Hormonal factors in rheumatoid arthritis – Their impact on disease risk and severity

Pikwer, Mitra

2012

Hormonal factors in rheumatoid arthritis

-Their impact on disease risk and severity

Mitra Pikwer

Från avdelningen för Reumatologi,
Institutionen för Kliniska Vetenskaper, Malmö
Lunds Universitet

LUNDS UNIVERSITET
Medicinska fakulteten

Akademisk avhandling
som med vederbörligt tillstånd av Medicinska Fakulteten vid Lunds Universitet för avläggande av doktorsexamen i medicinsk vetenskap kommer att offentligen försvaras i Medicinska klinikens aula, Inga Marie Nilssons gata 46,
Skånes Universitetssjukhus, Malmö,
Fredagen den 5 Oktober 2012 klockan 09.00.

Fakultetsopponent:
Alan Silman, professor, University of Manchester.

Handledare:
Carl Turesson, docent, Sektionen för Reumatologi,
Institutionen för kliniska vetenskaper, Malmö
Skånes Universitetssjukhus, Malmö.

Biträdande handledare:
Lennart Jacobsson, professor, Sektionen för Reumatologi,
Institutionen för kliniska vetenskaper, Malmö
Skånes Universitetssjukhus, Malmö, samt Institutionen för medicin, avdelning för reumatologi och inflammationsforskning, Sahlgrenska akademin, Göteborg.

Organization
LUND UNIVERSITY

Unit of Rheumatology
Department of Clinical Sciences
Malmö, Sweden

Document name
DOCTORAL DISSERTATION

Date of issue
2012-10-05

Sponsoring organization

Author(s)
Mitra Pikwer

Title and subtitle

Hormonal factors in rheumatoid arthritis -Their impact on disease risk and severity

Abstract

Rheumatoid arthritis (RA) is 4-6 times more common in women than men during the fertile years. For women, the incidence peaks shortly after menopause, and for men the risk is greater with higher age, when androgen levels drops. Sex hormones have been suggested to play a part in the pathogenesis, since low testosterone levels have been noted in men with RA and pregnancy has an ameliorating effect of the disease in women. Breastfeeding and use of exogenous hormones have been suggested to protect against the disease as well as being associated with a milder phenotype. Our aim was to further investigate associations between hormonal factors and RA.

Two large community based cohorts were established in Malmö between 1974 and 1992 (Malmö preventive medicine programme, (MPMP)) and 1991-1996 (Malmö diet and cancer study (MDCS), respectively). Participants answered a questionnaire and blood samples were collected. We identified incident cases of RA by linking the cohorts to four different RA registers. In nested case-control studies, we studied hormonal predictors in women from the MDCS cohort, and analysed androgens in males from the MPMP. By a structured review of female incident cases in the MDCS, clinical outcomes were collected, with the purpose of classifying the severity of the disease. Three clusters were identified; severe RA, mild/moderate RF negative RA and mild/moderate RF positive RA.

Longer duration of breastfeeding was associated with a reduced risk of RA (Odds ratio (OR)=0.46, 95% Confidence Interval (CI)=0.24-0.91), and menopause at 45 years of age or earlier was associated with an increased risk of mild/moderate Rheumatoid factor (RF) negative RA (OR=2.42, 95% CI=1.32-4.45). In multivariate analysis, there was a negative association between levels of testosterone and future development of RF negative RA in men (OR=0.31, CI=0.12-0.85). These results may improve our understanding about the impact hormones have in the complex pathogenesis of RA.

Key words: Rheumatoid arthritis, hormonal predictors, brest-feeding, early menopause and tesosterone.

Classification system and/or index termes (if any):

Supplementary bibliographical information:

Language
English

ISSN and key title:
1652-8220

ISBN
978-91-87189-39-5

Recipient's notes

Number of pages
150

Price

Security classification

Hormonal factors in rheumatoid arthritis

-Their impact on disease risk and severity

Mitra Pikwer

LUND UNIVERSITY
Faculty of Medicine

Department of Clinical Sciences, Malmö,
Unit of Rheumatology, Lund University, Sweden

Thesis Autumn 2012

Cover art by Annalisa Unkuri, © 2012

Faculty and Department

ISBN 978-91-87189-39-5

ISSN 1652-8220

Printed in Sweden by Media-Tryck, Lund University

Lund 2012

"With an open mind, seek and listen to all the highest ideals. Consider the most enlightened thoughts. Then choose your path, person by person, each for one self."

- Zarathustra

Contents

Abstract

Rheumatoid arthritis (RA) is 4-6 times more common in women than men during the fertile years. For women, the incidence peaks shortly after menopause, and for men the risk is greater with higher age, when androgen levels drops. Sex hormones have been suggested to play a part in the pathogenesis, since low testosterone levels have been noted in men with RA and pregnancy has an ameliorating effect of the disease in women. Breastfeeding and use of exogenous hormones have been suggested to protect against the disease as well as being associated with a milder phenotype. Our aim was to further investigate associations between hormonal factors and RA.

Two large community based cohorts were established in Malmö between 1974 and 1992 (Malmö preventive medicine programme, (MPMP)) and 1991-1996 (Malmö diet and cancer study (MDCS), respectively). Participants answered a questionnaire and blood samples were collected. We identified incident cases of RA by linking the cohorts to four different RA registers. In nested case-control studies, we studied hormonal predictors in women from the MDCS cohort, and analysed androgens in males from the MPMP. By a structured review of female incident cases in the MDCS, clinical outcomes were collected, with the purpose of classifying the severity of the disease. Three clusters were identified; severe RA, mild/moderate RF negative RA and mild/moderate RF positive RA.

Longer duration of breastfeeding was associated with a reduced risk of RA (Odds ratio (OR)=0.46, 95% Confidence Interval (CI)=0.24-0.91), and menopause at 45 years of age or earlier was associated with an increased risk of RA (OR=2.42, 95% CI=1.32-4.45), in particular with a mild/moderate Rheumatoid factor (RF) negative phenotype. In multivariate analysis, there was a negative association between levels of testosterone and future development of RF negative RA in men (OR=0.31, CI=0.12-0.85). These results may improve our understanding about the impact hormones have in the complex pathogenesis of RA.

Abbreviations

α	Alpha
ACPA	Anti-citrullinated protein antibody
ACTH	Adrenocorticotropic hormone
AS	Ankylosing spondylitis
β	Beta
BMD	bone mineral density
CI	Confidence interval
COMP	Cartilage oligomeric matrix protein
CRH	Corticotrophin-releasing hormone
DHEA	Dehydroepiandrosterone
DMARDs	Disease modifying anti rheumatic drugs
ECLI	ElectroChemiLuminiscence Immunoassay
ER-α	Estrogen receptor-alpha
ER-β	Estrogen receptor-beta
ESR	Erythrocyte sedimentation rate
Fc	Fragment crystallisable region, the tail region of an antibody
FSH	Follicle-stimulating hormone
HAQ	Health assessment questionnaire
HLA	Human leukocyte antigen
HPA	Hypothalamic-pituitary adrenal axis
HPG	Hypothalamic –pituitary-gonadal axis
HRT	Hormone replacement therapy

IgG	Immunoglobulin G
Il	Interleukin
JIA	Juvenile idiopathic arthritis
LH	Luteinizing hormone
MMP-3	Matrix metalloproteinase 3
NK	Natural killer
OC	Oral contraceptives
OR	Odds ratio
POF	Premature ovarian failure
PTPN22	Protein tyrosine phosphatase, non-receptor type 22
RA	Rheumatoid arthritis
RF	Rheumatoid factor
SLE	Systemic lupus erythematosus
STAT4	Signal transducer and activator of transcription protein number 4
Th-1	T-helper cell, type 1
Th-2	T-helper cell, type 2
Th-17	T-helper cell, type 17
TNF-α	Tumor necrosis factor alpha
Treg	Regulatory T cells

List of papers

This thesis is based on the following studies, referred to in the text by their Roman numerals. The papers have been reprinted with the permission of the publishers.

I *M Pikwer, U Bergström, J-Å Nilsson, L Jacobsson, G Berglund, C Turesson.* **Brestfeeding, but not use of oral contraceptives is associated with a reduced risk of rheumatoid arthritis**. *Ann Rheum Dis 2009; 68:526-530*

II *M Pikwer, U Bergström, J-Å Nilsson, L Jacobsson, C Turesson.* **Early menopause is an independent predictor of rheumatoid arthritis.** *Ann Rheum Dis 2012;71:378-381*

III *M Pikwer, J-Å Nilsson, U Bergström, L Jacobsson, C Turesson.* **Early menopause and severity of rheumatoid arthritis in women over 45 years of age.** *Arthritis Res & Ther 2012; 14:R190 [Epub ahead of print]*

IV *M Pikwer, A Giwercman, U Bergström, J-Å Nilsson, L Jacobsson, C Turesson.* **Association Between Testosterone Levels and Risk of Future Rheumatoid Arthritis in Men –a Population Based Case-control Study.** *Submitted*

Thesis at a glance

Paper I

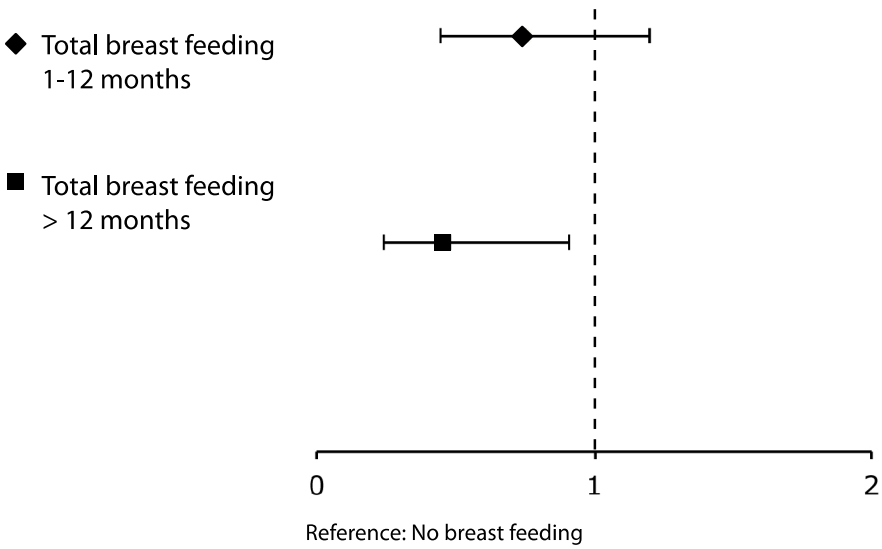


Figure 1. Longer duration of breastfeeding was associated with a reduced risk of RA, with an OR of 0.46 (95 % CI= 0.24-0.91) for women breastfeeding more than 12 months and an OR of 0.74 (95 % CI= 0.45-1.20) for women breastfeeding up to one year compared to women with no breastfeeding-history.

Paper II

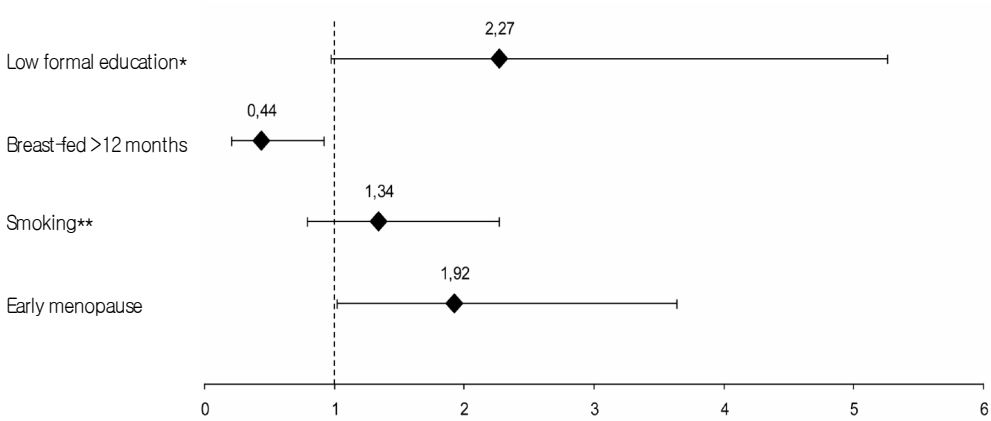


Figure 2. In a multivariate analysis of predictors of RA, early menopause (≤ 45 years of age) was significantly associated with the development of RA, and long term breast-feeding (>12 months) was associated with a reduced risk of RA.

Paper III

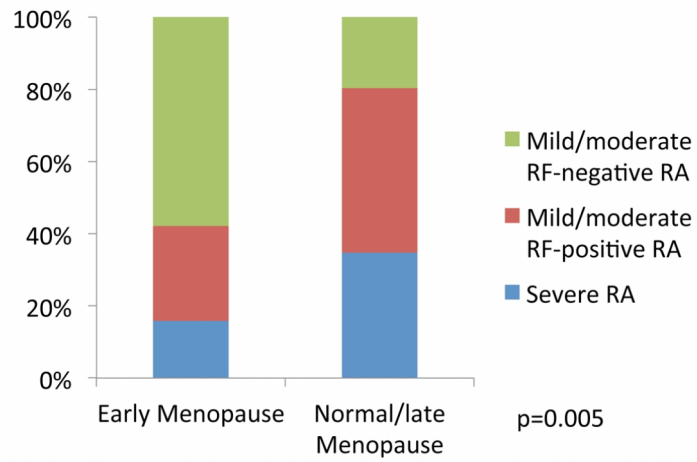


Figure 3. Early menopause was associated with the development of a mild type of RF negative RA (58 % vs. 20% for those with early menopause compared with those with normal/late menopause).

Paper IV

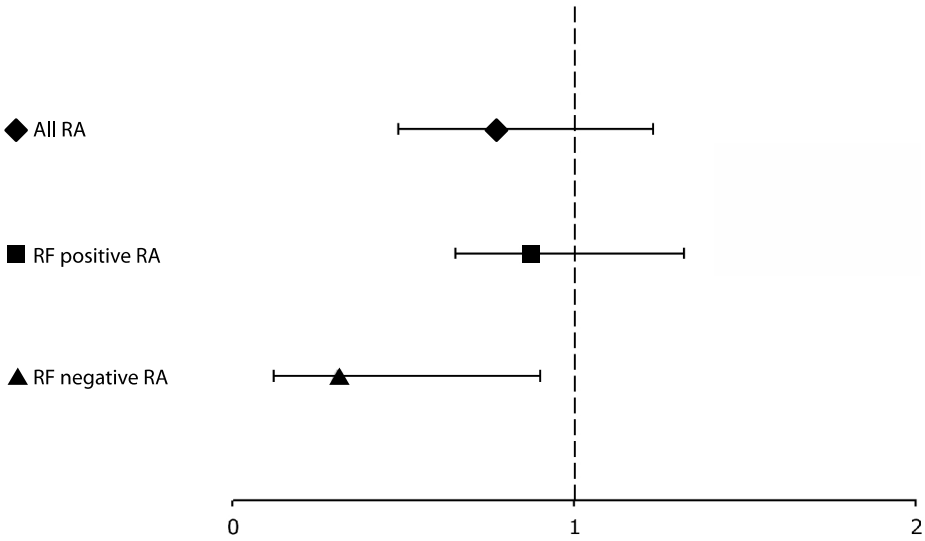


Figure 4. Testosterone levels were negatively associated with the development of RF negative RA (OR: 0.31 per standard deviation; 95 % CI: 0.12-0.85) in blood samples collected a median of 12.7 years prior to RA-diagnosis in men. This figure illustrates a multivariate analysis, adjusted for BMI, smoking and socioeconomic index.

Background

Rheumatoid arthritis

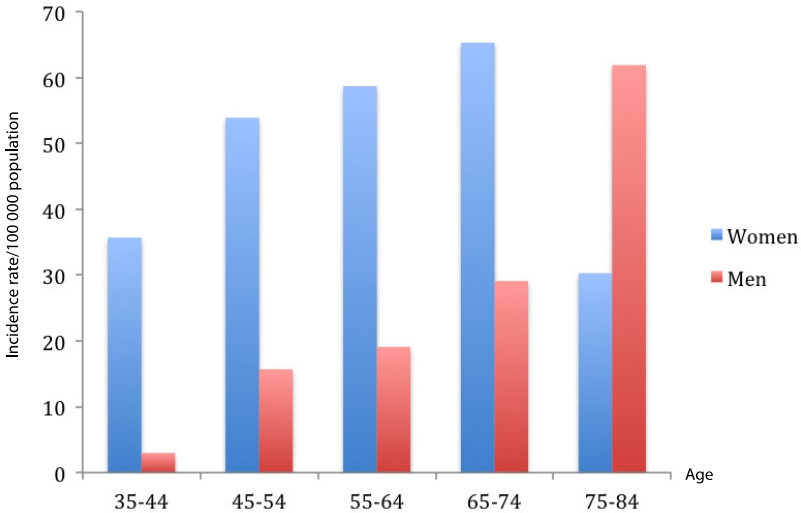
Epidemiology

Rheumatoid arthritis (RA) is a chronic inflammatory disease, associated with progressive disability, systemic complications and financial costs for the individual and for society. The term "rheumatoid arthritis" originates from the Greek words "rheum" and "arthro" and the suffix "-itis" which means flow, joint and inflammation, respectively. The terminology is based on the idea that human diseases originated from imbalance between body fluids. This was the basis for traditional treatments such as heat, bath, diet, laxation and bloodletting.

Some skeletal remains from Alabama, dated back to 3000-5000 years ago, have typical RA deformations [1]. A recent report describes the presence of the RA associated HLA-DRB1*0101 allele in a mummified Italian skeleton, with anatomical features of RA, dated to 1500 AD [2]. All this indicates that RA has been affecting humans for long time. Still the aetiology of RA remains unclear and it is only recently we have developed effective treatment, which can induce remission and prevent erosions in some patients.

The median annual incidence of RA in northern Europe has been estimated to be 29/100 000 [3-5]. A decline in RA incidence has been reported from the United States and Finland [6, 7]. On the other hand, a recent register based study estimated the overall annual incidence of RA to be 50/100 000 and prevalence to be 0.66 % in southern Sweden [8]. The disease can affect all ethnic groups throughout the world, but prevalence differ somewhat between races, ranging from 0.1% in native Africans to 5% in Pima Indians [9, 10], although methodological differences could explain some of the discrepancies. Overall, women have a more than twofold higher incidence of RA than men. This is mainly due to an increased risk for women during their reproductive years, when the incidence shows a female/male ratio of 4-6:1 [11-13]. Peak incidence of RA in women occurs after the menopausal age [11, 12]. RA is rare in men aged less than 45 years of age, but the incidence rises steeply with age [12] (Figure 5).

Figure 5. Incidence of Rheumatoid arthritis in 1990 in Norfolk, UK, based on a prospective population based register [12].



Diagnosis, clinical outcome and treatments

Rheumatoid arthritis is characterized by synovial inflammation and hyperplasia, autoantibody production as well as cartilage and bone destruction. Beside the joint symptoms, patients often experience fatigue and in some severe cases extra-articular manifestations. The disease is heterogeneous and instead of being a single disease, it has been regarded as a syndrome comprising several distinct phenotypes [14]. The most common subdivision is by the presence of anti-citrullinated protein antibodies (ACPA) and rheumatoid factor (RF). ACPA and RF positive patients tend to have more progressive erosive disease, and fewer reach remission [15].

The onset of RA is often insidious and may occur over weeks and months. The diagnosis is clinical and not based on any single diagnostic test. For the purpose of research on comparable patient samples, classification criteria have been developed and revised on several occasions. In this thesis, the 1987 American College of Rheumatology criteria for classification of RA have been used (table 1) [16].

However, recently (2010) new criteria have been established [17] to facilitate early diagnosis (Table 2).

Table 1. 1987 ACR classification criteria for RA [16].

Criterion	Definition
1. Morning stiffness	Morning stiffness in and around the joints, lasting at least 1 hour before maximal improvement
2. Arthritis of 3 or more joint areas	At least 3 joint areas simultaneously have had soft tissue swelling or fluid (not bony overgrowth alone) observed by a physician. The 14 possible areas are right or left PIP, MCP, wrist, elbow, knee, ankle, and MTP joints
3. Arthritis of hand joints	At least 1 area swollen (as defined above) in a wrist, MCP, or PIP joint
4. Symmetric arthritis	Simultaneous involvement of the same joint areas (as defined in 2) on both sides fo the body (bilateral involvement of PIPs, MCPs, or MTPs is acceptable without absolute symmetry)
5. Rheumatoid nodules	Subcutaneous nodules, over bony prominences, or extensor surfaces, or in juxtaarticular regions, observed by a physician
6. Serum rheumatoid factor	Demonstration of abnormal amounts of serum rheumatoid factor by any method for which the result has been positive in <5% of normal control subjects
7. Radiographic changes	Radiographic changes typical of rheumatoid arthritis on posteroanterior hand and wrist radiographs, which must include erosions or unequivocal bony decalcification localized in or most marked adjacent to the involved joints (osteoarthritis changes alone do not qualify)

For classification purposes, a patient shall be said to have RA if he/she has satisfied at least 4 or these 7 criteria. Criteria 1 through 4 must have been present for at least 6 weeks. Patients with 2 clinical diagnoses are not excluded. Designation as classic, definite, or probable RA is not to be made.

Table 2. Modified 2010 ACR-EULAR classification criteria for RA.

	Score
Target population (Who should be tested?): Patients who 1. have at least 1 joint with definite clinical synovitis (swelling)* 2. with the synovitis not better explained by another disease	
Classification criteria for RA (score-based algorithm: add score of categories A–D; a score of ≥6/10 is needed for classification of a patient as having definite RA)‡	
A. Joint involvement§	
1 large joint¶	0
2-10 large joints	1
1-3 small joints (with or without involvement of large joints)#	2
4-10 small joints (with or without involvement of large joints)	3
>10 joints (at least 1 small joint)**	5
B. Serology (at least 1 test result is needed for classification)††	
Negative RF *and* negative ACPA	0
Low-positive RF *or* low-positive ACPA	2
High-positive RF *or* high-positive ACPA	3
C. Acute-phase reactants (at least 1 test result is needed for classification)	
Normal CRP *and* normal ESR	0
Abnormal CRP *or* abnormal ESR	1
D. Duration of symptoms§§	
<6 weeks	0
≥6 weeks	1

Modified from Aletaha D, et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis Rheum 2010; 62: 2569–81.* [17]

**** The criteria are aimed at classification of newly presenting patients. In addition, patients with erosive disease typical of RA with a history compatible with prior fulfillment of the 2010 criteria should be classified as having RA. Patients with longstanding disease, including those whose disease is inactive (with or without treatment) who, based on retrospectively available data, have previously fulfilled the 2010 criteria should be classified as having RA.***

‡ Although patients with a score of <6/10 are not classifiable as having RA, their status can be reassessed and the criteria might be fulfilled cumulatively over time.

§ Joint involvement refers to any swollen or tender joint on examination, which may be confirmed by imaging evidence of synovitis. DIP joints , first carpometacarpal joints, and first MTP joint are excluded from assessment. Categories of joint distribution are classified according to the location and number of involved joints, with placement into the highest category possible based on the pattern of joint involvement.

¶ "Large joints" refers to shoulders, elbows, hips, knees, and ankles.

 # "Small joints" refers to the MCP joints, PIP joints, second through fifth MTP joints, thumb PIP or DIP joints, and wrists.

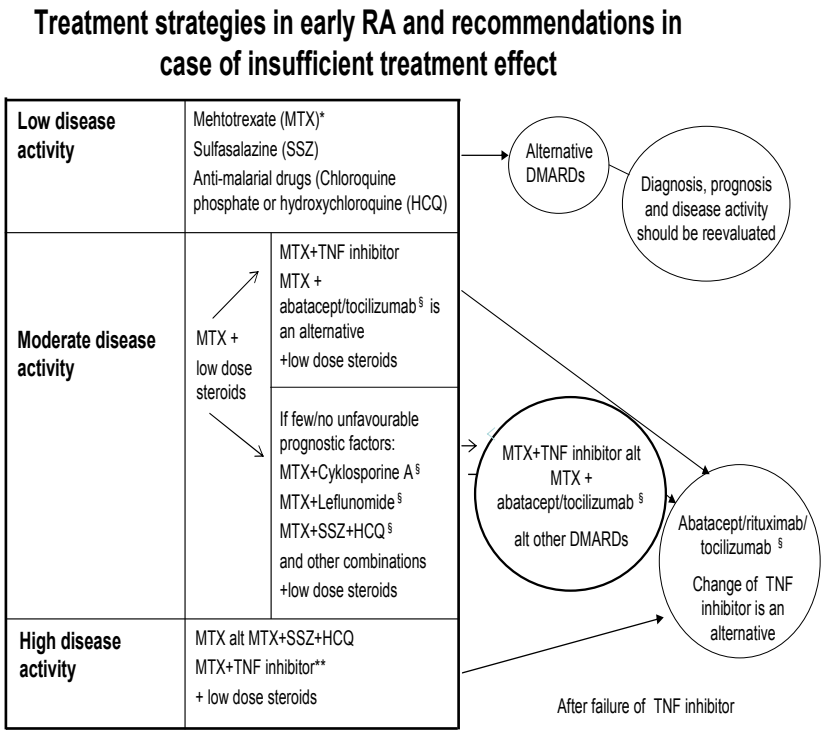
*** In this category, at least 1 of the involved joints must be a small joint; the other joints can include any combination of large and additional small joints, as well as other joints not specifically listed elsewhere (e.g., temporomandibular, acromioclavicular, sternoclavicular, etc.).*

†† Negative refers to IU values that are less than or equal to the upper limit of normal (ULN) for the laboratory and assay; low-positive refers to IU values that are higher than the ULN but ≤3 times the ULN for the laboratory and assay; high-positive refers to IU values that are >3 times the ULN for the laboratory and assay. Where RF information is only available as positive or negative, a positive result should be scored as low-positive for RF.

§§ Duration of symptoms refers to patient self-report of the duration of signs or symptoms of synovitis (e.g., pain, swelling, tenderness) of joints that are clinically involved at the time of assessment, regardless of treatment status.

There is an established notion that early medical interventions are crucial for future prevention of joint destruction [18]. In the 1980's and 1990's, the importance of treatment with disease modifying anti-rheumatic drugs (DMARDs) for the long term outcome were increasingly recognized, and methotrexate became the drug of choice for patients with severe disease. In the late 1990's the first biological treatments emerged, designed to target pathogenic mediators, mostly the pro-inflammatory cytokine Tumor necrosis factor alpha (TNF-α). The effect on symptoms and disease progression reformed the RA outcome. New biological treatments continue to develop. In Sweden, treatment with biologics for RA is based on national evidence based guidelines [19] and international recommendations [20, 21]) (Figure 6).

Figure 6. Guidelines for the pharmaceutical management of rheumatoid arthritis. Swedish Society of Rheumatology 2012.



*Above all with factors indicating unfavourable prognosis

**With several factors indicating unfavourable prognosis, e.g. verified early erosions, § listed in alphabetic order without indication of preference.

There is not one single test that can reflect the severity of the disease, no "golden standard". The assessment is a combination of the physician's examination and the patient's reported experience. As objective tests, we have radiographic damage and parameters of inflammation. One of the most frequent monitoring tools is the Disease Activity Score 28, which is a composite of the 28-joint swollen and tender joint counts, the ESR and the patient's global assessment, and therefore assesses inflammation and disease activity, but not disability. The Simplified Disease Activity Index is often used in clinical trials and the Clinical Disease Activity Index was constructed to be used in daily clinical practice [22]. The health assessment questionnaire (HAQ) is a self-administered questionnaire, consisting of questions

about 20 activities of daily living in 8 categories [23]. The HAQ is useful for assessing functional disability and also for monitoring the patient´s course and response to therapy. The HAQ scale range from 0 to 3, with higher scores being associated with worse disability and also predicting worse long-term outcome [24].

Pathogenesis

The pathogenesis of RA is complex and involves both the innate and adaptive immune system. Possible pathways include a genetic predisposition for abnormal T-cell selection, elevated cytokine production or enhanced protein citrullination that together with environmental exposure and chance trigger the innate immunity. Dendritic cells then migrate to central lymphoid organs and trigger the adaptive immune system, with T-cell activation and autoantibody production. The synovium is the primary localisation of inflammation, and a dominant cell at the site is the CD4+ T cell, also known as Th1 helper cell. Although RA generally is considered a Th 1 driven disease, new focus have been set on the role of type 17 helper T cells (Th17), which are characterized by production of IL-17 [25]. The synovial fluid increases dramatically in RA-patients due to increased leakage from the synovial microvasculature. In the fluid, cells from the innate immune system, like neutrophiles, NK-cells and macrophages, dominate. Growth factor β and interleukin-1β (IL-1β), IL-6, IL-21 and IL-23, all derived from macrophages and dendritic cells, stimulate Th 17 differentiation and suppress regulatory T cells (Treg). RA is also associated with the presence of circulating autoantbodies with a varying degree of specificity. Rheumatoid factors, which were first described in 1940, are autoantibodies directed against the Fc portion of IgG [25]. ACPA, which are more specific for RA, can be directed against several different proteins that include citrulline residues. Citrullination, i.e. the post translational modification of arginine into citrulline, is a physiologic process occurring during different conditions including inflammation [26].

Genetics

More than 30 risk alleles have been identified for seropositive RA. HLA-DRB1 alleles featuring the shared epitope [27] are found in most patients with seropositive RA [28], and are involved in the antigen presentation for T-cells. Other risk-genes that activate T-cells include the protein tyrosine phosphatase gene (PTPN22) and STAT4. Other known risk genes are associated with immune regulation, such as the NF-κB pathways [25]. One HLA-DRB1 allele, HLA-DRB1*13, have been found more frequently in healthy individuals than seropositive RA patients, and therefore appear to be associated with protection from RA [26].

There is an estimated concordance of 15% for RA in monozygotic twins and 5% for dizygotic twins [29]. This indicates that environmental factors also play an important role in the development of RA.

Early biomarkers

A biomarker is a variable that can be measured as an indicator of normal or pathological processes, as well as a response to treatment. Examples of biomarkers for RA are inflammatory variables such as C reactive protein (CRP) and erythrocyte sedimentation rate (ESR) and antibodies like ACPA and RF, which today are important as diagnostic tools, as well as prognostic. RF occurs in other rheumatic disorders, and also in some healthy individuals, whereas ACPA are highly specific. Studies have shown that both RF and ACPA can be detected in serum years before diagnosis [30].

Cartilage oligomeric matrix protein (COMP) is marker of cartilage turnover [31]. Elevated COMP levels have been found in RA patients, and correlate with large joint destruction [32, 33]. Raised COMP can be found in a subset of individuals a few years before RA diagnosis, in particular in ACPA negative pre-RA cases [33]. Matrix metalloproteinase 3 (MMP-3) is an enzyme that is highly expressed in the synovial cells of RA patients and involved in cartilage destruction. MMP-3 can also be measured in the serum and is known to correlate with disease activity. Increased levels of MMP-3 are, however, not RA specific [34].

Elevated levels of cytokines, such as IL-1α, IL-1β, IL-4, IL-10 and TNF-α have been detected in sera from pre-RA cases 5 years before the diagnosis [35].

Prognostic factors

A clinically relevant aspiration is to find prognostic biomarkers that enable the practice of personalized medicine, so that patients who are prone to develop a severe, destructive disease can receive appropriate treatment early in the disease course.

At group level, ACPA positive patients have worse radiological destruction [15]. Being female has been found to predict worse outcome, although some of the gender differences in RA prognosis may be explained by systematic differences in patient reported outcomes, rather than a true difference in objective measures of disease activity [36, 37]. Other prognostic factors for worse outcome include high CRP and ESR as well as the number of swollen joints at onset [38]. Early magnetic resonance imaging has been found to be able to predict radiographic erosions even better than RF and clinical features alone in two recent studies [39, 40].

Smoking is known to be associated with rheumatoid nodules and severe extra-articular manifestations in patients with early RA [41, 42], and a particularly strong association between smoking and RA associated vasculitis has been noted in several studies [43, 44].

Environmental predictors

Both genetic and environmental factors predispose to RA, and gene-environment interactions may occur years prior to clinical onset of the disease. Lower education and low socio-economic status are known to be associated with chronic diseases in general [45], and so also with RA [46], independently of smoking status. Socio-economic status could be a marker of infections, unfavourable dietary habits, poor dental health or other exposures.

It has been suggested that the so-called "Mediterranean diet", with high levels of olive oil and vegetables suppresses disease activity in RA patients [47], and vitamin D has been suggested to protect against RA [48]. Mixed results about the effect of caffeine on RA risk have been reported [49, 50]. Regarding infections, it has been proposed that Epstein Barr Virus could initiate the inflammatory process that leads to RA [51].

High birth weight has been found to be significantly associated with RA in a large cohort study of more than 87 000 women, including 619 women with confirmed incident RA [52] as well as in a case-control study of 77 RA cases in Sweden [53]. The mechanisms underlying the link between high birth weight and RA are not clear, but there are data connecting high birth weight and a less responsive Hypothalamus-Pituitary-Adrenal-axis (HPA) axis in adult life [54]. It has been

suggested that RA patients have a dysregulated HPA-axis [55], suggesting a biological connection between high birth weight and RA development.

A well-documented environmental predictor is smoking, which seems to be a stronger risk factor for men and mainly associated with RF and ACPA positive disease [56, 57]. It has been hypothesized that smoking leads to increased citrullination, which could lead to anti-citrulline autoimmunity in susceptible individuals [58]. Alcohol use has been associated with a reduced risk of RA in several studies [59, 60].

Stress related factors

Acute short-lived stress is known to enhance immune functions, and chronic long-lived stress is correlated to immunosuppression [61]. There seems to be fundamental differences between consequences of stress between healthy subjects and patients with chronic inflammatory diseases [61]. The effect of stress on younger individuals appears to be different compared to older persons. For instance, stress has been reported to be a disease-permissive factor for juvenile idiopathic arthritis (JIA) but not for RA [61]. Potential explanations include a stronger and more reactive immune system in younger individuals. There is however a recent study indicating that low job decision latitude, known to be associated with changes in the immune system, for instance increased IL-6 and fibrinogen levels, is associated with RA [62]. It remains a challenge to sort out such associations from other effects related to level of education or occupation.

Hormonal predictors

In 1978, results from the Royal College of General Practitioners´ Oral Contraception Study was published in the Lancet, saying that oral contraceptives (OC) protects against development of RA [63]. Results have thereafter been diverging, from reports confirming a protective effect to reports not reporting any association [64-66]. A study of healthy mothers of children with diabetes [67] showed that women who consumed OC had significantly lower prevalence of RF, which suggests that OC is protective against development of RF, but not necessarily against RA. RF-negative RA is often a milder disease and more likely to go into remission [68]. There are reports that have found that OC-use is associated with a milder type of RA with less disability, measured with HAQ [69, 70].

A Danish case-control study of 366 women, found that women who had a late menarche (≥ 15 years) had an almost doubled risk of RA compared with those who had menarche at 12 years or less [71]. This is in contrast to an American prospective study, which reported a non-significant opposite trend, where females

who entered menarche before the age of 11 had a higher risk of RA compared to menarche at 13 years of age [72].

During pregnancy, amelioration of the disease [73], and a high rate of remissions are seen, more often for ACPA-negative patients [74]. Possible explanations include a shift in the maternal immune system from Th1 to a more Th2 based defence and/or an increased number of regulatory T cells [75]. Several epidemiological studies have found parity to protect against RA [76-78], although some inconsistent results have been reported [72, 79]. A recent study argues that the discrepancies might be explained by differences in age and in the time that had elapsed since prior pregnancies. They demonstrated a strong protective effect of parity for RA with onset before age 45, but no significant reduction in disease with later onset [80]. Fetal microchimerism, when fetal cells, in this case including protective HLA alleles, remains in the mother after pregnancy, have been suggested as a possible explanation for the protective effect. The protective effect of parity was stronger closer to the childbirth [80].

Menopause is defined as cessation of a woman's menstrual period [81]. The mean age of natural menopause is 51 years in Sweden [82]. Premature menopause is menopause before 40 years of age. If the menopause has occurred naturally before age 40, it is referred to as premature ovarian failure (POF) [83]. Early menopause, often defined by menopause before the age of 45 [84], is a predictor of several autoimmune diseases, such as systemic sclerosis [85], SLE [86], type 1 diabetes [87] and giant cells arteritis [88]. A prospective cohort study from Iowa, USA, showed that women with menopause after the age of 51 had a relative risk of 0.64 to develop RA, compared to women with menopause before the age of 45 [89].

Hormonal replacement therapy (HRT) was introduced in 1941, to reduce clinical symptoms of menopause. HRT is primarily composed of estrogens, but also progesterone, the latter to diminish the risk of endometrium cancer. It has been suggested that HRT could have a protective effect against development of RA [90] and an ameliorating effect on established disease [91].

Gonadal steroid hormones

In general

All steroids are derived from cholesterol. The gonadal steroids consist of estrogen, androgens and progesterone. Estrogen is mainly produced by the granulosa cells of the ovary, but also to some degree by the adrenal cortex, adipose tissue and testicles. There are two types of estrogen receptors (ER), α and β, which estrogen binds to. After binding, the complex translocates to the nucleus and activate estrogen response elements in gene promoters [92].

Estrogen levels vary in premenopausal women depending on their menstrual cycle, are markedly increased during pregnancy and drop significantly in the postmenopausal state. It has an important role in the maturation of the reproductive system, plays a part in the preservation of the skeleton, and influences the immune system.

Estrogen can have opposite effect on immune system at high verses low levels and distinct effects on different cell types [93]. For instance a stimulatory effect on B-cells and both inhibitory and stimulatory effects on T-cells have been observed [94]. Estrogen has been shown to stimulate IFN-γ production from T-cells but inhibit IFN-γ production from macrophages and dendritic cells [95]. Studies of murine models of RA have shown amelioration of disease during pregnancy [96] and blocking of ER α and ER β has been noted to enhance the disease [97]. A recent Swedish study of a postmenopausal murine model noted that the positive effect of estrogen was conducted via ER α signalling pathways, and not ER β [98]. Proposed mechanisms of estrogen related immunomodulation with a positive effect on RA include upregulation of regulatory T cells, a shift towards Th2 differentiation and modulation of Th17 cells [95].

Progesterone is the second female sex hormone, and its main purpose is to maintain pregnancy. It has been reported to down-regulate the production of the pro-inflammatory cytokine IL-8 in rabbits [99].

Testosterone is the principal androgen, secreted mainly from the testis, but also from the adrenal cortex and the ovaries in women. In general, androgens tend to suppress immune responses [100], both the humoral and the cell-mediated immunity [101], and stimulate lymphocytes to a Th2 shift, although the interactions are complex and not fully established [102]. Mouse models of the human demyelinating disease multiple sclerosis, which is a Th1 driven autoimmune disease like RA, showed a protective effect of testosterone via a shift from Th1 to Th2 helper response [103].

In rheumatoid arthritis and other rheumatic diseases

The fact that the peak of incidence of RA in women coincides with the drop of estrogen [104], whereas for men the incidence continues to increase with age when testosterone levels decrease, indicates that gonadal steroids play a role in RA pathogenesis. Since estrogen has differential effect on the immune system, it is understandable that it could have varying effects on different autoimmune disorders. For instance, estrogen substitution has been seen to induce flares and increased antibody production in SLE-patients [105] but have an ameliorating effect on RA-patients [94].

A Swedish 2-year prospective, randomized controlled study analysed the effect of HRT on clinical and laboratory activity of RA, and reported a significant improvement in the group receiving HRT [91]. Both decrease in DAS28 and laboratory parameters like ESR and serum orosomucoid, as well as an increase in bone mineral density were demonstrated. A French study found that HRT might reduce the risk of RA, by protecting against anti-CCP production [90]. The results from the Women's Health Initiative randomized controlled trials did however not confirm a positive effect on RA severity [106]. A shorter, more recent clinical trial of 12 weeks, examining the effect of selective estrogen receptor β agonist, ERB-041, on RA patients, did not demonstrate any significant clinical effect [107], although one may argue that the follow-up time was rather short. Interestingly, one observational study showed a significantly elevated risk of RA in former HRT-users compared to none-users [89]. When considering HRT for patients with RA, the risk of cardiovascular complications [108], which is known to be increased in RA patients *per se* [109], also needs to be taken into account.

Tengstrand et al found that men with early RA had on average lower bioavailable testosterone [110]. Low levels of testosterone in both female and male RA patients compared to controls have been demonstrated in other cross-sectional studies [111-113]. Since chronic inflammation is known to lower testosterone through conversion to estrogen, it is difficult to know if the inflammation is an effect of low androgens, or vice versa [114]. A prospective study of female pre-RA cases found no evidence of lower androgen levels, measured at a single time point prior to RA onset, compared to matched controls [115].

Treatment with testosterone has been reported to have a positive but modest disease modifying effect amongst postmenopausal women with RA [116]. One randomised study of 30 male patients found no beneficial effect [117] and an even smaller study of 7 men suggested a positive effect of testosterone [118].

Gonadotrophins have been found to be altered in male RA-patients compared to controls, with diverse results [119, 120]. Tengstrand et al found low levels of Luteinizing Hormone (LH) in male patients compared to controls, even though

testosterone levels were low, indication a central dysfunction. Gordon et al. did, however, find opposite results, with high levels of LH [121].

Ankylosing Spondylitis (AS) is an autoimmune disease, which is mostly seen in men. A role for sex steroids in the pathogenesis has been suggested, because of the male predominance, increased number of flares post partum in women, and the fact that sex steroids can modulate immune functions. Some studies have reported slightly higher testosterone levels in patients with AS compared with controls, but these differences did not reach statistical significance [112]. Taken together, up to now there are no convincing data suggesting a role of sex steroids in AS [101].

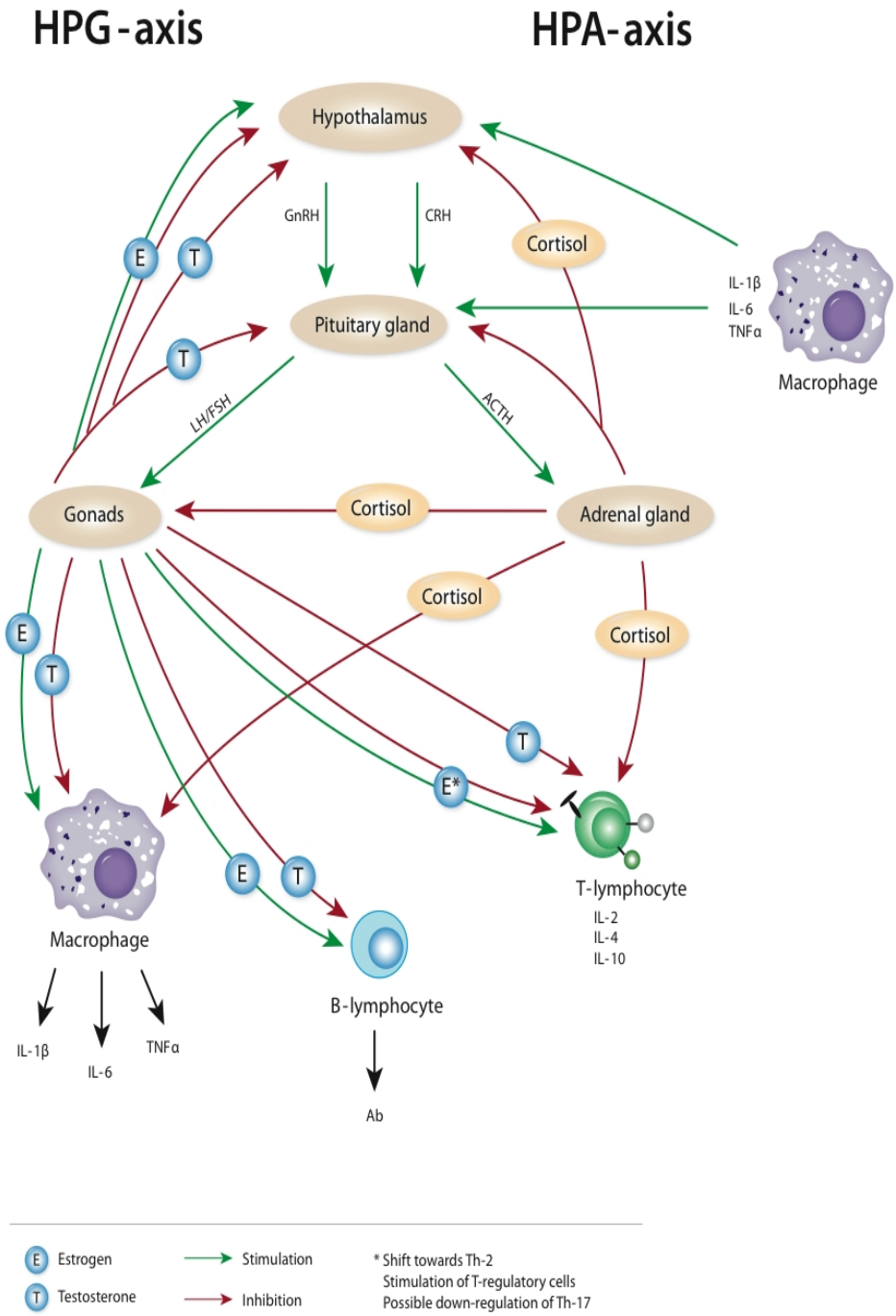
Hypothalamic-pituitary-adrenal axis

In general

The HPA-axis is a complex set of direct influence and feedback interactions between the hypothalamus, the pituitary gland and the adrenal gland (Figure 7). Corticotropin-releasing Hormone (CRH) is secreted from the hypothalamus, and stimulate the anterior pituitary gland to release Adrenocorticotropic hormone (ACTH), which in turn stimulate the adrenal cortex to release its hormones, which include mineralocorticoids, glucocorticoids, and androgens (mainly dehydroepiandrosterone (DHEA) and its sulphate derivative (DHEAs). DHEA is the active hormone, which can be converted to downstream androgens in cells like the macrophages [100]. Cortisol is the most potent antiphlogistic and immunosuppressive hormone, and its presence in peripheral blood has diurnal variation, with lowest levels at night/early morning. DHEA and DHEAS down regulate inflammation by inhibition of inflammatory cytokines like IL-1, IL-6 and TNF α [100].

IL-1 is a potent stimulator of the HPA-axis [122] and also an important cytokine in the pathogenesis of RA.

Figure 7. Illustration of the HPA and HPG axis.



In rheumatoid arthritis

In 1948 the rheumatologist Philip Hench administered Cortisol as treatment for RA for the first time, and 1950 he received the Nobel Prize for his achievement, together with Kendall and Reichstein [123]. The immune system can, through cytokines like IL-1, IL-6 and TNF α, activate the HPA axis in all three levels in the feedback system [124]. However, in RA patients the response to stressors are inadequately low, and lower adrenal products, such as DHEA and glucocorticoids have been measured in RA patients compared to healthy controls [102, 124]. Cortisol was the first treatment for RA with great success, although side effects like hyperglycaemia, hypertension and obesity were soon noticed, in particular with higher doses. Cortisol is still frequently used in RA treatment, in particular in low doses (5-7.5 mg/day) added to traditional DMARD therapy. Evidence of quick suppression of inflammation as well as inhibition of destruction has been noted in studies over two years of treatment [125, 126].

Aims

- To investigate if hormonal patterns during a woman's life, like menarchal age, use of OC, breast-feeding history and menopausal age, have any effect on the risk of RA.
- To examine the effect of hormone related factors on the severity of RA.
- To investigate if hormonal predictors differ between rheumatoid factor positive and negative disease.
- To explore androgen and gonadotropin levels in pre-RA men.

Patients and methods

Paper I and II

The Malmö Diet and Cancer Study

The Malmö Diet and Cancer Study (MDCS) is a population-based prospective cohort study performed in Malmö, Sweden, between 1991 and 1996. The main objective of the MDCS was to study the effect of diet on cancer. Baseline examination included a self-administered questionnaire, anthropometric measuring and blood sampling, stored in a biobank. All individuals living in Malmö and aged 44-74 on 1 January 1991 were invited. 30 477 subjects (18 326 women) were recruited; language problems and mental retardation were the only exclusion criteria.

Cases and controls

By linking the MDCS to 4 registers, a community based RA-register, the local outpatient clinic administrative register, the National Hospital Discharge Register and the National Cause of Death Register, we identified individuals that had developed RA after their participation in the MDCS. The medical records for all patients were reviewed and those patients that fulfilled the 1987 American College of Rheumatology (ACR) criteria for RA up until December 31, 2004 (n=172; 136 women) were included in our nested case control study. Four controls for each validated case, matched for sex, age and year of screening, who were alive and free of RA when the index person was diagnosed with RA, were selected from the MDCS.

Variables

Data on reproductive factors, such as OC use (including number of years taken and when started), number of births, whether each child was breast-fed and for how many months, age at menarche and if applicable age at menopause was obtained

using a self-administered questionnaire. OC use was stratified into never used, 1-5 years of use and >5 years of use. Breast-feeding history was stratified into never breast-fed, 1-12 months of cumulative breast-feeding and more than 12 months of cumulative breast-feeding (independent of the number of children). Early menarche was defined as menarche before the age of twelve, and early menopause as reported menopause at the age of 45 or younger. Since all cases and controls were 45 years or older when participating in the MDCS, those who reported that they had not yet reached menopause were classified in the normal/late group, with a menopause later than 45 years of age.

The stress-related factors studied included sleeping disorders, which were investigated using an instrument constructed on basis of the DSM-IV diagnostic criteria of insomnia [127]. The questions concerned problems with falling asleep, with waking up in the middle of night or waking up early in the morning. Each question had 4 level answers, from having no problem (1) to having major problems (4). For each question, individuals reporting a score of 3 or 4 were compared to those reporting a score of 1 or 2. In a previous study, individuals who reported moderate to major problems in several of these areas had an increased risk of developing musculoskeletal pain [14].

Physical and mental wellbeing was reported using a validated 7-grade scale question [128]. Responders were stratified into those reporting a lower level of wellbeing (1-4) compared to those reporting higher values (5-7). A question about stress due to problems outside work (yes vs. no), which has been used in previous studies of the MDCS cohort [129], was also included.

Paper III

The medical records of the patients identified in the MDCS and included in paper I and II were subjected to a structured review. Clinical outcomes, including erosions on x-rays, RF status, use of DMARDs and disability measured with HAQ were collected. The variables with best availability and clinical plausibility were added to the SPSS TwoStep Cluster analysis, an algorithm that allows use of both categorical and continuous variables in the same model. The algorithm first identified six clusters, which however became difficult to interpret from a clinical standpoint, and the small sample for each cluster was a concern. We limited the algorithm to maximum three clusters, and the subsequent analysis identified three clusters of clinically relevant RA severity: severe RA, mild/moderate RF-positive RA and mild/moderate RF negative RA (See table 3). Hormonal exposures were retrieved from the questionnaire described in paper 1, and only after the cluster definition was established, was the statistical analysis of cluster distribution by exposure category performed.

Table 3. Distribution of clusters of clinical outcomes.

	Cluster I: Severe RA	Cluster II: Mild/moderate RF-positive RA	Cluster III: Mild/moderate RF-negative RA
N	26	25	39
Treated with biologics	100%	0%	0%
RF positive	88.5%	100%	0%
Documented erosions on x-ray (ever)	84.6%	56.4%	52.0%
HAQ after 5 years -mean (SD) -median (IQR)	 1.17 (0.61) 1.00 (0.72-1.66)	 0.74 (0.61) 0.63 (0.38-0.88)	 0.88 (0.66) 0.88 (0.25-1.25)

RF=rheumatoid factor, HAQ=Health assessment questionnaire, SD=Standard deviation, IQR=interquartile range.

Paper IV

Malmö Preventive Medicine Program

Between 1974 and 1992, the Malmö Preventive Medicine Program (MPMP), a preventive case finding program for cardiovascular risk factors, alcohol abuse and breast cancer, was conducted, including a total of 22 444 males born between 1949 and 1921 and 10 902 females born between 1938 and 1925, in Malmö, Sweden (population 235 000 in 1974) [130]. The aim was to screen large strata of the adult population in order to identify individuals for preventive intervention. The overall attendance rate was 71.2%. The subjects underwent physical examination including height and weight measurements and laboratory tests, and completed a self-administered questionnaire on health and life style factors [131]. The subjects were invited to leave blood samples in the morning, between 8.00 and 10.00 a.m., after an overnight fast. The samples were stored at -20 degrees Celsius [132].

Selection of cases and controls

We have identified individuals who developed RA after inclusion in this cohort and up to December 31, 2004, by linking the MPMP register four different RA

registers (see methods for paper I and II). In a structured review of all medical records, possible cases were validated and classified according to the 1987 American College of Rheumatology criteria for RA [16]. Four matched controls for each validated case were selected from the MPMP cohort. Vital status and information on emigration were retrieved from the national census, and controls who were not alive or living in Sweden through index date were excluded.

For the present study, serum samples from two controls per case were collected from the MPMP bio bank. For various reasons, samples were missing from a subset of cases as well as controls. For cases with available serum, the retrieval of control samples was extended to include the two remaining matched controls.

Socioeconomic background and co-morbidities

Data on socioeconomic status was derived from self-reported job titles in the Swedish national censuses, as previously described [46]. Briefly, occupations were coded and converted into standardized social class categories, and subjects were classified as "blue-collar workers" (manual workers, both skilled and unskilled), "white collar workers" (non-manual employees and self employed professionals) and "others". Housewives, students and unemployed without any other self-reported job title during the study period were excluded from this classification [133].

Data on self reported overall health and self reported cancer, diabetes and cardiovascular disease (the latter classified as self report of either hospitalization for stroke, physician diagnosis of angina pectoris or current use of heart medication) at baseline were extracted from the self-administered questionnaire.

Laboratory tests

Serum total testosterone, sex-hormone binding globulin (SHBG), LH and follicle-stimulating hormone (FSH) concentrations were quantified by ElectroChemiLuminiscence Immunoassay (ECLI) based on Rutenium derivate according to routine methods used at the Department of Laboratory Medicin, Skåne University Hospital. Free testosterone was calculated from total testosterone and SHBG levels using the Vermeulen formula [134]. The erythrocyte sedimentation rate (ESR) was measured at screening according to the standard Westergren method.

Data on RF tests were collected from the databases of the two clinical immunology laboratories in the area. RF has been found to be a stable biomarker in follow-up studies [135]

Statistics

Paper I and II

Predictors were examined with chi-square test and logistic regression models, taking into account the matched design of the study. Each case and the corresponding controls were assigned a group number, which was entered into the logistic regression model as a categorical variable. Multivariate models were used to adjust for potential confounders. Statistical significance was set at $p < 0.05$ in all papers.

Paper III

To identify degree of severity, we used the SPSS TwoStep clustering algorithm, as mentioned in the methods section. This algorithm allows the use of both categorical and continuous variables, and selected outcomes measures chosen were based on data availability and clinical plausibility. Natural groupings were explored, and three clusters were chosen since these clusters were considered to reflect clinically relevant phenotypes of RA.

Cluster distribution was examined based by hormonal exposures with Chi-square test. To avoid multiple testing, we refrained from formal statistical comparisons of the individual outcome parameters, and instead presented them as descriptive data.

Analysis of Covariance (ANCOVA) was used to compare age-adjusted mean HAQ between subsets defined by hormonal predictors.

Paper IV

The influence of hormone levels on the risk of RA was examined in logistic regression analysis, taking into account the matched design of the study, as mentioned above. For comparability of the impact of hormones with different concentrations, OR for RA were calculated per SD of testosterone, FSH, LH and SHBG, respectively. Multivariate logistic regression analysis was used to adjust for potential confounders, such as BMI, smoking and socioeconomic status. Analyses

were stratified by RF status at diagnosis or later (ever positive vs. negative) and also by time from screening to RA diagnosis (above vs. below the median).

Ethics

Paper I-IV

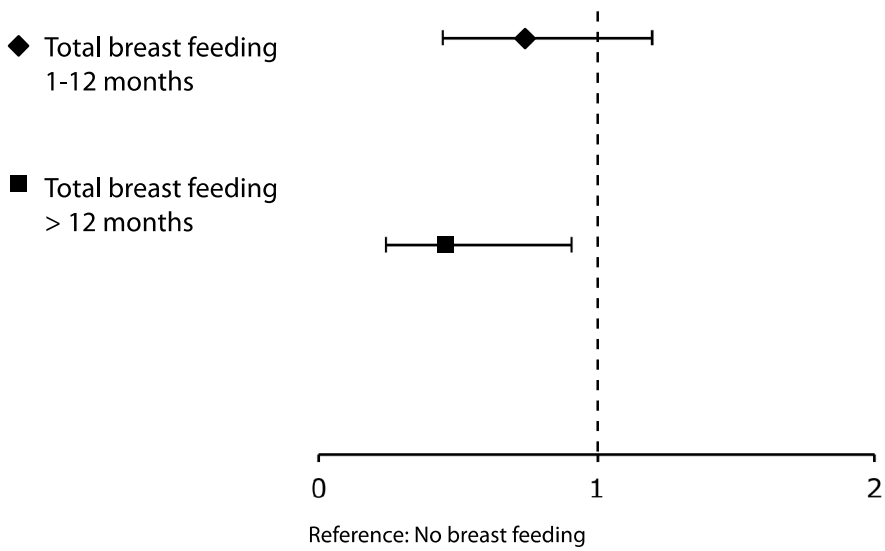
The regional research ethics committee for southern Sweden have approved all included studies in this thesis. The participants gave their informed consent to be included in the MDCS, the MPMP and the Malmö RA register, respectively. No informed consent was obtained specifically for the studies included in this thesis. The planned studies of predictors of RA was advertised in two local newspapers with good coverage, with a notice saying that the studied subjects could contact the investigators if they did not want to participate.

Results and discussion

Paper I and II

Baseline characteristics of cases and controls are described in Table 4. We saw a trend of reduced risk of RA for each child born, although this did not reach significance. Longer history of breast-feeding (> 12 months) was associated with a reduced risk of RA (OR: 0.46, 95% CI: 0.24-0.91), and OR for those who breast-fed 1-12 months was 0.74 (CI: 0.45-1.20) compared to women who did not breastfeed (figure 8).

Figure 8. Breast-feeding has a dose-dependent negative association with RA.

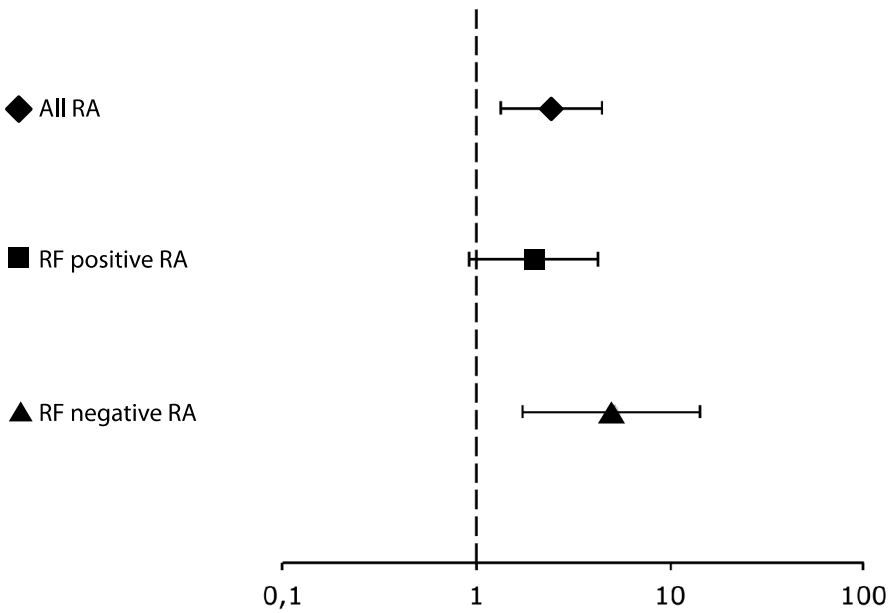


The associations were stronger for RF negative RA, although confidence intervals did overlap (OR for breast-feeding > 12 months vs. no breast-feeding: 0.30, 95 % CI 0.08-1.17 for RF negative RA; 0.61, 95 % CI 0.28-1.36 for RF positive RA).

We did not see any significant effect of OC use, or stress-related factors, on the risk of RA. Odds ratios with 95 % confidence intervals from bivariate analyses of all potential predictors are listed in Table 5.

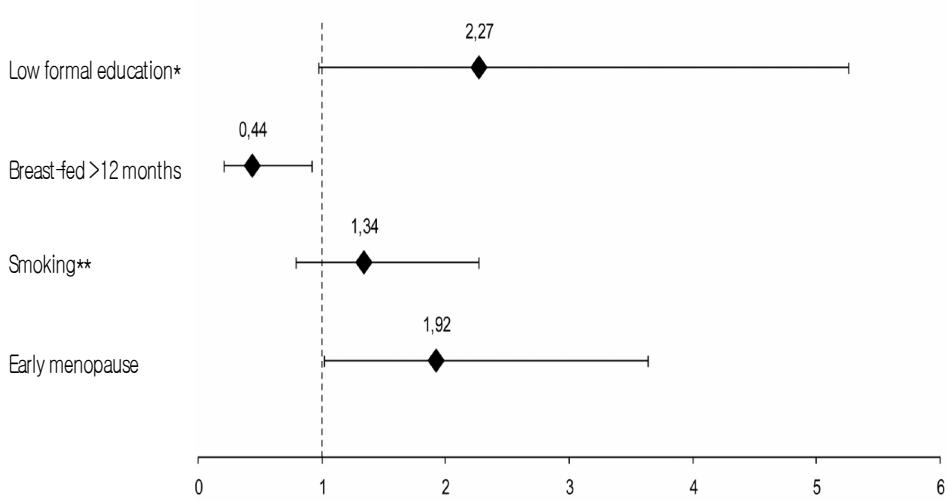
Early menopause (≤ 45 years) was a significant predictor of RA, and when stratifying by RF status at diagnosis or later, a stronger association was seen for RF negative RA (figure 9).

Figure 9. Early menopause was significantly associated with RF negative RA (OR: 5.00; 95% CI: 1.72-14.5), with an association of borderline significance with RF positive RA (OR: 1.98; 95% CI: 0.91-4.31). OR for all cases: 2.42; 95 % CI: 1.32-4.45).



In multivariate analysis, the impact of early menopause and long term breast-feeding on the risk of RA were both statistically significant (figure 10).

Figure 10. Multivariate analysis with hormonal and environmental predictors.



These results indicate that hormones are involved in the complex pathogenesis of RA. Possible explanations for the protective effect of breast-feeding could be long-term immunomodulation, a dysregulated HPA axis or unmeasured confounders. Although the association remained significant in a model adjusted for smoking and level of formal education, we cannot exclude that other life style factors or exposures associated with long term breast-feeding could play a role. Lankarani-Fard et al. noted significant higher levels of cortisol in post-menopausal women who had breast-fed compared to women who did not breast-feed. Lower cortisol-levels could indicate a dysfunctional HPA-axis. Taken together, women with breast-feeding problems could have a dysregulated HPA-axis as an explanation to both their breast-feeding problems and elevated risk of RA.

Our findings confirm the results of Karlson et al, who in 2004 reported a dose-dependent protective effect of breast-feeding in a large cohort (674 incident cases) of American nurses [136]. We observed a similar effect in a more heterogeneous population of women, with a wider range of socioeconomic backgrounds.

An impaired hormone axis could possible also manifest itself with higher occurrence of early menopause. However, the fact that duration of breastfeeding and age at menopause had independent effects in multivariate analyses suggest that the underlying mechanisms behind these associations may be different. There have been reports about a higher risk of RA in nulliparous women [76], which could be a confounder in the analysis of the effect of breast-feeding. However, we did not see

any significant effect of parity on development of RA in our material, and when limiting the analysis to women who ever had given birth, the protective effect of breast-feeding remained, although the CI was wider. In our material, the mean age at diagnosis was 63 years, with a range of 47 to 80 years. Therefore, we have no data on women with onset at a younger age than 47. It is reasonable to suspect that predictors could differ depending on age at onset, limiting our conclusions to women in their middle-age or older, who however represent the largest patient group.

We saw an inverse association between early menarche and RA, although the number of patients was limited. There was not any strong trend with OC-use observed, but there was a long lag period between OC-use and RA-development in this sample.

Estrogen is suggested to supress cellular immunity and stimulates a shift towards T-helper 2 subset. An early drop of estrogen levels, i.e. early menopause, may therefore enhance the risk of RA, since Th1 cells have been implicated to be an important part of the pathogenesis of RA. There are other prior studies that have found similar results about the effect of menopausal age on the risk of developing RA. Merlino et al reported that women who reached menopause at the age of 51 had a reduced risk of RA [89].

The exposure data were collected prior to outcome, which reduced the risk of recall bias or disease related effect on lifestyle. There may be recall error, because of the rather long time between breast-feeding as well as OC use, and answering the questionnaire. There is however no reason to believe that there would be a differential effect in this respect between cases and controls.

Table 4. Characteristics and baseline exposure information on RA cases and matched controls.

	Cases n=136	Controls n=544
Characteristics		
Mean age at RA diagnosis (SD)	63.3 (8.3)	NA
Mean age at screening (SD; Range)	57.9 (7.3; 45-72)	58.0 (7.3; 45-73)
Anti-CCP positive at diagnosis; %	56.0**	NA
RF positive at diagnosis; %	68.4†	NA
Baseline exposure information		
Current smoking; %	35.3	27.2
Low level of formal education	50.8	40.1
Reproduction related factors		
Mean age at menopause (SD; Range)	47.9 (6.0; 26-57)	49.2 (4.5; 19-59)
Early Menopause, ≤ 45 years; n (%)	26 (19.1)	62 (11.4)
Normal to late Menopause; n (%)	103 (75.7)	444 (81.6)
Mean age at menarche (SD; Range)	13.7 (1.44; 10-18)	13.5 (1.5; 10-20)
Early menarche, <12 years; n (%)	3 (2.2)	46 (8.5)
Normal to late menarche; n (%)	125 (97.7)	465 (91.0)
Number of fertile years; mean (SD)*	34.2 (6.4)	35.6 (4.7)
Ever used OCs (%)	63 (48.5)	254 (49,4)
Never used OCs (%)	67 (51.2)	259 (51.2)
Used OCs 1-5 years (%)	18 (14.0)	110 (21.7)
Used OCs >5 years	44 (34.1)	137 (27.1)
Given birth to ≥ 1 child; n (%)	106 (77.9)	445 (81.8)
Breast-fed 0 months; n (%)	43 (31.6)	137 (25.2)
Breast-fed 1-12 months; n (%)	74 (54.4)	297 (54.6)
Brest-fed >12 months; n (%)	19 (14.0)	110 (20.2)
Self reported stress related factors		
Lack of physical and mental wellbeing; n (%)	39 (30.0)	161 (31.3)
Problems falling a sleep at night; n (%)	32 (23.5)	105 (19.3)
Problems waking up in the night; n (%)	32 (23.5)	136 (25)
Problems waking up early; n (%)	13 (9.6)	85 (15.6)

*Data on 487 out of 680; ** 26/46 tested; † 91/133 tested.

Table 5. Odds ratios with 95 % confidence intervals (CI) from bivariate analyses of all potential predictors analysed in paper I an II.

	OR (95% CI)
No breast-feeding	Referent
1-12 months of breast-feeding	0.74 (0.45-1.20)
> 12 months of breast-feeding	0.46 (0.24-0.91)
No OC use	Referent
Ever used OCs	1.03 (0.63-1.67)
Each child born	0.87 (0.71-1.06)
Normal/late menopause	Referent
Early menopause (≤ 45 years)	2.42 (1.32-4.45)
Normal/late menarche	Referent
Early menarche (< 12 years)	0.21 (0.06-0.70)
Lack of physical and mental wellbeing	0.93 (0.58-1.49)
Problems falling asleep	1.63 (0.89-2.96)
Problems waking up in the night	0.92 (0.51-1.66)
Problems waking up early	0.52 (0.24-1.10)
Recent experience of stress or mental pressure because of problems outside work	0.84 (0.50-1.40)

Paper III

Individual outcome measures by age at menopause are listed in Table 6. Early menopause was significantly associated with a milder type of RF negative RA (Cluster III) (Figure 11), with fewer erosions on x-rays, no history of biological treatment and lower HAQ. Age adjusted HAQ scores were lower in women with early menopause, compared to women with normal/late menopause, at all time points, with borderline significance (p=0.06) at diagnosis (Table 7). HAQ is an important patient reported outcome measures, and also an established predictor of long-term outcome in RA-patients.

Table 6. Clinical outcomes - by age at menopause.

	Early Menopause (<46 years) (N: 25)*	Normal/late Menopause (≥ 46 years) (N: 102)*
Mean age at diagnosis; years (SD)	65.2 (6.94)	62.8 (8.48)
Mean follow-up time; years (SD)	10.5 (3.0)	10.1 (3.6)
>3 DMARDs used (%)	5/25 (20.0)	23/101 (22.8)
DMARDs in combination (%)	8/25 (32.0)	45/101 (44.6)
Biological treatment ever (%)	3/25 (12.0)	33/101 (32.7)
Documented radiographic erosions (ever) (%)	14/23 (60.9)	52/96 (54.2)
RF positive (%)	11/25 (44.0)	78/100 (78.0)

RF=rheumatoid factor, HAQ=Health assessment questionnaire, SD=Standard deviation, CI=Confidence Interval, *In case of missing data, numbers (n) indicated for each variable. **Adjusted for age at diagnosis.

Figure 11. Distribution of clusters of clinical outcomes defining disease severity; by age at menopause.

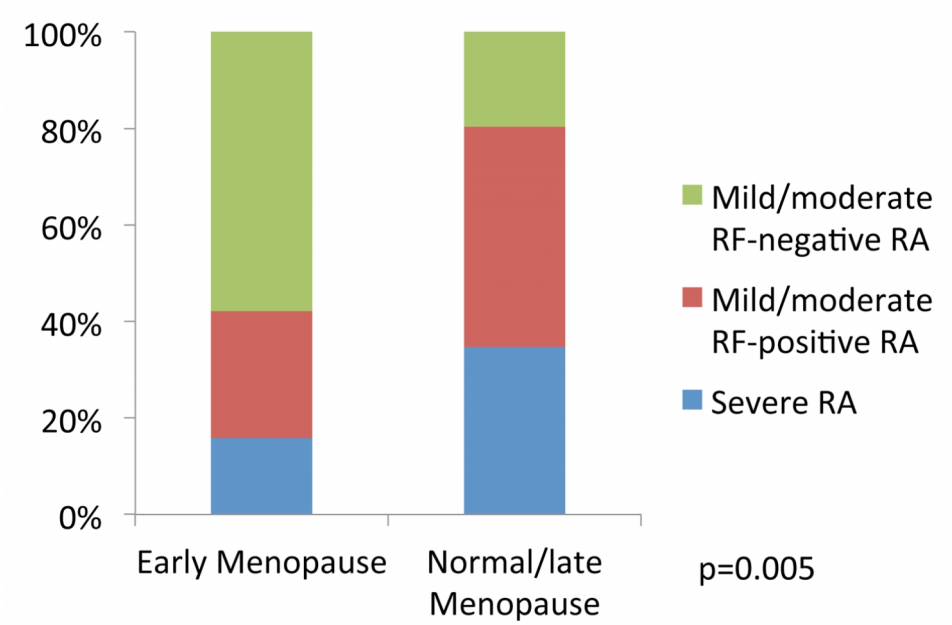


Table 7. Age-adjusted HAQ values depending on menopause status.

	Early Menopause (<46 years) (N: 25)*	Normal/late Menopause (≥ 46 years) (N: 102)*
Mean HAQ at diagnosis, (CI)**	0.58 (0.22-0.93) (n: 12)	0.96 (0.79-1.12) (n: 55)
Mean HAQ 1 year after diagnosis, (CI)**	0.45 (0.08-0.83) (n: 12)	0.86 (0.70-1.02) (n: 65)
Mean HAQ 5 years after diagnosis, (CI)**	0.88 (0.60-1.15) (n: 19)	0.90 (0.76-1.05) (n: 68)
Mean HAQ 10 years after diagnosis, (CI)**	0.67 (0.34-1.00) (n: 12)	0.98 (0.82-1.14) (n: 49)

These results are in accordance with paper I-II, where hormonal factors were stronger predictors for RF negative RA, pointing at the possibility that there is different pathogenesis behind various RA phenotypes. Hormones may be important in a subgroup of pre-RA cases, and not necessarily those who develop the more typical erosive RF-positive RA.

Early menopause is a known predictor of other types of autoimmune diseases [85, 86, 88], but also osteoporosis, mood disorders and premature death [137]. This support the idea that early menopause might not be a specific predictor for RA, and instead an indicator of general morbidity, a HPG-axis dysfunction or premature aging. It has been suggested that patients with RA have immunologic abnormalities indicating immunosenescence [138] due to premature aging of the immune system [139].

Individual outcome measures by length of reported breast-feeding and duration of previous OC use are listed in Tables 8 and 9, respectively. There was no significant effect of breast-feeding or OC-use on the severity of RA, measured as the distribution of clusters I-III (Figures 12 and 13). The individuals in our study had on average long time-span between OC use and RA diagnosis (20 years), which may explain the lack of effect on RA severity. Menopause is a predictor that occurs closer to diagnosis, which means that differences in severity related to menopause may be more likely to be detected in this type of study.

Table 8. Clinical outcomes-by duration of breast-feeding.

	*Breast-feeding 0 months (N:43)**	*Breast-feeding 1-12 months (N:72)**	*Brest-feeding >12 months (N:19)**
Mean age at diagnosis; years (SD)	63.6 (8.4)	62.8 (8.3)	64.2 (8.3)
Mean follow-up time; years (SD)	9.6 (3.4)	10.3 (3.7)	10.5 (2.8)
> 3 DMARDs used (%)	8 /43(18.6)	17/71 (23.9)	5/19 (26.3)
DMARDs in combination (%)	14/43 (32.6)	32/71 (45.1)	8/19 (42.1)
Biological treatment ever (%)	10/43(23.3)	21/71 (29.6)	6/19 (31.6)
Documented radiographic erosions (ever) (%)	25/39 (64.1)	36/70 (51.4)	10/16 (62.5)
RF positive (%)	28/43 (65.1)	53/71 (74.6)	13/18 (72.2)
Mean HAQ 5 years after diagnosis, (CI)**	0.91 (0.68-1.14) (n: 31)	0.90 (0.72-1.09) (n: 49)	0.85 (0.48-1.22) (n: 12)

Figure 12. Distribution of clusters of clinical outcomes defining disease severity; by breast-feeding history.

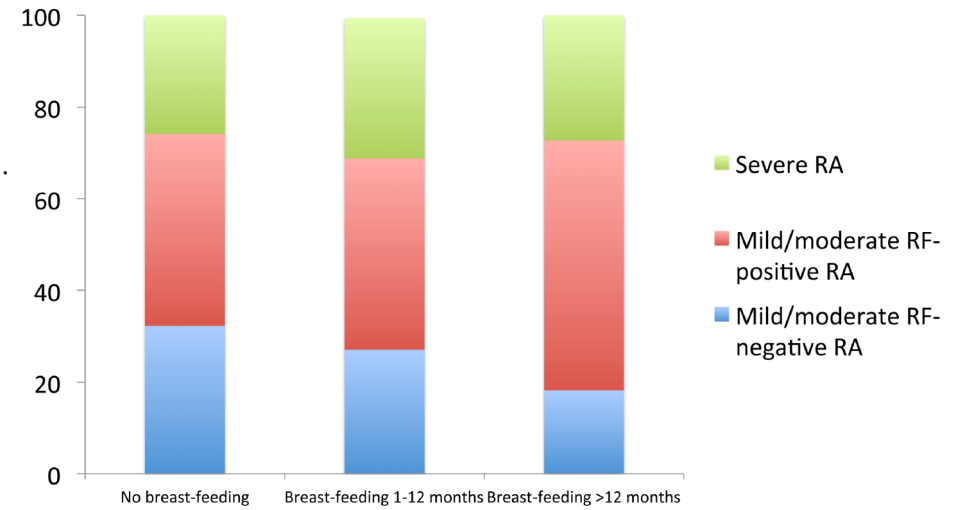
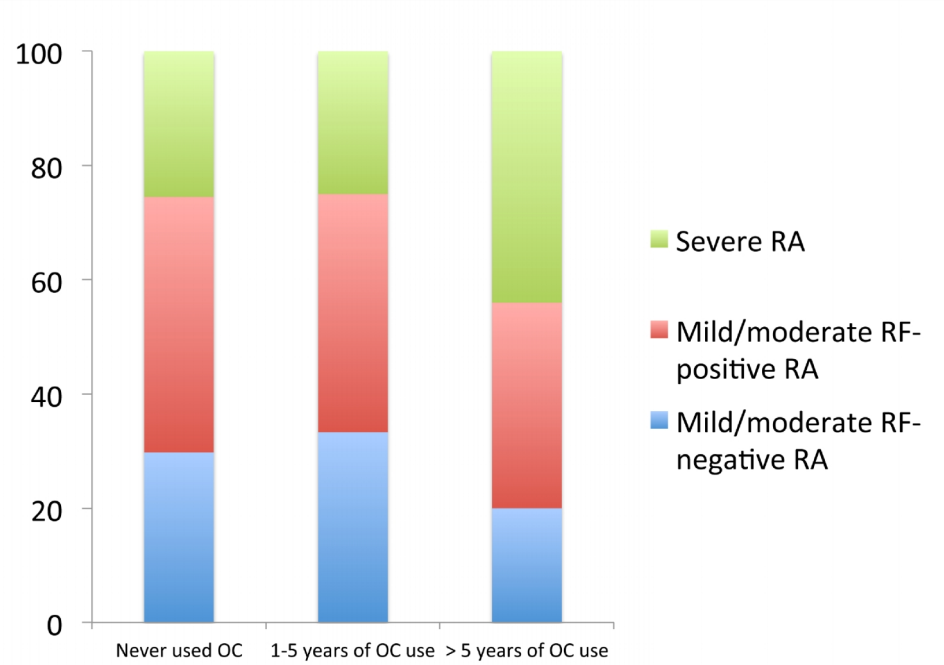


Table 9. Clinical outcomes-by history of use of oral contraceptives.

	Never used (N:67)*	1-5 years of use (N:17)*	> 5 years of use (N:43)*
Mean age at diagnosis; years (SD)	66.2 (7.8)	58.8 (8.2)	60.1 (7.2)
Mean follow-up time; years (SD)	10.4 (3.3)	9.9 (3.1)	10.0 (3.8)
> 3 DMARDs used (%)	11/67 (16.4)	4/17 (23.5)	14/42 (33.3)
DMARDs in combination (%)	26/67 (38.8)	8/17 (47.1)	20/42 (47.6)
Biological treatment ever (%)	14/67 (20.9)	4/17 (23.5)	19/42 (45.2)
Documented radiographic erosions (ever) (%)	37/62 (59.7)	10/17 (58.8)	20/40 (50.0)
RF positive (%)	46/66 (69.7)	10/16 (62.5)	34/43 (79.1)
Mean HAQ 5 years after diagnosis, (CI) **	0.93 (0.75-1.11) (n: 47)	0.80 (0.46-1.15) (n: 13)	0.86 (0.62-1.10) (n: 26)

Figure 13. Distribution of clusters of clinical outcomes defining disease severity; by use of Oral Contraceptives.



To our knowledge, this is the first report on the effect of menopausal age on the severity, and the results need to be confirmed in other studies.

Paper IV

One hundred and four incident male patients and 174 matched controls with available stored serum were identified. Characteristics and measured hormone levels are listed in Table 10. RF status was available for 83 patients. There were no differences between this subset and the entire group of incident male RA cases in age at screening (mean 45 vs. 46 years), age at RA diagnosis (59 years in both groups) or the proportion of blue-collar workers (52 vs. 54%).

Table 10. Characteristics and hormone levels of pre-RA cases and controls.

	Cases (104)	Controls (174)
Mean age at screening (SD)	46.0 (6.9)	46.0 (7.4)
Mean age at diagnosis (SD)	59.3 (9.4)	NA
RF positive at diagnosis or later	61/83 (73%)	NA
Median time to diagnosis (IQR, Range)	12.7 (8.4-19.4, 1-28)	NA
BMI kg/m^2 mean (SD)	24.3 (2.53)	25.0 (3.58)
Current smokers	58/104 (56 %)	79/174 (45 %)
ESR (mm) median (IQR, Range)	4 (3-8, 1-50)	5 (3-7, 1-61)
Mean testosterone nmol/L (SD)	21.5 (6.05)	21.6 (7.46)
Mean free testosterone nmol/L (SD)	0.42 (0.09)	0.42 (0.12)
Mean LH IU/L (SD)	5.31 (2.78)	5.44 (2.61)
Mean FSH IU/L (SD)	5.95 (4.70)	6.79 (5.69)
Mean SHBG nmol/L (SD)	36.08 (14.6)	35.05 (15.4)

[†] From analyses of testosterone, results were available from 104 cases and 174 controls; for FSH 101 cases and 169 controls; for LH 102 cases and 167 controls; for SHBG 104 cases and 173 controls; for free testosterone 104 cases and 173 controls.

Cases tended to have marginally lower mean values of all measured hormones except SHBG compared to controls (Table 10), with the main differences observed among those with a longer time from screening to diagnosis (Table 11).

Individuals who developed RA >12.7 years after screening were older (mean 65 vs. 54 years) and more likely to be RF negative (65 % vs. 35 %) at diagnosis.

Table 11. Mean hormonal levels in cases and controls. Stratified by median time to diagnosis (12.7 years).

	<12.7 years from diagnosis		>12.7 years from diagnosis	
	Cases	Controls	Cases	Controls
Mean testosterone nmol/L (SD)	22.1 (6.43)	22.0 (7.99)	20.8 (5.58)	21.3 (6.90)
Mean free testosterone nmol/L (SD)	0.43 (0.10)	0.43 (0.12)	0.41 (0.09)	0.42 (0.11)
Mean LH IU/L (SD)	5.57 (3.37)	5.26 (2.65)	5.00 (1.85)	5.66 (2.58)
Mean FSH IU/L (SD)	6.59 (5.98)	7.23 (6.62)	5.21 (2.40)	6.42 (4.54)
Mean SHBG nmol/L (SD)	36.6 (15.8)	35.9 (16.5)	35.5 (13.3)	34.5 (14.3)

Table 12 shows hormone levels by RF status at diagnosis or later, indicating that the differences between cases and controls in testosterone are greater in the RF negative cases.

Table 12. Mean hormonal levels in cases and controls. Stratified by Rheumatoid factor (RF) status.

	RF positive cases N =61		RF negative cases N =22	
	Cases	Controls	Cases	Controls
Mean testosterone nmol/L (SD)	21.9 (6.23)	21.7 (7.77)	19.4 (5.08)	21.6 (7.29)
Mean free testosterone nmol/L (SD)	0.42 (0.09)	0.42 (0.12)	0.40 (0.08)	0.44 (0.12)
Mean LH IU/L (SD)	5.11 (2.22)	5.27 (2.72)	5.40 (1.83)	5.73 (2.73)
Mean FSH IU/L (SD)	5.61 (2.22)	7.03 (6.29)	6.49 (3.69)	6.00 (4.27)
Mean SHBG nmol/L (SD)	37.9 (14.2)	36.4 (15.8)	30.8 (14.5)	33.1 (12.9)

In bivariate analyses, without adjustment for potential confounders, there was a trend towards a negative association between total and free testosterone levels and

RF negative RA (Table 13). There was a statistically significant positive association between FSH levels and risk of RF negative RA (Table 13).

Table 13. Unadjusted associations between levels of hormones, smoking, BMI and the risk of RA. Bivariate logistic regression analysis; Odds Ratio (95% Confidence Interval).

	All (104 cases)	RF positive (61cases)	RF negative (22 cases)
Testosterone per SD (6.95 nmol/l)	1.00 (0.72-1.38)	1.11 (0.73-1.70)	0.56 (0.26-1.16)
Free testosterone per SD (0.11 nmol/l)	0.92 (0.66-1.28)	1.00 (0.64-1.55)	0.58 (0.28-1.18)
FSH per SD (5.36 IU/l)	0.80 (0.56-1.16)	0.54 (0.27-1.03)	13.1 (1.70-100)
LH per SD: (2.67 IU/l)	1.00 (0.72-1.40)	0.89 (0.56-1.39)	1.14 (0.44-3.00)
SHBG per SD (15.1 nmol/l)	1.16 (0.85-1.58)	1.24 (0.82-1.88)	0.75 (0.34-1.63)
Current smokers (vs. non-smokers)	1.86 (0.94-3.68)	2.82 (1.16-6.89)	0.37 (0.09-1.48)
BMI per SD (3.24 kg/m^2)	0.73 (0.52-1.03)	0.74 (0.50-1.14)	0.57 (0.24-1.31)

Pre-RA cases had lower BMI than controls, a relationship that has recently been noted in male patients with early seronegative RA [140]. Smoking is a known risk factor of RA, which has been reported from this material earlier [46]. When adjusting for these potential confounders in a multivariate logistic analysis, lower free and total testosterone levels were associated with subsequent development of RF negative RA (Table 13). Low testosterone is a known consequence of inflammation, but there is no indication that these pre-patients would have any nascent inflammation related to arthritis, since there were no differences in ESR or self-reported health status between cases and controls.

We also noted differences in gonadotropin levels between pre-RA cases and controls, with higher FSH levels in the group of males that later developed a RF negative RA and lower FSH levels among those who later develop a RF positive disease, indicating partly different patho-mechanisms behind these phenotypes of RA in men. As mentioned earlier, there have been suggestions that patients with RA have a dysregulated HPA-axis, and also an altered HPG-axis [141]. Our study supports that this may be the case, at least in the pre-clinical phase for male patients with subsequent RF positive RA. The relation between FSH levels in patients with

RA and other inflammatory disorders and other hormonal pathways involving the pituitary should be further studied.

Low testosterone has previously been noted in men with RA, but since inflammation could reduce testosterone by acting both on the HPG-axis and stimulate the conversion of testosterone to estrogens by aromatase activity, it has been difficult to distinguish, based on cross sectional studies, whether low testosterone could be a part of the pathogenesis of RA in men or merely a consequence. Beside a small negative study from Finland [142], our study is the first study to explore hormone levels in men prior to RA diagnosis. Ours was the first prospective study with adjustment for confounders and with the possibility to study different phenotypes separately.

Testosterone has been seen to reduce TNF-α response in endothelial cells in atherosclerosis, and men with low testosterone levels are at increased risk of coronary artery disease [143]. It is possible that it could be the same mechanism that causes the association between RF negative RA and lower testosterone levels. Furthermore, since testosterone has been shown to contribute to a shift towards a Th2 response in an experimental model [103], testosterone may influence the risk of Th1 driven diseases, such as RA, through an effect on T cell differentiation in the pre-clinical phase.

Table 14. Associations between levels of measured hormones and risk of RA adjusted for smoking and/or body mass index. Stratified by rheumatoid factor (RF) status at diagnosis or later. Multivariate Logistic Regression; Odds Ratio (95% Confidence Interval).

	All (104 cases)	RF positive (61 cases)	RF negative (22 cases)
Testosterone per SD*	0.77 (0.52-1.12)	0.87 (0.53-1.43)	0.31 (0.12-0.85)
Free testosterone per SD*	0.75 (0.52-1.09)	0.83 (0.51-1.35)	0.38 (0.15-0.92)
FSH per SD**	0.77 (0.53-1.13)	0.42 (0.20-0.88)	11.5 (1.46-91.1)
LH per SD**	0.96 (0.68-1.36)	1.07 (0.53-2.15)	0.36 (0.08-1.69)
SHBG per SD**	1.89 (0.93-3.85)	1.11 (0.72-1.71)	0.83 (0.38-1.82)

*Adjusted for smoking and BMI, **adjusted for smoking.

Conclusions

- Cumulative long-term breast-feeding (total of > 12 months) was negatively associated with subsequent development of RA after the age of 45.
- We did not observe any effect of former use of OC on the risk of RA
- Early menopause was a predictor of RA, with an OR of 2.42.
- Early menopause was associated with a mild type of RF negative RA.
- Hormone related predictors had stronger associations with the development of RF negative disease in both men and women.
- Lower levels of testosterone were predictive of RF negative RA in men.
- Lower levels of FSH were found in men that later developed RF positive RA, and higher levels were found in those who later developed RF negative RA.

Final comments and future perspectives

Breast-feeding

Trends in breast-feeding habits have been reported, with very high levels in the 1940s, gradually declining to the 1970s, at which time 5 % of mothers breast-fed for ≥ 6 months [144]. It is difficult to know what influences mothers to breast-feed or not, but UNICEF launched a global programme in 1990, Baby Friendly Hospitals Initiative, in response to the low breast-feeding occurrence. The main purpose was to prevent perinatal malnutrition and mortality in developing countries, but also to educate individuals in more developed countries about the benefits of breast-feeding for both the child and the mother. During the last decades a steady increase in the proportion who breast-fed for ≥ 6 months has occurred. This figure has reached 73 % in the new millennium in Sweden [144], and similar trends have been seen in many other countries throughout the world [145, 146]. At the same time, a decline in the incidence of RA, and a shift towards later onset of RA have been reported [7], which of course could have many possible explanations. In Sweden there has been a debate about the frequent encouragements to breastfeed, as it could bring pressure to mothers who for different reasons cannot breast-feed. A thesis about mothers with breast-feeding difficulties, concluded that having breast-feeding problems lead to a both internal and external questioning (i.e. both the mother herself and others question her role as a mother and ability to care for the child) [147].

Established benefits of breastfeeding for the mother include breast cancer [148] and ovarian cancer [149]. Aside from our study, there was a report from a large prospective study from the USA in 2004, which found that longer duration of breastfeeding is associated with a lower risk of RA. Diverging results have however been published from northern Sweden [150]. In that retrospective study, the women were younger at diagnosis, indicating that hormones could have different short term and long-term effect. The information on breast-feeding was collected after the disease had occurred, which could have influenced the answers.

Throughout this thesis, the exposures were collected prior to the outcome, limiting the risk of disease influence on life style or of recall bias. Further prospective studies on the impact of breast-feeding on the risk of RA in large samples from different populations would be of interest.

In addition to the previously discussed potential associations between HPA-axis and breast-feeding, possible mediators of the protective effect of breast-feeding include a long-term effect of prolactin, the hormone that stimulates lactation. High levels of prolactin is associated with different autoimmune conditions, particularly SLE, sjogrens syndrome and systemic sclerosis, but the effect on RA is not clear [151]. Another potentially interesting hormone, which is released during lactation, is oxytocin. Oxytocin is known to lower blood pressure [152], protect tissues from oxidative stress [153] and induce wellbeing [154]. In addition, the role of this mediator in the pre-clinical phase of RA should be investigated.

Menopausal age as predictor of RA

Early menopause is a known predictor of morbidity, for instance it is associated with an elevated risk of SLE [86] and giant cells arteritis [88], as well as higher mortality overall [43, 155]. Whether it is the hormonal changes at a younger age that trigger the autoimmune process, or if having a menopause earlier is a consequence of another pathological process, is of course impossible to answer with an epidemiological study.

There have been some promising studies on an ameliorating effect of HRT on postmenopausal RA-women. Since severe side effects have been identified with the old type of HRT, new studies are being conducted with selective estrogen receptor modulators, which are molecules that mediate estrogen-like effects in some tissues, but antagonistic effects in other, thereby reducing the side effects. This could be an interesting adjuvant therapy for women with RA.

Menopausal age as predictor of disease severity

Prognostic indicators are of major interest for clinicians as well as patients, and information about menopausal age is something that most female patients remember and is easy to assess. Since the present study is the first one to investigate the relation between disease severity and menopausal age, more studies need to be conducted to be able to draw any conclusions that may be used as a basis for disease management. However, our studies suggest that hormone related factors may be more important for development of a milder RA phenotype in women (i.e. the association between early menopause and mild/moderate RF negative RA) as well

as in men (i.e. the association between lower testosterone levels and subsequent RF negative RA). The relative contribution of non-hormone related factors might be greater for classic, RF positive RA. This may be the basis for improved understanding of the mechanisms underlying different disease subtypes, and in the future influence therapeutic strategies.

Androgens

It is a interesting and relevant notion that low testosterone levels could play a part in the pathogenesis of RA, since the disease is less common in men and the risk of RA increase as they get older and the androgen levels drops. Testosterone is easy to measure, and does not fluctuate as much as estrogen. It is however known to be influenced by smoking [156], as was also noted in our material. Smokers had higher levels of testosterone, but also LH and SHBG, which could indicate insufficient peripheral effect of testosterone. It has been suggested that testosterone receptors could develop resistance when exposed to smoking. Testosterone could possibly also be influenced by various other environmental factors that we are unaware of, and therefore could not adjust for.

High BMI is associated with lower testosterone, and the reason for this is not fully established. Possible explanation include an increased conversion of testosterone to estradiol in adipose tissues, influence of adiposity on testosterone and SHBG via the HPG-axis as well as the notion that testosterone may mobilize abdominal fat deposits in males [157].

The differences in testosterone in pre-RA men noted in our material were only apparent in models adjusted for relevant confounders. This makes it more complicated to measure testosterone as a predictor of RA in the clinical situation.

More studies need to be done on the effect of androgens on the immune cells, as well as the effect of the gonadotrophins LH and FSH on the immune system. In principle, low testosterone could merely be an indicator of a sluggish HPG-axis, rather than having a direct effect on the risk of RA. The hormonal patterns observed in our study of pre-RA men suggest that RF positive disease is influenced by a sluggish HPG-axis, whereas testicular dysfunction with impaired testosterone production is a predictor of RF negative disease. Based on this, androgen substitution may be more helpful in men with RF negative RA. Except for the possible positive effect on disease symptom, there might be benefits in areas of osteoporosis, cardiovascular disease and erectile dysfunction [158], problems that are already present in men with RA [109, 159]. Potential side effects, such as negative effect on undiagnosed prostate cancer need to be taken into account. If

our results are confirmed in other studies, controlled clinical trials of androgen substitution for RA may be indicated, in particular for men with RF negative RA.

Concluding remarks

RA is a very complex systemic rheumatic disease, with a dynamic and changing process, and with great variations and heterogeneity between individuals and within the same patient during his/her lifetime. Epidemiologic data indicate that hormones are important in RA, and more research has been conducted over the latest decades about the mechanism behind these patterns. The diverging results could partly be a consequence of the need to separate the disease in different subgroups and also by sex, as the underlying pathological mechanisms seem to be different, as indicated in this thesis. More studies need to be conducted on the cellular mechanism behind the hormonal effect on the immune system and patterns of early biomarkers in relation to different hormonal predictors. Expanding knowledge about the importance of hormones in RA may further improve our understanding of the disease and the prospects of successful management.

Populärvetenskaplig sammanfattning

Ledgångsreumatism är en kronisk autoimmun sjukdom, som framföra allt angriper lederna, och orsakar smärta, stelhet och svullnad. Sjukdomens svårighetsgrad kan variera kraftigt och ett vanligt sätt att värdera svårighetsgraden av sjukdomen är att mäta antikroppar (till exempel reumatoid faktor) i blodet. De som har antikroppar har oftast en svårare sjukdom än de som inte har det (reumatoid faktor negativa). I fertil ålder är sjukdomen är 4-6 gånger vanligare hos kvinnor i än hos män. Män kan också drabbas, och risken ökar ju äldre de är, samtidigt som produktion av det manliga hormonet testosteron minskar. Orsaken till sjukdomen är inte klarlagd, men det pågår forskning och flera riskfaktorer har blivit mer etablerade, som t.ex. rökning och ärftlighet. Att hormoner kan påverka vårt immunförsvar är känt sedan tidigare, och könsfördelning gör att man misstänker att könshormoner kan påverka risken att få ledgångsreumatism samt hur svår sjukdom man får.

Sedan sjuttiotalet har man i Malmö genomfört två stora hälsoundersökningar, Malmö Förebyggande Medicin (MFM) mellan 1974-1992 talet och Malmö Kost Cancer (MKC) 1991-1996. Syftet med dessa var att finna riskfaktorer för hjärt-och kärlsjukdom samt cancer. Genom att söka i våra register med patienter med ledgångsreumatism har vi i vår forskning identifierat män och kvinnor som medverkade i dessa hälsoundersökningar och sedan utvecklade ledgångsreumatism. I hälsoundersökningarna har de medverkande svarat på frågeformulär, samt lämnat blodprover, som har sparats nedfrysta.

I de första två artiklarna beskriver vi hur vi i vår forskning undersökt huruvida hormonella faktorer påverkar risken att insjukna i ledgångsreumatism. Vi fann 136 kvinnor som var med i MKC, och senare utvecklade ledgångsreumatism. För varje fall valde vi sedan ut 4 kontroller som medverkade i MKC samma år och som var i samma ålder som fallet. Samtliga medverkande har givit sitt tillstånd att delta i forskning, som är godkänd av etiska kommittén. Genom att jämföra dessa grupper noterade vi att de kvinnor som ammade sina barn en längre tid, hade lägre risk att få ledgångsreumatism senare i livet. Ju längre tid de hade ammat, desto mindre var risken att få sjukdomen. Amning mer än ett år halverade risken. Vi såg ingen tydlig påverkan på risken att få sjukdomen om man hade ätit p-piller eller fött flera barn. Vi såg också att kvinnor som kom tidigt i klimakteriet, vid 45 år eller tidigare, hade en ökad risk att få sjukdomen jämfört med de kvinnor som kom i klimakteriet senare. Detta samband kvarstod även när vi tagit hänsyn till rök-vanor och

utbildningsnivå, och vi vet från tidigare studier att detta är faktorer som påverkar både risken att få ledgångsreumatism och att komma i tidigt klimakterium.

Genom att studera patienternas journaler undersökte vi om dessa hormonella faktorer, som påverkade risken att få ledgångsreumatism, också påverkade hur svår sjukdom man får. Vi noterade att tidigt klimakterium ökade risken att få en mildare typ av ledgångsreumatism, som oftast var reumatoid faktor negativ.

I tidigare studier har man funnit att män med ledgångsreumatism har lägre nivåer av könshormonet testosteron. Man vet också av tidigare forskning, att den inflammation som finns kroppen när man har ledgångsreumatism kan sänka nivåerna av testosteron. Det är därför svårt att veta om de uppmätta låga nivåerna hos patienterna är en konsekvens av sjukdomen eller en del av orsaken.

Vi fann i våra register 104 män som utvecklat RA efter att ha medverkat i MFM, och för vilka blodprov fanns sparade. När vi mätte hormonnivåerna fann vi att män som utvecklade en mildare typ av ledgångsreumatism, reumatoid faktor negativ sjukdom, hade lägre nivåer av testosteron än matchade kontroller, efter att man tagit hänsyn till deras rökvanor, vikt och längd. Denna studie är den första som har visat ett sådant samband, och fler studier behövs för att bekräfta det.

Sammantaget har vi i vår forskning funnit att hormonella faktorer kan påverka risken att få en mildare variant av ledgångsreumatism. Amning skyddar kvinnor från att insjukna. Att komma tidigt i klimakteriet är kopplat till en ökad risk för att få ledgångsreumatism, men den typ man får är en mildare variant. Män som utvecklar den mildare typen av sjukdomen har lägre nivåer av testosteron i blodet innan sjukdomen utvecklas.

Acknowledgements

Carl Turesson, my supervisor, who took me under his wings, and opened the door for science. The most reliable and hardworking supervisor I could ever hope for. Thank you for believing in me, and for your patience. It has been fun and I hope to continue our collaboration in some way in the future.

Lennart Jacobsson, my co-supervisor, and a big source of inspiration, both clinically and in science. It is a great privilege to have gotten to know you and work with you.

Jan-Åke Nilsson, my statistician, whom without I would be really lost.

Ulf Bergström, co-PhD-student and friend. Thank you for identifying all patients, and by that making my thesis possible. And for all giving discussions.

Aleksander Giwercman, professor in reproductive medicine and co-writer in paper IV, great scientist with an inspiring drive.

Bjarne Hansen, for mentor-ships and philosophical guidance. My clinical role model.

Minna Willim, my data-manager and a wonderful person, who gave paper III structure and credibility.

Anders Dahlin, data-manager, who helped us with data-extractions.

Gabriella Olsson and Susanne Skog, for their hospitality and generosity when I was gathering information for paper III.

Christina Book, Ylva Lindroth, Lida Marsal, rheumatologist who contributed with patients to my research.

Elke Theander, chief, but most of all, a friend. Thank you for the support.

Elisabeth Lindqvist, understanding chief.

Tore Saxne and **Pierre Geborek** for advice and encouragement.

Viola Toth Sörensson, graphic designer, for helping me with layout during the whole process.

Staff at the rheumatology department, for always being helpful and sending me good vibes and being wonderful company.

Annalisa Unkuri, best friend and artist who let me use her magnificent artwork in this thesis and several posters.

Family (Solveig, Daryush, Kave and Sam), always there, always supportive. My backbone.

Andreas, the love of my life, for believing in me before I did, and generously sharing his knowledge and love for science.

Nina and Fred for reminding me daily what is important in life, and making me forget everything else.

References

1 Rothschild BM, Woods RJ. Symmetrical erosive disease in Archaic Indians: the origin of rheumatoid arthritis in the New World? Semin. Arthritis Rheum. 1990;19(5):278-84.

2 Fontecchio G, Fioroni MA, Azzarone R, et al. Genetic predisposition to rheumatoid arthritis in a Tuscan (Italy) ancient human remain. Int J Immunopathol Pharmacol 2007;20(1):103-9.

3 Alamanos Y, Voulgari PV, Drosos AA. Incidence and prevalence of rheumatoid arthritis, based on the 1987 American College of Rheumatology criteria: a systematic review. Semin. Arthritis Rheum. 2006;36(3):182-8.

4 Uhlig T, Kvien TK, Glennas A, Smedstad LM, Forre O. The incidence and severity of rheumatoid arthritis, results from a county register in Oslo, Norway. J. Rheumatol. 1998;25(6):1078-84.

5 Soderlin MK, Borjesson O, Kautiainen H, Skogh T, Leirisalo-Repo M. Annual incidence of inflammatory joint diseases in a population based study in southern Sweden. Ann. Rheum. Dis. 2002;61(10):911-5.

6 Helmick CG, Felson DT, Lawrence RC, et al. Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part I. Arthritis Rheum. 2008;58(1):15-25.

7 Kaipiainen-Seppanen O, Kautiainen H. Declining trend in the incidence of rheumatoid factor-positive rheumatoid arthritis in Finland 1980-2000. J. Rheumatol. 2006;33(11):2132-8.

8 Englund M, Joud A, Geborek P, Felson DT, Jacobsson LT, Petersson IF. Prevalence and incidence of rheumatoid arthritis in southern Sweden 2008 and their relation to prescribed biologics. Rheumatology (Oxford). 2010;49(8):1563-9.

9 Del Puente A, Knowler WC, Pettitt DJ, Bennett PH. High incidence and prevalence of rheumatoid arthritis in Pima Indians. Am. J. Epidemiol. 1989;129(6):1170-8.

10 Jacobsson LT, Hanson RL, Knowler WC, et al. Decreasing incidence and prevalence of rheumatoid arthritis in Pima Indians over a twenty-five-year period. Arthritis Rheum. 1994;37(8):1158-65.

11 Doran MF, Pond GR, Crowson CS, O'Fallon WM, Gabriel SE. Trends in incidence and mortality in rheumatoid arthritis in Rochester, Minnesota, over a forty-year period. Arthritis Rheum. 2002;46(3):625-31.

12 Symmons DP, Barrett EM, Bankhead CR, Scott DG, Silman AJ. The incidence of rheumatoid arthritis in the United Kingdom: results from the Norfolk Arthritis Register. Br. J. Rheumatol. 1994;33(8):735-9.
13 Kvien TK, Uhlig T, Odegard S, Heiberg MS. Epidemiological aspects of rheumatoid arthritis: the sex ratio. Ann. N. Y. Acad. Sci. 2006;1069:212-22.
14 Scott IC, Steer S, Lewis CM, Cope AP. Precipitating and perpetuating factors of rheumatoid arthritis immunopathology: linking the triad of genetic predisposition, environmental risk factors and autoimmunity to disease pathogenesis. Best practice & research. Clinical rheumatology 2011;25(4):447-68.
15 van der Helm-van Mil AH, Verpoort KN, Breedveld FC, Toes RE, Huizinga TW. Antibodies to citrullinated proteins and differences in clinical progression of rheumatoid arthritis. Arthritis research & therapy 2005;7(5):R949-58.
16 Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum. 1988;31(3):315-24.
17 Aletaha D, Neogi T, Silman AJ, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum. 2010;62(9):2569-81.
18 Klareskog L, Catrina AI, Paget S. Rheumatoid arthritis. Lancet 2009;373(9664):659-72.
19 Turesson C, Baecklund E, Forsblad d'Elia H.Guidlines for the pharmaceutical management of rheumatoid arthritis.The Swedish Society of Rheumatology.http://www.svenskreumatologi.se/riktlinjer
20 Smolen JS, Landewe R, Breedveld FC, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs. Ann. Rheum. Dis. 2010;69(6):964-75.
21 Saag KG, Teng GG, Patkar NM, et al. American College of Rheumatology 2008 recommendations for the use of nonbiologic and biologic disease-modifying antirheumatic drugs in rheumatoid arthritis. Arthritis Rheum. 2008;59(6):762-84.
22 Anderson JK, Zimmerman L, Caplan L, Michaud K. Measures of rheumatoid arthritis disease activity: Patient (PtGA) and Provider (PrGA) Global Assessment of Disease Activity, Disease Activity Score (DAS) and Disease Activity Score With 28-Joint Counts (DAS28), Simplified Disease Activity Index (SDAI), Clinical Disease Activity Index (CDAI), Patient Activity Score (PAS) and Patient Activity Score-II (PASII), Routine Assessment of Patient Index Data (RAPID), Rheumatoid Arthritis Disease Activity Index (RADAI) and Rheumatoid Arthritis Disease Activity Index-5 (RADAI-5), Chronic Arthritis Systemic Index (CASI), Patient-Based Disease Activity Score With ESR (PDAS1) and Patient-Based Disease Activity Score Without ESR (PDAS2), and Mean Overall Index for Rheumatoid Arthritis (MOI-RA). Arthritis Care Res (Hoboken) 2011;63 Suppl 11:S14-36.
23 Fries JF, Spitz PW, Young DY. The dimensions of health outcomes: the health assessment questionnaire, disability and pain scales. J. Rheumatol. 1982;9(5):789-93.

24 Pincus T, Callahan LF. Quantitative measures to assess, monitor and predict morbidity and mortality in rheumatoid arthritis. Baillieres Clin. Rheumatol. 1992;6(1):161-91.
25 McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. N. Engl. J. Med. 2011;365(23):2205-19.
26 Bax M, van Heemst J, Huizinga TW, Toes RE. Genetics of rheumatoid arthritis: what have we learned? Immunogenetics 2011;63(8):459-66.
27 Gregersen PK, Silver J, Winchester RJ. The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. Arthritis Rheum. 1987;30(11):1205-13.
28 Turesson C, Matteson EL. Genetics of rheumatoid arthritis. Mayo Clin. Proc. 2006;81(1):94-101.
29 Silman AJ, MacGregor AJ, Thomson W, et al. Twin concordance rates for rheumatoid arthritis: results from a nationwide study. Br. J. Rheumatol. 1993;32(10):903-7.
30 Rantapaa-Dahlqvist S, de Jong BA, Berglin E, et al. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. Arthritis Rheum. 2003;48(10):2741-9.
31 Saxne T, Heinegard D. Cartilage oligomeric matrix protein: a novel marker of cartilage turnover detectable in synovial fluid and blood. Br. J. Rheumatol. 1992;31(9):583-91.
32 Mansson B, Carey D, Alini M, et al. Cartilage and bone metabolism in rheumatoid arthritis. Differences between rapid and slow progression of disease identified by serum markers of cartilage metabolism. J. Clin. Invest. 1995;95(3):1071-7.
33 Turesson C, Bergstrom U, Jacobsson LT, Truedsson L, Berglund G, Saxne T. Increased cartilage turnover and circulating autoantibodies in different subsets before the clinical onset of rheumatoid arthritis. Ann. Rheum. Dis. 2011;70(3):520-2.
34 Ribbens C, Andre B, Kaye O, et al. Synovial fluid matrix metalloproteinase-3 levels are increased in inflammatory arthritides whether erosive or not. Rheumatology (Oxford). 2000;39(12):1357-65.
35 Jorgensen KT, Wiik A, Pedersen M, et al. Cytokines, autoantibodies and viral antibodies in premorbid and postdiagnostic sera from patients with rheumatoid arthritis: case-control study nested in a cohort of Norwegian blood donors. Ann. Rheum. Dis. 2008;67(6):860-6.
36 Carmona L, Cross M, Williams B, Lassere M, March L. Rheumatoid arthritis. Best practice & research. Clinical rheumatology 2010;24(6):733-45.
37 Forslind K, Hafstrom I, Ahlmen M, Svensson B. Sex: a major predictor of remission in early rheumatoid arthritis? Ann. Rheum. Dis. 2007;66(1):46-52.
38 Combe B, Landewe R, Lukas C, et al. EULAR recommendations for the management of early arthritis: report of a task force of the European Standing Committee for International Clinical Studies Including Therapeutics (ESCISIT). Ann. Rheum. Dis. 2007;66(1):34-45.

39 Tanaka N, Sakahashi H, Ishii S, Sato E, Hirose K, Ishima T. Synovial membrane enhancement and bone erosion by magnetic resonance imaging for prediction of radiologic progression in patients with early rheumatoid arthritis. Rheumatol. Int. 2005;25(2):103-7.
40 Hetland ML, Ejbjerg B, Horslev-Petersen K, et al. MRI bone oedema is the strongest predictor of subsequent radiographic progression in early rheumatoid arthritis. Results from a 2-year randomised controlled trial (CIMESTRA). Ann. Rheum. Dis. 2009;68(3):384-90.
41 Nyhall-Wahlin BM, Jacobsson LT, Petersson IF, Turesson C. Smoking is a strong risk factor for rheumatoid nodules in early rheumatoid arthritis. Ann. Rheum. Dis. 2006;65(5):601-6.
42 Nyhall-Wahlin BM, Petersson IF, Nilsson JA, Jacobsson LT, Turesson C. High disease activity disability burden and smoking predict severe extra-articular manifestations in early rheumatoid arthritis. Rheumatology (Oxford). 2009;48(4):416-20.
43 Struthers GR, Scott DL, Delamere JP, Sheppeard H, Kitt M. Smoking and rheumatoid vasculitis. Rheumatol. Int. 1981;1(3):145-6.
44 Turesson C, Schaid DJ, Weyand CM, et al. Association of HLA-C3 and smoking with vasculitis in patients with rheumatoid arthritis. Arthritis Rheum. 2006;54(9):2776-83.
45 Pincus T, Callahan LF, Burkhauser RV. Most chronic diseases are reported more frequently by individuals with fewer than 12 years of formal education in the age 18-64 United States population. J. Chronic Dis. 1987;40(9):865-74.
46 Bergstrom U, Jacobsson LT, Nilsson JA, Berglund G, Turesson C. Pulmonary dysfunction, smoking, socioeconomic status and the risk of developing rheumatoid arthritis. Rheumatology (Oxford). 2011;50(11):2005-13.
47 Skoldstam L, Hagfors L, Johansson G. An experimental study of a Mediterranean diet intervention for patients with rheumatoid arthritis. Ann. Rheum. Dis. 2003;62(3):208-14.
48 Merlino LA, Curtis J, Mikuls TR, Cerhan JR, Criswell LA, Saag KG. Vitamin D intake is inversely associated with rheumatoid arthritis: results from the Iowa Women's Health Study. Arthritis Rheum. 2004;50(1):72-7.
49 Heliovaara M, Aho K, Knekt P, Impivaara O, Reunanen A, Aromaa A. Coffee consumption, rheumatoid factor, and the risk of rheumatoid arthritis. Ann. Rheum. Dis. 2000;59(8):631-5.
50 Karlson EW, Mandl LA, Aweh GN, Grodstein F. Coffee consumption and risk of rheumatoid arthritis. Arthritis Rheum. 2003;48(11):3055-60.
51 Carty SM, Snowden N, Silman AJ. Should infection still be considered as the most likely triggering factor for rheumatoid arthritis? J. Rheumatol. 2003;30(3):425-9.
52 Mandl LA, Costenbader KH, Simard JF, Karlson EW. Is birthweight associated with risk of rheumatoid arthritis? Data from a large cohort study. Ann. Rheum. Dis. 2009;68(4):514-8.
53 Jacobsson LT, Jacobsson ME, Askling J, Knowler WC. Perinatal characteristics and risk of rheumatoid arthritis. BMJ 2003;326(7398):1068-9.

54 Phillips DI, Barker DJ, Fall CH, et al. Elevated plasma cortisol concentrations: a link between low birth weight and the insulin resistance syndrome? J. Clin. Endocrinol. Metab. 1998;83(3):757-60.
55 Eijsbouts AM, van den Hoogen FH, Laan RF, Hermus AR, Sweep CG, van de Putte LB. Hypothalamic-pituitary-adrenal axis activity in patients with rheumatoid arthritis. Clin. Exp. Rheumatol. 2005;23(5):658-64.
56 Uhlig T, Hagen KB, Kvien TK. Current tobacco smoking, formal education, and the risk of rheumatoid arthritis. J. Rheumatol. 1999;26(1):47-54.
57 Kallberg H, Ding B, Padyukov L, et al. Smoking is a major preventable risk factor for rheumatoid arthritis: estimations of risks after various exposures to cigarette smoke. Ann. Rheum. Dis. 2011;70(3):508-11.
58 Makrygiannakis D, Hermansson M, Ulfgren AK, et al. Smoking increases peptidylarginine deiminase 2 enzyme expression in human lungs and increases citrullination in BAL cells. Ann. Rheum. Dis. 2008;67(10):1488-92.
59 Kallberg H, Jacobsen S, Bengtsson C, et al. Alcohol consumption is associated with decreased risk of rheumatoid arthritis: results from two Scandinavian case-control studies. Ann. Rheum. Dis. 2009;68(2):222-7.
60 Di Giuseppe D, Alfredsson L, Bottai M, Askling J, Wolk A. Long term alcohol intake and risk of rheumatoid arthritis in women: a population based cohort study. BMJ 2012;345:e4230.
61 Straub RH, Dhabhar FS, Bijlsma JW, Cutolo M. How psychological stress via hormones and nerve fibers may exacerbate rheumatoid arthritis. Arthritis Rheum. 2005;52(1):16-26.
62 Bengtsson C, Theorell T, Klareskog L, Alfredsson L. Psychosocial stress at work and the risk of developing rheumatoid arthritis: results from the Swedish EIRA study. Psychother. Psychosom. 2009;78(3):193-4.
63 Reduction in incidence of rheumatoid arthritis associated with oral contraceptives. Royal College of General Practitioners' Oral Contraception Study. Lancet 1978;1(8064):569-71.
64 Linos A, Worthington JW, O'Fallon WM, Kurland LT. Case-control study of rheumatoid arthritis and prior use of oral contraceptives. Lancet 1983;1(8337):1299-300.
65 Doran MF, Crowson CS, O'Fallon WM, Gabriel SE. The effect of oral contraceptives and estrogen replacement therapy on the risk of rheumatoid arthritis: a population based study. J. Rheumatol. 2004;31(2):207-13.
66 Drossaers-Bakker KW, Zwinderman AH, van Zeben D, Breedveld FC, Hazes JM. Pregnancy and oral contraceptive use do not significantly influence outcome in long term rheumatoid arthritis. Ann. Rheum. Dis. 2002;61(5):405-8.
67 Bhatia SS, Majka DS, Kittelson JM, et al. Rheumatoid factor seropositivity is inversely associated with oral contraceptive use in women without rheumatoid arthritis. Ann. Rheum. Dis. 2007;66(2):267-9.

68 Katchamart W, Johnson S, Lin HJ, Phumethum V, Salliot C, Bombardier C. Predictors for remission in rheumatoid arthritis patients: A systematic review. Arthritis Care Res (Hoboken) 2010;62(8):1128-43.
69 Jorgensen C, Picot MC, Bologna C, Sany J. Oral contraception, parity, breast feeding, and severity of rheumatoid arthritis. Ann. Rheum. Dis. 1996;55(2):94-8.
70 Camacho EM, Lunt M, Farragher TM, Verstappen SM, Bunn DK, Symmons DP. The relationship between oral contraceptive use and functional outcome in women with recent-onset inflammatory polyarthritis: results from the Norfolk Arthritis Register. Arthritis Rheum. 2011;63(8):2183-91.
71 Pedersen M, Jacobsen S, Klarlund M, et al. Environmental risk factors differ between rheumatoid arthritis with and without auto-antibodies against cyclic citrullinated peptides. Arthritis research & therapy 2006;8(4):R133.
72 Hernandez Avila M, Liang MH, Willett WC, et al. Reproductive factors, smoking, and the risk for rheumatoid arthritis. Epidemiology 1990;1(4):285-91.
73 Nelson JL, Ostensen M. Pregnancy and rheumatoid arthritis. Rheum. Dis. Clin. North Am. 1997;23(1):195-212.
74 de Man YA, Bakker-Jonges LE, Goorbergh CM, et al. Women with rheumatoid arthritis negative for anti-cyclic citrullinated peptide and rheumatoid factor are more likely to improve during pregnancy, whereas in autoantibody-positive women autoantibody levels are not influenced by pregnancy. Ann. Rheum. Dis. 2010;69(2):420-3.
75 Forger F, Marcoli N, Gadola S, Moller B, Villiger PM, Ostensen M. Pregnancy induces numerical and functional changes of CD4+CD25 high regulatory T cells in patients with rheumatoid arthritis. Ann. Rheum. Dis. 2008;67(7):984-90.
76 Hazes JM, Dijkmans BA, Vandenbroucke JP, de Vries RR, Cats A. Pregnancy and the risk of developing rheumatoid arthritis. Arthritis Rheum. 1990;33(12):1770-5.
77 Reckner Olsson A, Skogh T, Wingren G. Comorbidity and lifestyle, reproductive factors, and environmental exposures associated with rheumatoid arthritis. Ann. Rheum. Dis. 2001;60(10):934-9.
78 Spector TD, Roman E, Silman AJ. The pill, parity, and rheumatoid arthritis. Arthritis Rheum. 1990;33(6):782-9.
79 Pope JE, Bellamy N, Stevens A. The lack of associations between rheumatoid arthritis and both nulliparity and infertility. Semin. Arthritis Rheum. 1999;28(5):342-50.
80 Guthrie KA, Dugowson CE, Voigt LF, Koepsell TD, Nelson JL. Does pregnancy provide vaccine-like protection against rheumatoid arthritis? Arthritis Rheum. 2010;62(7):1842-8.
81 Research on the menopause in the 1990s. Report of a WHO Scientific Group. World Health Organ. Tech. Rep. Ser. 1996;866:1-107.
82 Hagstad A, Johansson S, Wilhelmsson C, Janson PO. Gynaecology of middle-aged women--menstrual and reproductive histories. Maturitas 1985;7(2):99-113.
83 Nelson HD. Menopause. Lancet 2008;371(9614):760-70.

84 Cassou B, Derriennic F, Monfort C, Dell'Accio P, Touranchet A. Risk factors of early menopause in two generations of gainfully employed French women. Maturitas 1997;26(3):165-74.
85 Bhadauria S, Moser DK, Clements PJ, et al. Genital tract abnormalities and female sexual function impairment in systemic sclerosis. Am. J. Obstet. Gynecol. 1995;172(2 Pt 1):580-7.
86 Costenbader KH, Feskanich D, Stampfer MJ, Karlson EW. Reproductive and menopausal factors and risk of systemic lupus erythematosus in women. Arthritis Rheum. 2007;56(4):1251-62.
87 Dorman JS, Steenkiste AR, Foley TP, et al. Menopause in type 1 diabetic women: is it premature? Diabetes 2001;50(8):1857-62.
88 Larsson K, Mellstrom D, Nordborg E, Oden A, Nordborg E. Early menopause, low body mass index, and smoking are independent risk factors for developing giant cell arteritis. Ann. Rheum. Dis. 2006;65(4):529-32.
89 Merlino LA, Cerhan JR, Criswell LA, Mikuls TR, Saag KG. Estrogen and other female reproductive risk factors are not strongly associated with the development of rheumatoid arthritis in elderly women. Semin. Arthritis Rheum. 2003;33(2):72-82.
90 Salliot C, Bombardier C, Saraux A, Combe B, Dougados M. Hormonal replacement therapy may reduce the risk for RA in women with early arthritis who carry HLA-DRB1 *01 and/or *04 alleles by protecting against the production of anti-CCP: results from the ESPOIR cohort. Ann. Rheum. Dis. 2010;69(9):1683-6.
91 D'Elia HF, Larsen A, Mattsson LA, et al. Influence of hormone replacement therapy on disease progression and bone mineral density in rheumatoid arthritis. J. Rheumatol. 2003;30(7):1456-63.
92 Pettersson K, Grandien K, Kuiper GG, Gustafsson JA. Mouse estrogen receptor beta forms estrogen response element-binding heterodimers with estrogen receptor alpha. Mol. Endocrinol. 1997;11(10):1486-96.
93 Straub RH. The complex role of estrogens in inflammation. Endocr. Rev. 2007;28(5):521-74.
94 Islander U, Jochems C, Lagerquist MK, Forsblad-d'Elia H, Carlsten H. Estrogens in rheumatoid arthritis; the immune system and bone. Mol. Cell. Endocrinol. 2011;335(1):14-29.
95 Fairweather D, Frisancho-Kiss S, Rose NR. Sex differences in autoimmune disease from a pathological perspective. Am. J. Pathol. 2008;173(3):600-9.
96 Bond A, Ratkay LG, Waterfield JD, Hay FC. Post-partum flare in MRL-lpr/lpr mice is associated with a parallel increase of N-acetylglucosamine on serum IgG. Br. J. Rheumatol. 1997;36(2):174-7.
97 Jansson L, Holmdahl R. Enhancement of collagen-induced arthritis in female mice by estrogen receptor blockage. Arthritis Rheum. 2001;44(9):2168-75.
98 Engdahl C, Jochems C, Windahl SH, et al. Amelioration of collagen-induced arthritis and immune-associated bone loss through signaling via estrogen receptor alpha, and not estrogen receptor beta or G protein-coupled receptor 30. Arthritis Rheum. 2010;62(2):524-33.

99 Ito A, Imada K, Sato T, Kubo T, Matsushima K, Mori Y. Suppression of interleukin 8 production by progesterone in rabbit uterine cervix. Biochem. J. 1994;301 (Pt 1):183-6.
100 Straub RH, Cutolo M. Involvement of the hypothalamic--pituitary--adrenal/gonadal axis and the peripheral nervous system in rheumatoid arthritis: viewpoint based on a systemic pathogenetic role. Arthritis Rheum. 2001;44(3):493-507.
101 Giltay EJ, van Schaardenburg D, Gooren LJ, Popp-Snijders C, Dijkmans BA. Androgens and ankylosing spondylitis: a role in the pathogenesis? Ann. N. Y. Acad. Sci. 1999;876:340-64; discussion 65.
102 Cutolo M, Seriolo B, Villaggio B, Pizzorni C, Craviotto C, Sulli A. Androgens and estrogens modulate the immune and inflammatory responses in rheumatoid arthritis. Ann. N. Y. Acad. Sci. 2002;966:131-42.
103 Dalal M, Kim S, Voskuhl RR. Testosterone therapy ameliorates experimental autoimmune encephalomyelitis and induces a T helper 2 bias in the autoantigen-specific T lymphocyte response. J. Immunol. 1997;159(1):3-6.
104 Goemaere S, Ackerman C, Goethals K, et al. Onset of symptoms of rheumatoid arthritis in relation to age, sex and menopausal transition. J. Rheumatol. 1990;17(12):1620-2.
105 Gompel A, Piette JC. Systemic lupus erythematosus and hormone replacement therapy. Menopause international 2007;13(2):65-70.
106 Walitt B, Pettinger M, Weinstein A, et al. Effects of postmenopausal hormone therapy on rheumatoid arthritis: the women's health initiative randomized controlled trials. Arthritis Rheum. 2008;59(3):302-10.
107 Roman-Blas JA, Castaneda S, Cutolo M, Herrero-Beaumont G. Efficacy and safety of a selective estrogen receptor beta agonist, ERB-041, in patients with rheumatoid arthritis: a 12-week, randomized, placebo-controlled, phase II study. Arthritis Care Res (Hoboken) 2010;62(11):1588-93.
108 Rossouw JE, Anderson GL, Prentice RL, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. JAMA 2002;288(3):321-33.
109 Turesson C, Jacobsson LT, Matteson EL. Cardiovascular co-morbidity in rheumatic diseases. Vascular health and risk management 2008;4(3):605-14.
110 Tengstrand B, Carlstrom K, Hafstrom I. Gonadal hormones in men with rheumatoid arthritis--from onset through 2 years. J. Rheumatol. 2009;36(5):887-92.
111 Cutolo M, Balleari E, Giusti M, Monachesi M, Accardo S. Sex hormone status of male patients with rheumatoid arthritis: evidence of low serum concentrations of testosterone at baseline and after human chorionic gonadotropin stimulation. Arthritis Rheum. 1988;31(10):1314-7.
112 Spector TD, Ollier W, Perry LA, Silman AJ, Thompson PW, Edwards A. Free and serum testosterone levels in 276 males: a comparative study of rheumatoid arthritis, ankylosing spondylitis and healthy controls. Clin. Rheumatol. 1989;8(1):37-41.
113 Spector TD, Perry LA, Tubb G, Silman AJ, Huskisson EC. Low free testosterone levels in rheumatoid arthritis. Ann. Rheum. Dis. 1988;47(1):65-8.

114 Cutolo M. Androgens in rheumatoid arthritis: when are they effectors? Arthritis research & therapy 2009;11(5):126.
115 Karlson EW, Chibnik LB, McGrath M, et al. A prospective study of androgen levels, hormone-related genes and risk of rheumatoid arthritis. Arthritis research & therapy 2009;11(3):R97.
116 Booji A, Biewenga-Booji CM, Huber-Bruning O, Cornelis C, Jacobs JW, Bijlsma JW. Androgens as adjuvant treatment in postmenopausal female patients with rheumatoid arthritis. Ann. Rheum. Dis. 1996;55(11):811-5.
117 Hall GM, Larbre JP, Spector TD, Perry LA, Da Silva JA. A randomized trial of testosterone therapy in males with rheumatoid arthritis. Br. J. Rheumatol. 1996;35(6):568-73.
118 Cutolo M, Balleari E, Giusti M, Intra E, Accardo S. Androgen replacement therapy in male patients with rheumatoid arthritis. Arthritis Rheum. 1991;34(1):1-5.
119 Tengstrand B, Carlstrom K, Hafstrom I. Bioavailable testosterone in men with rheumatoid arthritis-high frequency of hypogonadism. Rheumatology (Oxford). 2002;41(3):285-9.
120 Gordon D, Beastall GH, Thomson JA, Sturrock RD. Prolonged hypogonadism in male patients with rheumatoid arthritis during flares in disease activity. Br. J. Rheumatol. 1988;27(6):440-4.
121 Gordon D, Beastall GH, Thomson JA, Sturrock RD. Androgenic status and sexual function in males with rheumatoid arthritis and ankylosing spondylitis. Q. J. Med. 1986;60(231):671-9.
122 Del Rey A, Chrousos GP, Besedovsky HO. *The hypothalamus-pituitary-adrenal axis*. 1st ed. Amsterdam ; Boston: Elsevier; 2008.
123 Raju TN. The Nobel chronicles. 1950: Edward Calvin Kendall (1886-1972); Philip Showalter Hench (1896-1965); and Tadeus Reichstein (1897-1996). Lancet 1999;353(9161):1370.
124 Neeck G. Fifty years of experience with cortisone therapy in the study and treatment of rheumatoid arthritis. Ann. N. Y. Acad. Sci. 2002;966:28-38.
125 Svensson B, Boonen A, Albertsson K, van der Heijde D, Keller C, Hafstrom I. Low-dose prednisolone in addition to the initial disease-modifying antirheumatic drug in patients with early active rheumatoid arthritis reduces joint destruction and increases the remission rate: a two-year randomized trial. Arthritis Rheum. 2005;52(11):3360-70.
126 Kirwan JR, Bijlsma JW, Boers M, Shea BJ. Effects of glucocorticoids on radiological progression in rheumatoid arthritis. Cochrane Database Syst Rev 2007(1):CD006356.
127 Ohayon MM. Prevalence of DSM-IV diagnostic criteria of insomnia: distinguishing insomnia related to mental disorders from sleep disorders. J. Psychiatr. Res. 1997;31(3):333-46.

128 Eriksson I, Unden AL, Elofsson S. Self-rated health. Comparisons between three different measures. Results from a population study. Int. J. Epidemiol. 2001;30(2):326-33.
129 Ohlin B, Berglund G, Nilsson PM, Melander O. Job strain, decision latitude and alpha2B-adrenergic receptor polymorphism significantly interact, and associate with higher blood pressures in men. J. Hypertens. 2007;25(8):1613-9.
130 Nilsson PM, Nilsson JA, Berglund G. Population-attributable risk of coronary heart disease risk factors during long-term follow-up: the Malmo Preventive Project. J. Intern. Med. 2006;260(2):134-41.
131 Berglund G, Nilsson P, Eriksson KF, et al. Long-term outcome of the Malmo preventive project: mortality and cardiovascular morbidity. J. Intern. Med. 2000;247(1):19-29.
132 Berglund G, Eriksson KF, Israelsson B, et al. Cardiovascular risk groups and mortality in an urban swedish male population: the Malmo Preventive Project. J. Intern. Med. 1996;239(6):489-97.
133 Nilsson PM, Nilsson JA, Ostergren PO, Berglund G. Social mobility, marital status, and mortality risk in an adult life course perspective: the Malmo Preventive Project. Scandinavian journal of public health 2005;33(6):412-23.
134 Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. J. Clin. Endocrinol. Metab. 1999;84(10):3666-72.
135 Mjaavatten MD, van der Heijde DM, Uhlig T, et al. Should anti-citrullinated protein antibody and rheumatoid factor status be reassessed during the first year of followup in recent-onset arthritis? A longitudinal study. J. Rheumatol. 2011;38(11):2336-41.
136 Karlson EW, Mandl LA, Hankinson SE, Grodstein F. Do breast-feeding and other reproductive factors influence future risk of rheumatoid arthritis? Results from the Nurses' Health Study. Arthritis Rheum. 2004;50(11):3458-67.
137 Shuster LT, Rhodes DJ, Gostout BS, Grossardt BR, Rocca WA. Premature menopause or early menopause: long-term health consequences. Maturitas 2010;65(2):161-6.
138 Schmidt D, Goronzy JJ, Weyand CM. CD4+ CD7- CD28- T cells are expanded in rheumatoid arthritis and are characterized by autoreactivity. J. Clin. Invest. 1996;97(9):2027-37.
139 Hohensinner PJ, Goronzy JJ, Weyand CM. Telomere dysfunction, autoimmunity and aging. Aging and disease 2011;2(6):524-37.
140 Wesley A, Bengtsson C, Elkan AC, Klareskog L, Alfredsson L, Wedren S. Association between overweight, obesity, ACPA positive and ACPA negative Rheumatoid Arthritis-, results from the EIRA case-control study. Arthritis Care Res (Hoboken) 2012.
141 Cutolo M, Sulli A, Pizzorni C, Craviotto C, Straub RH. Hypothalamic-pituitary-adrenocortical and gonadal functions in rheumatoid arthritis. Ann. N. Y. Acad. Sci. 2003;992:107-17.

142 Heikkila R, Aho K, Heliovaara M, et al. Serum androgen-anabolic hormones and the risk of rheumatoid arthritis. Ann. Rheum. Dis. 1998;57(5):281-5.
143 Malkin CJ, Pugh PJ, Jones RD, Jones TH, Channer KS. Testosterone as a protective factor against atherosclerosis--immunomodulation and influence upon plaque development and stability. J. Endocrinol. 2003;178(3):373-80.
144 Hofvander Y. Breastfeeding and the Baby Friendly Hospitals Initiative (BFHI): organization, response and outcome in Sweden and other countries. Acta Paediatr. 2005;94(8):1012-6.
145 Merten S, Dratva J, Ackermann-Liebrich U. Do baby-friendly hospitals influence breastfeeding duration on a national level? Pediatrics 2005;116(5):e702-8.
146 Ryan AS, Wenjun Z, Acosta A. Breastfeeding continues to increase into the new millennium. Pediatrics 2002;110(6):1103-9.
147 Zwedberg S. *I wanted to breastfeed! A hermeneutical Study of Mothers with Breastfeeding Problems; Their Experiences, Coping Strategies, and Consultative Meeting with Midwives*: Stockholms university; 2010.
148 Breast cancer and breastfeeding: collaborative reanalysis of individual data from 47 epidemiological studies in 30 countries, including 50302 women with breast cancer and 96973 women without the disease. Lancet 2002;360(9328):187-95.
149 Jordan SJ, Cushing-Haugen KL, Wicklund KG, Doherty JA, Rossing MA. Breast-feeding and risk of epithelial ovarian cancer. Cancer Causes Control 2012;23(6):919-27.
150 Berglin E, Kokkonen H, Einarsdottir E, Agren A, Rantapaa Dahlqvist S. Influence of female hormonal factors, in relation to autoantibodies and genetic markers, on the development of rheumatoid arthritis in northern Sweden: a case-control study. Scand. J. Rheumatol. 2010;39(6):454-60.
151 Shelly S, Boaz M, Orbach H. Prolactin and autoimmunity. Autoimmunity reviews 2012;11(6-7):A465-70.
152 Handlin L, Jonas W, Ransjo-Arvidson AB, Petersson M, Uvnas-Moberg K, Nissen E. Influence of common birth interventions on maternal blood pressure patterns during breastfeeding 2 days after birth. Breastfeeding medicine : the official journal of the Academy of Breastfeeding Medicine 2012;7(2):93-9.
153 Ahmed MA, Elosaily GM. Role of Oxytocin in deceleration of early atherosclerotic inflammatory processes in adult male rats. International journal of clinical and experimental medicine 2011;4(3):169-78.
154 Heinrichs M, Baumgartner T, Kirschbaum C, Ehlert U. Social support and oxytocin interact to suppress cortisol and subjective responses to psychosocial stress. Biol. Psychiatry 2003;54(12):1389-98.
155 Svejme O, Ahlborg HG, Nilsson JA, Karlsson MK. Early menopause and risk of osteoporosis, fracture and mortality: a 34-year prospective observational study in 390 women. BJOG : an international journal of obstetrics and gynaecology 2012;119(7):810-6.
156 Shiels MS, Rohrmann S, Menke A, et al. Association of cigarette smoking, alcohol consumption, and physical activity with sex steroid hormone levels in US men. Cancer Causes Control 2009;20(6):877-86.

157 Derby CA, Zilber S, Brambilla D, Morales KH, McKinlay JB. Body mass index, waist circumference and waist to hip ratio and change in sex steroid hormones: the Massachusetts Male Ageing Study. Clin. Endocrinol. (Oxf). 2006;65(1):125-31.
158 Nieschlag E, Behre HM, Bouchard P, et al. Testosterone replacement therapy: current trends and future directions. Hum. Reprod. Update 2004;10(5):409-19.
159 Keller JJ, Lin HC. A population-based study on the association between rheumatoid arthritis and erectile dysfunction. Ann. Rheum. Dis. 2012;71(6):1102-3.

Paper I

Breast feeding, but not use of oral contraceptives, is associated with a reduced risk of rheumatoid arthritis

M Pikwer,[1] U Bergström,[1] J-Å Nilsson,[1] L Jacobsson,[1] G Berglund,[2] C Turesson[1]

[1] Department of Rheumatology, Malmö University Hospital, Malmö, Sweden; [2] Department of Medicine, Malmö University Hospital, Malmö, Sweden

Correspondence to: Dr M Pikwer, Department of Rheumatology, Malmö University Hospital, 205 02 Malmö, Sweden; mitrakes@gmail.com

Accepted 8 March 2008

ABSTRACT

Objective: To determine whether breast feeding or the use of oral contraceptives (OCs) affects the future risk of rheumatoid arthritis (RA) in a community-based prospective cohort.

Methods: A community-based health survey (18 326 women) was linked to regional and national registers, and incident cases of RA were identified. All women with a diagnosis of RA after inclusion in the health survey (n = 136) and four female controls for every case, who were alive and free from RA when the index person was given a diagnosis of RA, were included in a case–control study. Data on lifestyle factors at baseline were derived from a self-administered questionnaire. Potential predictors were examined in logistic regression models.

Results: 136 women with incident RA were compared with 544 age-matched controls. A longer history of breast feeding was associated with a reduced risk of RA (OR 0.46 (95% CI 0.24 to 0.91) for women who had breast fed for ⩾13 months and OR 0.74 (95% CI 0.45 to 1.20) for those who had breast fed for 1–12 months, compared with those who had never breast fed). The protective effect of longer breast feeding remained significant after adjustment for smoking and level of education in multivariate models, and point estimates were protective also when the analyses were restricted to parous women. Neither parity nor OC use had any significant effect on the risk of RA.

Conclusion: In this study, long-term breast feeding, but not OC use, was associated with a significant reduction in the risk of RA.

Both genetic and environmental factors can predispose to rheumatoid arthritis (RA). HLA-DRB1 alleles featuring the shared epitope[1] are found in most patients with RA, and genetic factors have an effect on disease progression.[2] On the other hand, only an estimated 16% concordance of RA in monozygotic twins has been found,[3] indicating that environmental factors also play an important role in the development of RA. Suggested predictors include smoking,[4] level of education,[5] and certain occupational exposures.[6 7] Furthermore, it has been hypothesised that infectious agents, especially Epstein–Barr virus, could initiate the inflammatory process leading to RA.[8]

Overall, women have more than a twofold higher incidence of RA than men.[9 10] This is mainly due to an increased risk in women during their reproductive years, when the incidence shows a female/male ratio of 5:1.[9] This difference may partly be explained by hormonal factors.

A number of studies have shown that use of oral contraceptives (OCs), which contain hormones that are also raised during pregnancy, may protect against RA,[11] and be associated with milder disease and less frequent hospital referrals,[12 13] but some studies have failed to show any protective effect of OC use.[14–16] Methodological differences may explain these discrepancies, and a biological explanation has never been presented, leading to the hypothesis that OC use is more likely to be a confounder or a marker for other protective variables.

It has been known for a long time that amelioration of RA is often seen in pregnancy,[17–19] possibly because of increased immune tolerance and a shift towards Th_2 differentiation of T cells, driven by high concentrations of circulating sex hormones and endogenous corticosteroids.[18]

There is often a flare of the disease during the postpartum period, which is associated with a sudden fall in cortisol concentration and, during breast feeding, a high concentration of prolactin.[20] It has been recognised that postpartum flares of the disease tend to be more pronounced in women who breast feed.[21 22]

In contrast, previous studies have suggested that extended breast feeding is associated with a reduced risk of future onset of RA.[14 23] The underlying mechanisms are unknown, and there is limited information on the relative effect of breast feeding and other suggested predictors of RA, such as smoking[4] and low level of formal education.[5]

The purpose of this study was to determine whether breast feeding and OC use affect the future risk of RA in a community-based cohort, with adjustment for confounders where applicable.

PATIENTS AND METHODS

This nested case–control study used information from the Malmö Diet and Cancer Study (MDCS), a community-based health survey performed between 1991 and 1996 in Malmö, Sweden. The MDCS included 30 477 subjects (18 326 women) aged 44–74. The main objective was to study the effect of diet on cancer incidence and mortality. Information on lifestyle factors, such as smoking and level of education, and previous and current health status was obtained using a self-administered questionnaire. Data were collected on reproductive factors, such as history of OC use (including number of years and when started), number of births, and whether each child was breast fed and for how many months. This survey has been extensively described previously.[24]

The MDCS cohort was linked to a community-based RA register,[25] the local outpatient clinic administrative register, the National Hospital Discharge Register and the National Cause of Death Register. The medical records of patients

Table 1 Characteristics of cases and controls

	Cases (n = 136)	Controls (n = 544)
Age at RA diagnosis*	63.3 (8.25)	NA
RF positive	68.4% (91/133 tested)	NA
ANA positive	30.5% (25/82 tested)	NA
Anti-CCP positive	66.7% (16/24 tested)	NA
Age at screening*	57.9 (7.33)	58.0 (7.32)
Ever used OCs†	63 (48.5%)	254 (49.4%)
Never used OCs	67 (51.2%)	259 (51.2%)
Used OCs 1–5 years	18 (14.0%)	110 (21.7%)
Used OCs >5 years	44 (34.1%)	137 (27.1%)
Given birth to ⩾1 child	106 (77.9%)	445 (81.8%)
Breast fed for 0 months	43 (31.6%)	137 (25.2%)
Breast fed for 1–12 months	74 (54.4%)	297 (54.6%)
Breast fed for >13 months	19 (14.0%)	110 (20.2%)

*Values are mean (SD).
†Data on OC use were available for 130 cases and 514 controls.
ANA, anti-nuclear antibodies; CCP, cyclic citrullinated peptides; NA, not applicable; OC, oral contraceptive; RA, rheumatid arthritis; RF, rheumatoid factor.

identified through these sources were subjected to a structured review, and all women given a diagnosis of RA, according to the 1987 American College of Rheumatology (ACR) criteria for RA,[26] after inclusion in the MDCS were included in our case–control study. Four female controls for every case, matched according to age and year of screening, who were alive and free of RA when the index person was given a diagnosis of RA, were selected from the MDCS survey population.

Patients were stratified according to OC use: never, 1–5 years of use, and >5 years of use. We also compared women who never used OCs with "ever users" and stratified them into groups depending on which decade they started OC use, from 1960 to after 1990, and by breast-feeding history as follows: never, total breast feeding for 1–12 months, total breast feeding for ⩾13 months. Non-responders were excluded from the analysis, with one exception: women who did not respond to the question "How many children have you given birth to?" were considered nulliparous.

Women who reported current daily smoking were compared with those who did not smoke. Level of formal education was stratified into five levels (elementary school only (⩽8 years of school), 9–10 years of school, 11–12 years of school, >12 years of school (but no university degree), and university degree).

Potential predictors were examined in χ^2 test and logistic regression models, taking into account the matched design of the study. Each case and the corresponding controls were assigned a group number, and this was entered into the logistic regression models as a categorical variable. To evaluate trends favouring a dose-dependent effect of breast feeding, the breast-feeding categories (no breast feeding vs 1–12 months vs ⩾13 months) were entered as an interval level variable. Multivariate models were used to adjust for potential confounders, such as smoking, level of education, and parity. Statistical significance was identified as $p<0.05$.

This study was approved by the regional research ethics committee for southern Sweden.

RESULTS

We identified 136 women with incident RA (table 1). The total follow-up for the cohort was 188 969 person-years, which gives an estimated incidence of 72/100 000 person-years. The mean age at RA onset was 63.3 years with a median duration of 5.5 years (range 1–13 years) from enrolment in the health survey to RA onset. These were compared with 544 age-matched female controls. OC use did not have any significant effect on the risk of RA onset (table 2). Longer reported OC use did not reduce the risk of RA (data not shown). Most OC users among the cases (81.7%) and the controls (84.5%) had started taking OCs between 1960 and 1970.

Although parity did not differ significantly between cases and controls (odds ratio (OR) 0.75, 95% CI 0.45 to 1.24), there was a trend towards a reduced risk of RA for each child born (OR 0.87, 95% CI 0.71 to 1.06). A history of breast feeding was less common in the RA group (table 1). A longer history of breast feeding was associated with a reduced risk of RA (OR 0.46 (95% CI 0.24 to 0.91) for women who had breast fed for ⩾13 months, and OR 0.74 (95% CI 0.45 to 1.20) for those who had breast fed for 1–12 months, compared with those who had never breast fed; fig 1) (p for trend 0.025; no breast feeding vs 1–12 months vs ⩾13 months). The trend was seen in both rheumatoid factor (RF)-positive and RF-negative women, but was stronger in the RF-negative group (table 2).

When the number of children and breast feeding were included as covariates in the same multivariate model, the results indicate that it is breast feeding, rather than the number of children, that is inversely associated with RA (table 3). The protective effect of longer breast feeding remained significant after adjustment for potential confounders such as smoking and level of education in multivariate models (table 4).

When the analysis was restricted to parous women, the estimated effect of long-term breast feeding was similar, although the confidence intervals were wider (OR 0.46 (95% CI 0.17 to 1.25) for women who had breast fed for ⩾13 months, and OR 0.78 (95% CI 0.33 to 1.83) for those who had breast fed

Table 2 Reproductive factors and the risk of rheumatoid arthritis for all cases and rheumatoid factor (RF)-positive and RF-negative cases in univariate analyses

		Person-years	OR (95% CI)		
	Cases (n)	at risk	All (n = 136)	RF +ve (n = 91)	RF −ve (n = 42)
No breast feeding	43	926	Referent	Referent	Referent
Breast feeding 1–12 months (vs not)	74	2065	0.74 (0.45 to 1.20)	1.07 (0.58 to 1.95)	0.40 (0.16 to 0.97)
Breast feeding ⩾13 months (vs not)	19	704	0.46 (0.24 to 0.91)	0.61 (0.28 to 1.36)	0.30 (0.08 to 1.17)
No OC use	63	1711	Referent	Referent	Referent
Ever used OCs	67	1795	1.03 (0.63 to 1.67)	1.07 (0.58 to 1.97)	0.98 (0.42 to 2.27)
Each child born	NA	NA	0.87 (0.71 to 1.06)	0.87 (0.68 to 1.11)	0.77 (0.54 to 1.11)

NA, not applicable; OC, oral contraceptive; +ve, positive; -ve, negative.

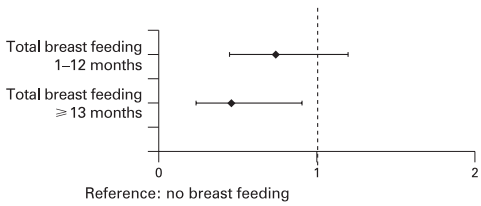


Figure 1 Risk of rheumatoid arthritis in women who reported total breast feeding for 1–12 months or ⩾13 months compared with no breast feeding (136 cases and 544 controls). Odds ratios with 95% CI are shown.

for 1–12 months, compared with those who had never breast fed; fig 2). Fifty-one women (13 cases) had no reported history of breast feeding, although they had given birth to one or more children (median 2; range 1–4). Compared with these, nulliparous women (n = 129) tended to have a lower risk of RA (OR 0.48; 95% CI 0.13 to 1.72), although the difference did not reach significance and statistical power was limited for this comparison.

DISCUSSION

Breast feeding is known to have multiple health benefits for the baby[27–30] and may protect mothers against breast cancer[31] and ovarian cancer.[32] In addition, we show that women who had breast fed for 13 months or more had a significantly reduced risk of developing RA. Furthermore, our results indicate that breast feeding may have a dose-dependent protective effect against RA, although the difference did not reach significance for women who had breast fed for 1–12 months.

Although it is difficult to separate the effect of breast feeding from that of childbirth, our data suggest that RA is inversely associated with long-term breast feeding, rather than with the number of children born. This should be confirmed in a larger study. Nulliparity has in previous studies been found to be a risk factor for RA,[33] but not all studies have been able to confirm this relationship.[11 34] We found no significant association between pregnancy and RA, suggesting that nulliparity did not confound our results. A Norwegian prospective study on risk of RA mortality[28] showed that total time of breast feeding was associated with a decreased risk of RA, but this study had major limitations. RA cases were identified via the death register, and therefore long-term survivors and patients with RA who did not have a diagnosis of RA registered on the death certificate were excluded. Selection bias was thus a major problem with this early study. More recently, Karlson *et al*[14] confirmed that there is a dose-dependent inverse correlation between breast feeding and RA. They showed a significant protective effect, adjusted for smoking, when total breast feeding exceeded 24 months, with a mean duration between breast feeding and RA onset of 25 years. These observations were based on the Nurses' Health Study, a large cohort of women that included 674 incident cases of RA. Our study confirms and extends these findings. It shows that the negative association between long-term breast feeding and RA is not limited to one defined social group (ie, nurses), but extends to a mixed cohort from an urban area. A third prospective study[16] of 158 cases of incident RA in Iowa found a modest non-significant protective effect of the number of children breast fed, after adjustment for age and smoking. As no data were available, the effect of the duration of breast feeding could not be studied.

Table 3 Effect of number of births and length of breast feeding on the risk of rheumatoid arthritis

	Odds ratio (95% CI)
Number of children (per child)	1.00 (0.78 to 1.27)
Breast feeding for 1–12 months vs 0 months	0.74 (0.41 to 1.35)
Breast feeding for ⩾13 months vs 0 months	0.47 (0.19 to 1.14)

The analysis was adjusted for the other variables in the table by multivariate analysis.

Possible explanations for the protective effect of breast feeding include long-term immunomodulation, such as the development of progesterone receptors on lymphocytes, dysregulated hypothalamic–pituitary–adrenal axis, and differences in cortisol concentrations. Lankarani-Fard *et al*[35] measured cortisol concentrations in postmenopausal women, and noted significantly higher concentrations in those who had breast fed. This requires further study.

Our findings may seem to contrast with the reported increased risk of RA during breast feeding.[15] Oxytocin, one of the hormones that is raised in women who breast feed, is known to reduce cortisol concentrations,[36] induce well-being, and lower blood pressure in the mothers.[37] Prolactin, which also is increased during breast feeding, is a known immunostimulator,[38] and high concentrations of prolactin are seen in patients with RA.[39 40] Taken together, these findings suggest different short-term and long-term effects of breast feeding on the immune system and on susceptibility to RA.

In our study, OC use was not associated with a reduced risk of RA regardless of which decade it was started (from the 1960s to the 1990s) or its duration. A meta-analysis of nine studies concluded that OCs may prevent the progression of RA to severe disease by modifying the disease process, rather than having a protective effect.[41] On the other hand, a population-based study did show a protective effect of OC use.[11] Compared with that study, OC use was substantially more common in our sample. We cannot exclude the possibility that changes in the pattern of OC use explains these discrepancies. OC use could only have an effect in women who where sexually active when the pill became available in the 1960s, although this would probably not influence our results in any particular direction. As our study included women who were aged 45–73 when entering the study, we have mainly investigated previous, rather then current, OC use.

The strengths of our study include the community-based approach, the well-defined catchment area, and the comprehensive effort to identify incident RA cases using multiple sources. The estimated incidence of RA in this cohort of 72/100 000 person-years is slightly higher than recent findings in corresponding age groups in a population-based study from the UK (annual incidence 54–65 per 100 000 in women aged 50–70 and 36/100 000 in women overall).[42] This suggests that we

Table 4 Predictors of rheumatoid arthritis

	Odds ratio (95% CI)
Breast feeding for 1-12 months vs 0 months	0.62 (0.36 to 1.07)
Breast feeding for ⩾13 months vs 0 months	0.40 (0.20 to 0.83)
Smoking*	1.38 (0.83 to 2.31)
Low level of formal education†	2.14 (0.96 to 4.80)

Analyses were adjusted for the other variables in the table by multivariate analysis.
*Current smokers vs non-smokers.
†⩽8 years of school vs university degree.

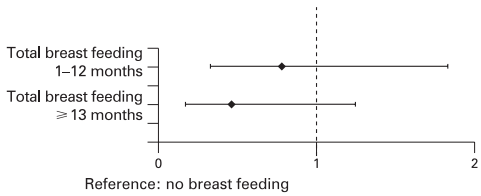


Figure 2 Risk of rheumatoid arthritis in women who reported total breast feeding for 1–12 months or ⩾13 months compared with no breast feeding. The analysis was restricted to parous women (106 cases and 445 controls). Odds ratios and 95% CI are shown.

identified virtually all cases of incident RA in the cohort, indicating that our cases are likely to be a representative sample of patients with RA in the area. Data on predictors were collected before disease onset, which means that the effect of RA on lifestyle and recall bias could not influence our results. There may be recall errors in reported duration of breast feeding and duration of OC use, but there is no reason to believe that there is a differential effect between cases and controls. Limitations include the relatively low number of cases, especially when the analysis was limited to parous women. The number of women who gave birth but did not breast feed was too small for a reliable estimate of their risk compared with nulliparous women. Furthermore, our data are only from women with onset of RA in their 40s or later, and, because of the study design, the short-term effect of breast feeding could not be assessed. It is possible that unmeasured confounders influenced our analysis, in particular as there is a long lag period between the reported breast feeding and the development of RA.

A decline in incidence over time and a shift towards later onset of RA has been reported.[43] Doran *et al*[11] calculated that, on the basis of their data, the increased OC use can only explain a small portion of the decrease in RA. The breast-feeding rate in Sweden has increased steadily since the beginning of the 1970s, from less than 5% of women breast feeding for ⩾6 months to about 73%.[44] A similar trend can be seen in the USA,[45] and UNICEF launched a global programme, Baby Friendly Hospitals Initiative, in 1990 to promote breast feeding throughout the world.[46] Whether there is a connection between the decreasing incidence of RA and higher rates of breast feeding cannot be concluded from the present data, but our findings suggest yet another reason for women to continue breast feeding.

In conclusion, our data show a decreased risk of RA in postmenopausal women with a history of long-term breast feeding. The effect was dose-dependent and remained significant after adjustment for smoking and level of education.

Acknowledgements: We thank Anders Dahlin for excellent help with data extraction from the MDCS database.

Funding: This study was funded by Lund University, The Craaford Foundation and the Swedish Rheumatism Association.

Competing interests: None declared.

REFERENCES

1. **Gregersen PK,** Silver J, Winchester RJ. The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. *Arthritis Rheum* 1987;**30**:1205–13.
2. **Turesson C,** Matteson EL. Genetics of rheumatoid arthritis. *Mayo Clin Proc* 2006;**81**:94–101.
3. **Silman AJ,** MacGregor AJ, Thomson W, Holligan S, Carthy D, Farhan A, *et al*. Twin concordance rates for rheumatoid arthritis: results from a nationwide study. *Br J Rheumatol* 1993;**32**:903–7.
4. **Hutchinson D,** Shepstone L, Moots R, Lear JT, Lynch MP. Heavy cigarette smoking is strongly associated with rheumatoid arthritis (RA), particularly in patients without a family history of RA. *Ann Rheum Dis* 2001;**60**:223–7.
5. **Pedersen M,** Jacobsen S, Klarlund M, Frisch M. Socioeconomic status and risk of rheumatoid arthritis: a Danish case-control study. *J Rheumatol* 2006;**33**:1069–74.
6. **Olsson AR,** Skogh T, Axelson O, Wingren G. Occupations and exposures in the work environment as determinants for rheumatoid arthritis. *Occup Environ Med* 2004;**61**:233–8.
7. **Stolt P,** Kallberg H, Lundberg I, Sjogren B, Klareskog L, Alfredsson L. Silica exposure is associated with increased risk of developing rheumatoid arthritis: results from the Swedish EIRA study. *Ann Rheum Dis* 2005;**64**:582–6.
8. **Carty SM,** Snowden N, Silman AJ. Should infection still be considered as the most likely triggering factor for rheumatoid arthritis? *J Rheumatol* 2003;**30**:425–9.
9. **Masi AT.** Incidence of rheumatoid arthritis: do the observed age-sex interaction patterns support a role of androgenic-anabolic steroid deficiency in its pathogenesis? *Br J Rheumatol* 1994;**33**:697–9.
10. **Linos A,** Worthington JW, O'Fallon WM, Kurland LT. The epidemiology of rheumatoid arthritis in Rochester, Minnesota: a study of incidence, prevalence, and mortality. *Am J Epidemiol* 1980;**111**:87–98.
11. **Doran MF,** Crowson CS, O'Fallon WM, Gabriel SE. The effect of oral contraceptives and estrogen replacement therapy on the risk of rheumatoid arthritis: a population based study. *J Rheumatol* 2004;**31**:207–13.
12. **Symmons D,** Harrison B. Early inflammatory polyarthritis: results from the Norfolk Arthritis Register with a review of the literature. I. Risk factors for the development of inflammatory polyarthritis and rheumatoid arthritis. *Rheumatology (Oxford)* 2000;**39**:835–43.
13. **Drossaers-Bakker KW,** Zwinderman AH, van Zeben D, Breedveld FC, Hazes JM. Pregnancy and oral contraceptive use do not significantly influence outcome in long term rheumatoid arthritis. *Ann Rheum Dis* 2002;**61**:405–8.
14. **Karlson EW,** Mandl LA, Hankinson SE, Grodstein F. Do breast-feeding and other reproductive factors influence future risk of rheumatoid arthritis? Results from the Nurses' Health Study. *Arthritis Rheum* 2004;**50**:3458–67.
15. **Moskowitz MA,** Jick SS, Burnside S, Wallis WJ, Dickson JF, Hunter JR, *et al*. The relationship of oral contraceptive use to rheumatoid arthritis. *Epidemiology* 1990;**1**:153–6.
16. **Merlino LA,** Cerhan JR, Criswell LA, Mikuls TR, Saag KG. Estrogen and other female reproductive risk factors are not strongly associated with the development of rheumatoid arthritis in elderly women. *Semin Arthritis Rheum* 2003;**33**:72–82.
17. **Nelson JL,** Hughes KA, Smith AG, Nisperos BB, Branchaud AM, Hansen JA. Maternal-fetal disparity in HLA class II alloantigens and the pregnancy-induced amelioration of rheumatoid arthritis. *N Engl J Med* 1993;**329**:466–71.
18. **Ostensen M,** Villiger PM. Immunology of pregnancy: pregnancy as a remission inducing agent in rheumatoid arthritis. *Transpl Immunol* 2002;**9**:155–60.
19. **Hench PS.** The ameliorating effect of pregnancy on chronic atrophic (infectious rheumatoid) arthritis, fibrositis, and intermittent hydrarthrosis. *Proc Staff Meet Mayo Clin* 1938;**13**:161–7.
20. **Neidhart M,** Gay RE, Gay S. Prolactin and prolactin-like polypeptides in rheumatoid arthritis. *Biomed Pharmacother* 1999;**53**:218–22.
21. **Barrett JH,** Brennan P, Fiddler M, Silman A. Breast-feeding and postpartum relapse in women with rheumatoid and inflammatory arthritis. *Arthritis Rheum* 2000;**43**:1010–15.
22. **Brennan P,** Silman A. Breast-feeding and the onset of rheumatoid arthritis. *Arthritis Rheum* 1994;**37**:808–13.
23. **Brun JG,** Nilssen S, Kvale G. Breast feeding, other reproductive factors and rheumatoid arthritis. A prospective study. *Br J Rheumatol* 1995;**34**:542–6.
24. **Manjer J,** Carlsson S, Elmstahl S, Gullberg B, Janzon L, Lindstrom M, *et al*. The Malmo Diet and Cancer Study: representativity, cancer incidence and mortality in participants and non-participants. *Eur J Cancer Prev* 2001;**10**:489–99.
25. **Soderlin MK,** Lindroth Y, Jacobsson LT. Trends in medication and health-related quality of life in a population-based rheumatoid arthritis register in Malmo, Sweden. *Rheumatology (Oxford)* 2007;**46**:1355–8.
26. **Arnett FC,** Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, *et al*. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;**31**:315–24.
27. **Scariati PD,** Grummer-Strawn LM, Fein SB. A longitudinal analysis of infant morbidity and the extent of breastfeeding in the United States. *Pediatrics* 1997;**99**:E5.
28. **Oddy WH,** Holt PG, Sly PD, Read AW, Landau LI, Stanley FJ, *et al*. Association between breast feeding and asthma in 6 year old children: findings of a prospective birth cohort study. *BMJ* 1999;**319**:815–19.
29. **Howie PW,** Forsyth JS, Ogston SA, Clark A, Florey CD. Protective effect of breast feeding against infection. *BMJ* 1990;**300**:11–16.
30. **Gartner LM,** Morton J, Lawrence RA, Naylor AJ, O'Hare D, Schanler RJ, *et al*. Breastfeeding and the use of human milk. *Pediatrics* 2005;**115**:496–506.
31. **Collaborative Group on Hormonal Factors in Breast Cancer**. Breast cancer and breastfeeding: collaborative reanalysis of individual data from 47 epidemiological studies in 30 countries, including 50 302 women with breast cancer and 96 973 women without the disease. *Lancet* 2002;**360**:187–95.
32. **Danforth KN,** Tworoger SS, Hecht JL, Rosner BA, Colditz GA, Hankinson SE. Breastfeeding and risk of ovarian cancer in two prospective cohorts. *Cancer Causes Control* 2007;**18**:517–23.

33. **Hazes JM,** Dijkmans BA, Vandenbroucke JP, de Vries RR, Cats A. Pregnancy and the risk of developing rheumatoid arthritis. *Arthritis Rheum* 1990;**33**:1770–5.
34. **Pope JE,** Bellamy N, Stevens A. The lack of associations between rheumatoid arthritis and both nulliparity and infertility. *Semin Arthritis Rheum* 1999;**28**:342–50.
35. **Lankarani-Fard A,** Kritz-Silverstein D, Barrett-Connor E, Goodman-Gruen D. Cumulative duration of breast-feeding influences cortisol levels in postmenopausal women. *J Womens Health Gend Based Med* 2001;**10**:681–7.
36. **Heinrichs M,** Baumgartner T, Kirschbaum C, Ehlert U. Social support and oxytocin interact to suppress cortisol and subjective responses to psychosocial stress. *Biol Psychiatry* 2003;**54**:1389–98.
37. **Gutkowska J,** Jankowski M, Mukaddam-Daher S, McCann SM. Oxytocin is a cardiovascular hormone. *Braz J Med Biol Res* 2000;**33**:625–33.
38. **McMurray RW.** Estrogen, prolactin, and autoimmunity: actions and interactions. *Int Immunopharmacol* 2001;**1**:995–1008.
39. **Chikanza IC,** Petrou P, Chrousos G, Kingsley G, Panayi GS. Excessive and dysregulated secretion of prolactin in rheumatoid arthritis: immunopathogenetic and therapeutic implications. *Br J Rheumatol* 1993;**32**:445–8.
40. **Zoli A,** Lizzio MM, Ferlisi EM, Massafra V, Mirone L, Barini A, *et al*. ACTH, cortisol and prolactin in active rheumatoid arthritis. *Clin Rheumatol* 2002;**21**:289–93.
41. **Spector TD,** Brennan P, Harris P, Studd JW, Silman AJ. Does estrogen replacement therapy protect against rheumatoid arthritis? *J Rheumatol* 1991;**18**:1473–6.
42. **Symmons DP,** Barrett EM, Bankhead CR, Scott DG, Silman AJ. The incidence of rheumatoid arthritis in the United Kingdom: results from the Norfolk Arthritis Register. *Br J Rheumatol* 1994;**33**:735–9.
43. **Kaipiainen-Seppanen O,** Kautiainen H. Declining trend in the incidence of rheumatoid factor-positive rheumatoid arthritis in Finland 1980–2000. *J Rheumatol* 2006;**33**:2132–8.
44. **Hofvander Y.** Breastfeeding and the Baby Friendly Hospitals Initiative (BFHI): organization, response and outcome in Sweden and other countries. *Acta Paediatr* 2005;**94**:1012–16.
45. **Ryan AS,** Wenjun Z, Acosta A. Breastfeeding continues to increase into the new millennium. *Pediatrics* 2002;**110**:1103–9.
46. **Merten S,** Dratva J, Ackermann-Liebrich U. Do baby-friendly hospitals influence breastfeeding duration on a national level? *Pediatrics* 2005;**116**:e702–8.

Paper II

ARD Online First, published on October 4, 2011 as 10.1136/ard.2011.200059

CONCISE REPORT

Early menopause is an independent predictor of rheumatoid arthritis

Mitra Pikwer, Ulf Bergström, Jan-Åke Nilsson, Lennart Jacobsson, Carl Turesson

► Additional data are published online only. To view the files please visit the journal online (http://ard.bmj.com)

Department of Clinical Sciences, Malmö, Section of Rheumatology, Lund University and Skåne University Hospital, Malmö, Sweden

Correspondence to
Dr Mitra Pikwer, Department of Rheumatology, Skåne University Hospital, SE-205 02 Malmö, Sweden;
mitra.pikwer@med.lu.se

Accepted 10 September 2011

ABSTRACT

Background As rheumatoid arthritis (RA) occurs more often in women than in men, it has been suggested that reproductive hormones may play an important role in the pathogenesis.

Methods Between 1991 and 1996, 30 447 subjects (18 326 women) were included in a community-based health survey. Information on female hormonal changes and stress-related factors was obtained using a self-administered questionnaire. This population was linked to four different local and national RA registers. The medical records for patients with a diagnosis of RA were subjected to a structured review and all women with incident RA according to the 1987 American College of Rheumatology criteria after inclusion in the health survey were included in a nested case–control study. Matched controls (1:4) were selected from the health survey population.

Results Early age at menopause (≤45 years) was associated with the subsequent development of RA (OR 2.42, 95% CI 1.32 to 4.45). The effect of early menopause remained significant after adjusting for smoking, level of education and length of breastfeeding (OR 1.92, 95% CI 1.02 to 3.64)

Conclusion RA was predicted by an early age at menopause. This implicates an influence of hormonal changes during the fertile period on the development of RA in postmenopausal women.

Throughout the reproductive years, the incidence of rheumatoid arthritis (RA) in women is more than twofold the incidence in men.[1 2] Based on this and on observations of amelioration of RA during pregnancy, an important role of reproductive hormones has been implicated in the pathophysiology. There are conflicting data on the impact of age at menopause[3 4] and age at menarche[5 6] on the risk of RA.

The relation between reproductive factors, the hypothalamic–pituitary–adrenal (HPA) axis and inflammation is complex and not fully understood. The HPA axis may be altered in individuals who later develop RA in the uterus[7 8] or it may be secondarily modified by later hormonal events, for instance breast-feeding or pregnancy.[9] Furthermore, stress-related factors may strongly influence breastfeeding habits,[10] and hypothetically, stress-related elevation of proinflammatory cytokines[11] could lead to increased RA susceptibility.

The aim of this study was to examine the impact of age at menopause, age at menarche and a set of stress-related factors on the risk of RA with peri or postmenopausal onset and to explore further the effect of long-term breast-feeding in this context.

PATIENTS AND METHODS

The Malmö Diet and Cancer Study (MDCS), a community-based health survey performed in Malmö, Sweden, between 1991 and 1996, included 30 477 subjects (18 326 women) aged 44–74 years.[12] Information on lifestyle factors, such as smoking, level of education, reproductive factors and previous and current health status was obtained using a self-administered questionnaire.

As previously described,[13] incident cases of RA were identified by linking the MDCS cohort to several local and national registers, followed by a structured review of these cases. All women diagnosed with RA who fulfilled the 1987 American College of Rheumatology criteria for RA[14] after inclusion in the MDCS were included in this nested case–control study. Four female controls for every case, matched according to age and year of screening, who were alive and free of RA diagnosis when the index person was diagnosed with RA, were selected from the MDCS survey population.

The women were stratified into two groups, early (≤45 years) or normal to late (>45 years) menopausal age.[15] As all cases and controls were 45 years or older when answering the questionnaire, those who reported that they had not yet reached menopause were classified in the later group but were excluded when analysing the total number of fertile years. Early menarche was defined as menarche before the age of 12 years. Breast-feeding history was stratified as previously described.[13] The stress-related factors and the definition of smoking history and level of formal education are described in the supplementary text, available online only.

Non-responders to each question were excluded from the corresponding analysis. Data on rheumatoid factor (RF) and anti-cyclic citrullinated peptide status at RA diagnosis or later were extracted in a structured review of all medical records.

Potential predictors were examined in conditional logistic regression models. Each case and the corresponding controls were assigned a group number, which was entered into the logistic regression models as a categorical variable. Multivariate models were used to adjust for potential confounders. Bivariate analyses were stratified by RF status (positive/negative) after RA diagnosis among the cases. The regional research ethics committee for southern Sweden approved the study.

Table 1 Characteristics and baseline exposure information on RA cases and matched controls

	Cases n=136	Controls n=544
Characteristics		
Mean age at RA diagnosis (SD)	63.3 (8.3)	NA
Mean age at screening (SD; range)	57.9 (7.3; 45–72)	58.0 (7.3; 45–73)
Anti-CCP positive at diagnosis, %	56.0†	NA
RF positive at diagnosis, %	68.4‡	NA
Baseline exposure information		
Reproduction-related factors		
Menopause-related factors		
Mean age at menopause (SD; range)	47.9 (6.0; 26–57)	49.2 (4.5; 19–59)
Early menopause, ≤45 years, n (%)	26 (19.1)	62 (11.4)
Normal to late menopause, n (%)	103 (75.7)	444 (81.6)
Menarche-related factors		
Mean age at menarche (SD; range)	13.7 (1.44; 10–18)	13.5 (1.5; 10–20)
Early menarche, <12 years, n (%)	3 (2.2)	46 (8.5)
Normal to late menarche, n (%)	125 (97.7)	465 (91.0)
Other factors		
No of fertile years, mean (SD)*	34.2 (6.4)	35.6 (4.7)
Given birth to ≥1 child, n (%)	106 (77.9)	445 (81.8)
Breast-fed 0 months, n (%)	43 (31.6)	137 (25.2)
Breast-fed 1–12 months, n (%)	74 (54.4)	297 (54.6)
Brest-fed >13 months, n (%)	19 (14.0)	110 (20.2)
Self-reported stress–related factors		
Lack of physical and mental wellbeing, n (%)	39 (30.0)	161 (31.3)
Problems falling a sleep at night, n (%)	32 (23.5)	105 (19.3)
Problems waking up in the night, n (%)	32 (23.5)	136 (25)
Problems waking up early, n (%)	13 (9.6)	85 (15.6)

*Data on 487 out of 680.
†26/46 tested.
‡91/133 tested.
CCP, cyclic citrullinated peptide; NA , not applicable; RA, rheumatoid arthritis; RF, rheumatoid factor.

RESULTS

One hundred and thirty-six women with incident RA were compared with 544 age-matched female controls (table 1). The median duration from enrolment in the health survey to RA diagnosis was 5.5 years. The estimated crude incidence was 72/100 000 persons-years, based on the 188 969 person-years follow-up of the cohort. There was missing data on menopause for 45 women (6.6%; seven cases, 38 controls). Those with normal/late menopause were more likely to report breast-feeding more than 12 months compared with those with early menopause (21.4% vs 12.5%; p=0.007).

Bivariate analyses

Early age at menopause (≤45 years) was a predictor of the subsequent development of RA (table 2). Early menopause was significantly associated with RF-negative RA, whereas there was a trend for RF-positive RA that did not reach significance (table 2). Early menarche, at less than 12 years, was significantly associated with a reduced risk of RA, with similar patterns for RF-positive and RF-negative RA when analysed separately (table 2). In accordance with these results, a longer fertile period was protective against developing RA (OR 0.92 per year, 95% CI 0.87 to 0.97).

Parity (table 2) and stress-related factors (see supplementary table S1, available online only) had no significant effect on the risk of RA.

Multivariate analyses

Early menopause (p=0.007) and early menarche (p=0.013) had a similar influence on the risk of RA in models adjusted for parity. In multivariate analysis, long-term breastfeeding (>12 months; OR 0.45, 95% CI 0.22 to 0.92) and early menopause (OR 2.22, 95% CI 1.20 to 4.12) had independent effects on the risk of RA. The association with early menopause remained significant in a model adjusted for the length of breastfeeding, smoking and the level of formal education (OR 1.92, 95% CI 1.02 to 3.64).

In a multivariate model including all three variables related to reproduction as covariates and further adjusted for smoking and level of education, the estimated effects of early menarche, early menopause and the length of breastfeeding were similar to the bivariate analyses, although CI were wider (table 3).

DISCUSSION

In this nested case–control study based on a prospective survey, we found that early menopause was significantly associated with RA. This may suggest a link between hormonal changes and chronic inflammation or factors specifically related to RA (see supplementary text, available online only).

Smoking and a low level of formal education are known predictors of early menopause,[16] but adjusting for these did not change our estimates, indicating that they were not confounders in our sample. Our results support the study by Merlino *et al*,[3] who found that women who reached menopause after the age of 51 years had a reduced risk (adjusted RR 0.64) of developing RA compared with women whose menopause occurred before the age of 45 years. In a Swedish case–control study, early menopause was protective against RA.[4] The retrospective nature and the limited sample size of that study could explain the discrepancy from our results.

Potential explanations for our findings include endocrine factors such as an insufficient HPA axis with a sluggish response to stress.[17] On the pathway to RA development such sluggish HPA axis could possibly manifest itself as a lower frequency of early menarche, a higher frequency of breastfeeding problems

Table 2 Reproductive factors and the risk of RA for all cases and by RF status at inclusion: bivariate analysis

	Cases n=136	Controls n=544	All OR (CI)	RF-positive OR (CI)	RF-negative OR (CI)
Nulliparous	30	99	1.00	1.00	1.00
Given birth to ≥1 child	106	445	0.75 (0.45–1.24)	0.84 (0.44–1.60)	0.64 (0.27–1.52)
Normal/late menopause	103	444	1.00	1.00	1.00
Early menopause	26	62	2.42 (1.32–4.45)	1.98 (0.91–4.31)	5.00 (1.72–14.51)
Normal/late menarche	125	465	1.00	1.00	1.00
Early menarche, <12 years	3	46	0.21 (0.06–0.70)	0.18 (0.04–0.81)	0.28 (0.32–2.52)
No breast-feeding	43	137	1.00	1.00	1.00
Breast-feeding 1–12 months	74	297	0.74 (0.45–1.20)	1.07 (0.58–1.95)	0.40 (0.16–0.97)
Breast-feeding >13 months	19	110	0.46 (0.24–0.91)	0.61 (0.28–1.36)	0.30 (0.08–1.17)

RA, rheumatoid arthritis; RF, rheumatoid factor.

Table 3 Multivariate analysis, adjusted for all variables in the table

	OR (95% CI)
Early menopause*	1.92 (1.00 to 3.67)
Smoking†	1.32 (0.77 to 2.26)
Low level of formal education‡	2.30 (0.97 to 5.43)
Breastfeeding >12 months versus 0 months	0.49 (0.21 to 1.03)
Early menarche§	0.29 (0.06 to 0.77)

*≤45 Years versus normal/late menopause (>46 years).
†Current smokers versus non-smokers.
‡≤8 Years versus university degree.
§<12 Years versus normal/late menarche (>12 years).

and more frequent early menopause. Other mechanisms could hypothetically also be involved including premature ovarian failure (see supplementary text, available online only).

We have previously shown that cumulative breastfeeding of more than 12 months was associated with a significantly reduced risk of developing RA.[13] This is in accordance with a previous report,[18] although a recent retrospective study reported opposing results.[19] As expected, women with early menopause in the present study were less likely to report a history of long-term breastfeeding. However, age at menopause and history of breastfeeding were found to affect the risk of RA independently.

Early menarche (<12 years) was inversely associated with RA, although the number of patients was limited. This is in accordance with a Danish study,[5] but an American study reported contradictory results, with an increased risk of RA in subjects with early menarche (<11 years).[6] Geographical and ethnic differences, as well as differences in the case mix, may explain these discrepancies.

Hormonal factors may have different effects on disease presenting in the middle-aged/older individuals compared with those with a younger onset, as well as between RF-positive and RF-negative RA. In our material the effect of early menopause was statistically significant for RF-negative RA, although there also was a similar trend for RF-positive RA. This is in contrast to other risk factors for RA, for instance smoking, which is mainly a predictor of RF-positive RA.[20]

We did not find any association between the risk of RA and a set of stress-related factors, including sleeping disorders and self-reported health. We can not exclude that such factors may have an impact on the risk of RA, as our questions were asked several years before diagnosis. Furthermore, psychosocial aspects not investigated in the present study may be important.

Limitations of the present study include the lack of information on polycystic ovary syndrome, endometriosis or hysterectomy and the low number of subjects with early menarche. It is possible that unmeasured confounders influenced our analysis; in particular as there is a long lag period between some of the exposures (ie, menarche and breast-feeding) and the development of RA. Furthermore, due to the lack of screened subjects aged less than 44 years, our results are only relevant for disease starting after this age.

The strengths of our study consist of the community-based approach and the fact that data on predictors were collected before RA diagnosis. This means that the effect of RA on lifestyle and recall bias could not influence our results.

In conclusion, early age at menopause was one of several hormonal factors that were associated with an increased risk of RA. This suggests an influence of hormonal changes during the fertile period on the development of RA in postmenopausal women.

Acknowledgements The authors would like to thank Professor Göran Berglund for his contributions to the study design and to the first draft of this paper. The authors also thank Anders Dahlin for excellent help with data extraction from the MDCS database and Per-Olof Östergren for valuable comments on the questionnaire regarding stress-related factors.

Funding This study was funded by the Swedish Research Council, The Craaford Foundation, Lund University and the Swedish Rheumatism Association.

Competing interest None.

Ethics approval The regional research ethics committee for southern Sweden approved the study.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1. **Englund M**, Jöud A, Geborek P, *et al.* Prevalence and incidence of rheumatoid arthritis in southern Sweden 2008 and their relation to prescribed biologics. *Rheumatology (Oxford)* 2010;**49**:1563–9.
2. **Gabriel SE**, Crowson CS, O'Fallon WM. The epidemiology of rheumatoid arthritis in Rochester, Minnesota, 1955–1985. *Arthritis Rheum* 1999;**42**:415–20.
3. **Merlino LA**, Cerhan JR, Criswell LA, *et al.* Estrogen and other female reproductive risk factors are not strongly associated with the development of rheumatoid arthritis in elderly women. *Semin Arthritis Rheum* 2003;**33**:72–82.
4. **Olsson AR**, Skogh T, Wingren G. Aetiological factors of importance for the development of rheumatoid arthritis. *Scand J Rheumatol* 2004;**33**:300–6.
5. **Pedersen M**, Jacobsen S, Klarlund M, *et al.* Environmental risk factors differ between rheumatoid arthritis with and without auto-antibodies against cyclic citrullinated peptides. *Arthritis Res Ther* 2006;**8**:R133.
6. **Hernández Avila M**, Liang MH, Willett WC, *et al.* Reproductive factors, smoking, and the risk for rheumatoid arthritis. *Epidemiology* 1990;**1**:285–91.
7. **Jacobsson LT**, Jacobsson ME, Askling J, *et al.* Perinatal characteristics and risk of rheumatoid arthritis. *BMJ* 2003;**326**:1068–9.
8. **Mandl LA**, Costenbader KH, Simard JF, *et al.* Is birthweight associated with risk of rheumatoid arthritis? Data from a large cohort study. *Ann Rheum Dis* 2009;**68**:514–18.
9. **Tu MT**, Lupien SJ, Walker CD. Multiparity reveals the blunting effect of breastfeeding on physiological reactivity to psychological stress. *J Neuroendocrinol* 2006;**18**:494–503.
10. **Zanardo V**, Gasparetto S, Giustardi A, *et al.* Impact of anxiety in the puerperium on breast-feeding outcomes: role of parity. *J Pediatr Gastroenterol Nutr* 2009;**49**:631–4.
11. **Hansel A, Hong S**, Camara RJ, *et al.* Inflammation as a psychophysiological biomarker in chronic psychosocial stress. *Neurosci Biobehav Rev* 2010;**35**:115–21.
12. **Manjer J, Carlsson S**, Elmståhl S, *et al.* The Malmö Diet and Cancer Study; Representativity, cancer incidence and mortality in participants and non-participants. *Eur J Cancer Prev* 2001;**10**:489–99.

13. **Pikwer M**, Bergström U, Nilsson JA, *et al.* Breast feeding, but not use of oral contraceptives, is associated with a reduced risk of rheumatoid arthritis. *Ann Rheum Dis* 2009;**68**:526–30.
14. **Arnett FC**, Edworthy SM, Bloch DA, *et al.* The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;**31**:315–24.
15. **Cassou B**, Derriennic F, Monfort C, *et al.* Risk factors of early menopause in two generations of gainfully employed French women. *Maturitas* 1997;**26**:165–74.
16. **Nilsson P**, Möller L, Köster A, *et al.* Social and biological predictors of early menopause: a model for premature aging. *J Intern Med* 1997;**242**:299–305.
17. **Cutolo M**, Villaggio B, Foppiani L, *et al.* The hypothalamic–pituitary–adrenal and gonadal axes in rheumatoid arthritis. *Ann N Y Acad Sci* 2000;**917**:835–43.
18. **Karlson EW**, Mandl LA, Hankinson SE, *et al.* Do breast-feeding and other reproductive factors influence future risk of rheumatoid arthritis? Results from the Nurses' Health Study. *Arthritis Rheum* 2004;**50**:3458–67.
19. **Berglin E**, Kokkonen H, Einarsdottir E, *et al.* Influence of female hormonal factors, in relation to autoantibodies and genetic markers, on the development of rheumatoid arthritis in northern Sweden: a case-control study. *Scand J Rheumatol* 2010;**39**:454–60.
20. **Costenbader KH**, Feskanich D, Mandl LA, *et al.* Smoking intensity, duration, and cessation, and the risk of rheumatoid arthritis in women. *Am J Med* 2006;**119**:503.e1–9.

Paper III

Early menopause and severity of rheumatoid arthritis in women over 45 years of age

Mitra Pikwer[1]*, Jan-Åke Nilsson[1], Ulf Bergström[1], Lennart TH Jacobsson[1,2] and Carl Turesson[1]

[1]Department of Rheumatology, Institution of Clinical Sciences, Inga Marie Nilssons gata 32, 205 02, Malmö, Sweden

[2]Deptartment of Rheumatology & Inflammation Research, Institution of Medicin, University of Gothemburg, Guldhedsgatan 10 A, 405 30 Göteborg, Sweden

* Corresponding author: mitra.pikwer@med.lu.se

Abstract

Introduction: We aimed to investigate if recognised hormonal predictors of Rheumatoid arthritis (RA) also influence the severity of RA.

Methods: One hundred and thirty-four incident RA cases identified by four different local and national registers, which had participated in a community-based health survey between 1991 and 1996, were included. By a retrospective structured review of the medical records, information on the use of disease modifying anti rheumatic drugs, (DMARDs), erosions on x-rays, rheumatoid factor (RF) status and disability measured using the health assessment questionnaire (HAQ) were collected. The variables were added to the SPSS TwoStep Cluster Analysis to reveal natural groupings of RA severity. Known hormonal predictors analysed were breastfeeding history, history of oral contraceptive (OC) use and menopausal age.

Results: The mean age at RA diagnosis was 63.4 years; 72 % were RF positive and 28 % had received biological treatment. Three clusters were identified, one with severe RA, one with mild/moderate RF positive RA and one with mild/moderate RF negative RA. There was a significant difference (p= 0.005) in the distribution of clusters between patients with a history of early menopause compared with those with menopause after 45 years, with a higher proportion with mild/moderate RF negative RA in the early menopause subset. There was no major difference in severity of the disease depending on OC use or history of breast-feeding.

Conclusions: Early menopause was associated with a milder form of RA. Hormonal changes may influence pathways that are distinct from those leading to severe, progressive disease.

Introduction

Rheumatoid arthritis (RA) is a chronic, inflammatory disease, with a female predominance [1, 2]. Hormonal factors have been suggested to influence the risk of RA. There is a reduced incidence during pregnancy, and also an increased rate of clinical remission among women with RA who become pregnant [3]. Low testosterone levels have been described in both women and men with RA [4-6] and there is a high incidence of RA during the peri- and post-menopausal period in women [7], suggesting that hormones and hormonal changes may influence pathogenesis and disease progression. The disease is heterogeneous and nowadays often regarded as a syndrome of several distinct phenotypes instead of a being a single disease [8]. Hormonal factors could have diverse influence on older onset of RA compared to younger onset, as well as on seropositive and seronegative disease.

The effect of oral contraceptives (OC) on RA has been the subject of many studies and meta-analyses [9-11] with diverging results. Variances in methods, doses and types of OC, as well as population differences, have been proposed to explain some of these controversies. It has been suggested that OC alter the course of RA instead of acting as a predictor and is associated with a milder type of disease and less disability [12, 13]. In a previous study, we did not find any impact of a history of OC use on the risk of RA [2]. We did, however, demonstrate an association for early menopause (≤45 years) with increased risk of RA, and a protective effect of long term breast-feeding [2, 14] on postmenopausal onset of RA. Jorgensen et al reported that the mean duration of breast-feeding was longer in women who subsequently developed a severe RA phenotype [13]. To our knowledge, there have been no studies on the influence of menopausal age on the long-term outcome of RA.

The aim of this study was to investigate if hormonal factors, such as breast-feeding history, OC use and menopausal age, influence the severity of RA.

Materials and methods

Malmö Diet and Cancer Study

The Malmö Diet and Cancer Study (MDCS) is a population-based prospective cohort study performed in Malmö, Sweden, between 1991 and 1996. Baseline examination included a self-administered questionnaire, anthropometric measuring and blood sampling, stored in a biological bank. All individuals living in Malmö and aged 44-74 in 1 January 1991 were invited. A total of 30 477 subjects (18 326 women) were recruited. Language problems and mental retardation were the only exclusion criteria [15].

By linking the MDCS to 4 registers (a community based RA-register, the local outpatient clinic administrative register, the National Hospital Discharge Register and the National Cause of Death Register) we identified individuals who had developed RA after their participation in the MDCS (≥ 1 year before RA diagnosis). Based on a review of the medical records, patients were classified according to the 1987 American College of Rheumatology (ACR) criteria for RA [16]. Female patients who been diagnosed before December 31, 2004, and fulfilled the ACR criteria from 1987, were included in the present study.

Clinical outcome measures

In a separate, structured review of all medical records, performed by one of the authors (MP), data were collected on clinical outcome measures up to the date of the review

(between February and October 2010) or to the last day of follow up. Information on treatment with Disease Modifying Anti Rheumatic Drugs, (DMARDs), including biologics, was collected. Since treatment with biologics for RA in Sweden is to a great extent based on national evidence based guidelines. These were first formulated in 2002, and have been updated in 2004 and since 2008 on an annual basis [17]. Although there have been changes in the guidelines over the years, biologics are still mainly recommended for patients with severe RA that is refractory to traditional DMARD therapy [17]. Based on this, ever use of biologics can be used as a surrogate marker for severe disease. Data on exposure to biologics were also derived from linkage to a regional follow-up system for patients with rheumatologic diseases receiving such treatment, the South Swedish Arthritis Treatment Group (SSATG) register [18]. The SSATG was established in 1999 and includes patients from a total of ten rheumatology centres, of which six are in the Region Skåne area in southern Sweden (~ 1.3 million inhabitants). The register has been compared to pharmaceutical sales data and found to cover over 90% of anti-TNF treated patients in the area [18]. Further clinical information collected from medical records included data on prednisolone treatment at diagnosis (or within one month before or after the time of diagnosis), severe extra-articular manifestations (pericarditis, pleuritis, vasculitis, Felty's syndrome, glomerulonephritis and interstitial lung disease) [19], and disability measured using the Health Assessment Questionnaire (HAQ) [20] at diagnosis and after 1 year (the closest value within 6-18 months was accepted), 5 years (4-6 years accepted) and 10 years (8-12 years accepted) from diagnosis. The HAQ is a self-administered questionnaire, consisting of questions about of 20 activities of daily living in 8 categories. This instrument is useful in monitoring the patient´s course and response to therapy and to assess functional disability. The HAQ form is distributed to all patients with RA before visiting the outpatient clinic at the

University hospital in Malmö. The scale ranges from 0 to 3, with higher scores indicating greater disability. To be able to classify HAQ scores in relation to disease duration, the exact date of diagnosis was set as the day a rheumatologist gave a definitive (ICD-10) diagnosis of RA, independently of the beginning of symptoms. Data on rheumatoid factor (RF) tests and tests for antibodies to cyclic citrullinated peptides (anti-CCP) were retrieved from the databases of the two clinical immunology laboratories in the area. Patients with at least one positive test for RF or anti-CCP were considered positive for this marker.

All available reports on radiographs of hands and feet were reviewed. Based on the radiology reports, patients were classified as ever having arthritic erosions, typical of RA, or not. Data on smoking history, time of cessation, if applicable, number of children given birth to and ever use of Hormonal Replacement Therapy (HRT) were collected.

Hormonal exposure variables

Data on age at menopause, breast-feeding history, and use of OC and level of formal education were retrieved from the self-administered questionnaire used in the MDCS, assessed on average 5.5 years before RA diagnosis. All female individuals in the present sample who answered the MDCS questionnaire were 45 years of age, or older. If they reported a menopause after the age of 45, or answered that they had not yet reached menopause, they were classified in the group “normal/late menopause”. If they had reached menopause before the age of 46, they were included in the group “early menopause”.

Breast-feeding history was stratified into never having had breastfed, cumulative breastfeeding of 1-12 months or cumulative breastfeeding of $\geq$ 13 months, as previously described [2]. Based on information on use of OC, cases were divided into 3 groups; never-

users, 1-5 years of use and > 5 years of use. Non-responders were excluded from the analyses.

Statistics

Clusters of RA severity were identified using SPSS TwoStep Clustering algorithm (version 19). This algorithm allows the use of both categorical and continuous variables in the same model. Selected outcome measures were based on data availability and clinical plausibility: biological treatment, presence of radiographic erosions, RF status and disability 5 years after diagnosis measured using the HAQ. The HAQ score at five year was included in the model since data were available in a greater number of patients at this time point. These outcome measures were added to the algorithm and different number of natural groupings was explored. The algorithm initially identified six clusters, but these were considered difficult to interpret from a clinical standpoint, and the small sample for each cluster was a concern. In the subsequent analysis, the algorithm was pre-set to identify three clusters. The model identifying three clusters was chosen since these clusters were considered to reflect clinically relevant phenotypes. Only after this decision had been made was the distribution of such clusters by baseline hormonal exposures examined using the Chi-square test.

For the individual outcome parameters, only descriptive data without formal statistical comparisons were presented. Analysis of Covariance (ANCOVA) was used to compare mean HAQ between subsets defined by hormonal predictors, adjusted for age.

The regional research ethics committee for southern Sweden approved the study. All patients gave their informed consent to be included in the Malmö RA registers and the MDCS database. No informed consent was obtained specifically for the present study.

Results

Of the 136 incident female patients previously described [2], two patients on long term follow-up were found to develop a phenotype compatible with erosive osteoarthritis rather than RA, and were therefore excluded from this study. Median time from inclusion in MDCS to RA diagnosis was 5.5 years. The mean age at RA diagnosis was 63.4 years and the mean disease duration at the last follow-up was 10.1 years (SD 3.5). Ninety-four (71.2 %) were RF positive. Twenty-eight percent received biological treatment during follow-up, and the median number of DMARDs used was 2. Almost 60 % of the patients had documented radiographic erosions, and 5 % had severe extra-articular manifestations (Table 1). Availability of data on HAQ was limited and varied for different time points (Table 1), but there was no major differences in the proportions with missing HAQ at different time points between those with early and normal/late menopause (52 % vs. 46 % at diagnosis, 52 % vs. 36 % at 1 year, 24 % vs. 33 % at 5 years, 52 % vs. 52 % at 10 years).

All patients with complete data for the relevant outcome variables (n= 90) were included in the cluster analysis. Three different clusters of RA-severity were identified, of which cluster I represented a severe RA phenotype, cluster II a moderate/mild RF-positive RA phenotype and cluster III a moderate/mild RF negative RA phenotype (Table 2).

Since HAQ score values had a normal distribution among patients in cluster I, and a skewed distribution in clusters II and III, means with standard deviations as well as medians with interquartile ranges are presented (Table 2). In the group with severe RA, all patients had been treated with biologics, 89 % were RF positive, 85 % had documented erosions and the

mean HAQ score after 5 years was 1.17. In the mild/moderate RF positive and RF negative clusters, no one had received biologics, the proportions with documented erosions was lower, and mean HAQ scores after 5 years were lower than in the severe RA cluster (Table 2).

Hormonal factors and disease outcome

The duration of follow-up was similar in patients with a history of early vs. normal/late menopause (Table 3) and also similar across strata of length of breast-feeding (Table 4) and use of OC (Table 5). There was no major difference in the mean age at diagnosis between patients with early menopause (65.2 years) and normal/late menopause (62.8 years). There was a significant difference in the distribution of patients between the clusters depending on menopausal age (p=0.005), with more women with early menopause developing RF negative mild/moderate RA (Figure 1). The age-adjusted mean HAQ scores were lower among women with early menopause at all time points (Table 3). There was no significant interaction between age at RA diagnosis and early vs. normal/late menopause status in analyses of their impact on HAQ (data not shown). In analyses adjusted for age, there was a borderline association between early menopause and lower HAQ at diagnosis (β= -0.38; 95% CI:-0. 78 to 0.01), with a similar tendency at other time points (data not shown).

No significant differences in the distribution of clusters of clinical outcome measures were seen in analysis by breastfeeding history (p=0.88) or previous use of OC (p=0.56) (Tables 4 and 5). Women who had used OC > 5 years tended to be in the cluster of severe RA, and the mean age at RA diagnosis was lower in this subset (Table 5). Overall, individuals who were younger at diagnosis were more likely to be treated with biologics (data not shown). There were no major differences in age at diagnosis depending on breast-feeding history (Table 4).

Women who hade normal/late menopause were more likely to receive treatment with biologics and DMARDS in combination, but there were no major differences in the number of different DMARDS used during the follow-up (Table 3).

Discussion

We have previously shown that hormonal factors such as early menopause and breastfeeding history influence the risk of RA [2, 14]. In particular, early menopause was a robust risk factor for RA [14]. In this study, we demonstrate that early menopause predicts a milder type of RA.

Oestrogen is suggested to directly suppress cellular immunity but stimulate the humoral immune system [21]. A drop in oestrogen levels may thus contribute to a T cell differentiation skewed towards the T-helper 1 (Th1) subset. This has previously been implicated as an important part of the pathogenesis of RA [22, 23]. Further studies should examine the impact of the timing of menopause on immunologic and inflammatory pathways leading to RA.

Dysfunctions of the Hypothalamic-Pituitary- Adrenocortical-axis (HPA-axis), the Hypothalamic-Pituitary- Gonadal axis (HPG) and the autonomic nervous system have all been suggested to be a part of the complex pathogenesis of RA [24, 25]. Similar observations have been made in primary Sjögren's syndrome [26]. A gradual decline in the function of the HPG axis is considered a key element in the development of menopause [27, 28]. We propose that women with a less responsive HPG axis, leading to an increased risk of early menopause, may also have a primarily malfunctioning or "sluggish" HPA- axis. A reduced

response of the HPA axis to inflammation would make them more susceptible to develop a chronic inflammatory disease. In support of this, it has been found that women who later in life develop RA have higher birth weight than normal controls [29, 30], and there is data linking high birth weight with a less responsive HPA function later in life [31]. This may reflect an impact of HPA function on chronic inflammation in general, rather than on the specific pathogenesis of erosive arthritis. In this study, women with a history of early menopause who developed RA were more likely to have a mild disease course, and less likely to progress to severe RA. This suggests that different predictors may be associated with distinct clinical outcomes. For example, other known risk factors for RA, such as the shared epitope of HLA-DRB1, and smoking, may, in comparison with early menopause, be more specifically associated with a severe phenotype of RA [32, 33].

A recent study [12] suggested that OC use is associated with mild RA, and demonstrated a reduction of disability, measured by HAQ, over time in former OC users compared with non-users. Furthermore, current OC users had lower HAQ scores at baseline and over time than previous users. Jorgensen et al [13], found that OC is both protective for RA and associated with a mild phenotype in individuals who develop RA. Yet another study did not find a protective effect of current use of OC for RA [34]. Since our patients were older, with a mean age at diagnosis of 63 years (range 47-80), only one patient reported current use of OC at diagnosis, and we could therefore not address the effect of current use of OC. The mean time between OC use and RA diagnosis was 20 years, which may explain the limited impact of former OC use on the severity of RA. In our sample, the former users rather tended to develop a more severe RA, at least by some outcome measures. This could be biased by their younger age at diagnosis. In particular, the difference in the proportion treated with

biologics may be due to more extensive prescription of such drugs for younger individuals with RA, as was the case in the present study and also reported in a recent national survey [35].

Barrett et al [36] suggested that breast-feeding is a risk factor for a more severe inflammatory polyarthritis in a small group of genetically susceptible women, based on the linkage between HLA-DRB1 alleles and the prolactin gene on chromosome 6 [37]. In a prospective short-term study of pregnant women with inflammatory polyarthritis, they found that women who breast-fed after a first pregnancy had a more severe disease 6 months postpartum compared to non-breast-feeders and previous breast-feeders [36]. However, in the present study, we could not demonstrate any significant long-term effect of breast-feeding on the severity of RA. Our study design excludes the smaller group of women who develop RA during their younger years, which could affect our results. The immune-stimulating effect of prolactin could also be short-term, and breast-feeding could have a different long-term immune-modulating effect, suggested by the results from the Nurses Health study [38] and our previous results [2].

Limitations of this study include the retrospective data collection, since information in medical records is sometimes incomplete and not always coherent, due to diverse assessments by different physicians. Since risk factor information and clinical records are two completely unrelated sources of information, it is unlikely that such incompleteness is differential between risk factor exposure categories. Nevertheless, the relatively large proportion of missing data of HAQ at specific time points is a limitation. Since some physicians may be less prone to routinely collect HAQ, and this may also change over time, we cannot exclude a systematic bias based on this. There were, however, no major

differences in the proportions with missing HAQ between those with early and normal/late menopause Due to differences in management practice, the methods used to define clusters of disease severity may not be valid in other populations. Another limitation is the relatively small sample size, which affects analyses of subsets with different risk factor profiles.

Strengths include the prospective assessment of hormone related factors before disease onset, which limits the risk of recall bias, and the populations based approach, which reduces the risk of selection bias.

Conclusions

We report that early menopause is associated with mild type of RA among women with disease onset after 45 years of age. Hormonal changes may influence pathways that are distinct from those leading to severe, progressive disease. To our knowledge, this is the first study on the impact of menopausal age on the severity of RA, and our findings must be confirmed in other studies of larger samples. Such studies require that data on the exposure (i.e. age at menopause) have been collected in a consistent and valid manner. If our results are confirmed, it may in the future be relevant to check for early menopause in women with RA.

Key messages:

- Early menopause is associated with a mild seronegative RA phenotype.
- Breastfeeding history and former use of oral contraceptives did not influence the severity of RA.

- Hormonal changes may influence pathways leading to a mild type of rheumatoid arthritis.

Abbreviations

ACR, American College of Rheumatology; Anti-CCP, antibodies to cyclic citrullinated peptides; ANCOVA, Analysis of Covariance; DMARDs, Disease Modifying Anti Rheumatic Drugs; HAQ, Health Assessment Questionnaire; HRT, Hormonal Replacement Therapy; HPA-axis, Hypothalamic-Pituitary- Adrenocortical-axis; HPG-axis, Hypothalamic-Pituitary- Gonadal axis; MDCS, Malmö Diet and Cancer Study; OC, Oral Contraceptives; RA, Rheumatoid Arthritis; RF, Rheumatoid Factor; SSATG, South Swedish Arthritis Treatment Group; Th-1 cell, T-helper 1 cell.

Competing interests

The authors have no potential conflicts of interest regarding this paper.

Authors' contributions

MP carried out data collection, participated in the design of the study, the statistical analysis and wrote the paper. J-ÅN contributed to the statistical analysis and writing. UB identified cases and controls and contributed to the writing of the paper. LJ participated in the design

of the study and writing. CT contributed to the study design, statistical analysis, research supervision and writing. All authors read and approved the final version.

Acknowledgements

We thank Anders Dahlin for excellent help with data extraction from the MDCS. Database, Pierre Geborek for providing data from the SSATG register, Lennart Truedsson and Gunnel Henriksson for providing information from databases of RF and anti-CCP tests, Minna Willim, for excellent help with data management and Göran Berglund for his contributions to the study design. This study was funded by The Swedish Research Council, Lund University, The County of Skåne and the Swedish Rheumatism Association.

References

1. Englund M, Joud A, Geborek P, Felson DT, Jacobsson LT, Petersson IF: **Prevalence and incidence of rheumatoid arthritis in southern Sweden 2008 and their relation to prescribed biologics**. *Rheumatology (Oxford)* 2010, **49**:1563-1569.

2. Pikwer M, Bergstrom U, Nilsson JA, Jacobsson L, Berglund G, Turesson C: **Breast feeding, but not use of oral contraceptives, is associated with a reduced risk of rheumatoid arthritis**. *Ann Rheum Dis* 2009, **68**:526-530.

3. Hazes JM, Coulie PG, Geenen V, Vermeire S, Carbonnel F, Louis E, Masson P, De Keyser F: **Rheumatoid arthritis and pregnancy: evolution of disease activity and**

pathophysiological considerations for drug use. *Rheumatology (Oxford)* 2011, **50**:1955-1968.

4. Cutolo M, Balleari E, Giusti M, Monachesi M, Accardo S: **Sex hormone status of male patients with rheumatoid arthritis: evidence of low serum concentrations of testosterone at baseline and after human chorionic gonadotropin stimulation**. *Arthritis Rheum* 1988, **31**:1314-1317.

5. Tengstrand B, Carlstrom K, Hafstrom I: **Gonadal hormones in men with rheumatoid arthritis--from onset through 2 years**. *J Rheumatol* 2009, **36**:887-892.

6. Masi AT, Chatterton RT, Aldag JC: **Perturbations of hypothalamic-pituitary-gonadal axis and adrenal androgen functions in rheumatoid arthritis: an odyssey of hormonal relationships to the disease**. *Ann N Y Acad Sci* 1999, **876**:53-62; discussion 62-53.

7. Doran MF, Pond GR, Crowson CS, O'Fallon WM, Gabriel SE: **Trends in incidence and mortality in rheumatoid arthritis in Rochester, Minnesota, over a forty-year period**. *Arthritis Rheum* 2002, **46**:625-631.

8. Scott IC, Steer S, Lewis CM, Cope AP: **Precipitating and perpetuating factors of rheumatoid arthritis immunopathology: linking the triad of genetic predisposition, environmental risk factors and autoimmunity to disease pathogenesis**. *Best practice & research Clinical rheumatology* 2011, **25**:447-468.

9. Vandenbroucke JP, Valkenburg HA, Boersma JW, Cats A, Festen JJ, Huber-Bruning O, Rasker JJ: **Oral contraceptives and rheumatoid arthritis: further evidence for a preventive effect**. *Lancet* 1982, **2**:839-842.

10. Drossaers-Bakker KW, Zwinderman AH, van Zeben D, Breedveld FC, Hazes JM: **Pregnancy and oral contraceptive use do not significantly influence outcome in long term rheumatoid arthritis**. *Ann Rheum Dis* 2002, **61**:405-408.

11. Oliver JE, Silman AJ: **Risk factors for the development of rheumatoid arthritis**. *Scand J Rheumatol* 2006, **35**:169-174.

12. Camacho EM, Lunt M, Farragher TM, Verstappen SM, Bunn DK, Symmons DP: **The relationship between oral contraceptive use and functional outcome in women with recent-onset inflammatory polyarthritis: results from the Norfolk Arthritis Register**. *Arthritis Rheum* 2011, **63**:2183-2191.

13. Jorgensen C, Picot MC, Bologna C, Sany J: **Oral contraception, parity, breast feeding, and severity of rheumatoid arthritis**. *Ann Rheum Dis* 1996, **55**:94-98.

14. Pikwer M, Bergstrom U, Nilsson JA, Jacobsson L, Turesson C: **Early menopause is an independent predictor of rheumatoid arthritis**. *Ann Rheum Dis* 2011.

15. Manjer J, Carlsson S, Elmstahl S, Gullberg B, Janzon L, Lindstrom M, Mattisson I, Berglund G: **The Malmo Diet and Cancer Study: representativity, cancer incidence and mortality in participants and non-participants**. *Eur J Cancer Prev* 2001, **10**:489-499.

16. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, Healey LA, Kaplan SR, Liang MH, Luthra HS, Medsger Jr TA, Mitchell DM, Neustadt DH, Pinals RS, Schaller JG, Sharp JT, Wilder RL, Hunder GG: **The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis**. *Arthritis Rheum* 1988, **31**:315-324.

17. Turesson C, Baecklund E, Forsblad d'Elia H: **Guidlines for the pharmaceutical management of rheumatoid arthritis.** In.: The Swedish Society of Rheumatology.; 2011.

18. Geborek P, Nitelius E, Noltorp S, Petri H, Jacobsson L, Larsson L, Saxne T, Leden I: **Population based studies of biological antirheumatic drug use in southern Sweden: comparison with pharmaceutical sales**. *Ann Rheum Dis* 2005, **64**:1805-1807.

19. Turesson C, Jacobsson LT: **Epidemiology of extra-articular manifestations in rheumatoid arthritis**. *Scand J Rheumatol* 2004, **33**:65-72.

20. Fries JF, Spitz PW, Young DY: **The dimensions of health outcomes: the health assessment questionnaire, disability and pain scales**. *J Rheumatol* 1982, **9**:789-793.

21. Straub RH: **The complex role of estrogens in inflammation**. *Endocr Rev* 2007, **28**:521-574.

22. McInnes IB, Schett G: **Cytokines in the pathogenesis of rheumatoid arthritis**. *Nat Rev Immunol* 2007, **7**:429-442.

23. Schulze-Koops H, Kalden JR: **The balance of Th1/Th2 cytokines in rheumatoid arthritis**. *Best practice & research Clinical rheumatology* 2001, **15**:677-691.

24. Wahle M, Krause A, Pierer M, Hantzschel H, Baerwald CG: **Immunopathogenesis of rheumatic diseases in the context of neuroendocrine interactions**. *Ann N Y Acad Sci* 2002, **966**:355-364.

25. Cutolo M, Sulli A, Pizzorni C, Craviotto C, Straub RH: **Hypothalamic-pituitary-adrenocortical and gonadal functions in rheumatoid arthritis**. *Ann N Y Acad Sci* 2003, **992**:107-117.

26. Johnson EO, Skopouli FN, Moutsopoulos HM: **Neuroendocrine manifestations in Sjogren's syndrome**. *Rheum Dis Clin North Am* 2000, **26**:927-949.

27. Wu JM, Zelinski MB, Ingram DK, Ottinger MA: **Ovarian aging and menopause: current theories, hypotheses, and research models**. *Exp Biol Med (Maywood)* 2005, **230**:818-828.

28. Downs JL, Wise PM: **The role of the brain in female reproductive aging**. *Mol Cell Endocrinol* 2009, **299**:32-38.

29. Jacobsson LT, Jacobsson ME, Askling J, Knowler WC: **Perinatal characteristics and risk of rheumatoid arthritis**. *BMJ* 2003, **326**:1068-1069.

30. Mandl LA, Costenbader KH, Simard JF, Karlson EW: **Is birthweight associated with risk of rheumatoid arthritis? Data from a large cohort study**. *Ann Rheum Dis* 2009, **68**:514-518.

31. Phillips DI, Barker DJ, Fall CH, Seckl JR, Whorwood CB, Wood PJ, Walker BR: **Elevated plasma cortisol concentrations: a link between low birth weight and the insulin resistance syndrome?** *J Clin Endocrinol Metab* 1998, **83**:757-760.

32. de Rooy DP, van der Linden MP, Knevel R, Huizinga TW, van der Helm-van Mil AH: **Predicting arthritis outcomes--what can be learned from the Leiden Early Arthritis Clinic?** *Rheumatology (Oxford)* 2011, **50**:93-100.

33. Nyhall-Wahlin BM, Petersson IF, Nilsson JA, Jacobsson LT, Turesson C: **High disease activity disability burden and smoking predict severe extra-articular manifestations in early rheumatoid arthritis**. *Rheumatology (Oxford)* 2009, **48**:416-420.

34. Brennan P, Bankhead C, Silman A, Symmons D: **Oral contraceptives and rheumatoid arthritis: results from a primary care-based incident case-control study**. *Semin Arthritis Rheum* 1997, **26**:817-823.

35. Neovius M, Simard JF, Askling J: **Nationwide prevalence of rheumatoid arthritis and penetration of disease-modifying drugs in Sweden**. *Ann Rheum Dis* 2011, **70**:624-629.

36. Barrett JH, Brennan P, Fiddler M, Silman A: **Breast-feeding and postpartum relapse in women with rheumatoid and inflammatory arthritis**. *Arthritis Rheum* 2000, **43**:1010-1015.

37. Brennan P, Ollier B, Worthington J, Hajeer A, Silman A: **Are both genetic and reproductive associations with rheumatoid arthritis linked to prolactin?** *Lancet* 1996, **348**:106-109.

38. Karlson EW, Mandl LA, Hankinson SE, Grodstein F: **Do breast-feeding and other reproductive factors influence future risk of rheumatoid arthritis? Results from the Nurses' Health Study**. *Arthritis Rheum* 2004, **50**:3458-3467.

Figure 1. Distribution of clusters of clinical outcomes defining disease severity; by age at menopause. p=0.005.

Table 1. Patient characteristics

	All patients. N= 134
Mean age at diagnosis; years (SD) (range)	63.2 (8.3) (47-80)
Mean follow-up time; years (SD)	10.1 (3.5)
RF positive (%)	94/132 (71.2)
Anti-CCP positive (%)	34/57 (59.6)
Smoking at diagnosis (%)	39/118 (33.1)
Low formal education (<8 years of school)(%)	66/128 (49.3)
Prednisolone at diagnosis (%)	39/120 (32.5)
Biological treatment ever (%)	37/133 (27.8)
Number of used DMARDS; median (IQR)	2 (1-3)
>3 DMARDs used (%)	30 (22.4)
DMARDS in combination (%)	54 (40.3)
Documented radiographic erosions (ever) (%)	71/125 (56.8)
Severe extra-articular manifestations (%)	7 (5.2)
Median HAQ at diagnosis*	0.87
Median HAQ 1 year after diagnosis*	0.66
Median HAQ 5 years after diagnosis*	0.64
Median HAQ 10 years after diagnosis*	0.56

SD=Standard deviation, RF=Rheumatoid factor, CCP= cyclic citrullinated peptides, DMARD= Disease modifying ant-rheumatic drug, IQR= Inter Quartile Range, HAQ= Health assessment questionnaire. * Data available for HAQ at diagnosis in 70 cases, for HAQ at 1 year in 82 cases, for HAQ at 5 years in 92 cases and for HAQ at 10 years in 63 cases.

Table 2. Distribution of clusters of clinical outcomes

	Cluster I: Severe RA (N:26)	**Cluster II: Mild/moderate seropositive RA (N:25)**	**Cluster 3: Mild/moderate seronegative RA (N:39)**
Treated with biologics (ever)	100%	0%	0%
RF positive	88.5%	100%	0%
Documented erosions on x-ray (ever)	84.6%	56.4%	52.0%
HAQ after 5 years			
-mean (SD)	1.17 (0.61)	0.74 (0.61)	0.88 (0.66)
-median (IQR)	1.00 (0.72-1.66)	0.63 (0.38-0.88)	0.88 (0.25-1.25)

RF=rheumatoid factor, HAQ=Health assessment questionnaire, SD=Standard deviation,

IQR=interquartile range.

Table 3. Clinical outcomes - by age at menopause

	Early Menopause (<46 years) (N: 25)*	**Normal/late Menopause (≥ 46 years) (N: 102)***
Mean age at diagnosis; years (SD)	65.2 (6.94)	62.8 (8.48)
Mean follow-up time; years (SD)	10.5 (3.0)	10.1 (3.6)
>3 DMARDs used (%)	5/25 (20.0)	23/101 (22.8)
DMARDs in combination (%)	8/25 (32.0)	45/101 (44.6)
Biological treatment ever (%)	3/25 (12.0)	33/101 (32.7)
Documented radiographic erosions (ever) (%)	14/23 (60.9)	52/96 (54.2)
RF positive (%)	11/25 (44.0)	78/100 (78.0)
Mean HAQ at diagnosis, (CI)**	0.58 (0.22-0.93) (n: 12)	0.96 (0.79-1.12) (n: 55)
Mean HAQ 1 year after diagnosis, (CI)**	0.45 (0.08-0.83) (n: 12)	0.86 (0.70-1.02) (n: 65)
Mean HAQ 5 years after diagnosis, (CI)**	0.88 (0.60-1.15) (n: 19)	0.90 (0.76-1.05) (n: 68)
Mean HAQ 10 years after diagnosis, (CI)**	0.67 (0.34-1.00) (n: 12)	0.98 (0.82-1.14) (n: 49)
Cluster distribution (p=0.005)		
-severe RA (%)	3 (15.8%)	23 (34.8%)
-mild/moderate seropos RA (%)	5 (26.3%)	30 (45.5%)
-mild/moderate seroneg RA (%)	11 (57.9%)	13 (19.7%)

RF=rheumatoid factor, HAQ=Health assessment questionnaire, SD=Standard deviation, CI=Confidence Interval, *In case of missing data, numbers (n) indicated for each variable.

**Adjusted for age at diagnosis.

Table 4. Clinical outcomes-by duration of breast-feeding

	Breast-feeding 0 months (N:43)*	**Breast-feeding 1-12 months (N:72)***	**Brest-feeding >12 months (N:19)***
Mean age at diagnosis; years (SD)	63.6 (8.37)	62.8 (8.30)	64.2 (8.27)
Mean follow-up time; years (SD)	9.6 (3.4)	10.3 (3.7)	10.5 (2.8)
> 3 DMARDs used (%)	8 /43(18.6)	17/71 (23.9)	5/19 (26.3)
DMARDs in combination (%)	14/43 (32.6)	32/71 (45.1)	8/19 (42.1)
Biological treatment ever (%)	10/43(23.3)	21/71 (29.6)	6/19 (31.6)
Documented radiographic erosions (ever) (%)	25/39 (64.1)	36/70 (51.4)	10/16 (62.5)
RF positive (%)	28/43 (65.1)	53/71 (74.6)	13/18 (72.2)
Mean HAQ at diagnosis, (CI)**	0.91 (0.64-1.18) (n: 21)	0.89 (0.69-1.09) (n: 39)	0.86 (0.47-1.25) (n: 10)
Mean HAQ 1 year after diagnosis, (CI)**	0.76 (0.49-1.03) (n: 24)	0.86 (0.66-1.06) (n: 44)	0.68 (0.32-1.03) (n: 14)
Mean HAQ 5 years after diagnosis, (CI)**	0.91 (0.68-1.14) (n: 31)	0.90 (0.72-1.09) (n: 49)	0.85 (0.48-1.22) (n: 12)
Mean HAQ 10 years after diagnosis, (CI)**	0.78 (0.50-1.06) (n: 16)	0.94 (0.76-1.13) (n: 38)	1.08 (0.70-1.45) (n:9)
Cluster distribution (p=0.88)			
-severe RA (%)	8/31 (25.8)	15/48 (31.2)	3/11 (27.3)
-mild/moderate seropos RA (%)	13/31 (41.9)	20/48 (41.7)	6/11 (54.5)
-mild/moderate seroneg RA (%)	10/31 (32.3)	13/48 (27.1)	2/11 (18.2)

RF=rheumatoid factor, HAQ=Health assessment questionnaire, SD=Standard deviation, CI=Confidence Interval, *In case of missing data, numbers (n) indicated for each variable. **Adjusted for age at diagnosis.

Table 5. Clinical outcomes-by history of use of oral contraceptives

	Never used (N:67)*	**1-5 years of use (N:17)***	**> 5 years of use (N:43)***
Mean age at diagnosis; years (SD)	66.2 (7.82)	58.8 (8.19)	60.1 (7.17)
Mean follow-up time; years (SD)	10.4 (3.3)	9.9 (3.1)	10.0 (3.8)
> 3 DMARDs used (%)	11/67 (16.4)	4/17 (23.5)	14/42 (33.3)
DMARDs in combination (%)	26/67 (38.8)	8/17 (47.1)	20/42 (47.6)
Biological treatment ever (%)	14/67 (20.9)	4/17 (23.5)	19/42 (45.2)
Documented radiographic erosions (ever) (%)	37/62 (59.7)	10/17 (58.8)	20/40 (50.0)
RF positive (%)	46/66 (69.7)	10/16 (62.5)	34/43 (79.1)
Mean HAQ at diagnosis, (CI) **	0.75 (0.53-0.97) (n:34)	0.93 (0.42-1.43) (n: 6)	1.06 (0.81-1.30) (n: 27)
Mean HAQ 1 year after diagnosis, (CI) **	0.83 (0.61-1.05)(n: 39)	0.64 (0.20-1.09) (n: 9)	0.81 (0.56-1.06) (n: 29)
Mean HAQ 5 years after diagnosis, (CI) **	0.93 (0.75-1.11) (n:47)	0.80 (0.46-1.15)(n: 13)	0.86 (0.62-1.10) (n: 26)
Mean HAQ 10 years after diagnosis, (CI) **	0.79 (0.59-1.00) (n: 33)	1.20 (0.77-1.63) (n: 7)	1.00 (0.75-1.26) (n: 21)
Cluster distribution (p=0.56)			
-severe RA (%)	12/47 (25.5)	3/12 (25.0)	11/25 (44,0)
-mild/moderate seropos RA (%)	21/47 (44.7)	5/12 (41.7)	9/25 (36.0)
-mild/moderate seroneg RA (%)	14/47 (29.8)	4/12 (33.3)	5/25 (20.0)

RF=rheumatoid factor, HAQ=Health assessment questionnaire, SD=Standard deviation, CI=Confidence Interval, *In case of missing data, numbers (n) indicated for each variable, **Adjusted for age at diagnosis.

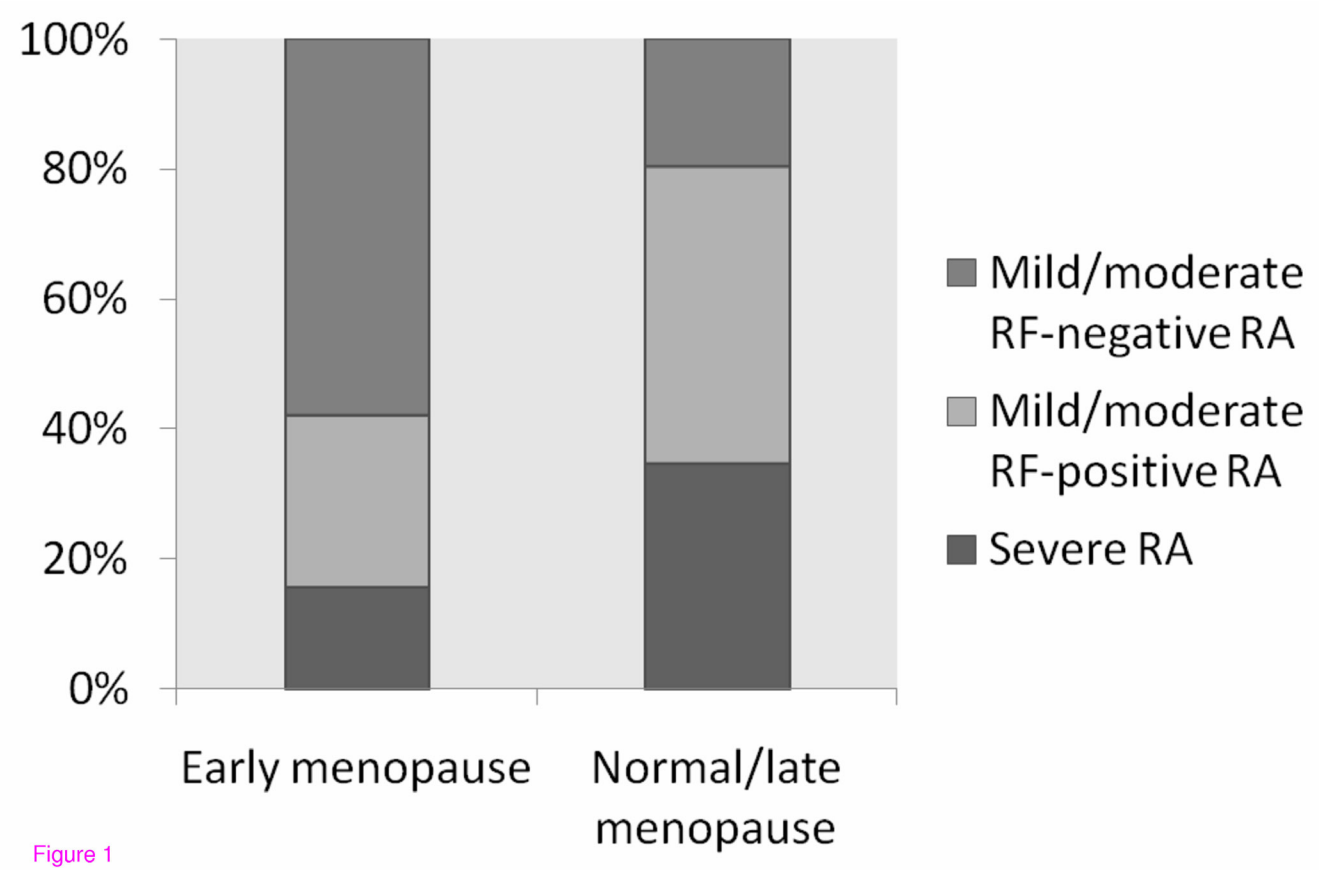

100%
80%
60%
40%
20%
0%
Early menopause
Normal/late menopause
Mild/moderate RF-negative RA
Mild/moderate RF-positive RA
Severe RA

Figure 1

Paper IV

Association Between Testosterone Levels and Risk of Future Rheumatoid Arthritis in Men – a Population Based Case-control Study.

Mitra Pikwer[1], Aleksander Giwercman[2], Ulf Bergström[1], Jan-Åke Nilsson[1], Lennart TH Jacobsson[1,3], Carl Turesson[1].

[1]Section of Rheumatology, Department of Clinical Sciences, Malmö, Lund University, Malmö, Sweden.

[2]Reproductive Medicine Centre (RMC), Skåne University Hospital, Malmö, Lund University, Malmö, Sweden.

[3]Department of Rheumatology and Inflammation Research, The Sahlgrenska Academy, University of Gothenburg, Sweden

Correspondence to

Dr Mitra Pikwer, Department of Rheumatology,

Lund University, Skåne University Hospital,

SE-205 02

Malmö, Sweden

Mitra.pikwer@med.lu.se

Abstract

Background

Rheumatoid arthritis (RA) is less common among men than women, and sex hormones have been suggested to play a part in the pathogenesis. Lower levels of testosterone have been demonstrated in men with RA, but it is not known if these changes precede the disease.

Methods

In a nested case-control study, using information and blood samples from a population based health survey, incident cases of RA were identified by linking the cohort to local and national RA registers. Two controls for each validated case, matched for age, sex and year of screening, were selected from the health survey. Using stored blood samples, collected between 8.00 and 10.00 a.m. after an overnight fast, testosterone and other reproductive hormone levels were analysed.

Results

Serum was available from 104 cases (median time from screening to RA diagnosis 12.7 years (range 1-28); 73 % rheumatoid factor [RF] positive at diagnosis or later) and 174 matched controls. In conditional logistic regression models, adjusted for smoking and body mass index, lower levels of testosterone were associated with subsequent development of RF negative RA (Odds Ratio [OR]: 0.31 per standard deviation (SD), 95% Confidence Interval [CI]: 0.12-0.85), with a weaker association with RF positive RA (OR per SD 0.87; 95% CI: 0.53-1.43). Levels of follicle-stimulating hormone were significantly increased in pre-RF negative RA (p=0.02), but decreased in pre-RF positive RA (p=0.02).

Conclusions

Lower levels of testosterone were predictive of RF negative RA, suggesting that hormonal changes precede the onset of RA and affect the disease phenotype.

Introduction

Risk factors for rheumatoid arthritis (RA) include genetic,[1] environmental [2] and hormonal factors.[3] Autoimmunity affects men to a lesser extent than women [4] and, during the fertile years, RA has a female:male incidence ratio of 4-6:1.[5] With increasing age, the sex difference in incidence narrows.[6] In cross-sectional studies, lower levels of serum testosterone have been found in both male and female patients with RA compared to healthy controls.[7-10] Pro-inflammatory cytokines are known to stimulate the hypothalamic-pituitary- adrenal axis but suppress the hypothalamic-pituitary-gonadal axis, suggesting that low testosterone levels might be a consequence of the inflammatory disease.[11] Alternatively, the measured low testosterone levels could reflect a role of androgens in the pathogenesis of RA.

A large prospective study of women did not find any association between androgen levels measured at a single time point or polymorphism in the sex hormone receptors and risk of RA. The only prospective study on men, based on 32 incident male RA cases from Finland, did not show any significant differences in testosterone levels prior to RA onset compared to controls.[12] However, this study had limited power and did not adjust for potential confounders or stratification by different phenotypes of RA. In particular, stratification by rheumatoid factor (RF) status may be relevant, since risk factors and outcomes have been reported to differ for RF positive and RF negative disease[13], and we have previously demonstrated stronger associations between RF negative disease and hormonal predictors in women.[3] Taken together, there is limited data on the importance of androgen levels for the development of RA, in particular in men.

The aim of this study was to measure testosterone and other sex hormones in a larger sample of men who subsequently developed RA, to investigate if differences in hormone

concentrations from matched controls could be detected years before diagnosis and if such patterns differed between subtypes of RA.

Patients and methods

Source population: The Malmö Preventive Medicine Program

Between 1974 and 1992, the Malmö Preventive Medicine Program (MPMP), a preventive case finding program was conducted in Malmö, Sweden (population 235 000 in 1974).[14] The program included a total of 22 444 males born between 1949 and 1921 and 10 902 females born between 1938 and 1925. The aim of the health survey was to screen large strata of the adult population in order to identify individuals for preventive intervention. The overall attendance rate was 71.2%. The subjects underwent physical examination including height and weight measurements and laboratory tests, and completed a self-administered questionnaire on health and life style factors.[15] The subjects were invited to leave blood samples in the morning, between 8.00 and 10.00 a.m., after an overnight fast. The samples were stored at -20 degrees Celsius.[16]

Selection of cases and controls

In a previous survey [17], we identified individuals who developed RA after inclusion in this cohort and up to December 31, 2004, by linking the MPMP register to a community based RA register, [18,19] the local outpatient clinic administrative register for Malmö University Hospital, the National Hospital Discharge Register and the National Cause of Death Register.[17] The community based RA register has been shown to include more than 90 % of patients in the catchment area.[19] The Swedish national inpatient register includes more than 99 % of all hospital discharges and has a high validity for RA and many other diagnoses. [20]

In a structured review of all medical records, possible cases were validated and classified according to the 1987 American College of Rheumatology criteria for RA.[21] Four controls for each validated case, matched for sex, year of birth and year of screening, who were alive and free of RA when the index person was diagnosed with RA, were selected from the MPMP cohort. Vital status and information on emigration were retrieved from the national census, and controls who were not alive or living in Sweden through the index date were excluded.

For the present study, serum samples from two controls per case were collected from the MPMP bio bank. For various reasons, samples were missing from a subset of cases as well as controls. For cases with available serum, the retrieval of control samples was extended to include the two remaining matched controls, when such were available.

This study was approved by the regional research ethics committee for southern Sweden. All participants gave their informed consent to be included in the MPMP and the Malmö RA register, respectively. No informed consent was obtained specifically for the present study.

Socioeconomic background and co-morbidities

Data on socioeconomic status was derived from self-reported job titles in the Swedish national censuses, as previously described.[17] Briefly, occupations were coded and converted into standardized social class categories, and subjects were classified as "blue-collar workers" (manual workers, both skilled and unskilled), "white collar workers" (non-manual employees and self employed professionals) and "others". Housewives, students and unemployed without any other self-reported job title during the study period were excluded from this classification.[22]

Data on self reported overall health and self reported cancer, diabetes and cardiovascular disease (the latter classified as self report of either hospitalization for stroke, physician diagnosis of angina pectoris or current use of heart medication) at baseline were extracted from the self-administered questionnaire.

Laboratory tests

Serum total testosterone, sex-hormone binding globulin (SHBG), luteinizing hormone (LH) and follicle-stimulating hormone (FSH) concentrations were quantified by ElectroChemiLuminiscence Immunoassay (ECLI) based on Rutenium derivate according to routine methods used at the Department of Laboratory Medicin, Skåne University Hospital. Free testosterone was calculated from total testosterone and SHBG levels using the Vermeulen formula.[23] The detection limits for testosterone, SHBG, LH and FSH were 0.0087 nmol/L, 0.35 nmol/L, 0.10 international units (IU)/L and 0.10 IU/L respectively. Imprecision levels for low and high levels were 2.4% and 4.0% for testosterone, 1.0% and 1.1% for SHBG, 2.0 and 2.2% for LH 3.3% and 2.2% for FSH respectively. The erythrocyte sedimentation rate (ESR) was measured at screening according to the standard Westergren method.

Data on RF tests were collected from the databases of the two clinical immunology laboratories in the area.

Statistics

The impact of baseline hormone levels on the risk of RA was examined in bivariate conditional logistic regression analysis, taking into account the matched design of the study. For comparability of the impact of hormones with different concentrations, OR for RA were calculated per SD of testosterone, FSH, LH and SHBG, respectively. Potential

confounders were examined in a similar manner. Correlations between BMI and testosterone were examined using Pearson´s test. Multivariate logistic regression analysis was used to adjust for potential confounders. Analyses were stratified by RF status at diagnosis or later (ever positive vs. negative) and also by time from screening to RA diagnosis (above vs. below the median). Statistical significance was set at $p < 0.05$. Mitra Pikwer and Carl Turesson performed all analyses using SPSS version 19, and results were discussed with all the authors.

Results

Cases and controls

As previously reported, 151 male cases of incident RA were identified.[17] For the present study, stored serum was available from 104 males who subsequently developed RA and 174 matched controls. RF status at diagnosis or later was available for 83 patients. Age at screening (mean age 45 vs. 46 years), age at RA diagnosis (mean 59 years in both groups) and the proportion of blue-collar workers (52 vs. 54%) were similar in this subset with known RF status and in the entire population of incident male RA cases. Characteristics of pre-RA cases and controls are described in Table 1. Cases who developed RF negative RA were older at screening (mean 48 vs. 45 years) and at RA diagnosis (mean 64 vs. 58 years) compared to those who developed RF positive RA**.** There were no substantial differences in ESR between cases and controls (table 1). Co-morbidities and self-reported health status were similar in cases and controls (Table 1). Comparison between those with and without available sera showed similar frequencies of full self-reported health among both cases (76 % vs. 70 %) and controls (70 % vs. 74

%). Age at screening and age at diagnosis were similar in cases with and without serum available.

Confounders: smoking and BMI

Pre-RA cases had on average lower BMI than controls (Table 1), and there was a negative correlation between BMI and testosterone levels ($r=-0.46$; $p<0.01$) as well as free testosterone levels ($r=-0.36$; $p<0.01$), but not with other hormones (SHBG, LH and FSH). As previously reported[17], smoking was associated with RA (Table 1), and smokers had higher levels of all measured hormones (testosterone: mean 23.0 vs. 20.2 nmol/L, $p=0.01$; free testosterone: mean 0.44 vs. 0.41 nmol/L, $p=0.01$; FSH; mean 7.10 vs. 5.86 IU/L, $p=0.06$; LH: mean 7.72 vs. 5.06 IU/L, $p=0.05$; SHBG: mean 38.4 vs. 32.6 nmol/L, $p<0.01$; for levels in all cases and controls, see Table 2). In bivariate logistic regression analysis, smoking was associated with increased risk of RF positive RA, and lower BMI tended to be associated with in particular RF negative RA (Table 3).

Hormone levels and the risk of RA

Cases tended to have lower mean values of all measured hormones except SHBG compared to controls, with the main differences observed among those with a longer time from screening to diagnosis (Table 2). Individuals who developed RA >12.7 years after screening were older (mean 65 vs. 54 years) and more likely to be RF negative (65 % vs. 35 %) at diagnosis.

In bivariate analyses, without adjustment for potential confounders, there was a trend towards a negative association between total and free testosterone levels and RF negative RA. There was statistically significant positive association between FSH levels and risk of RF negative RA (Table 3).

In multivariate logistic regression analysis, adjusted for BMI and smoking, there was a trend towards a negative association between testosterone levels (both total and free testosterone) and subsequent development of RA, which was statistically significant for RF-negative cases (Table 4).

Serum levels of FSH were positively associated with future development of RF negative RA and negatively associated with future RF positive RA, in both bivariate (Table 3) and multivariate analysis, adjusted for smoking (Table 4). When stratifying by smoking or median BMI, similar associations between hormone levels and RA were seen in all strata (data not shown).

To exclude nascent inflammation in pre-RA cases as an explanation for the differences in testosterone levels, we limited the analysis to cases that were diagnosed with RA more than 5 years after screening (n=89; 21 RF negative; 51 RF positive; 17 unknown) and their controls, with similar results for testosterone (adjusted ORs with 95 % CIs: for all cases 0.82 [0.55-1.21], for RF negative RA 0.31 [0.11-0.84] and for RF positive RA 0.89 [0.53-1.50]) and for the other hormones (data not shown).

Blue-collar worker status was also a potential confounder, being associated with both RA (Table 1), as previously reported [17], and also with a tendency towards higher serum levels of testosterone (mean 22.2 nmol/l vs. 20.9 nmol/l in white-collar workers; p=0.17). Further adjustment for this factor, in addition to smoking and BMI, gave similar point estimates for the associations between total serum testosterone and risks for RA development (Figure 1).

Discussion

This study demonstrated a negative association between levels of testosterone and free testosterone and subsequent development of RF negative RA in men. There were also differences in gonadotropin levels between pre-RA cases and controls, with distinct and different patterns for pre-RF negative (higher FSH) and pre-RF positive RA (lower FSH). With the exception of a small study from Finland, by Heikkila et al.,[12] this is the first study to explore hormone levels in men prior to RA diagnosis.

Low testosterone could be a consequence of primary testicular dysfunction, a primary dysfunction in the hypothalamus-pituitary part of the HPG axis, but also a result of inflammation.[24] However, the lack of differences in ESR or self-reported health status between pre-RA cases and controls does not indicate inflammation due to early arthritis as the main explanation for the observed differences. Furthermore, when excluding men who developed RA within five years of screening, similar results were obtained.

The associations between smoking, BMI and hormone levels in the present study are supported by previous findings.[25,26] Smoking is a well-known risk factor for RA,[13] and a recent case-control study demonstrated an association between low BMI and sero-negative RA in men.[27] The rational for adjusting for smoking and BMI in the multivariate analysis is therefore not only based on data from the present sample, but also compatible with the literature.

Testosterone has been proposed to have anti-inflammatory functions, by suppressing both the cellular and the humoral immune system.[28] Male sex has been found to be an independent predictor of remission in early RA.[29] This has led to the hypothesis that androgen supplementation may be useful in the management of RA. Testosterone as treatment for RA has however so far been investigated in limited samples, with different results [30,31]

It has been suggested that RA patients have an altered hypothalamic-pituitary- adrenal axis with inappropriately low cortisol and gonadotropin levels compared to the expected, given the state of inflammation.[32] A Swedish case-control study of men with early RA diagnosis identified lower testosterone levels in cases, and noted that older men with RA (>50 years old) had low luteinizing hormone (LH) levels despite low testosterone, indicating a central genesis to the deficiency.[33] Furthermore, they showed a negative correlation between testosterone levels and disease activity.[34] In our material, LH levels were also lower in pre-RA cases compared to controls despite lower testosterone, although the differences for LH did not reach statistical significance. We also identified discrepancies in FSH levels between RF negative and positive RA, indicating different patho-mechanisms behind the two subtypes of the disease. FSH levels were higher before onset of RF negative RA, indicating an increased hypothalamus-pituitary response to testicular dysfunction. By contrast, lower FSH levels were found before onset of RF positive RA, implicating an impaired hypothalamus-pituitary function as a possible factor in the pathogenesis of RF positive RA.

RA is a heterogeneous disease, which may be subdivided into different phenotypes, of which RF status is the most widely recognised and considered a stable phenotype based on follow-up studies.[35]. Predictors may differ between the sexes, geographical area [36] and phenotypes. For instance, smoking is a predictor of RF positive disease, with a stronger impact in men,[13] whereas in women, age at menopause and breast-feeding history predominantly affect the risk of RF negative disease with onset after age 45.[3,37] In the present study, the average age at diagnosis of RF negative RA was substantially higher compared to RF positive disease. Taken together, this suggests that changes in sex hormones mainly influence onset of RF negative RA in older individuals of both sexes.

This is a unique material of fasting morning blood samples obtained from a relatively large number of men who subsequently developed RA. Limitations include the fact that samples were stored for a number of years before analysis. However, this is unlikely to have had a systematic effect on our comparison, since cases and controls were matched for year of screening. Furthermore, due to missing blood samples and missing data on RF status in a subset of the cases, statistical power is limited for some of the sub analyses.

Strengths include the population based approach, and the fact that we used a validated local register, together with the Swedish national inpatient register. Previously reported incidence estimates indicate that we captured virtually all incident RA cases during the study period,[17] suggesting that our cases are representative of RA cases in the community. On the other hand, the cases were mainly Caucasians of Scandinavian heritage, and the results may not apply to other ethnic groups or other geographic settings.

A further strength is the availability of data on confounders and comorbidities from the health survey. Our analyses indicate that differences in comorbidities and socioeconomic status did not explain the associations between baseline hormone levels and future risk of RA.

In conclusion, we report a negative association of testosterone and free testosterone levels with the risk of developing RF negative RA in men. Since this is the first major study of testosterone and related hormones in the pre-clinical phase of RA, our findings should be verified in other populations.

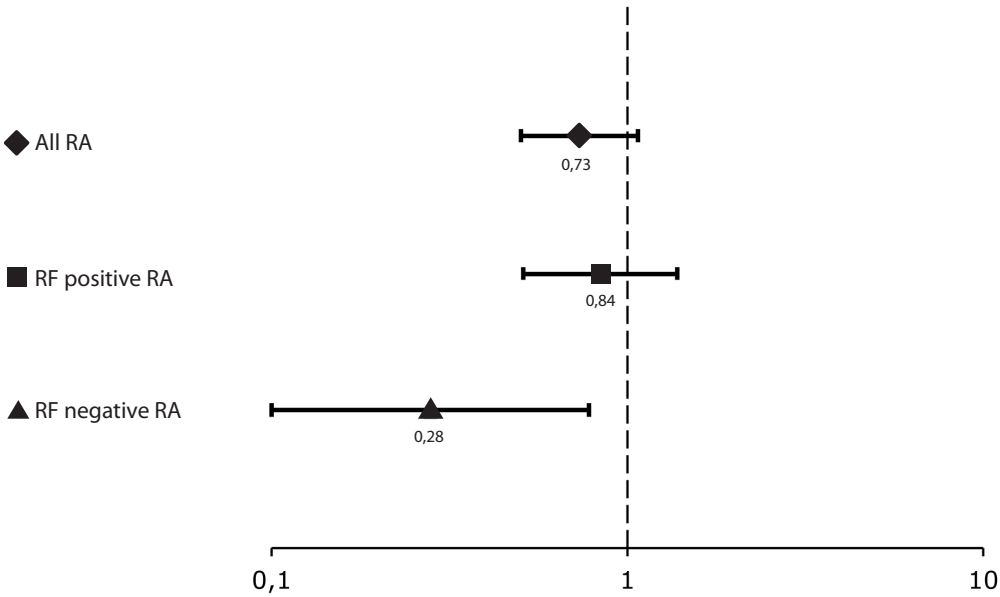
Acknowledgements

The authors would like to thank Göran Berglund and collaborators in the MPMP steering committee for excellent advice on the study, Anders Dahlin and Håkan Andersson for excellent help with data extraction from the MPMP database, Yvonne Bengtsson, Lisbet Andreassen and Charlotte Becker from the Department of Laboratory Medicin, Skåne University Hospital, for their work with analyses of testosterone, FSH, LH and SHBG, Yvonne Giwercman for helpful comments on the study and Gunnel Henriksson and Lennart Truedsson for providing information from the clinical immunology databases on RF tests.

This study was supported by the Swedish Research Council, the Swedish Rheumatism Association, Lund University and the County of Skåne.

Figures legends

Figure 1. Impact of total testosterone levels on the risk of RA overall, RF negative RA and RF positive RA. Conditional logistic regression analysis, adjusted for smoking, socioeconomic status and body mass index. Odds Ratios with 95 % Confidence Intervals.



Tables

Table 1. Characteristics of pre-RA cases and controls.

	Cases (104)	Controls (174)
Mean age at screening, years (SD)	46.0 (6.9)	46.0 (7.4)
Mean age at diagnosis, years (SD)	59.3 (9.4)	NA
RF positive at diagnosis or later (%)	61/83 (73)	NA
Median time (years) to diagnosis (IQR, Range)	12.7 (8.4-19.4, 1-28)	NA
BMI kg/m^2 mean (SD)	24.3 (2.53)	25.0 (3.58)
Current smokers (%)	58/104 (56)	79/174 (45)
ESR (mm) median (IQR, Range)	4 (3-8, 1-50)	5 (3-7, 1-61)
Socioeconomic index, - blue-collar workers (%) - white collar-workers (%)	 53/104 (51) 37/104 (36)	 80/174 (46) 71/174 (41)
Full self reported health (%)	73/104 (70)	128/174 (74)
Self reported diabetes (%)	3/84 (3.6)	2/140 (1.4)
Self reported cancer (current or previous) (%)	2/84 (2.4)	0/140 (0.0)
Self reported cardiovascular disease (%)	2/84(2.4 %)	0 /140(0.0 %)

Table 2. Hormone levels† in all cases and controls, stratified by RF status in the case at diagnosis or later, and by median time from inclusion to RA diagnosis.

	All		<12.7 years from diagnosis		>12.7 years from diagnosis		RF positive cases		RF negative cases	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
Mean testosterone nmol/L (SD)	21.5 (6.05)	21.6 (7.46)	22.1 (6.43)	22.0 (7.99)	20.8 (5.58)	21.3 (6.90)	21. 9 (6.23)	21.7 (7.77)	19.4 (5.08)	21.6 (7.29)
Mean free testosterone nmol/L (SD)	0.42 (0.09)	0.42 (0.12)	0.43 (0.10)	0.43 (0.12)	0.41 (0.09)	0.42 (0.11)	0.42 (0.09)	0.42 (0.12)	0.40 (0.08)	0.44 (0.12)
Mean LH IU/L (SD)	5.31 (2.78)	5.44 (2.61)	5.57 (3.37)	5.26 (2.65)	5.00 (1.85)	5.66 (2.58)	5.11 (2.22)	5.27 (2.72)	5.40 (1.83)	5.73 (2.73)
Mean FSH IU/L (SD)	5.95 (4.70)	6.79 (5.69)	6.59 (5.98)	7.23 (6.62)	5.21 (2.40)	6.42 (4.54)	5.61 (2.22)	7.03 (6.29)	6.49 (3.69)	6.00 (4.27)
Mean SHBG nmol/L (SD)	36.08 (14.6)	35.05 (15.4)	36.6 (15.8)	35.9 (16.5)	35.5 (13.3)	34.5 (14.3)	37.9 (14.2)	36.4 (15.8)	30.8 (14.5)	33.1 (12.9)

† From analyses of testosterone, results were available from 104 cases and 174 controls; for FSH 101 cases and 169 controls; for LH 102 cases and 167 controls; for SHBG 104 cases and 173 controls; for free testosterone 104 cases and 173 controls.

Table 3. Unadjusted associations between levels of hormones, smoking, BMI and the risk of RA. Bivariate logistic regression analysis; Odds Ratio (95% Confidence Interval)

	All (104 cases)	RF positive (61cases)	RF negative (22 cases)
Testosterone per SD (6.95 nmol/l)	1.00 (0.72-1.38)	1.11 (0.73-1.70)	0.56 (0.26-1.16)
Free testosterone per SD (0.11 nmol/l)	0.92 (0.66-1.28)	1.00 (0.64-1.55)	0.58 (0.28-1.18)
FSH per SD (5.36 IU/l)	0.80 (0.56-1.16)	0.54 (0.27-1.03)	13.1 (1.70-100)
LH per SD: (2.67 IU/l)	1.00 (0.72-1.40)	0.89 (0.56-1.39)	1.14 (0.44-3.00)
SHBG per SD (15.1 nmol/l)	1.16 (0.85-1.58)	1.24 (0.82-1.88)	0.75 (0.34-1.63)
Current smokers (vs. non-smokers)	1.86 (0.94-3.68)	2.82 (1.16-6.89)	0.37 (0.09-1.48)
BMI per SD (3.24 kg/m^2)	0.73 (0.52-1.03)	0.74 (0.50-1.14)	0.57 (0.24-1.31)

Table 4. Associations between levels of measured hormones and risk of RA adjusted for smoking and/or body mass index. Stratified by rheumatoid factor (RF) status at diagnosis or later. Multivariate Logistic Regression; Odds Ratio (95% Confidence Interval)

	All (104 cases)	RF positive (61 cases)	RF negative (22 cases)
Testosterone per SD*	0.77 (0.52-1.12)	0.87 (0.53-1.43)	0.31 (0.12-0.85)
Free testosterone per SD*	0.75 (0.52-1.09)	0.83 (0.51-1.35)	0.38 (0.15-0.92)
FSH per SD**	0.77 (0.53-1.13)	0.42 (0.20-0.88)	11.5 (1.46-91.1)
LH per SD**	0.96 (0.68-1.36)	1.07 (0.53-2.15)	0.36 (0.08-1.69)
SHBG per SD**	1.89 (0.93-3.85)	1.11 (0.72-1.71)	0.83 (0.38-1.82)

*Adjusted for smoking and BMI, **adjusted for smoking

References

1. Silman AJ, MacGregor AJ, Thomson W, et al. Twin concordance rates for rheumatoid arthritis: results from a nationwide study. Br J Rheumatol 1993;32:903-7.
2. Karlson EW, Lee IM, Cook NR, Manson JE, Buring JE, Hennekens CH. A retrospective cohort study of cigarette smoking and risk of rheumatoid arthritis in female health professionals. Arthritis Rheum 1999;42:910-7.
3. Pikwer M, Bergstrom U, Nilsson JA, Jacobsson L, Turesson C. Early menopause is an independent predictor of rheumatoid arthritis. Ann Rheum Dis 2011.
4. Fairweather D, Frisancho-Kiss S, Rose NR. Sex differences in autoimmune disease from a pathological perspective. Am J Pathol 2008;173:600-9.
5. Kvien TK, Uhlig T, Odegard S, Heiberg MS. Epidemiological aspects of rheumatoid arthritis: the sex ratio. Ann N Y Acad Sci 2006;1069:212-22.
6. Englund M, Joud A, Geborek P, Felson DT, Jacobsson LT, Petersson IF. Prevalence and incidence of rheumatoid arthritis in southern Sweden 2008 and their relation to prescribed biologics. Rheumatology (Oxford) 2010;49:1563-9.
7. Cutolo M, Balleari E, Giusti M, Monachesi M, Accardo S. Sex hormone status of male patients with rheumatoid arthritis: evidence of low serum concentrations of testosterone at baseline and after human chorionic gonadotropin stimulation. Arthritis Rheum 1988;31:1314-7.
8. Spector TD, Ollier W, Perry LA, Silman AJ, Thompson PW, Edwards A. Free and serum testosterone levels in 276 males: a comparative study of rheumatoid arthritis, ankylosing spondylitis and healthy controls. Clin Rheumatol 1989;8:37-41.
9. Spector TD, Perry LA, Tubb G, Silman AJ, Huskisson EC. Low free testosterone levels in rheumatoid arthritis. Ann Rheum Dis 1988;47:65-8.
10. Tengstrand B, Carlstrom K, Hafstrom I. Gonadal hormones in men with rheumatoid arthritis--from onset through 2 years. J Rheumatol 2009;36:887-92.
11. Jones TH, Kennedy RL. Cytokines and hypothalamic-pituitary function. Cytokine 1993;5:531-8.
12. Heikkila R, Aho K, Heliovaara M, et al. Serum androgen-anabolic hormones and the risk of rheumatoid arthritis. Ann Rheum Dis 1998;57:281-5.
13. Uhlig T, Hagen KB, Kvien TK. Current tobacco smoking, formal education, and the risk of rheumatoid arthritis. J Rheumatol 1999;26:47-54.
14. Nilsson PM, Nilsson JA, Berglund G. Population-attributable risk of coronary heart disease risk factors during long-term follow-up: the Malmo Preventive Project. J Intern Med 2006;260:134-41.
15. Berglund G, Nilsson P, Eriksson KF, et al. Long-term outcome of the Malmo preventive project: mortality and cardiovascular morbidity. J Intern Med 2000;247:19-29.
16. Berglund G, Eriksson KF, Israelsson B, et al. Cardiovascular risk groups and mortality in an urban swedish male population: the Malmo Preventive Project. J Intern Med 1996;239:489-97.
17. Bergstrom U, Jacobsson LT, Nilsson JA, Berglund G, Turesson C. Pulmonary dysfunction, smoking, socioeconomic status and the risk of developing rheumatoid arthritis. Rheumatology (Oxford) 2011;50:2005-13.
18. Soderlin MK, Lindroth Y, Jacobsson LT. Trends in medication and health-related quality of life in a population-based rheumatoid arthritis register in Malmo, Sweden. Rheumatology (Oxford) 2007;46:1355-8.

19. Hekmat K, Jacobsson L, Nilsson JA, et al. Decrease in the incidence of total hip arthroplasties in patients with rheumatoid arthritis--results from a well defined population in south Sweden. Arthritis research & therapy 2011;13:R67.
20. Ludvigsson JF, Andersson E, Ekbom A, et al. External review and validation of the Swedish national inpatient register. BMC public health 2011;11:450.
21. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-24.
22. Nilsson PM, Nilsson JA, Ostergren PO, Berglund G. Social mobility, marital status, and mortality risk in an adult life course perspective: the Malmo Preventive Project. Scandinavian journal of public health 2005;33:412-23.
23. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab 1999;84:3666-72.
24. Maggio M, Basaria S, Ceda GP, et al. The relationship between testosterone and molecular markers of inflammation in older men. J Endocrinol Invest 2005;28:116-9.
25. Shiels MS, Rohrmann S, Menke A, et al. Association of cigarette smoking, alcohol consumption, and physical activity with sex steroid hormone levels in US men. Cancer Causes Control 2009;20:877-86.
26. Gautier A, Bonnet F, Dubois S, et al. Associations between visceral adipose tissue, inflammation and sex steroid concentrations in men. Clin Endocrinol (Oxf) 2012.
27. Wesley A, Bengtsson C, Elkan AC, Klareskog L, Alfredsson L, Wedren S. Association between overweight, obesity, ACPA positive and ACPA negative Rheumatoid Arthritis-, results from the EIRA case-control study. Arthritis Care Res (Hoboken) 2012.
28. Dalal M, Kim S, Voskuhl RR. Testosterone therapy ameliorates experimental autoimmune encephalomyelitis and induces a T helper 2 bias in the autoantigen-specific T lymphocyte response. J Immunol 1997;159:3-6.
29. Forslind K, Hafstrom I, Ahlmen M, Svensson B. Sex: a major predictor of remission in early rheumatoid arthritis? Ann Rheum Dis 2007;66:46-52.
30. Hall GM, Larbre JP, Spector TD, Perry LA, Da Silva JA. A randomized trial of testosterone therapy in males with rheumatoid arthritis. Br J Rheumatol 1996;35:568-73.
31. Cutolo M, Balleari E, Giusti M, Intra E, Accardo S. Androgen replacement therapy in male patients with rheumatoid arthritis. Arthritis Rheum 1991;34:1-5.
32. Cutolo M, Sulli A, Pizzorni C, Craviotto C, Straub RH. Hypothalamic-pituitary-adrenocortical and gonadal functions in rheumatoid arthritis. Ann N Y Acad Sci 2003;992:107-17.
33. Tengstrand B, Carlstrom K, Hafstrom I. Bioavailable testosterone in men with rheumatoid arthritis-high frequency of hypogonadism. Rheumatology (Oxford) 2002;41:285-9.
34. Tengstrand B, Carlstrom K, Fellander-Tsai L, Hafstrom I. Abnormal levels of serum dehydroepiandrosterone, estrone, and estradiol in men with rheumatoid arthritis: high correlation between serum estradiol and current degree of inflammation. J Rheumatol 2003;30:2338-43.
35. Mjaavatten MD, van der Heijde DM, Uhlig T, et al. Should anti-citrullinated protein antibody and rheumatoid factor status be reassessed during the first year of followup in recent-onset arthritis? A longitudinal study. J Rheumatol 2011;38:2336-41.

36. Oliver JE, Silman AJ. What epidemiology has told us about risk factors and aetiopathogenesis in rheumatic diseases. Arthritis research & therapy 2009;11:223.
37. Pikwer M, Bergstrom U, Nilsson JA, Jacobsson L, Berglund G, Turesson C. Breast feeding, but not use of oral contraceptives, is associated with a reduced risk of rheumatoid arthritis. Ann Rheum Dis 2009;68:526-30.