From THE INSTITUTE OF ENVIRONMENTAL MEDICINE,
UNIT OF CARDIOVASCULAR EPIDEMIOLOGY
Karolinska Institutet, Stockholm, Sweden

THE ASSOCIATION BETWEEN HORMONAL/REPRODUCTIVE FACTORS AND THE RISK OF DEVELOPING RHEUMATOID ARTHRITIS

Cecilia Orellana Pozo

Karolinska Institutet

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To my mother

To Rasmus and Sebastián

‘All species capable of grasping this fact manage better in the struggle for existence than those which rely upon their own strength alone: the wolf, which hunts in a pack, has a greater chance of survival than the lion, which hunts alone’.

- *Christian Lous Lange*

‘Always remember that striving and struggle precede success, even in the dictionary’.

- *Sarah Ban Breathnach*

The association between hormonal/reproductive factors and the risk of developing rheumatoid arthritis.
THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Cecilia Orellana Pozo

Principal Supervisor:
Camilla Bengtsson
Karolinska Institutet
Institute of Environmental Medicine
Unit of Cardiovascular Epidemiology

Co-supervisor(s):
Lars Alfredsson
Karolinska Institutet
Institute of Environmental Medicine
Unit of Cardiovascular Epidemiology

Lars Klareskog
Karolinska University Hospital
Department of Medicine
Rheumatology Unit

Elizabeth Karlson
Harvard University
Brigham and Women's Hospital
Department of Medicine

Opponent:
Professor Helena Forsblad d'Elia
Umeå University
Department of Public Health and Clinical Medicine
Division of Medicine

Examination Board:
Associate Professor Christopher Sjöwall
Linköping University
Department of Clinical and Experimental Medicine
Section of Rheumatology

Professor Jan Ernerudh
Linköping University
Department of Clinical and Experimental Medicine
Clinical Immunology

Assistant Professor Karin Modig
Karolinska Institutet
Institute of Environmental Medicine
Unit of Epidemiology

ABSTRACT

Rheumatoid arthritis (RA) is a chronic inflammatory disease which leads to joint damage and bone destruction, with a complex interplay of genetic and environmental factors involved in its etiology. RA is more common among women than men at all ages, but the gender difference seems to be highest before menopause. It has been hypothesized that changes in female hormonal levels might have a role in RA pathogenesis. The overall aim of this thesis was to study the association between hormonal/reproductive factors and the risk of RA and to determine whether these factors were differently associated with serological phenotypes of the disease (according to the presence/absence of anti-citrullinated peptides antibodies (ACPA) and rheumatoid factor (RF)).

This thesis is based on information from two large studies. Three articles were based on the Swedish Epidemiological Investigation of Rheumatoid Arthritis (EIRA), a population-based case-control study comprising incident RA cases. The study population were people aged 18 and above, living in diverse geographical parts of Sweden from 1996. Controls were randomly selected from the population register and matched to the cases by age, sex and residential area. Cases and controls completed an extensive questionnaire, collecting information about life-style/environmental exposures. One article was based on the Nurses' Health Study (NHS), which consists of two prospective cohorts of female nurses in the USA. Data collection started in 1976 (women aged 30-55 years) and 1989 (women aged 25-42 years). Both cohorts of the NHS were followed via biennial questionnaires about diseases, lifestyle and health practices.

According to our results, parous women had an increased risk of ACPA-negative RA compared with nulliparous women, aged 18-44 years. The increased risk was attributable to an elevated risk during the postpartum period, and to a young age at first birth. Older age at first birth seemed to be associated with a decreased risk of ACPA-positive RA. Parous women who breastfed for more than a year had a decreased risk of ACPA-positive RA compared with parous women who breastfed for up to 6 months. This decreased risk was non-significant after adjustment for smoking. Ever oral contraceptive use was significantly associated with a decreased risk of ACPA-positive RA, while a longer duration of use was significantly associated with a decreased risk for both RA subsets. Postmenopausal women had an increased risk of seronegative RA, but they had no association with the onset of seropositive RA. Women with a long duration of postmenopausal hormone therapy (PMH) had an increased risk of seropositive RA in the NHS. Finally, in the EIRA study, postmenopausal women who were currently using PMH at onset of their disease had a decreased risk of ACPA-positive RA. This decreased risk was mainly observed among women aged 50-59 years, with a short duration of use (<7 years), and only among users of a combined therapy of estrogen and progestogens.

Further research is required to explore the biological mechanisms behind our findings, but our results contribute to the knowledge of hormonal/reproductive factors, and their impact on the serological phenotypes of RA.

LIST OF SCIENTIFIC PAPERS

I. **Orellana C**, Wedrén S, Källberg H, Holmqvist M, Karlson EW, Alfredsson L, Bengtsson C; EIRA Study Group.
Parity and the risk of developing rheumatoid arthritis: results from the Swedish Epidemiological Investigation of Rheumatoid Arthritis study.
Ann Rheum Dis. 2014 Apr;73(4):752-5.

II. **Orellana C**, Saevarsdottir S, Klareskog L, Karlson EW, Alfredsson L, Bengtsson C.
Breastfeeding, oral contraceptives and the risk of developing rheumatoid arthritis: results from the Swedish EIRA study.

III. Bengtsson C, Malspeis S, **Orellana C**, Sparks JA, Costenbader K, Karlson EW.
Menopausal factors are associated with seronegative RA in large prospective cohorts: results from the Nurses' Health Studies.

IV. **Orellana C**, Saevarsdottir S, Klareskog L, Karlson EW, Alfredsson L, Bengtsson C.
Postmenopausal hormone therapy and the risk of rheumatoid arthritis: results from the Swedish EIRA population-based case-control study.
Eur J Epidemiol. 2015 May;30(5):449-57.

CONTENTS

LIST OF ABBREVIATIONS

ACPA	Anti-citrullinated Protein Antibodies
ACR	American College of Rheumatology
ATC	Anatomical Therapeutic Chemical classification system
BF	Breastfeeding
BMI	Body Mass Index
CI	Confidence Interval
CRP	C-reactive protein
CSQ	Connective Tissue Disease Screening Questionnaire
EIRA	Epidemiological Investigation of Rheumatoid Arthritis
ERS	Erythrocyte Sedimentation Rate
EULAR	European League Against Rheumatism
HLA	Human Leukocyte Antigen
HR	Hazard Ratio
NHS	Nurses' Health Study
OC	Oral Contraceptive
OR	Odds Ratio
PMH	Postmenopausal Hormone Therapy
RA	Rheumatoid Arthritis
RF	Rheumatoid Factor
SAS	Statistical Analysis System
SE	Shared Epitope

1 INTRODUCTION

1.1 RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is one of the most common autoimmune diseases, a criteria-based syndrome with a multifactorial etiology. It is characterized by symmetrical inflammation of the small joints and eventual bone destruction. [1] The introduction of biological treatment, which has replaced the classical disease modifying anti-rheumatic drugs, has greatly improved the management of the disease. [2] Nevertheless, RA remains an important chronic disorder, with reduced life expectancy and increased mortality from infections and cardiovascular and respiratory diseases. [3]

The occurrence of RA seems to have important geographical variation. The median annual incidence has been found to range from 16.5 in southern European countries, to 29 in northern European countries, and up to 38 cases per 100,000 in North America. [4] Using data from a nationwide register-based study in Sweden, the incidence of RA was estimated to be 41 per 100,000 (56 and 25 per 100,000 for women and men respectively). [5]

RA is two to three times more common among women, with an estimated disease prevalence of 2.0-2.7 percent in women above 60 years of age. [6] A higher incidence of RA is seen among women compared to men across all ages, [7-9] but the difference is greater during the reproductive years. [7, 8] The highest incidence among women has been reported between 55-64 years of age, during the peri- or postmenopausal stages. [7, 9] These gender differences have led to the hypothesis that hormonal factors are important in disease development.

1.2 RISK FACTORS FOR RA

1.2.1 Autoantibodies in RA

Autoantibodies, mostly detected in the serum, are useful in the diagnosis, prognosis and follow-up of patients with rheumatic diseases. [10]

Rheumatoid factor (RF) can be found in approximately 75% of RA patients, but its specificity is limited as it can also be present in patients with other autoimmune diseases (e.g. Sjögren's syndrome), infectious diseases, and even in healthy population. The presence of RF has been widely used as a diagnostic marker of RA in spite of its low specificity, [11] and was part of the 1987 American College of Rheumatology (ACR) criteria for RA diagnosis (Table 1). [12]

Table 1. The 1987 revised criteria for the classification of rheumatoid arthritis* [12]

1. Morning stiffness
2. Arthritis of 3 or more joint areas
3. Arthritis of hand joints
4. Symmetric arthritis
5. Rheumatoid nodules
6. Serum rheumatoid factor
7. Radiographic changes

* The patient should satisfy at least 4 of these 7 criteria to be classified as an RA case

Anti-citrullinated protein antibodies (ACPA) are among the latest markers for the diagnosis of RA, showing a higher specificity than the classic RF. [13] Moreover, emerging data suggest that ACPAs would be able to predict the development of early or undifferentiated RA, the severity in established RA, and the onset of RA in certain high-risk populations. [14] In 2010, new criteria were introduced which included ACPA-status, together with RF (Table 2). [15] RF and ACPAs overlap to a considerable extent. [16, 17]

Table 2. The 2010 ACR/EULAR European League against Rheumatism classification criteria for rheumatoid arthritis [15]

Classification criteria for RA (score-based algorithm: add scores of categories A-D; a score of ≥6/10 is needed for classification of patient as having definite RA	Score
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 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

ACR= American college of Rheumatology; EULAR= European League against Rheumatism; CRP=C-reactive protein, ESR=erythrocyte sedimentation rate

According to emerging evidence, ACPA-positive and ACPA-negative RA have different environmental and genetic risk factors; this supports the notion of RA as two different disease entities with different/distinct etiologies. Few risk factors have been identified for the ACPA-negative subgroup of RA, except for obesity. [18, 19]

1.2.2 Genetic risk factors

Twin studies in RA have demonstrated a low concordance in monozygotic twins, ranging between 12% and 30%. [20-23] This suggests that environmental factors play a fundamental role in the etiology of the disease.

Genetic risk factors for RA include the human leukocyte antigen (HLA) region, specifically the *HLA-DRB1* shared epitope (SE) alleles, and the PTPN22 gene. It is interesting that these genetic factors have been mainly associated with the risk of ACPA-positive RA. [24-26]

1.2.3 Environmental risk factors

Apart from the well-described association between smoking and increased risk of developing RA, [17, 27-29] several other environmental exposures have been explored with regard to the risk of developing RA, including for example alcohol consumption, [30, 31] body mass index (BMI), [18, 32, 33] and exposure to silica. [34-36] The striking gender difference in the occurrence of disease has led to the hypothesis that hormonal/reproductive factors are involved in the etiology of RA. Several studies have investigated these factors, especially oral contraceptive (OC) use; however it is notable that most have not taken into consideration ACPA-status or genetic factors.

1.2.3.1 Parity

The gender difference in RA incidence seems to be higher during the reproductive years, with a female/male ratio of 3-6:1. [7, 8] In pre-established RA an amelioration of symptoms during pregnancy followed by a postpartum flare has been well described, [37-39] suggesting an involvement of reproductive factors in the etiology. A decreased RA incidence has been observed during pregnancy, followed by an increase after delivery. The increased risk of RA has been observed during the first three months up to 2 years postpartum, [40-42] while parous women seem to have no increased, [43-46] or even decreased risk of RA in the long term. [47-49] With regard to the effect of number of children [43-45, 47, 49] and age at first birth on RA risk, [43-45, 47, 48] no consensus has been reached.

1.2.3.2 Breastfeeding and OC use

Breastfeeding (BF) has been associated with a decreased risk of RA, [44, 46, 50] with the strongest association among those with a long history of BF. [44] Other studies, however, have reported an elevated disease risk. [51, 52] A recent meta-analysis showed a relative risk of 0.68 (95% CI 0.49-0.92) based on data from six published studies. [44, 46, 50, 51, 53, 54] The different impact of BF on the subsets of RA has not been investigated.

No association between OC use and the risk of RA could be demonstrated in the majority of studies, [42, 44, 46, 49, 51, 55-59] including two recent meta-analyses. [60, 61] A few studies have shown an inverse association, [62-67] including one study in which a larger effect with a longer duration of use was demonstrated. [51] To our knowledge, no study has included ACPAs in a stratified manner but only as a confounder, when investigating the association between OC use and RA.

1.2.3.3 Menopausal factors

It has been suggested that the menopausal transition, a time with notorious hormonal changes, might be involved in RA pathogenesis though the evidence is limited. [68] An early menopause (<45 years of age) has been associated with an increased risk of RA, which was more pronounced for seronegative RA than for seropositive RA. [69] As with other environmental factors, menopausal factors may also be associated differently with the two subgroups of disease, but most previous studies were conducted without stratification into seronegative/seropositive RA phenotypes.

1.2.3.4 Postmenopausal hormone therapy

The use of postmenopausal hormone therapy (PMH) for menopause-related symptoms in relation to RA risk has been explored in several studies, most of them showing no association [44, 59, 62, 70-76] while a few have reported an increased [57] or decreased risk of developing RA. [77, 78] In one study, findings indicated that the use of PMH among women carrying the HLA-DRB1 SE alleles may protect against the development of ACR criteria-defined RA in a population with early undifferentiated arthritis, and that this protection is associated with a reduction in ACPA levels. [77] Nevertheless, to our knowledge, no study has investigated the association between PMH and the risk of ACPA-positive as compared to ACPA-negative RA in a setting in which exposure to PMH was ascertained in a healthy population.

2 AIMS

2.1 GENERAL AIM

The general aim of this thesis was to study the association between hormonal/reproductive factors and the risk of developing RA among women. A further aim was to determine whether these factors were differently associated with serological phenotypes of the disease (ACPA-positive/-negative RA and RF-positive/-negative RA). Specific factors of interest were investigated in four separate studies (Papers I-IV).

2.2 SPECIFIC AIMS

- **Paper I:** To study the association between parity and the risk of developing RA.
- **Paper II:** To study the association between both BF history and OC use and the risk of developing RA.
- **Paper III:** To investigate whether menopausal factors are associated with subsequent development of serological RA phenotypes.
- **Paper IV:** To study the association between use of PMH and the risk of developing RA.

3 MATERIALS AND METHODS

3.1 STUDY BASE

Three out of four studies included in this thesis (Papers I, II and IV) were based on the Swedish Epidemiological Investigation of Rheumatoid Arthritis (EIRA), a population-based case-control study comprising the population, aged 18 years and above, living in central and southern Sweden. Data collection started in 1996 and is still on-going. So far, 3724 cases and 5935 controls have participated in EIRA; of these, 2809 cases and 4250 controls are women (data until September 2014). The participation rate among women for the complete study period is 95% among cases and 80% among controls. The observation periods ended in 2009 (Paper I), 2011 (Paper IV) and 2014 (Paper II).

For one of the studies (Paper III) we utilized data from the Nurse's Health study (NHS), which consists of two prospective cohorts. Data collection in the first cohort (NHS) started in 1976 and included 121,700 female nurses, aged 30-55 years, and followed through 2010. The second cohort (NHSII) started in 1989 and included 116,430 female nurses, who were younger at baseline (aged 25-42 years, born between 1947 and 1964) and followed through 2011. In total, 1096 incident RA cases have so far been identified.

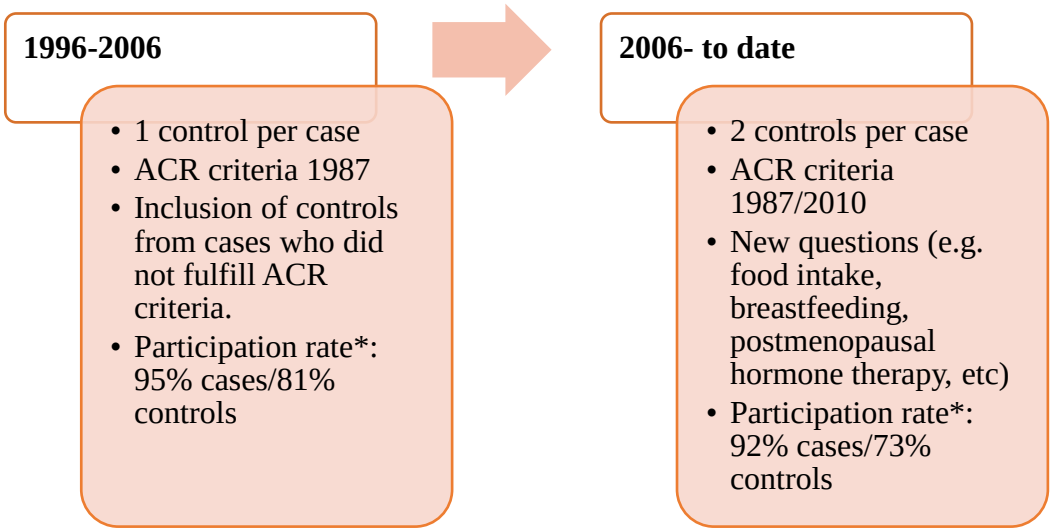
3.2 STUDY DESIGN

3.2.1 Case identification and selection of controls

Papers I, II and IV: Incident cases were diagnosed by rheumatologists according to the 1987 ACR criteria for RA. [12] During the conduction of the studies included in this thesis, new criteria for case diagnosis were published in 2010; however, only a small fraction of cases in our studies (Papers II and IV) have been diagnosed with only these more recent criteria [15] and not with the original criteria. [12]

At the beginning of the EIRA study (1996-2006), one control was selected for each case, matched by sex, age and residential area. In a second phase (2006 to the present), two controls were selected for each case (Figure 1). Controls were randomly selected from the population, using the national population register which is continuously updated and covers the total population in Sweden. All study subjects were required to speak in Swedish. If a selected control could not be contacted or refused to participate, another control was invited to participate. At an early stage, some cases not fulfilling the ACR criteria were included with the purpose of investigating undifferentiated arthritis. Although these cases were eventually excluded, their controls were still included in order to increase power.

Figure 1. Phases of the EIRA study over time.



* Overall participation rate for men and women combined.
EIRA=Epidemiological Investigation of Rheumatoid Arthritis.

Paper III: The identification of RA cases has previously been described in detail. [44] Briefly, case identification was a two-stage procedure in which a Connective Tissue Disease Screening Questionnaire (CSQ) [79] was sent to individuals with a physician's diagnosis of RA based on self-reported information. The medical records of those who screened positive

were reviewed by two board-certified rheumatologists in order to confirm RA according to the 1987 ACR classification criteria. [12]

3.2.2 Data collection

For the EIRA study, an identical questionnaire was given to the cases shortly after diagnosis and sent to the controls by mail, in order to collect information on a broad range of environmental and life-style factors. In 2006, a new version of the questionnaire was released, including new questions for example on food intake (Figure 1). Participating cases and controls were also asked to provide a blood sample for serological and genetic analyses.

Both cohorts of the NHS were followed via biennial questionnaires regarding diseases, lifestyle and health practices. The participation rate has been high with <10% of cases lost to follow-up. [80] The questions on hormonal/reproductive factors are extensive and include OC use, menopausal status, parity, miscarriage, age at menarche and regularity of menses, among many others. Participants were asked to provide blood samples for serological analyses.

3.2.3 Serological analyses

Papers I, II and IV: Blood samples were assayed for ACPA-status using the Immunoscan-RA Mark2 ELISA test (Euro-Diagnostica, Malmö, Sweden). [81, 82] The cut-off value for ACPA-positive RA was 25 U/ml. Cases that lacked information on ACPA-status were excluded from the analyses (28 and five cases for Papers I and IV respectively; for Paper II, 35 and 13 cases were excluded for OC use and BF analyses respectively).

Paper III: Information on RF and ACPA (available since 1990) was collected from medical records reviewed from the date of RA diagnosis. The DIASTAT CCP (Axis-Shield Diagnostics, Dundee, UK) second-generation test, a semiquantitative/qualitative ELISA, was used for the detection of ACPAs. [83] A titer >5 U/ml was considered positive according to the manufacturer's established threshold. Seropositive RA was then defined as RF-positive *or* ACPA-positive, while seronegative RA was defined as RF-negative *and* ACPA-negative.

3.3 ENVIRONMENTAL EXPOSURES AND THE RISK OF RA

Papers I, II and IV: For each case, the year when the first symptoms of RA occurred was defined as the index-year and the same index-year was used for the corresponding control.

3.3.1 Parity

Parous women were defined as those who had given birth before or during the index-year. Women who had not given birth before or during the index-year were considered nulliparous. The postpartum period was defined as 0 (if both childbirth and RA onset occurred during the index-year), 1, or 2 (if there were 1 year or 2 years, respectively, between the most recently born child and the index-year).

Age at first birth was obtained for women in their reproductive years (aged 18-44 years) and was categorized according to the quartiles among the controls (≤22, 23-26, 27-30 and ≥31 years). The number of children was categorized as 1, 2, 3 and ≥4.

3.3.2 BF and OC use

Total BF history among parous women was calculated as the sum of the duration of BF for each delivered child and categorized as 0-6, 7-12 and ≥13 months. Parous women who did not breastfeed (two cases and 14 controls) were included in the reference category.

'Current users' of OCs were defined as those who were currently using OCs during the index-year and started at least 1 year before symptom onset. A total of four cases and seven controls had started using OCs during the index-year and they were excluded from the analyses. 'Past users' were defined as those who used OCs in the past and had stopped at least the year before the index-year. 'Ever users' were defined as current and past users while 'never users' included women who had not used OCs before the index-year.

3.3.3 Menopausal factors

In both cohorts, participants were asked as part of each questionnaire (until 2002 in NHS) whether their menstrual periods had ceased permanently and, if so, at what age and the type of menopause they had experienced (natural, radiation-induced, or surgical). Menopausal status was categorized into premenopausal, postmenopausal or unclear. Age at menopause was categorized as ≤44 years, 45-49 years, ≥50 years. We further stratified type of menopause at different ages into the following categories: natural ≤44 years, natural ≥45 years, surgical ≤44 years, surgical ≥45 years, and missing. Finally, after excluding women who had undergone hysterectomy and removal of one ovary (as their age at menopause is unknown), we calculated total ovulatory years as the age at natural menopause or age at surgical menopause (if both ovaries were removed), subtracting the age at menarche, number of children born (12 months each), and years of OC use. Ovulatory years were then categorized as <24 years, 24-29 years, 30-34 years, ≥35 years, and missing.

In each cohort, information on PMH use was collected at baseline and at each biennial questionnaire. In the analyses of PMH, we investigated never/past/current PMH use, age at initiation (never, ≤44 years, 45-49 years, ≥50 years, and missing), and total duration of PMH use (never, <4 years, 4 to ≤8 years, ≥8 years, missing). We analyzed PMH use only among postmenopausal women.

3.3.4 PMH

The questions regarding PMH use included the type of medication and the time (years) of initiation and end of the therapy. Medications were later coded according to the Anatomical Therapeutic Chemical (ATC) classification system [84] and classified as estrogen alone or a combination of estrogen plus progestogen. The latter group represents a broad classification

including the natural hormone, progesterone, and the synthetic form, progestin and included both combined and sequential regimens.

3.4 CONFOUNDING FACTORS

For all of the studies including EIRA data (Papers I, II and IV) we adjusted for the matching variables (age and residential area). Although sex is an important matching variable in EIRA, we did not include it as all of our analyses were restricted to women. We performed additional adjustments for potential confounders for each one of the four studies, as follows:

Cigarette smoking, BMI, level of education (university degree yes/no), BF and OC use for all studies including EIRA material (Papers I, II and IV); parity, number of children, age at menarche, age at first birth and post-partum period for papers II and IV; and menopausal status, PMH and alcohol consumption for Paper II. Of these, only pack-years of cigarette smoking (0 to <10, ≥10 to <20 and ≥20) affected the estimates and were included in adjusted models.

For the NHS (Paper III), hazard ratios (HRs) were adjusted in a multivariate analysis for age (updated at each cycle of the NHS questionnaire), questionnaire cycle, median household income in quintiles, BMI, pack-years of cigarette smoking, and parity/BF (nulliparous, none to <1 month, 1-11 months, and ≥12 months). Additional variables, including alcohol consumption, OC use, age at menarche, and irregular menses, were considered as potential confounders, but because they did not substantially alter the hazard ratio estimates they were not included in the final models.

3.5 STATISTICAL ANALYSIS

Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated for ACPA-positive and ACPA-negative RA, by means of unconditional logistic regression for all studies including EIRA material (Papers I, II and IV). We conducted both unmatched and matched analyses (unconditional/conditional logistic regression) but only presented unconditional results as they were in close agreement with the conditional analyses, but had a higher precision. All analyses were carried out using the Statistical Analysis System (SAS).

3.5.1 Paper I

Parous women were compared with nulliparous in different age groups. We also investigated the effect of number of children, age at first birth and postpartum period on RA risk.

3.5.2 Paper II

Total lifetime duration of BF for 7-12 and ≥13 months were compared with the shortest duration of BF (0-6 months).

We also explored the effect of BF according to number of children breastfed (one, two and three or more). With regard to OC use, current/past/ever users were compared with never users. A short (≤7 years) or long (>7 years) duration of OC use was compared with no use at all (never OC users).

3.5.3 Paper III

The relative risks of three outcomes, seropositive, seronegative and overall RA, were analyzed by calculating the incidence rate ratios in different age-groups compared with women aged 25-44 years.

Cox proportional hazards models were used to obtain HRs with 95% CIs of seropositive or seronegative RA associated with each factor in separate models including menopausal status, age at menopause, type of menopause, and ovulatory years. We censored women at first self-report of cancer, RA, or other connective tissue disease if not confirmed as RA, as well as RA diagnosis or death, whichever came first. Premenopausal women were considered as the reference group, except in the analysis of ovulatory years (reference group: <24 years).

We further analyzed risk of RA according to PMH among only postmenopausal women in separate models including current/past PMH use, age at initiation and duration of PMH, with never users as the reference category. The analyses were performed separately for NHS and NHSII and pooled by meta-analysis using the random effects methods of DerSimonian and Laird. [85] Two-sided p-values <0.05 were considered statistically significant. All analyses were performed using SAS version 9.3.

3.5.4 Paper IV

Current, past and ever PMH users were compared with never users. We also analyzed different age groups, duration of PMH use (1-6 and ≥7 years) and type of preparation.

Table 3. Overview of pepers included in this thesis

Paper	**I**	**II**	**III**	**IV**
Short title	**Parity and risk of RA**	**BF, OC and risk of RA**	**Menopausal factors and risk of RA**	**PMH and risk of RA**
Study design	Case-control	Case-control	Cohort	Case-control
Study population	2035 cases, 2911 controls. Women aged 18-70 years, living in Sweden, between 1996 and 2009	2641 cases, 4251 controls, between 1996 and 2014 (OC use). 884 cases, 1949 controls, between 2006 and 2014 (BF)	1,096 incident RA cases. In NHS 120,700 female nurses aged 30-55 (1976-2010) and in NHSII 116,430 female nurses aged 25-42 (1989-2011) were followed	523 cases, 1057 controls. Women aged 50-70 years, living in Sweden, between 2006 and 2011
Main exposures	Parity, postpartum period, age at first birth	BF (time in months), oral contraceptive use (duration)	Menopausal factors (menopausal status, age at menopause, type of menopause, ovulatory years and PMH use)	Postmenopausal hormone therapy (type of therapy and duration)
Main outcome	ACPA+/- RA	ACPA+/- RA	Seropositive/seronegative RA	ACPA+/- RA
Potential confounders	Matching variables (age and residential area). Additional adjustments: smoking (pack-years), BMI, oral contraceptive use, breastfeeding and university degree	Matching variables (age and residential area) and smoking (pack-years). Additional adjustments: parity, number of children, BMI, menopausal status, PMH use, age at menarche, age at first birth, postpartum period, alcohol consumption and university degree.	Age, questionnaire cycle, median household income in quintiles, BMI, smoking (pack-years), parity/BF, alcohol consumption, oral contraceptive use, age at menarche, and irregular menses	Matching variables (age and residential area) and smoking (pack-years). Additional adjustments: parity, number of children, BMI, OC use, BF, age at menarche, age at first birth, postpartum period, and university degree
Statistical method	Logistic regression	Logistic regression	Cox proportional hazards models	Logistic regression

RA=rheumatoid arthritis, BF=breastfeeding, OC= oral contraceptive, PMH=postmenopausal hormone therapy, ACPA=anti-citrullinated protein antibody, BMI=body mass index, NHS=Nurses' Health Study, NHSII=second cohort of the NHS.

4 RESULTS

A concise overview of the most important findings of this thesis will be provided in this section. For further details, please see the individual publications included at the end of the thesis.

4.1 PARITY AND THE RISK OF RA (PAPER I)

In total, 2035 cases and 2911 controls were included in the analyses; of these, 603 cases and 906 controls were aged 18-44 years. In all, 64% of cases were ACPA-positive and the mean time period between symptom onset and diagnosis was 10 months for both ACPA-positive and ACPA-negative RA cases.

4.1.1 Parity and the risk of ACPA-positive/-negative RA

Parous women had an increased risk of developing ACPA-negative RA compared with nulliparous women in the younger age-group (18-44 years) (OR=2.1, 95% CI 1.4-3.2), but not in the older age-group (45-70) (OR=0.9, 95% CI 0.7-1.3). There was no association between parity and the risk of developing ACPA-positive RA in either age-group (Table 4) nor were there any differences in the risk of ACPA-positive and ACPA-negative RA according to the number of children.

Table 4. Relative risk of ACPA-positive and ACPA-negative RA according to parity, in different age-groups. EIRA, Sweden, 1996-2006

		18-44 years		45-70 years	
ACPA status	Parous	Cases/Controls	OR[a] 95% CI	Cases/Controls	OR[a] 95% CI
ACPA-positive	No	165/360	1.0	112/238	1.0
	Yes	237/546	0.9 (0.7-1.2)	797/1766	1.0 (0.8-1.2)
ACPA-negative	No	65/360	1.0	65/238	1.0
	Yes	136/546	2.1 (1.4-3.2)	458/1766	0.9 (0.7-1.3)
RA overall	No	230/360	1.0	177/238	1.0
	Yes	373/546	1.1 (0.9-1.5)	1255/1766	1.0 (0.8-1.2)

ACPA= anti-citrullinated protein antibodies, RA= rheumatoid arthritis, OR= odds ratio, CI= confidence interval, EIRA=Epidemiological Investigation of Rheumatoid Arthritis.
[a] Adjusted for matching variables (age and residential area).

4.1.2 Postpartum period and risk of ACPA-positive/-negative RA

An increased risk of ACPA-negative RA was found in women, aged 18-44 years, who had their last child the same year as the index-year (OR=2.6, 95% CI 1.4-4.8). The OR was lower among those whose last child was born within 1 year before the index-year (OR=1.8, 95% CI 0.9-3.6) and reached the null value within 2 years before disease onset (Table 5). The estimates decreased after adjustment for age at first birth.

Table 5. Relative risk of ACPA-positive and ACPA-negative RA according to postpartum period for the last delivered child in women aged 18-44 years. EIRA, Sweden, 1996-2006

ACPA status	Years between last delivered child and index-year	Cases/Controls	OR[a] 95% CI	OR[b] 95% CI
ACPA-positive	Nulliparous	165/360	1.0	1.0
	0	29/59	1.1 (0.7-1.8)	0.8 (0.4-1.6)
	1 year	27/57	1.1 (0.6-1.8)	0.8 (0.4-1.5)
	2 years	20/49	0.9 (0.5-1.5)	0.6 (0.3-1.3)
	p for trend[c]	-	0.9136	0.6316
ACPA-negative	Nulliparous	65/360	1.0	1.0
	0	23/59	2.6 (1.4-4.8)	2.1 (0.9-4.8)
	1 year	14/57	1.8 (0.9-3.6)	1.4 (0.6-3.6)
	2 years	6/49	1.0 (0.4-2.5)	0.8 (0.2-2.3)
	p for trend[c]	-	0.0093	0.1336

ACPA= anti-citrullinated protein antibodies, RA= rheumatoid arthritis,
OR= odds ratio, EIRA=Epidemiological Investigation of Rheumatoid Arthritis.
[a] Adjusted for matching variables (age and residential area).
[b] Adjusted for age, residential area and age at first birth.
[c] Wald chi-squared test for trend.

4.1.3 Age at first birth and risk of ACPA-positive/-negative RA

Among women aged 18-44 years who had their first child before 23 years of age the OR of ACPA-negative RA was 2.5 (95% CI 1.5-4.1). The OR decreased by increasing age at first birth. A moderately decreased risk of ACPA-positive RA was found among women who had their first child after 30 years of age (OR=0.7, 95% CI 0.4-1.0) Adjustment for the postpartum period increased the risk estimates for ACPA-negative RA, and decreased the estimates for ACPA-positive RA.

4.2 BF, OC USE AND THE RISK OF RA (PAPER II)

In total, 2637 cases and 4244 controls were included in the analyses. In all, 1753 (66.5%) cases were ACPA-positive and the mean time period between symptom onset and diagnosis was 10 months for both serological phenotypes of RA.

4.2.1 BF and the risk of RA

Compared with women who breastfed for 0-6 months, those who breastfed their children for 7-12 months had an OR of 0.93 (95% CI 0.75-1.14) of developing RA, while BF for 13 months or more significantly reduced the risk of RA (OR=0.77, 95% CI 0.63-0.94). The trend was significant for ACPA-positive, but not for ACPA-negative RA. These estimates were attenuated after adjustment for pack-years of smoking (Table 6).

Table 6. Relative risk of ACPA-positive, ACPA-negative and RA overall according to breastfeeding. EIRA, Sweden, 2006-2014

ACPA-status	Breastfeeding	Cases/Controls	OR (95% CI)[a]	OR (95% CI)[b]
ACPA-positive	≤6 months	194/533	1.0	1.0
	7-12 months	192/574	0.91 (0.72-1.15)	0.99 (0.78-1.26)
	≥13 months	234/842	0.74 (0.59-0.93)	0.88 (0.70-1.11)
	p-value trend	-	0.0086	0.2644
ACPA-negative	≤6 months	81/533	1.0	1.0
	7-12 months	83/574	0.97 (0.70-1.35)	1.02 (0.73-1.43)
	≥13 months	100/842	0.83 (0.60-1.15)	0.92 (0.66-1.28)
	p-value trend	-	0.2405	0.5951
RA overall	≤6 months	275/533	1.0	1.0
	7-12 months	275/574	0.93 (0.75-1.14)	0.99 (0.80-1.22)
	≥13 months	334/842	0.77 (0.63-0.94)	0.89 (0.72-1.09)
	p-value trend	-	0.0075	0.2366

[a] Adjusted for age and residential area

[b] Adjusted for age, residential area and smoking (pack-years)

ACPA= anti-citrullinated protein antibodies, RA= rheumatoid arthritis, OR= odds ratio, CI= confidence interval, EIRA=Epidemiological Investigation of Rheumatoid Arthritis.

4.2.2 RA risk according to number of children breastfed

Among women who only breastfed one child, we observed a non-significant decreased risk of developing RA overall (OR=0.86, 95% CI 0.57-1.29), and especially of ACPA-negative RA.

This decrease in risk was not as large among women who breastfed a total of two children (OR=0.91, 95% CI 0.71-1.71), almost reaching the null value among those who breastfed three or more children (OR=1.08, 95% CI 0.78-1.49).

4.2.3 OC use and the risk of RA

Ever users of OCs had a decreased risk of developing RA compared with never users (OR=0.88, 95% CI 0.79-0.98). The ORs were 0.86 (95% CI 0.69-1.07) and 0.88 (95% CI 0.80-0.98) for current and past users respectively. The association between ever and past OC use was significant for ACPA-positive but not for ACPA-negative RA. The estimates for ever and past OC use remained significant after adjustment for smoking (pack-years) (Table 7).

A longer duration of ever OC use (>7 years) was significantly associated with a decreased risk of RA overall (OR=0.83, 95% CI 0.73-0.94) and ACPA-positive RA (OR=0.82, 95% CI 0.71-0.95), while a non-significant association was found for ACPA-negative RA (OR=0.83, 95% CI 0.69-1.01).

Table 7. Relative risk of ACPA-positive, ACPA-negative and RA overall according to oral contraceptive use among women. EIRA, Sweden, 1996-2014

ACPA status	OC use	Cases/Controls	OR 95% CI[a]	OR 95% CI[b]
ACPA-positive	Ever	1135/2862	0.85 (0.75-0.96)	0.81 (0.71-0.92)
	Current	134/331	0.87 (0.68-1.12)	0.85 (0.66-1.10)
	Past	1001/2531	0.85 (0.75-.096)	0.80 (0.70-0.91)
	Never	572/1267	1.0	1.0
	Missing	46/115	-	-
ACPA-negative	Ever	582/2862	0.94 (0.80-1.11)	0.91 (0.77-1.07)
	Current	61/331	0.83 (0.59-1.17)	0.78 (0.55-1.11)
	Past	521/2531	0.95 (0.81-1.12)	0.92 (0.78-1.09)
	Never	289/1267	1.0	1.0
	Missing	13/115	-	-
RA overall	Ever	1717/2862	0.88 (0.79-0.98)	0.84 (0.75-0.94)
	Current	195/331	0.86 (0.69-1.07)	0.83 (0.67-1.04)
	Past	1522/2531	0.88 (0.80-0.98)	0.84 (0.75-0.94)
	Never	861/1267	1.0	1.0
	Missing	59/115	-	-

[a] Adjusted for age and residential area

[b] Adjusted for age, residential area and smoking (pack-years)

ACPA= anti-citrullinated protein antibodies, RA= rheumatoid arthritis, OC= oral contraceptives, OR= odds ratio, CI= confidence interval, EIRA=Epidemiological Investigation of Rheumatoid Arthritis.

4.3 MENOPAUSAL FACTORS AND THE RISK OF RA (PAPER III)

The study population for Paper III was 109,443 women contributing 2,498,323 person-years in NHS from 1976-2010, and 112,523 women contributing 1,987,756 person-years in NHSII from 1989-2011. A total of 1,096 cases were included in the analyses (729 in NHS, 367 in NHSII; 401 seronegative/695 seropositive cases).

4.3.1 Age and risk of RA

Women aged 45 years or older had an increased risk of RA in all age-groups, compared with women aged 25-44 years, with peak HR at 55-59 years. For all RA, the pooled HRs were 1.5 (95% CI 1.2-1.9) at ages 45-49 years, 2.0 (95% CI 1.6-2.5) at ages 50-54 years, 2.3 (95% CI 1.7-3.2) at ages 55-59 years and 1.9 (95% CI 1.4-2.6) at ages 60-64 years. There were no large differences between NHS and NHSII; however there were very few cases aged 60 years or greater in NHSII. For seronegative RA, the pattern was similar, with a peak HR at ages 55-59 in the pooled analysis. Women aged 50 or more had an increased risk of seropositive RA, with peak HR at ages 55-59.

4.3.2 Menopausal factors and risk of seropositive/seronegative RA

Postmenopausal women had an increased risk of seronegative RA, compared with premenopausal women, in a multivariate analysis (pooled HR=2.1, 95% CI 1.4-3.0) (Table 8). Any age at menopause was associated with an increased risk of seronegative RA, with the highest HR observed among women with natural menopause at early age (<45 years) (pooled HR=2.4, 95% CI 1.5-4.0). Longer duration of ovulatory years appeared to be associated with a decreased risk, at least in NHSII. None of the menopausal factors were significantly associated with seropositive RA (for postmenopausal women compared with premenopausal women, pooled HR=1.2, 95% CI 0.9-1.6).

4.3.3 PMH use and risk of seropositive/seronegative RA

Current PMH use was modestly associated with a non-significantly increased risk of seronegative RA (pooled HR=1.3, 95% CI 0.9-1.8), while there was no association with past PMH use. Long duration of PMH use (≥8 years) was related to a non-significantly increased risk of seronegative RA (pooled HR=1.4, 95% CI 1.0-2.0), but age at PMH initiation or time since last use were not associated with this sub-group of disease.

Regarding seropositive RA, current PMH users had an increased risk only in NHS (HR=1.4, 95% CI 1.1-1.9), but not in NHSII (HR=0.9, 95% CI 0.5-1.7) with a pooled HR of 1.3 (95% CI 0.9-1.8). There was no association with past PMH use in either cohort. Long duration of PMH use (≥8 years) was significantly associated with risk of seropositive RA (pooled HR=1.4, 95% CI 1.1-1.9). However, age at initiation of PMH was not associated with either type of RA.

Table 8. Menopausal status and the relative risk of seropositive RA and seronegative RA in the NHS (1976-2010) and NHSII (1989-2011) cohorts

	Seronegative RA						
	NHS			NHSII			Pooled (NHS+NHSII)
Factors	Cases	Person-years	HR 95%CI[1]	Cases	Person-years	HR 95%CI[1]	HR 95%CI[1]
Menopausal status							
Pre-menopausal	52	755,275	1.0	71	1479200	1.0	1.0
Postmenopausal	201	1,600,561	1.8 (1.1-3.0)	53	423261	2.4 (1.4-3.9)	2.1 (1.4-3.0)
Unclear[b]	16	130,556	1.5 (0.8-2.9)	8	80277	2.1 (1.0-4.8)	1.7 (1.0-2.9)
Type of menopause							
Pre-menopausal	52	755,275	1.0	71	1479200	1.0	1.0
Natural	165	1,281,514	2.0 (1.2-3.4)	30	281384	1.9 (1.0-3.6)	2.0 (1.3-2.9)
Surgical	36	319,046	1.6 (0.9-2.8)	23	141876	2.7 (1.6-4.7)	2.1 (1.2-3.5)
Age at menopause							
Pre-menopausal	52	755,275	1.0	71	1479200	1.0	1.0
≤ 44 years	33	223,806	1.9 (1.1-3.2)	18	132428	2.4 (1.3-4.2)	2.1 (1.4-3.1)
45-49 years	55	412,947	1.7 (1.0-3.0)	11	125915	1.8 (0.8-3.8)	1.7 (1.1-2.7)
≥ 50 years	77	658,661	1.7 (1.0-2.9)	18	138738	2.7 (1.2-6.1)	2.0 (1.2-3.1)
Type of/age at menopause							
Pre-menopausal	52	755,275	1.0	71	1,479,200	1.0	1.0
Natural≤44	15	74.935	2.7 (1.4-5.3)	6	41,718	2.6 (1.1-6.2)	2.4 (1.5-4.0)
Natural≥45	116	919,120	1.8 (1.1-3.1)	23	222,463	2.0 (1.0-4.0)	1.8 (1.2-2.8)
Surgical≤44	18	148,871	1.6 (0.8-2.9)	12	90,711	2.2 (1.1-4.2)	1.8 (1.0-3.0)
Surgical≥45	16	152,488	1.4 (0.7-2.8)	6	42,190	2.6 (1.0-6.8)	1.6 (0.9-2.9)
Ovulatory years							
< 24 years	42	465,671	1.0	25	505367	1.0	1.0
24-29 years	53	518,520	0.9 (0.6-1.4)	27	545347	0.4 (0.2-0.8)	0.6 (0.2-1.5)
30-34 years	57	589,439	0.7 (0.5-1.1)	35	415482	0.4 (0.1-0.9)	0.6 (0.3-1.1)
≥ 35 years	47	412,547	0.8 (0.5-1.3)	21	228713	0.4 (0.1-1.1)	0.7 (0.3-1.3)

	Seropositive RA						
	NHS			NHSII			Pooled (NHS+NHSII)
Factors	Cases	Person-years	HR 95%CI[1]	Cases	Person-years	HR 95%CI[1]	HR 95%CI[1]
Menopausal status							
Pre-menopausal	101	750,055	1.0	141	1,447,465	1.0	1.0
Postmenopausal	327	1,594,188	1.3 (0.9-1.9)	83	417,058	1.1 (0.7-1.7)	1.2 (0.9-1.6)
Unclear[b]	32	129,495	1.3 (0.8-2.1)	11	78,782	0.8 (0.4-1.6)	1.3 (0.9-1.8)
Type of menopause							
Pre-menopausal	101	750,055	1.0	141	1,447,465	1.0	1.0
Natural	265	1,277,316	1.3 (0.9-2.0)	53	278,836	1.0 (0.6-1.6)	1.2 (0.9-1.6)
Surgical	62	316,872	1.3 (0.8-1.9)	30	138,222	1.3 (0.8-2.0)	1.3 (0.9-1.7)
Age at menopause							
Pre-menopausal	101	750,055	1.0	141	1,447,465	1.0	1.0
≤ 44 years	51	222,262	1.4 (0.9-2.1)	19	128,843	0.9 (0.6-1.6)	1.2 (0.9-1.7)
45-49 years	87	411,303	1.2 (0.8-1.9)	26	124,646	1.1 (0.7-1.9)	1.2 (0.9-1.7)
≥ 50 years	126	656,583	1.2 (0.8-1.9)	30	137,833	1.1 (0.6-2.0)	1.2 (0.9-1.7)
Type of/age at menopause							
Pre-menopausal	101	750,055	1.0	141	1,447,465	1.0	1.0
Natural≤44	21	74,559	1.6 (1.0-2.8)	5	40,939	0.8 (0.3-1.9)	1.4 (0.9-2.3)
Natural≥45	184	916,366	1.3 (0.8-1.9)	44	220,848	1.0 (0.6-1.7)	1.1 (0.8-1.6)
Surgical≤44	30	147,703	1.2 (0.8-2.0)	14	87,904	1.0 (0.6-1.8)	1.1 (0.8-1.7)
Surgical≥45	29	151,519	1.3 (0.8-2.1)	12	41,631	1.6 (0.8-3.1)	1.4 (0.9-2.1)
Ovulatory years							
< 24 years	67	462,049	1.0	34	493,200	1.0	1.0
24-29 years	88	516,119	0.9 (0.6-1.3)	51	531,987	1.1 (0.6-2.2)	1.1 (0.8-1.5)
30-34 years	121	586,979	1.0 (0.7-1.4)	62	408,867	1.5 (0.7-3.3)	1.1 (0.8-1.5)
≥ 35 years	75	410,846	0.9 (0.6-1.3)	42	226,651	1.4 (0.6-3.2)	1.2 (0.8-1.7)

Footnote for Table 8:

[a]Cox proportional hazards models adjusted for age, questionnaire cycle, median household income, BMI, smoking pack-years, breastfeeding, parity. Reference category is premenopausal women for menopausal variables.

[b]Unclear includes women whose date of menopause is unclear due to hysterectomy with unilateral oophorectomy, or menopause due to radiation.

Missings values for each model (cases/person years):

Seronegative, Type of menopause: NHS 16/130,556, NHSII 8/80,277; Age at menopause: NHS 52/435,703, NHSII 14/106,456; Type of/age at menopause: NHS 52/435,703 NHSII 14/106,456; Ovulatory years: NHS 70/500,215 NHSII 24/287,830.

Seropositive, Type of menopause: NHS 32/129,495 NHSII 11/78,782; Age at menopause: NHS 95/433,535 NHSII 19/104,518; Type of/age at menopause: NHS 95/433,535 NHSII 19/104,518; Ovulatory years: NHS 109/497,745 NHSII 46/282,600.

4.4 PMH AND THE RISK OF RA (PAPER IV)

In total, 467 cases and 935 controls were included in the analyses. In all, 303 (64.9%) cases were ACPA-positive and the mean duration of disease at inclusion in the study was 10 months for both ACPA-positive and ACPA-negative RA. Cases were more likely to be ever smokers, overweight, and to have a lower level of education.

4.4.1 Current/past use of PMH and risk of RA

Compared with never users, current users of PMH had a decreased risk of developing ACPA-positive RA (OR=0.6, 95% CI 0.3-0.9) in the adjusted model, but no association was observed for past users. No association was found between ever, current or past use of PMH and the risk of ACPA-negative RA (Table 9).

Table 9. Relative risk of ACPA-positive, ACPA-negative and RA overall according to ever, current and past use of PMH among women aged 50-70 years. EIRA, Sweden, 2006-2011

ACPA status	Use of PMH	Cases/Controls	OR 95% CI[a]	OR 95% CI[b]
ACPA-positive	Ever[c]	90/304	0.9 (0.7-1.2)	0.9 (0.6-1.2)
	Current	22/105	0.6 (0.4-1.0)	0.6 (0.3-0.9)
	Past	68/197	1.0 (0.7-1.4)	1.1 (0.8-1.5)
	Never	209/626	1.0	1.0
	Missing[d]	4/5	-	-
ACPA-negative	Ever	55/304	1.1 (0.7-1.5)	1.0 (0.7-1.4)
	Current	18/105	1.0 (0.6-1.7)	0.9 (0.5-1.5)
	Past	37/197	1.1 (0.7-1.7)	1.1 (0.7-1.7)
	Never	109/626	1.0	1.0
	Missing§	0/5	-	-
RA overall	Ever	145/304	0.9 (0.7-1.2)	0.9 (0.7-1.2)
	Current	40/105	0.7 (0.5-1.1)	0.7 (0.4-1.0)
	Past	105/197	1.1 (0.8-1.4)	1.1 (0.8-1.4)
	Never	318/626	1.0	1.0
	Missing[d]	4/5	-	-

[a] Adjusted for age and residential area.

[b] Adjusted for age, residential area and smoking (pack-years).

[c] Two controls only had information on year of initiation and type of therapy and were defined as ever users.

[d] Missing information on PMH use.

ACPA= anti-citrullinated protein antibodies, PMH= postmenopausal hormone, RA= rheumatoid arthritis, OR= odds ratio, CI= confidence interval, EIRA=Epidemiological Investigation of Rheumatoid Arthritis.

4.4.2 Duration of PMH and risk of RA

A shorter duration of PMH (1-6 years) was associated with a decreased risk of ACPA-positive RA among current users (adjusted OR=0.3, 95% CI 0.1-0.7). The association was not statistically significant for ACPA-negative RA (adjusted OR=0.4, 95% CI 0.1-1.3). A longer duration of PMH among current as well as past users was not associated with ACPA-positive RA. A longer duration was associated with a non-significantly increased risk of ACPA-negative RA among current (OR=1.3, 95% CI 0.7-2.4) but not among past PMH users (OR=0.9, 95% CI 0.5-1.7).

4.4.3 Current/past use of PMH and risk of RA in different age groups

The decreased risk of ACPA-positive RA among current users of PMH was observed mainly in the group of women aged 50-59 years (OR=0.3, 95% CI 0.1-0.8), while no significant effect was observed in those aged 60-70 years (OR=0.8, 95% CI 0.4-1.4). No association between past PMH use and the risk of ACPA-positive RA was observed. No association between past/current PMH use and risk of ACPA-negative RA was observed in any of the age groups.

4.4.4 Type of therapy and risk of RA

Among current users of a combined PMH therapy (estrogen plus progestogens) an OR of 0.3 (95% CI 0.1-0.7) of developing ACPA-positive RA was observed. There was no significant association between current PMH use and ACPA-positive RA among women who used estrogen alone (OR=0.8, 95% CI 0.5-1.6). For the ACPA-negative subset, no association was found for ever, current, or past use of either type of PMH therapy (Table 10).

Table 10. Relative risk of ACPA-positive and ACPA-negative RA according to type of medication, among women aged 50-70 years. EIRA, Sweden, 2006-2011

ACPA status	Use of PMH	Estrogen only		Estrogen + progestogens[a]	
		Cases/Controls	OR 95% CI[b]	Cases/Controls	OR 95% CI[b]
ACPA-positive	Ever	57/165	1.1 (0.7-1.5)	33/139	0.7 (0.5-1.1)
	Current	15/50	0.8 (0.5-1.6)	7/55	0.3 (0.1-0.7)
	Past	42/114	1.2 (0.8-1.8)	26/83	1.0 (0.6-1.6)
	Never	209/626	1.0	209/626	1.0
	Missing	4/5	-	4/5	-
ACPA-negative	Ever	31/165	1.0 (0.7-1.6)	24/139	1.0 (0.6-1.6)
	Current	9/50	0.8 (0.3-1.8)	9/55	0.9 (0.4-2.0)
	Past	22/114	1.1 (0.7-1.9)	15/83	1.1 (0.6-2.0)
	Never	109/626	1.0	109/626	1.0
	Missing	0/5	-	0/5	-

[a] The estrogen plus progestogen group includes both combined and sequential regimens.

[b] Adjusted by age, residential area and smoking (pack-years).

ACPA= anti-citrullinated protein antibodies, RA= rheumatoid arthritis, PMH= postmenopausal hormone therapy, OR= odds ratio, CI= confidence interval, EIRA=Epidemiological Investigation of Rheumatoid Arthritis.

Table 11. Summary of main results obtained and presented in this thesis

Paper	Specific results	Seropositive RA	Seronegative RA
Parity and risk of RA (Paper I)	Parity among young women (18-44 years)	NA	↑
	Postpartum period	NA	↑
	Young age at first age	NA	↑
BF, OC and risk of RA (Paper II)	BF ≥13 months (among parous women)	↓	NA
	Ever OC use	↓	NA
	Current OC use	NA	NA
	Past OC use	↓	NA
	Long duration (≥8y) among ever and past OC users	↓	↓
Menopausal factors and risk of RA (Paper III)	Older age	↑	↑
	Postmenopausal women compared with premenopausal	NA	↑
	Natural menopause at early age (≤44 years)	NA	↑
	Long duration of PMH (≥8y)	↑	↑
PMH and risk of RA (Paper IV)	Current PMH use among women aged 50-70 years	↓	NA
	Current PMH use among women aged 50-59 years	↓	NA
	Shorter duration of PMH use	↓	NA
	Use of a combined PMH therapy (estrogen plus progestogens)	↓	NA

↑= increased risk, ↓= decreased risk, NA= No association

BF= breastfeeding, OC= oral contraceptive, PMH= postmenopausal hormone therapy

Results from Papers I, II and IV are specific for ACPA-status, while results from Paper III are for either RF or ACPA.

5 DISCUSSION

5.1 COMMENTS ON PRESENT RESULTS AND PREVIOUS STUDIES

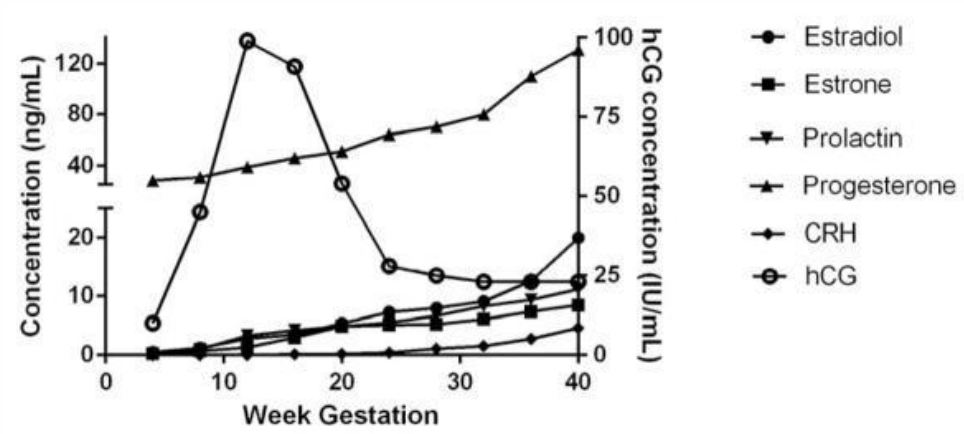
5.1.1 Parity and the risk of RA (Paper I)

Our results have shown that parous women of reproductive age (18-44 years) had an increased risk of ACPA-negative RA. We found an elevated risk during the postpartum period, and among women who had a young age at first birth. We found no association between either parity or the postpartum period and the risk of ACPA-positive RA, but older age at first birth appeared to be associated with a decreased risk of this RA subset.

In general, parity has previously been described as a risk factor for RA close to delivery [39-42] but after some years this risk might weaken [43-49]. Our results provide more detailed information, showing that the increased postpartum risk is restricted to ACPA-negative RA. Inconsistent findings with regard to other factors, such as age at first birth and number of children, might be due to methodological issues, such as inclusion of prevalent cases, [49] inclusion of non-population-based controls [48, 49] or relatively few cases [45-49]. The decreased risk of ACPA-negative RA with increasing age remained even after adjustments for potential confounders.

Pregnancy entails considerable immunological adaptation. The high concentrations of various circulating hormones (e.g. cortisol, estrogen) (Figure 2), might account for the lower RA incidence in the months of pregnancy. [86] The considerable drop in hormonal levels after delivery together with high prolactin levels during BF might explain the increased postpartum RA risk. [87]

Figure 2. Hormone changes in week of hormones important for the regulation of gestation in healthy pregnant women. CRH= corticotropin releasing hormone, hCG= human chorionic gonadotropin. [88]



5.1.2 BF, OCs and the risk of RA (Paper II)

We found a decreased risk of developing ACPA-positive RA among parous women who breastfed more than 1 year, compared with parous women who breastfed for up to 6 months. This decreased risk was non-significant after adjustment for smoking. Women who had breastfed one child had a lower risk of RA, and especially ACPA-negative RA, compared with women who had breastfed three or more children. Ever and past use of OCs was significantly associated with a decreased risk of developing ACPA-positive RA. The decrease in risk was greater for a longer duration of use.

From reviewing the literature on the association between BF and RA, it is clear that a consensus has not been reached. Some authors have described an increased risk of RA with increasing time of BF [51] or mainly related to the first pregnancy. [52] Consistent with our results, other studies have found a protective effect of BF. In a large prospective cohort study, Karlson *et al* found a decreased risk of RA among women who breastfed for more than 12 months, with a significant trend with increased duration of BF. [44] In this study, a similar pattern was observed for RF-positive cases. Similar results have been found in Swedish, [46] British, [54] and Asian populations, [50] including a recently published systematic review and meta-analysis that includes conflicting results. [89] Our results confirm and extend these findings by adding the stratification according to ACPA-status, which has not been previously explored.

Most studies investigating OC use and the risk of RA have not been able to prove an association [42, 44, 46, 49, 51, 55-59] including two recently published meta-analyses. [60, 61] Those that have shown a significant association are in line with our results, [62-67] including a report of a protective effect with a longer duration of OC use. [51] A decreased risk of RA has been associated with OC use especially in early years when preparations contained higher doses of estrogen. [62] Several studies have shown no associations between low-dose estrogens and RA. [44, 46, 61] Different methodologies (e.g. study design) as well as insufficient sample size might also explain these disparate previous results.

Prolactin, which is the hormone related to lactation, has been mostly linked with an increased risk of RA due to its immunostimulating properties. [90] Recent findings, however, suggest that prolactin might act more as a regulator of inflammation, with protective and regenerative functions. [91] Other potential biological mechanisms that could explain our results regarding BF might be an anti-inflammatory effect given by prolonged effect of progesterone in the postpartum period. [92] Finally, elevated levels of cortisol, which has been found to be significantly higher among post-menopausal women with a history of BF, might also explain our results. [93] The finding of an inverse association between BF and the risk of ACPA-positive, but not ACPA-negative RA is in line with our results from Paper I, in which we observed an increased risk of ACPA-negative but not ACPA-positive RA during the post-partum period.

With regard to our results on OC use and the decreased risk of RA, we found that the protective effect was limited to ACPA-positive RA and that a longer duration of OC use had a stronger effect, supporting the hypothesis of a dose-response effect.

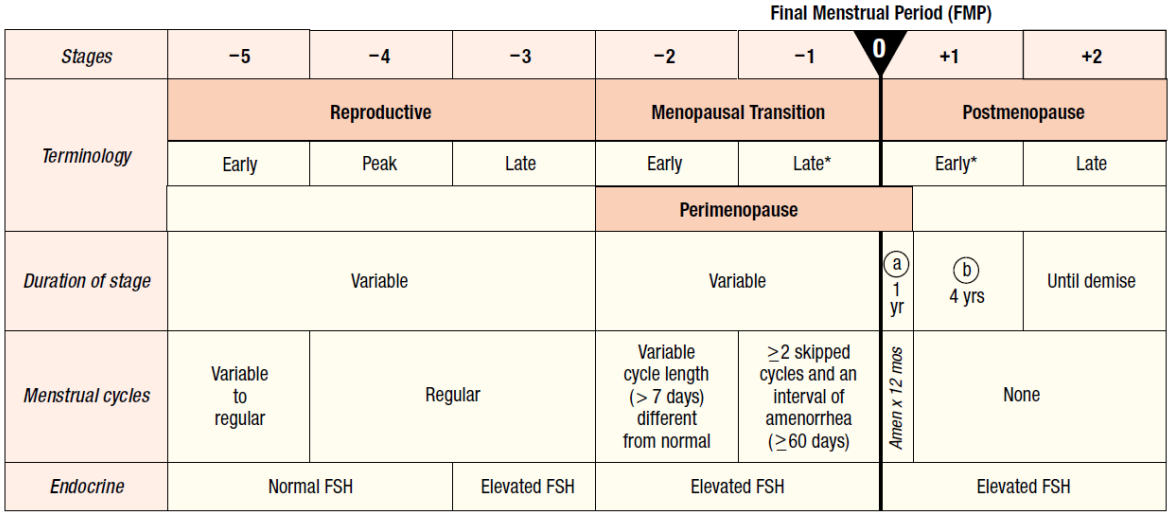
Furthermore, these results are in line with our findings from Paper IV (reduced risk of developing RA among women who used PMH), demonstrating a similar effect of current use of exogenous sex hormones on the risk of RA, but at different stages in life (pre and postmenopausal women). Notably, a combined therapy of estrogen and progestogens had the strongest protective effect, supporting the hypothesis that progesterone exerts an anti-inflammatory effect in menopause.

5.1.3 Menopausal factors and the risk of RA (Paper III)

Menopausal factors were strongly associated with risk of seronegative, but not seropositive RA in these large prospective cohorts. Postmenopausal women had more than a two-fold increased risk of seronegative disease, compared with premenopausal women. Those in whom a natural menopause occurred at an early age (≤44 years of age) had an HR of 2.4 of seronegative RA. We observed no associations between PMH use and the risk of either serological phenotype of RA, except for an increased risk of seropositive RA among women with a long duration of PMH. Moreover, the peak risk of developing RA was observed at ages 55-59 years for both serological phenotypes, which is after the menopausal transition in most women.

To our knowledge, this is the first study to demonstrate that menopausal factors are mainly associated with seronegative RA. Menopausal transition is a dynamic process, which can occur at different ages among women [94] (Figure 3).

Figure 3. Stages of reproductive aging. *Stages most likely to be characterized by vasomotor symptoms; FSH, follicle stimulating hormone; ↑, elevated; amen., amenorrhea. [94]



The menopausal transition and the underlying hormonal changes have been related to an increased risk of RA, although the literature has been scarce. [68, 69] Our findings suggest that postmenopausal women and those with an early natural menopause are at increased risk of RA, but that this risk is restricted to the seronegative phenotype.

It remains unclear whether the menopausal RA risk is increased due to falling estrogen or progesterone levels, but the immunomodulatory effect given by progesterone [95-98] together with its anti-inflammatory effect during pregnancy [99] might exert an analogous role during the menopausal phase.

An association between PMH use and the risk of seronegative or seropositive RA could not be confirmed in this study. However, the different results observed for NHS and NHSII, especially for seropositive RA, are in line with the findings from the EIRA study (Paper IV) and might indicate that different strategies over the decades have different impact on disease development.

Apart from one Swedish study in which the RA incidence peaked at 70-79 years of age, [5] most previous studies have observed a peak in RA incidence at 45-64 years. [7-9, 100] In our study, we observed that disease incidence peaked later than the mean age at menopause in these cohorts (51-52 years), at 55-59 years of age, for both serological RA phenotypes. Therefore, menopausal factors might be involved in later development of RA, especially seronegative RA, but other factors might promote the peak incidence after menopause.

Finally, most menopausal factors affected RA risk in a similar way in the two NHS cohorts, strengthening the conclusion that these factors are involved in the development of seronegative RA.

5.1.4 PMH and the risk of RA (Paper IV)

Postmenopausal women who were currently using PMH at onset of their disease had a decreased risk of ACPA-positive RA. This decreased risk was mainly observed among women aged 50-59 years and only among users of a combined therapy of estrogen and progestogens. Women with a short duration of PMH use (≤7 years) also had a decreased risk of ACPA-positive RA, which might indicate that the initial time of PMH use has an effect on development of ACPA-positive disease. We were not able to further explore PMH duration due to the low number of observations.

Previous studies on the association between PMH use and the onset of RA have reported inconclusive results. [44, 59, 62, 70-76] Our results are in accordance with previous reports of a decreased risk of RA among current PMH users [59, 78] and among current users of a combined therapy [72] More recently, it has been proposed that the use of hormone replacement therapy in women with early undifferentiated arthritis protects against RA in individuals carrying HLA-DRB1 SE alleles (OR=0.43, 95% CI 0.24-0.77) by reducing the risk for the presence of ACPA. [77] Nevertheless, to the best of our knowledge, our study is

the first to investigate this exposure separately by ACPA-status and by type of therapy (estrogen only or estrogen plus progestogens).

One of the most interesting findings from this study was the different result for current users of PMH including estrogen alone, compared with users of a combination of estrogens and progestogens. This might be explained by the immunomodulatory effect of the natural hormone progesterone, which may differ from the effects of estrogens and androgens. [95-98] The anti-inflammatory milieu as a result of elevated concentrations of various circulating hormones during pregnancy, [86] together with inhibition of T-helper (Th)1 and Th17 pathways and induction of anti-inflammatory molecules given by progesterone [99] may explain the reduced RA incidence during pregnancy. When progesterone levels fall in the postpartum period, a higher incidence of RA has been observed; this was confirmed by the results of our first study (Paper I), but interestingly confined to ACPA-negative RA.

Finally, it is important to note that in spite of the protective effect of PMH use found in this study, the associations between this therapy and the occurrence of endometrial cancer, breast cancer and cardiovascular diseases, among other conditions, should not be ignored. [101, 102]

5.2 METHODOLOGICAL CONSIDERATIONS

A number of issues could be considered in this section, but I have chosen to restrict the discussion to several topics that in my opinion are the most relevant for this thesis.

5.2.1 Study design

We utilized data from two different study designs, namely a case-control and a cohort study. A case-control study using incident cases of disease has the advantage of being more cost-efficient and of obtaining specific exposure information with regard to RA. To our knowledge, EIRA is the largest population-based case-control study that has been conducted to date, with a substantial number of cases and controls with detailed information on environmental/genetic risk factors. By selecting the controls continuously over time, and from the same population in which the cases originated, the ORs estimate the rate ratio that would be obtained from a cohort study in the same study base. [103] One drawback with this study design is its retrospective nature when it comes to exposure assessment.

The cohort design of the NHS is ideal in terms of obtaining data prior to disease onset from the entire source population. The strengths of this design include the repeated and prospective assessment of most exposures/confounders, the possibility to adjust for several confounders (including BMI, cigarette smoking, alcohol consumption, income, parity and BF) and the long follow-up period. Its main weakness is a lower number of incident RA cases, compared to the large sample size from a case-control study; the latter is more efficient when studying a rare disease such as RA.

5.2.2 Selection bias

The EIRA study has the advantage of being a large, population-based case-control study including incident cases of RA. In order to minimize the risk of selection bias that frequently threatens the validity of case-control studies, we selected controls randomly and continuously from the same study base as the cases. Although the general participation rates have decreased over the years (from 95% to 92% among cases and from 81% to 73% among controls), we still have a good participation rate when it comes solely to women (95% and 80% for cases and controls respectively). In addition, the frequency of exposure among controls was similar to that in the population. (e.g. BF habits and PMH use). For example, BF among controls was very similar to the high frequency of BF observed in Sweden, [104] and we found approximately the same frequency of current PMH use among the controls as reported in a previous Swedish study. [105]

One of the disadvantages of the NHS material is the lack of blood samples for the entire study population to assess ACPA-status. However, it has been shown that the demographic and exposure characteristics of participants who provided blood samples are similar to those in the overall cohorts. [106, 107] The high participation rate in this cohort minimizes the risk of selection bias.

5.2.3 Misclassification of exposure

It is very unlikely that a misclassification occurred in the analysis of parity (Paper I). However, misclassification of the postpartum period during the index-year might have occurred as we did not collect detailed information on month of delivery. This misclassification is possibly non-differential (generally leading to a dilution of the results), which is supported by the different results according to ACPA status. Similarly, we lacked detailed information regarding the exact time of the absence of menses (Paper IV), which we minimized by excluding all women who did not report a specific age at menopause and by restricting our analyses to women aged 50-70 years when the menopause is more likely to have already occurred.

In a case-control study, bias originating from differential recall between cases who have RA symptoms at the time of reporting exposures and controls cannot be ruled out. However, we do not believe that the main hormonal/reproductive factors (e.g. parity, ever use of OCs or PMH) included in our study may suffer from a differential-misclassification of exposure. We decided not to include detailed information on PMH and OC use (specific preparations and doses), mainly because of the lack of completeness and resulting loss of power for any stratified analyses.

For the NHS, a limitation is the self-reported exposure data, which might lead to misclassification of the menopausal factors. However, because the data were collected prospectively this potential misclassification of exposure would be non-differential and the results would be diluted.

5.2.4 Misclassification of disease

An important requirement for any epidemiological study is a set of well-defined diagnostic criteria. Cases included in this thesis, whether from EIRA or from NHS, were mostly diagnosed according to the 1987 ACR-criteria [12] which are widely used in clinical practice. The limitation of these criteria might be the inability to detect early cases of RA. Cases could then be classified as non-cases, which may have occurred predominantly for seronegative RA. However, since our analyses were conducted stratifying the cases by serological phenotype, we believe that this potential source of bias was minimized.

It is unlikely that the exposures under study would have had any impact on the inclusion of cases. Therefore, this source of misclassification is likely to be non-differential, leading to a dilution of our results.

We do not believe that the emergence of new criteria for RA had an impact on our results. Most of the cases in our studies fulfilled both criteria, with only a small fraction (one and 24 cases in Papers IV and II respectively) having been diagnosed with these more recent but not the original criteria.

For the NHS, the reliance on medical record documentation rather than physical examination for the classification of RA cases could result in a misclassification of cases as non-cases. This was minimized by censoring those cases with self-reported RA or other connective tissue diseases at the date of first report if RA diagnosis could not be confirmed by medical records.

Another issue related to the NHS is that ACPA was not available for all individuals diagnosed with RA in the 1980s, prior to its clinical availability. This might lead to a potential for misclassification of ACPA-positive RA cases as seronegative cases, which would underestimate the associations between menopausal factors and the risk of seronegative RA.

5.2.5 Confounding

Cases for the EIRA study were matched according to age and residential area. These variables were always included in the analyses. Of the potential confounders considered in our analyses using material from the EIRA study, smoking was the only factor that affected our estimates and therefore adjusted results are shown in most of the tables contained in the papers using this material (Papers I, II and IV). As smoking habits may change over time, it is difficult to evaluate this variable in relation to the exposures under study, especially for parity and BF practices.

The strategy used for analyzing the NHS data consisted of multivariate analyses, excluding those potential confounders that did not affect the estimates.

Both types of studies are limited by the ability to adjust only for confounders that were measured in the parent study. Thus unmeasured confounders cannot be included in multivariable models, and could explain some of the different results.

5.3 FINAL REMARKS AND FUTURE RESEARCH

RA is one of the most common autoimmune diseases, with a higher prevalence among women. The involvement of hormonal/reproductive factors has long been suspected to be related to the pathogenesis of the disease. Not much is known about ACPA-status or genetic variables in relation to these hormonal factors. Our study contributes to the knowledge in this area by providing a thorough analysis of some of the most important reproductive factors using data from two of the largest ongoing studies.

Our results showed that: parity, the postpartum period and a young age at first birth were associated with an increased risk of ACPA-negative RA; both BF and OC use were associated with a decreased risk of ACPA-positive RA; postmenopausal women had an increased risk of seronegative RA, and women with a history of PMH use had an increased risk of seropositive RA in the NHS; and postmenopausal women who were using PMH at disease onset had a decreased risk of ACPA-positive RA in the EIRA study.

The results of this thesis highlight the importance of discriminating between serological phenotypes when evaluating risk factors for RA. It would be interesting to explore whether some of the hormonal/reproductive factors may also have an impact on the severity of the disease. This might be possible because a follow-up study of the cases from the EIRA study is currently being conducted.

A recent study of different specificities in RA has shown that antibodies might be present even for the subgroup of ACPA-negative disease. [108] Further studies looking at the hormonal/reproductive factors investigated in this thesis in relation to the different specificities, beyond the broader group of ACPA-status, would be of interest.

Why the hormonal/reproductive factors have a different impact depending on the serological RA phenotypes remains to be elucidated, and we can only hypothesize about the exact biological mechanisms underlying our findings. For these theories to be confirmed, more studies including laboratory measurements are required in order to determine the real hormonal changes associated with major events in the life of every woman and their impact on the onset of RA.

6 CONCLUSIONS

- Parous women of reproductive age had an increased risk of ACPA-negative RA. The increased risk was mainly due to an increased risk in the postpartum period and young age at first birth.
- Parous women who breastfed their children for more than a year had a decreased risk of developing ACPA-positive RA, compared with parous women who breastfed for a period of six months or less.
- Ever and past use of OCs were significantly associated with a decreased risk of developing ACPA-positive RA, and we found that this association was stronger for a longer duration of use.
- The association between PMH use among and the risk of seropositive/-negative RA is inconclusive. However, a long duration of PMH use might be related to an increased risk of seropositive RA. The inconclusive results might be due to unmeasured confounders
- Our findings of a different impact of hormonal/reproductive factors on the two subsets of RA (ACPA-positive and ACPA-negative) add further support to the notion that RA comprises two different disease entities with different etiologies.
- Menopausal factors, are strongly associated with seronegative RA, but not seropositive RA, suggesting differences in disease etiology according to serotype.
- Further research is needed in order to explore the biological mechanisms behind our findings; nevertheless, our results regarding hormonal/reproductive factors can contribute to understanding the complex etiology of RA and the higher incidence among women.

7 SAMMANFATTNING PÅ SVENSKA

Reumatoid artrit (RA) är en inflammatorisk sjukdom som kännetecknas av kronisk inflammation i kroppens leder. Etiologin består av ett komplext samspel mellan genetiska och miljömässiga faktorer. RA är vanligare bland kvinnor än bland män och kan uppstå i alla åldrar, men skillnaden mellan könen tycks vara störst före klimakteriet. Det har antagits att förändringar i kvinnliga hormonnivåer kan vara inblandade i RA-patogenesen. Det övergripande syftet med min avhandling var att studera sambandet mellan hormonella/reproduktiva faktorer och risken för RA och huruvida sambandet med dessa faktorer skiljer sig mellan olika serologiska fenotyper av sjukdomen (baserat på närvaro/frånvaro av antikroppar mot citrullinerade peptider (ACPA) och reumatoid-faktor (RF)).

Denna avhandling bygger på data från två stora studier. Tre artiklar baserades på den svenska EIRA (epidemiologisk undersökning av riskfaktorer för reumatoid artrit) studien, en befolkningsbaserad fall-kontrollstudie med incidenta RA-fall. Studiepopulationen bestod av befolkningen i åldern 18 år och uppåt, boende i vissa geografiska delar av Sverige från 1996. Kontrollerna valdes slumpmässigt ur befolkningsregistret med hänsyn tagen till ålder, kön och bostadsort. Fall och kontroller besvarade ett detaljerat frågeformulär rörande livsstils- och miljöexponeringar. En artikel bygger på Nurses' Health Study (NHS) som består av två prospektiva kohorter av kvinnliga sjuksköterskor i USA. Datainsamlingen i NHS startade 1976 (kvinnor i åldern 30-55 år) och 1989 (kvinnor i åldern 25-42 år). Båda kohorter följdes upp vartannat år via frågeformulär avseende sjukdomar, livsstil och hälsa.

Enligt våra resultat hade kvinnor, som fött barn i åldrarna 18-44 år, en ökad risk för ACPA-negativ RA jämfört med kvinnor i samma ålder som inte fött barn. Den ökade risken kunde främst förklaras av en förhöjd risk vid tiden efter förlossningen samt ung ålder vid första barnets födelse. Äldre förstagångsföderskor hade en minskad risk för ACPA-positiv RA. Kvinnor som fött barn och som ammat i över ett år hade en minskad risk för ACPA-positiv RA jämfört med kvinnor som ammat sex månader eller mindre. Efter justering för rökning var den skyddande effekten inte längre signifikant.

Kvinnor som någon gång använt p-piller hade en lägre risk för ACPA-positiv RA medan en längre duration var signifikant associerad med en minskad risk för båda RA-subtyper. Postmenopausala kvinnor hade en fördubblad risk för seronegativ RA jämfört med premenopausala kvinnor, men de hade inget samband med insjuknande i seropositiv RA. I NHS hade kvinnor med lång postmenopausal hormonbehandling (PMH) en ökad risk för seropositiv RA. Slutligen, i EIRA hade postmenopausala kvinnor som använt PMH vid sjukdomsdebut en lägre risk för ACPA-positiv RA. Den minskade risken gällde för kvinnor i åldrarna 50-59 år med kortvarig användning (<7 år) och endast bland dem med en kombinerad östrogen- och gestagenbehandling.

Ytterligare forskning behövs för att utforska de biologiska mekanismerna bakom våra fynd men våra resultat bidrar till kunskapen om hormonella/reproduktiva faktorer och deras inverkan på serologiska fenotyper av RA.

8 RESUMEN EN ESPAÑOL

La artritis reumatoide (AR) es una enfermedad crónica inflamatoria que conduce a daño articular y destrucción ósea, con una compleja interacción entre factores genéticos y ambientales involucrados en su etiología. La AR es más frecuente en mujeres que en hombres en todas las edades, pero esta diferencia parece ser mayor antes de la menopausia. Se ha propuesto como hipótesis que los cambios en los niveles hormonales en el género femenino podrían estar involucrados en la patogénesis de la AR. El objetivo general de esta tesis fue estudiar la asociación entre factores hormonales/reproductivos y el riesgo de AR y determinar si estos factores se asocian de manera diferente con los fenotipos serológicos de la enfermedad (de acuerdo a la presencia o ausencia de anticuerpos contra péptidos citrulinados (ACPA) y contra el factor reumatoide (FR)).

Esta tesis está basada en información obtenida de dos grandes estudios. Tres artículos fueron basados en la Investigación Epidemiológica Sueca de AR (Swedish Epidemiological Investigation of RA – EIRA), un estudio de casos y controles de base poblacional que comprende casos incidentes de AR. La población de estudio fueron personas de 18 o más años de edad, con vivienda en diferentes regiones geográficas de Suecia a partir de 1996. Los controles fueron elegidos del registro poblacional de manera aleatoria y emparejados a los casos según edad, sexo y área residencial. Los casos y controles respondieron un extenso cuestionario a través del cual se recolectó información acerca de su estilo de vida y exposiciones ambientales. Un artículo está basado en el Estudio sobre la Salud de las Enfermeras (Nurses' Health Study – NHS) el cual consiste en dos cohortes prospectivas de enfermeras en Estados Unidos. La recolección de los datos comenzó en 1976 (mujeres entre 30 y 55 años de edad) y 1989 (mujeres entre 25 y 42 años de edad). Ambas cohortes del NHS fueron seguidas a través de cuestionarios bienales con relación a enfermedades, estilo de vida y prácticas sanitarias.

De acuerdo con nuestros resultados, las mujeres que tuvieron hijos presentaron un riesgo incrementado de AR ACPA-negativa comparadas con las mujeres nulíparas, entre los 18 y 44 años de edad. Este resultado fue atribuido a un riesgo elevado durante el postparto y a una edad temprana al nacimiento del primer(a) hijo(a). Una edad más avanzada al momento del primer nacimiento pareció estar asociada con un riesgo menor de AR ACPA-positiva. Las mujeres que amamantaron a su(s) hijo(s) por más de un año tuvieron un riesgo menor de AR ACPA-positiva comparadas con las mujeres que amamantaron por un periodo menor o igual a seis meses. Esta disminución del riesgo no fue significativa al ajustar el análisis por el consumo de cigarrillo.

El uso de anticonceptivos orales estuvo significativamente asociado con una disminución en el riesgo de AR ACPA-positiva, mientras que una larga duración en el uso de los mismos estuvo significativamente asociada con una disminución del riesgo para ambos fenotipos de AR. Las mujeres postmenopáusicas presentaron un riesgo incrementado de desarrollar AR seronegativa, pero no tuvieron asociación con el desarrollo de la AR seropositiva. Las mujeres postmenopáusicas, usuarias de terapia de reemplazo hormonal (TRH) de manera prolongada, tuvieron un riesgo incrementado de AR seropositiva en el NHS. Finalmente, en el estudio EIRA, las mujeres postmenopáusicas que se encontraban usando TRH al inicio de su enfermedad tuvieron un riesgo disminuido de AR ACPA-positiva. Esta disminución en el riesgo se presentó

principalmente en mujeres entre los 50 y 59 años de edad, con un uso corto de TRH (menor a 7 años) y sólo entre usuarias de terapia combinada de estrógenos y progestágenos.

Se requiere investigación adicional para explorar los mecanismos biológicos detrás de nuestros hallazgos, pero nuestros resultados contribuyen al entendimiento de los factores hormonales/reproductivos y su impacto en los fenotipos serológicos de la AR.

9 APPENDIX

Questions about hormonal/reproductive factors included in the EIRA study.

C. Om du är kvinna

1. **Om du har barn, har du själv ammat ditt/dina barn?** ☐ Nej ☐ Ja
   Om "Ja", försök att ange hur länge?
   1:a barnet .....
   2:a barnet .....
   3:e barnet .....
   4:e barnet .....

2. **Har du haft någon graviditet som varat mindre än 6 mån, dvs slutat med missfall eller abort?** ☐ Nej ☐ Ja
   Om "Ja", hur många .....

3. **Vid vilken ålder hade du din första menstruation?** .....

4. **Har din menstruation upphört?** ☐ Nej ☐ Ja
   Om "Ja", vid vilken ålder .....

5. **Hur regelbunden är/var din menstruation mellan 20–35 års ålder, när du inte använt p-piller eller varit gravid?**

Regelbunden	Oftast Regelbunden	Oftast Oregelbunden	Mycket Oregelbunden
☐	☐	☐	☐

6. **Har du någon gång under livet tagit p-piller regelbundet?** (ta även med p-piller som du tar för närvarande)

   ☐ Nej ☐ Ja Om "Ja", ange vilka perioder och (om du minns) vilket/vilka preparat:

Fr o m år	T o m år	Preparat
.....	.....	.....
.....	.....	.....

7. **Har du någon gång använt andra typer av hormonella preventivmedel än p-piller (p-spruta, hormonspiral, p-stav, p-ring)?** Ange även preparat som du tar för närvarande.

   ☐ Nej ☐ Ja

   Om "Ja":

Fr o m år	T o m år	Preparat
.....	.....	.....
.....	.....	.....

8. **Har du någon gång fått hormonersättningspreparat innehållande östrogen och/eller progesteron (gulkroppshormon) i tablettform eller som plåster?** Ange även preparat som du tar för närvarande.

   ☐ Nej ☐ Ja

   Om "Ja":

Fr o m år	T o m år	Preparat
.....	.....	.....
.....	.....	.....

10 ACKNOWLEDGMENTS

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