTX3 on CV events in our patient group. Thus, further research is warranted to clarify the impact of PTX3 on CV morbidity.

5.2.2 ED

5.2.2.1 ED in patients with IA

Among IA patients in need of antirheumatic treatment due to active disease, those with AS had the most pronounced alteration of EF and the highest CVD morbidity although they were younger, had similar disease duration and were more likely to use statins than the RA and PsA groups. In theory, this might be due to some AS-specific effects on ECs and/or due other factors such as increased occurrences of certain CV risk factors or higher use of drugs altering EF. For example, AS patients had the highest proportion of men and smokers, and the highest use of NSAIDs and coxibs [178].

5.2.2.2 ED, inflammation and CV risk

In IA patients with ED, both MTX monotherapy and anti-TNF regimens were associated with significant improvement of EF after 6 weeks of treatment. Between 6 weeks and 6 months, EF continued to improve only in the MTX group while it slightly declined in the anti-TNF group, leading to a significant difference in EF between the two groups at 6 months. The reduction of the effect of the anti-TNF regimens might perhaps be caused by the well-known secondary non-response effect due to the development of anti-drug antibodies [179]. Nonetheless, the level of EF at 6 months visit significantly exceeded its baseline levels also in the anti-TNF regimens (Figure 8).

The exact mechanism behind the protective effect of antirheumatic treatment on ED is not known [50]. It is commonly believed that the EF preserving effects of antirheumatic drugs could be mediated by inhibition of systemic inflammation and the associated metabolic abnormalities. However, this explanation is not supported by our findings as the EF improvement was independent of changes in systemic markers of disease activity, including

ESR and CRP. Moreover, we did not find any significant relationships between EF and any of these inflammatory markers at baseline.

In theory antirheumatic treatment could ameliorate EF through inhibition of local vascular inflammation (and the vascular inflammation might not be reliably reflected by levels of systemic inflammatory factors). Indeed, IA patients with CVD have been reported to have more inflammation, involving overexpression of TNF, in their vascular media and adventitia compared to their counterparts without IA [178, 180]. It might be that even inflammation located in deep vascular layers could affect the luminal part of the artery, including the function of the ECs [181]. In support of this notion, 8 weeks anti-TNF treatment was reported to reduce signs of vascular inflammation in RA patients, and this effect was related to improvement in EF [182].

Nevertheless, it is important to point out that our results cannot definitely rule out the potential involvement of inflammatory pathways in pathophysiology of ED and its reversal in IA.
ED, which occurs when the endothelium is activated, is characterized by overexpression of adhesion molecules such as ICAM-1, E-selectin and VCAM-1 [48]. These molecules facilitate migration of leucocytes into the vessel wall (including atherosclerotic lesions) and serve as circulating markers of ED [48, 183]. Previous studies have demonstrated the ability of both MTX and anti-TNF to downregulate the expression of these adhesion molecules on EC [17, 57-59].

Antirheumatic drugs could influence vascular health (including the integrity of ECs) also by other pathways, such as through improvement of cell cholesterol transport. Indeed, both MTX and anti-TNF therapy has been reported to improve lipoprotein functions and cell cholesterol handling, independently of their anti-inflammatory effects [184, 185].

Although there has been most focus on the importance of impaired cell cholesterol handling in the development of foam cells from macrophages in atherosclerotic plaques, the same mechanism can also underlie disturbances in ECs, leading to reduction of their vasodilating and anti-inflammatory functions [186]. In fact, increased cholesterol efflux from ECs is associated with higher NO expression and prostacyclin release (prostacyclin is a vasodilator

and it inhibits platelet activation) [187-189]. Thus, the improved cell cholesterol efflux due to antirheumatic treatment might protect from atheroma formation as well as from ED.

Taken together, there is still need for more research to determine the exact signaling pathways involved in pathogenesis of ED in IA, and how it can be mitigated by treatment.

We cannot definitely rule out the possibility that the observed differences in the effects of the two antirheumatic regimens on EF might be based on differences in patient populations or other factors. E.g., it might be that patients with longer and more therapy-resistant IA (which are features typical of patients requiring anti-TNF therapy) have a higher CV risk and are less likely to improve their EF by antirheumatic treatment than other IA patients.
Our results showed that the beneficial effects of antirheumatic treatment on EF were more pronounced in patients with shorter rheumatic disease duration than in those with longer disease duration. One could speculate that this phenomenon could therefore at least partly explain why patients treated with MTX monotherapy (who had shorter disease duration) experienced greater EF improvement than those treated with anti-TNF regimens. However, the difference in the effect of MTX monotherapy and anti-TNF regimens was independent of disease duration as well as gender distribution and other relevant factors.

Our observations indicating that women could be more susceptible to the EF-ameliorating effects of antirheumatic drugs than men warrant clarification in further studies.

5.2.3 Selenium

5.2.3.1 Selenium levels in patients with IA

In the present study, Norwegian patients with chronic IA had a median s-selenium level at 72 µg/L which was within the reference range (50-120 µg/L), but below the level that appears to be necessary for optimal protection against CVD (80-85 µg/L) [116, 190, 191].

In general, reference ranges reflect levels of the given parameters in the given populations. Thus, reference ranges for s-selenium levels mirror the actual situation in the given area, dependent on the local selenium intake, but not automatically the range that is necessary for maintenance of optimal health.

It is becoming increasingly apparent that the lowest levels of the current selenium reference ranges might not be sufficient for maintenance of ideal health [192]. Thus, there is a need to clearly define the recommended range of s-selenium, in order to secure appropriate selenium supplementation to counteract deficiencies.

Our results indicate that s-selenium levels in RA, PsA and AS are similar. Previous studies demonstrated that RA and PsA patients had statistically significant lower selenium levels than healthy individuals [193-196].
In 1978 Aaseth et al. reported that Norwegian patients with RA had s-selenium mean level at 93 µg/L, while the control group had 129 µg/L [193]. A study from USA reported that patients with RA had mean s-selenium level at 148 µg/L, while the control group had s-selenium levels of 160 µg/L. The discrepancies regarding s-selenium levels in RA patients between these studies may be caused by the fact that participants in the American study came from an area with relatively high selenium intake [194].

Further, the aforementioned studies were performed before the decrease in selenium content in some foods in Norway. Norwegians have become more self-sufficient on flour, growing their own grain that contains substantially less selenium than the previously imported flour from North-America, presumably partly explaining the difference in s-selenium levels between the patient group in the study by Aaseth et al. (93 µg/L) and our patient group (72µg/L) [197, 198].

To the authors knowledge, there is no information about selenium levels in AS.

5.2.3.2 Selenium and inflammation

The cause of lower selenium levels in IA patients compared to healthy individuals is unclear. Interestingly, there appears to be a reciprocal relationship between inflammation and selenium: while inflammation may lead to a decrease in selenium levels, low selenium levels may promote inflammation [109, 199, 200]. In fact, a study of 18 709 healthy subjects revealed that low s-selenium level was a risk factor for development of rheumatoid factor-negative RA [201].

Intriguingly, s-selenium levels in our study increased in all diagnostic groups with antirheumatic treatment already within 6 weeks and remained relatively stable for the remainder of the 6 month study period.
The underlying mechanism of this phenomenon is unknown. In theory, inflammation might reduce s-selenium levels due to increased turnover of selenoproteins and subsequent selenium depletion, or due to their reduced synthesis. Selenoprotein P, which constitutes the greatest part of s-selenium (60%), is synthesized in the liver, similar to acute phase reactants such as CRP [202-205]. Theoretically, the increased production of acute phase proteins in liver might inhibit the production of selenoprotein P during inflammation. In keeping with this notion, our results revealed that changes in s-selenium were negatively related to changes in systemic inflammatory biomarkers including CRP and ESR. Similar findings have been reported by others [200, 206].
It is known that selenoprotein P expression is down-regulated at the transcriptional level by proinflammatory cytokines. Thus, the increase in s-selenium levels upon anti-inflammatory treatment may be due to augmented selenoprotein P synthesis [207, 208].

Both MTX monotherapy and anti-TNF±MTX treatment were associated with increased s-selenium levels after 6 weeks as well as 6 months of therapy, but the improvements were statistically significant in the MTX group only.

Although we cannot rule out the possibility of a weaker effect of the anti-TNF regimens on selenium levels compared to MTX monotherapy, the lack of statistical significance might also be due to type-II error. In support of this notion, p values for differences between s-selenium levels at baseline and 6 weeks and between baseline and 6 months in the anti-TNF±MTX group were close to statistical significance ($p=0.075$ and $p=0.080$, respectively) (Figure 10). Additionally, the mean difference in s-selenium from baseline to 6 months was even greater in the TNF±MTX group than the MTX group.

One previous study examining Turkish RA patients did not find any effect of 1 month MTX treatment on selenium levels. However, the baseline mean s-selenium level was much higher in their study group (131.4 µg/dl) compared to ours. Indeed, it might be that antirheumatic treatment may improve s-selenium status only in patients with low s-selenium levels, while no further improvement is possible in individuals who already have

high s-selenium levels. However, the Turkish study should be interpreted with caution as the selenium levels were either extremely high or given in wrong units (µg/dl compared to µg/L in our study) [209].

Further studies are needed to elucidate if antirheumatic drugs improve selenium levels through a shared mechanism, e.g. through their anti-inflammatory effects, or if they convey specific actions that influence selenium homeostasis.

5.2.3.3 Selenium and CV risk

We did not find any significant associations between s-selenium and CV parameters such as CVD co-morbidity, traditional CV risk factors and EF and proportion of patients with ED. However, although our results do not indicate that low selenium levels are related to CV risk in IA, this notion cannot be definitely ruled out by our study.

Previously, low selenium levels have been reported to be associated with high CV and all-cause mortality risk [190, 210]. Selenium is suspected to exhibit its cardioprotective effects through various mechanisms, including the anti-platelet, anti-inflammatory and antioxidative functions. Of the antioxidative selenoproteins, glutathione peroxidase, thioredoxine reductase and selenoprotein P appear to have particularly important cardioprotective roles [211-213]. The potential role of selenium against atherosclerosis is also supported by a study demonstrating that supplementation with selenium and coenzyme Q10 for four years lead to persistent reduction in CV mortality even 12 years after the study ended [214].

The amount of selenium available in diets varies with geographic area, due to quality of soil where they are produced. Since the basic selenium intake in North-America is above 120 µg/day, further selenium supplementation is not expected to result in CV protection [215]. Thus, it might not be surprising that an American randomized control trial with 1250 participants, showed no statistically significant association between selenium supplementation and CVD morbidity and mortality [216]. In contrast, selenium and coenzyme Q10 supplementation lead to reduction of CV mortality in Swedish healthy

elderly individuals who had an estimated basic selenium intake as low as about 35 µg/day [116].

A recent meta-analysis based on 16 RCTs concluded that selenium supplementation might reduce inflammation, but is not sufficient to reduce CVD mortality [217]. However, most of the analyzed studies included participants from selenium-rich populations.

The discrepancies in interventions examining the effect of selenium supplementation on CV risk might be caused by differences in the examined groups, and the general selenium nutritional status. Furthermore, they may be due to differences in efficiency of the chosen selenium supplements, lack of a standardized method for measurement of selenium, or errors due to low sample power [71, 218]. Additionally, the U-shaped relationship between selenium and CV risk factors might also partly explain some of the conflicting results, as the cardioprotective effects of selenium decreases both with very low (below 40µg/L) and very high levels (over 150 µg/L) [106, 219].

It is not clear what is the clinical significance of the observed relatively small increase in s-selenium level in our group, particularly in individuals without any pronounced selenium deficit. Nevertheless, the observed statistically significant treatment-related changes in s-selenium levels may be of substantial importance as they may improve insights into the pathophysiological pathways in IA, and the pharmacological actions of antirheumatic drugs.

6. Prospective for further studies

As a common rule, it is important to validate findings and the generated hypotheses from an observational study in further studies designed for that purpose. There is a chance that both statistically significant findings as well as lack of significance might not be in accordance with the truth. Therefore, there is usually need for multiple studies examining the same topic.

Moreover, through our work we have identified some new questions that would be interesting to answer through future research:

- What is the trigger of s-PTX3 excess in IA, and does it mirror subclinical, residual inflammatory activity that is not detectable by other current inflammatory biomarkers?
- Could s-PTX3 be related to CV risk in IA, although we did not find any association with EF and other CV risk characteristics? For example, is s-PTX3 level related to prothrombotic factors or to development of CV events and mortality?
- Does s-PTX3 protect from CV risk in IA?
- Does MTX have a more sustained effect on EF than anti-TNF?
- Since EF improvement was independent of changes in inflammatory activity, which non-inflammatory processes mediate ED in IA?
- How does antirheumatic treatment induce inflammation-independent improvement in EF? Is it mediated by changes in cell cholesterol handling or other specific effects on vessel walls?
- If women are really more susceptible to improvement of EF with antirheumatic drugs, what is the underlying mechanism? Might it be used in development of new therapies?
- Does early efficient DMARD treatment protect from atherosclerosis through early beneficial effects on EF? What are the reasons that reversibility of ED is greater in IA patients with short disease duration than in those with longstanding disease?
- Does antirheumatic treatment influence selenium levels by their effect on inflammation, or by other mechanisms?

- Could s-selenium, possibly in a panel including other biomarkers, be used as a marker of response to antirheumatic therapy?
- What is the ideal level of s-selenium in order to convey optimal cardioprotective effects?
- Are levels below 80 µg/L, such as that found in our study, really insufficient for optimal protection against CVD?
- Does low s-selenium level contribute to accelerated CVD in IA?
- Would selenium supplementation have a protective role in IA patients with insufficient selenium levels?
- Which role does selenium play in the inflammatory process in IA?
- Does antirheumatic treatment convey specific actions that influence selenium homeostasis? Detailed insights into the pathophysiology of IA and the pharmacological actions of the current antirheumatic drugs can help to more fully utilize their potential, as well as define other therapeutic opportunities.

6.1 Conclusion

- Median s-PTX3 levels in patients with active IA was 3.9 ng/mL, i.e. above the upper limit of the reference range (1-3ng/mL). There were no differences in baseline s-PTX3 levels between RA, PsA and AS patients in our study.
- Levels of s-PTX3 in IA patients did not change statistically significantly neither with 6 weeks nor 6 months of antirheumatic treatment, and there was no difference between MTX monotherapy and anti-TNF regimens.
- Levels of s-PTX3 were not related to any of the examined IA characteristics (including CRP and ESR) and CV risk factors (including EF and ED). Further, changes in s-PTX3 were not related to changes in any of these markers.

- ED at baseline was observed in 35% of our IA patients. Patients with AS had the highest frequency of ED, even though they were the youngest.

- In patients with ED, EF improved already after 6 weeks of antirheumatic treatment and remained statistically significantly better also at 6 months visit. The improvement was statistically significant in the total IA and RA group.
- While MTX had a sustained beneficial effect on EF throughout the whole study period, the effect of anti-TNF regimens of EF slightly declined at 6 months of treatment.
- Among IA patients with ED, antirheumatic treatment induced greater EF improvement in women than in men, and in patients with shorter IA duration than in those with longer disease duration.
- There were no other significant relationships between EF and any of the relevant CV risk factors and IA characteristics including CRP and ESR, or between the respective changes in these parameters.

- Median s-selenium levels in patients with active IA were 72 µg/L, i.e. within the reference range (50-120 µg/L), but below the level of 80-85 µg/L that has been suggested as necessary for optimal protection against CVD. There were no significant differences in the baseline s-selenium levels between RA, PsA and AS patients.
- In the total IA group, levels of s-selenium increased statistically significantly already after 6 weeks of antirheumatic treatment and remained statistically significantly higher than at baseline for the remainder of the 6 month treatment period.
- The improvements in s-selenium levels were statistically significant in those treated with MTX monotherapy, but not in those treated with anti-TNF regimens.
- The s-selenium changes between baseline and 6 months were related to corresponding changes in CRP and ESR.
- There were no significant relationships between s-selenium and any of the selected CV risk factors.

6.2 Clinical implications

If the increased s-PTX3 levels in IA really reflect disease activity not inhibited by antirheumatic treatment and not reflected by traditional inflammatory biomarkers, PTX3 might have potential as a valuable biomarker of the residual disease activity.

Although we did not find any significant relationship between PTX3 and EF, the association between PTX3 and risk for CV events in IA cannot be ruled out by our study, e.g. due to possibility of type-2 error. Furthermore, PTX3 might reflect other components of the atherosclerotic process than ED (e.g., plaque inflammation and vulnerability, and development of cardiovascular events). Indeed, a large body of evidence indicates that PTX3 predicts CV prognosis if the general population. If this is true also for IA, increased levels of circulating PTX3 might help to estimate the real CV risk in IA.

Nevertheless, it is important to realize that in spite of its documented direct association to CV risk, PTX3 is likely to play a protective role in CVD.

Better insights into the role of PTX3 and the related biological pathways in IA and CVD may have implications for development of new targets for therapy.

Our study may help to increase awareness about the potential of antirheumatic treatment to improve EF, and therefore hinder atherosclerosis development in IA. If MTX really has a particularly sustained beneficial effect on EF, it might have implications for decision-making regarding treatment strategies.

Since our data indicate that antirheumatic drugs have greater effect on EF in IA patients with shorter than longer disease duration, we emphasize a need for early efficient antirheumatic treatment in order to minimize ED (while it is easily reversible), which could slow down atherosclerosis development.

Selenium levels below 80-85 µg/L have been suggested to augment CVD risk. If true, it might be reasonable to screen IA patients for their selenium levels (as their selenium levels might be low due to chronic inflammation), and potentially prescribe selenium supplements to those with selenium insufficiency. However, s-selenium levels in our cohort improved with

antirheumatic treatment, without any selenium supplementation. Thus, optimal selenium levels in IA might be achieved primarily by adequate disease control.

The association between inflammation and low s-selenium levels, and the s-selenium increasing effect of antirheumatic treatment, may help to better understand the pathophysiology of IA as well as modes of actions of antirheumatic drugs, and potentially help to develop new therapeutic options.

Similar to PTX3, in spite of the lack of a significant relationship between s-selenium and EF, our study does not definitely exclude the role of selenium deficiency in CVD. If selenium deficiency promotes development of CVD in IA as it appears to do in the general population, it might have a potential as a CV risk marker.

Further studies may clarify if s-selenium might help to estimate inflammatory activity and response to therapy in IA, e.g. as a part of a test panel including multiple parameters.

Moreover, improved insights into the selenium dependent pathophysiologic processes may help to direct development of new CV protective strategies.

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Papers I-III

Paper I

Gia Deyab, Ingrid Hokstad, Jon Elling Whist, Milada Cvancarova Smastuen, Stefan Agewall, Torstein Lyberg, Barbara Bottazzi, Pier Luigi Meroni, Robert Leone, Gunnbjorg Hjeltnes and Ivana Hollan. **Anti-rheumatic treatment is not associated with reduction of pentraxin 3 in rheumatoid arthritis, psoriatic arthritis and ankylosing spondylitis**. *PloS one. 2017;12: e0169830.*
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The 14.sentence page 14. Typing error: should state 450 **nm** instead of 450 **mm**.

Paper 2

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Paper 3

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Paper I

Gia Deyab, Ingrid Hokstad, Jon Elling Whist, Milada Cvancarova Smastuen, Stefan Agewall, Torstein Lyberg, Barbara Bottazzi, Pier Luigi Meroni, Robert Leone, Gunnbjorg Hjeltnes and Ivana Hollan. **Anti-rheumatic treatment is not associated with reduction of pentraxin 3 in rheumatoid arthritis, psoriatic arthritis and ankylosing spondylitis**. *PloS one. 2017;12:e0169830. DOI: 10.1371/journal.pone.0169830*

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RESEARCH ARTICLE

Anti-rheumatic treatment is not associated with reduction of pentraxin 3 in rheumatoid arthritis, psoriatic arthritis and ankylosing spondylitis

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Abstract

Background

Pentraxin 3 is proposed to be a marker of inflammation and cardiovascular risk, but its role in inflammatory rheumatic diseases (IRDs) is still uncertain. Therefore, we wanted to examine if anti-rheumatic treatment reduced serum PTX3 (s-PTX3) levels in IRDs, and if s-PTX3 levels were related to other markers of inflammation and to endothelial function (EF).

Methods

We examined s-PTX3, EF and established inflammatory biomarkers in 114 IRD patients from the PSARA study before and after 6 weeks and 6 months of treatment with methotrexate (MTX) or anti-tumor necrosis factor alpha (anti-TNF) therapy with or without MTX co-medication.

Results

s-PTX3 levels in all IRD diagnoses were above the upper limit of the reference range. In contrast to established inflammatory markers, in particular CRP and ESR, s-PTX3 levels did not change significantly after 6 weeks and 6 months of anti-rheumatic therapy. There was no difference in change in s-PTX3 levels from baseline to 6 weeks and 6 months between MTX

Competing Interests: The authors have declared that no competing interests exist.

monotherapy and anti-TNF regimens. CRP, ESR and EF were not related to changes in s-PTX3 neither in crude nor adjusted analyses.

Conclusion

IRD patients have increased s-PTX3 levels, which, in contrast to other inflammatory markers, do not seem to improve within 6 months of therapy with MTX and/or anti-TNF. Thus, s-PTX3 might reflect a persisting immune process, even a causal factor of inflammation, not inhibited by the standard anti-rheumatic treatment. Furthermore, even though s-PTX3 is thought to be a strong predictor of cardiovascular prognosis, it was not related to EF.

Introduction

Patients with inflammatory rheumatic diseases (IRDs) have increased cardiovascular (CV) morbidity and mortality, predominantly due to accelerated atherosclerosis. The reason to premature cardiovascular disease in IRDs has not been fully clarified, but immune dysregulation and inflammation appear to play important roles [1, 2].

Inflammation is known to be involved in the pathogenesis of all stages of the atherothrombotic process, from initiation of endothelial dysfunction (ED), to atheroma formation, plaque destabilization and thrombogenesis [3]. It is well known that increased levels of systemic inflammatory biomarkers, such as C-reactive protein (CRP), predict cardio vascular disease (CVD) development and are related to CVD severity [4]. During the last years, there has been increasing interest in another protein from the pentraxin family (which CRP belongs to), i.e. pentraxin 3 (PTX3). There is evidence suggesting that PTX3 might be at least as good independent predictor of CV risk as CRP [5–7]. In contrast to CRP, which is produced in the liver upon stimulation by interleukin-6 (IL-6), PTX3 is produced directly in the inflamed tissue. Furthermore, it is stored in granules of neutrophils, ready to be rapidly released upon microbial stimulation [8–10]. Thus, the PTX3 response is faster than the CRP response, and PTX3 is thought to more accurately reflect the actual inflammatory situation [11].

PTX3 is produced in the vessel wall in response to pro-inflammatory cytokines such as interleukin-1beta (IL-1β) and tumor necrosis factor alpha (TNF) [12]. For example, PTX3 has been observed in atherosclerotic plaques, and there are theories suggesting that systemic PTX3 levels might be a useful indicator of acute coronary syndrome, because of its reflection of vascular inflammation [3, 13–15].

Similar to CRP, PTX3 is a pattern recognition molecule of the immune system, and has multiple important functions, including anti-microbial effects, participation in clearance of apoptotic cells, and regulation of inflammation [8]. Several studies have reported increased PTX3 levels in IRDs. Some of these studies suggested that PTX3 might be related to the increased CV risk in IRD [8, 16, 17]. However, the real role of PTX3 in inflammation and premature CVD in IRD has not been fully elucidated yet. For instance, it is still unknown how PTX3 responds to anti-rheumatic treatment, and whether it might be used as a biomarker of IRD activity and CV risk.

Therefore, the aim of this study was to examine if anti-rheumatic treatment in form of methotrexate (MTX) and/or anti-TNF (anti-TNF) reduced serum PTX3 (s-PTX3) levels in IRDs, and if s-PTX3 levels were related to other inflammatory markers, and to endothelial function (EF).

Patients and methods

Patients

A total of 140 patients, 74 with rheumatoid arthritis (RA), 40 with psoriatic arthritis (PsA) and 26 with ankylosing spondylitis (AS) were enrolled in the PSoriatic arthritis, Ankylosing spondylitis, Rheumatoid Arthritis (PSARA) study at Lillehammer Hospital for Rheumatic Diseases between October 2008 and May 2010. The study was retrospectively registered with the following trial registrations: Clinicaltrials (NCT00902005); The Norwegian Regional Ethical Committee (S-07377b) and the Norwegian Biobank register (2054). Written consents were obtained from all patients included in the study.

Inclusion criteria were as follows: males and females with age range 18–80 years; PsA according to Moll and Wright 1973 criteria [18], AS according to the modified New York diagnostic criteria for ankylosing spondylitis [19] or RA according to the ACR 1987 criteria [20]; clinical indication for starting with either MTX monotherapy or anti-TNF treatment with or without MTX co-medication (anti-TNF±MTX). Women with childbearing potential had to use a reliable method of contraception.

Exclusion criteria included lack of co-operability, any contraindication for MTX and anti-TNF, any significant infection (including subclinical tuberculosis), immunodeficiency, pregnancy or breastfeeding, congestive heart failure, uncontrolled diabetes mellitus, recent stroke (within 3 months), demyelinating disease, use of systemic glucocorticoid $>$ 10 mg/day during the last 2 weeks or anti-TNF during the last 4 weeks before the inclusion, malignancy and any chronic inflammatory disease other than RA, AS or PsA.

The patients were examined at baseline and after 6 weeks and 6 months of treatment. Of all included patients (140), 114 completed the 6 months follow-up. The reasons for dropout were as follows: side-effects in 12 patients, insufficient treatment response in 11 patients, hepatitis C in 1 patient, failure in logistics in 2 patients (patients were not summoned for follow- up).

Treatment

The type and doses of anti-rheumatic treatment were decided by clinical rheumatologists not involved in the study, upon clinical judgment, and in accordance with the Norwegian guidelines. Doses were as follows: etanercept 50 mg subcutaneous (SC) injection once a week, adalimumab 40 mg SC injection every other week, infliximab 3–5 mg/kg intravenous injection at baseline, then following standard dosing regimen. MTX doses were 15–25 mg orally once a week.

Clinical guidelines consider MTX as first line of anti-rheumatic treatment in RA and some other IRDs, especially in those with peripheral joint arthritis [21]. On the other hand, due to limited effect of conventional disease modifying anti-rheumatic drugs (DMARDs), including MTX, in axial spondyloarthritis (including AS and PsA), TNF inhibition is the treatment of choice in most patients with axial spondylarthritis who do not sufficiently respond to non-steroidal anti- inflammatory drugs (NSAIDs) [22, 23].

Clinical tests

The data collection included demographic data, medical history, life-style information and medication (including previous and current use of DMARDs and systemic glucocorticosteriods, NSAIDs, statins and other drugs known to affect the cardiovascular system).

At all three visits, EF was assessed by the Reactive Hyperemia Index (RHI) measured by a fingertip plethysmograph (EndoPAT 2000; Itamar). A finger probe were placed on the index fingers of each hand and a blood pressure cuff was placed on the right upper arm, while the

other arm functioned as the control arm. The right upper arm was occluded for 5 min and then released. The RHI was calculated as the ratio between the average post-obstructive pulse wave amplitude (PWA) and the average of pre-occlusion PWA. This is described in more details in Hjeltnes et al, and Onkelinx et al [24, 25]. Endothelial dysfunction was defined as RHI <1.67, in accordance with the cut-off level determined for patients at risk for coronary artery disease [26]. Furthermore, the patients were examined by several self-reported and clinical instruments for evaluation of their disease activity and severity adequate for their condition such as Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), Bath Ankylosing Spondylitis Patients Global Score (BAS-G), Bath Ankylosing Spondylitis Metrology Index (BASMI), Bath Ankylosing Spondylitis Functional Index (BASFI), Medical health Assessment Questionnaire (MHAQ), Disease Activity Score for 28 joints (DAS28), Physicians' Global Assessment Score of disease activity (PGA) and Patients' Global Assessment Score of disease activity (PtGA) (Table 1).

Blood samples

Blood samples were drawn after fasting for 8 hours (including non-allowance of smoking). Routine hematological and biochemical tests including erythrocyte sedimentation rate (ESR), white blood cells (WBC) and CRP, were performed at all visits using test standards of the local hospital laboratory. Furthermore, small aliquots of serum and plasma were stored at -80˚C for later analyses (including PTX3 analysis). PTX3 levels were determined in serum by a home-made sandwich enzyme-linked immunosorbent assay based on the murine monoclonal antibody MNB4 as capturing antibody, and a rabbit antiserum (pAb) raised against human PTX3, affinity purified and biotinylated, as detection antibody [8, 27]. Samples were assessed in batches and in random order, by assessor blinded for clinical data. The procedure was performed as described for human plasma, with the only addition of a preincubation step of serum samples with Polybrene-EDTA. Briefly 1μl Polybrene-EDTA (2.5% polybrene; 2.5% EDTA in phosphate buffered saline without calcium and magnesium, pH 7.4) was added to 50μl of undiluted serum samples and incubated for 10 minutes at room temperature. Human serum samples were then diluted and added to MNB4 coated wells. Absorbance at 450 mm was measured after incubation with pAb and then streptavidin-horse radish peroxidase. Mean PTX3 content was calculated converting Abs450 values to protein concentration by means of a standard curve with recombinant purified human PTX3 (range from 75 pg/ml to 2.4 ng/ml). The assay has a sensitivity of 100 pg/ml and interassay variability ranges from 8% to 10%. No cross-reaction was observed with CRP and serum amyloid P component.

Statistical analyses

As all continuous variables were not normally distributed, non-parametric tests such as Mann-Whitney U test and Wilcoxon sign test were applied for comparisons between and within the examined groups. Chi-square test was used for comparison of categorical data between the study groups. Linear regression analyses were used to assess associations between s-PTX3 and selected laboratory and clinical variables. The multiple regression models were adjusted for the central variables of interest and for age and gender (as gender and age are known to influence PTX3 levels) and for baseline characteristics that were statistically significantly related to PTX3 in simple regression analysis and in analysis adjusted for age and gender only [7]. We performed two multiple linear regression models: one to investigate if s-PTX3 was independently related to EF (Model I) and one to examine if s-PTX3 was independently related to established inflammatory markers (CRP and ESR) (Model II).

All analyses are considered exploratory; however due to multiple testing, P-values ≤ 0.01 were considered statistically significant, and all statistical tests were two-sided.

Table 1. Baseline patient characteristics.

Characteristics	RA (n = 64)	PsA (n = 30)	AS (n = 20)
Age	57 (28–79)	*50 (23–78)**	*49 (30–72)¥*
Rheumatic disease duration, years	2 (0–30)	3 (0–37)	3 (0–40)
Male (gender), n (%)	17 (27)	*17 (57)**	*16 (80)¥*
Current smokers, n (%)	20 (31)	7 (23)	10 (50)
Hypertension, n (%)	17 (27)	7 (23)	6 (30)
Diabetes mellitus, n (%)	3 (5)	0 (0)	1 (5)
Methotrexate, n (%)	34 (53)	16 (53)	*0 (0)ϕ¥*
Endothelial dysfunction, n (%)	23 (36)	8 (27)	9 (45)
PTX 3 (ng/mL)	3.8 (1.2–22.1)	4.2 (1.1–14.7)	4.0 (1.9–8.5)
CRP (mg/L)	8 (1–78)	5 (1–99)	10 (1–157)
WBC (10^9/L)	7.25 (3.7–11.3)	6.3 (4.2–11.3)	*7.9 (4.7–12.3)ϕ*
ESR (mm/h)	18.5 (1–81)	*7 (2–48)**	*9.5 (2–87) ¥*
Total Cholesterol (mmol/L)	5.2 (2.8–8.7)	5.3 (3.8–7.1)	4.9 (2.9–7.9)
LDL (mmol/L)	3.2 (1.1–5.8)	3.4 (2.4–4.9)	2.8 (1.6–5.2)
HDL (mmol/L)	1.4 (0.9–2.8)	1.3 (0.7–2.9)	1.25 (0.8–2.5)
Triglycerides (mmol/L)	1.2 (0.5–2.8)	0.96 (0.6–3)	1.25 (0.7–2.1)
HbA1C (%)	5.7 (4.9–8.9)	5.5 (4.6–6.4)	5.6 (4.9–6.9)
Fasting serum glucose (mmol/L)	5.1 (4.2–8.6)	5.1 (4.4–6.9)	5 (4.5–8.4)
Uric acid μmol/L	271 (151–499)	296 (152–566)	310 (182–410)
BMI (kg/m^2)	26 (19–41)	26 (19–39)	28 (22–36)
Previously used DMARDs, n (%)	40 (63)	15 (50)	2 (10)
MHAQ	0.65 (0–1.45)	0.40 (0.05–1.55)	0.43 (0–1.40)
PGA ¤	38(7–73)	*21 (0–57)**	*26 (3–60)¥*
PtGA ¤	52 (5–98)	44 (2–96)	56 (6–96)
Number of swollen joints	6 (0–28)	*2 (0–8)**	*0 (0–6)¥ϕ*
BASDAI	-	4.7 (0.3–9.5)	5.1 (0.9–9.6)
BASFI	-	3,1(0–7.2)	4.1 (1.1–7.6)
BASMI	-	-	3 (0–10)
DAS 28	4.98 (2.6–7.3)	-	-

Unless indicated otherwise, values are given as median (range).

RA, rheumatoid arthritis; PsA, psoriatic arthritis; AS, ankylosing spondylitis; PTX3, pentraxin 3; CRP, C-reactive protein; WBC, white blood cells; ESR, erythrocyte sedimentation rate; LDL, low-density lipoprotein; HDL, High-density lipoprotein; HbA1C, glycated haemoglobin; BMI, body mass index; MHAQ, Medical Health Assessment Questionnaire; PGA, Physicians' Global Assessment Score of disease activity; PtGA, Patients' Global Assessment Score of disease activity; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; BASMI, Bath Ankylosing Spondylitis Metrology Index; DAS 28, Disease Activity Score for 28 joints.

* $p < 0.01$ for comparisons between the RA group and the PsA group.

¥ $p < 0.01$ for comparisons between the RA group and the AS group.

ϕ $p < 0.01$ for comparisons between the PsA group and the AS group.

¤ On a 100-mm visual analog scale.

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All analyses were performed in IBM SPSS statistics, Version 23.

Results

Patient characteristics

Baseline characteristics are described in Table 1. Gender and age in the RA group differed significantly from the AS group and PsA group. AS patients had the highest proportion of

patients with ED, and PsA patients the highest level of s-PTX3, but none of these differences were statistically significant. RA patients had significantly higher levels of PGA than PsA and AS patients. Number of swollen joints (NSJ) differed significantly in all patient groups (highest in RA and lowest in AS).

MTX was initiated in about half of the RA and PsA patients, but in none of the AS patients (as a result of guidelines for treatment of these diseases).

Changes in established markers of disease activity and PTX3 during anti-rheumatic treatment

Fig 1 shows the changes in s-PTX3, WBC, CRP, ESR, PGA and PtGA at all visits. In the entire patient sample, the median s-PTX3 levels were above the upper limit of the reference range (1–3 ng/ml) at all visits. For the total IRD group and for all the three diagnostic groups, there was a tendency towards decrease in s-PTX3 levels after 6 weeks of treatment, and towards increase from 6 weeks to 6 months of treatment; however, none of these changes reached the level of statistical significance.

In the total IRD group and in the RA group, CRP, ESR, WBC, PGA and PtGA levels decreased significantly after 6 weeks of therapy ($p<0.001$ for all). Of these variables, only the PGA and PtGA in RA and in the total IRD group ($p<0.003$ for both) decreased further from 6 weeks to 6 months, while no additional decrease in CRP, WBC and ESR levels was observed in any of the examined groups. In the PsA group, no statistically significant changes in any of the examined markers were revealed, except for the PtGA (Fig 1). The AS group had a statistically significant decrease in all of the examined markers from baseline to 6 weeks, except for CRP and s-PTX3 (Fig 1).

Effects of MTX monotherapy and anti-TNF±MTX treatment on s-PTX3

There were no statistically significant changes in the s-PTX3 levels between patients treated with MTX and anti-TNF±MTX in the total IRD group and in the RA and PsA groups (not shown).

As all AS patients were treated with anti-TNF, no comparison was possible between the two treatment regimens in this group.

Relationship of other clinical and laboratory factors to PTX3

In univariate regression analyses, there were no associations between s-PTX3 and CRP, ESR, RHI, PGA, PtGA, NSJ, and WBC (Table 2).

Furthermore, s-PTX3 was not related to hemoglobin, thrombocyte count and neutrophil count, IRD duration or CV risk factors, neither in crude analyses nor in analyses adjusted for age and gender and inflammatory markers. There were no statistically significant associations between s-PTX3 and DAS28 for RA patients, and BASDAI and BASFI for AS and PsA patients, in neither univariate analyses nor analyses adjusted for age and gender.

Model I: Neither there were significant relationships between s-PTX3 and RHI after adjustments only for age and gender. RHI was not related to s-PTX3 in analyses adjusted for age, gender, IRD and traditional CV risk factors (smoking, BMI, hypertension, hyperlipidemia and hypercholesterolemia). Similar results were obtained when we evaluated EF in terms of ED (dichotomous variable) instead of RHI (continuous variable).

Model II: Neither there were significant relationships between s-PTX3 and ESR and CRP in analyses adjusted only for age and gender. s-PTX3 was not statistically significantly related to neither CRP nor ESR in analyses adjusted for age, gender and ESR and CRP.

IRD was not related to s-PTX3 in any of the multiple regression models.

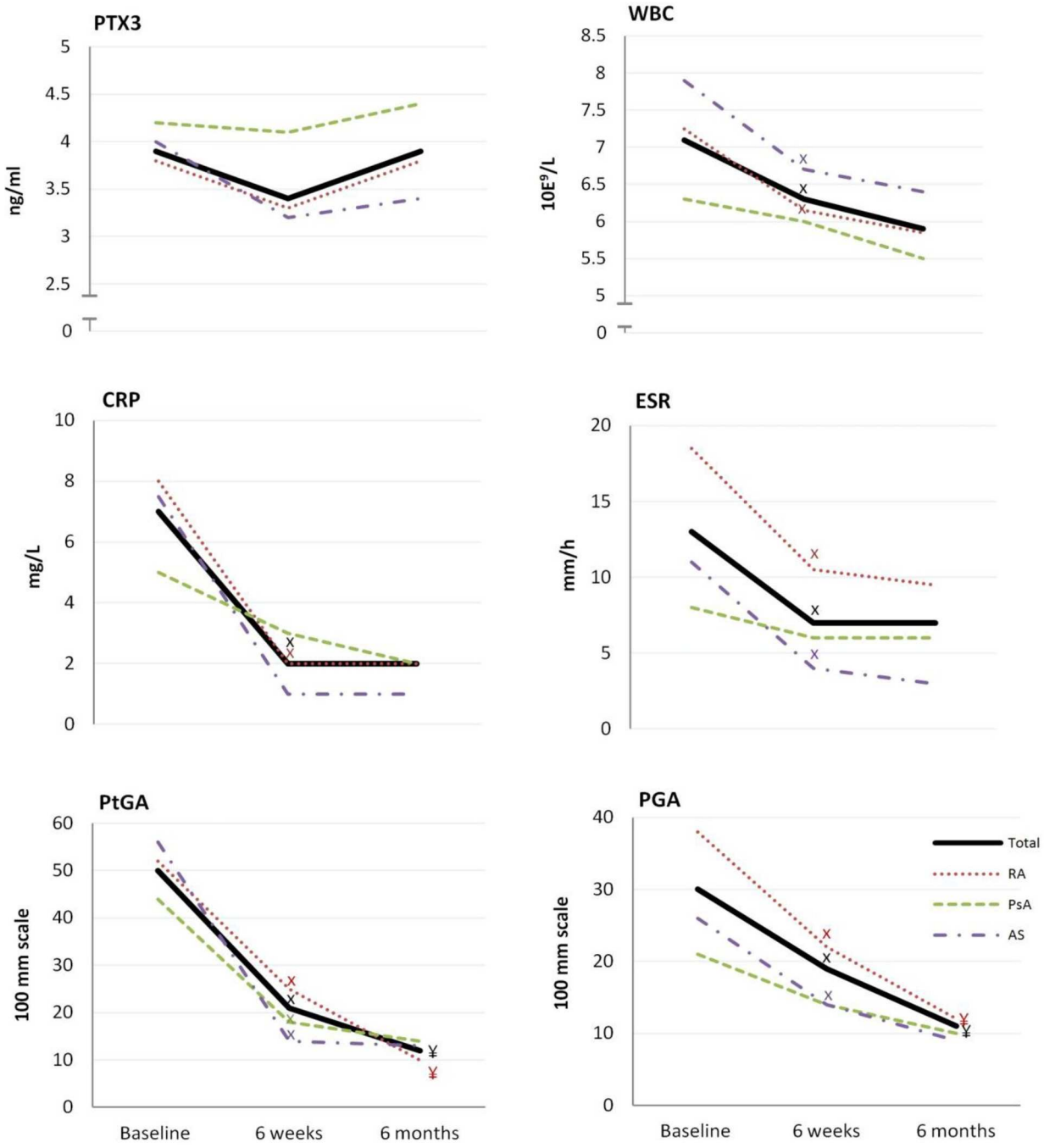

Fig 1. PTX3 and established markers of disease activity at baseline, 6 weeks and 6 months of treatment. Values are given in median. IRD, inflammatory rheumatic disease; RA, rheumatoid arthritis; PsA, psoriatic arthritis; AS, ankylosing spondylitis; PTX3, pentraxin 3; WBC, white blood cells; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; PGA, Physicians Global Assessment Score of disease activity; PtGA, Patients' Global Assessment Score of disease activity. X $p<0.01$ for difference between the evaluation at baseline and at 6 weeks ¥ $p<0.01$ for difference between the evaluation at 6 weeks and 6 months.

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Discussion

The main findings in this novel study were as follows: 1) s-PTX3 levels did not change significantly with anti-rheumatic treatment, in contrast to other inflammatory markers and clinical

Table 2. Predictors of PTX3.

	Unadjusted analyses			Adjusted analyses			Adjusted analyses		
				Model 1			Model 2		
	B	95% CI	p-value	B	95% CI	p-value	B	95% CI	p-value
Age	-0.033	-0.070 to 0.005	0.090	-0.037	-0.075 to 0.002	0.062	-0.032	-0.073 to 0.009	0.126
Gender	0.656	-0.187 to 1.500	0.126	0.789	-0.078 to 1.656	0.074	1.086	0.180 to 1.992	*0.019*
CRP	0.004	-0.017 to 0.025	0.709				0.023	-0.005 to 0.052	0.109
ESR	-0.013	-0.038 to 0.012	0.305				-0.037	-0.074 to 0.000	0.048
WBC	-0.003	-0.271 to 0.265	0.982						
RHI	-0.231	-1.230 to 0.768	0.647	-0.070	-1.064 to 0.925	0.890			
PGA	0.014	-0.011 to 0.039	0.270						
PtGA	-0.005	-0.023 to 0.013	0.575						
NSJ	0.145	-0.075 to 0.365	0.192						

Predictors of PTX3. CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; RHI, reactive hyperaemic index; PGA, Physician's global assessment of disease activity; PtGA, Patient's global assessment of disease activity; NSJ, number of swollen joints.
Model 1; $R^2 = 0.048$, Model 2; $R^2 = 0.054$.

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disease activity measures. 2) There was no difference in the effect of MTX monotherapy and anti-TNF ±MTX treatment (in MTX failures) on s-PTX3 levels in RA and PsA patients. 3) s-PTX3 was not statistically significantly related to other systemic inflammatory markers. 4) s-PTX3 was not statistically significantly related to EF.

The reference range of s-PTX3 applied by the laboratory was 1–3 ng/mL [7]. The median s-PTX3 baseline value in the studied IRD group was 3.9 ng/mL, and it stayed above the upper limit at all visits. Other studies have shown that PTX3 levels are higher in several IRDs (RA, PsA, AS, polymyalgia rheumatica, giant cell arteritis, systemic lupus erythematosus and small vessel vasculitis) compared to control groups [8, 16, 17, 28, 29]. A recent systematic review also confirmed that both serum and plasma levels of PTX3 in autoimmune diseases were significantly higher than in normal controls [30].

However the s-PTX3 values in the present study were different from those observed in other studies. Compared to the IRD patients in the study of Hollan et al. (PTX3 mean = 2.1 ng/ml), our patients had higher s-PTX3 values, even though the s-PTX3 analyses were performed in the same laboratory, using the same method [8]. This might possibly be attributed to a higher disease activity in the current patient group, as all patients, recruited from a rheumatology clinic, were in need of initiation of anti-rheumatic treatment due to active IRD, while the IRD patients in Hollan et al. previous study were recruited with connection to their coronary artery bypass surgery, during a period of a relatively low disease activity.

Compared to Okan et al., who measured PTX3 values in PsA (median = 11.21 ng/ml), our patients had lower s-PTX3 levels [16]. These differences might most likely be due to different laboratory techniques, because also the control group had higher PTX3 values than the reference range in our laboratory.

The median s-PTX3 levels in our patient population were comparable to the patients with small vessel vasculitis (mean = 3.24 ng/ml) in the study by Fazzini et al. [29].

There were no statistically significant differences in s-PTX3 at baseline between RA, PsA and AS patients (Fig 1), despite the differences in their demographic characteristics and measures of IRD disease activity, including ESR and PGA (Table 1).

These differences might be accidental, but they may also be due to a real difference in these systemic inflammatory markers at the point of time when these patients were in need of initiation or intensification of their anti-rheumatic therapy.

Several markers of disease activity (CRP, ESR, WBC, PGA and PtGA) decreased rapidly after 6 weeks and 6 months of therapy indicating reduction of inflammation, while there was no statistically significant change in s-PTX3 levels in the total IRD group, nor in the different diagnostic groups after 6 weeks and 6 months of anti-rheumatic treatment.

The PsA group had the highest s-PTX3 levels at all visits, and at 6 weeks, CRP, ESR, WBC and PGA levels had not decreased significantly, in contrast to the RA and AS group (except for CRP in AS group; see limitations. Thus, inflammation, in terms of these parameters appeared to be less susceptible to MTX or anti-TNF±MTX treatment in PsA than in RA and AS [31]. This remains to be examined in further studies.

Notably, s-PTX3 levels were not positively related to traditional markers of disease activity, such as CRP and ESR, at baseline. Furthermore, the changes in s-PTX3 between baseline and 6 weeks and 6 months visits were not related to changes in CRP and ESR during the same period of time. Our findings are in accordance with previous studies that did not find any positive correlation between PTX3 and other inflammatory markers including CRP, ESR and WBC in patients with PsA, small vessel vasculitis, Takayasu's arteritis, acute myocardial infarction patients and AS [17, 29, 32–34]. However, although PTX3 does not seem to be related to CRP in psoriasis, it is reportedly positively related to the extent and severity of skin psoriasis [35].

In a study of PsA patients, their PTX3 baseline levels did not differ from healthy controls.

However, after 24 months of anti-TNF treatment, the PTX3 levels significantly increased within the PsA group compared to the baseline[36].

Taken together, our results indicate that s-PTX3 does not reflect the actual systemic disease activity in RA, PsA and AS patients, and disease activity amelioration after anti-rheumatic treatment, determined by the traditional clinical and biochemical measures of disease activity.

However, it is important to keep in mind that the anti-rheumatic treatment does not target the cause of these IRDs, and that it usually does not lead to a total and permanent remission of the disease activity (characterized by a total absence of any inflammatory activity).

Thus, in hypothesis, s-PTX3 might reflect an active inflammatory pathway that sustains PTX3 production even in patients apparently responding to the traditional anti-rheumatic therapy. Hence, further studies are needed to explore if PTX3 might be a marker of the residual inflammatory activity in patients with apparently satisfying therapeutic response.

It is not clear what are the principal sources and triggers of the increased PTX3 levels in IRDs. PTX3 might originate from various cells involved in local inflammatory processes, e.g. from circulating neutrophils, in joints and in the cardiovascular system. In theory, the residual production of PTX3 in IRD might occur in vessels, as there are indications that inhibition of inflammatory disease activity does not prevent progression of vascular damage in PsA [37].

Further studies are needed to clarify the roles of different possible triggers for the increased PTX3 production, such as autoimmune inflammation, infections (including latent infection due to IRD-related dysregulation or immunosuppressive therapy), and atherogenic lipoproteins [12, 13, 38, 39].

Based on our results, we cannot say with certainty what is the reason to persistent high PTX3 levels in patients with apparent low disease activity as determined by the common measures, such as CRP. However, one might speculate that the local/neutrophilic PTX3 production may persists, e.g. due to persistent stimulation by certain triggers or by reduced inhibition of the PTX3 response, even in patients with low levels of systemic inflammation (not sufficient to induce high production of CRP in the liver and marked inflammatory symptomatology). It is possible that PTX3 mirrors an underlying pathology of IRD, which is not well-reflected by the established disease activity markers, such as CRP. Hence, PTX3 might have an advantage in reflecting subtle pathologic, and even pathogenetic, changes in IRD compared to CRP.

Though, this hypothesis has to be tested in further studies as our study is not designed to give an answer to this question.

PTX3 is thought to be a strong predictor of CV risk. Consequently, we expected to find a positive association between s-PTX3 and EF [6–8]. To our knowledge, the association between PTX3 and EF has not been examined before. Our data do not support the notion that PTX3 might be a good biomarker for ED and the associated CV risk. A hypothetical explanation for the lack of association between s-PTX3 and EF, could be that damaged endothelial cells increase and maintain their PTX3 production. According to Bjorklund et al., endothelial cells from irradiated human artery express PTX3 even years after the irradiation [40]. In theory, this could suggest that damage to endothelial cells may result in prolonged PTX3 induction, perhaps to protect the vasculature. However, as mentioned above, PTX3 may also stem from other cells and tissues, such as from inflamed joints.

MTX and anti-TNF inhibit inflammatory activity through different modes of action [41, 42]. TNF is one of the key pro-inflammatory cytokines involved in the pathogenesis of RA and other IRDs, and TNF inhibition represents one of the most efficient and common types of current anti-rheumatic therapy [41, 43]. MTX, a folate-antagonist, is an anchor anti-rheumatic drug given as the drug of choice to most patients with newly detected chronic peripheral arthritis. It appears to reduce disease activity by multiple actions, such as by inhibition of secretion of IL-1β and other pro-inflammatory molecules [42]. Thus, one might speculate that the high s-PTX3 levels in our IRD patients with active disease might be partly secondary to their high levels of pro-inflammatory cytokines, e.g., TNF and IL-1β. However, as neither TNF-inhibition nor MTX treatment seemed to reduce s-PTX3 levels, other factors than these cytokines are likely to significantly contribute to the excess PTX3 formation and secretion in our patient sample [44].

Because PTX3 is an important molecule of the innate immunity response, protecting against pathogens, its increase might even reflect an underlying, currently unknown, cause of IRDs, such as an ongoing infection. Thus, there is a need to examine in further studies how PTX3 levels influence long-term outcomes in IRD, and what it is induced by.

In theory, the high CV risk associated with high PTX3 might be secondary to the trigger of PTX3 production and release, rather than to PTX3 itself (i.e., PTX3 might be just a bystander of this relationship). It is possible that the trigger of PTX3 expression might be involved in the pathogenesis of CVD and/or IRD [27].

In fact, in animal models, PTX3 administration has been observed to protect from CVD, probably via modulation of the complement cascade and immuno-inflammatory balance and other mechanisms [45, 46]. Hence, it is possible that the maintenance of high PTX3 production in IRDs might be beneficial, counteracting negative effects of the traditional and non-traditional CV risk factors [45, 46].

Limitations

Our study is burdened by common disadvantages of an observational study, such as differences in baseline characteristics between the groups as the patients were not randomly selected. However, it has been increasingly recognized that observational studies also possess advantages compared to randomized control trials, e.g., increased reproducibility due to a real-life population, better safe-guarding of ethical principles as they allow for providing optimal individualized treatment for the patients, etc. [47].

To compensate for baseline differences between the groups, we adjusted for several baseline characteristics in multiple regression models.

It is important to keep in mind that there are essential differences between IRD patients starting with MTX monotherapy and those starting with anti-TNF. For example, as MTX is

the drug of choice in most patients with peripheral chronic arthritis, patients with these conditions who receive anti-TNF treatment are likely to have longer disease duration, and a more severe disease, more refractory to anti-rheumatic therapy (Table 1).

We cannot evaluate differences in monotherapy with MTX or anti-TNF as most of the patients using anti-TNF also used MTX co-medication. Nevertheless, all the PsA and RA patients using anti-TNF were MTX-failures, i.e. they did not get a sufficient effect of MTX prior to the initiation of anti-TNF treatment. Thus, it is likely that the MTX effect on disease activity in the IRD group is relatively poor, and that the MTX is provided first of all to reduce side-effects of anti-TNF therapy.

The p-value was set to 0.01, to decrease the risk of Type-one error (false positive findings). On the other hand, this approach increases the chance of Type-two error (false negative findings). For example, the lack of significant decrease in CRP in the PsA and AS group during the treatment is likely to be due to this phenomenon (as the findings would be significant at 5% level of significance).

A great advantage of our novel study is a well-characterized study population, and design that makes it possible to compare the effect of two of the main anti-rheumatic treatment regimens on s-PTX3 in three common IRDs.

Conclusion

In conclusion, our data revealed that anti-rheumatic treatment with MTX and TNF±MTX did not affect the increased s-PTX3 levels in IRD patients. s-PTX3 was not related to any established markers of disease activity. It is therefore possible that s-PTX3 might reflect a persisting immune process, even a causal factor of the inflammation, not inhibited by the standard anti-rheumatic treatment.

Furthermore our data do not support the notion that s-PTX3 might be a good biomarker of CV risk in IRD as it was not related to EF.

Supporting information

S1 File. SPSS file. SPSS file containing all data underlying the statistical analysis performed in this study.
(SAV)

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Paper 2

Gia Deyab, Ingrid Hokstad, Jon Elling Whist, Milada Cvancarova Smastuen, Stefan Agewall, Torstein Lyberg, Nicoletta Ronda, Knut Mikkelsen, Gunnbjorg Hjeltnes and Ivana Hollan. **Methotrexate and anti-tumor necrosis factor treatment improves endothelial function in patients with inflammatory arthritis.** *Arthritis Research & Therapy (2017) 19:232. DOI: 10.1186/s13075-017-1439-1*

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Methotrexate and anti-tumor necrosis factor treatment improves endothelial function in patients with inflammatory arthritis

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Abstract

Background: Inflammatory arthritis (IA), including rheumatoid arthritis (RA), ankylosing spondylitis (AS) and psoriatic arthritis (PsA), leads to increased cardiovascular disease occurrence probably due to atherosclerosis. One of the first stages in atherogenesis is endothelial dysfunction (ED). Therefore, we aimed to compare endothelial function (EF) in patients with IA, and to examine the effects of methotrexate (MTX) monotherapy and antitumor necrosis factor (anti-TNF) treatment with or without MTX comedication (anti-TNF ± MTX) on EF.

Methods: From the PSARA observational study, all patients with RA ($n = 64$), PsA ($n = 29$), and AS ($n = 20$) were evaluated for EF. In patients with ED at baseline ($n = 40$), we evaluated changes in the Reactive Hyperemic Index (RHI) after 6 weeks and 6 months of antirheumatic therapy.

Results: In IA patients with ED, RHI significantly improved after 6 weeks ($p < 0.001$) and 6 months ($p < 0.001$) of treatment, independent of changes in disease activity parameters. After 6 months, RHI had improved more in the MTX group than in the anti-TNF ± MTX group, and the difference remained statistically significant after adjustments for potential confounders. Among patients with active RA, AS, and PsA, those with AS appeared to have the worst endothelial function, although they were the youngest.

Conclusion: Treatment with MTX and anti-TNF ± MTX was associated with a relatively fast improvement of EF in IA patients with ED, independent of change in disease activity. Therefore, modes of action other than the anti-inflammatory effect may contribute to the EF improvement. After 6 months, the EF improvement was more pronounced in the MTX group than in the anti-TNF ± MTX group.

Trial registration: Clinicaltrials, NCT00902005. Registered on 13 May 2009.

Keywords: Inflammatory arthritis, Methotrexate, Anti-tumor necrosis factor, Rheumatic arthritis, Spondyloarthritis

Background

Inflammatory arthritis (IA), including rheumatoid arthritis (RA), ankylosing spondylitis (AS), and psoriatic arthritis (PsA), has increased cardiovascular (CV) morbidity and mortality, probably due to cardiovascular disease (CVD) caused by atherosclerosis [1–5]. The first step in the development of atherosclerosis is endothelial dysfunction (ED) which is initially a reversible process [6]. Thus, improving endothelial function (EF) might be of great importance in preventing atherosclerosis. The endothelium has several vital homeostatic functions, including regulation of vascular tone and growth, thrombogenesis and thrombolysis, and interactions between platelets and leukocytes and the vessel wall. The endothelium secretes vasorelaxing (e.g., nitric oxide) and vasoconstricting (e.g., endothelin-1) substances in response to mechanical stress [6, 7]. ED is characterized by impaired ability of the artery to dilate in response to physical and chemical stimuli [8, 9].

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Assessment of EF has been used to estimate the CV risk in IA patients [10, 11].

Clinical studies indicate that antirheumatic treatment, including methotrexate (MTX) and antitumor necrosis factor (anti-TNF) treatment, not only ameliorates disease activity but also reduces CV morbidity and mortality in RA patients [12, 13]. There is also evidence that anti-TNF treatment improves EF in RA, and reduces arterial stiffness and intima-media thickness in patients with RA, PsA, and AS [14, 15].

However, information on the effect of antirheumatic drugs on EF in AS and PsA patients is still limited. Therefore, the aim of this study was to compare EF in RA, AS, and PsA patients, and to examine the effect of antirheumatic treatment (MTX and/or anti-TNF) on EF in these patient groups.

Methods

Patients

We examined patients from the PSoriatic arthritis, Ankylosing spondylitis, Rheumatoid Arthritis (PSARA) study who completed 6 months of follow-up and in whom EF was measured ($n = 113$). Of the 114 patients who completed the study, one PsA patient was excluded because she was not able to adhere to the requirements of the EF measurement (smoked and did not sit still).

All patients in PSARA, an observational study, had been included at the Lillehammer Hospital for Rheumatic Diseases as described elsewhere [16]. Briefly, the inclusion criteria were: males and females with an age range 18–80 years; and PsA according to the Moll and Wright 1973 criteria [17], AS according to the modified New York diagnostic criteria for AS [18], or RA according to the ACR 1987 criteria [19], and clinical indication for starting with either MTX monotherapy or anti-TNF treatment with or without MTX comedication (anti-TNF ± MTX).

Exclusion criteria included lack of cooperability, any contraindication for MTX and anti-TNF, any significant infection (including subclinical tuberculosis), pregnancy or breastfeeding, congestive heart failure, use of systemic glucocorticoids > 10 mg/day during the last 2 weeks or anti-TNF during the last 4 weeks before the inclusion, and any chronic inflammatory disease other than RA, AS, or PsA.

All patients were Caucasian and were examined at baseline and after 6 weeks and 6 months of treatment.

Treatment

Patients were either treated with MTX monotherapy or with anti-TNF ± MTX. The type and doses of antirheumatic treatment were decided by rheumatologists not involved in the study upon clinical judgment and in accordance with Norwegian guidelines. Doses were as follows: etanercept 50 mg subcutaneous injection once a week; infliximab 3–5 mg/kg intravenous injection at baseline, then following standard dosing regimen; adalimumab 40 mg subcutaneous injection every other week; MTX 15–25 mg orally once a week.

Norwegian guidelines consider MTX as a first-line antirheumatic treatment in patients with chronic peripheral arthritis, in particular RA [20]. Due to limited effects of conventional disease-modifying antirheumatic drugs (DMARDs) in axial spondyloarthritis (SpA), including AS and PsA, TNF inhibition is used in SpA patients with axial disease who do not sufficiently respond to nonsteroidal anti-inflammatory drugs (NSAIDs) [21, 22]. Throughout the study period, patients using glucocorticoids were kept on a steady dose (10 mg or less per day).

Clinical and laboratory tests

Data collection included demographic data, medical history, physical findings, lifestyle information and medication.

EF was examined, and blood samples were drawn after fasting for 8 h (including nonallowance of smoking), and hospital routine blood tests were consecutively performed.

EF was evaluated using a reactive hyperemia peripheral arterial tonometry (RH-PAT) examination which evaluates the overall health of the endothelium by measurment of finger arterial pulsatile volume changes as described previously [23]. The Reactive Hyperemic Index (RHI) was calculated as the ratio between the magnitude of the average postobstructive pulse wave amplitude (PWA) and the average of baseline PWA (preocclusion). ED was defined as RHI ≤ 1.67 as recommended by the manufacturer and in accordance with findings from a population at risk for ischemic heart disease [23]. RHI results for a subgroup of our RA sample have been published previously [24].

We evaluated improvement in RHI only in patients with ED, as a significant improvement in RHI could not be expected in patients with normal EF.

Statistics

For comparisons of continuous independent variables between and within the examined groups, nonparametric tests (Mann-Whitney U test and Wilcoxon sign test) were applied, since the continuous variables of interest were not normally distributed (according to normality plots). For comparison of categorical data between the study groups, the Chi-square test was used. Linear regression analyses were used to assess associations between RHI change modeled as the dependent variable (baseline and 6 months) and selected disease activity markers. Age, gender, rheumatic diagnosis, and variables that showed a significant association with the dependent variable in simple regression analyses were included in multiple linear regression models.

P values ≤ 0.05 were considered statistically significant, and all statistical tests were two-sided. Our analyses

were considered exploratory so no correction for multiple testing was performed.

All analyses were performed using IBM SPSS statistics, version 23.

Results

Baseline patient characteristics

Baseline clinical and cardiovascular characteristics of all patients who completed the 6 months of follow-up are described in Tables 1 and 2.

The anti-TNF ± MTX and MTX groups had similar characteristics except for a significantly shorter rheumatic disease duration and higher Physicians' Global Assessment (PGA) score in the MTX group ($p = 0.043$ and $p = 0.002$, respectively). The proportion of patients with ED was similar in both treatment groups.

Although patients with AS were the youngest (statistically significantly younger than the RA group), they had higher frequency of ED, angina pectoris, myocardial infarction, and use of some cardiovascular drugs (beta blockers, statins and warfarin) compared to the RA and PsA groups (these differences did not reach the level of statistical significance).

The AS group had the lowest median RHI value, which was significantly different from the PsA group ($p = 0.040$; Fig. 1). The proportion of women was highest in the RA group and lowest in the AS group (Table 1).

When evaluating only patients with ED, there were no statistically significant differences in RHI baseline values between any of the three diagnostic groups.

RHI improvement in patients with ED

In the total IA group with ED ($n = 40$), RHI significantly improved from baseline to 6 weeks ($RHI_{6weeks} = 1.86$, $p < 0.001$), and from baseline to 6 months ($RHI_{6months} = 1.80$, $p < 0.001$) (Fig. 1). RHI baseline levels are described in Table 2.

The RHI improvement was most pronounced at 6 weeks. At 6 months, the RHI median level slightly, but statistically nonsignificantly, decreased again (Fig. 1).

In analyses of all three diagnostic groups with ED, only RA patients showed statistically significant RHI improvement from baseline to 6 weeks (RHI at 6 weeks = 1.96, $p < 0.001$) and baseline to 6 months (RHI at 6 months = 1.86, $p = 0.001$; Fig. 1). The PsA group showed RHI improvement at both visits (RHI at 6 weeks = 1.67 and RHI at 6 months = 1.80). In the AS group the RHI levels slightly decreased from baseline to 6 weeks (RHI at 6 weeks = 1.50). However, after 6 months of treatment, the RHI levels increased again (RHI at 6 months = 1.68). None of the RHI changes in the PsA and AS groups reached statistical significance.

Effect of MTX and anti-TNF ± MTX on RHI in patients with ED

In both treatment groups, RHI significantly improved at both follow-up visits compared to baseline (MTX: baseline to 6 weeks $p = 0.002$, baseline to 6 months $p = 0.001$; anti-TNF ± MTX: baseline to 6 weeks $p = 0.004$, baseline to 6 months $p = 0.024$). After 6 months of treatment, RHI values in the MTX group continued to increase compared to 6 weeks. However, in the anti-TNF ± MTX group RHI values at 6 months were lower than at 6 weeks, resulting in a statistically significant difference in RHI values between the two groups at 6 months (Fig. 2).

Within the RA and PsA groups there were no significant differences in RHI between patients treated with MTX and anti-TNF ± MTX.

Linear regression analysis

Our data did not reveal any statistically significant associations between RHI and inflammatory markers, including C-reactive protein (CRP), white blood cell (WBC) count, erythrocyte sedimentation rate (ESR), pentraxin (PTX)3, Modified Health Assessment Questionnaire (MHAQ), PGA, or Patients' Global Assessment Score of Disease Activity (PtGA), at baseline (data not shown).

In simple regression analyses, only female gender and rheumatic disease duration were significantly related to RHI change from baseline to 6 months, while age, IA diagnosis, changes in markers of IA activity and severity (CRP, WBC count, ESR, PTX3, MHAQ, PGA, and PtGA) (Table 3), traditional CV risk factors (smoking, hypertension, diabetes, body mass index, established CVD (history of previous myocardial infarctions and presence of angina) and medications (statins, angiotensin converting enzyme inhibitors, and calcium antagonists); data not shown) were not.

Female gender was related to a greater improvement in RHI compared to male gender, and the association remained statistically significant in several multiple regression models including models adjusted for age, rheumatic disease duration, and IA diagnosis. Rheumatic disease duration was negatively related to RHI change from baseline to 6 months and it stayed statistically significant in several multiple regression models (adjusted for age, gender, and IA diagnosis and age, gender, and treatment).

The difference in RHI change from baseline to 6 months between the MTX group and the anti-TNF ± MTX group remained statistically significant after adjustments for age, female gender, rheumatic disease duration, and IA diagnosis (Table 3).

Corrections for baseline RHI values

In analyses adjusted for baseline RHI values, MTX was associated with a greater improvement in RHI than anti-TNF ± MTX after 6 months in patients with ED ($p = 0.007$).

Table 1 Baseline clinical characteristics for all patients

	RA (*n* = 64)	PsA (*n* = 29)	AS (*n* = 20)	MTX (*n* = 49)	anti-TNF ± MTX (*n* = 64)
Age (years)	**57 (28–79)**	**50 (23–78)**[¥]	**49 (30–72)**[ϕ]	56 (28–79)	55 (23–75)
Women, *n* (%)	**47 (73)**	**12 (41)**[¥]	**4 (20)**[ϕ€]	30 (61)	33 (52)
Rheumatic disease duration (years)	2 (0–30)	3 (0–37)	3 (0–40)	**0.10 (0–25)**	**3.7 (0–40)***
Disease activity					
CRP (mg/L)	8 (1–78)	5 (1–99)	10 (1–157)	8 (1–99)	6.5 (1–157)
ESR (mm/h)	**18.5 (1–81)**	**7 (2–48)**[¥]	**9.5 (2–87)**[ϕ]	14 (1–81)	13 (2–87)
Anti-CCP, *n* (%)	39 (61)	–	–	17 (35)	22(34)
Rheumatoid factor IgA, *n* (%)	32 (50)	–	–	15 (31)	17 (27)
Rheumatoid factor IgM, *n* (%)	45 (70)	–	–	22(45)	23(36)
BASDAI	–	4.73 (0.3–9.5)	5.1 (0.9–9.6)	5.5 (0.3–9.3)	5.1 (0.9–9.7)
BASFI	–	3.2(0–9)	4.1 (1.1–7.6)	3.0 (0–9)	3.8 (0.4–8.6)
BASMI	–	–	3 (0–10)	–	3 (0–10)
DAS28	4.98 (2.6–7.3)	–	–	5.2 (3.1–7.3)	5.1(2.6–7.1)
PtGA	52 (5–98)	44 (2–96)	56 (6–96)	52 (2–96)	49 (6–98)
PGA	**38 (7–73)**	**21 (0–57)**[¥]	**26 (3–60)**[ϕ]	**38 (11–73)**	**27(0–73)***
MHAQ	0.65 (0–1.45)	0.40 (0.05–1.55)	0.43 (0–1.40)	0.45 (1–1.55)	0.50 (0–1.40)
Treatment, n (%)					
Anti-TNF monotherapy	**0 (0)**	**4 (14)**[¥]	**20 (100)**[ϕ€]	**0**	**24 (38)***
MTX monotherapy	**34 (53)**	**15 (52)**	**0 (0)**[ϕ€]	**49 (100)**	**0 (0)***
Anti-TNF ± MTX	**30 (47)**	**10 (34)**	**0 (0)** [ϕ€]	**0 (0)**	**40 (62)***
Beta-blockers	5 (8)	1 (3)	4 (20)	4 (8)	6 (9)
Calcium antagonists	5 (8)	2 (7)	2 (10)	2 (4)	7 (11)
ACE inhibitors	6 (9)	1 (3)	2 (10)	4 (8)	5 (8)
NSAIDs	**47 (73)**	**14 (48)**[¥]	**14 (70)**	35 (71)	40 (62)
Coxibs	0 (0)	0 (0)	1 (5)	0 (0)	1 (2)
Statins	**12 (19)**	**1 (3)**[¥]	**7 (35)**[€]	7 (14)	13 (20)
Acetyl salicylic acid	6 (9)	2 (7)	3 (15)	6 (12)	5 (8)
Warfarin	0 (0)	0 (0)	**1 (5)**[€]	0 (0)	1 (2)
Glucocorticoids	**17 (27)**	**3 (10)**[¥]	**2 (10)**[ϕ]	**8 (16)**	**15 (23)***

Unless indicated otherwise, values are given as median (range)
Statistically significant differences are shown in bold typeface
*$P < 0.05$, versus MTX
[¥]$P < 0.05$, versus RA
[€]$P < 0.05$, versus PsA
[ϕ]$P < 0.05$, versus RA
ACE angiotensin converting enzyme, *anti-TNF* antitumor necrosis factor, *AS* ankylosing spondylitis, *BASDAI* Bath Ankylosing Spondylitis Disease Activity Index, *BASFI* Bath Ankylosing Spondylitis Functional Index, *BASMI* Bath Ankylosing Spondylitis Metrology Index, *CRP* C-reactive protein, *DAS28* Disease activity score for 28 joints, *ED* endothelial dysfunction, *ESR* erythrocyte sedimentation rate, *Ig* immunoglobulin, *MHAQ* Medical Health Assessment Questionnaire, *MTX* methotrexate, *NSAID* nonsteroidal anti-inflammatory drug, *NSJ* number of swollen joints, *PGA* Physicians' Global Assessment Score of Disease Activity, *PsA* psoriatic arthritis, *PtGA* Patients' Global Assessment Score of Disease Activity, *RA* rheumatoid arthritis, *RHI* Reactive Hyperemic Index, *WBC* white blood cell

The RHI change from baseline to 6 months was not related to RHI baseline values in patients with ED.

RHI mean values in patients with normal EF did not change at any of the control points of time (data not shown).

Discussion

To our knowledge, this is the first study to compare the effect of MTX monotherapy and anti-TNF ± MTX treatment on EF in IA patients, and to compare levels of RHI between RA, PsA, and AS patients with active disease.

In IA patients with ED, antirheumatic treatment was associated with improvement in EF both at 6 weeks and 6 months of follow-up compared to baseline. However, after 6 weeks, EF continued to improve only in the MTX group.

Table 2 Baseline cardiovascular characteristics for all patients

	RA (*n* = 64)	PsA (*n* = 29)	AS (*n* = 20)	MTX (*n* = 49)	anti-TNF ± MTX (*n* = 6)
Cardiovascular risk factors					
Hypertension	17 (27)	7 (24)	6 (30)	9 (18)	21 (33)
BMI (kg/m^2), median (range)	26 (19–41)	26 (19–39)	28 (22–36)	26 (20–39)	27 (20 – 41)
Hyperlipidemia	11 (17)	3 (10)	3 (15)	9 (18)	8 (12)
Current smokers	20 (31)	6 (21)	10 (50)	15 (31)	21 (33)
Family history of CVD or death	33 (52)	13 (45)	10 (50)	24(50)	32 (50)
Diabetes	3 (5)	0 (0)	1 (5)	0 (0)	4 (6)
Medical history					
Previous myocardial infarction	5 (8)	0 (0)	2 (10)	2 (4)	5 (8)
Angina pectoris	2 (3)	1 (3)	2 (10)	2 (4)	3 (5)
Endothelial dysfunction					
RHI, median (range)	1.89 (1.24–2.94)	**2.06 (1.45–2.94)**	**1.81 (1.37–2.72)**[€]	1.93 (1.24–2.76)	1.82 (1.37–2.94)
ED	22 (34)	9 (31)	9 (45)	18 (37)	22 (34)
RHI, median (range) for patients with ED	1.47 (1.24–1.65)	1.56 (1.45–1.64)	1.52 (1.37–1.64)	1.49 (1.24–1.63)	1.52 (1.37–1.65)

Unless indicated otherwise, values are given as number (percentage)
Statistically significant differences are shown in bold typeface
[€]$P < 0.05$, versus PsA
anti-TNF antitumor necrosis factor, *AS* ankylosing spondylitis, *BMI* body mass index, *CVD* cardiovascular disease, *ED* endothelial dysfunction, *MTX* methotrexate, *PsA* psoriatic arthritis, *RA* rheumatoid arthritis, *RHI* Reactive Hyperemic Index

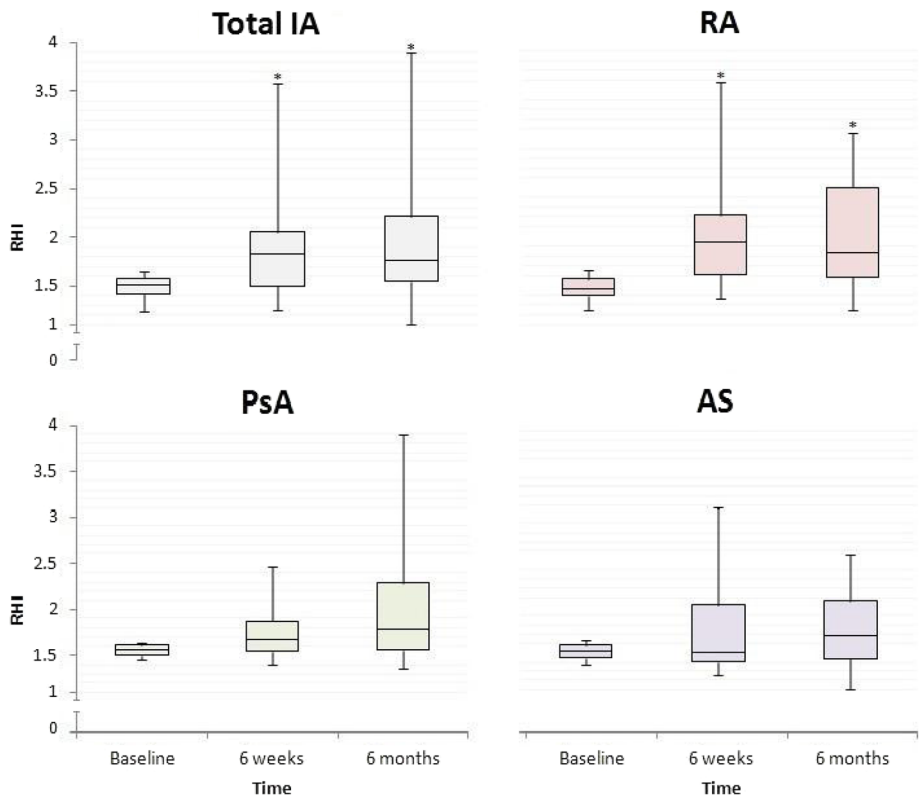

Fig. 1 RHI values in RA, PsA, and AS patients with ED at all visits. *$P < 0.05$, versus baseline. The lines inside of the boxes show the median; the whiskers of the boxes show upper and lower values. *AS* ankylosing spondylitis, *IA* inflammatory arthritis, *PsA* psoriatic arthritis, *RA* rheumatoid arthritis, *RHI* Reactive Hyperemic Index

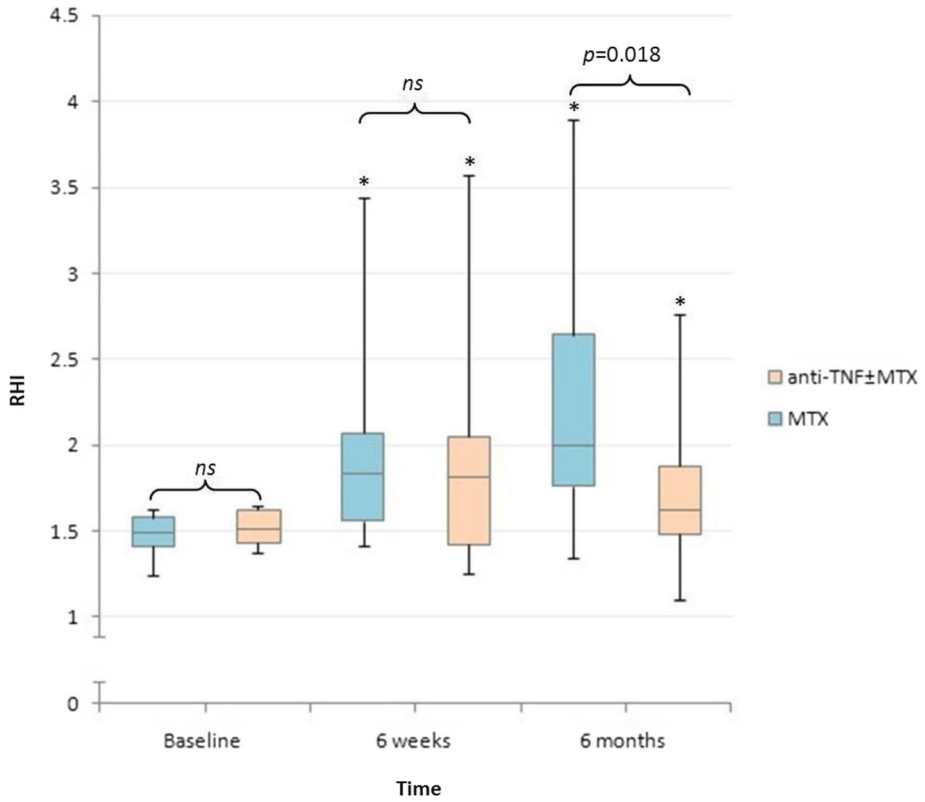

Fig. 2 RHI values for patients with ED between and within the MTX and anti-TNF ± MTX groups. *$P < 0.05$ compared to baseline value. *anti-TNF* anti-tumor necrosis factor, *MTX* methotrexate, *ns* not statistically significant, *RHI* Reactive Hyperemic Index

Table 3 Predictors of RHI change after 6 months of antirheumatic treatment in patients with ED

	Unadjusted			Adjusted		
	Beta	*P*	95% CI	Beta	*P*	95% CI
Female gender	**0.492**	**0.011**	**0.118 to 0.865**	**0.621**	**0.004**	**0.220 to 1.022**
Age	−0.004	0.669	−0.024 to 0.016	−0.001	0.919	−0.021 to 0.019
Anti-TNF ± MTX	**−0.505**	**0.008**	**−0.866 to −0.143**	**−0.448**	**0.032**	**−0.855 to −0.041**
RDD	**−0.026**	**0.033**	**−0.049 to −0.002**	−0.024	0.068	−0.050 to 0.002
PsA	−0.067	0.779	−0.550 to 0.416	0.064	0.733	−0.386 to 0.514
AS	−0.267	0.242	−0.722 to 0.188	0.219	0.419	−0.327 to 0.765
CRP	−0.004	0.523	−0.017 to 0.009			
ESR	−0.002	0.771	−0.017 to 0.013			
PTX3	−0.071	0.255	−0.194 to 0.053			
PGA	0.008	0.160	−0.003 to 0.019			
PtGA	−0.001	0.824	−0.008 to 0.006			
MHAQ	−0.328	0.332	−1.006 to 0.349			
NSJ	−0.150	0.050	−0.299 to 0.000			

Comparators: female gender versus male gender, anti-TNF ± MTX versus MTX monotherapy
Statistically significant differences are shown in bold typeface
anti-TNF anti-tumor necrosis factor, *AS* ankylosing spondylitis, *CI* confidence interval, *CRP* C-reactive protein, *ESR* erythrocyte sedimentation rate, *MHAQ* Medical Health Assessment Questionnaire, *MTX* methotrexate, *NSJ* number of swollen joints, *PGA* Physicians' Global Assessment Score of Disease Activity, *PsA* psoriatic arthritis, *PtGA* Patients' Global Assessment Score of Disease Activity, *PTX3* pentraxin 3, *RDD* rheumatic disease duration, *RHI* Reactive Hyperemic Index

Because MTX monotherapy was initiated only in MTX-naive patients, and the combination therapy only in patients who had previously used MTX without sufficient effect, our findings may indicate that MTX treatment in MTX-naive patients has a greater and more sustained vasculoprotective effect than anti-TNF monotherapy, or anti-TNF added to MTX treatment in MTX nonresponders. It is likely that, in MTX nonresponders, MTX also exhibited a poor response on disease activity after the addition of anti-TNF (MTX in this group was provided first of all to reduce side-effects of anti-TNF therapy). One might speculate that the poor response of MTX on inflammation is associated also with a poor effect on EF.

The exact mechanism behind the protective effect of antirheumatic treatment on ED is not known [25]. Theoretically, it might be mediated by inhibition of systemic inflammatory factors and the corresponding metabolic abnormalities. However, this explanation is not supported by our findings since the improvement in RHI was not related to systemic markers of disease activity, such as ESR and CRP. Moreover, we did not find any significant relationships between RHI levels and inflammatory markers at baseline.

Another explanation might be that the examined drugs might have a direct beneficial effect on the vessel walls, including the endothelium. It has been shown that MTX and anti-TNF treatments are associated with improvements in reverse cholesterol transport by various mechanisms [26, 27]. For example, MTX increases high-density lipoprotein (HDL) capacity to promote cholesterol efflux from cells [28]. Anti-TNF agents counteract the deleterious effects of TNF on the expression of genes involved in cholesterol efflux and reduce cell cholesterol accumulation through amelioration of serum lipoprotein functions and through reverse signaling following direct interaction with cell membrane-bound TNF [26].

Although most focus has been on the importance of impaired cell cholesterol efflux in the development of foam cells from macrophages in atherosclerotic plaques, the same mechanism may also underlie disturbances in endothelial cells, with reduction of their vasodilating and anti-inflammatory functions [29]. In fact, increased cholesterol efflux through the membrane transporters ATP-binding cassette A1 and G1 and Scavenger Receptor class B type I in endothelial cells is associated with promotion of eNOS expression and PGI2 production [30–32]. Moreover, serum HDL capacity to promote cell cholesterol efflux is directly correlated to flow mediated dilation [33]. Thus, the improved cell cholesterol efflux due to antirheumatic treatment might both protect from atheroma formation and from ED.

IA patients have been reported to have more inflammation, involving overexpression of TNF, in their vascular media and adventitia compared to non-IA patients with CVD [34, 35]. It might even be that inflammation located in deep vascular layers might affect the luminal part of the artery, including the phenotype of the endothelial cells [36]. Thus, in theory, antirheumatic treatment, such as anti-TNF, could also ameliorate EF by inhibition of vascular inflammation.

ED occurs when the endothelium is activated and is characterized by cytokine production, loss of vascular integrity, and expression of adhesion molecules [37]. Adhesion molecules such as intercellular adhesion molecule (ICAM)-1, E-selectin, and vascular cell adhesion molecule (VCAM)-1 make the endothelium surface more adhesive to leukocytes and facilitate their migration into the vessel wall (including atherosclerotic lesions) [37, 38].

In keeping with our results, both MTX and anti-TNF have been previously reported to downregulate expression of adhesion molecules on endothelial cells, i.e., circulating markers of ED [39–43]. Also, a recent review and meta-analysis article concluded that anti-TNF treatment might improve EF in RA patients [44].

Our previous article based on the same patient sample demonstrated that MTX and anti-TNF ± MTX treatment significantly reduced inflammatory activity (determined by ESR, CRP, WBC count, PGA, and PtGA) both at 6 weeks and at 6 months compared to baseline [16]. This may indicate that both treatment regimens have a longstanding effect on inflammation, but only MTX (in patients potentially responding to it) has a prolonged beneficial effect on the endothelial cells.

We cannot definitely rule out the possibility that the observed differences in the effect of the antirheumatic treatments on EF might be based on differences in patient populations or other factors. For example, it might be that patients with longer and more therapy-resistant IA (i.e., features typical for the anti-TNF ± MTX group; Table 1) had a higher CV risk and were less likely to improve their EF by antirheumatic treatment than the remaining IA patients (Table 2). Nevertheless, the differences in RHI change between baseline and 6 months in the two treatment groups were independent of rheumatic disease duration, IA diagnosis, and age. Moreover, there were no statistically significant differences in the examined traditional CV risk factors, the occurrences of clinical CVD and ED, and median RHI values at baseline between the two treatment groups (Table 2).

As different immune and other mechanisms are involved in the pathogenesis of RA, PsA, and AS, it might be that ED in these diseases is also mediated partly by different factors. Consequently, the effect of different antirheumatic drugs on ED in these particular diseases might also be different.

When comparing the three diagnostic groups, the AS group were the most likely to have ED and CV comorbidity

(Table 2), in spite of being younger, having a similar disease duration as the RA and PsA groups, and being less likely to use systemic glucocorticoids than the RA group (Table 1). It is possible that the increased occurrence of certain CV risk factors, such as a high proportion of men and smokers, could partly explain the impaired EF in the AS group [45].

The improvements in RHI from baseline to 6 weeks and 6 months were apparent in all diagnostic groups, but it was statistically significant only in the total IA and in the RA group. The lack of statistically significant differences in RHI improvement in the other groups might be due to their relatively low sample size. Indeed, other studies indicate that antirheumatic treatment (anti-TNF) also improves EF in patients with PsA and AS [46, 47]. We cannot exclude the possibility that AS patients experienced less protection from antirheumatic treatment because they were treated only with anti-TNF and not MTX.

The cause of the observed decreased effect of anti-TNF on EF at 6 months is unclear. Among other factors, it might be caused by the well-known secondary nonresponse effect due to the development of antidrug antibodies [48].

Interestingly, women had statistically greater RHI improvement after 6 months of treatment than men (Table 2). Thus, our results may indicate that women have a better ability to reverse ED than men, independently of IA diagnosis, when treated with MTX or anti-TNF ± MTX. We do not know the molecular mechanism behind this phenomenon.

Rheumatic disease duration showed a stable negative association with RHI change from baseline to 6 months in several multiple regression models. It seems more difficult to achieve an EF improvement in patients with longer rheumatic disease duration, and this applies for both treatment regimens. Thus, these data support the notion that early antirheumatic treatment is important not only for prevention of joint damage, but also for protection from atherosclerosis. However, our results have to be confirmed in larger studies.

As in most studies, ours has several limitations. First, due to ethical reasons (to avoid prescribing MTX to patients in need of anti-TNF, and to avoid overtreatment in patients that might be sufficiently treated with MTX monotherapy) we conducted an observational study instead of a randomized controlled trial (RCT). Thus, we could not secure the same level of similarity between study groups at baseline as in a RCT, nor conduct double-blinded evaluation. On the other hand, observational studies have other advantages, e.g., they can more accurately reflect real life, and therefore have increasingly been called for over the last years. To compensate for baseline differences between the groups, we adjusted for several baseline characteristics in multiple regression models.

As MTX is the drug of choice in most patients with peripheral chronic arthritis, patients with these conditions who receive anti-TNF treatment are likely to have a longer and more severe IA. Nevertheless, the anti-TNF group did not differ from the MTX group when comparing several disease activity markers. In fact, the MTX group had statistically significantly higher PGA scores than the anti-TNF ± MTX group (Table 1).

Second, we were not able to evaluate differences in monotherapies with MTX and anti-TNF as most of the patients using anti-TNF also used MTX comedication.

Third, we evaluated RHI change only in patients with ED because we could not expect substantial RHI improvement in patients with normal EF. Therefore, regression to the mean might be questioned. However, in contrast to patients with low RHI, RHI mean values in patients with normal EF did not change towards the RHI mean at any of the control points of time. Taken together, these observations diminish the suspicion that the observed RHI differences in the ED group could be explained by regression to the mean only.

Finally, owing to a relatively small sample size, the apparent lack of some differences and associations may be due to type II errors and insufficient statistical power. Still, as this is to our knowledge the first study comparing the effect of MTX and anti-TNF regimens in IAs on EF, and comparing EF in RA, PsA, and AS, it brings new important insights into CVD in IAs, and indicates the need for further research.

An advantage of our study is a well-characterized study sample, and a design that makes it possible to examine the effect of two of the main antirheumatic treatment regimens on EF in three common IAs.

Conclusions

In conclusion, treatment with MTX and anti-TNF ± MTX appears to improve EF relatively quickly in IA patients with ED. After 6 months, the EF improvement was more pronounced in the MTX users than in the anti-TNF ± MTX users. Among other factors, this might be due to a more sustained beneficial effect of MTX on the vasculature.

Because the EF improvement was independent of improvement in rheumatic disease activity, modes of action other than the anti-inflammatory effect might play a role.

Among patients with active RA, AS, and PsA, those with AS had the worst endothelial function (the difference was statistically significantly different compared to those with PsA), although they were the youngest.

Abbreviations

AS: Ankylosing spondylitis; CRP: C-reactive protein; CV: Cardiovascular; CVD: Cardiovascular disease; DMARD: Disease-modifying antirheumatic drug; ED: Endothelial dysfunction; EF: Endothelial function; ESR: Erythrocyte sedimentation rate; HDL: High-density lipoprotein; IA: Inflammatory arthritis; ICAM: intercellular adhesion molecule; MHAQ: Modified Health Assessment Questionnaire; MTX: Methotrexate; NSAID: Nonsteroidal anti-inflammatory

drug; PGA: Physicians' Global Assessment Score of Disease Activity; PsA: Psoriatic arthritis; PSARA: PSoriatic arthritis, Ankylosing spondylitis, Rheumatoid Arthritis; PtGA: Patients' Global Assessment Score of Disease Activity; PTX: Pentraxin; PWA: Pulse wave amplitude; RA: Rheumatoid arthritis; RCT: Randomized controlled trial; RHI: Reactive Hyperemic Index; RH-PAT: Reactive hyperemia peripheral arterial tonometry; SpA: Spondyloarthritis; TNF: Tumor necrosis factor; VCAM: Vascular cell adhesion molecule; WBC: White blood cell

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Availability of data and materials
All the supporting data are available upon request.

Authors' contributions
IHok contributed with acquisition of data and drafting the manuscript. IHol, TL, SA, and GH were the founders of the PSARA study and contributed to conception and design, and analysis and interpretation of data. IHol was also involved in drafting the manuscript. MCS and GD performed the statistical analysis and interpreted the results. GD was the major contributor to writing the manuscript. KM, NR, and JEW contributed to interpretation of data. All authors have been involved in critically revising the manuscript. All authors read and approved the final manuscript and agreed to be accountable for all aspects of the work.

Authors' information
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Ethics approval and consent to participate
The Norwegian Regional Ethical Committee approved the study protocol and all patients gave informed written consent.

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Paper 3

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Clinical studies

Effect of anti-rheumatic treatment on selenium levels in inflammatory arthritis

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ABSTRACT

Objectives: The reason for increased cardiovascular risk in inflammatory arthritis (IA) is unclear. Interestingly, selenium-deficiency is suspected to contribute to the development of cardiovascular disease (CVD) in the general population. Although the reference range of serum selenium (s-selenium) is 50–120 μg/L, there are indications that levels up to 85 μg/L might not be sufficient for optimal cardioprotection. Our aim was to examine s-selenium levels in rheumatoid arthritis (RA), psoriatic arthritis (PsA) and ankylosing spondylitis (AS), to evaluate the effect of anti-rheumatic treatment on s-selenium levels, and to assess relationships between s-selenium levels and clinical and laboratory parameters including markers of disease activity and CVD risk.

Methods: We examined 64 patients with RA, 40 with PsA and 26 with AS starting with methotrexate (MTX) monotherapy or anti-tumor necrosis factor therapy (anti-TNF) with or without methotrexate (anti-TNF ± MTX) due to active disease. S-selenium, inflammatory biomarkers, endothelial function (EF) and other variables were examined at baseline and after 6 weeks and 6 months of treatment.

Results: In the total IA group, s-selenium increased within 6 weeks of anti-rheumatic treatment, and thereafter the levels remained stable until the end of the 6 months follow-up period. There were no significant differences in s-selenium changes between the three diagnostic groups and between the two treatment regimens. Changes in s-selenium were negatively related to changes in C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), but there were no significant relationships to any other of the examined risk parameters for CVD including EF.

Conclusion: IA patients had s-selenium within the reference range, but below the level that might be necessary for optimal CVD protection.

Anti-rheumatic treatment had a relatively rapid and sustained effect on s-selenium levels. The increase in s-selenium was related to reduction in inflammatory activity. In theory, anti-rheumatic drugs might improve s-selenium levels through inhibition of pro-inflammatory processes or through other mechanisms. Although we have not revealed any significant relationships between s-selenium and CVD risk parameters, the role of sub-optimal s-selenium levels in pathogenesis of premature CVD in IA cannot be ruled out.

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1. Introduction

Inflammatory arthritis (IA), including rheumatoid arthritis (RA), psoriatic arthritis (PsA) and ankylosing spondylitis (AS), are associated with increased risk of cardiovascular disease (CVD) that cannot be fully explained by traditional CVD risk factors [1]. Intriguingly, there are indications that selenium deficiency might enhance CVD risk, and that serum selenium (s-selenium) levels may be decreased in inflammatory conditions [2,3]. Theoretically, low s-selenium could contribute to the accelerated CVD in IA [4,5].

Selenium, mainly through its incorporation into various selenoproteins, is involved in a wide range of biological processes in the human body. For example, selenium has anti-oxidative, anti-inflammatory and intracellular signaling effects, and influences differentiation, activation and proliferation of immune cells [6–9].

Selenium intake varies with geographic area, mainly because selenium concentrations in foods are highly dependent on the quality of soil where they are produced. Selenium content in soil in several parts in Europe including Norway appears to be low compared to the USA, i.e. due to the high soil acidity and the complexation of selenium with iron and aluminum that decreases selenium uptake [10–12].

It is still unclear which s-selenium levels are optimal. The reference range in our laboratory is 50–120 μg/L, while reference ranges in the USA are as high as 70–150 μg/L [13]. Importantly, it appears that levels below 80–85 μg/L might be insufficient for optimal protection against CVD [14–16].

The data on s-selenium in IA, particularly in PsA and AS are sparse and it is still unknown if inhibition of inflammation in IA improves selenium levels [2,3,5,17]. Therefore, in this study, we compared s-selenium levels in Norwegian patients with RA, PsA and AS, and evaluated if the selenium levels were influenced by methotrexate (MTX) and/or anti-tumor necrosis factor (anti-TNF) treatment. Furthermore, we searched for associations between s-selenium levels and markers of disease activity and CVD risk.

2. Material and methods

2.1. Patients

From the PSoriatic arthritis, Ankylosing spondylitis, Rheumatoid Arthritis (PSARA) study, a total of 140 patients, 74 with RA, 40 with PsA and 26 with AS were included at Lillehammer Hospital for Rheumatic Diseases as described elsewhere [18]. We analyzed serum from all patients who completed 6-months follow-up (n = 114). In brief, the inclusion criteria were: age 18–80 years; RA according to the ACR 1987 criteria, AS according to the modified New York diagnostic criteria for AS or PsA according to Moll and Wright 1973 criteria and clinical indication for starting with either MTX monotherapy or anti-TNF treatment with or without MTX co-medication (anti-TNF ± MTX) due to active disease[19–21].

Exclusion criteria included poor co-operability, pregnancy, breast-feeding, contraindications for MTX and anti-TNF, significant infections (including subclinical tuberculosis), congestive heart failure, chronic inflammatory disease other than RA, AS or PsA, and use of systemic glucocorticoids > 10 mg/day during the previous 2 weeks or anti-TNF during the previous 4 weeks before the inclusion.

The study was conducted in accordance with the Declaration of Helsinki. All patients were Caucasian and gave informed written consent. The patients were examined at baseline and after 6 weeks and 6 months of treatment.

The trial was registered in Clinicaltrials (NCT00902005) and the Norwegian Regional Ethical Committee approved the study protocol.

This study has been supported by a grant from Innlandet Hospital Trust. Abbot Norway financed the establishment of PSARA biobank.

2.2. Treatment

Included patients were either treated with MTX monotherapy or with anti-TNF ± MTX. Following a clinical assessment, a rheumatologist not involved in the study determined the type and doses of anti-rheumatic treatment, according to the Norwegian guidelines. These guidelines consider MTX as first line anti-rheumatic treatment in patients with chronic peripheral arthritis, in particular in RA [22]. However, in patients with axial spondyloarthritis, anti-TNF is commonly used as first-line treatment in patients not responding to non-steroidal anti-inflammatory drugs (NSAIDs) [23]. While MTX monotherapy was used in MTX naïve patients only, MTX in addition to anti-TNF was used in patients who had previously used MTX monotherapy without sufficient effect. These MTX non-responders continued with MTX mainly to reduce the risk of side effects of anti-TNF therapy.

The doses were as follows: MTX 15–25 mg orally once a week; etanercept 50 mg subcutaneous injection once a week; infliximab 3–5 mg/kg intravenous injection at baseline, then following standard dosing regimen; adalimumab 40 mg SC injection every other week.

Throughout the study period, patients using glucocorticoids were kept on a steady dose (10 mg or less per day) and no dietary change or selenium supplementation were introduced.

2.3. Clinical and laboratory tests

Data collection included demographic data, physical findings, lifestyle information, medical history and medication.

Endothelial function (EF) was assessed by Reactive hyperemia index (RHI) as described earlier [24]. Frozen serum samples were shipped to Lab1, Sandvika, Norway (SYNLAB group; SYNLAB International GmbH) where s-selenium was analyzed by atomic absorption spectrometry (Varian AA 240Z Zeeman-GFAAS) in batch and in random order, by assessor blinded for clinical data. A total of 400 μl serum were mixed with 200 μl triton 2%-solution and homogenized by shaking well. Then, 10 μl of the sample was added to 10 μl modifier and 10 μl Triton 1%. Atomization found place at 2300 °C and a hollow cathode lamp was used to measure the absorption at 196 nm. As external quality controls, the Round Robin tests by INSTAND e.V. Düsseldorf were used. The intra-assay coefficient of variation at 53.1 μg/L was 5.1%. The inter-assay coefficient of variation and the assays accuracy at 118 μg/L were 5.2% and 2%, respectively.

2.4. Statistical analyses

Categorical variables were described with number and percentages whereas continuous variables were expressed with median and range. Crude differences between the groups were assessed by chi-square test (categorical data) or non-parametric tests (Mann-Whitney Wilcoxon test or Wilcoxon signed rank test for comparisons of continuous variables between or within the examined groups).

Linear regression analyses were used to assess associations between baseline s-selenium levels as well as changes in s-selenium during follow-up and selected demographic, clinical and laboratory variables (including IA characteristics and CV parameters). Age, gender, inflammatory markers, RHI and variables that showed significant associations with baseline s-selenium or s-selenium change during treatment in univariate linear regression analyses were included in the multivariate models. All statistical tests were two-sided, and p-values ≤ 0.05 were considered statistically significant. Our analyses were considered exploratory, therefore no correction for multiple testing was performed.

All analyses were completed using IBM SPSS statistics, Version 23.

Table 1
Baseline characteristics for the study group.

	RA n = 64	PsA n = 30	AS n = 20	MTX n = 50	anti-TNF ± MTX n = 64
Age, yrs	*57 (28-79)*	*50 (23-78)*[¥]	*49 (30-72)*[Φ]	56 (28-79)	55 (23-75)
Women, n (%)	47 (73)	*13 (43)*[¥]	*4 (20)*[Φ,€]	31 (62)	33 (52)
Selenium (μg/L)	*72 (46-139)*	*67 (41-115)*	*74(56-132)*	*68 (50-115)*	*73(41-139)*
Disease duration, years	2 (0-30)	2.5 (0-37)	3 (0-40)	*0.1 (0-25)*	*3.7 (0-40)*[*]
CRP (mg/L)	8 (1-78)	5 (1-99)	10 (1-157)	8 (1-99)	6.5 (1-157)
ESR (mm/h)	*18.5 (1-81)*	*7 (2-48)*[¥]	*9.5 (2-87)*[Φ]	14 (1-81)	13 (2-87)
Anti-CCP, n (%)	39 (61)	–	–	14 (34)	22(34)
HbA1C (%)	5.7 (4.9-8.9)	*5.5 (4.6-6.4)*	*5.6 (4.9-6.9)*	5.6 (4.9-6.4)	5.6(4.6-8.9)
Rheumatoid factor IgM, n(%)	45 (70)	–	–	22 (44)	23(36)
BASDAI	–	4.3 (0.3-9.5)	5.1 (0.9-9.6)	5.4 (0.3-9.3)	5.1 (0.9-9.7)
BASFI	–	3.1 (0-9)	4.1 (1.1-7.6)	2.8 (0-9)	3.8 (0.4-8.6)
BASMI	–	–	3 (0-10)	–	3 (0-10)
DAS 28	4.98 (2.6-7.3)	–	–	5.2 (3.1-7.3)	5.1(2.6-7.1)
PtGA	52 (5-98)	69 (2-98)	56 (6-96)	52 (2-96)	49 (6-98)
PGA	*38 (7-73)*	*23 (0-57)*[¥]	*26 (3-60)*[Φ]	*37 (11-73)*	*27(0-73)*[*]
MHAQ	0.65 (0-1.45)	0.4 (0-1.55)	0.43 (0-1.40)	0.45 (0-1.55)	0.50 (0-1.40)
ED, n (%)	22 (34)	9 (30)	9 (45)	18 (36)	22 (34)
Hypertension, n (%)	17 (27)	7 (24)	6 (30)	9 (18)	21 (33)
BMI (kg/m^2)	26 (19-41)	26 (19-39)	28 (22-36)	26 (20-39)	27 (20 - 41)
Hyperlipidemia, n (%)	11 (17)	3 (10)	3 (15)	9 (18)	8 (12)
Current smokers, n (%)	20 (31)	7 (23)	10 (50)	16 (32)	21 (33)
Family history of CVD, n (%)	33 (52)	14 (47)	10 (50)	25 (50)	32 (50)
Diabetes, n (%)	3 (5)	0(0)	1 (5)	0 (0)	4 (6)
Previous MI, n (%)	5 (8)	0 (0)	2 (10)	2 (4)	5 (8)
Angina pectoris, n (%)	2 (3)	1 (3)	2 (10)	2 (4)	3 (5)
Anti-TNF monotherapy, n (%)	0 (0)	4 (13)	20 (100)[Φ,€]	0 (0)	24 (38)[*]
MTX mono therapy, n (%)	34 (53)	16 (53)	0 (0)[Φ,€]	50 (100)	0 (0)[*]
Anti-TNF ± MTX, n (%)	30 (47)	10 (33)	0(0)[Φ,€]	0 (0)	40 (62)[*]
NSAIDs, n (%)	*47 (73)*	*14 (47)*[¥]	14 (70)	35 (70)	40 (62)
Coxibs, n (%)	0 (0)	0(0)	1 (5)	5 (10)	1 (2)
Statins, n (%)	*12 (19)*	*1 (3)*[¥]	7 (35)[€]	7 (14)	13 (20)
Acetyl salicylic acid	6 (9)	2 (7)	3 (15)	6 (12)	5 (8)

Unless otherwise indicated, values are given as median (range). RA, rheumatoid arthritis; PsA, psoriatic arthritis; AS, ankylosing spondylitis; ED, endothelial dysfunction; CRP, C-reactive protein; WBC, white blood cells; ESR, erythrocyte sedimentation rate; NSJ, number of swollen joints;HbA1C, glycosylated Hemoglobin; BASDAI, bath ankylosing spondylitis disease activity index; BASFI, bath ankylosing spondylitis functional index; BASMI, bath ankylosing spondylitis metrology index; DAS 28, Disease activity score for 28 joints; PtGA, Patients' Global Assessment Score of disease activity; PGA, Physicians' Global Assessment Score of disease activity; MHAQ, medical health assessment questionnaire; BMI, body mass index; MI, myocardial infarction; RHI, reactive hyperaemic index; Anti-TNF, anti-tumor necrosis factor; MTX, methotrexate; ACE, angiotensin converting enzyme; NSAIDs, non-steroidal anti-inflammatory drugs.

[*] $p < 0.05$ between MTX and Anti-TNF ± MTX group.
[¥] $p < 0.05$ between RA and PsA.
[€] $p < 0.05$ between PsA and AS.
[Φ] $p < 0.05$ between AS and RA.

3. Results

3.1. Patient baseline characteristics

Baseline characteristics of the study group are described in Table 1.

The anti-TNF ± MTX group had significantly longer rheumatic disease duration and lower physicians' global assessment (PGA) score that the MTX group ($p < 0.001$ and $p = 0.002$, respectively).

The AS group and PsA group had significantly lower median age, lower proportion of women (the percentage of women was the lowest in the AS group), and lower erythrocyte sedimentation rate (ESR) levels and PGA score than the RA group. RA and PsA patients were treated either with MTX or anti-TNF ± MTX regimens, while all AS patients were treated with anti-TNF mono-therapy. Fewer patients with PsA used statins compared to the two other groups, and fewer used NSAIDs compared to RA group.

Baseline s-selenium levels were similar between the diagnostic and treatment groups (median baseline s-selenium values were below 80 μg/L in all groups) (Table 1)

3.2. Changes in s-selenium levels during anti-rheumatic treatment

Changes in s-selenium levels at baseline and follow-up are shown in Fig. 1. In all diagnostic groups an increasing trend of s-selenium was noted. In the total IA group, s-selenium level increased significantly at 6 weeks and 6 months, as compared to baseline values.

The improvement was statistically significant after 6 weeks of treatment in the RA group, and after 6 months of treatment in the PsA group. And there were an increase in s-selenium levels in RA group also after 6 months and in PsA after 6 weeks. The AS group had the largest absolute s-selenium increase after 6 months, compared to the RA and PsA groups, although this increase did not attain statistical significance at any time points (Fig. 1).

3.3. Effects of MTX monotherapy and anti-TNF ± MTX treatment on s-selenium

In both treatment groups s-selenium levels increased from baseline to the follow-up visits, however the improvements were statistically significant only for the MTX group (Fig. 2).

There were no statistically significant changes in s-selenium levels between these two treatment groups at any time point.

3.4. Association between s-selenium and other selected clinical and laboratory factors

We performed univariate and multivariate linear regression analyses to identify variables associated with baseline s-selenium levels,

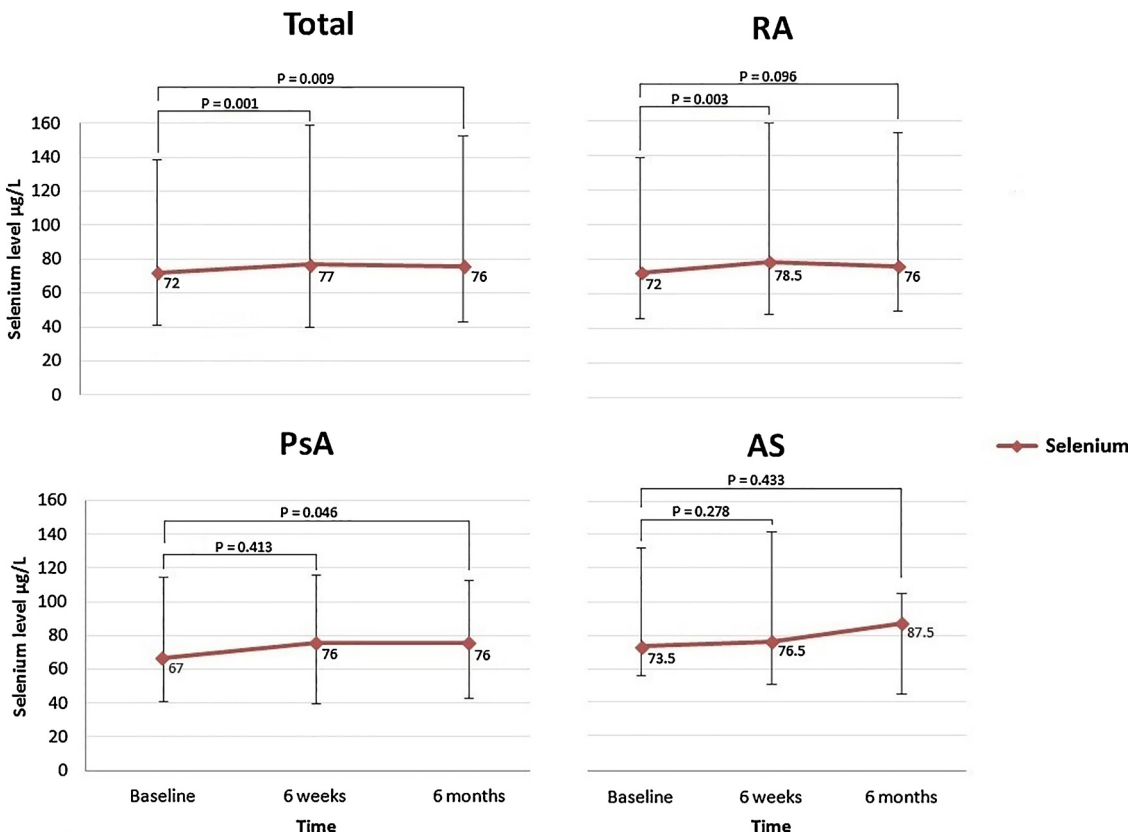

Fig. 1. Changes in serum selenium levels during anti-rheumatic treatment in RA, PsA and AS. Data are given as median and range (minimum-maximum). IA, Inflammatory arthritis; RA, Rheumatoid arthritis; PsA, Psoriatic arthritis; AS, Ankylosing spondylitis.

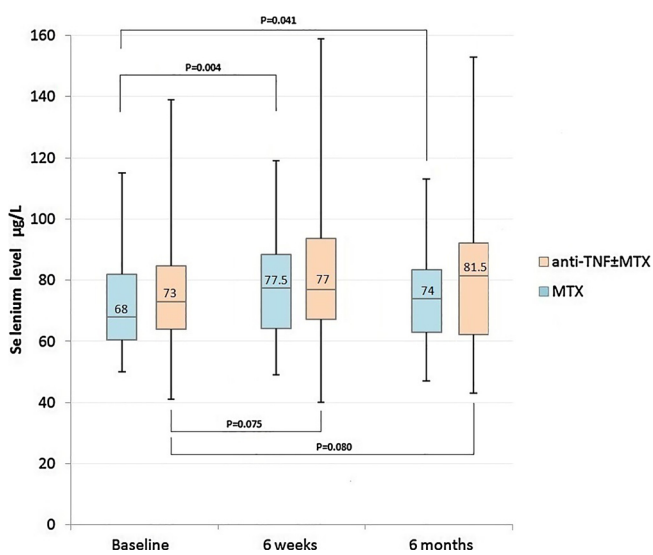

Fig. 2. Boxplot showing s-selenium levels at all points of time in MTX and anti-TNF ± MTX groups. Midline represents median Bottom and top of the box represent 25 and 75 percentile and whiskers represent minimum and maximum values. anti-TNF, anti-tumor necrosis factor; MTX, methotrexate.

and with change in s-selenium from baseline to 6 weeks and 6 months.

3.4.1. Predictors of baseline s-selenium levels

In unadjusted analyses, baseline s-selenium levels were not statistically significantly associated with demographic data (age, gender, education), circulating inflammatory biomarkers (ESR, C-reactive protein (CRP) and pentraxin 3) or other characteristics of IA (Bath ankylosing spondylitis disease activity index (BASDAI), disease activity score for 28 joints (DAS28), number of swollen joints (NSJ), patients' Global Assessment Score of disease activity (PtGA), medical health assessment questionnaire (MHAQ) and rheumatic disease duration), CVD comorbidity and CVD risk factors (angina pectoris, myocardial infarction, peripheral artery disease, hypertension, family history of CVD, diabetes, hyperlipidemia, body mass index (BMI), alcohol use, smoking, exercise serum glucose, glycosylated hemoglobin (HbA1C), high density lipoprotein (HDL), low density lipoprotein (LDL), total cholesterol and lipoprotein a (Lp(a)) and medications (statins, calcium blockers, ACE inhibitors, beta-blockers, anti-platelet agents, systemic glucocorticoids and NSAIDs and coxibs).

Further, our data did not reveal any significant associations between baseline s-selenium and the inflammatory (ESR, CRP) and CVD parameters (including RHI) in models adjusted for age and gender (data not shown).

3.5. Predictors of change in s-selenium after treatment

In univariate analyses, change in s-selenium after 6 months of treatment was inversely associated with corresponding changes in CRP and ESR (Table 2). After 6 weeks, this association was statistically significant for CRP only ($p = 0.025$) (data not shown).

The association between change in s-selenium and changes in ESR and CRP during 6 months of therapy remained statistically significant also in the age and gender adjusted models (Table 2 - Model 1 and 2).

There were no other significant associations between changes in s-selenium levels during the 6 weeks and 6 months of treatment and any of the examined variables including demographic data as well as corresponding changes in other markers of IA activity and in modifiable CV risk factors and RHI (Table 2 - Model 3) (neither in unadjusted analyses nor in analyses adjusted for age and gender).

Table 2
Variables associated with s-selenium change after 6months of anti-rheumatic treatment in patients with IA.

	Unadjusted			Adjusted Model 1			Adjusted Model 2			Adjusted Model 3		
	B	95% CI	p	B	95% CI	p	B	95% CI	p	B	95% CI	p
Female gender	2.808	-3.300 - 8.915	0.364	-0.070	-6.567 - 6.427	0.983	0.455	-5.968 - 6.877	0.889	2.577	-4.073 - 9.228	0.444
Age	0.089	-0.181 - 0.360	0.514	0.025	-0.267 - 0.316	0.868	0.045	-0.243 - 0.334	0.757	0.070	-0.211 - 0.350	0.624
CRP	−0.181	−0.317 - -0.450	0.010				−0.190	−0.334 - -0.045	0.011			
ESR	−0.201	−0.397 - 0.005	0.045	−0.289	−0.511 - - 0.068	0.011						
Glucose	-5.742	-11.762 - 0.279	0.061									
RHI	0.001	-4.764 - 4.766	1.000							-0.527	-5.572 - 4.518	0.836

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; RHI, reactive hyperaemic index.

4. Discussion

In the present study, Norwegian patients with chronic IA had median s-selenium level (72 μg/L), i.e. within the reference range (50–120 μg/L), but below the level (80–85 μg/L) that appears to be necessary for optimal protection against CVD [14–16]. In general, the reference ranges reflect s-selenium levels in the given populations, depending on the local selenium intake. Thus, reference ranges mirror the actual situation in the given area, but not necessarily the range that is required for maintenance of optimal health.

It is becoming increasingly apparent that the lowest levels of the current selenium reference ranges might not be sufficient for maintenance of ideal health [25]. Thus, there is a need to clearly define the recommended range of s-selenium, in order to secure appropriate selenium supplementation.

To our knowledge, this is the first study to compare s-selenium between patients with RA, PsA and AS: our results indicate that s-selenium levels in these diseases are similar.

Previous studies have demonstrated that RA and PsA patients have statistically significant lower selenium levels than healthy individuals [17,26–28]. In 1978 Aaseth et al., reported that Norwegian patients with RA had s-selenium mean levels of 93 μg/L, while the control group had 129 μg/L [26]. Another study from USA reported that patients with RA had mean s-selenium levels of 148 μg/L, while the control group had s-selenium levels of 160 μg/L. The discrepancies regarding s-selenium levels in RA patients between these studies might be caused by the fact that participants in the American study came from an area of relatively high selenium intake [27]. Further, these Norwegian and American studies were performed in the 1990's or earlier, and selenium intake has changed since then due to changes in food sources and fertilizers. Norwegians have become more self-sufficient on flour, growing their own grain that contains substantially less selenium than the previously imported flour from North-America, presumably partly explaining the difference in s-selenium levels between the patient group of Aaseth et al, (93 μg/L) and our patient group (72 μg/L) [29,30].

However, the cause of lower selenium-levels in RA patients compared to healthy individuals is still unclear. Interestingly, there appears to be a reciprocal relationship between inflammation and selenium: while inflammation may lead to a decrease in selenium levels, low selenium levels may promote inflammation [9,31,32]. In fact, a study of 18,709 healthy subjects revealed that low s-selenium level was a risk factor for development of rheumatoid factor-negative rheumatoid arthritis [33].

Intriguingly, s-selenium levels in our study increased in all diagnostic groups with anti-rheumatic treatment already within 6 weeks, and remained relatively stable for the remainder of the 6 months study period. We observed a non-statistical tendency towards further increase in s-selenium levels between 6 weeks and 6 months in the anti-TNF ± MTX group as well as in the AS group. The underlying mechanism is unknown. In theory, inflammation might reduce s-selenium levels due to increased turnover of selenoproteins and subsequent selenium depletion, or due to their reduced synthesis. Selenoprotein P, which represents the majority of s-selenium (60% of the total s-selenium), is synthesized in the liver, similar to acute phase reactants such as CRP [34–37]. Theoretically, the increased production of acute phase proteins in liver might inhibit the production of selenoprotein P during inflammation. In keeping with this notion, our results revealed that changes in s-selenium were negatively related to changes in systemic inflammatory biomarkers including CRP and ESR. Similar findings have been reported by others [32,38]. Further, it is known that selenoprotein P expression is down-regulated at the transcriptional level by pro-inflammatory cytokines, and thus it is more likely that the higher s-selenium levels upon anti-inflammatory treatment are due to improved biosynthesis of the selenoprotein P [39,40].

To our knowledge, this is the first study that evaluates the effect of MTX and anti-TNF ± MTX on s-selenium in IA: both MTX monotherapy and anti-TNF ± MTX treatment was associated with increased s-selenium levels after 6 weeks as well as 6 months of therapy, but the improvements were statistically significant in the MTX group only. Although we cannot rule out the possibility of a weaker effect of anti-TNF ± MTX regimen on selenium levels compared to MTX monotherapy, the lack of statistical significance might also be due to Type-2 Error. In support of this notion, p values for differences between s-selenium levels at baseline and 6 weeks and between baseline and 6 months in the anti-TNF ± MTX group were 0.075 and 0.080, respectively. Additionally, the mean difference in s-selenium from baseline to 6 months was greater for the TNF ± MTX group than the MTX group (Fig. 2).

One previous study examining Turkish RA patients revealed that MTX had no effect on selenium levels after one month of treatment. However, the baseline mean s-selenium level was much higher in their study group (131.4 μg/dl) compared to ours. Indeed, it might be that anti-rheumatic treatment may improve s-selenium status only in patients with low s-selenium levels, while no further improvement is possible in individuals who already have high s-selenium levels. Of note, selenium levels in the Turkish study were either extremely high or given in wrong units (μg/dl) [41].

We did not observe any significant differences between the effect of MTX and anti-TNF ± MTX on s-selenium in the examined IA diagnoses. However, no comparisons between the two regimens were possible in patients with AS since all of them received anti-TNF ± MTX (always in the form of anti-TNF monotherapy).

Further studies are needed to elucidate if anti-rheumatic drugs improve selenium levels through a shared mechanism, e.g. through their anti-inflammatory effects, or if they convey specific actions that influence selenium homeostasis.

We did not find any significant associations between s-selenium and CV parameters such as traditional CV risk factors and CVD co-morbidity, and EF and proportion of patients with endothelial dysfunction (ED), although anti-rheumatic treatment was associated with reduced ED, as reported earlier [24]. However, although our results do not support the notion that low selenium levels are related to CV risk in IA, this notion cannot be definitely ruled out by our study.

Indeed, low selenium levels have been reported to be associated

with high CV and all-cause mortality risk [15,42]. Selenium is suspected to exhibit its cardio-protective effects through various mechanisms, including the anti-platelet, anti-inflammatory and anti-oxidant functions. Of the antioxidative selenoproteins, glutathione peroxidase, thioredoxine reductase and selenoprotein P appear to have particularly important cardio-protective roles [43–45].

Since the basic intake in populations in North-America is above about 120 μg/day, further selenium supplementation is not expected to result in CV protection [46]. In contrast, in Swedish healthy elderly individuals with an estimated basic selenium intake as low as about 35 μg/day, CV mortality was reduced by selenium and coenzyme Q10 supplementation [14]. Therefore, it might not be surprising that an American randomized control trial with 1250 participants, showed no statistically significant association between selenium supplementation and CVD morbidity and mortality [47].

Recently a meta-analysis conducted on 16 RCTs concluded that selenium supplementation might reduce inflammation, but is not sufficient to reduce CVD mortality [48].However, most of the included studies in the meta-analysis included participants from selenium rich populations.

The discrepancies found in intervention studies might be caused by differences in the populations, and the general selenium nutritional status. Furthermore, they may be due to differences in efficiency of the chosen selenium supplements, lack of a standardized method for measurement of selenium, or errors due to low sample power [49,50]. Additionally, the U-shaped relationship between selenium and CV risk factors might also partly explain some of the conflicting results, as the cardio-protective effect of selenium decreases both with very low (below 40 μg/L) and very high levels (over 150 μg/L) [3,13].

It is not clear what is the clinical significance of the observed relatively small increase in s-selenium level, in particular in individuals without any pronounced selenium deficit. Nevertheless, the observed statistically significant treatment-related changes in s-selenium levels may be of substantial importance as they may improve insights into the pathophysiological pathways in IA, and into the pharmacological actions of anti-rheumatic drugs.

5. Limitations

Because of ethical reasons (to avoid prescribing anti-TNF to patients that could be sufficiently treated with MTX monotherapy, and to avoid prescribing MTX monotherapy in patients in need of anti-TNF agents), we conducted an observational study. Although observational studies are burdened with some disadvantages compared to randomized control trials (RCTs), they have also some advantages. For example, they more accurately reflect real life, and have higher external validity than RCTs. Therefore, observational studies have been increasingly called for during the last years. To minimize effects of potential confounders, we adjusted for them by statistical methods.

It is important to keep in mind that there are essential differences between IA patients starting with MTX monotherapy and those starting with anti-TNF. As MTX is the drug of choice in most patients with peripheral chronic arthritis, patients with these conditions who receive anti-TNF treatment are likely to have longer disease duration, and a more severe disease (Table 1). Moreover, we could not evaluate differences in monotherapy with MTX and monotherapy with anti-TNF as most of the patients using anti-TNF also used MTX co-medication. However, all PSA and RA patients using anti-TNF were MTX-failures, and MTX was provided primarily to reduce side-effects of anti-TNF therapy: therefore, the MTX effect on disease activity in patients receiving anti-TNF + MTX was likely to be poor.

Our study was not designed to compare selenium levels in IA compared to healthy individuals as no healthy individuals were included in our study. On the other hand, our study provides information regarding differences in selenium levels between different rheumatic diseases as well as during anti-rheumatic therapy. Moreover, it gives an opportunity to compare the levels to the relevant reference range.

6. Conclusion

Patients with active IA had s-selenium levels within the normal range, but below the levels that might be necessary for optimal CVD protection (80–85 μg/L). Anti-rheumatic treatment was associated with increase in s-selenium levels that was apparent already after 6 weeks and lasted for 6 months. The increase in s-selenium was related to reduction in inflammatory activity. There were no statistically significant differences in s-selenium levels and their changes between RA, AS and PsA patients and between the MTX and anti-TNF ± MTX groups at any points of time.

Further research is necessary to clarify if the improvement in s-selenium levels is mainly due to inhibition of pro-inflammatory processes or through other mechanisms. The gained knowledge about s-selenium behavior may improve insights into pathophysiological processes in IA and pharmacological actions of anti-rheumatic drugs.

Although our data did not reveal any associations between s-selenium levels and CVD parameters including EF, the role of suboptimal s-selenium levels in pathogenesis of premature CVD in IA cannot be definitely ruled.

Competing interests statement

All authors declare that they have no competing interests.

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