CARDIOVASCULAR CO-MORBIDITY IN RHEUMATOID ARTHRITIS- WHO, WHEN, HOW MUCH, AND WHY?

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Jag sjunger som jag vill!
KSMB *En slemmig torsk*

Humboldt räknade lössen i deras flätade hår. (---) Han (Bonplant) frågade vad en statistik över löss skulle vara bra för. *Man sökte kunskap därför att man sökte kunskap, sade Humboldt.* Ingen hade tidigare undersökt förekomsten av dessa anmärkningsvärt motståndskraftiga djur på huvudena av människor i områdena nära ekvatorn.
Daniel Kehlmann *Die Vermessung der Welt*

Till minne av farsan

ABSTRACT

This thesis is based on four different studies, all focusing on co-morbidities in rheumatoid arthritis. Diabetes mellitus is assessed as a risk factor for rheumatoid arthritis, the temporal relationship between ischemic heart disease and rheumatoid arthritis, and the extent of coronary stenosis in rheumatoid arthritis, is studied. The rationale for this is that patients with rheumatoid arthritis suffer an increased risk of ischemic heart disease that cannot be explained by traditional risk factors for cardiovascular disease but is hypothesized to be related to rheumatoid arthritis specific factors, that patients with rheumatoid arthritis have been reported to have a more severe atherosclerosis and that autoimmunity seem to aggregate.

First, we studied diabetes mellitus and its relationship to rheumatoid arthritis using a population-based case-control study of rheumatoid arthritis. 1,419 patients with newly diagnosed rheumatoid arthritis and 1,674 age-, sex-, and residential area- matched controls were compared with respect to having diabetes mellitus prior to study inclusion. After validating self-reported information, 20 cases and 5 controls were classified as having type 1 diabetes mellitus and 42 cases and 46 controls as having type 2 diabetes mellitus. This study demonstrated that having type 1 diabetes mellitus conferred a seven-fold increased risk of developing a specific subset of rheumatoid arthritis defined by the presence of anti-citrullinated protein antibodies. This association depended to some extent on a genetic variant known to be involved in the pathogenesis of both type 1 diabetes and rheumatoid arthritis.

We then studied the temporal relationship between ischemic heart disease and rheumatoid arthritis in two population-based cohorts of patients with rheumatoid arthritis and age-, sex-, and residential area-matched controls from the general population. The occurrence of ischemic heart disease before first symptom of rheumatoid arthritis (controls were given the same date as their matched case) in two population-based cohorts of rheumatoid arthritis ($n_{cohort\ 1}$= 8,454, $n_{cohort2}$=2,025) was compared to the occurrence of ischemic heart disease among controls ($n_{cohort\ 1}$=42,267, $n_{cohort2}$=2,760) and revealed that having a history of ischemic heart disease before onset of first symptom of rheumatoid arthritis was as common among cases as controls; approximately 6% of cases and 6% of controls in cohort 1 had experienced an ischemic heart disease before first symptom of rheumatoid arthritis. After excluding those who had had any ischemic heart disease at diagnosis of rheumatoid arthritis, we followed cohort 1 over time and found that there indeed was an increased risk of ischemic heart disease in patients with rheumatoid arthritis (n=7,469) compared with the general population (n=37,024) and that this increased risk was apparent and manifest already within a few years following rheumatoid arthritis diagnosis. Among individuals included in cohort 1 who underwent angiography with indication acute coronary syndrome ($n_{rheumatoid\ arthritis}$=168, $n_{comparators}$=534) we found that although the development of ischemic heart disease was much more rapid in patients with rheumatoid arthritis, the extent of coronary stenosis was not related to rheumatoid arthritis.

In summary, these results indicate that type 1 diabetes mellitus increases the risk of developing a specific type of rheumatoid arthritis defined by a specific auto-antibody, that the risk of ischemic heart disease in rheumatoid arthritis goes from not being elevated to 60% increased compared to the general population within a few years following diagnosis of rheumatoid arthritis, and that the extent of coronary stenosis in rheumatoid arthritis with acute coronary syndromes is very similar to that in controls with the same clinical symptoms of acute coronary syndrome. The rapid increase in risk of ischemic heart disease could be related to factors associated with rheumatoid arthritis.

LIST OF PUBLICATIONS

This thesis is based four original papers. They are listed below and will be referred to in Roman numerals.

I. Liao KP, **Gunnarsson M**, Källberg H, Ding B, Plenge RM, Padyukov L, Karlson EW, Klareskog L, Askling J, and Alfredsson L.

Specific association of type 1 diabetes mellitus with anti-cyclic citrullinated peptide-positive rheumatoid arthritis.

Arthritis Rheum 2009 Mar;60(3):653-60.

II. **Holmqvist ME**, Wedrén S, Jacobsson LT, Klareskog L, Nyberg F, Rantapää-Dahlqvist S, Alfredsson L, and Askling J.

No increased occurrence of ischemic heart disease prior to the onset of rheumatoid arthritis: results from two Swedish population-based rheumatoid arthritis cohorts.

Arthritis Rheum. 2009 Oct;60(10):2861-9.

III. **Holmqvist ME**, Wedrén S, Jacobsson LT, Klareskog L, Nyberg F, Rantapää-Dahlqvist S, Alfredsson L, and Askling J.

Rapid increase in myocardial infarction risk following diagnosis of rheumatoid arthritis amongst patients diagnosed between 1995 and 2006

J Intern Med. 2010 Aug 4. [Epub ahead of print]

IV. **Holmqvist ME**, Stenestrand U, Jacobsson LTH, Klareskog L, Alfredsson L, James S, and Askling J.

Patients with rheumatoid arthritis and acute coronary syndrome have an angiographic pattern similar to other individuals with acute coronary syndrome.

In manuscript

CONTENTS

1 INTRODUCTION

This thesis includes some of the results from a broad research program aiming to study co-morbidities and their etiology in rheumatoid arthritis. My work in this research program has primarily dealt with cardiovascular disease, and three of the four studies herein presented are investigating factors related to this. The study not specifically related to cardiovascular disease is focused on another co-morbid condition etiologically related to rheumatoid arthritis and a strong risk factor for ischemic heart disease in the general population; diabetes mellitus. Within the same research program, but not presented in this thesis, are ongoing studies of stroke in rheumatoid arthritis, statins and their effect on the risk and prognosis of rheumatoid arthritis as well as studies on the genetic risk factors for cardiovascular disease in rheumatoid arthritis.

Autoimmune diseases such as rheumatoid arthritis and type 1 diabetes mellitus have been observed to co-occur within individuals and families. Although the exact etiologies of rheumatoid arthritis and type 1 diabetes mellitus are unknown, they are both likely due to a combination of genetic susceptibility and interactions between environmental risk factors and genes in both cases. Lately, it has become clear that this is also the case with rheumatoid arthritis and atherosclerosis, one of the vessel diseases known to be closely related to ischemic heart disease. The development of atherosclerosis shares features with that of rheumatoid arthritis. Understanding disease etiology is a complex task. Research fields and methods must often be combined in order to reach an increased understanding of the pathways altered in the diseased. Epidemiology constitutes one such research field, and in the last decades epidemiologic research based on clearly specified biologic hypotheses and carefully designed studies have provided important insights in disease etiology, not only disease distribution.

This thesis is based on four studies where epidemiologic methods are used to study disease etiology.

In order for the reader to gain a greater understanding of the results presented and discussed in this thesis, I have put together a short introduction to the problems studied.

1.1 RHEUMATOID ARTHRITIS

Rheumatoid arthritis is a chronic inflammatory joint disease. First symptoms are often fatigue, pain, morning stiffness and symmetric inflammation of the small joints of hands and feet. The clinical course is difficult to predict at onset, but evidence of aberrations in the humoral immune system, such as antibodies to citrullinated protein antigens (ACPA), has been associated with a more progressive disease with a higher disease activity and a higher burden of co-morbidities. Worldwide, the reported prevalence estimates of rheumatoid arthritis based on the American College of Rheumatology (ACR) criteria (see section 1.1.1 for a presentation of the criteria) ranges from 0.2% to 0.85% in populations above 16 years of age [1]. The reported annual incidence rates of rheumatoid arthritis vary between 0.2 and 0.4 cases per 1,000 inhabitants in populations above 16 years of age [1].

1.1.1 Classification and subsets

To classify rheumatoid arthritis different sets of criteria have been used over the years. Today, the 1987 revised ACR criteria for rheumatoid arthritis are in use (table 1)[2], but new criteria have been presented [3]. To be classified as having rheumatoid arthritis at least four of the seven criteria presented in table 1 must be met. The first four must have been present for at least six weeks to be

regarded as met. Present criteria have been criticized for making it too difficult to classify, and thus initiate appropriate treatment, individuals early in the disease course. Since rheumatoid factor (RF) is one of the criteria, they have also been criticized for primarily classifying individuals with a potentially severe and progressive disease. RF is prevalent in up to 80% of patients with rheumatoid arthritis and has historically been used to divide rheumatoid arthritis into two subsets; RF positive and RF negative disease. Although the sensitivity of RF is rather high, 80-90%, its specificity is rather low, 60-70% [4, 5]. Recently, a marker with 80% sensitivity and 98% specificity has been identified; ACPA, sometimes referred to as anti-cyclic citrullinated peptides [6, 7]. ACPAs are present in approximately 60% of all patients with rheumatoid arthritis. Both RF and ACPA have a role as prognostic factors [4, 7]. Being ACPA and/or RF positive at diagnosis is predictive of developing a more severe rheumatoid arthritis. With increasing knowledge about the fine specificity of the humoral response related to rheumatoid arthritis, the need for stratification based on serostatus has been highlighted. In etiologic research conducted the last decade it has been indicated that the two subsets of rheumatoid arthritis have different risk factors, genetic as well as environmental (see section 1.1.2 for more details on this).

Table 1. The 1987 revised ACR criteria for rheumatoid arthritis.

	Criterion	Definition
1	Morning stiffness	In and around the joints for more than one hour before maximum improvement
2	Arthritis of three or more joint areas	At least three joint areas simultaneously with soft tissue swelling
3	Arthritis of joints in hand	At least one area swollen in a wrist, metacarpophalangeal joint, or proximal interphalangeal joint
4	Symmetric arthritis	Involvement of the same joint areas on both sides of the body at the same time
5	Rheumatoid nodules	Subcutaneous nodules over bony prominences, extensor surfaces, or close to joints
6	Rheumatoid factor positivity	Rheumatoid factor levels above the 95th percentile of what's measured in controls.
7	Radiographic changes	Changes typical of rheumatoid arthritis on postero-anterior hand and wrist radiographies

1.1.2 Risk factors for rheumatoid arthritis

The patterns of risk factors for ACPA positive and negative rheumatoid arthritis differ from each other. HLA-*DRB1* is the strongest genetic contributor to rheumatoid arthritis, and as early as the 1980s the "shared epitope" alleles and their influence on risk of rheumatoid arthritis were discovered. The shared epitope alleles are all based within the HLA-*DRB1* gene on chromosome 6 and share one common feature – they all have a conserved amino-acid sequence at position 70-74 in the third hypervariable region [8, 9]. The risk alleles are HLA-*DRB1*01*, HLA-*DRB1*04* and HLA-*DRB1*10* and have been found to only confer an excess risk of ACPA/RF positive rheumatoid arthritis, not ACPA/RF negative rheumatoid arthritis [10, 11]. HLA-*DRB1* shared epitope has also been associated with more severe disease [12, 13]. Apart from these risk alleles, several other susceptibility genes have been

identified; the R620W allele in the *PTPN22* gene [14-16] *TRAF1/C5, STAT4* and *PADI4* [17-19] are all novel genetic risk factors. The R620W allele in *PTPN22* has also been found to exclusively be related to ACPA/RF positive rheumatoid arthritis, and also to other autoimmune diseases, such as type 1 diabetes mellitus [20]. ACPA negative rheumatoid arthritis has been associated with HLA-*DR3* [21, 22], and there are reports of other genes, such as *IRF5* and *DCIR* primarily associated with ACPA negative rheumatoid arthritis [23, 24].

Among the environmental risk factors, smoking is the most established. The evidence for a causal influence of smoking on risk of developing ACPA positive rheumatoid arthritis is overwhelming (extensively reviewed in a meta-analysis by Sugiyama *et al.* in [25]). Recently, smoking was found to interact with shared epitope alleles, indicating that carriers of shared epitope who smoke are subjected to an even greater risk of rheumatoid arthritis than smokers who are not carriers of shared epitope, and than non-smokers who are carriers are subjected to combined [26]. These findings further strengthen the idea of, at least, two distinct subsets of rheumatoid arthritis with different genetic and environmental risk factors and presumably different pathogenetic pathways [23]. In addition to smoking, alcohol consumption has been shown to modify the risk for RA [27-30]. First demonstrated in a US population, it has been suggested that high alcohol consumption compared to no alcohol consumption decreases the risk of rheumatoid arthritis. Other suggested risk factors are exposure to mineral oil [31, 32], inhalation of silica dust [33-36], low socio-economic status [37], and hormonal factors [38-40].

1.1.3 All-cause mortality in rheumatoid arthritis

Rheumatoid arthritis was for long considered to be a disease of great suffering, but with little or no impact on survival. It was a disease you died with, not from. In 1953, Cobb *et al.* [41] changed that view by presenting data that indicated that patients with rheumatoid arthritis died earlier in life than individuals without rheumatoid arthritis. In table 2 some of the published studies on mortality in rheumatoid arthritis are summarized. A large majority of these studies indicate that there is an increased mortality, even in contemporary cohorts of patients with rheumatoid arthritis. It seems like mortality indeed was increased in patients with rheumatoid arthritis diagnosed before 1995 and who were followed for a long time. In contemporary population-based patients with rheumatoid arthritis, followed from first diagnosis and for less than 10 years there are studies indicating that there is no increased mortality (REF). The cause-specific mortality has been extensively studied and the main causes of death are cardiovascular disease, infections and malignancies [42-48].

Table 2. Studies reporting all-cause mortality in cohorts of patients with rheumatoid arthritis

	Increased mortality	SMR*	Country
Cobb *et al.* 1953 [41]	Yes	1.32	US
Duthie *et al.* 1964 [49]	Yes	2.14	UK
Uddin *et al.* 1970 [50]	Yes	1.29	Canada
Monson and Hall 1976 [51]	Yes	1.86	US
Linos *et al.* 1980 [52]	No	1.16	US
Lewis *et al.* 1980 [53]	Yes	1.28	UK
Allebeck 1982 [42]	Yes	2.50	Sweden
Prior *et al.* 1984 [54]	Yes	3.00	UK
Pincus *et al.* 1984 [55]	Yes	1.31	US
Mitchell *et al.* 1986 [56]	Yes	1.51	Canada
Jacobsson *et al.* 1993 [57]	Yes	1.28	US
Wolfe *et al.*1994 [43]	Yes	2.26	US
Wållberg-Jonsson *et al.* 1997 [45]	Yes	1.57	Sweden
Symmons *et al.* 1998 [58]	Yes	2.7	UK
Sokka *et al.* 1999 [46]	No	1.28	Finland
Riise *et al.* 2001[47]	Yes	2.0	Norway
Björnådal *et al.* 2002 [48]	Yes	2.03	Sweden
Goodson *et al.* 2002 [59]	No	0.99	UK
Doran *et al.* 2002 [60]	Yes	1.27	US
Thomas *et al.* 2003 [61]	Yes	Women 1.97, Men 2.07	UK
Goodson *et al.* 2005 [62]	Yes	Women 1.84, Men 1.45	UK
Young *et al.* 2007 [63]	Yes	1.27	UK
Gonzalez *et al.* 2007 [64]	Yes	1.35	US
Radovits *et al.* 2010 [65]	Yes, after 10 years	1.40	Holland
Puolakka *et al.* 2010 [66]	No	0.97	Finland

*SMR- standardized mortality ratio

1.2   ISCHEMIC HEART DISEASE IN RHEUMATOID ARTHRITIS

Ischemic heart disease in rheumatoid arthritis has been extensively researched. Of the mortality studies published, many report increased ischemic heart disease mortality rates in rheumatoid arthritis compared to the general population. Already in 1976, Monson and Hall presented a study indicating that patients with rheumatoid arthritis treated at the Brigham Hospital in Boston between 1930 and 1960 suffered an almost doubled risk of dying from atherosclerotic heart disease compared to the risk in the general population [51]. This finding has since been replicated, with few exceptions (table 3). However, a high mortality from ischemic heart disease does not automatically mean that the incidence of ischemic heart disease is higher in the rheumatoid arthritis population; it could be that individuals with rheumatoid arthritis suffer more severe events leading to a higher case-fatality. Although there were studies indicating that ischemic heart disease was rather common among patients with rheumatoid arthritis [45], the first reports of ischemic heart disease morbidity in comparison with the general population were presented in 2001, when del Rincón *et al.* showed that patients with rheumatoid arthritis suffered an almost four-fold increased risk of cardiovascular events, incidence rate ratio 3.96 (95% confidence interval 1.86, 8.43) [67]. They used an outcome definition including cerebrovascular disease, as well as ischemic heart disease, but in 2003 Solomon *et al.* [68] presented data from the Nurses' Health Study, suggesting that there indeed was an increased risk of ischemic heart disease (in this case, myocardial infarction) in the rheumatoid arthritis population. Since then, this result has been replicated in many studies in different populations, a vast majority of them with established longstanding rheumatoid arthritis and unknown symptom duration at diagnosis who were diagnosed before 1995 (table 4).

Also contributing to the higher ischemic heart disease mortality is an increased case-fatality; in 2006 Solomon *et al.* [69] reported an increased 30-day mortality following myocardial infarction or stroke for patients with rheumatoid arthritis, relative risk 1.89 (95% confidence interval 1.56, 2.30). When Södergren *et al.* [70] in 2007 investigated the 28-day mortality following myocardial infarction they failed to replicate this finding, but instead report a hazard ratio of 1.67 (95% confidence interval 1.02, 2.71) for death within 10 years after acute myocardial infarction comparing patients with RF positive rheumatoid arthritis with controls. In accordance with these results is a study presented by Douglas *et al.* in 2006 [71]. In this study, authors followed 40 patients with rheumatoid arthritis and 40 controls from the date they had an acute coronary event and onwards. The 30-day mortality was not increased but there was an increased risk of dying from any cause at the end of the approximately 6.5 year long follow-up. The risk of dying from a cardiovascular event was also increased in patients with rheumatoid arthritis, as was the risk of having recurrent cardiac events.

In light of the different risk factor profiles in RF/ACPA positive/negative rheumatoid arthritis, it is interesting to note that researchers have stratified their analysis by RF status in only two of the studies of ischemic heart disease [62, 72]. Goodson *et al.* [62] reported an increased risk of dying from ischemic heart disease in RF positive as well as RF negative patients while Gonzalez *et al.* [72] reported of an increased mortality in RF positive but not RF negative patients with rheumatoid arthritis.

Table 3. Ischemic heart disease mortality in rheumatoid arthritis

	Country	Setting	Enrollment period	Follow-up	Outcome definition	Relative risk
Monson and Hall 1976 [51]	US	Hospital-based	1930-1960	From enrollment until death or 1 January 1972	Atherosclerotic heart disease	1.99
Lewis *et al.* 1980 [53]	UK	Clinic-based	1966-1976	1966-1976	Ischemic heart disease	1.43[*]
Linos *et al.* 1980 [52]	US	Population-based	1950–1974	Diagnosis until death, emigration, or 1 Jan 1975	Ischemic heart disease	No difference
Mutru *et al.* 1985 [73]	Finland	Clinic-based	1959–1968	1970-1980	Coronary heart disease	No difference
Erhardt *et al.* 1989 [74]	UK	Clinic-based	1976–1979	Inclusion until end of 1985	Myocardial infarction	4.7
Reilly *et al.* 1990 [75]	UK	Clinic-based	1957–1963	Inclusion and 25 years onwards	Cardiovascular disease	No difference
Wållberg-Jonsson *et al.* 1997 [45]	Sweden	Population-based	Up to1979	From diagnosis until death or end of 1994	Ischemic heart disease	1.54
Turesson *et al.* 1999 [76]	Sweden	Population-based	1990–1994	From first visit after 1 Jan 1990 until death or 30 June 1994	Heart disease	1.9[*]
Björnådal *et al.* 2002 [48]	Sweden	Hospital-based	1964–1994	First discharge with RA until death or 31 Dec 1995.	Coronary heart disease	1.79
Goodson *et al.* 2002 [59]	UK	Population-based	1990-1994	Started at inclusion and ended in 1999, at death or emigration	Heart disease	No difference
Watson *et al.* 2003 [77]	UK	Population-based	1987 to last encounter	Started at age 40, first visit to practice, registration, or first date of RA diagnosis. Ended at last visit, death or event	Myocardial infarction	Women 1.7, men 1.5[#]

Table 3 continued.

	Country	Setting	Enrollment period	Follow-up	Outcome definition	Relative risk
Goodson *et al.* 2005 [62]	UK	Clinic-based	1981–1996	From inclusion to death, or 31 Dec 2002	Ischemic heart disease	Women 1.95, men 1.46
Maradit-Kremers *et al.* 2005 [78]	US	Population-based	1955-1995	From first fulfillment of ACR criteria until death, migration or 1 Jan 2001	Sudden death	1.94
Young *et al.* 2007 [63]	UK	Population-based	1986-1997	From inclusion until death, emigration or1997	Cardiovascular disease	1.49
Gonzalez *et al.* 2008 [72]	US	Population-based	1955-1995	From first fulfillment of ACR criteria until death, migration or 1 Jan 2006	Ischemic heart disease	RF+ 1.4, RF- 1.0
Bergström *et al.* 2009 [79]	Sweden	Population-based	1978 and 1996	1978-1985, 1995-2002	Ischemic heart disease	1.75 and 1.72
Radovits *et al.* 2010 [65]	Holland	Clinic-based	1985-2008	From inclusion until death or 15 March 2008	Coronary heart disease	No difference

[*]Crude estimates calculated by dividing the observed number of deaths given in the report with the expected [#]Incidence rate ratio calculated by dividing the rate in rheumatoid arthritis with that in individuals without rheumatoid arthritis.

Table 4. Ischemic heart disease morbidity in rheumatoid arthritis

	Country	Setting	Enrollment period	Outcome definition	Follow-up	Absolute risk RA/non-RA	Relative risk (95% CI)
del Rincón *et al.* 2001 [67]	US	Clinic-based	1996	Self-reported hospitalized CV event, chart verified	1996-1997	0.3/ 0.06/1,000 py	Incidence rate ratio: 3.96 (1.86, 8.43)
Solomon *et al.* 2003[68]	US	Population-based	1977-1996	Self-reports of MI, chart verified	Started at confirmed diagnosis of RA, ended at death, event of 31 May 1996	2.7/1.0/1,000 py	Incidence rate ratio: 2.0 (1.23, 3.29)
Turesson *et al.* 2004 [80]	Sweden	Population-based	1997	Hospitalizations listing MI	1 July 1997 until 31 December 1999	Cumulative incidence: 3.5%/1.2%*	Standardized morbidity ratio: 1.76 (1.23, 2.44)
Watson *et al.* 2003 [77]	UK	Clinic-based	1987-? (at the latest 2000)	MI by chart review	Started at age 40, first visit, registration or RA diagnosis, ended last visit, death or event, mean 4.7 years	Incidence rate: 10.1/7.1/1,000py, (men), 5.8/3.5/ 1,000 py (women)	Incidence rate ratio: 1.3 (1.2, 1.4)
Fisher *et al.* 2004 [81]	UK	Clinic-based	1995-April 2002	MI in the GPRD, exposure RA	N/A	N/A	Odds ratio: 1.47 (1.23, 1.76)
Goodson *et al.* 2005 [62]	UK	Clinic-based	1981-1996	Hospitalizations listing MI, IHD	1 April 1994 or immigration to area until death, admission, emigration, 31 March 2002	N/A	MI: 1.3 (0.6, 2.5), IHD 0.8 (0.5, 1.3) (women). 0.7 (0.3, 1.9), 0.6 (0.3, 1.1)
Maradit-Kremers *et al.* 2005 [82]	US	Population-based	1955-1994	Hospitalized MI, unrecognized MI detected via chart review	Started at first fulfillment of ACR criteria, ended at death, event, Jan 2001, mean ~15 years	Hospitalized: 4.0/4.6/1,000py, unrecognized 2.7/1.5/1,000 py	Hazard ratio: Hospitalized 1.09 (0.71, 1.68), unrecognized 2.13 (1.13, 4.03)
Solomon *et al.* 2006 [69]	Canada	Hospital-based	1999-2003	Hospitalizations listing MI	From third admission for RA until event, emigration, death or end of 2003	Incidence rate: 5.3/2.9/1,000 py	Incidence rate ratio: 1.9 (1.7, 2.0)

Table 4 continued

	Country	Setting	Enrollment period	Outcome definition	Follow-up	Absolute risk RA/non-RA	Relative risk
Han *et al.* 2006 [83]	US	Clinic-based	2001-2002	Ischemic heart disease listings in insurance database	1 Jan 2001-31 Dec 2002	N/A	Prevalence ratio: 1.5 (1.4, 1.6)
Södergren *et al.* 2007 [70]	Sweden	Population-based	Up to 1979	Myocardial infarction, WHO criteria	1985-2003	N/A	Standardized incidence ratio: 2.9 (1.9, 4.1)
Kremers *et al.* 2008 [84]	US	Population-based	1955-1994	Revascularization, silent +non-fatal MI, congestive heart failure, CV death from chart review	Started at first fulfillment of ACR criteria, ended at death, event, Jan 2001, mean ~15 years	Incidence rate: 49.5/ 31.7/1,000 py.	Incidence rate ratio: 1.6[#]
Wolfe and Michaud 2008 [85]	US	Clinic-based	1999-2006	Self-reports of MI, chart verified	Started at first questionnaire received, ended at last received. Mean 3 years.	*RA: 4.4/1,000 p-years, non-RA: 3.0/1,000 p-years	Hazard ratio: 1.9 (1.2, 2.9)
Bergström *et al.* 2009 [79]	Sweden	Population-based	1:1978 and 2:1996	Ischemic heart disease	1:1978-1985, 2:1995-2002	N/A	Standardized morbidity ratio: elevated in both cohorts

[*]Person-years calculated by multiplying the number of individuals with the mean length of follow-up. Cumulative incidence was calculated by dividing the number of events during follow-up with the number of individuals who were followed in each group. [#]calculated by dividing the rate among patients with rheumatoid arthritis with the rate among controls. MI- myocardial infarction, CV-cardiovascular, RA-rheumatoid arthritis, N/A- not assessed/not applicable, GPRD- general practice research database

1.2.1 Risk factors for ischemic heart disease

1.2.1.1 Traditional risk factors and their impact in rheumatoid arthritis

In the general population, several risk factors for ischemic heart disease have been identified and established. They are often referred to as "traditional" risk factors, and include age, male sex, smoking, overweight, dyslipidemia, hypertension, family history of ischemic heart disease and diabetes mellitus [86]. In rheumatoid arthritis, reports on the prevalence of these traditional risk factors compared to that in the general population are inconclusive, and differences in study design and study population make many of them difficult to compare [67, 68, 71, 87-97]. There are studies that have assessed the effect of each of the traditional risk factors on risk of ischemic heart disease in patients with rheumatoid arthritis and controls [97-99]. Wållberg-Jonsson *et al.* demonstrated in 1999 [98] that adding one year to age at diagnosis of rheumatoid arthritis increased the risk of cardiovascular disease by 7%, being hypertensive at baseline by 150%, and being male by 90% [98]. Bergenström *et al.* [79] also demonstrated that hypertension was associated with an increased risk of cardiovascular events and Gonzalez *et al.* [97] demonstrated that being hypertensive increased the risk of cardiovascular events by 97%, but in their study male sex was not associated with an increased risk. In a study by Naranjo *et al.* [99] authors studied not only cardiovascular disease, but also myocardial infarction in particular, and observed that being a woman was protective, adding one year to age at baseline increased the risk by 4% and being hypertensive was not associated with an increased risk. Having ever smoked was associated with a three-fold increased risk of myocardial infarction in the study by Naranjo *et al.* but in the studies by Wållberg-Jonsson *et al.* and Gonzalez *et al.* smoking was not associated with an increased risk of cardiovascular disease (including stroke in the study from Sweden and heart failure in the study from the US). Diabetes mellitus was not associated with an increased risk of myocardial infarction in the study by Naranjo *et al.* or of cardiovascular disease in the study by Wållberg-Jonsson *et al.* In the study by Gonzalez *et al* it was though. Overweight, defined as a body mass index ≥25 kg/m2 (a body mass index between 20 and 25 is considered normal), has been associated with an increased risk of ischemic heart disease in the general population [100]. In rheumatoid arthritis, the opposite has been found; underweight rather than overweight seem to infer a greater risk of cardiovascular disease. In two studies from the Mayo Clinic, Maradit-Kremers *et al.* [101] reported in 2004 and Gonzalez *et al.* reported in 2008 [97] that having a body mass index below 20 kg/m2 at diagnosis of rheumatoid arthritis compared with a normal body mass index was significantly associated with cardiovascular death and morbidity. A high body mass index has not been associated with cardiovascular disease or myocardial infarction [97, 99]. A few studies have adjusted for traditional risk factors for cardiovascular disease and found that the increased risk of ischemic heart disease is not markedly modified [67, 97]. For example, del Rincón *et al.* published a study in 2001 [67] where they found that even after adjusting for the traditional risk factors age, sex, diabetes mellitus, systolic blood pressure, body mass index, cigarette smoking, and hypercholesterolemia having rheumatoid arthritis was still associated with a three-fold increase in the incidence rate of cardiovascular disease, incidence rate ratio 3.17 (95% confidence interval 1.33, 6.36).

1.2.1.2 Rheumatoid arthritis related risk factors for ischemic heart disease

Since traditional risk factors for ischemic heart disease cannot explain the increased risk of ischemic heart disease in rheumatoid arthritis, other risk factors have also been investigated and identified. Severe extra-articular manifestations have been associated with an increased risk of first ever cardiovascular event [102], first myocardial infarction [99] and cardiovascular mortality [78]. Disability,

measured by the Health Assessment Questionnaire, has also been identified as a predictor for cardiovascular mortality [63, 103], increased atherosclerosis [104], and cardiovascular events [105]. The fact that disability and severe extra-articular manifestations are predictors of cardiovascular disease correlates well with the findings first published by Wållberg-Jonsson *et al.* in 1999 [98], indicating that an increased erythrocyte sedimentation rate one year after the diagnosis of rheumatoid arthritis was associated with an increased risk of cardiovascular disease.

The association between inflammatory activity and cardiovascular disease in this population has since been replicated. Maradit-Kremers *et al.* [78] demonstrated an association between cardiovascular death and three or more measurements of erythrocyte sedimentation rate above or equal to 60 mm/hour at any time before the event, Goodson *et al.* [106] demonstrated that c-reactive protein levels above four at baseline were associated with an increased risk of cardiovascular death during follow-up, and Gonzalez-Gay *et al.* published in 2007 a study where they demonstrated that both increased erythrocyte sedimentation rate and c-reactive protein were associated with cardiovascular mortality and morbidity [107]. Although convincingly replicated, it must be said that Bergenström *et al.* [79] failed to demonstrate an association between erythrocyte sedimentation rate and cardiovascular disease, as did Kapetanovic *et al.* [108].

RF positivity has been associated with an increased cardiovascular mortality [109-111] and morbidity [110, 112] in the general population. Liang *et al.* [113] also demonstrated this and tried to assess the effect of ACPA positivity in both patients with rheumatoid arthritis and individuals without any rheumatic disease but were underpowered to make any conclusions. Goodson *et al.* [106] investigated RF positivity and its effect on cardiovascular death in rheumatoid arthritis and found that it indeed increased the risk three-fold after adjusting for age, sex, disease severity score, and smoking, while Bergenström *et al.* [79] failed to detect any associations between RF positivity and cardiovascular events.

In 2007, the first study assessing genetic risk factors for rheumatoid arthritis and their effect on the risk of cardiovascular disease was published. Gonzalez-Gay *et al.* investigated the effect of HLA-*DRB1* on both cardiovascular mortality and morbidity and found that carrying one or two copies of HLA-*DRB1*0404* was associated with an increased risk of first time cardiovascular events and cardiovascular mortality. In 2003, the same group, published data indicating that HLA-*DRB1*04* increased risk of endothelial dysfunction in the same population [114]. Farragher *et al.* published a study in 2008 assessing the influence of HLA-*DRB1* alleles on cardiovascular mortality. They also assessed the influence of *PTPN22* on the same outcomes. They found that carriers of two copies of HLA-DRB1*01/*04 were at a two-fold increased risk of cardiovascular death compared to those who had no or one shared epitope allele, but found no influence of *PTPN22* on cardiovascular death [115]. *TRAF1/C5* was also assessed as a risk factor for cardiovascular death in rheumatoid arthritis, and there was no association between the minor allele and the risk of cardiovascular disease [116]. Recently, Palomino-Morales *et al.* reported that patients with rheumatoid arthritis with a certain polymorphism in the *MTHFR* gene had a statistically significant increased risk of suffering cardiovascular events compared with those not carrying any copy of the specific polymorphism [117].

1.3 ATHEROSCLEROSIS

In recent years, it has become evident that atherosclerosis is an inflammatory process. The first step in this process is endothelial dysfunction, characterized by an increased expression of endothelial

leukocyte adhesion molecules and a change in endothelial permeability allowing the leukocytes and other inflammatory cells to migrate into the intima of the vessel wall [118-120]. These changes are often caused by external stimuli, such as chemical factors related to smoking or diabetes mellitus. Hypertension has also been found to lead to endothelial dysfunction, as well as oxidized low-density lipoproteins and elevated plasma homocystein levels [118, 120]. In the intima, macrophages differentiate into foam cells, and fatty streaks are formed. Fatty streaks are pre-cursor lesions populated by activated T-cells [118, 120]. If the inflammatory processes continue, smooth muscle cells in the vessel walls are stimulated to migrate into the intima and proliferate. Also, the production of extra-cellular matrix is stimulated, altogether resulting in a thickened intima with populations of inflammatory cells, a core of lipids and necrotic tissue covered by a fibrous cap mainly consisting of collagen, an atheroma [118, 120]. Metalloproteinases expressed by activated macrophages degrade the collagen in the fibrous cap causing the plaque to rupture, and thereby trigger a thrombotic event resulting in an infarction [118, 120]. In about 30% of the myocardial infarctions there is no plaque rupture. Instead pro-thrombotic inflammatory cells cause a thrombus resulting in an infarction [118].

1.3.1   Atherosclerosis in rheumatoid arthritis

In 2001, the first study indicating that patients with rheumatoid arthritis have an increased prevalence of atherosclerosis, compared to the general population, was published [121]. In that study, the intima-media thickness of the common carotid arteries was measured in patients with rheumatoid arthritis and age-, and sex- matched general population controls. This has been replicated and confirmed in established rheumatoid arthritis [90, 94, 122-124]. A majority of studies investigating atherosclerotic changes in coronary arteries have used computerized tomography to detect coronary artery calcification [95, 125-128]. Several indicate that the prevalence of coronary artery calcification is increased in established rheumatoid arthritis [95, 125-127] and some that the severity is [95, 127, 128].

Interestingly, patients with newly diagnosed rheumatoid arthritis are no different from age-, and sex-matched controls [129] but the intima-media thickness [129] and the prevalence and severity of coronary artery calcification [95, 125] is increased compared to controls after some time with rheumatoid arthritis. To my knowledge, only one study has been published that uses angiography to assess clinically significant coronary stenosis in patients with coronary symptoms [130]. The results from that study suggested that patients with rheumatoid arthritis and symptoms of angina pectoris who underwent angiography had a more widespread coronary stenosis than individuals without rheumatoid arthritis. In an autopsy study by Aubry *et al.,* published in 2007 [131], authors studied the extent and severity of coronary stenosis in patients with rheumatoid arthritis and age-, sex-, and autopsy date-matched controls. In a subgroup analysis, only individuals who had a history of cardiovascular disease were included in analysis. They then found that patients with rheumatoid arthritis were less likely to have multiple vessel disease, defined as two or more coronaries with grade 4 stenosis in left anterior descending, left circumflex and right coronary artery or grade 3 or 4 in the left main artery, than controls (32% and 61% respectively, p=0.0018). They also found that patients with rheumatoid arthritis and cardiovascular disease had a less severe coronary atherosclerosis, both in terms of extent (number of vessels with stenosis) and grade (obstruction of cross-sectional area in percent).

1.3.2   Inflammation in atherosclerosis and in rheumatoid arthritis

The inflammatory events leading to atherosclerosis are similar to those seen in the pathogenesis of rheumatoid arthritis; T-cell and mast cell activation, production of tumor necrosis factor-α and

interleukin-6, and an increased expression of leukocyte adhesion molecules. Further, patients with rheumatoid arthritis have elevated levels of acute phase reactants, such as c-reactive proteins, a marker of inflammation that is associated with an increased cardiovascular risk. They also have an increased intima-media thickness which correlates with markers of systemic inflammation in both patients with rheumatoid arthritis and the general population. What causes the actual rupture of the plaques responsible for approximately 70% of the events occurring, is yet to be discovered. It has been suggested that tumor necrosis factor is involved in not only the development of plaques but also the rupture and there are tissue studies indicating that inflammatory cells are lining the borders of the atherosclerotic plaques, probably promoting plaque rupture.

1.4   RATIONALE FOR STUDIES INCLUDED IN THIS THESIS

1.4.1   Study I

Existing studies investigating the association between rheumatoid arthritis and diabetes mellitus have done so without stratifying their analyses by ACPA status among the patients with rheumatoid arthritis which, considering the different patterns of risk factors behind the two subsets of disease is pivotal. Nor have they been able to assess the two types of diabetes mellitus separately in relation to rheumatoid arthritis. In comprehensive assessments of autoimmune co-morbidity taking established common genetic risk factors, as well as geno- and pheno-typic aspects of the diseases under study into account is important. We aimed to do so and hypothesized that type 1 diabetes mellitus, an autoimmune disease that shares *PTPN22* as a susceptibility gene, may be associated with rheumatoid arthritis, and that this association may be dependent on the phenotype defined by the presence or absence of ACPA.

1.4.2   Study II

One way to assess the relative importance of a cause-and-effect association vs. shared etiology for ischemic heart disease in rheumatoid arthritis is to assess the timing of ischemic heart disease in relation to rheumatoid arthritis onset. Ischemic heart disease risks exclusively increased after onset of rheumatoid arthritis would support a cause-and-effect association between rheumatoid arthritis and ischemic heart disease. By contrast, increased occurrence of ischemic heart disease already before onset of rheumatoid arthritis would/could indicate that there is an impact of shared risk factors or etiologies. Some support for an increased occurrence and an altered spectrum of ischemic heart disease already before diagnosis of rheumatoid arthritis was presented by Maradit-Kremers *et al.* [82]. They studied 603 rheumatoid arthritis patients diagnosed 1955-1995 and 603 controls with respect to the occurrence of ischemic heart disease before first fulfilment of the ACR criteria, and a higher than expected occurrence of hospitalized myocardial infarction and unrecognized myocardial infarction prior to diagnosis of rheumatoid arthritis was reported. In that study, however, the occurrence of ischemic heart disease was assessed up until the time of fulfilment of the ACR criteria for rheumatoid arthritis, without any restrictions on the duration of rheumatoid arthritis symptoms before fulfilment of the ACR-criteria. As a consequence and as concluded by the authors, the increased occurrence of ischemic heart disease before rheumatoid arthritis might have been attributed to ongoing inflammatory activity predating the time-point of fulfilment of the ACR-criteria, thus precluding the distinction between shared etiology and cause-and-effect association. To assess the relative contributions of a causal association and shared etiologies to the increased risk of ischemic heart disease in patients with rheumatoid arthritis, and to circumvent confounding of *pre* rheumatoid arthritis occurrence by true *post* rheumatoid arthritis risks, we therefore used two large cohorts of patients diagnosed with

rheumatoid arthritis shortly after onset of rheumatoid arthritis symptoms (median latency = 6 months), and two general population control cohorts. In these cohorts, we set out to (i) confirm an increased risk of ischemic heart disease after rheumatoid arthritis diagnosis, and (ii) to investigate the relative risk and phenotype of ischemic heart disease up until onset of symptoms of rheumatoid arthritis. Since there have been indications of marked etiologic heterogeneity in different rheumatoid arthritis subsets [132], we assessed the occurrence of ischemic heart disease prior to onset of rheumatoid arthritis symptoms in RF/ACPA positive and negative rheumatoid arthritis separately. We also stratified by presence or absence of any shared epitope allele, for which an independent link with cardiovascular disease risk in rheumatoid arthritis has been reported [107, 115].

1.4.3 Study III

The next step in distinguishing between shared etiology and rheumatoid arthritis specific factors influencing the risk of ischemic heart disease was to study the evolution of ischemic heart disease risks following diagnosis of rheumatoid arthritis. In contrast to the wealth of literature on risks among patients with longstanding rheumatoid arthritis, only four studies have assessed risks of cardiovascular disease in inception cohorts of rheumatoid arthritis. Goodson *et al.* [62] reported an increased standardized mortality ratio for ischemic heart disease in both women and men diagnosed with rheumatoid arthritis1981-1996. Solomon *et al.* [69] reported an increased incidence rate ratio for myocardial infarction among patients first classified with rheumatoid arthritis 1999-2003 compared to controls. Young *et al.* [63] reported an increased standardized mortality ratio cardiovascular disease in patients diagnosed 1986-1997, and Maradit-Kremers *et al.* [84] reported a higher cardiovascular disease incidence rate among rheumatoid arthritis patients diagnosed 1955-1995 than in the general population. Based on this, and on the findings in *study II* (indicating no increased occurrence of ischemic heart disease before first symptom of rheumatoid arthritis), we wanted to explore the temporal evolution of ischemic heart disease risks following onset of rheumatoid arthritis. Because of the etiologic heterogeneity in the different subsets of rheumatoid arthritis, we also stratified by RF status.

1.4.4 Study IV

The results from *study II* and *study III,* indicating that the risk of ischemic heart disease increases rapidly from diagnosis of rheumatoid arthritis, gave rise to questions regarding the development of coronary artery disease in this population. The etiology behind the increased occurrence of acute coronary syndromes in rheumatoid arthritis is not established. It has been suggested that an increased prevalence of atherosclerosis, a key factor in the development of clinical manifest acute coronary syndrome in the general population, could be one part of the explanation. For instance, several studies have reported increased carotid intima-media thickness in patients with established rheumatoid arthritis [90, 94, 121-124]. Studies using computerized tomography to detect coronary artery calcification [95, 125-128] indicate that the prevalence [95, 125-127] and possibly also severity [95, 127, 128] of coronary artery calcification is increased in established rheumatoid arthritis. Interestingly, in newly diagnosed rheumatoid arthritis, neither intima-media thickness [129] nor the prevalence or severity of coronary artery calcification [95, 125] is different from that among age-, and sex-matched controls, whereas it increased later during the course of rheumatoid arthritis [129]. With respect to coronary angiography, only one study has assessed the occurrence of clinically significant coronary stenosis in patients with coronary symptoms [130]. The results from that study, including 75 patients with rheumatoid arthritis complicated by angina leading to angiography and 128 controls with the same coronary symptoms but without rheumatoid arthritis, suggested that more patients with rheumatoid arthritis had significant coronary artery involvements than controls [130]. Studies of the distribution of

coronary stenoses in rheumatoid arthritis complicated by other acute coronary syndromes manifestations are lacking, at least coronary angiography patterns among patients with rheumatoid arthritis subject to angiography at the time of the acute coronary syndromes event.

The primary aim of our study was therefore to study, in the same large rheumatoid arthritis inception cohort from which we have previously reported acute coronary syndromes risks (*study III*), the extent and distribution of coronary stenosis in patients with rheumatoid arthritis undergoing angiography due to manifest acute coronary syndromes compared to that in the population comparators with acute coronary syndromes. We also wanted to compare the distribution of coronary artery stenoses between patients with rheumatoid arthritis who develop acute coronary syndromes early vs. later during the rheumatoid arthritis disease course, and to study whether the pattern of coronary stenosis differ between RF positive or negative rheumatoid arthritis.

2 OBJECTIVES

2.1 OVERALL OBJECTIVE

The overall objective of this thesis is to gain greater knowledge of how other diseases are related to rheumatoid arthritis and to increase our understanding of what mechanisms underlie the increased ischemic heart disease risk seen in rheumatoid arthritis.

2.2 SPECIFIC OBJECTIVES

We have addressed four specific questions:

1. Is diabetes mellitus a risk factor for rheumatoid arthritis?
2. Do patients with rheumatoid arthritis have an increased history of ischemic heart disease at the time of first symptom of rheumatoid arthritis compared to population-based controls?
3. When, after diagnosis of rheumatoid arthritis, is there an increased risk of ischemic heart disease?
4. Among patients with acute coronary syndrome, is the extent of significantly stenoses higher in patients with rheumatoid arthritis compared to population-based controls?

3 METHODS

3.1 SETTING

All of the studies included in this thesis were conducted in Sweden, which has a long tradition of keeping track of its inhabitants. Already in the 17th century, ministers registered information about their parishioners, and ever-since the inhabitants of Sweden have, from an international perspective, been conspicuously willing to have potentially sensitive information registered about them in different registers and to participate in studies by answering questionnaires and even provide researchers with biological samples.

This long tradition of keeping registers has been a fertile soil for registers to grow. Today we have completely covering census registers, including nationwide registers on hospitalization and healthcare usage with nearly 100% coverage. Recently, a new type of register has evolved; the quality registers. The quality registers in Sweden have typically been formed by interested parties within the medical profession itself. The registers were initiated to evaluate the quality of the care given on a group level. There is much hope to their potential in research, with more detailed information on the diagnoses and more clinical information on each patient treated for this disease. Chronic conditions with a long follow-up, inpatient treatment and a need for continuous evaluation of disease progress, such as in rheumatoid arthritis, are aptly suited for quality register follow-up. This is largely due to the fact that the entries made by the treating physician also facilitate the physician's longitudinal follow-up of his/her patients.

These factors, in combination with the national registration number [133], unique to each resident in Sweden, have been of great importance to the epidemiologic research conducted in Sweden. To have population-based cohorts of individuals with the disease of interest, the ability to sample controls from the general population in a completely non-biased manner, and to be able to follow these individuals over time with very little loss of follow-up (at least with respect to events that are fatal or lead to hospitalization) provides a solid ground for epidemiologic research with high external and internal validity. With this said, it must be emphasized that even the best of data can give rise to false results when used and analyzed carelessly.

3.2 DATA SOURCES USED

3.2.1 The Epidemiologic Investigation of Rheumatoid Arthritis

3.2.1.1 EIRA in short

The Epidemiologic Investigation of Rheumatoid Arthritis (EIRA) is a population-based case-control study of incident rheumatoid arthritis. It was initiated in 1996 and is still enrolling new cases and controls. In May 2006, 1998 cases, all fulfilling the 1987 revised ACR criteria for rheumatoid arthritis, and 2,252 controls had been included. Of those, 71% were women, 62% were ACPA positive and 66% RF positive. More information on the EIRA study design can be found in several publications, e.g. Stolt *et al.* published in 2003 [134].

3.2.1.2 Identification of cases and controls

Cases are recruited from private and hospital-based rheumatology units throughout mid- and south Sweden and must be diagnosed with rheumatoid arthritis by a rheumatologist, be between 18 and 70

years old at inclusion, speak Swedish, and give their consent to be included. Typically, each patient with rheumatoid arthritis is included in the study by a rheumatologist upon diagnosis, a blood sample is drawn for serologic analysis and DNA extraction, and an extensive questionnaire is distributed to each case to be filled out. When a patient with rheumatoid arthritis has been included, a control is randomly selected using The Swedish Register of Population and Population Changes (see section 3.2.4 for more information on this register). The control is matched to its case by age, sex, and residential area. Since controls are selected continuously as cases are enrolled, cases and controls are also, in practice, matched on calendar year. A questionnaire, identical to the one given to the cases, is sent to each control. If an identified control does not return the filled out questionnaire after being reminded four times within eight weeks, a new control is drawn from the Swedish Register of Population and Population Changes. This process is re-iterated until there is questionnaire data available for one control per case. When EIRA was initiated it included cases with undifferentiated poly-arthritis, and controls were selected for each of these individuals as well. These cases are excluded from all analyses but the controls selected are not. This is why, although EIRA is a 1:1 matched case-control study, there are more controls than cases in the analyses reported. The participation proportion is 95% among eligible cases. Among controls, 81% of the individuals first asked to participate answered the questionnaire. The controls that consented to participate were also asked to provide a blood sample and 60% of all participating controls did so.

3.2.1.3   *Questionnaire information*

The questionnaire that is used to gather information on cases and controls includes a wide range of questions, all aiming to assess different kinds of environmental exposures occurring before inclusion in EIRA. Examples of the exposures assessed are weight at inclusion, weight at 20 years of age, height, food habits, work-place exposures, smoking habits, alcohol consumption, and family composition. Special effort is put into specifying the approximate time of first symptom of RA in the cases.

3.2.1.4   *Serology and genetic information*

Rheumatoid factor status was determined using nephelometry and the cutoff for being classified as rheumatoid factor positive was specified at each respective laboratory performing the nephelometry. ACPAs were detected with an Immunoscan-RA Mark2 enzyme-linked immunosorbent assay. Levels above 25 units/ml were considered positive for antibodies to citrullinated protein antigens. HLA–*DRB1* and *PTPN22* typing and sub-typing were performed using polymerase chain reaction–based methods. *DRB1*01, *04, or *10* alleles constituted the shared epitope.

3.2.2   The Swedish Rheumatology Register

3.2.2.1   *The Swedish Rheumatology Register in short*

The Swedish Rheumatology Register (SRR, sometimes referred to as the Swedish Rheumatoid Arthritis Register [RAR]) was initiated in 1995 and provides rheumatologists in Sweden with a clinical tool to evaluate and monitor their patients, and a useful basis for assessing quality of care in this population. Initially, it included patients newly diagnosed with rheumatoid arthritis and patients on biologic treatment only but has, in recent years, expanded to also include patients with established disease, patients with long symptom duration at diagnosis and other rheumatologic diseases, regardless of treatment. Today the register is used throughout Sweden. By September 1st 2009, more than 30.000 patients with rheumatic diseases (predominantly rheumatoid arthritis) were followed using the register and 191.861 visits had been entered [135].

3.2.2.2   Inclusion and coverage

Typically, a patient with rheumatoid arthritis is included in Swedish Rheumatology Register at the first visit after debut of symptoms, when biologic treatment is initiated, or when a physician would like to use the register to follow all of his/her patients, regardless of the two previous criteria.
The Swedish Rheumatology Register has been estimated to include 51% of all newly diagnosed patients with rheumatoid arthritis in Sweden (personal communication Jonas Eriksson, unpublished results). This is based on an analysis where incident patients with rheumatoid arthritis were defined as individuals who presented in outpatient specialist care at least twice, were treated with anti-rheumatic therapies, and had no previous inpatient visits for rheumatoid arthritis. Exposure to anti-rheumatic treatment, including DMARDs, biologics and steroids, for the analysis was ascertained from the Prescribed Drug Register and the Swedish Biologics Register ARTIS, while data on inpatient and outpatient specialist visits were retrieved from the National Patient Register (partly described in *section 3.2.4.1*).

3.2.2.3   Information found in the Swedish Rheumatology Register

When the decision has been made to use the Swedish Rheumatology Register, the physician enters information on diagnosis, debut of symptoms, ACR criteria, RF status, disease activity, and previous treatment into the database at the first visit. At each follow up visit, which is typically held at pre-specified intervals, information on disease activity parameters, such as c-reactive protein and erythrocyte sedimentation rate, number of swollen and tender joints, current treatment (including biologic treatment), and the physicians global assessment of disease activity is collected. The patient also fills out the Health Assessment Questionnaire and assesses his/her pain and overall well-being by using a visual analog scale.

3.2.3   The Swedeheart Register

In 1991, a national quality register for percutaneous coronary interventions was initiated, aiming to register information on all percutaneous coronary interventions performed in Sweden. Parallel to this register, the Swedish Coronary Angiography Register registered information on all coronary angiographies performed. In 1998, these two registers were joined together, forming the Swedish Coronary Angiography and Angioplasty Register (referred to as SCAAR in study IV). Since 2001, close to 100% of all angiographies performed in Sweden are entered into this register. Information available in this register is date of investigation, indication for and findings on investigation, and other clinical parameters' such as treatment before angiography. Recently, the Swedish Coronary Angiography and Angioplasty Register was merged with the Register of Information and Knowledge about Swedish Heart Intensive care Admissions, and is now referred to as the Swedeheart Register. For this thesis, only information regarding angiographies was used.

3.2.4   National health registers and demographic registers

3.2.4.1   The Swedish Inpatient Register

In Sweden, nearly all inpatient care is public and virtually equally accessible to all residents. The small discrepancies in accessibility noted are not due to socio-economic factors, but rather to differences in referral practices across counties in Sweden. For administrative purposes, and lately also for remuneration purposes, data on hospitalizations have been registered by county and computerized since 1964, and nationwide with complete coverage since 1987. These data have been gathered in the

Inpatient Register, also referred to as the Hospital Discharge Register, and include, among other things, the national registration number of the individual hospitalized, date of admission, date of discharge, as well as primary and secondary discharge diagnoses (coded according to *International Classification of Diseases*, seventh through tenth revision). Registrations indicating hospitalizations for acute myocardial infarction have been validated using the medical records of each hospitalization and with international criteria for myocardial infarction as the gold standard. The positive predictive value of being identified with a myocardial infarction was found to be 95%[136][137]. Since reporting to the Inpatient Register is mandatory, approximately 99% of all hospitalizations are reported.

3.2.4.2   *The Swedish Cause of Death Register*

Information on deaths and causes of deaths in Swedish residents has been recorded since the 18th century. In the 20th century, this information was gathered in a structured way and since 1961 there is computerized information on all deaths of Swedish residents. The national registration number for the deceased, date of birth, date of death, and sex are registered annually for each death occurring. The cause of death, with one underlying cause of death and several contributory causes of death (coded according to *International Classification of Diseases*, seventh through tenth revision), is available for 99.5% of all deaths occurring (including deaths occurring abroad). The information found in this register is based on data found on the death certificate filled out by the physician who declared the individual dead.

3.2.4.3   *The Swedish Myocardial Infarction Register*

The Swedish Myocardial Infarction Register was initiated in 1987, and includes information on all hospitalizations listing a primary or secondary discharge diagnosis of myocardial infarction from the above described inpatient register, and all individuals with myocardial infarction as underlying or contributory cause of death in their death certificate, including sudden out of hospital deaths, as well as cases who died upon or during hospitalization.

3.2.4.4   *The Swedish Register of Population and Population Changes*

The Swedish Register of Population and Population Changes comprises all official Swedish census data since 1961. The information found in this register is based on data from the Tax authorities, which receives information on all Swedish residents from other public authorities and agencies. To be entitled to social benefits and to be able to vote, correct information must be given to the Tax authorities. All individuals residing in Sweden who are still alive at the end of the year are included with information on the national registration number, place of birth, citizenship, marital status, and immigration and emigration from Sweden (since 1968).

3.3   STUDY POPULATIONS AND STUDY DESIGNS

By linking the above described data sources, two population-based cohorts of rheumatoid arthritis (the Swedish Rheumatology Register cohort and the EIRA cohort) could be followed prospectively, starting at the very date of diagnosis with respect to mortality (using the Swedish Cause of Death Register), hospitalizations (using the Swedish Inpatient Register) and with respect to angiographies performed (using the Swedeheart Registers). We were also able to study the pattern of hospitalization prior to diagnosis of rheumatoid arthritis. For individuals included in the EIRA study, both cases and controls, questionnaire information on exposures occurring before first symptom of rheumatoid arthritis in the cases and before inclusion in the controls, as well as serology and genetic information was available.

Using the Swedish Register of Population and Population Changes, controls were identified to each of the patients with rheumatoid arthritis. As stated in section 3.2.1.2, the controls for the EIRA cases were matched (1:1) with respect to variables age, sex, county, and calendar year of inclusion in EIRA. The controls for the Swedish Rheumatology Register cohort were matched, 5 controls to each case, with respect to age, sex, county, marital status, and calendar year of rheumatoid arthritis diagnosis. By linking the controls to the same registers as the patients with rheumatoid arthritis we were able to follow all individuals included in all studies in a similar fashion (figure 1). This enabled us to conduct two case-control studies (studies I and II), one cohort study (study III), and one cross-sectional study (study IV).

Figure 1. Linkage of the two rheumatoid arthritis cohorts and their population-based controls to the different outcome registers used in study I-IV.
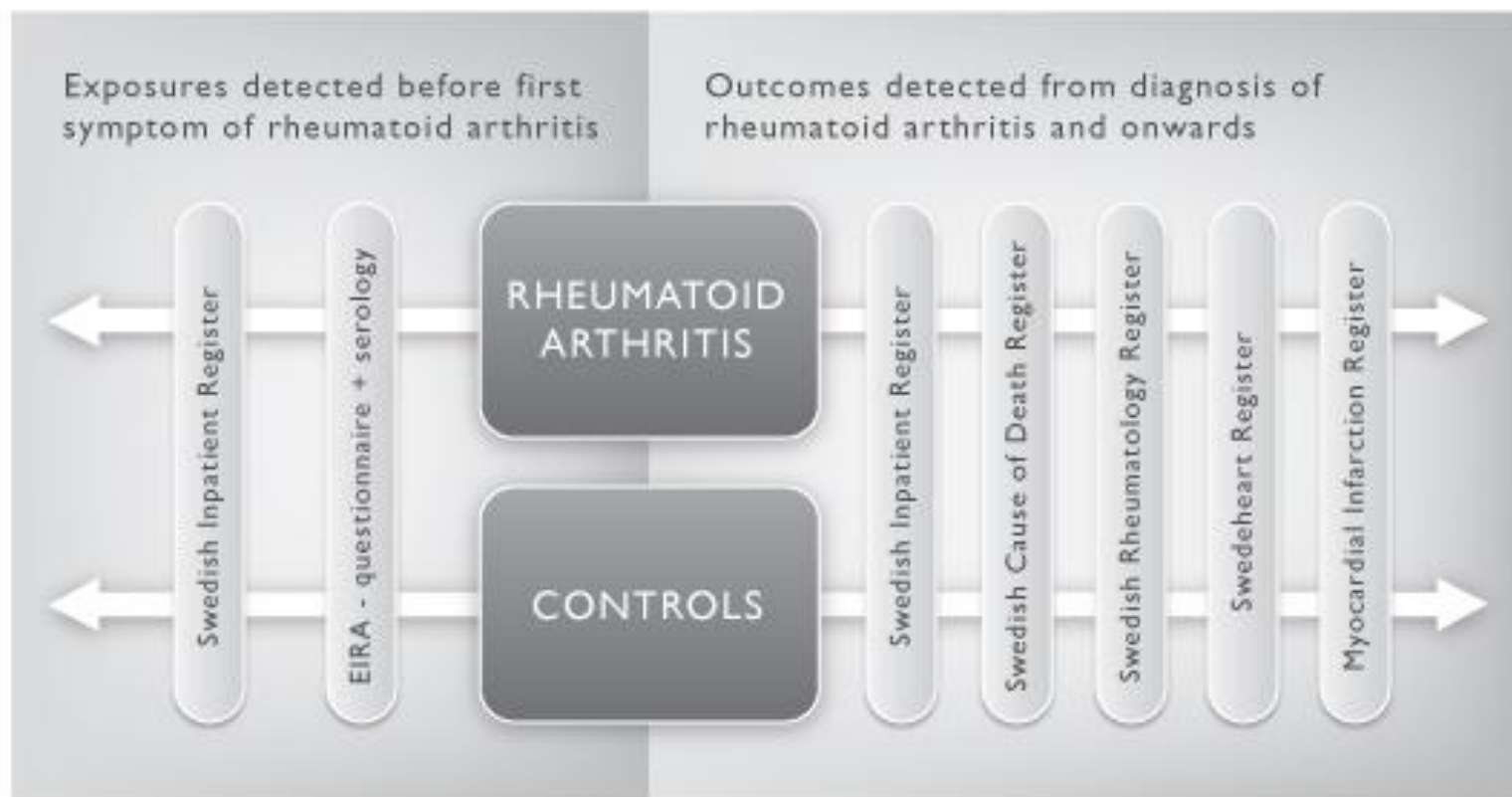
3.3.1 Study I

In this population-based matched case-control study, we used the EIRA study to assess the association between diabetes mellitus and rheumatoid arthritis. Between May 1996 and December 2003, 1,419 cases and 1,674 controls were included in the EIRA study.

3.3.1.1 Exposure classification

In the EIRA questionnaire, 62 cases and 51 controls answered 'yes' to a specific question regarding diabetes mellitus ('Do you have diabetes mellitus? Yes/No'). The year of diabetes mellitus onset and anti-diabetic treatment at inclusion in EIRA was also requested. The participants were not requested to state whether they had type 1 or type 2 diabetes mellitus. To confirm and classify these self-reports a validation study was performed. The medical records of 44 of the 113 cases and controls with self-reported diabetes mellitus were available; their diagnosis was confirmed and classified as type 1 or type 2 diabetes mellitus. In addition, we contacted the 96 individuals who self-reported diabetes mellitus and were still alive when study I was conducted (including those who were still alive of the 44 on whom we had medical record information). 86 of those answered a diabetes questionnaire, either via telephone or via paper questionnaire (see section 10.1 for the questionnaire in full). Based on the information given in the diabetes questionnaire, two independent reviewers with medical training classified each individual as having type 1 or type 2 diabetes mellitus. The reviewers were blinded to the case-control status of the individuals and unaware of the research question at hand. For two subjects, there was discordance between reviewers in the assigned diagnosis. Final classification of diabetes mellitus was then reached by consensus. Hypothesizing that patients with type 1 diabetes mellitus could be identified as those who were receiving insulin mono-therapy at EIRA inclusion and were < 30 years of age when diagnosed with diabetes mellitus, we calculated the positive predictive value of this method for classifying type 1 diabetes mellitus against the two other methods. The positive predictive value of using the age cutoff in combination with insulin mono-therapy compared with medical records was 100% (sensitivity 69%, specificity 100%), and compared to the results from the diabetes questionnaire, 100% (sensitivity 72%, specificity 100%). The agreement between the results from the review of medical records and from the diabetes questionnaire was high (κ= 0.94). Based on this, the diabetes classification was done using one of the three different methods:

(i)   if information was available from the diabetes questionnaire, the classification was done based on this. 52 cases and 34 controls were classified using this method.

(ii)   If no information was available from the diabetes questionnaire, information from the medical records was used. 5 cases and 6 controls were classified using this method.

(iii)   For the remaining individuals with self-reported diabetes mellitus (5 cases and 11 controls), for whom information from method (i) and (ii) was unavailable, we classified as having type 1 diabetes mellitus those who were younger than 30 years when diagnosed with diabetes mellitus and who were treated with insulin mono-therapy by the time of inclusion in EIRA.

Figure 2. Validation and classification of self-reports of diabetes mellitus in EIRA. T1D- type 1 diabetes mellitus, T2D- type 2 diabetes mellitus
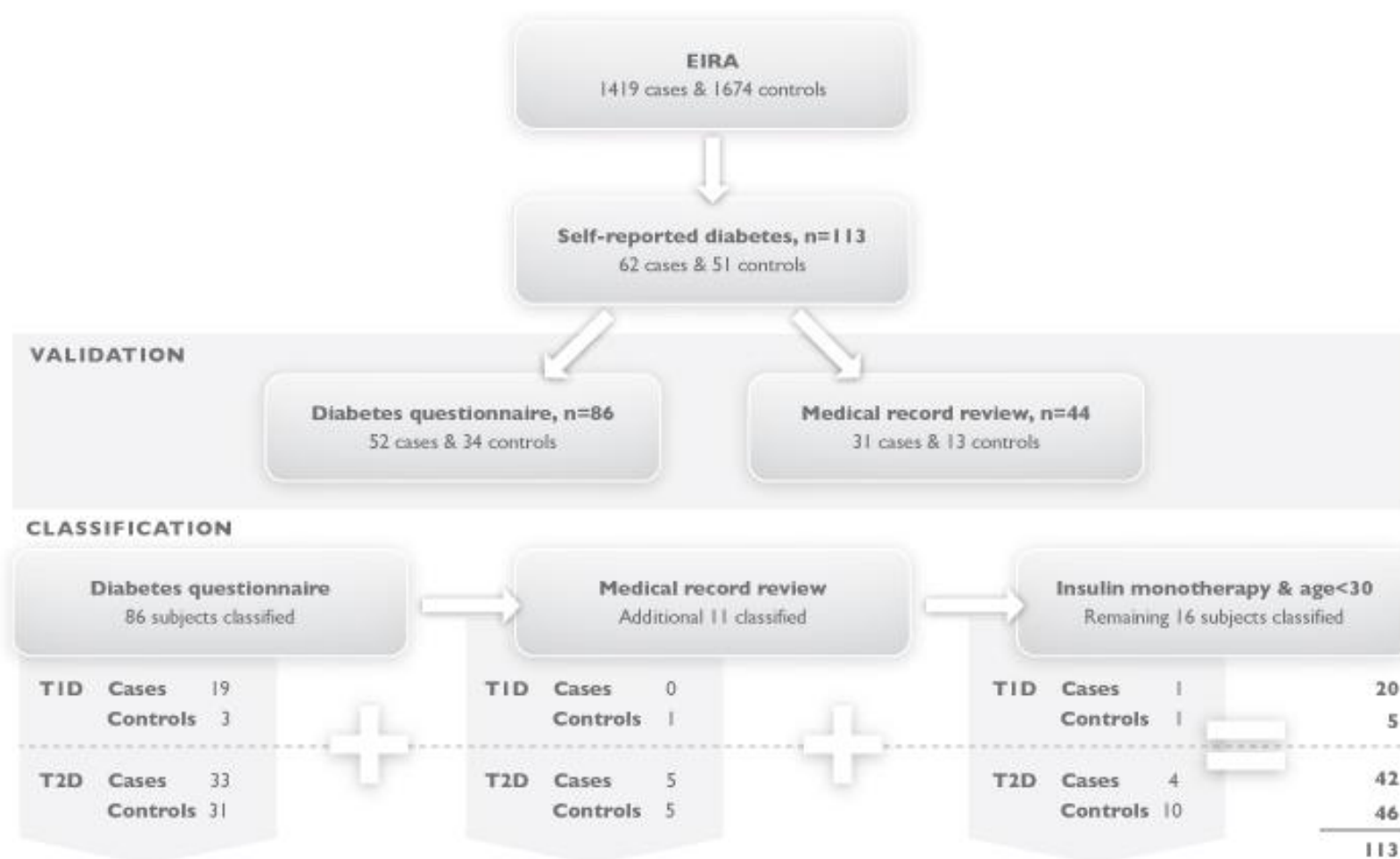
3.3.1.2   Other covariates of interest

All other covariates of interest were assessed at inclusion in EIRA using the questionnaire. Smoking was classified as ever/never, meaning those who reported to never have smoked before first symptom of rheumatoid arthritis were classified as never smokers. Those who had smoked at some point before first symptom were classified as ever smokers. Body mass index (kg/m^2) was calculated using self-reported weight in kilograms and height in meters and was classified in four groups; <20, 20-25, 26-30, >30. Having one or two alleles of the 620W variant of *PTPN22* was classified as *PTPN22* positivity. RF and ACPA were analyzed and classified as described in section 3.2.1.4.

3.3.2   Study II

In study II, the two population-based cohorts of patients with incident rheumatoid arthritis and their controls were included and analyzed separately with respect to the occurrence of ischemic heart disease before first symptom of rheumatoid arthritis. Between January 1995 and December 2007, 8,454 patients with rheumatoid arthritis from the Swedish Rheumatology Register with symptom duration of less than 18 months by the time of diagnosis and 42,267 controls were identified. In the EIRA study, 2,025 patients with rheumatoid arthritis and 2,760 controls were included between May 1996 and December 2007.

3.3.2.1   Exposure classification

Among EIRA participants, history of ischemic heart disease was assessed using self-reported history from the EIRA questionnaires and the Swedish Inpatient Register. The latter was also source was also used for the Swedish Rheumatology Register cases and their corresponding controls. In the EIRA questionnaire, items pertaining to ischemic heart disease before rheumatoid arthritis onset specifically asked "Do you have, or have you had any cardiovascular disease which has been treated by a physician?" (yes/no) "If yes, of what kind, and when?" The answer to this question was given verbatim, and coded according to the *International Classification of Diseases*, tenth revision by the study secretariat.

In table 5 below codes from which revision of the *International Classification of Disease* was used during which periods and how the codes are grouped are presented. These were used to classify exposure in the Swedish Inpatient Register. To classify self-reports in EIRA, only the tenth revision was used.

Table 5. *International Classification of Disease* versions and codes used to classify exposure

	ICD-7: 1964-1967	ICD-8 and ICD-9 1968-1996	ICD-10 1997-
Ischemic heart disease	420.10, 420.17, 420.18, 420.20, 420.28	410, 411, 413	I20-I22, I24
Myocardial infarction	420.10, 420.17, 420.18	410	I21
Angina pectoris	420.20, 420.28	413	I20

Each of these three groups, ischemic heart disease, myocardial infarction, and angina pectoris were assessed as exposures in both case-control sets.

3.3.2.2   Other covariates of interest

In the EIRA participants, smoking was classified as ever vs. never. Body mass index was calculated using the same variables as in study I but was classified somewhat differently; <25, 25-29, >29. Hypertension status (1/0) was classified based on self-reports; as was diabetes mellitus status (1/0). RF and ACPAs were analyzed and classified as described in section 3.2.1.4. Having one or two alleles of the HLA-*DRB1* shared epitope was classified as shared epitope positivity (1/0). In the Swedish Rheumatology Register cohort and its controls, hypertension and diabetes mellitus status was based on hospitalization listings of hypertension and diabetes mellitus and were classified according to these listings (1/0).

3.3.3   Study III

In the third study, we assessed the relative risk of ischemic heart disease after the diagnosis of rheumatoid arthritis. We also wanted to assess *when*, after start of follow-up, an increased risk could be detected. We identified 7,469 patients with rheumatoid arthritis from the Swedish Rheumatology Register and their matched comparators, 37,024 individuals. They were diagnosed between 1st of January 1995 and 31st of December 2006. Only individuals who had not had any ischemic heart disease before diagnosis of rheumatoid arthritis (for the patients with rheumatoid arthritis) or the matching date (for the comparators) were included in analysis.

3.3.3.1   Exposure classification

Rheumatoid arthritis was the exposure of interest in this cohort study. Individuals who had been diagnosed with rheumatoid arthritis by a rheumatologist and entered into the Swedish Rheumatology Register were classified as exposed. The individuals included in the population-based matched comparator cohort were classified as unexposed.

3.3.3.2   Follow-up

Follow-up started at the date of rheumatoid arthritis diagnosis. Individuals in the comparator cohort started their follow-up on the same date as their corresponding patients with rheumatoid arthritis. End of follow-up was defined as whichever of the following occurred first: the outcome of interest, death, date of first emigration, of 31st of December 2006.

3.3.3.3   Outcome definition

All individuals were followed over time, regardless of exposure status. The outcomes of interest, corresponding *International Classification of Diseases*- codes used to detect these outcomes, and the register source used for detection, are given in table 6 below.

Table 6. *International Classification of Diseases*- codes used to detect outcome, their register source and how they were grouped.

	ICD-9 1987-1996	ICD-10 1997-	Register source
Acute myocardial infarction	410	I21	Swedish Myocardial Infarction Register
Fatal myocardial infarction	410	I21	Swedish Myocardial Infarction Register
Coronary revascularization	Intervention code: 3080	Intervention code: FNG02, FNG03, FNG05, FNG06	Swedish Inpatient Register
Angina pectoris	413	I20	Swedish Inpatient Register
Any ischemic heart disease	410, 3080, 413	I21, FNG02, FNG03, FNG05, FNG06, I20	Swedish Myocardial Infarction Register, Swedish Inpatient Register

3.3.4 Study IV

In the fourth study, the extent of coronary stenosis at the time of clinical manifestations of acute coronary syndrome in patients with rheumatoid arthritis was compared to that in the general population. To do this we identified all patients with rheumatoid arthritis, and their comparators, who were entered in the Swedeheart Register and underwent angiography with the indication unstable angina, unspecified central chest pain, silent ischemia, or acute myocardial infarction after start of follow up. 168 patients with rheumatoid arthritis and 534 comparators underwent angiography and were entered into the Swedish Coronary And Angioplasty Register with any of the above specified indications after start of follow-up, between 1st of January 1995 and 31st of August 2008.

3.3.4.1   Definition of significantly stenosed artery

Coronaries were classified as significantly stenosed if a > 50% stenosis could be detected and considered clinically important or if the fractional flow reserve was < 75%. The classification was performed by the angiographer and is below simply refered to as stenosis. All individuals who underwent angiography were classified in groups based on which and how many coronaries that were significantly stenosed; those who had one coronary afflicted (and not the left main stem), two coronaries (and not the left main stem), three coronaries (and not the left main stem), those who had stenosis in the left main stem only or in combination with other coronaries (one, two, or three). The different categories were mutually exclusive. We also constructed a composite outcome- left stem stenosis overall- including all with a left stem stenosis regardless of the status of the other coronaries.

3.4   STATISTICAL ANALYSIS

All analyses conducted and reported in this thesis were performed in SAS statistical software, 9.1 and 9.2 (Cary, NC, USA).

3.4.1 Important concepts

3.4.1.1 Parametric and non-parametric tests

To compare differences in two groups there are two main groups of tests to be chosen from; parametric tests and non-parametric tests. Parametric tests are based on the assumption that data are sampled from a normal distribution, and non-parametric tests do not make any assumptions about the distribution of the variable in the sampled population. If the variable of interest is continuous, and the assumption of normality is not violated, the most common test to use when comparing two unmatched groups is the parametric two-tailed Student t-test. If normality cannot be assumed the non-parametric Mann-Whitney U test is used. Non-parametric tests of continuous variables rank data and compare the ranks. When two unmatched groups are compared with respect to the distribution of a categorical variable the chi-square test or Fisher's exact test is used.

3.4.1.2 Logistic regression

Binary logistic regression is a form of regression that is used when the dependent variable is a dichotomous variable, for example rheumatoid arthritis (1/0), and the independent variables are of any type, for example blood pressure as a continuous variable or categorized into high/normal, low/normal. Logistic regression applies maximum likelihood estimation after transforming the dependent variable into a logit variable (the natural log of the odds of the dependent variable occurring or not). In this way, logistic regression estimates the odds of a certain event occurring. Two main groups of logistic regression models are used; conditional and unconditional. Conditional logistic regression is used when analyzing data where every case is matched to a control by certain variables. These specific pairs are then compared to each-other, with the implication that if one of the two is missing information on a certain variable entered into the model the entire pair is removed from the analyses. In unconditional logistic regression all cases are compared to all controls. If matched data is analyzed using unconditional logistic regression, the matching variables must be entered into the model.

3.4.1.3 Cox' proportional hazards models

Cox regression, which uses the *proportional hazards model*, is designed for analysis of time to event data. One or more predictor variables are used to predict an event variable. In medicine, it was originally used to estimate time from diagnosis with a lethal disease until the event of death, and this method is therefore sometimes referred to as *survival analysis*. The Cox model estimates the hazard ratio, i.e. the ratio of the instantaneous probability of a given event occurring in a given time period comparing two groups. This model assumes that the hazard is proportional throughout the follow-up period.

3.4.2 Analyses performed in the included studies

Unconditional logistic regression models were used to estimate odds ratios and corresponding 95% confidence intervals in three of the studies included in this thesis; *studies I-II and IV*. In studies I-II we also used conditional logistic regression models and found that the estimates based on these models were similar to the unconditional logistic regression models, but had lower precision.
Cox' proportional hazards models were used to estimate the hazard ratio, expressed as a relative risk, and to calculate 95% confidence intervals in *study III*. Tests of differences between means of continuous

variables were done using two-tailed student t-tests when the values of the variable in the underlying population could be assumed to be normally distributed. Mann-Whitney U tests were performed when normality could not be assumed. Tests of differences in proportions were performed using the chi-square test or Fisher's exact test, whichever was suitable based on the number of counts in each cell.

The EIRA study is a 1:1 matched study, but we chose to break the matching and analyze it frequency matched. Doing so allowed us to include all controls with questionnaire information and to increase the number of individuals used in each model, thus increasing the efficiency of the models. In all EIRA analyses (*Studies I and II*), the dependent variable was rheumatoid arthritis (1/0). Subgroup analyses based on serostatus (RF/ACPA positive or negative) were performed with all controls, regardless of the serostatus in controls or the status of their matched case, as reference. The matching factors age, sex, and residential area were included as covariates in all models.

3.4.2.1   Study I

In *study I*, the exposure of interest was diabetes mellitus (1/0). Stratifications based on type of diabetes mellitus, described in section 3.3.1.1., were also performed. In addition to the crude model including only the matching variables and the exposure of interest, we also adjusted for smoking (never/ever) and body mass index (in four categories, with 20-25 as reference). A third model was fitted by adding a dichotomous variable indicating absence or presence of any *PTPN22* risk variant to the model which included smoking and body mass index.

3.4.2.2   Study II

In *study II*, the exposure of interest was different classifications of ischemic heart disease, see section 3.3.2.1 for details. In the analyses based on EIRA, we adjusted for self-reported hypertension (1/0) and diabetes mellitus (1/0). We performed analyses stratified on shared epitope status and on timing of the exposure in relation to rheumatoid arthritis onset (exposure occurring > 5 years before onset and 5 years or less before onset). In the analyses based on the Swedish Rheumatology Register cohort and their controls, the dependent variable was also rheumatoid arthritis (1/0), and the matching variables, age, sex, county, calendar year of rheumatoid arthritis diagnosis, and marital status, were included in all models. Further adjustments were made using listings of hypertension (1/0) and diabetes mellitus (1/0) in the Swedish Inpatient Register.

3.4.2.3   Study III

In *study III*, rheumatoid arthritis (1/0) was the exposure of interest and ischemic heart disease in different categories (see section 3.3.3.3 for details) was the outcome. The association between each of the outcomes was assessed separately, with the implication that one individual could contribute an event to more than one outcome, but only one event to each outcome. The time scale was time since study entry, which meant time since diagnosis of rheumatoid arthritis in those exposed and the corresponding matching date in the unexposed. The matching variables were included in all models. To assess the time-dependent effect of duration of rheumatoid arthritis on outcome, we introduced three time-dependent covariates for the duration of exposure: the first year of follow-up, 1-4 years after start of follow-up, and 5-12 years after start of follow-up. To assess potential differences in secular trends, we stratified the analysis on calendar period of study entry (year of rheumatoid arthritis diagnosis); 1995-1997, 1998-2001, and 2002-2006. The proportional hazards assumption was tested by introducing an interaction term between rheumatoid arthritis and time since study entry. This was

done for all models constructed. Since all of these interaction terms were non-significant we concluded that we were not in violation of the proportional hazards assumption in any of the models. Crude incidence rates were calculated by dividing the number of events during follow-up by the corresponding person-time. All individuals with any type of ischemic heart disease before study entry were excluded from the analyses.

3.4.2.4 Study IV

In *study IV*, we wanted to compare patients with rheumatoid arthritis and comparators with respect to the odds of having a certain number of coronaries with significant stenosis, and therefore fit unconditional logistic regression models with rheumatoid arthritis (1/0) as the independent variable, and the outcome of interest (1/0) as the dependent variable. All models were adjusted for age at angiography and sex. Adjustment for indication for angiography was done in all models with each indication (1/0) included in the model. Stratification on age at angiography in patients with rheumatoid arthritis and the corresponding date in the comparators was performed. Four subsets were constructed based on age quartiles (<61, 62-68, 69-76, >77). We also performed an internal comparison within the patients with rheumatoid arthritis. We compared those who underwent angiography >5years after diagnosis of rheumatoid arthritis to those who underwent angiography <1 year after diagnosis of rheumatoid arthritis. Patients with RF positive rheumatoid arthritis were also compared with patients with RF negative rheumatoid arthritis with respect to the same outcomes.

All studies included in this thesis were approved by the ethics committee at the Karolinska Institutet.

4 RESULTS

4.1 STUDY I

Sixty-two cases (4.4% of all cases included in analyses) and 51 controls (3.1% of all controls included in analyses) self-reported to have a pre-existing diabetes mellitus when included in the EIRA study. After applying the algorithm of classification described in section 3.3.1.1, 20 cases and 5 controls were classified as having type 1 diabetes mellitus, and 42 cases/46 controls were classified as having type 2 diabetes mellitus. The association between diabetes mellitus overall and risk of developing rheumatoid arthritis overall, was moderate (crude odds ratio 1.4, 95% confidence interval 1.0, 2.1), and, after adjusting for body mass index and smoking, non-significant, odds ratio 1.3 (95% confidence interval 0.9, 2.0). When type 1 and type 2 diabetes mellitus were assessed separately, the increased risk of rheumatoid arthritis was confined to those with type 1 diabetes mellitus, adjusted odds ratio 4.8 (95% confidence interval 1.8, 12.9), rather than to those with type 2 diabetes mellitus, adjusted odds ratio 1.0 (95% confidence interval 0.6, 1.5). Stratifications by ACPA status among the cases resulted in following two-by-two tables:

Table 7A. Relationship between type 1 diabetes mellitus and rheumatoid arthritis

Type 1 DM	Exposed	Unexposed	∑		Exposed	Unexposed	∑
ACPA positive cases	18	839	857	ACPA negative cases	2	542	544
Controls	5	1,669	1,674	Controls	5	1,669	1,674
∑	23	2,508		∑	7	2,211	

Crude odds ratio: (18/839)/(5/1,669)=7.2   (2/542)/(5/1,669)=1.2

Table 7B. Relationship between type 2 diabetes mellitus and rheumatoid arthritis

Type 2 DM	Exposed	Unexposed	∑		Exposed	Unexposed	∑
ACPA positive cases	22	835	857	ACPA negative cases	20	524	544
Controls	46	1,628	1,674	Controls	46	1,628	1,674
∑	68	2,463		∑	66	2,152	

Crude odds ratio: (22/835)/(46/1,628)=0.9   (20/542)/ (46/1,628)=1.3

After adjusting for the matching factors, body mass index, and smoking, type 1 diabetes mellitus conferred an increased risk of developing ACPA positive rheumatoid arthritis, adjusted odds ratio 7.3 (95% confidence interval 2.6, 20.2). Additionally adjusting for any *PTPN22* allele attenuated the odds ratio of developing ACPA positive rheumatoid arthritis when exposed to type 1 diabetes mellitus, but left all other estimates unchanged. Similar results were seen when analyses were stratified based on rheumatoid factor status. There was no association between type 1 diabetes mellitus and ACPA negative rheumatoid arthritis, or between type 2 diabetes mellitus and ACPA positive or negative rheumatoid arthritis. In table 8, estimates based on all models and from all stratifications are given.

Table 8. Association between diabetes mellitus by type and risk of developing rheumatoid arthritis, stratified by antibody to citrullinated protein antigen status and rheumatoid factor status with successive adjustments for potential confounders

Type of diabetes mellitus, ACPA/RF status	N exposed cases /n exposed controls	Odds ratio (95% confidence interval)		
		Model 0*	Model 1	Model 2
Type 1				
All	20/5	4.9 (1.8,13.1)	4.8 (1.8, 12.9)	3.5 (1.0, 12.1)
ACPA positive	18/5	7.3 (2.7, 20.0)	7.3 (2.6, 20.2)	5.3 (1.5, 18.7)
ACPA negative	2/5	1.3 (0.3, 7.0)	1.3 (0.2, 6.9)	1.1 (0.2, 6.7)
RF positive	19/5	7.1 (2.6, 19.2)	7.0 (2.6, 19.2)	5.1 (1.5, 17.8)
RF negative	1/5	0.7 (0.1, 5.8)	0.7 (0.1, 5.7)	0.6 (0.1, 5.6)
Type 2				
All	42/46	1.1 (0.7, 1.6)	1.0 (0.6, 1.5)	1.1 (0.6, 1.8)
ACPA positive	22/46	0.9 (0.6, 1.6)	0.9 (0.5, 1.6)	1.0 (0.6, 1.9)
ACPA negative	20/46	1.3 (0.7, 2.2)	1.1 (0.6, 1.9)	1.1 (0.6, 2.2)
RF positive	27/46	1.1 (0.7, 1.8)	1.0 (0.6, 1.7)	1.2 (0.7, 2.1)
RF negative	15/46	1.0 (0.6, 1.9)	0.9 (0.5, 1.7)	0.9 (0.5, 1.9)

*Model 0 included the matching factors, Model 1= model 0+ smoking and body mass index, Model 2= Model 1+ *PTPN22* status

4.2 STUDY II

In the Swedish Rheumatology Register cohort and their matched control group, 490 cases (5.8% of all cases) and 2,397 controls (5.7% of all controls) had been discharged from hospital with a diagnosis of ischemic heart disease before first symptom of rheumatoid arthritis, resulting in an adjusted odds ratio of 1.0 (95% confidence interval 0.9, 1.1). Two hundred and thirty-three (2.8%) cases and 1,198 (2.9%) controls had a history of having been hospitalized with a diagnosis of myocardial infarction before first symptom of rheumatoid arthritis (adjusted odds ratio for myocardial infarction=1.0, 95% confidence interval 0.9, 1.1), and 373 (4.4%) patients with rheumatoid arthritis and 1,811 (4.3%) controls had been diagnosed with angina pectoris (adjusted odds ratio 1.0, 95% confidence interval 0.9-1.2). The lack of differences in the occurrence of ischemic heart disease, myocardial infarction, or angina pectoris between patients with rheumatoid arthritis and controls was not modified by age, sex, or RF status among the cases. The results from defining exposure as a history of ischemic heart disease occurring 5 years or less before index date and as a history of ischemic heart disease occurring >5 years before index date separately were similar. The crude odds ratio for having undergone revascularization processes before onset of symptoms was 1.2 (95% confidence interval 0.8, 1.6, n=42 cases, 178 controls).

The baseline distribution of traditional cardiovascular risk factors in the EIRA cohort participants is found in table 9. In the EIRA cohort results pertaining to history of ischemic heart disease were similar to those based on the Swedish Rheumatology Register cohort and their matched control group. 48 cases (2.4 % of all cases) and 60 controls (2.2 % of all controls) were hospitalized with a diagnosis of ischemic heart disease before first symptoms of rheumatoid arthritis, corresponding to a crude odds ratio of 1.1 (95% confidence interval 0.7-1.6). Adjusting for body mass index, smoking, hypertension, and diabetes mellitus yielded similar results. Stratifying cases on RF and ACPA status yielded some elevated point estimates with low precision. 52 cases (2.6% of all cases) and 57 controls (2.1% of all

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controls) self-reported a history of any ischemic heart disease before first symptom of rheumatoid arthritis, adjusted odds ratio 0.9 (95% confidence interval 0.6-1.4). In table 10 the results based on different exposure classifications are given.

When we assessed exposures occurring > 5 years before onset and 5 years or less before onset a somewhat higher statistically non-significant occurrence of ischemic heart disease noted in patients with rheumatoid arthritis, compared to the controls, in the 5 years immediately before the onset of rheumatoid arthritis symptoms was noted.

Analyses stratified by RF or ACPA status among the cases or by shared epitope status did not reveal any heterogeneity. No major sex- or age specific association emerged.

Table 9. Baseline distribution of traditional cardiovascular risk factors in the EIRA cohort.

	Cases, n= 2,025	Controls, n=2,760
	Number (%)*	
Ever smokers	1,370 (69.4)	1,404 (62.5)
BMI <25	1,063 (53.9)	1,203 (54.3)
BMI 25-29	653 (33.1)	744 (33.4
BMI ≥30	256 (13.0)	274 (12.3)
Diabetes Mellitus	85 (4.4)	66 (3.0)
Hypertension	182 (9.0)	230 (8.3)

*Percentages reflect proportion of all in each category where data was available. BMI-body mass index.

Table 10. Ischemic heart disease before first symptom of rheumatoid arthritis in the EIRA cohort

Exposure assessment, serologic status	No. of events		OR (95% CI)*	OR (95% CI)
	Cases	Controls		
Ischemic heart disease				
Self-reported, any	52	57	1.2 (0.8,1.7)	0.9 (0.6-1.4)
Hospital Discharge Register				
Any	48	60	1.1 (0.7, 1.6)	1.1 (0.7, 1.7)
ACPA positive	23	60	0.9 (0.6, 1.5)	1.0 (0.6, 1.7)
ACPA negative	23	60	1.4 (0.8, 2.3)	1.5 (0.9, 2.6)
RF positive	31	60	1.1 (0.7, 1.7)	1.1 (0.6, 1.7)
RF negative	17	60	1.0 (0.6, 1.8)	1.2 (0.6, 2.1)
Myocardial Infarction				
Self-reported, any	28	24	1.6 (0.9, 2.7)	1.1 (0.7, 2.0)
Hospital Discharge Register				
Any	25	35	1.0 (0.6, 1.6)	1.0 (0.6, 1.8)
ACPA positive	16	35	1.2 (0.6, 2.1)	1.2 (0.6, 2.2)
ACPA negative	9	35	0.9 (0.4, 2.0)	1.0 (0.5, 2.3)
RF positive	18	35	1.1 (0.6, 1.9)	1.1 (0.6, 2.1)
RF negative	7	35	0.7 (0.3, 1.7)	0.8 (0.6, 1.9)
Angina Pectoris				
Self-reported, any	28	36	1.0 (0.6, 1.6)	0.8 (0.5, 1.4)
Hospital Discharge Register				
Any	36	42	1.1 (0.7, 1.8)	1.2 (0.7, 2.0)
ACPA positive	18	42	1.1 (0.6, 1.9)	1.2 (0.6, 2.1)
ACPA negative	16	42	1.3 (0.7, 2.4)	1.5 (0.8, 2.7)
RF positive	25	42	1.2 (0.7, 2.1)	1.3 (0.7, 2.2)
RF negative	11	42	1.0 (0.5, 1.9)	1.0 (0.5, 2.1)
Ischemic heart disease occurring >5 years before onset of symptoms	28	43	0.9 (0.5, 1.4)	1.0 (0.6, 1.9)
Ischemic heart disease occurring 5 years or less before onset of symptoms	20	17	1.5 (0.8, 3.0)	1.2 (0.6, 2.4)

*OR- odds ratio, CI-confidence interval,

4.3   STUDY III

7,469 patients with rheumatoid arthritis and 37,024 comparators were followed for a median of 4.1 years (range 0-12 years), accumulating 33,436 person-years in the rheumatoid arthritis cohort and 166,510 person-years in the comparison cohort. By January 1st 2007, 233 vs. 701 subjects had developed a myocardial infarction. The relative risk for myocardial infarction was 1.6 (95% confidence interval 1.4, 1.9). We noted significantly increased risks for all ischemic heart disease outcomes except for death from acute myocardial infarction. No major sex differences with respect to relative risk could be detected. During the first year following study entry, point estimates were above 1.0 for all outcome definitions apart from angina pectoris. After the first year following study entry, significantly increased relative risks were noted for all outcomes in all follow-up intervals except for death from acute myocardial infarction, which was not increased in any of the follow-up intervals, and for angina pectoris 5-12 years after study entry. In table 11 we present all of the relative risk estimates of all outcomes in all follow-up intervals. Stratifying by calendar year of study entry, and thus diagnosis of rheumatoid arthritis, did not modify the observed association between ischemic heart disease and rheumatoid arthritis. The relative risk for hospitalized myocardial infarction was significantly increased for rheumatoid factor positive rheumatoid arthritis (relative risk 1.7, 95% confidence interval 1.4, 2.0) as well as for rheumatoid factor negative rheumatoid arthritis (relative risk 1.4, 95% confidence interval 1.1, 1.9). The difference between these two subgroups of rheumatoid arthritis was not statistically significant (p=0.4). Age-specific incidence rates, and incidence rates stratified by time since study entry, are given in table 12.

Table 11. Relative risk and 95% confidence interval, including number of events among patients with rheumatoid arthritis and comparators, of all outcomes under study in all follow-up intervals.

Outcome	<1 year since study entry	1-4 years since study entry	5-12 years since study entry	Entire follow-up
Myocardial infarction	1.4 (0.9, 2.1) 34/115	1.6 (1.3, 2.0) 134/388	1.6 (1.2, 2.2) 65/198	1.6 (1.4, 1.9) 233/701
Any ischemic heart disease	1.1 (0.8, 1.5) 52/215	1.5 (1.2, 1.7) 197/650	1.5 (1.2, 1.9) 92/315	1.4 (1.2, 1.6) 341/1,180
Fatal myocardial infarction	1.3 (0.6, 2.7) 9/33	1.1 (0.7, 1.8) 19/92	1.1 (0.6, 2.3) 10/56	1.1 (0.8, 1.6) 38/181
Coronary revascularization	1.5 (0.7, 3.1) 10/32	1.4 (1.0, 2.0) 46/149	2.0 (1.3, 3.2) 27/75	1.6 (1.2, 2.1) 83/256
Angina pectoris	0.9 (0.5, 1.5) 16/87	1.3 (1.0, 1.8) 67/241	1.2 (0.8, 1.9) 29/111	1.2 (1.0, 1.5) 112/439

Table 12. Age-specific and follow-up interval specific incidence rates (n events/1,000 person-years) of being hospitalized with a myocardial infarction in the Swedish Rheumatology Register cohort and its comparators.

		<51 years old	51-70 years old	>70 years old	<1 year since study entry	1-4 years since study entry	5-12 years since study entry
Incidence rate (95% CI)	Patients with RA	0.81 (0.3, 1.7)	4.82 (3.8, 6.0)	16.7 (14.2, 19.7)	4.90 (3.4, 6.9)	7.18 (6.0, 8.5)	8.10 (6.3, 10.3)
	Comparators	0.28 (0.2, 0.5)	2.7 (2.4, 3.1)	10.8 (9.9, 11.7)	3.35 (2.8, 4.0)	4.19 (3.8, 4.6)	4.88 (4.2, 5.6)
Crude incidence rate ratio		2.9	1.8	1.5	1.5	1.7	1.7

RA- rheumatoid arthritis, CI-confidence interval

4.4 STUDY IV

6,919 patients with rheumatoid arthritis and 34,638 population based comparators without any previous hospitalization for ischemic heart disease were identified. Mean age at index year was 57 years and 71% were women. 168 (2.4% of all) patients with rheumatoid arthritis and 534 (1.5% of all) comparators underwent an angiography and were found in Swedeheart after index date, relative risk 1.5 (95% CI 1.2, 1.7) comparing patients with rheumatoid arthritis to their matched comparators. The sex distribution (49% females), and the mean age at angiography (68 years) was similar in patients with rheumatoid arthritis and comparators (Table 13). There was no difference between patients with rheumatoid arthritis and comparators with respect to indication for angiography (2% vs. 2% had silent ischemia, 10 % vs. 9% central chest pain, 33% vs. 26% ST- elevation myocardial infarction listed as indication, 57% vs. 64% had unstable coronary artery disease listed as indication).

There was no difference in the distribution of significant coronary stenoses (table 14) among those who underwent an angiography due to acute coronary syndrome; age- and sex -adjusted odds ratio of having three coronaries with significant coronary stenosis when comparing patients with rheumatoid arthritis to comparators 0.9 (95% CI 0.6, 1.4). Among patients with RF negative rheumatoid arthritis, the odds ratio for having a significant stenosis in the left stem regardless of the status of other arteries was 2.6 (95% CI 1.1, 6.4) (table 4). The corresponding odds ratio among RF positive patients with rheumatoid arthritis was 0.6 (95% CI 0.2, 1.8). The odds ratio of having a significant stenosis in the left stem overall among RF positive patients compared to RF negative patients was 0.2 (95% CI 0.1, 0.9). Additionally adjusting for indication did not alter any of the results. Stratifying by sex or age at angiography did not reveal any heterogeneities in relative risks. Comparing patients with rheumatoid arthritis with a disease duration of >5 years by the time of the angiography to those with <1 year did not indicate that patients with early rheumatoid arthritis who experienced an acute coronary syndrome had a more severe case of coronary stenosis than patients with rheumatoid arthritis with longer disease duration.

Table 13. Baseline characteristics of patients with rheumatoid arthritis and comparators who underwent angiography after study entry.

	Patients with RA, n=168	Comparators, n=534	
Age at angiography in years, mean (SD)	67.9 (10.1)	68.0 (9.8)	N/S
Proportion women of all who underwent angiography, n (%)	83 (49.4)	256 (47.9)	N/S
RF positives[*]	104 (64)	N/A	
Indication for angiography			
Silent ischemia, n (%)	2 (1.2)	11 (2.1)	N/S
Unspecified central chest pain, n (%)	16 (9.5)	47 (8.8)	N/S
STEMI, n (%)	55 (32.7)	137 (25.7)	N/S
Unstable coronary artery disease, n (%)	95 (56.6)	339 (63.5)	N/S

6 patients with RA were missing on RF status

Table 14. Table 3. Odds ratios (OR) and 95% confidence interval (CI) for each dependent variable comparing patients with rheumatoid arthritis and comparators. Adjusted for age at angiography and sex.

Dependent variable of interest	N (%) rheumatoid arthritis with dependent variable of interest	N (%) comparators with dependent variable of interest	Age- and sex- adjusted OR (95%CI)
Coronary artery with significant stenosis			
Left stem overall[#]	13 (8.2)	30 (5.9)	1.4 (0.7, 2.8)
-Left stem only	0 (0)	1 (0.2)	---
- Left stem +1 coronary	1 (0.6)	4 (0.8)	0.7 (0.08, 6.8)
- Left stem +2 coronary	3 (1.9)	6 (1.2)	1.6 (0.4, 6.4)
- Left stem + 3 coronaries	9 (5.7)	19 (3.8)	1.5 (0.7, 3.4)
1 coronary	47 (29.8)	162 (32.0)	0.9 (0.6, 1.3)
2 coronaries	37 (23.4)	110 (21.7)	1.1 (0.7, 1.7)
3 coronaries	26 (16.5)	90 (17.8)	0.9 (0.6, 1.4)
Normal coronaries	35 (22.2)	115 (22.7)	1.0 (0.6, 1.6)

[#]Includes all with afflicted left main stem regardless of status of other vessels. 10 patients with rheumatoid arthritis and 27 comparators were missing on findings on angiography. Percentages were calculated based on all with information on angiography findings. OR-odds ratio, CI-confidence interval

5 DISCUSSION

There are, of course, many interesting issues to address when discussing the studies herein reported. I have chosen to discuss a few of these in the text below. Some concerns are of methodological interest and some are related to the diseases studied.

5.1 LIMITATIONS AND STRENGTHS

The studies included in this thesis have a few obvious caveats. In *studies I and II* potential misclassification of the exposures under study is the major threat to the internal validity of the studies. In *study III* surveillance bias might have been introduced, which also could have affected the interval validity, and there is a potential for a skewed selection of patients with rheumatoid arthritis to study, which might have had an impact on the external validity. In *study IV*, the choice of study population might also have affected the external validity. The inability to adjust for additional potential confounders might also have rendered biased estimates. The population-based setting and the size of the study population in all four studies included are some of the major strengths of our studies. The ability to detect outcome in an unbiased way and to have very little loss-of-follow-up are others.

5.1.1 Misclassification of exposure

In *study I* we have relied mainly on self-reported information to detect presence of the exposure of interest (diabetes mellitus). We then used three different ways of classifying this self-reported exposure. The positive predictive value for classifying type 1 diabetes mellitus by requiring that a patient be receiving insulin mono-therapy and be age <30 years at diabetes mellitus diagnosis was 100% when compared with classification by medical record review or by telephone interview. However, the sensitivity of this classification was 69% as compared with classification by medical record review, and 72% as compared with classification by telephone interview. This could lead to misclassification, in which type 1 diabetes mellitus might be classified as type 2 diabetes mellitus. Given that this misclassification struck equally hard on both cases and controls, in other words that this misclassification is likely non-differential with respect to the outcome, this would have lead to a dilution of the true gradient between the two groups and thus an underestimation of the true odds ratio. To explore this possibility, we conducted a sensitivity analysis including only individuals with available information from the medical record review, where the probability of misclassification was the lowest. In this sensitivity analysis, the odds ratio of developing ACPA positive rheumatoid arthritis among patients diagnosed with type 1 diabetes mellitus by medical record review was 11.7, 95% confidence interval 2.6, 52.7. These results could be interpreted as suggesting that we indeed were underestimating the true odds ratio in the analyses of all with diabetes mellitus. In that case, the odds ratio we report is conservative and the true association stronger than we could detect. However, the odds ratio and its increase could also be due to lower power when analysing a data set with few exposed. In *study II* this type of non-differential misclassification could explain the null results we report. However, a validation study of 2,065 randomly selected hospitalizations listing ischemic heart disease, performed within the Swedish Inpatient Register may suggest otherwise. All medical records of these hospitalizations were retrieved, and patients were classified as either certain myocardial infarction, possible myocardial infarction or not myocardial infarction. In total, 86% of the 713 patients listed with a myocardial infarction were found to fulfil criteria for myocardial infarction; 9% were classified as possible myocardial infarction and 5% as not having had a myocardial infarction. Of the 1,135 patients listed with other ischemic heart disease, 3% fulfilled the criteria for

myocardial infarction [4]. The acute ischemic heart disease events in this particular rheumatoid arthritis population have also been validated against the medical records of each respective patient. Only 4% were classified as unlikely events. These validations and the use of two different sources of exposure information (self-reports in EIRA and in the Swedish Inpatient Register) demonstrating similar results, reduce the probability of exposure misclassification.

Recall bias is a potential threat to validity one has to consider in any case-control study using self-reported exposure information. This form of bias is due to the fact that cases and controls report their exposure status differently which gives rise to a differential misclassification of exposure whose direction and magnitude of bias is difficult to predict. In *study I* it could be that cases with diabetes mellitus to a larger extent than controls accurately reported their diabetes mellitus. Although our validation study showed that 100% of cases and controls who reported diabetes as a pre-existing disease at the time of diagnosis of rheumatoid arthritis and who underwent medical record review and/or completed the diabetes questionnaire actually had diabetes does not change the fact that we do not know the proportion of true diabetes mellitus individuals among cases and controls. What we should have done in this situation is to validate a sample of the individuals not reporting to be exposed to see how many cases and controls in fact had diabetes mellitus. In the absence of this additional evaluation, we did find that the prevalence of diabetes mellitus in our control population is similar to that in the Swedish population [138, 139]. This also suggests that the prevalence of diabetes observed in our study population is not a consequence of selection bias skewed toward healthy participants. In *study II,* we did see signs of differential reporting of ischemic heart disease events when analyzing the EIRA study participants. When we compared with the information retrieved from the Swedish Inpatient Register we found that controls under-reported their history of myocardial infarction. In table 13 the relationship between self-reported and Inpatient Register information is depicted.

Table 13. Relationship between self-reported and Inpatient Register information

Cases	Inpatient Myocardial infarction	No Inpatient Myocardial infarction	∑	Controls	Inpatient Myocardial infarction	No Inpatient MI	∑
Self-reported MI,	23* (82, 92)	5 (18, 0.3)	28	Self-reported MI	20 (83, 57)	4 (17, 0.1)	24
No self-reported MI	2 (0.1, 8)	1,995 (100, 100)	1,997	No self-reported MI	15 (0.5, 43)	2,721 (99, 100)	2,736
∑	25	2,000	2025	∑	35	2,725	2,760

*n, (row percentage, column percentage)

From this table we can deduce that of the cases who were hospitalized with a myocardial infarction before first symptom of rheumatoid arthritis, 23 self-reported to have had a myocardial infarction, rendering a sensitivity of 92% ("true" positives/self-reported positives), and a 100% specificity (self-reported negatives/"true" negatives). Among the controls the sensitivity was 57% and the specificity 100%. We could be seeing results from this underestimation of exposure among controls when we use self-reported information of myocardial infarction, which renders an odds ratio of 1.6 (95% confidence interval 0.9, 2.7), in contrast to an odds ratio of 1.0 (95% confidence interval 0.6, 1.6)

when using hospitalization data to assess exposure. Although interesting from a methods perspective, this does not alter the conclusions drawn from *study II*.

5.1.2 Selection of study population and surveillance bias

In most studies, the main goal is to produce results that can be inferred on the rest of the population with the disease under study. For example, if you study ischemic heart disease in rheumatoid arthritis you want the results from your study to be generalizable to any patients with rheumatoid arthritis, not just the individuals in your study population. The best way to do this is to design and aim for a population-based study. Both of the cohorts used in this study were recruited from university hospital clinics, private clinics, and smaller hospital clinics. This decreases the risk of choosing individuals with a specific rheumatoid arthritis phenotype (for example, only those who need hospitalization for their disease, and therefore could be assumed to have a more severe disease) or who are different from the average patient with rheumatoid arthritis in some demographic aspect (for example, by recruiting from specific private clinics only we might be studying only those with rheumatoid arthritis and a higher education which might result in an earlier diagnosis of disease). By using this source of study participants with rheumatoid arthritis we aimed to study the magnitude of the problems reported in this thesis in patients met by rheumatologists in Sweden every day. In the EIRA study, the risk of including patients in a way that could threaten the study's validity is low. The participation proportion among eligible cases is high, 95%, and an analysis of those who declined to participate in EIRA showed that patients with rheumatoid arthritis who did not participate were only slightly different those that were included; they were somewhat older, less educated and more often born outside of Sweden, but there was no difference in terms of sex distribution, marital status or residential area [140]. This is not very likely to introduce any bias in the results based on the EIRA study. In the Swedish Rheumatology Register, no such analysis has been performed, and it might be so that individuals who are diagnosed with rheumatoid arthritis are not included in the register if the probability of them surviving long enough for a systematic follow-up to be useful is very low. This could have effects on the external validity of the studies based on the Swedish Rheumatology Register, and could result in an underestimation of the ischemic heart disease mortality during the first year after diagnosis of rheumatoid arthritis (*study III*). The opposite might also be true; it could be that patients with very mild symptoms at onset, thus being considered to have a low probability of needing follow-up, are not entered.

The use of nationwide quality registers and nationwide 100% covering registers of inpatient care helps us to detect outcomes and clinical findings in an unbiased fashion. All information that is registered in these data sources are registered regardless of the exposure status of the patient in question and all investigators are unaware of the study hypotheses being tested when the angiography is performed, as in *study IV,* or when a decision to hospitalize for ischemic heart disease is made, as in *studies I-III*. The fact that the outcome under study after diagnosis of rheumatoid arthritis is myocardial infarction, an acute event which is virtually always hospitalized, if not immediately fatal, makes it unlikely that the structured follow-up patients with rheumatoid arthritis are under would introduce surveillance bias.

5.1.3 Study question-related limitations and strengths

One of the strengths of *study I* is the high resolution of information in a large number of incident cases of rheumatoid arthritis. To validate and classify the exposure information (self-reports of diabetes mellitus), we used two methods, medical record review and contacting all available subjects with self-reported diabetes by telephone or by administering a diabetes questionnaire. In addition,

we discriminated ACPA positive rheumatoid arthritis from negative rheumatoid arthritis; previous studies have shown this distinction to be of importance, because the two subgroups are associated with specific, but different, genetic and environmental risk factors and interactions between them [11, 17, 26, 33, 37, 134, 141-143]. Finally, we incorporated genotype information on *PTPN22*, a genetic susceptibility loci shared by RF/ACPA positive rheumatoid arthritis and type 1 diabetes mellitus, into our model and assessed its influence on the risk of developing rheumatoid arthritis when exposed to diabetes mellitus. In addition to shared genetic risk factors for type 1 diabetes mellitus and rheumatoid arthritis, there are major clinical differences between patients with type 1 diabetes mellitus and those with type 2 diabetes mellitus that could potentially explain the association observed in our study. Patients with type 1 diabetes mellitus are exposed to elevated glucose levels and exogenous insulin much longer than those with type 2 diabetes mellitus. To test this alternative explanation, we performed a subset analysis that assessed the risk of rheumatoid arthritis in individuals exposed to insulin as treatment for type 2 diabetes mellitus. No increased risk of rheumatoid arthritis was observed in this group, although the numbers of exposed subjects were small. A smaller percentage of controls than cases were genotyped for *PTPN22*. If the probability of being genotyped was related to diabetes, this may result in biased relative risk estimates. To assess this, we compared characteristics of the control group of subjects who were genotyped with those of the controls who were not genotyped. No significant differences were seen, in general, for sex, age, area of residence, smoking, and body mass index, and there was no relationship between diabetes mellitus and the probability of being genotyped.

Although we, in *study II*, used two different way of assessing exposure, hospitalized ischemic heart disease detected via International *Classification of Diagnosis* -coding in a nationwide register and self-reported events of ischemic heart disease, we could not study the entire range of ischemic heart disease morbidity. For example, we were not able to detect and study the occurrence of unrecognized myocardial infarctions before first symptom of rheumatoid arthritis, as was done in a study based on the Rochester Epidemiology Project [82], or after diagnosis of rheumatoid arthritis. In both *study II and III*, myocardial infarction was diagnosed clinically by physicians, and we did not have access to individual electrocardiograms or to results from laboratory analyses for blinded diagnostic review. Although the ischemic heart disease diagnoses have been shown to have high validity [4][137], and although we have no reason to believe that this validity would depend on the rheumatoid arthritis status of each individual, a lack of standardized and systematic characterization of each individual event is a limitation of *studies I and II*. Also in *study IV,* the lack of characterization and individual assessment of severity of atherosclerosis and the distribution of unstable plaques is a limitation.

In the general population, smoking has been associated with an increased risk of ischemic heart disease [144]. Smoking has also been associated with rheumatoid factor positive but not rheumatoid factor negative rheumatoid arthritis in our study population [134]. If smoking has the same effect in patients with rheumatoid arthritis as in the general population, it might thus have confounded the association between rheumatoid factor positive rheumatoid arthritis and myocardial infarction. It is therefore interesting to note that in a study published in 1999 Wållberg-Jonsson *et al.* [98] could not find an association between having ever smoked and risk of cardiovascular disease in rheumatoid arthritis. Similar results were noted Gonzalez *et al.* [97] in 2008, where authors demonstrated that having ever smoked was not significantly associated with an increased risk of cardiovascular disease among patients with rheumatoid arthritis. In addition to this we recently reported, in a study partly based on the same study population as in this report, that the overall risk of myocardial infarction after rheumatoid

arthritis diagnosis remained unaltered after adjustment for smoking [145]. In the same study, we noted increased risks of myocardial infarction in the RF negative subset of rheumatoid arthritis, a subset not associated with smoking in our study population [134]. These previously reported findings in relation to our null- findings in *study IV*, makes it unlikely that our inability to investigate smoking in relation to ischemic heart disease and atherosclerosis could have, in any major way, damaged the internal validity of our studies. With this said, it could be that the somewhat higher relative risk estimates observed in RF positive (than in RF negative) patients with rheumatoid arthritis could in fact be due to the higher prevalence of smoking in this subgroup of rheumatoid arthritis. The distribution of traditional risk factors for cardiovascular disease in the EIRA study was similar among cases and controls, except for the proportion of ever smokers and diabetes mellitus that was somewhat larger among cases than controls (table 9). Although we have not assessed each of these factors separately with respect to risk of ischemic heart disease in the EIRA population, we could conclude that adjusting for all of the factors did not alter our estimates.

5.2   FINDINGS AND IMPLICATIONS

In this thesis, results regarding potential risk factors for rheumatoid arthritis, the temporal relationship between rheumatoid arthritis and ischemic heart disease, and the extent of coronary artery disease in rheumatoid arthritis at the time of clinical manifestations of ischemic heart disease have been reported. In *study I*, we reported a significant association between type 1 diabetes mellitus and rheumatoid arthritis. This association was for a particular subset of rheumatoid arthritis, ACPA positive rheumatoid arthritis. Part of this association, but not all, could be attributed to the presence or effect of the 620W *PTPN22* allele, which corroborates the findings from previous studies in which the *PTPN22* polymorphism has been determined to be a risk factor for type 1 diabetes mellitus and ACPA positive rheumatoid arthritis. Although the risk of ACPA positive rheumatoid arthritis was attenuated after adjusting for the presence of *PTPN22* (odds ratio decreasing from 7.3 to 5.3), our data suggest that other genetic and/or environmental factors contribute to the association between type 1 diabetes mellitus and rheumatoid arthritis. Since RF positivity and ACPA positivity are highly correlated in rheumatoid arthritis, the association between type 1 diabetes mellitus and rheumatoid arthritis was also found for RF positive rheumatoid arthritis, but not for RF negative rheumatoid arthritis. There was no association between any subset of rheumatoid arthritis and type 2 diabetes mellitus. These results emphasize that further studies of co-morbidities and shared susceptibility factors between different immune-mediated inflammatory diseases are warranted, and that the fine specificity of the immune-reactions need to be taken into account when performing these studies. Further investigation of the susceptibility genes and other risk factors for type 1 diabetes mellitus are also needed to identify potential risk factors for seropositive rheumatoid arthritis, and may ultimately provide more insight into the etiology of autoimmunity.

In *study II* and *study III*, we demonstrated that the risk of ischemic heart disease goes from null before the first symptom of rheumatoid arthritis to a 60% increased risk, compared to the general population, within just a few years after diagnosis of rheumatoid arthritis. Our results provide support for the hypothesis that rheumatoid arthritis is a risk factor for ischemic heart disease in rheumatoid arthritis. Rheumatoid arthritis could be a risk factor independent of other risk factors or genetic susceptibilities shared by rheumatoid arthritis and the inflammatory processes important in the development of atherosclerosis, but it could also be a risk factor interacting with inherent susceptibilities, genetic or environmental. Another possibility is that there are shared susceptibilities that are associated with premature death causing patients to die before they actually develop their rheumatoid arthritis. We also demonstrated that this increased risk was manifest in rheumatoid

factor positive as well as negative rheumatoid arthritis and that patients with rheumatoid arthritis diagnosed in recent years were at increased risk compared to the general population in spite of the recent advances in treatment of rheumatoid arthritis. By studying ischemic heart disease before the first symptoms of rheumatoid arthritis, we reduced the possible impact of inflammation, or other factors, associated with rheumatoid arthritis having already exerted adverse effects on the risk of ischemic heart disease. The fact that the increased risk of myocardial infarction is evident earlier in the course of rheumatoid arthritis than previously thought raises further questions regarding the etiology of ischemic heart disease in this population.

In *study IV*, we studied the extent of coronary stenosis among patients with rheumatoid arthritis and comparators who all underwent angiography due to acute coronary syndrome. We found that the extent of significant stenoses on angiography was similar comparing patients with rheumatoid arthritis with comparators overall. In RF negative patients, the stenoses seemed to be more widespread compared to that in the comparators. This could not be observed in RF positive patients, and when we compared RF positive patients to RF negative, RF positive rheumatoid the odds ratio for a significant stenosis in the left stem overall was 0.2 (95% confidence interval 0.1, 0.9). Thus, although the process of atherosclerotic development in rheumatoid arthritis is accelerated, and although patients with rheumatoid arthritis are suggested to have an altered spectrum of acute coronary syndrome, the mechanisms of action behind the increased acute coronary syndrome risk in rheumatoid arthritis manifest themselves similarly to that of acute coronary syndrome in the general population.

Studies of the association between rheumatoid arthritis and diabetes mellitus are few and they have been designed in a way that answers a different research question than the one posed in our study. In a cross-sectional study conducted by Simard and Mittleman published in 2007 [146], a non-significant association between diabetes mellitus overall and rheumatoid arthritis overall was reported. The point estimates presented ranged from 1.1 to 1.5 but none of them reached statistical significance. In a study by Solomon *et al.* published in 2010 [147], the incidence rate of diabetes mellitus in a population of patients with rheumatoid arthritis was higher than in the general population, 8.6 per 1,000 person-years (95% confidence interval 8.5, 8.7) and 5.8 per 1,000 person-years (95% confidence interval 5.8, 5.8) respectively corresponding to a relative risk of 1.5 (95% confidence interval 1.4, 1.5). Gonzalez *et al.* presented in 2008 [97] a similar analytic approach but found no increased rate of diabetes mellitus in rheumatoid arthritis compared to the non-rheumatoid arthritis population. If the research question posed is to assess the effect of diabetes mellitus on risk of developing rheumatoid arthritis, one of the most important factors is of course the temporal relationship between the exposure (diabetes mellitus) and the outcome (rheumatoid arthritis). In the studies by Solomon and Gonzalez, the relationship is the opposite: the exposure is rheumatoid arthritis and the outcome diabetes mellitus. The result from their studies thus assesses the association between rheumatoid arthritis and diabetes mellitus but with a reversed causal direction. In the study by Simard and Mittleman, it is not known which of the two diseases occurs first. Therefore it is impossible to make any causal inferences about the association described in their report (which the authors also refrain from doing).

 In *study II*, we found that there was no increased occurrence of ischemic heart disease in patients who later develop rheumatoid arthritis. This finding is the first of its kind, and to my knowledge only one study addressing this issue has been presented previously. In 2005 Maradit-Kremers *et al.* [82] presented a study of 603 patients with rheumatoid arthritis and 603 non-rheumatoid arthritis subjects matched on birth-year, sex, and duration of medical history followed in the Rochester Epidemiology Project. Patients with rheumatoid arthritis were identified and fulfilled ACR criteria for rheumatoid

arthritis for the first time between1955 and 1995. Authors found that patients with rheumatoid arthritis had a several-fold increased occurrence of hospitalization for myocardial infarction (17 events) and of unrecognized myocardial infarction (11 events) by the time they fulfilled the ACR criteria for rheumatoid arthritis, compared with the controls (5 hospitalizations, 2 unrecognized), odds ratio for hospitalized myocardial infarction 3.40 (95% confidence interval 1.25, 9.22), for unrecognized myocardial infarction 5.50 (95% confidence interval 1.22, 24.81). Unrecognized myocardial infarction was defined as the presence of characteristic ECG findings in a non-acute setting, or a recorded physician diagnosis of a characteristic ECG finding in a patient with no documented history of previous myocardial infarction. Besides small numbers, one explanation for the apparent discrepancy compared with our results (odds ratio of 1.0) may be the fact that the study by Maradit-Kremers *et al.* assessed ischemic heart disease events occurring prior to fulfillment of the ACR criteria rather than first symptom of rheumatoid arthritis. It could be that the patients with rheumatoid arthritis already had had a clinically manifest inflammatory disease for a considerable period of time before fulfillment of the ACR criteria and that the increased occurrence in fact was due caused by the factors increasing risk of ischemic heart disease in established rheumatoid arthritis. In contrast, our study populations encompasses patients with a latency of less than18 months between the first symptom of rheumatoid arthritis and the date of the rheumatoid arthritis diagnosis (median 6.3 months) and in whom ischemic heart disease events were assessed up until the first occurrence of symptoms rather than until rheumatoid arthritis diagnosis. The somewhat higher statistically non-significant occurrence of ischemic heart disease noted in patients with rheumatoid arthritis, compared to the controls, in the 5 years right before the onset of rheumatoid arthritis symptoms, supports this line of argument.

HLA-*DRB1*0404*, one of the variants included in the shared epitope, has been suggested to be an independent risk factor for ischemic heart disease in patients with established rheumatoid arthritis[107]. Apart from being associated with clinically manifest ischemic heart disease, it has also been associated with endothelial dysfunction [114] and shared epitope overall has been associated with carotid plaques[148]. When we stratified our cases and controls based on shared epitope status, we could not detect any heterogeneity between shared epitope positives and negatives, which could indicate that the shared epitope *per se*, although a marker of ischemic heart disease risk after the onset of rheumatoid arthritis, does not constitute a risk factor for ischemic heart disease independently of rheumatoid arthritis or that any such risk would be small in magnitude. Not favouring this interpretation of our results is a study by Palikhe *et al.* published in 2007[149] indicating that the HLA-*DRB1*01*, also one of the variants included in the shared epitope, indeed is significantly associated with acute coronary syndrome in the general population. In this study, the authors assessed the impact of shared epitope on coronary artery disease in individuals who had clinical manifestations of acute coronary syndrome (n=100) compared with age- and sex-matched blood-donor controls without acute coronary syndrome (n=74). They found that HLA-*DRB1*01* was associated with acute coronary syndrome (odds ratio 2.34, 95% confidence interval 1.23, 4.43). In 2010, Björkbacka *et al.* [150] reported that HLA-*DRB1*0101* indeed was associated with myocardial infarction in the general population; 8.7% of all 1,188 cases of first-time myocardial infarction and 7.1% of the 1,191 age- and sex- matched controls without myocardial infarction were carriers of the specific genetic variant. This corresponded to an odds ratio of 1.24 (95% confidence interval 1.00, 1.53, p=0.056). With this said it seems likely, but not certain, that this genetic variant associated with rheumatoid arthritis and ischemic heart disease in rheumatoid arthritis in fact is associated with ischemic heart disease also in the general population. What makes our results differ could be found in differences in study design and the sample size. In both of the studies here referred to, the sex distribution differed from in our study; 70% of the study participants were men in the two studies,

while 70% were women in our study. It could also be an issue of statistical power. In the study by Björkbacka *et al*, where they compared 1,188 cases with 1,191 population based controls and the exposure prevalence among the controls was 7.1%, the authors were able to detect a borderline statistically significant odds ratio of 1.24. Based on this it could be that any potential differences in risk with respect to shared epitope had to be larger than they presented to be able to be detected in our study. It might also be that we need to stratify based on shared epitope variant (HLA-*DRB1*01/*04/*10*) to be able to detect any heterogeneities.

The results from *study III,* together with the demonstration of the non-elevated occurrence of ischemic heart disease before first symptom of rheumatoid arthritis discussed above, indicate that the risk of ischemic heart disease in rheumatoid arthritis arises rather quickly; the relative risk goes from null to a 1.6 within a few years. This may indicate that any, presumably rheumatoid arthritis related, factors exerting their effects on clinical ischemic heart disease do so very soon after the development of the first symptoms of rheumatoid arthritis. This has obvious implications both for further studies on the molecular pathogenesis of ischemic heart disease in rheumatoid arthritis and for prevention strategies to address the increased risk of ischemic heart disease in patients with rheumatoid arthritis. It may be that more emphasis should be put on studies of coagulation and plaque rupture rather than the slow progression of atherogenesis. Aberrations in the levels of both fibrinogen and von Willebrand factor have been associated with increased risk of ischemic heart disease in the general population [151, 152], and levels of these molecules are increased in patients with active rheumatoid arthritis [88, 153]. Furthermore, there are remarkable similarities in the inflammatory responses in the development of atherosclerosis and in the development of rheumatoid arthritis; there is T-cell and mast cell activation, an increased production of pro-inflammatory cytokines (for example tumour necrosis factor-alpha and interleukin-6), as well as an increased expression of leukocyte adhesion molecules in both processes [154]. These findings suggest that systemic inflammation, as in rheumatoid arthritis, contributes to accelerated atherogenesis.

Atherosclerosis and its impact on the hard end-points such as myocardial infarction is well investigated in the general population and in the last decades researchers have demonstrated that the complications of atherosclerosis are not a consequence of coronary stenosis leading to narrowing of coronaries that prevent adequate amounts of oxygenated blood to reach all parts of the myocardium, resulting in myocardial ischemia. Instead, we have learned that the plaques that cause acute myocardial infarctions often do not produce high-grade stenosis and that events often are caused by ruptures of atheromatous plaques rather than high grade stenosis [155, 156]. Inflammation is not only an important component in the progression of atheromatous plaques but also promotes the development of complicated lesions[157]. In rheumatoid arthritis, some studies have indicated that laboratory markers of systemic inflammation, for example c-reactive protein and erythrocyte sedimentation rate, correlate with intima-media thickness [89, 158, 159], while some studies have failed to replicate this finding [94, 121, 160]. In the study by Warrington *et al.*, where 75 patients with rheumatoid arthritis complicated by angina were compared to 128 controls with angina, the authors presented results indicating that patients with rheumatoid arthritis had a worse coronary engagement than controls. To be included in that study, participants had to have had a clinical manifestation of angina and an angiography within the first year of that clinical manifestation. The patients with RA included in their study all had established RA with long disease duration at first angiography. Mean (SD) RA disease duration at angiography was 17.6 (11.0) years. In our study, we have assessed the angiographic pattern in individuals who underwent angiography because they had

acute on-setting symptoms classified as ACS quite shortly after they were diagnosed with RA (mean [SD] disease duration at first angiography 4.2 [2.7] years). With this in mind, it might well be that the seemingly conflicting results reported in the study by Warrington *et al.* and ours, merely reflect a different population under study. In an autopsy study by Aubry *et al.,* published in 2007 [131], authors studied the extent and severity of coronary atherosclerosis in patients with rheumatoid arthritis and age-, sex-, and autopsy date-matched controls. In a subgroup analysis, only individuals who had a history of cardiovascular disease were included. In this subgroup, patients with rheumatoid arthritis were less likely to have multiple vessel disease, defined as two or more coronaries with grade 4 stenosis in left anterior descending, left circumflex and right coronary artery or grade 3 or 4 in the left main artery, than controls (32% and 61% respectively, p=0.0018). They also found that patients with rheumatoid arthritis and cardiovascular disease had a less severe coronary atherosclerosis, both in terms of extent (number of vessels with stenosis) and grade (obstruction of cross-sectional area in percent). These results are, if not corroborating, then at least not contradicting the results we present in *study IV* indicating no difference in extent of atherosclerosis at the time of the event and the discrepancies noted could be due to differences in selection of study subjects. It could be that patients with rheumatoid arthritis who die with a history of cardiovascular disease in fact have a less complicated coronary disease profile and die from sudden deaths due to rupture of vulnerable plaques, which is also suggested by the authors. Other studies comparing prevalence or severity of atherosclerosis measuring the carotid intima-media thickness in patients with rheumatoid arthritis and comparators have done so in individuals without a history of acute coronary syndrome [94, 95, 104, 122, 159] rather than at the time point of acute coronary syndrome. They have done so in order to study the influence of rheumatoid arthritis itself on the development of atherosclerosis rather than to investigate whether those who actually experience acute coronary syndrome have a more widespread atherosclerosis. Interestingly, in the study by Aubry *et al.* authors reported of a higher degree of inflammatory infiltrates in the coronary walls and an increased frequency of vulnerable plaques (fibrous cap <65μm and >25 inflammatory cells/high power field)[131] among patients with rheumatoid arthritis compared to controls. This together with the fact that they also reported that patients with rheumatoid arthritis did not have an increased extent or grade of stenosis suggests that vulnerable plaques are of greater importance in clinical outcomes than the actual amount of coronary atherosclerosis.

 With regard to clinical strategies, we may need to reconsider timing of clinical trials as well as current daily practice to prevent ischemic heart disease in rheumatoid arthritis. In 2009, recommendations on cardiovascular risk management in patients with rheumatoid arthritis (among other diseases) were reported by a multidisciplinary expert committee comprising 18 members including rheumatologists, cardiologists, internists and epidemiologists [161]. The aim of the report was to present recommendations for cardiovascular risk management in rheumatoid arthritis based on the highest evidence available. In short, the recommendations stated:
- that adequate control of disease activity was necessary to lower the cardiovascular disease risk,
- that risk assessments was to be performed once a year using national guidelines for all patients with rheumatoid arthritis,
- that the total cholesterol/high-density lipoprotein ratio should be used when the SCORE model was used to assess cardiovascular risk,
- that all interventions should be carried out according to national guidelines,
- that statins, ACE-inhibitors and/or AT-II blockers were preferred treatment options,
- that NSAID and COXIBs should be used carefully,

- that the lowest dose of corticosteroids was to be prescribed,
- that smoking cessation was to be recommended and
- that the risk score reached when assessing the cardiovascular risk should be multiplied by a factor of 1.5 if the patient with rheumatoid arthritis fulfilled two of the following three criteria: disease duration of more than 10 years; rheumatoid factor or antibodies to citrullinated protein antigen positivity; presence of certain extra-articular manifestations.

In light of our findings of a rapid increase in ischemic heart disease risk, and given that the results are replicated, it might be that these clinical recommendations need to be modified to include patients with rheumatoid arthritis early after diagnosis and without RF.

In recent years, the treatment of early rheumatoid arthritis has been intensified. It is therefore a concern that we observed little difference in the elevated short-term risk of myocardial infarction between patients diagnosed during the periods 1995–1997 and 2002–2006. This suggests that factors other than inflammation may be of importance, that inflammation is still insufficiently treated or that the treatments used affect cardiovascular risk negatively. A systematic review of studies assessing the association between cardiovascular risk and the use of low-dose corticosteroid, defined as an average daily dose below 10 mg per day, in rheumatoid arthritis was recently presented [162]. The authors of this review investigated original reports where patients with rheumatoid arthritis treated with < 10 mg corticosteroids per day were compared to patients with rheumatoid arthritis without corticosteroid treatment with respect to hard end-points (for example cardiovascular mortality, myocardial infarction, stroke, or heart failure) and cardiovascular surrogate markers such as atherosclerosis, arterial stiffness, and traditional cardiovascular risk factors such as high blood pressure, diabetes mellitus, lipids concentrations, homocysteine levels or metabolic syndrome. The authors conclude that there is a weak association between low-dose corticosteroid treatment and cardiovascular surrogate markers/traditional cardiovascular risk factors, and that there is a trend of increased risk of hard end-points among those treated with low-dose corticosteroids. Most of the studies included in this review are observational and although the authors of a majority of the studies included have tried to adjust for disease severity and/or activity, the possibility that the results presented are results from confounding by indication is not to be neglected. In 2002, Choi *et al.* [163] presented data indicating that methotrexate treatment lowered the overall mortality and the cardiovascular mortality in rheumatoid arthritis. 1,240 patients with rheumatoid arthritis were followed for a mean of six years, and methotrexate-use assessed through-out the follow-up was the exposure. To adjust for confounding by indication the authors used a weighted Cox proportional hazards model. Based on this model the cardiovascular mortality hazard ratio comparing methotrexate users with non-methotrexate users was 0.3 (95% confidence interval 0.2, 0.7). Since then, and to my knowledge, four reports on risk of myocardial infarction in relation to methotrexate treatment in rheumatoid arthritis have been published; one presents a reduction in risk, one a non-significant reduction in risk, and two reports show no impact of methotrexate on risk of myocardial infarction (extensively reviewed by Westlake *at al.*[164]). Further, results from the British Society from Rheumatology Biologics Register indicate that the incidence of myocardial infarction in individuals with a EULAR good or moderate response to anti-TNF treatment may be lower than that in individuals who do not respond to anti-TNF treatment [165]. Interestingly, yet unpublished results partly based on data analyzed in this thesis indicate that EULAR response good or moderate does not associate with acute coronary events [166]. Thus, the impact of rheumatoid arthritis treatment on the risk of acute coronary syndrome is yet to be established. However, the lack of reduction in short-term risk of myocardial infarction presented in *study III* in more recently diagnosed patients

does not negate the possibility of an improving long-term outlook in these patients, but raises the possibility that myocardial infarctions that occur either early or late during the course of rheumatoid arthritis may have different aetiologies.

Although we demonstrated increased risks of myocardial infarction across all calendar periods and in most follow-up intervals, and despite adequate statistical power (84% overall power to detect a relative risk of death from myocardial infarction of 1.15 and 82% to detect an relative risk of ischemic heart disease of 1.05), we did not observe any increased risk of death from myocardial infarction. The reason for this is unclear. It could be due to a skewed selection of patients into the Register. It might be that patients with a poor predicted survival (meaning those with a high probability to actually die following a myocardial infarction) are less likely to be included in prospective clinical rheumatoid arthritis registers, for example the Swedish Rheumatology Register, and therefore that short-term mortality in our cohorts is under-estimated, or that patients with rheumatoid arthritis have less severe myocardial infarctions. It could also be that the actual risk of dying from ischemic heart disease is not elevated within the follow-up period we have studied in patients with such short duration of symptoms at diagnosis and thus start of follow-up. There are several studies published that report of an increased mortality due to cardiovascular diseases, acute coronary syndrome, or myocardial infarction in rheumatoid arthritis [48, 51, 54, 57, 59, 61-63, 69, 72, 73, 79, 80, 84, 107, 167-170]. Many of these studies are based on cohorts where patients with rheumatoid arthritis have been identified using inpatient registers, meaning individuals had to be hospitalized in order to be identified and followed [48, 51, 61, 167]. This selection of patients, presumably with a rheumatoid arthritis more severe than the average rheumatoid arthritis, could have an effect on the generalizability of the results based on these cohorts. It would be difficult to make any inferences about the cardiovascular mortality in rheumatoid arthritis as a whole, based on these studies. Some of the other studies reporting of an increased cardiovascular mortality are hospital-based cohorts where the enrolling clinics are not the only rheumatology clinics in the area [54, 73, 169]. As in the example above, it could be that by choosing individuals to be included in the follow-up based on hospital clinics the generalizability of the results is limited. Of the population-based studies [57, 59, 62, 63, 69, 72, 79, 80, 84, 107, 168, 170], only one study includes inception cohorts where duration of symptoms is known and below 18 months by the time of diagnosis and thus follow-up [63]. This study had a longer follow-up than ours; Young *et al.* reported a standardized ischemic heart disease mortality ratio of 1.49 (95% confidence interval1.21, 1.77) during a median follow-up of 9.1 years. The median follow-up in our study was 4.1 years. This could explain the discrepancy noted between our results and the results presented by Young *et al.* Notable in this context, are the results from the British Norfolk Arthritis Register [59] and from the Dutch Nijmegen inception cohort of early rheumatoid arthritis [65]. The Norfolk Arthritis Register includes patients who have had swelling of at least 2 joints for at least 4 weeks with onset after January 1st 1989. Based on this Register, Goodson *et al.* presented in 2002 a study where 575 individuals were classified as patients with rheumatoid arthritis at baseline between 1990 and 1994. They were followed for a median time of 6.9 years and there was no increased overall mortality due to cardiovascular disease, standardized mortality ratio in women 1.10, 95% confidence interval 0.67, 1.69 and in men 0.70, 95% confidence interval 0.36, 1.22 during follow-up. The Dutch Nijmegen inception cohort started recruiting patients with short disease duration (<1 year) in 1985 and has since been enrolling new patients via two hospital-based outpatient-clinics. In the mortality study here referred to, patients with rheumatoid arthritis included between 1985 and October 2007 were analyzed. Radovits *et al.* found that there was no increased overall or ischemic heart disease mortality, during the 10 first years of follow-up, but that after 10 years with rheumatoid arthritis the

mortality increased, which in light of the results presented by Young *et al.* (increased risk with ~9 years of median follow-up), by Goodson *et al.* (no increased risk with ~7 years of median follow-up) and by us (no increased risk is with ~4 years of median follow-up) is particularly interesting. Further, Radovits *et al.* reported that there was no apparent calendar effect, indicating that those diagnosed with rheumatoid arthritis in 1985 were as well off as those diagnosed 1995 or 2000, results that well corroborate with our findings. Both the studies by Goodson *et al.* and Radovits *et al.* were performed on inception cohorts of rheumatoid arthritis, with study subjects similar to ours (no increased mortality from ischemic heart disease with a median follow-up of ~4 years).

Stratifying the study population in *study III* by RF status did not alter any of the relative risks presented, except for increasing the uncertainty of the estimates. RF positivity has been associated with an increased risk of mortality, compared to RF negativity in the general population [110] and compared to controls in studies of patients with rheumatoid arthritis [45]. In both studies cardiovascular death was increased among those who were RF positive, and in the study by Thomasson *et al.* [110] there was a borderline significant increased risk of cardiovascular disease. Results for the RF negative subset of patients with rheumatoid arthritis have been less conclusive [59, 62, 72]. Our study may resolve some of these previous ambiguities. We had sufficient power, as well as detailed data on RF status, to enable an analysis of ischemic heart disease risk in the two major subsets of rheumatoid arthritis. The increased risk of ischemic heart disease also in the rheumatoid factor-negative subgroup as well as over the entire 10-year period of follow-up, indicate that we need to search for biomarkers and mechanisms in addition to RF, to understand phenotypic differences in ischemic heart disease risk between different subsets of patients with rheumatoid arthritis. Surprisingly, in *study IV*, we noted a more widespread coronary stenosis in patients with RF negative rheumatoid arthritis, compared to controls, who experience an event. This could be due to RF negative patients having a lower disease activity (than RF positive rheumatoid arthritis) over time and therefore accumulates more coronary atherosclerosis over time and then eventually experiencing a ruptured plaque while patients with rheumatoid factor positive rheumatoid arthritis have high disease activity, a constant high degree of systemic inflammation and therefore experience a plaque rupture before they have a significantly larger extent of stenosis.

6 CONCLUSIONS

Taking all the considerations discussed above into account, we have conducted four studies of high internal and external validity within the field of co-morbidities in rheumatoid arthritis.

(i)   We have shown that type 1 diabetes mellitus and seropositive rheumatoid arthritis are strongly associated and that this association to some extent but not entirely is carried by a shared genetic risk factor, the 620W *PTPN22* allele. The temporal relationship between the exposure (type 1 diabetes mellitus) and the outcome (seropositive rheumatoid arthritis), indicates that type 1 diabetes mellitus is a risk factor for seropositive rheumatoid arthritis.

(ii)   The development of ischemic heart disease risk is a lot more rapid than previously thought (going from none before first symptom of rheumatoid arthritis to significantly increased within a few years of diagnosis of rheumatoid arthritis). Although the temporal relationship between ischemic heart disease and rheumatoid arthritis supports the idea that rheumatoid arthritis factors rather than shared genetic or environmental risk factors drive this association, shared etiologies cannot be excluded. It also seems as although patients with rheumatoid arthritis are more prone to myocardial infarctions they are not more likely to die from myocardial infarctions during the follow-up period here studied.

(iii)   With this altered ischemic heart disease spectrum in mind, it is interesting to note that among individuals who have developed acute ischemic events, the extent of coronary stenosis is no different among patients with rheumatoid arthritis compared to the general population.

7 FUTURE RESEARCH

With the findings discussed in section 5.2 in mind several questions are raised, some of clinical concern and some pertaining to future studies of the etiology of ischemic heart disease in rheumatoid arthritis. All few of the implications of the results presented are found in the discussion.

(i) To be able to identify high-risk patients in the clinical every day work, there is a need for an assessment tool that could be used by the clinicians actually treating the patients. Today, modified versions of SCORE and the Framingham score, developed to identify high-risk individuals in the general population, are recommended but since the influence of traditional risk factors is not the same in rheumatoid arthritis as in the general population, and since the rheumatoid arthritis specific risk factors are not specifically considered in these assessment tools the need for a rheumatoid arthritis specific tool is evident. To do this we are initiating a study to combine information on clinical factors (from the Swedish Rheumatology Register, and from an extensive medical record review), life style factors (from the EIRA study) and genetic factors (also from the EIRA study) in patients with rheumatoid arthritis who experience an acute ischemic event after diagnosis of rheumatoid arthritis and in patients with rheumatoid arthritis who have not by the time they are chosen as controls. By doing so we will be able to assess genetic and environmental factors their impact on ischemic heart disease risk in this population. After having assessed each and every possible such we will be combine the results into a scoring model based on which we will be able to identify individuals in need of primary prevention interventions.

(ii) After having identified high-risk individuals how do we treat them to reduce risk? Again, with the altered spectrum of risk factors for ischemic heart disease in rheumatoid arthritis it might be that interventions aimed to reduce risk in high-risk individuals in the general population are not as efficient in patients with rheumatoid arthritis. This needs to be assessed in long-term clinical trials specifically assessing hard end-points such as myocardial infarction.

(iii) Since the extent of coronary artery stenosis is not different comparing patients with rheumatoid arthritis and controls one must try to figure out what is. First, the characteristics of the atherosclerotic plaque need to be investigated more in terms of physiology and composition than anatomy. Is it so that atherosclerotic plaques in rheumatoid arthritis are different in their composition or stability than in the general population? Is the fibrous cap thinner? Are the cells infiltrating (and possibly causing?) unstable plaques different from the cell populations seen in stable plaques? To do this large studies including in vivo imaging with scintigraphy of radioactively marked cells are needed.

(iv) As discussed above, we have shown that the risk of ischemic heart disease in rheumatoid arthritis is increased by approximately 60% already within a few years. Another outcome, often grouped together with ischemic heart disease and then called cardiovascular disease, is stroke. There are fewer studies assessing the relationship between stroke and rheumatoid arthritis and the different set of risk factors underlying ischemic heart disease and hemorrhagic stroke (for example) makes it obvious that to study "cardiovascular disease overall" is not very informative from an etiologic perspective. We are planning to study the distribution of stroke in rheumatoid arthritis compared to that in the general population after having classified the outcome by type of stroke.

8 SVENSK SAMMANFATTNING FÖR LEKMÄN

8.1 INTRODUKTION

Ledgångsreumatism är en sjukdom som kännetecknas av förhöjd inflammatorisk aktivitet, delvis lokalt i vissa leder, vilket leder till att dessa leder svullnar och ömmar, men även i blodet vilket påverkar hela kroppen något som manifesteras av en förhöjd förekomst av sjukdomar man inte primärt förknippas med ledgångsreumatism. Man brukar dela in patienter som har ledgångsreumatism i två grupper; seropositiva eller seronegativa. Dessa två grupper har visat sig ha något olika riskfaktorprofil och har dessutom något olika sjukdomsförlopp. Seropositiv ledgångsreumatism kännetecknas av en immunologisk reaktion med bildning av antikroppar och leder ofta till en svårare sjukdom, medan seronegativ ledgångsreumatism ofta har ett något mildare förlopp och inte bildar antikroppar.

8.1.1 Mortalitet vid ledgångsreumatism

Man trodde länge att ledgångsreumatism var en sjukdom man dog *med,* inte *av*. I mitten på 50-talet började den uppfattningen ifrågasättas då det publicerades en studie som visade att dessa patienter löpte en dubbelt så hög risk att dö som individer som inte hade ledgångsreumatism. Sedan dess har flertalet studier publicerats som visat ungefär detsamma; patienter med ledgångsreumatism som haft sin sjukdom ett tag riskerar i högre grad än andra att dö i förtid. De främsta dödsorsakerna i flertalet studier var infektioner, cancersjukdomar och hjärt-kärlsjukdomar. Senare studier visade att hjärt-kärlsjukdomar, i synnerhet sådana som påverkade hjärtmuskelns syresättning, så kallade ischemiska hjärtsjukdomar, var den huvudsakliga dödsorsaken. Att fler dör av ischemisk hjärtsjukdom är inte detsamma som att fler faktiskt drabbas av ischemisk hjärtsjukdom. Det kan ju vara så att de infarkter som drabbar patienter med ledgångsreumatism inte är flera men allvarligare och att de därför i högre grad än i andra leder till döden.

8.1.2 Ischemisk hjärtsjukdom vid ledgångsreumatism

För att ta reda på hur det förhöll sig med detta började man i början av 2000-talet studera hur ofta individer med ledgångsreumatism drabbades av hjärtkärlsjukdom i allmänhet och av ischemisk hjärtsjukdom i synnerhet. De studier som då publicerades visade att bland patienter som haft ledgångsreumatism en längre tid alternativt som man inte visste hur länge de varit sjuka faktiskt oftare än individer som inte hade ledgångsreumatism drabbades av ischemisk hjärtsjukdom. I normalbefolkningen har man identifierat riskfaktorer för ischemisk hjärtsjukdom och genom att kartlägga enskilda individers riskfaktorprofil kan man initiera insatser för att sänka risken för ischemisk hjärsjukdom på individnivå. Dessa riskfaktorer har dock visats vara av mindre vikt bland patienter med ledgångsreumatism. Rökning och diabetes, till exempel, som är en starka riskfaktor för hjärtinfarkt i normalbefolkningen har visats ha mindre inflytande på risken att få hjärtinfarkt bland patienter med ledgångsreumatism. Faktorer relaterade till själva ledgångsreumatismen har i stället identifierats som riskfaktorer. Exempel på sådana är bland annat en genetisk riskfaktor för ledgångsreumatism, inflammatorisk aktivitet och svår funktionsnedsättning.

8.1.3 Åderförkalkning och ledgångsreumatism

Åderförkalkning är förändringar i kärlen som har visats vara delvis orsakade av inflammatoriska processer. Åderförkalkning av kärlen som försörjer hjärtmuskeln med syresatt blod leder till att blodförsörjningen till hjärtat påverkas och risken att drabbas av en hjärtinfarkt ökar. Bland patienter

som har ledgångsreumatism har man sett att de är hårdare drabbade av åderförkalkning än lika gamla kontroller med samma kön.

8.2 SYFTE MED STUDIERNA I DENNA AVHANDLING

8.2.1 Delarbete I

Det finns en del studier som har tittat på förhållandet mellan diabetes och ledgångsreumatism. Anledningen till att man har velat göra det är att det finns rapporter som beskriver att patienter med ledgångsreumatism ofta har diabetes. Det finns även studier som har visat att seropositiv ledgångsreumatism och typ 1 diabetes har gemensamma genetiska riskfaktorer. Man har inte sett några likanande samband mellan typ 2 diabetes och ledgångsreumatism, varken seropositiv eller seronegativ. De studier som hittills har publicerats som undersökt förhållandet mellan ledgångsreumatism och diabetes har inte studerat typ 1 och typ 2 diabetes separat, vilket troligen är av stor betydelse. De har heller inte undersökt om den gemensamma genetiska riskfaktorn ger upphov till kliniskt detekterbara uttryck av sjukdom i samma individ. Av dessa anledningar ville vi studera hur typ 1 och typ 2 diabetes påverkade risken att drabbas av ledgångsreumatism. Vi ville dessutom studera effekten av den genetiska variant som funnits vara associerad med både typ1 diabetes och seropositiv ledgångsreumatism.

8.2.2 Delarbete II och III

För att närmare undersöka om det är gemensamma riskfaktorer som ligger bakom den ökade risken för ischemisk hjärtsjukdom bland patienter med ledgångsreumatism eller om det är faktorer som är specifika för sjukdomsaktiviteten vid ledgångsreumatism ville vi undersöka när, i förhållande till insjuknande i ledgångsreumatism, den ökade risken för ischemisk hjärtsjukdom kan detekteras. Om risken föreligger redan innan första symptom har gjort sig gällande så kan detta innebära att gemensamma riskfaktorer för ledgångsreumatism och ischemisk hjärtsjukdomar har ett starkt inflytande. Om det är så att det inte föreligger någon ökad risk innan första symptom så kan det vara så att det som leder till en ökad risk är något som är nära förknippat med aktiv ledgångsreumatism. Om risken dessutom stiger efter diagnos ter det sig ännu troligare att ledgångsreumatism-relaterade faktorer är vad om leder till den ökade risken. För Därför utförde vi två studier där den ena undersökte förekomsten av ischemisk hjärtsjukdom före första symptom och en studie som tittade på risken för ischemisk hjärtsjukdom efter diagnos av ledgångsreumatism.

8.2.3 Delarbete IV

Eftersom det fanns en hel del studier som visade att patienter med ledgångsreumatism var hårdare drabbade av åderförkalkning, och eftersom vi funnit att dessa patienter fick flera hjärtinfarkter väldigt snart efter att de fått sin diagnos av ledgångsreumatism, ville vi undersöka om det var någon skillnad på kranskärlen, i fråga om förträngningar i kärlen, vid manifestation av ischemisk hjärtsjukdom bland patienter med ledgångsreumatism jämfört med individer utan ledgångsreumatism.

8.3 MATERIAL OCH METODER

Delarbetena i denna avhandling är baserade på datakällor som grundar sig på hela befolkningen, en stor fall-kontroll-studie av ledgångsreumatism och en databas som används av reumatologer för att följa sina patienter över tid.

Nedanstående datakällor har kopplats samman och nyttjats i denna avhandling:

(i) Fall-kontroll-studien EIRA. Denna studie initierades 1995 och innehåller information om 3500 individer med ledgångsreumatism (fall) och 4500 individer utan ledgångsreumatism (kontroller)

(ii) Svensk reumatologis kvalitetsregister. Innehåller klinisk information om patienter med ledgångsreumatism som följs i registret.

(iii) Slutenvårdsregistret. Innehåller information om alla sjukhusinläggningar i Sverige.

(iv) Angiografiregistret. Innehåller information om nästan alla angiografier som genomförs i Sverige.

(v) Dödsorsaksregistret. Innehåller uppgifter om dödsorsaker för alla i Sverige.

I samtliga delarbeten har jämförelser mellan patienter med ledgångsreumatism och individer utan ledgångsreumatism gjorts. Dessa jämförelser redovisas som en kvot beräknad baserat på jämförelsegruppen (individer utan ledgångsreumatism) som nämnare.

8.4 RESULTAT

8.4.1 Delarbete I

I delarbete I studerades risken att utveckla ledgångsreumatism om man tidigare i livet blivit diagnostiserad med diabetes. Eftersom typ 1 och typ 2 diabetes är två sjukdomar som har vitt skilda riskfaktorprofiler och eftersom de som skiljer sig från varandra med avseende på biologiska mekanismer som leder till sjukdom studerade vi de olika sjukdomarna var för sig. Med tanke på att även seropositiv och seronegativ ledgångsreumatism har vitt skilda biologiska mekanismer som leder till sjukdom ville vi även utvärdera effekten av typ 1 och typ 2 diabetes på seropositiv och seronegativ ledgångsreumatism var för sig. Därtill kom att vi ville se hur den genetiska riskfaktor som har visats vara gemensam för typ 1 diabetes och seropositiv ledgångsreumatism påverkar förhållandet mellan de två sjukdomarna.

4.4% av alla patienter med ledgångsreumatism och 3.1% av kontrollerna som var inkluderade i EIRA studien rapporterade att de hade diabetes innan de inkluderades. När vi delade upp grupperna på det sätt som nämnts ovan, fann vi att typ 1 diabetes ökade risken att utveckla seropositiv, men inte seronegativ ledgångsreumatism. Typ 2 diabetes ökade inte risken för varken seropositiv eller seronegativ ledgångsreumatism. När vi justerade för förekomst av den gemensamma genetiska riskfaktorn, sjönk risken för att drabbas av seropositiv ledgångsreumatism om man har typ 1 diabetes.

8.4.2 Delarbete II och III

I dessa delarbeten ville vi studera när i förhållande till fastställd ledgångsreumatism risken för ischemisk hjärtsjukdom kunde uppmätas. Först undersökte vi om patienter med ledgångsreumatism oftare än kontroller hade en sjukdomshistoria där ischemisk hjärtsjukdom kunde bekräftas. För att göra detta använde vi både EIRA-studien och svensk reumatologis kvalitetsregister (och kontroller valda från normalbefolkningen till dessa). Vi jämförde dels andelen personer som hade rapporterat i EIRA-studien att de hade haft någon typ av ischemisk hjärtsjukdom innan de fick sina första symptom av ledgångsreumatism, dels sjukhusinläggningar för ischemisk hjärtsjukdom innan första symptom av ledgångsreumatism i både EIRA-studien och svensk reumatologis kvalitetsregister. Circa 6% av patienterna med ledgångsreumatism i svensk reumatologis kvalitetsregister och 6% av deras kontroller hade varit varit inlagda på sjukhus för ischemisk hjärtsjukdom innan de diagnostiserades fick sina första symptom av ledgångsreumatism. I EIRA-studien var det cirka 2 % av fallen och lika stor andel av

kontrollerna som hade varit inlagda på sjukhus. Med andra ord, patienter med ledgångsreumatism hade inte oftare än kontroller en sjukdomshistoria som inkluderade ischemisk hjärtsjukdom. När vi konstaterat detta så uteslöt vi alla som haft någon typ av ischemisk hjärtsjukdom innan de fick ledgångsreumatism och följde dem som var kvar över tid från datumet då det inkluderades och framåt. Vi fann att redan inom några år var patienter med ledgångsreumatism hårdare drabbade av ischemisk hjärtsjukdom än kontrollerna. Även patienter som insjuknat med ledgångsreumatism under 2000-talet löpte en ökad risk för ischemisk hjärtsjukdom.

8.4.3   Delarbete IV

I delarbete IV ville vi undersöka förekomsten av kliniskt signifikanta förträngningar i koronarkärlen hos individer som undersöktes på grund av symptom på ischemisk hjärtsjukdom (hjärtinfarkt eller instabil angina pectoris). Detta gjorde vi genom att jämföra patienter med ledgångsreumatism och kontroller som undersökts med angiografi på grund av ischemisk hjärtsjukdom. Vi fann att utbredningen av kliniskt signifikanta stenoser var densamma i de två grupperna.

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10 APPENDIX

10.1 DIABETES QUESTIONNAIRE

1. Is it true that you have diabetes? ☐Yes ☐No
If not, you can stop here.

2. Which kind of diabetes do you have? ☐Type 1 ☐Type 2
☐Do not know

3. How old were you when the disease was discovered? ....years old

4. What treatment was you prescribed when the disease was discovered?
☐Dietary restrictions only
☐Dietary restrictions and oral
  medication
☐Oral medication only
☐Oral medication and insulin
☐Insulin only

5. What treatment are you on now? ☐Dietary restrictions only
☐Dietary restrictions and oral
  medication
☐Oral medication only
☐Oral medication and insulin
☐Insulin only
☐Byetta-injections

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