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*INCIDENCE, PREVALENCE, AND MORTALITY*

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# THE RISK OF RHEUMATOID ARTHRITIS

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**BY**
**BOLETTE GYLDEN SOUSSI**

DISSERTATION SUBMITTED 2023

AALBORG UNIVERSITY
DENMARK

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# CURRICULUM VITAE

**Academic degree**

January 2017, Master of Science in Medicine (MD), Aarhus University, Denmark.

**Research employment**

August 2019 enrolled as PhD student, Faculty of Medicine, Aalborg University, Denmark.

March 2019 to August 2023, Clinical Assistant, Center of Rheumatic Research Aalborg (CERRA), Department of Rheumatology, Aalborg University Hospital, Denmark.

**Research fields**

Main research field are patients with rheumatoid arthritis with focus on the occurrence, severity, and development of the disease from an epidemiological research perspective. The overall purpose is to increase the understanding of the disease rheumatoid arthritis and to reduce the disease burden in the society.

**Teaching experience**

2019, participated in the education of physiotherapy students from Aalborg University in joint examination and rheumatology cases.

2018 and onwards, participating in the education of medical students from Aalborg University in medical musculoskeletal diseases.

2017 to 2020, participated in the education of medical student from Aarhus University in leadership and management.

## Talks and poster presentations

2023, oral presentation at the Danish Rheumatology Society's annual meeting. **Soussi BG**, Duch K, Cordtz RL, Kristensen S, Lindhardsen J, Linauskas A, Lene Dreyer. Temporal trends in mortality in patients with rheumatoid arthritis: A Danish population-based matched cohort study [abstract].

2022, ignite talk and poster presentation at American College of Rheumatology Convergence. **Soussi B**, Duch K, Cordtz R, Bork C, Kristensen S, Schmidt E, Lindhardsen J, Dreyer L. Improvement in Excess Mortality in Patients with Rheumatoid Arthritis over the Last Two Decades: A Danish Population-based Matched Cohort Study [abstract]. *Arthritis Rheumatol* 2022; 74:suppl 9.

2021, poster presentation at the European Alliance of Associations for Rheumatology congress. **Soussi BG**, Cordtz RL, Kristensen S, Sørensen CB, Christensen J, Schmidt EB, Prieto-Alhambra D, Dreyer L. Incidence rates and point prevalence of seropositive and seronegative rheumatoid arthritis in Denmark: a nationwide register-based study from 1998 to 2018 using four different case criteria [abstract]. *Ann Rheum Dis* 2021;80:S1:215.

2021, poster presentation at the Danish Rheumatology Society's annual meeting. **Soussi BG**, Cordtz RL, Kristensen S, Sørensen CB, Christensen J, Schmidt EB, Prieto-Alhambra D, Dreyer L. Incidence rates and point prevalence of seropositive and seronegative rheumatoid arthritis in Denmark: a nationwide register-based study from 1998 to 2018 using four different case criteria [abstract].

2020, oral presentation at the European Alliance of Associations for Rheumatology congress. **Soussi BG**, Cordtz RL, Kristensen S, Sørensen CB, Christensen J, Schmidt EB, Prieto-Alhambra D, Dreyer L. Incidence and prevalence of rheumatoid arthritis in Denmark: a nationwide population-based study investigating the effect of four different case definitions [abstract]. *Ann Rheum Dis* 2020;79,S1:46.

## Publications with peer review (not included in this thesis)

Alzubaidi A, Cordtz R, Westermann R, **Soussi BG**, Lauridsen KB, Kristensen S, Dreyer L. SARS-CoV-2 test patterns in Danish patients with inflammatory rheumatic diseases during the COVID-19 pandemic. *Scand J Rheumatol* 2023;52:321-323.

Cordtz R, Lindhardsen J, **Soussi BG**, Vela J, Uhrenholt L, Westermann R, Kristensen S, Nielsen H, Torp-Pedersen C, Dreyer L. Incidence and severeness of COVID-19 hospitalisation in patients with inflammatory rheumatic disease: a nationwide cohort study from Denmark. *Rheumatology (Oxford)* 2021;60:SI59-SI67.

Cordtz R, Hawley S, Prieto-Alhambra D, Højgaard P, Zobbe K, Kristensen LE, Overgaard S, Odgaard A, **Soussi BG**, Dreyer L. Reduction in Upper Limb Joint Surgery among Rheumatoid Arthritis Patients: An Interrupted Time Series Analysis using Danish Health Care Registers. *Arthritis Care Res (Hoboken)* 2020;72:274-282.

# ENGLISH SUMMARY

Rheumatoid arthritis (RA) is a common chronic immune-mediated disease that untreated can lead to severe joint damage and disability. Based on the presence or absence of autoantibodies the disease is divided into seropositive and seronegative RA, respectively. RA is characterised by inflammatory arthritis and extraarticular involvement, and in many cases caused by the interaction between genes and environmental factors, though the aetiology is not yet fully understood.

The overall aims of this thesis were to study the epidemiology and burden of RA including temporal changes and provide contemporary estimates of the disease in Denmark. Existing studies concerning the epidemiology of RA have methodological differences, and this thesis explored the impact of some of these approaches. The improvement in the management of RA over the last decades and the introduction of new classification criteria for RA in 2010 were expected to be reflected in temporal trends.

Using two nationwide population-based cohort designs, the incidence and prevalence of RA were investigated and showed a relatively stable incidence and an increased prevalence over calendar time. The underlying temporal incidence pattern exposed an increased incidence of seropositive RA and a decreasing trend of seronegative RA, as autoantibody testing became more frequent. The thesis confirmed and emphasised the importance of methodological considerations including the definition of RA and seropositivity in register-based studies.

Next, the 5-year all-cause mortality for incident patients with RA was investigated in a nationwide population-based matched cohort. Over time, the 5-year mortality risk attenuated and men with RA attained similar mortality risk as the general population, while excess mortality persisted in women with RA.

Continued focus on the prevention of RA, its progression, and comorbidities is needed. Therefore, this thesis also included a protocol for investigation of the association between a potential inverse lifestyle risk factor, intake of marine n-3 polyunsaturated fatty acids, and subsequent development of RA.

In conclusion, improvement in RA management and changes in the Danish society was reflected in the occurrence and mortality of RA. The thesis uncovered sex-specific differences in the prognosis of RA disease, that need to be investigated further.

# DANSK RESUME

Leddegigt er en hyppigt forekommende kronisk autoimmun sygdom som ubehandlet kan føre til alvorlige ledskader og invaliditet. Leddegigt er karakteriseret ved inflammatorisk artrit og ekstraartikulær involvering og i mange tilfælde forårsaget af interaktionen mellem gener og miljøfaktorer, omend ætiologien endnu ikke er fuldt klarlagt. Baseret på tilstedeværelse eller fravær af autoantistoffer inddeles sygdommen i henholdsvis seropositiv og seronegativ leddegigt.

Det overordnede formål med denne afhandling var at belyse epidemiologien og byrden af leddegigt samt de tidsmæssige tendenser, og give nutidige estimater for forekomst og mortalitet af sygdommen i Danmark. Tidligere epidemiologiske studier af leddegigt har anvendt forskellige metodemæssige tilgange, og denne afhandling undersøgte betydningen af nogle af disse. Forbedringen i behandlingen af leddegigt i de seneste årtier og indførelsen af nye klassifikationskriterier for leddegigt i 2010 forventedes at have influeret de tidsmæssige tendenser.

I to landsdækkende befolkningsbaserede kohortestudier blev incidensen og prævalensen af leddegigt undersøgt. De viste en forholdsvis konstant incidens men en stigende prævalens af leddegigt over kalendertid, men en stigende incidens af seropositiv leddegigt og faldende tendens for seronegativ leddegigt, efterhånden som autoantistoftest blev mere tilgængeligt. Afhandlingen bekræftede og understregede vigtigheden af metodiske overvejelser herunder definitionen af leddegigt og seropositivitet i register-baserede studier.

Ydermere blev den 5-årige dødelighed for nyligt diagnosticerede patienter med leddegigt undersøgt i et landsdækkende befolkningsbaseret matchet kohortestudie. Over kalendertid faldt 5-års dødeligheden, men der var en kønsforskel i den observerede forbedring, hvor overdødelighed vedblev hos kvinder med leddegigt, mens den over tid for mænd med leddegigt nåede samme niveau som mænd i baggrundsbefolkningen.

Fortsat fokus på forebyggelse af sygdom, sygdomsprogression og komorbiditet er nødvendig. Derfor var næste skridt at identificere modificerbare risikofaktorer, hvorfor denne afhandling også inkluderede en protokol til undersøgelse af sammenhængen mellem en potentiel beskyttende livsstilsfaktor, n-3 flerumættede fedtsyrer, og efterfølgende udvikling af leddegigt.

I denne afhandling var forbedring i diagnosticering og behandling af patienter med leddegigt og ændringer i det danske samfund afspejlet i forekomsten og dødeligheden af leddegigt. Afhandlingen demaskerede kønsspecifikke forskelle i prognosen for leddegigt, som skal undersøges nærmere.

# ACKNOWLEDGEMENTS

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To the #GetYourShitTogether group (Line Uhrenholt, Katrine Gade, Jonathan Vela, René Cordtz and Rasmus Westermann) thank you for all the laughs and discussions (both professional and personal). Words cannot describe my gratitude for our friendship.

My warmest gratitude to the Department of Rheumatology, Aalborg University Hospital, Danish Rheumatism Association, Aase og Ejnar Danielsens Fond, Grosserer L. F. Foghts Fond, and Frimodt-Heineke Fonden for funding my research.

The learning experience and knowledge this PhD has given me will provide a solid foundation for future research. I look forward to continue conducting research with valuable collaborations.

Lastly, a special and heartfelt thanks to my loving and supportive family. With your unlimited encouragement, this has been a family project.

Bolette Gylden Soussi, June 2023

# PREFACE

This thesis contains scientific works carried out in collaborations between the Center of Rheumatic Research Aalborg at Aalborg University Hospital, and the Department of Cardiology at Aalborg University Hospital, the Department of Rheumatology at North Denmark Regional Hospital Hjørring, the Center for Rheumatology and Spine Diseases at Rigshospitalet and the CSM-NDORMS at Oxford University.

The overall purpose of this dissertation was to investigate the incidence, prevalence, and mortality of rheumatoid arthritis in Denmark. Further, a presentation of a planned investigation of n-3 polyunsaturated acids and the subsequent risk of developing rheumatoid arthritis will be presented.

The thesis consists of five chapters: Chapter 1 provides a broad overview of the research area; Chapter 2 presents the aims and hypotheses; Chapter 3 provides an overview of studies included in this dissertation; Chapter 4 contains a summarized discussion of the respective studies; and Chapter 5 states the conclusion of this dissertation alongside perspectives for future research in this area.

I hope you enjoy the thesis.

# LIST OF PAPERS

This thesis is based on the following four studies which are presented in full length in supplementary, Appendix A-D. Throughout the thesis, the papers are referenced to as presented below.

**Paper I (Study I)**

Soussi BG, Cordtz RL, Kristensen S, Bork CS, Christensen JH, Schmidt EB, Torp-Pedersen C, Prieto-Alhambra D, Dreyer L. Incidence and prevalence of rheumatoid arthritis in Denmark from 1998 to 2018: a nationwide register-based study. *Scand J Rheumatol* 2022;51(6):481-489.

**Paper II (Study II)**

Soussi BG, Cordtz RL, Duch K, Kristensen S, Linauskas A, Bork CS, Schmidt EB, Dreyer L. Incidence of seropositive and seronegative rheumatoid arthritis in Denmark: a nationwide population-based study. Submitted to Arthritis Care & Research (in review).

**Paper III (Study III)**

Soussi BG, Duch K, Cordtz RL, Lindhardsen J, Kristensen S, Bork CS, Linauskas A, Schmidt EB, Dreyer L. Temporal trends in mortality in patients with rheumatoid arthritis: A Danish population-based matched cohort study. *Rheumatology Oxford* (accepted for publication).

**Paper IV (Study IV)**

Soussi BG, Bork CS, Kristensen S, Lundbye-Christensen S, Duch K, Cordtz RL, Christensen JH, Schmidt EB, Dreyer L. Intake of marine n-3 polyunsaturated fatty acids and the risk of rheumatoid arthritis: protocol for a cohort study using data from the Danish Diet, Cancer and Health cohort and Danish health registers. *BMJ Open* 2021;11:e04798.

# ABBREVATIONS

Presented in alphabetical order:

ACPA, anti-citrullinated protein antibodies

ACR, American College of Rheumatology

Anti-CCP, anti-cyclic citrullinated peptide

ARA, arachidonic acid

ATC, Anatomical Therapeutic Chemical

csDMARD, conventional synthetic disease-modifying anti-rheumatic drug

CI, confidence interval

CIP, cumulative incidence proportion

DCH, Diet, Cancer and Health

DHA, docosahexaenoic acid

DMARD, disease-modifying anti-rheumatic drug

DNPR, Danish National Patient Registry

DPA, docosapentaenoic acid

EPA, eicosapentaenoic acid

EULAR, European Alliance of Associations for Rheumatology

ICD, International Classification of Diseases

Ig, immunoglobulin

IL, interleukin

IP, incidence proportion

IR, incidence rate

LA, linoleic acid

LR, lifetime risk

NPR, Danish National Prescription Registry

PP, point prevalence

PUFA, polyunsaturated fatty acid

PY, person-years

RA, rheumatoid arthritis

RD, risk difference

RF, rheumatoid factor

RR, relative risk

SSI, Statens Serum Institut

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# CHAPTER 1. BACKGROUND

## 1.1. RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a common chronic autoimmune and inflammatory disease (1–3). Manifestations mainly consist of symmetric polyarthritis (pain and swelling) of small joints, typically in hands and feet, but RA can affect any synovial joint (3). RA is a systemic disease and can therefore also present with extraarticular manifestations and are associated with multiple coexisting conditions (3,4). The disease process begins years before the clinical manifestation of the disease (3). Genetic research has implied that RA might be a heterogeneous group of diseases with overlapping syndromes but with different disease mechanisms, where especially seronegative RA pose a diagnostic challenge (5–8).

### 1.1.1. DIAGNOSTIC CLASSIFICATION AND CRITERIA

The 1987 American College of Rheumatology (ACR; formerly the American Rheumatism Association) criteria for classification of RA was introduced in 1987 and involved assessment of joint involvement, serology, and radiology (9). The 1987 ACR criteria for classification of RA were criticised for lack of sensitivity in early RA disease and therefore replaced in 2010 with new classification criteria established to identify RA at an earlier stage and patients at high risk for persistent and/or erosive RA (10). The 2010 ACR/European Alliance of Associations for Rheumatology (EULAR; formerly the European League Against Rheumatism) classification criteria for RA are the current guidelines for classification of RA and are based on four aspects of which each is weighted and assigned points: joint involvement, serology, acute-phase reactants, and duration of symptoms (10,11). Validation studies of the diagnostic performance of the 2010 ACR/EULAR classification criteria for RA compared with the 1987 ACR criteria for RA showed a higher sensitivity for the 2010 ACR/EULAR criteria (0.85 versus 0.76), but otherwise, the criteria performed similarly (e.g. in specificity), and the new criteria did not outperform the older criteria in the detection of erosive disease (12,13).

### 1.1.2. EPIDEMIOLOGY

The annual age- and sex-standardised incidence rate (IR) of RA in Scandinavian countries has previously been reported to be approximately 41 per 100 000 individuals

and 31 per 100 000 person-years (PY), which was similar or lower than observed in other Western countries (Table 1-1) (14–19).

Trends in temporal IR of RA have been inconsistent in cohort studies with decreasing trends (UK 1990 to 2014; Canada 1996 to 2010), an increasing trend (Denmark 1995 to 2001), or no consistent trend/stable trend (US 1985 to 2014; Canada 2001 to 2014) (15–19). The decreasing patterns consisted of a combination of periodic decline and stabilisation (16,19). However, the increasing trend observed in the Southern part of Denmark could be a result of changes in referral patterns introducing an artefact (15).

The disease is more frequent in women than in men with a two-fold higher IR (14–16,18). In the Swedish population, the IR peaked for both sexes in the age of 70 to 79 years with an IR of 102 per 100 000 individuals for women and 67 per 100 000 individuals for men (14). In Scandinavian countries women had higher IR across all age groups, however, the difference between the sexes decreased with higher age (14,15).

Overall age- and sex-standardised estimates of point prevalence (PP) for RA varied between 0.36% and 0.67% depending on the year in question (UK 1990 to 2014) (Table 1-2) (19). This was lower than the period prevalence of 0.70% (Sweden 2001 to 2007), the cumulative prevalence of 0.77% (Sweden 2008), and prevalence rates of 0.62% to 0.72% (US 1995 to 2005), although higher than other prevalence rates of 0.48% to 0.68% (Canada 2001 to 2015), and varying between lower or higher levels depending on the year (Canada 1996 to 2010) observed in other Western countries (16,17,20,21).

In line with the higher IR of RA in women than in men, a higher PP was also detected in women, with increasing PP with age for both sexes (Table 1-2) (19,22,23). Sex-specific age-standardised PP for both sexes in Denmark was lower than in the whole Nordic region in 1995, at the same level in 2015, and higher than global levels in both years (22).

Temporal trends in PP have also shown some inconsistency. In European countries an annual increasing trend was found until 2005 whereafter the trend remained stable (UK 1990 to 2014), though a complete agreement does not exist, as the trend was found stable in Denmark and decreasing in the Nordic Region based on two point estimates in 1995 and 2015, respectively (19,22). Evidence of an increasing trend was supported by increasing prevalence rates in other Western countries outside Europa (US 1995 to 2005; Canada 1996 to 2010; Canada 2001 to 2015) (16,17,21).

In general, some of the variations found in incidence and prevalence estimates can be due to the heterogeneity in the methodological approaches including RA case definitions and statistical methods, but differences in genetic disposition,

environmental factors and access to medical care may also be of importance (1,14,18,24–27).

**Table 1-1.** Overview of selected* cohort studies investigating the incidence rate of rheumatoid arthritis. Studies are listed in chronological order by year of publication.

| Author, year, and country | Study period | Setting | RA patient definition, Number (N) of patients | Sex, age | IR of RA** |
|---|---|---|---|---|---|
| Pedersen et al., 2007, Southern part of Denmark (15) | 1995 to 2001. | Population-based cohort study. | 1987 ACR criteria. Medical record screening.<br><br>N = 440 | Women and men, ≥15 years. | Overall: 30.6 (95%CI 27.8 to 33.6) per 100 000 PY.<br><br>Women: 39.1 (95%CI 34.7 to 44.0) per 100 000 PY.<br><br>Men: 21.8 (95%CI 18.5 to 25.6) per 100 000 PY. |
| Eriksson et al., 2013, Sweden (14) | 2006 to 2008. | Nationwide population-based cohort study. | Base: 1) first inpatient or nonprimary outpatient care visit for RA, 2) a second discharge or nonprimary outpatient care visit for RA within 1 year, 3) no DMARD >6 months before the first RA visit. N = 8826<br><br>Liberal: 1) and 2) form the base definition. N = 10 094 | Women and men, ≥18 years. | Overall (base): 40.6 (95%CI 39.7 to 41.4) per 100 000 individuals.<br><br>Women (base): 55.7 (95% CI 54.3 to 57.1) per 100 000 individuals. |

| | | | | | |
|---|---|---|---|---|---|
| | | | Strict: as base and ≥1 of the visits should be at department of rheumatology or internal medicine. N = 8009 | | Men (base): 25.0 (95% CI 24.0 to 25.9) per 100 000 individuals.<br><br>A 14% variation in estimates depending on the RA case definition. |
| Widdifield et al., 2014, Ontario, Canada (16) | 1996 to 2010. | Population-based cohort study. | ≥3 physician billing RA diagnoses with ≥1 by a rheumatologist, general internist, or orthopaedic surgeon, within 2 years, or ≥1 hospitalisation diagnosis for RA.<br><br>N = 87 452 | Women and men, ≥15 years. | Overall: ranging from 52 (95%CI 51 to 54) to 62 (95%CI 60 to 63) per 100 000 individuals depending on the year in question. |
| Abhishek et al., 2017, UK (19) | 1990 to 2014. | Nationwide population-based cohort study. | No medical code for RA prior to the latest of current registration date, and ≥1 year registration prior to date of first RA diagnosis in the Clinical Practice Research Datalink.<br><br>N = 1299 | Women and men, ≥18 years. | Overall: ranging from 3.73 (95%CI 3.54 to 3.92) to 7.81 (95%CI 6.93 to 8.68) per 10 000 PY depending on the year in question. |
| Nair et al., 2019, Saskatchewan, Canada (17) | 2001 to 2014. | Population-based cohort study. | ≥3 physician billing RA diagnoses with ≥1 by a rheumatologist, general internist, or orthopaedic surgeon, | Women and men, | Overall: ranging from 33.6 (95%CI 29.9 to 37.6) to 73.1 (95%CI 67.6 to 79.0) per 100 000 |

| | | | | | |
|---|---|---|---|---|---|
| | | | within 2 years, or ≥1 hospitalisation diagnosis for RA.<br><br>N = 5758 | ≥18 years. | individuals depending on the year question. |
| Myasoedova et al., 2020, Olmsted County, Minnesota, US (18) | 1985 to 2014. | Population-based cohort study. | 1987 ACR criteria. Medical record review.<br><br>N = 1011 | Women and men, ≥18 years. | 1985 to 1994: overall 40 (95%CI 35 to 46), women 48 (95%CI 41 to 56), men 32 (95%CI 25 to 40) per 100 000 individuals.<br><br>1995 to 2004: overall 43 (95%CI 38 to 48), women 55 (95%CI 48 to 63), men 30 (95%CI 24 to 36) per 100 000 individuals.<br><br>2005 to 2014: overall 41 (95%CI 37 to 45), women 53 (95%CI 47 to 59), men 29 (95%CI 24 to 34) per 100 000 individuals. |

RA, rheumatoid arthritis; IR, incidence rate; ACR, American College of Rheumatology; PY, person-years; 95%CI, 95% confidence interval; DMARD, disease-modifying anti-rheumatic drug.

* Systematic literature search on PubMed for existing evidence on RA and IRs in English and Scandinavian languages (January 2023). Titles and abstracts were screened, and relevant papers were read in full. Also, references included in relevant studies were

evaluated. Western cohort studies with age- and sex-standardised or sex-specific age-standardised estimates within the years 1998 to 2018 were included.

** Annual IR were age- and sex-standardised for overall RA and sex-specific estimates were age-standardised.

**Table 1-2.** Overview of selected* cohort studies investigating the point prevalence of rheumatoid arthritis. Studies are listed in chronological order by year of publication.

| Author, year, and country | Study period | Setting | RA patient definition, Number (N) of patients | Sex, age | PP of RA** |
|---|---|---|---|---|---|
| Englund et al., 2010, County of Skåne, Sweden (23) | 2008. | Population-based cohort study. | ICD-10 RA diagnosis on at ≥2 separate occasions with ≥1 from rheumatologist or general internist.<br><br>N = 5546 | Women and men, ≥20 years. | Women: 0.80%.<br><br>Men: 0.31%. |
| Abhishek et al., 2017, UK (19) | 1990 to 2014. | Nationwide population-based cohort study. | Individuals in the Clinical Practice Research Datalink with a medical Read code of RA on 1 July of each calendar year.<br><br>N = 26 385 | Women and men, ≥18 years. | Overall: ranging from 0.36% (95%CI 0.36 to 0.37) to 0.67% (95%CI 0.66 to 0.67) depending on the year in question. |
| Kiadaliri et al., 2018, Global (22) | 1995 to 2015. | Population-based cohort study. | RA in the Global Burden of Disease Study cohort where RA was based on the 1987 ACR criteria and ICD-10 codes of M05-M06.9 and M08.0-M08.89.<br><br>N = 159 853 | Women and men, NA. | Denmark, 1995: Women 0.60% (95%CI 0.54 to 0.66), men 0.24% (95%CI 0.22 to 0.27).<br><br>Denmark, 2015: Women 0.63% (95%CI 0.57 to |

| | | | | | |
|---|---|---|---|---|---|
| | | | | | 0.70), men 0.26% (95%CI 0.24 to 0.29).<br><br>Nordic region, 1995: Women 0.68% (95%CI 0.62 to 0.73), men 0.28% (95%CI 0.26 to 0.31).<br><br>Nordic region, 2015: Women 0.61% (95%CI 0.56 to 0.67), men 0.26% (95%CI 0.24 to 0.29).<br><br>Global, 1995: Women 0.49% (95%CI 0.45 to 0.53), men 0.21% (95%CI 0.19 to 0.23).<br><br>Global, 2015: Women 0.47% (95%CI 0.44 to 0.52), men 0.21% (95%CI 0.20 to 0.23). |

RA, rheumatoid arthritis; PP, point prevalence; ICD, International Classification of Diseases; 95%CI, 95% confidence interval.

* Systematic literature search on PubMed for existing evidence on RA and PP in English and Scandinavian languages (February 2023). Titles and abstracts were screened, and relevant papers were read in full. Also, references included in relevant studies were

evaluated. Western cohort studies with age- and sex-standardised or sex-specific age-standardised estimates within the years 2000 to 2018 were included.

** PP were age- and sex-standardised for overall RA and sex-specific estimates were age-standardised.

### 1.1.3. SEROLOGY

RA can be divided into seropositive and seronegative RA based on the presence or absence of autoantibodies. Seropositive RA is recognised as a phenotype of RA with the presence of rheumatoid factor (RF) and/or anti-citrullinated protein antibodies (ACPA) in the serum, whereas seronegative RA is characterised by the absence of both of these autoantibodies (7,28). Immunoglobulin (Ig)M-RF and IgA-RF are pathogenic markers directed against the Fc fragment of IgG, whereas the anti-cyclic citrullinated peptide (anti-CCP) is directed against the synthetic citrullinated peptide (5,28,29). Other autoantibodies have been identified, but only RF and ACPA were considered clinically useful and included in the 2010 ACR/EULAR classification criteria for RA (10,29). RF was the only serologic measure included in the 1987 ACR criteria for classification of RA (9). In Denmark, tests for ACPAs measure the level of anti-CCP.

Genetic traits and smoking have been associated with ACPA with the possibility of the two factors acting synergistic (3,5,28). Smoking is a risk factor for ACPA positive RA, especially in individuals that carry the HLA-DRB1 shared epitope alleles (5,30). The autoantibodies have been linked to the pathogenesis of RA, and seropositivity has been associated with more aggressive disease, and a higher risk of comorbidities and premature death (8,31–36).

In Scandinavian populations, the proportion of seropositive patients was 61 to 76% in the past decades, which tended to be higher than the proportion observed in other Western countries ranging between 53 and 64% (14,15,30,36–40). With tendencies of a larger range in the positive percentage in incident-determined seropositive RA (53 to 76%) than in cross-sectional (62 to 67%) and of a lower proportion of seropositive RA when using ICD-10 codes (53 to 67%) than with autoantibodies (61 to 76%) (14,15,36–40). The majority of Swedish patients with RA were double seropositive (57%), 9% were positive for one of the autoantibodies, and 25% negative for both with no differences between sexes (30). Age- and sex-standardised IRs ranged between 21 to 30 per 100 000 individuals for seropositive RA and between 12 to 20 per 100 000 individuals for seronegative RA depending on the time period (Table 1-3) (18).

Temporal trends in IR of seropositive and seronegative RA have shown some inconsistency. In Finland with serostatus based on RF, age-adjusted IRs showed a declining trend for seropositive and a somewhat more stable trend for seronegative RA between 1980 to 2000 (41). However, with serostatus defined with ICD-10 codes in Finland from 2000 to 2014, a stable trend for seropositive RA and a decreasing trend in seronegative RA were observed (37). In contrast, a decline in age- and sex-adjusted RF-positive RA and an increase in RF-negative RA were found in the US in 2005 to 2014 when compared to previous decades (Table 1-3) (18). Using ACPA the

crude IR showed an increasing trend for seronegative RA and a stable trend for seropositive RA in the Netherlands from 1994 to 2015 (42).

In general, the inconsistency in studies investigating the temporal trends in IRs of seropositive and seronegative RA may be due to differences in cohort sizes, methodological approaches including the RA case definition, the definition of seropositivity (ICD-10, RF, anti-CCP, ACPA), and time for serological status in term of the study design (incident cohort versus cross-sectional). Further, differences in genetic disposition, environmental factors including smoking, and autoantibody test sensitivity could also be of importance.

**Table 1-3.** Overview of selected* cohort studies investigating the incidence rate of seropositive and seronegative rheumatoid arthritis. Studies are listed in chronological order by year of publication.

| Author, year, and country | Study period | Setting | RA patient definition, Number (N) of patients | Definition of serostatus | Sex, age | IR of seropositive RA** | IR of seronegative RA** |
|---|---|---|---|---|---|---|---|
| Kaipiainen-Seppänen et al., 2006, Jyväskylä, Kotka, Kuopio, Lahti, and Tampere districts, Finland (41) | 1980 to 2000. | Population-based cohort study. | 1987 ACR criteria.<br><br>N = 1843 | RF. | Women and men, ≥16 years. | Women: 20.4 (95%CI 16.8 to 24.4) to 37.4 (95%CI 32.4 to 43.0) per 100 000 individuals depending on the year in question.<br><br>Men: 15.9 (95%CI 12.6 to 19.7) to 23.5 (19.4 to 28.2) per 100 000 individuals depending on the year in question. | Women: 9.4 (95%CI 7.0 to 12.3) to 14.4 (95%CI 11.5 to 17.9) per 100 000 individuals depending on the year in question.<br><br>Men: 1.4 (95%CI 0.6 to 2.8) to 6.0 (95%CI 4.9 to 8.5) per 100 000 individuals depending on the year in question. |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Myasoedova et al., 2020, Olmsted County, Minnesota, US (18) | 1985 to 2014. | Population-based cohort study. | 1987 ACR criteria. Medical record review.<br><br>N = 1011 | RF. | Women and men, ≥18 years. | 1985 to 1994: overall 28 (24 to 33), women 33 (95%CI 27 to 40), men 23 (95%CI 17 to 30) per 100 000 individuals.<br><br>1995 to 2004: overall 30 (26 to 33), women 39 (95%CI 33 to 45), men 19 (95%CI 15 to 24) per 100 000 individuals.<br><br>2005 to 2014: overall 21 (18 to 24), women 26 (95%CI 22 to 30), men 15 (95%CI 12 to 19) per 100 000 individuals. | 1985 to 1994: overall 12 (9 to 15), women 15 (95%CI 11 to 19), men 9 (95%CI 5 to 12) per 100 000 individuals.<br><br>1995 to 2004: overall 13 (11 to 16), women 16 (95%CI 13 to 20), men 10 (95%CI 7 to 14) per 100 000 individuals.<br><br>2005 to 2014: overall 20 (18 to 23), women 26 (95%CI 22 to 31), men 14 (95%CI 11 to 18) per 100 000 individuals. |

RA, rheumatoid arthritis; IR, incidence rate; ACR, American College of Rheumatology; RF, rheumatoid factor.

* Systematic literature search on PubMed for existing evidence on seropositive and seronegative RA and IRs in English and Scandinavian languages (February 2023). Titles and abstracts were screened, and relevant papers were read in full. Also, references

included in relevant studies were evaluated. Western cohort studies with age- and sex-standardised or sex-specific age-standardised estimates within the years 2000 to 2018 were included.

** Annual IR were age- and sex-standardised for overall RA and sex-specific estimates were age-standardised.

### 1.1.4. CHANGES IN TREATMENT STRATEGIES

Since 2010, the EULAR has provided recommendations for managing RA with updates every third year (last updated in 2022) (43). The Danish national treatment guidelines for RA have been based on these EULAR recommendations with modifications based on Danish experience and studies as well as cost-related prioritisations. Since the late 1990s and 2000s several biologic and targeted synthetic disease-modifying anti-rheumatic drugs (DMARDs) have become available for RA treatment, and the use of these drugs has increased over time (36). The treatment strategies aim to lower the inflammatory burden and have changed over time according to new knowledge and treatment options, resulting in a gradually improved management of patients with RA disease and comorbidities during the last decades. The current Danish national treatment guidelines advocate for early disease detection, and a treat-to-target strategy to obtain rapid remission or low disease activity with early initiation of DMARD treatment with methotrexate as the first-treatment strategy and short-term glucocorticoids initially or at flares (11,43).

### 1.1.5. SEVERITY OF RHEUMATOID ARTHRITIS

As RA is a systemic inflammatory disease, it can present with both articular and extraarticular manifestations. The inflammation of the synovial joints, particularly symmetric small joint involvement, can lead to irreversible joint damage due to erosion of cartilage and bone when the disease activity is persistent or at severe short-term arthritis flares. Joint destruction may lead to severe disability, which can reduce the quality of life for patients with RA and on a societal level increase health care costs. The extraarticular manifestations involve organs that are not part of the musculoskeletal system among others skin, eye, lung, heart, kidney, blood vessels, salivary glands, central and peripheral nervous systems, and bone marrow (4,44). Furthermore, extraarticular manifestations can also include parts of the musculoskeletal system such as bones and muscles (4). High levels of IgM-RF and anti-CCP have been associated with the presence of severe extraarticular manifestations, indicating that autoantibodies may be part of the pathogenesis of these manifestations (45).

#### 1.1.5.1 Comorbidity

Several comorbidities appear more frequently in patients with RA than in the general population (24,46–48). A systematic review and meta-analysis found higher prevalence of anxiety (62% versus 7%), depression (32% versus 7%), hypertension (38% versus 31%), heart failure (11% versus 1%), ischaemic heart disease (7% versus

2%), thyroid disease (15% versus 4%), diabetes mellitus (12% versus 9%), fibromyalgia (12% versus 7%), and bronchial asthma (9% versus 4%) among patients with RA than in the general population (46). There were large variations in the frequency of comorbidities between countries (24). Thus, Danish patients with RA had higher odds of diseases in the circulatory system, respiratory system (mainly chronic obstructive pulmonary disease and pneumonia), musculoskeletal system and connective tissue, endocrine system (mainly diabetes mellitus type 2), eyes and adnexa, blood and blood-forming organs and some disorders involving the immune system, certain infectious and parasitic diseases, digestive system, skin and subcutaneous tissue, and genitourinary system (47). These factors may influence health status and lead to an increased number of contacts with health services both before and after the RA diagnosis compared with the general population (47). Importantly, comorbidities may affect the choice of treatment for patients with RA (11,43,49).

#### 1.1.5.2 Mortality

Large observational studies in incident and prevalent Western populations of RA have shown excess mortality among patients with RA compared to the general population (16,35,56–60,36,47,50–55). The inflammatory burden, comorbidities, accelerated biological ageing, genes, and adverse effects to treatments have been described as possible causes of the higher mortality, but in general, the cause could be considered multifactorial (61,62). The life expectancy has increased in the general population and the medical treatment of patients with RA has improved substantially leading to hope of an increase in life expectancy in patients with RA as well. However, studies investigating temporal trends in absolute and relative all-cause mortality in patients with RA compared to the general population have pointed to both a decreasing trend (UK 1994 to 2014; Canada 1996 to 2006; Canada 1996 to 2009; Netherlands 1997 to 2012; Sweden 1997 to 2015; US 2000 to 2017; Canada 2001 to 2015; UK 2005 to 2009) and a stable or no clear trend (UK 1990 and 2004; Canada 2001 to 2019) in recent decades (35,50,53,54,57,58,60,63,64). As a consequence disagreement regarding development in the mortality gap exists with some data suggesting trends towards a widening (US 1955 to 2000; Netherlands 1985 to 2007), a narrowing or closing (UK 1994 to 2014; Canada 1996 to 2006), or an unchanged excess mortality between patients with RA and the general population (Canada 1996 to 2009; Canada 2001 to 2015; Canada 2001 to 2019) (54,57,58,60,63,65,66).

In the general population men have higher mortality rates than women. But studies on this within RA populations have shown ambiguous results, as studies also have presented an earlier onset of premature deaths in female patients and a similar mortality rate for both sexes (65,66). The level of sex-specific excess mortality occurred similarly, although few studies have pointed towards more pronounced

excess mortality in women with RA than in men with RA when compared to the same sex in the general population, and with persisting excess mortality in the majority of the studies (50,53–55,60,64,65,67). Temporal changes in sex-specific all-cause mortality are not well described, but indications of differences in the sex-specific temporal pattern have been found (16,58).

In general, the inconsistency in studies investigating temporal trends in all-cause mortality in patients with RA may partly be explained by differences in cohorts and study characteristics, although differences in comorbidities and other risk factors for death may also be of importance.

## 1.1.6. AETIOLOGY

The aetiology of RA is considered multifactorial but is not fully understood (2). In addition to a genetic predisposition to the disease, environmental and lifestyle factors seem to play an important role in the development of RA (2,3,68). Genetics is the most prominent risk factor for RA with a factor of 2 to 5 increased risk in first-degree relatives of patients with RA (3). Smoking is the best-established and the main environmental risk factor for the development of RA and both the duration, intensity, and years since smoking cessation are of importance (3,5,69). Lui et al. pointed towards a behaviour change of sustained smoking cessation may reduce the risk of seropositive RA, but after 30 years of smoking cessation, the risk was still elevated compared to never smokers, though the risk was approaching the risk for non-smokers (3,69). A synergistic effect of genetics and cigarette smoking appears to exist by interaction with the shared epitope alleles to increase the risk of seropositive RA (3,69). In addition, environmental risk factors that may increase the risk of RA include high alcohol consumption, low socioeconomic status, obesity, low vitamin D levels, early menopause and other hormonal factors (2,3,5,68,70,71). Though obesity and early menopause may also have a hereditary component. The impact of other lifestyle factors such as diet and physical activity is less clear (2,72,73). Demographic risk factors for RA include age and sex with a higher rate at increasing age and in women as described earlier, which also imply that sex-related risk factors may be involved in the development of RA (3).

### 1.1.6.1 Intake of marine n-3 polyunsaturated fatty acids and risk of rheumatoid arthritis

Polyunsaturated fatty acids (PUFAs) are organic compounds characterised by two or more double bindings in the carbon chain (74–76). PUFAs are classified according to the position, number, and configuration of the double binds, and can be divided into

n-3 and n-6 PUFAs (74). N-3 PUFAs can be divided into marine n-3 PUFAs (derived from seafood) and n-3 PUFAs derived from plants (74). In general, Western diets are typically low in n-3 PUFAs and high in n-6 PUFAs (74).

The major long-chain marine n-3 PUFAs, eicosapentaenoic acid (EPA; 20:5n3) and docosahexaenoic acid (DHA; 22:6n3) are mainly found in fatty fish, though a growing number of dietary products have been enriched with n-3 PUFAs (2,77). After ingestion, marine n-3 PUFAs become incorporated into cellular membranes, converted into lipid signalling molecules, or pooled for storage (2). EPA and DHA have been ascribed anti-inflammatory and pro-resolving properties mediated through direct and indirect pathways in the immune system of importance for RA (74). The anti-inflammatory properties includes interaction with macrophages with inhibition of the synthesis and secretion of matrix metalloproteinases, interleukin(IL)-1β, tumor necrosis factor-α and IL-6, and promotion of IL-10 secretion, anti-proliferative effect on T-helper lymphocytes mediated by inhibition of IL-2 secretion, completion of n-6 arachidonic acid leading to reduction in lipid derived pro-inflammatory compounds production (in particular leukotrienes), and reduction in the expression of adhesion molecules on immune cells and endothelium (74,75,78). Metabolism of EPA and DHA produces pro-resolving mediators (Resolvin D, E and protectin D1) responsible for restoring tissue and return to homeostasis after inflammation blocking neutrophil recruitment, promoting recruitment and activation of monocytes, and mediating non-phlogistic phagocytosis (74,75,78,79). Intake of seafood rich in EPA and DHA leads to the incorporation of these fatty acids into cell membranes at the expense of n-6 PUFAs with mostly proinflammatory properties and may therefore result in a relative anti-inflammatory state (74,75). The interaction between EPA and DHA and the immune system in relation to inflammation is not completely understood, and confirmation of other pathways may develop when new knowledge emerges.

Smaller cohort studies suggested that a high intake of long-chain n-3 PUFAs and fish were inversely associated with the development of RA, with the lowest risk observed among those consuming more than 0.21 g/day corresponding to at least one serving of fatty fish per week or four servings of lean fish per week (Table 1-4) (80,81)**.** However, in the Danish Diet, Cancer and Health (DCH) cohort no association between intake of EPA and DHA and the risk of RA was found, but a 49% lowering of the risk of RA was detected for each 30 g fatty fish (≥ 8 g fat/100 g fish) intake per day (81). An investigation of the dose-response relationship between long-chain n-3 PUFA and risk of RA showed a constant relative risk (RR) with intake >0.35 g/day of marine n-3 PUFA and increasing dose-response <0.35 g/day (80). A possible beneficial association between intake of marine n-3 PUFA and the risk of RA was supported by a dose-response meta-analysis, including cohort and case-control studies, which found a weak inverse association between total fish consumption and risk of development of RA (72). In contrast, a large cohort study with multiple food-frequency questionnaires found no association between marine n-3 PUFA intake and development of RA (Table 1-4) (82).

**Table 1-4.** Overview of cohort studies* investigating the association between EPA and DHA and risk of rheumatoid arthritis. Studies are listed in chronological order by year of publication.

| Author, year, and country | Study period | Setting | RA patient definition, Number (N) of patients | Sex, age | Duration of follow-up | Exposure assessment | Risk of RA |
|---|---|---|---|---|---|---|---|
| Pedersen et al., 2005, Area of greater Copenhagen and Aarhus, Denmark (81) | 1993 to 2001. | The Diet, Cancer and Health cohort.<br><br>Cohort study. | Record linkage to the Danish National Patient Registry, verified by a rheumatologist though medical record.<br><br>N = 69 | Women and men, 50 to 64 years. | 2.8 years (mean). | EPA and DHA from a single food frequency questionnaire. | No association. |
| Di Giuseppe et al., 2014, Uppsala and Västmanland counties, Sweden (80) | 2003 to 2010. | Swedish Mammography Cohort.<br><br>Cohort study. | ICD-10 code for RA (M05 or M06) in the Swedish Rheumatology Register or the Swedish National Board of Health and Welfare. | Women, 54 to 89 years. | NA. | EPA, DPA, and DHA in g/day from two food-frequency questionnaire (1987, 1997). | >0.21 g/day lowered the risk of RA with 35% compared with daily intake <0.21 g/day, and with long lasting intake >0.21 g/day a |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | N = 205 | | | | 52% lower risk. |
| Sparks et al., 2019, US (82) | 1984 to 2014 and 1991 to 2015. | Nurses' Health Study and Nurses' Health Study II.<br><br>Cohort study. | Self-reported RA, screened and verified with medical records by rheumatologists.<br><br>N = 1080 | Women, 25 to 55 years. | 3,863,909 person-years of follow-up. | Cumulative average frequency intake of EPA, DHA, and DPA (diet and supplements) from food frequency questionnaires at baseline and every 4 years. | No association. |

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; RA, rheumatoid arthritis; ICD, International Classification of Diseases; DPA, docosapentaenoic acid.

* Identified through systematic literature search on PubMed for existing evidence on RA and polyunsaturated fatty acids in English and Scandinavian languages (January 2023). Titles and abstracts were screened, and relevant papers were read in full. Also, references included in relevant studies were evaluated. Western cohort studies were included.

# CHAPTER 2. AIMS AND HYPOTHESES

The overall aim of this thesis was to study the occurrence and severity of RA in Denmark.

**Aim and hypothesis 1 (Study I):**

To study the overall and sex-specific incidence and prevalence of RA and to explore the impact of different register-based RA case definitions on the results, with an investigation of

a) Incidence rate (IR)
b) Incidence proportion (IP)
c) Lifetime risk (LR)
d) Point prevalence (PP)

The hypothesis: the incidence and prevalence of RA increased with calendar time, and the choice of RA case definition was of importance for the estimates.

**Aim and hypothesis 2 (Study II):**

To study the IRs including the temporal trend of seropositive and seronegative RA. Further, to explore the importance of methodological choices when defining seropositivity and RA in a register-based study using various combinations of Danish nationwide registers and laboratory information on autoantibodies.

The hypothesis: the incidence of seropositive RA was higher than for seronegative RA.

**Aim and hypothesis 3 (Study III):**

To study temporal trends in 5-year all-cause mortality among patients with RA.

The hypothesis: 5-year all-cause mortality decreased with calendar time as a reflection of improvements in therapeutic and organisational management of RA.

**Aim and hypothesis 4 (Study IV):**

To describe the design and methods of a study investigating EPA and DHA intake and subsequent development of RA.

The hypothesis: high levels of EPA and DHA were inversely associated with the rate of incident RA.

# CHAPTER 3. PRESENTATION OF STUDIES

This chapter provides a brief description of each study included in this dissertation, for in-depth study characteristics please see Papers I-IV (Appendix A to D) (1,2).

## 3.1. STUDY I (PAPER I. INCIDENCE AND PREVALENCE OF RHEUMATOID ARTHRITIS IN DENMARK)

### 3.1.1. STUDY OBJECTIVES

The objective of Study I was to estimate the IR, IP, LR, and PP in Denmark, and by using four different case definition of RA to explore and assess the impact of the choice of RA case definition in epidemiological studies.

### 3.1.2. STUDY DESIGN, POPULATION AND METHODS

Study I was designed as a nationwide population-based cohort study with patients with RA registered from 1998 to 2018 and identified by record linkage using Danish administrative registers (1). Using the Civil Personal Register number assigned to all Danish residents at birth or immigration, relevant register-based information from the Danish Civil Registration System, the Danish National Patient Registry (DNPR), and the Danish National Prescription Registry (NPR) were linked (83–86). Thereby, accurate and dynamic follow-up on an individual level was possible. A description of the used registers is available in Paper I (1).

Four case definitions of RA were used (Table 1 in Paper I; referred to as Criterion in Paper I):

- Case definition A: First-time registration of an M05 (seropositive RA) or M06 (other RA, excluding M06.1 adult-onset Still's disease) in the DNPR and subsequent a redeemed prescription of a conventional synthetic disease-modifying anti-rheumatic drug (csDMARD) in the following year (1). Anatomical Therapeutic Chemical (ATC) codes defining csDMARD use are available in supplemental material to Paper I (1). Case definition A was a slightly altered case definition of the one validated by Linauskas et al. with a positive predictive value of 88% for overall RA (1,87). Study I allowed for

redeemed prescriptions for gold and ciclosporin and had a time limit of one year from diagnosis to a redeemed prescription of a csDMARD, whereas Linauskas et. al. did not include the aforementioned drugs and had no requirement of a maximum duration from diagnosis to redeemed prescription (Appendix C) (87).

- Case definition B: Two diagnoses with M05 (seropositive RA) or M06 (other RA, excluding M06.1 adult-onset Still's disease) within 90 days of each other with both records in the DNPR originating from a department of rheumatology or general internal medicine (1). Case definition B was slightly altered from the validated case definition described by Ibfelt et al. with a positive predictive value of 79% (1,88). The case definitions differed in that Ibfelt et al. only allowed RA diagnoses to originate from a department of rheumatology and ICD-10 codes for other RA to be M06.0 (seronegative RA), M06.8 (other types of RA), and M06.9 (unspecified RA) (88).

- Case definition C: A registration of an M05 (seropositive RA) or M06 (other RA, excluding M06.1 adult-onset Still's disease) in the DNPR, and a redeemed prescription of a csDMARD in the year before or after the RA diagnosis (1). The M05/M06 diagnosis was not required to be the first one ever registered in the DNPR, but only to be the first one where the additional csDMARD requirement was met. This case definition has not been validated, but has been used in several Danish register-based studies, and was therefore included in Study I (1,89–91).

- Case definition D: Defined as case definition A with the additional requirement that there were no registrations for selected inflammatory diseases (psoriatic or enteropathic arthropathies, systemic connective tissue disorders, inflammatory bowel diseases, or sarcoidosis) prior to the RA diagnosis in the DNPR (1,87). Compared to the original paper by Linauskas et al., the same changes to the case definition were made as those mentioned under the description of case definition A above; and further, patients with RA were only excluded if they had registrations in the past, whereas Linauskas et al. excluded RA cases if had ever had a registration of any of the other diagnoses in the DNPR. Linauskas et al. obtained a positive predictive value of 96% using the originally proposed case definition for overall RA (1,87).

As illustrated in Figure 3-1 overlap between the four case definitions of RA existed. Case definition A was used as the primary case definition. Case definition C was the least restrictive and case definition D was the strictest case definition used for this study.

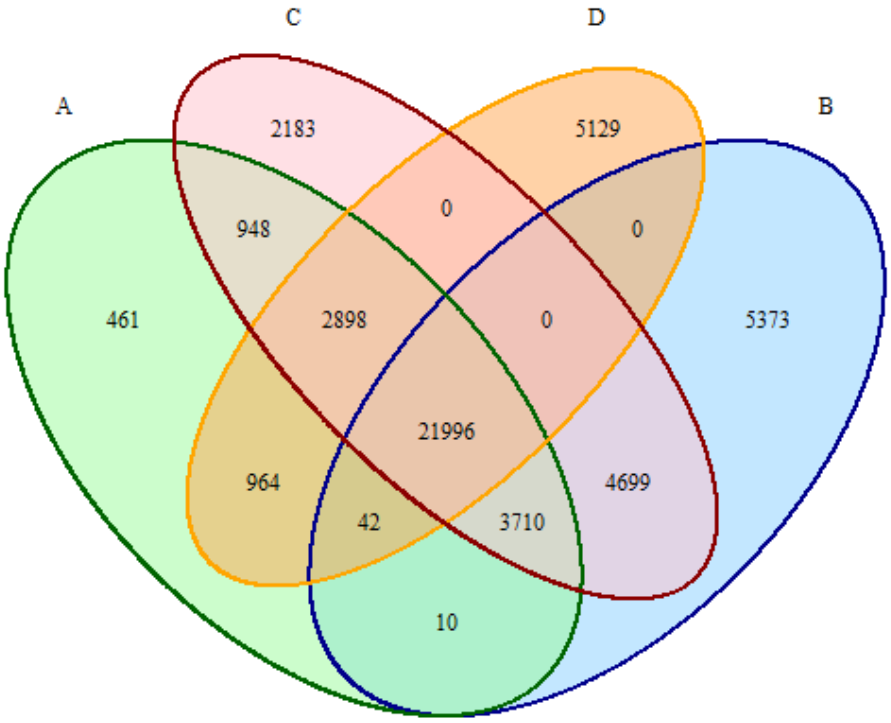


**Figure 3-1.** Venn diagram illustrating the overlap between the four applied case definitions of rheumatoid arthritis.

Age- and sex-standardised, age- and sex-specific, and age-standardised sex-specific IRs were calculated. Age- and sex-standardised IPs were calculated (Figure 3-2). Sex-specific LRs were calculated. Age- and sex-standardised and age-standardised sex-specific PP were calculated (Figure 3-2). All estimates were calculated with a 95% confidence interval (CI).

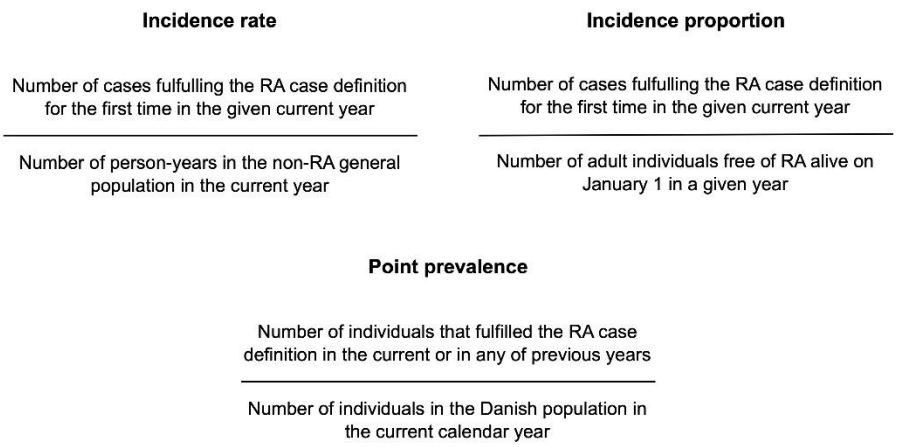


**Figure 3-2.** Graphic presentation of numerators and denominators in incidence rates, incidence proportions, and point prevalence calculations of rheumatoid arthritis (RA).

### 3.1.3. RESULTS

When case definition A was applied, the overall IR of RA from 1998 to 2018 was 35.5 (95%CI 35.1 to 35.9) per 100 000 PY (1). The age-standardised IR was higher for women than for men across all age groups with an age-standardised sex-specific IR for women of 47.1 (95%CI 46.5 to 47.8) per 100 000 PY and 23.6 (95%CI 23.1 to 24.1) per 100 000 PY for men (1).

**Table 3-1.** Incidence rates and proportions for rheumatoid arthritis in Denmark from 1998 to 2018.

| | **Incidence rate*** per 100 000 person-years | **Incidence proportion*** per 100 000 individuals |
|---|---|---|
| **Case definition A** | 35.5 (95%CI 35.1 to 35.9) | 35.2 (95%CI 34.8 to 35.5) |
| **Case definition B** | 41.1 (95%CI 40.7 to 41.5) | 40.6 (95%CI 40.2 to 41.0) |
| **Case definition C** | 41.8 (95%CI 41.3 to 42.2) | 41.3 (95%CI 40.8 to 41.7) |
| **Case definition D** | 29.6 (95%CI 29.3 to 30.0) | 29.3 (95%CI 29.0 to 29.7) |

** Age- and sex-standardised.*

Table 3-1 shows that all IP estimates were slightly lower than their corresponding IR (1). As a result, the choice of denominator did not impact the estimates substantially. However, results from this study did show the importance of the register-based RA case definition, as a difference of 29% from the lowest (case definition D) to the highest (case definition C) estimated overall IR was observed.

Analyses of LR showed similar age patterns but with a difference in the risk amplitude for women and men and between the case definitions of RA. Cumulative LR analyses reflected the age and sex patterns found in the IRs with an LR <1% before age 50, gradually increasing until age 80 after which a stationary risk, and a higher LR for women than men (Supplementary Figure 1 in Paper I) (1).

Like the IR estimates the PP also varied depending on the applied case definition. A significant increasing trend in overall and sex-specific PP was found from 2000 to 2018, with a twofold higher PP in women than men, regardless of the case definition (1). In 2018 the age- and sex-standardised PP was 0.55% (95%CI 0.54 to 0.56%), and the age-standardised sex-specific PP was 0.75% (95%CI 0.74 to 0.76%) for women and 0.34% (95%CI 0.33 to 0.35%) for men when defined according to the primary case definition of RA (A).

### 3.1.4. METHODOLOGICAL CONSIDERATIONS

The main strengths of this study included the large nationwide population-based design which accounted for demographic changes and had highly valid individual-based information (1,83).

In addition, the use of four different but supplementary epidemiological measurements for the occurrence of RA in Denmark was considered a strength of this study.

The main limitations of this study were the potential misclassification bias. By using four case definitions of RA the risk of misclassification was explored and reduced but not eliminated. Though it was a strength that four case definitions of RA were used for the study, it was at the same time a limitation that the altered case definitions were not validated prior to this study.

Case definition A was chosen as the primary RA case definition, based on an assumption that this definition was closest to the 'true' estimate. The assumption was supported by the positive predictive value of 88% in the original validation study; and the fact that the case definition included both a diagnosis and csDMARD treatment from two independent registers, though a modification of the case definition was made for Study I (87). Case definition D was the strictest case definition of RA, which may

have increased the risk of false-negative misclassification, whereas case definition C was the least strict.

### 3.1.5. CONCLUSION

The IRs of RA remained relatively stable over time with a peak in 2010, whereas the PP increased significantly. Similar temporal patterns in incidence and prevalence were observed for all four case definitions, but the level of IR and PP differed depending on the applied case definition. The findings of this study illustrated and emphasised the impact of the choice of case definition of RA applied in register-based studies.

## 3.2. STUDY II (PAPER II. INCIDENCE OF SEROPOSITIVE AND SERONEGATIVE RHEUMATOID ARTHRITIS IN DENMARK)

### 3.2.1. STUDY OBJECTIVES

The objective of Study II was to investigate the IRs including the temporal trends of seropositive and seronegative RA in Denmark and to explore the importance of methodological choices in the definition of seropositivity and RA in register-based studies.

### 3.2.2. STUDY DESIGN, POPULATION AND METHODS

In this nationwide population-based cohort study all patients with RA were identified from 2000 to 2018 through record linkage as described in Study I. By applying three outcome definitions of seropositivity and four case definitions for RA the importance of these methodological choices in register-based studies was explored. A description of the used registers is available in Paper II (Appendix B). Patients with RA were identified using case definitions A-D described in Study I, all of which were inspired by previously validated and/or commonly used case definitions of RA. Case definition A was the primary case definition of RA, whereas analyses of case definitions B-D were considered sensitivity analyses. The four case definitions of RA (A-D), previously described in section 3.1.2, are presented here with focus on the validation of seropositive and seronegative RA, respectively:

- Case definition A: The original case definition described by Linauskas et al. had a positive predictive value of 80% for seropositive RA and 41% for other RA (87).

- Case definition B: The original case definition described by Ibfelt et al. was validated for overall RA, but not for seropositive and seronegative RA separately (88).

- Case definition C: The case definition has not been validated.

- Case definition D: The original case definition described by Linauskas et al. had a positive predictive value of 85% for seropositive RA and 48% for other RA (87).

Three outcome definitions of seropositivity were pre-specified. The data sources used for each of these are shown in Table 3-2. The outcome definitions of seropositivity were the following:

- Laboratory- and physician-reported autoantibodies and ICD-10 codes: IgM-RF and/or anti-CCP registered in the Autoimmune Laboratory at Statens Serum Institut (SSI) or the Register of Laboratory Results for Research (92). For those with missing information on laboratory-reported autoantibodies, physician-reported autoantibodies of IgM-RF and/or anti-CCP in DANBIO were used (93). If still the serological marker was missing, imputation of serostatus according to ICD-10 codes from the DNPR when the case definition of RA was fulfilled was conducted.

- Laboratory-reported autoantibodies and ICD-10 codes: IgM-RF and/or anti-CCP registered in the Autoimmune Laboratory at SSI or the Register of Laboratory Results for Research, followed by imputation of serostatus according to ICD-10 codes from the DNPR for those with missing information on laboratory autoantibodies (92).

- Laboratory-reported autoantibodies: IgM-RF and/or anti-CCP registered in the Autoimmune Laboratory at SSI or the Register of Laboratory Results for Research (92).

Registration of a positive autoantibody within 15 years before and 14 days after the case definition of RA was fulfilled or an M05 diagnosis was classified as seropositive RA. The presence of a test for one or both autoantibodies with negative results or an M06 diagnosis qualified as seronegative RA.

The annual age- and sex-standardised IRs with 95%CIs for seropositive and seronegative RA were calculated.

**Table 3-2.** Overview of data sources used in each of the outcome definitions used to distinguish the serological subtypes of rheumatoid arthritis.

| **Laboratory- and physician-reported autoantibodies and ICD-10 codes** | **Laboratory-reported autoantibodies and ICD-10 codes** | **Laboratory-reported autoantibodies** |
|---|---|---|
| Autoimmune Laboratory at Statens Serum Institut<br><br>Register of Laboratory Results for Research<br><br>DANBIO<br><br>Danish National Patient Registry | Autoimmune Laboratory at Statens Serum Institut<br><br>Register of Laboratory Results for Research<br><br>Danish National Patient Registry | Autoimmune Laboratory at Statens Serum Institut<br><br>Register of Laboratory Results for Research |

*Information from the data sources was weighted as: laboratory-reported autoantibodies from Autoimmune Laboratory at Statens Serum Institut and Register of Laboratory Results for Research overruling physician-reported autoantibodies from DANBIO overruling ICD-10 codes from the Danish National patient Registry.*

### 3.2.3. RESULTS

The temporal trend in IR increased for seropositive RA and decreased for seronegative RA when outcomes definitions consisted of combinations of laboratory and nationwide register information (Figure 1 in Paper II) (Appendix B). The IRs were higher for seropositive RA than for seronegative RA from 2009 and onwards with a widening of the IR gap between 2009 and 2016, ending with an approximately twofold higher IR for seropositive RA than of seronegative RA, regardless of the definition of seropositivity used (Table 3-3). As testing for autoantibodies became more frequent over time (Table 1 in Paper II), the rate of RA cases with missing values for autoantibodies decreased significantly (Figure 2 in Paper II) (Appendix B).

**Table 3-3.** Age- and sex-standardised incidence rates per 100 000 person-years of seropositive and seronegative rheumatoid arthritis in 2018 according to the three outcome definitions of seropositivity.

| | **Seropositive rheumatoid arthritis** | **Seronegative rheumatoid arthritis** |
|---|---|---|
| **Laboratory- and physician-reported autoantibodies and ICD-10 codes** | 19.0 (95%CI 17.8 to 20.2) | 9.0 (95%CI 8.2 to 9.8) |
| **Laboratory-reported autoantibodies and ICD-10 codes** | 18.7 (95%CI 17.5 to 19.9) | 9.3 (95%CI 8.5 to 10.2) |
| **Laboratory-reported autoantibodies** | 17.1 (95%CI 16.0 to 18.3) | 8.0 (95%CI 7.3 to 8.8) |

The underlying temporal pattern for IRs of seropositive and seronegative RA depended on the applied definition for RA and seropositivity (Supplementary Figure S2 to S4 in Paper II) (Appendix B). Although, results were almost similar with and without DANBIO as a data source in the outcomes definitions using multiple (≥ 3) data sources.

### 3.2.4. METHODOLOGICAL CONSIDERATIONS

To investigate the IRs of seropositive and seronegative RA the main limitation of this study was the potential misclassification between these serological subtypes. To explore such misclassification, three outcome definitions were used to distinguish the serological subtypes of RA. The use of laboratory information on autoantibodies would be the preferred method to classify the serological subtypes of RA and study trends in register-based studies instead of ICD-10 codes. However, particularly in the study of trends, missing data in laboratory values (IgM-RF and anti-CCP) was a critical point with 77% of the RA cases having missing values before 2010 and 38% thereafter. Therefore, alternative data sources (DANBIO and DNPR) were added as data sources to define the serostatus of RA cases with missing values in laboratory autoantibodies. Even though these additional data contained uncertainty (12% misclassification in DANBIO and 27% misclassification in the DNPR), it was still considered a strength of the study, as it also reflected challenges in epidemiological

studies where definitions of seropositive and seronegative RA must be based on the available data (Table 2 and 3 in Paper II) (Appendix B). Further, the risk of misclassification of RA was explored using four case definitions of RA. Methodological considerations with regard to the case definitions for RA have been described in Study I (section 3.1.4), though with the important difference that only two of the original case definitions (A and D) were validated separately for seropositive and seronegative RA, which revealed low positive predictive values for seronegative RA when classified by ICD-10 codes (87). Overall, the risk of misclassification in both the serological subtypes and RA could have affected the observed IRs and temporal trends in this study and might have led to underestimation of seropositive RA and overestimation of seronegative RA particularly in early calendar years where a larger proportion of subtypes of RA were classified by ICD-10 codes.

Autoantibodies in the Register of Laboratory Results for Research from the Central Jutland Region were not available for this study. Assuming the distribution between positive and negative autoantibodies in that region was similar to the distribution in the rest of the country, it most likely did not affect the IR ratio between seropositive and seronegative RA.

Test patterns and test kits for autoantibodies changed during the study period with IgM-RF available from 2000 and onwards and anti-CCP from 2004 and onwards. Changes in procedures and workflows were reflected in the test data availability with the number of samples taken in the calendar periods, and therefore this study was strengthened by the transparency of this as it might have influenced the results (Table 1 in Paper II) (Appendix B).

### 3.2.5. CONCLUSION

In a period where the availability of autoantibody testing grew and where autoantibodies received greater weight in the classification criteria for RA, the IR of seropositive RA increased, while the IR of seronegative RA decreased. The findings of this study illustrated and emphasised the impact of the choice of how the serological subtypes of RA and patients with RA were identified in register-based studies.

## 3.3. STUDY III (PAPER III. TEMPORAL TRENDS IN MORTALITY IN PATIENTS WITH RHEUMATOID ARTHRITIS)

### 3.3.1. STUDY OBJECTIVES

The objective of Study III was to investigate the temporal trend in overall and sex-specific 5-year all-cause mortality in patients with RA compared to matched controls.

### 3.3.2. STUDY DESIGN, POPULATION AND METHODS

Study III was designed as a nationwide population-based matched cohort study with patients with RA diagnosed between 1996 and 2015 in Denmark (94). A description of the used registers is available in Paper III (Appendix C). Patients with RA ≥ 18 years of age, were identified using the previously described RA case definition A. Incident RA patients were matched 1:5 on year of birth and sex with non-RA individuals (Appendix C) (94). To reduce the risk of including prevalent RA cases in the study population individuals with an ICD-8 or ICD-10 code for RA before 1996, and those ≥ 18 years of age immigrating during the study period were excluded (Appendix C). The study cohort was categorised into 5-year calendar period groups chosen based on eras in RA management and classification.

Time-to-event analyses were performed using the pseudo-observation approach, whereby 5-year cumulative incidence proportion (CIP) could be estimated (Appendix C) (95,96). Risk difference (RD) and RR were calculated using generalised linear regression based on the pseudo-observations (Appendix C). The pseudo-observation analyses were stratified by case status (RA versus non-RA) and calendar period. Analyses were conducted using either all patients with RA or stratified by sex as crude and age-adjusted analyses (95,97,98). Assumptions of independent censoring were examined using Cox regression and the Kaplan-Meier estimator (Appendix C) (97).

### 3.3.3. RESULTS

The study population consisted of 28 958 incident patients with RA and 145 210 matched controls with characteristics presented in Table 1 in Paper III (Appendix C).

The crude overall 5-year mortality risk decreased over time for both patients with RA and matched controls but remained higher for patients with RA compared to matched controls in all calendar periods. Similar temporal patterns were observed in sex-specific analyses (Appendix C).

The overall age-adjusted 5-year CIP of death for a 60-year-old patient with RA decreased over time and remained higher for patients with RA compared to matched controls (Appendix C). This finding was driven by a continuously higher 5-year mortality risk among women with RA throughout the study period, whereas men with RA obtained a similar mortality risk to matched controls in year 2011 to 2015. A reduction in 5-year mortality risk was observed for both patients with RA and matched controls over calendar time, with a larger improvement in patients with RA, resulting in a narrowing of the mortality gap over time (Appendix C). Indications of a sex-specific difference in the declining RD were detected when patients with RA were compared to matched controls within the same calendar period, as women had a decrease in risk in early calendar periods and men had a steeper decrease in the last calendar period (Appendix C).

In sensitivity age-adjusted analyses, a lower 5-year mortality risk was detected when the 25 quantiles were used as reference age and a higher risk of death at the 75 quantiles.

### 3.3.4. METHODOLOGICAL CONSIDERATIONS

The main strengths of this study were the population-based cohort design and the applied statistical method of pseudo-observation. In addition, nearly complete follow-up of the population, age-adjusted analyses, and low risk of misclassification strengthened the study.

The main limitations of this study were the lack of data on disease activity and laboratory data to determine the serologic status in patients with RA, which may affect the mortality risk in patients diagnosed with RA (35,36,66,99). Most likely not all potential risk factors affecting RA patients' excess mortality have been identified. Further, it is important to be aware that patients with RA tend to have a higher prevalence of comorbidities which also may have affected the observed excess mortality. The results of the study were limited to 5-years of follow-up but might have been different with a longer follow-up time (66).

Investigation of the assumption of independent censoring with Cox regression and Kaplan-Meier estimator regarding death or emigration revealed a higher proportion of individuals who emigrated from the matched control groups than from the RA groups over time. This was presumably due to healthy individuals being more likely to emigrate than sick individuals. Therefore, the analyses needed to be stratified. An ANOVA test showed no interaction between age and case status.

All statistical methods have strengths and limitations which should be weighed against their utility and interpretability. Hazard based models have become the primary choice

of method in time-to-event regression analysis (96,97). However, the pseudo-observation method is a relevant alternative to the Cox proportional hazard regression in cohort studies. The major advantage of the pseudo-observation approach is the possibility to use cumulative risks on both the additive and multiplicative risk scale, while the Cox regression model is limited to the multiplicative scale (97,100). Another advantage of the pseudo-observation approach is the possibility to create informative graphical displays, which have been lacking in time-to-event analysis (96). In the extended model control in the pseudo-observation method, the stratification approach provides the possibility to handle violations in assumptions (97). Hazard ratios obtained from a Cox regression have to remain constant over time and assumptions to be met, otherwise, the interpretation of the hazard ratios becomes unclear (97,101). On the other hand, right-censoring can with ease be accounted for in hazard regression models (96). A hazard ratio can be difficult to interpret and has often been misinterpreted as a term of risk (risk ratio) although a quantified estimate is not retrieved from the model (100). Gabriel et al. recommended the use of pseudo-observation-based regression over other time-to-event methods e.g. Cox models, when the time of entry into large population-based registers studies was not associated with event time of interest, and risk differences were the desired estimate (100). Further, the pseudo-observation method is useful in prospective cohort studies where the exposure of interest exists at entry time and individuals are followed until the occurrence of the event of interest. As in our study, the analysis should be adjusted in censoring models when factors are associated with a loss of follow-up (Appendix C) (100). In summary, the pseudo-observation method is more flexible and approachable from a public point of view than the more commonly used Cox proportional hazard regression (Appendix C). Study III is the first study to use the pseudo-observation approach to investigate the mortality in patients with RA (Appendix C).

### 3.3.5. CONCLUSION

The 5-year all-cause mortality risk decreased over time for both patients with RA and matched controls with enhanced improvement in patients with RA. The results also indicated a sex difference in the positive development of mortality over time with a closing of the mortality gap in men with RA, but continuing excess mortality among women with RA.

## 3.4. STUDY IV (PAPER IV. INTAKE OF MARINE N-3 POLYUNSATURATED FATTY ACIDS AND THE RISK OF RHEUMATOID ARTHRITIS)

### 3.4.1. STUDY OBJECTIVES

The objective of Study IV was to present the study design and the theory behind the study investigating an association between intake of the n-3 marine PUFAs, EPA and DHA, and the risk of incident RA.

### 3.4.2. STUDY DESIGN, POPULATION AND METHODS

Study IV is a protocol paper describing a study designed as a cohort study based on the Danish DCH cohort.

The DCH cohort consisted of men and women from 50 to 65 years of age at inclusion in 1993 to 1997 from the area of greater Copenhagen and Aarhus in Denmark. In total, 57 053 participants with a slight overweight of women, who all underwent anthropometric measurements and completed detailed questionnaires including a 192-item semiquantitative food frequency questionnaire at baseline (2,102–104). The participants will be followed through record linkage to the DNPR and the NPR using the Civil Personal Register number. Further descriptions of the data sources are available in Paper IV (2).

The associations of interest will be investigated using time-to-event analyses with Cox proportional hazard regression models. The planned analyses will allow for differences in sex-specific baseline hazards, have attained age as the underlying timescale, and will be adjusted for baseline age to secure that the baseline covariates were of the same age (2). Exposures of interest will be the energy-adjusted intake of EPA and DHA determined from a validated food frequency questionnaire collected at baseline, and the outcome of interest will be incident RA (2). For this study, RA will be defined as the original case definition described by Linauskas et al.: first-time ICD-10 code of M05 or M06 (not including M06.1) in the DNPR and subsequent a redeemed prescription of a csDMARD in the NPR (2,87). Established demographic, lifestyle, and hormonal risk factors for RA will be included as covariates in the statistical models (Table 2 in Paper IV) (2).

### 3.4.3. METHODOLOGICAL CONSIDERATIONS

For this study, the Cox regression approach was chosen. The Cox regression model is an established analysis to use in the DCH cohort, and an excellent choice of method in studies of biology if the model assumptions are met.

The exposures will be energy-adjusted with the assumption that intake of marine n-3 PUFA are of more biological relevance in relation to total energy intake (2). However, the knowledge is limited in this field, and therefore this assumption was based on the theoretical hypothesis that total energy intake was not a strong risk factor for incident RA. Therefore, the residual method will be applied for energy adjustment of EPA and DHA intake, whereby the estimates become independent of total energy and measurement errors are reduced (105).

The DCH cohort has some major strengths that need to be addressed. As the peak onset in incidence for RA was between ages 70 to 79 for both sexes in Denmark, and DCH participants were aged 50 to 65 at inclusion in 1993 to 1997, the DCH cohort is expected to capture most incident RA patients during the follow-up (1,102). The DCH cohort is highly recognised for a detailed and a high data quality with the nationwide Danish registers allowing nearly complete follow-up. However, it also has some limitations that need to be addressed. A validated case definition for RA will be used, but misclassification on the outcome level cannot be ruled out. Also, assessing dietary intake of EPA and DHA is prone to random measurement error (104). A weakness is that exposures will be based on a single food frequency questionnaire collected from the participants' baseline. Thereby it represents a fixed moment in time and not necessarily a long-lasting dietary pattern.

### 3.4.4. CONCLUSION

In conclusion, Paper IV described in detail the methods which will be applied to investigate the study objective.

# CHAPTER 4. DISCUSSION

This dissertation shed light on the epidemiology and burden of a common immune-mediated disease in Denmark and provided nationwide contemporary estimates of RA.

## 4.1. INCIDENCE AND PREVALENCE OF RHEUMATOID ARTHRITIS

Aligned with findings from Scandinavian countries a peak in IR of RA in ages 70 to 79 for both sexes and a sex ratio of approximately 2:1 in IR and PP for women compared to men were found in Study I (1,14,15,23,106). The overall IR (35.5 per 100 000 PY) observed in Study I was slightly higher than what has been found in patients with RA fulfilling the 1987 ACR classifications criteria of RA in the Southern part of Denmark in 1995 to 2001 (31 per 100 000 PY) (1,15). The difference could be reflecting a true difference in IR due to geographic or calendar period variation but may also reflect an overestimation of the incidence of RA when identified through administrative health registers. The absolute number of both prevalent patients with RA and residents in the Danish population has increased, but with a more pronounced increase for patients with RA, causing a higher burden of RA from 2000 to 2018 (1). Population growth, population ageing, and declining mortality in the Danish population are factors influencing this development (22). Though there previously have been inconsistencies in the temporal trend of IR and PP for RA, Study I and an English study published afterwards found a relatively stable trend in IR and an increasing trend in PP (1,107). Changes observed in the temporal trends of RA may have reflected external factors and continual changes in exposures to risk factors. E.g., the shift in classification criteria for RA may have caused the peak in IR in 2010 as the new ACR/EULAR classification criteria of RA were designed, among others, to identify early RA (1,10,108). However, in England, no significant impact on annual IR was found after the introduction of the new classification criteria for RA (107). Further, data from Study I may also have reflected changes in registration practice after an introduction of a new healthcare information technology system in the Capital and Zealand Regions of Denmark, which may have caused the decrease in IR in 2017 and 2018 (Appendix B) (1). In the English data, a sudden decrease in IR was detected in 2020, which indirectly reflected the change in health care when the COVID-19 pandemic struck (107).

## 4.2. INCIDENCE OF SEROPOSITIVE AND SERONEGATIVE RHEUMATOID ARTHRITIS

With autoantibodies (IgM-RF and anti-CCP) from laboratory registers used to define seropositivity, the proportion of seropositive RA was 66% to 70% from 2010 and onwards (Appendix B). This tended to be slightly higher than observed in other Scandinavian populations (61% to 67%) when using RF and ICD-10 codes, but lower than found in a study from the Southern part of Denmark from 1995 to 2001 using RF (76%) (14,15,36,106). The difference is likely explained, at least in part, by heterogeneity in methodological approaches including study design (e.g., cross-sectional or incident cohort), information used to define the serological status (RF, anti-CCP, or ICD-10), and calendar time. IRs in 2018 varied from 17 to 19 and 8 to 9 per 100 000 PY for seropositive and seronegative RA, respectively, depending on the outcome definition of seropositivity applied. Both estimates were lower than reported in other Western countries except in Greece, though heterogenicity between the studies existed (18,106,109,110). Besides the heterogeneity in methodological approaches, these differences might be due to the suspected underestimation of IRs in 2017 and 2018 in Study II caused by delayed and suboptimal registration in the DNPR after the introduction of a new healthcare information technology system in two of the Danish regions (Appendix B) (1). Geographic variation as indications of lower IRs of RA in Southern European countries than in Northern European countries has also been reported (111). The temporal trend in IR was increasing for seropositive RA and decreasing for seronegative RA during the study period in Study II (Appendix B). The findings were partly in agreement with a study from Finland (ICD-10) showing a decreasing trend for seronegative RA, but a stable trend for seropositive RA (37). Whereas studies from other Western countries have indicated a decreasing or stable trend for seropositive RA and an increasing or stable trend for seronegative RA using either RF or ACPA to define seropositivity (18,42,110). Study II revealed that the peak in IR of overall RA in 2010 was driven by temporal trends in seropositive RA. After the introduction of the 2010 ACR/EULAR classification criteria for RA the differences between IRs of seropositive and seronegative RA increased, which could be an outcome of the increased number of autoantibody test performed and that ACPA was included as a serologic marker in the new criteria (Appendix B) (10). Further, changes in population demographic, genetics or exposures to risk factors may have contributed to the observed temporal trends (5,30,112). Study II assumed ‘one-time IgM-RF or anti-CCP positive always seropositive’ and therefore did not account for the possibility of seroconversion with a rate of up to 5% for RF and 9% for anti-CCP in early inflammatory arthritis or potential changes in sensitivity of the laboratory test kits (Appendix B) (113).

## 4.3. MORTALITY IN PATIENTS WITH RHEUMATOID ARTHRITIS

Previous studies investigating temporal trends in all-cause mortality in patients with RA have used different statistical approaches including calculation of standardised mortality rate, standardised mortality rate ratio, and hazard ratio. Study III used yet another statistical approach in the form of the pseudo-observation method to investigate that aim. Though the heterogeneity in the statistical approaches makes direct comparison of results difficult, the pseudo-observation approach holds some advantages over the more commonly known statistical methods (see section 3.3.4). Several temporal mortality studies did not allow for an equal window of opportunity for follow-up time for patients with RA included early and late in the study cohorts (35,53,54,60,64,65). Although the use of different methodologies, findings from Study III supported evidence from several previous studies showing improvement in mortality risk over time and narrowing or closing of the mortality gap between patients with RA and the general population (35,50,53,54,57,60,63,64). Indications of sex differences in temporal changes in mortality were also supported by Study III, and as a new finding an enhanced positive development in mortality for men with RA with closing of the mortality gap to their matched controls over time, while excess mortality persisted in women with RA (Appendix C) (16,58). In agreement with studies showing a higher presence of comorbidities in patients with RA compared to the general population, data from this dissertation showed a higher prevalence of chronic obstructive pulmonary disease, cardiovascular disease, diabetes mellitus and hip and knee replacement surgery prior to RA onset compared with matched controls (Table 1 in Paper III) (Appendix C) (24,46–48). With inflammation, smoking, genetic risk, and comorbidity being prognostic factors for death, this would be relevant to account for in the prediction of the individual RA patients' mortality risk (3,61,114). Improvement in the management of RA and dependent and independent comorbidities may have led to a lower burden of inflammation and comorbidities and thereby improved mortality risk for patients with RA. Combined with the improvement in mortality risk for the general population, these arguments could provide an explanation to the improvement found in mortality risk over time. However, the causes of the sex differences observed in the 5-year all-cause mortality remained unclear under the assumption that sex does not influence RA management (115). Therefore, the sex-specific differences in mortality risk need to be explored further.

## 4.4. POLYUNSATURATED FATTY ACIDS AND THE RISK OF RHEUMATOID ARTHRITIS

Identifying complex associations between self-reported behaviour and diseases in observational studies is difficult (116). Marine n-3 PUFA can be metabolised into lipid mediators involved in the immune system, which may influence the risk of developing RA (117). Estimated intakes of marine n-3 PUFA are prone to random

measurement error, and therefore the use of complementary information from objective biomarkers may provide important valuable information. Sex-related risk factors are likely involved in the predominance of the RA disease in women where effects of estrogen on the immune system could play a role (3,68,118). Therefore, potential hormonal risk factors were included in one of the models to account for some of the sex-specific risk differences in the population (2). Current research in the field of the association between n-3 PUFA and risk of incident RA has mainly been conducted in women, though a small Danish study also included men (81). A synergistic effect of environmental and behavioural influences and genetics may exist, and future research could benefit from combining genetic and environmental information to characterise the gene-environment interaction in RA development (3,68).

## 4.5. CASE DEFINITION OF RHEUMATOID ARTHRITIS IN OBSERVATIONAL STUDIES

In epidemiological research, the validity of the exposure and outcome is essential to draw valid conclusions from register-based studies. By using one diagnosis code of RA from the DNPR a validity of 46% could be obtained (119). Better diagnostic validity could be gained by adding additional eligibility criteria and restrictions e.g., more than one RA diagnosis, DMARD treatment, or registration from a department of rheumatology (87,88,119). All case definitions of RA used in this dissertation were inspired by previously validated definitions with high positive predictive values and/or commonly used case definitions of RA (87–91). Study I, II and III contained one or more case definitions of RA altered to increase the specificity (alterations were presented in section 3.1.2). The modifications secured that all individuals in the population had the same window of opportunity to become a RA case, and that the case status did not rely on future disease events, thereby avoiding a time-related bias (120). However, it could also be argued that the absence of applying validated case definitions of RA were a limitation. Preferably a sensitivity analysis to examine the impact of changes in the case definitions of RA should be performed to establish the validity and robustness of these.

Particularly seronegative RA poses a diagnostic challenge due to uncertainty in early disease and classification of the serological subtype of RA is conditional on the available and validated immunological assays (8,112,121,122). Autoantibody test results are not always available when conducting register-based studies. In Study II, the autoantibodies were included in the outcome definitions, although some calendar periods were significantly affected by missing information on autoantibodies (Appendix B). Other methods are frequently used to determine the serological subtype of RA. Curtis J. et al. described RA-specific ICD-10 codes as a reasonable proxy with high sensitivity to identify seropositive and seronegative RA when laboratory

autoantibodies test results were not available (123). Results from Study II indicated that when applying this algorithm in the Danish population an overestimation of seronegative and an underestimation of seropositive RA occurred. This finding was not surprising in light of the significantly lower positive predictive value for seronegative RA than for seropositive RA (41% versus 80%) found for the case definition used as inspiration for this thesis case definition A (87). Although the serological status from ICD-10 codes among Danish verified seropositive and seronegative RA cases could be confirmed with autoantibodies in 92% and 78%, respectively, with information on autoantibodies originating from blood test results for IgM-RF and/or ACPA taken prior to or at the latest one month after the first RA diagnosis in the DNPR (87). Study II indicated that a combination of data sources could be a reasonable solution to determine the serological subtypes of RA in register-based studies when information on autoantibodies is not available in all cases. However, the algorithms were not validated, which should be done before a final conclusion is drawn. Laboratory autoantibodies serve as the golden standard for determining the serological subtype of RA. With more autoantibody tests performed and better registration practices, the alternative approaches could become redundant. However, missing information on autoantibodies from laboratories still poses a challenge in epidemiological research.

When using the case definition of RA with time-dependent criteria, as presented in Study I-IV, the index date reflects the date of fulfilment of criteria set for RA and does not reflect disease onset or the date of diagnosis (Appendix A to D) (1,2). This could be of importance to results in cohort studies with RA as the exposure, as the case was in Study III, where an RA case could have been exposed up to one year before occurring as a patient with RA in the study population. On the other hand, the case definition ensures fewer false positive RA cases, and thus the definition is a trade-off of which definition is most trusted.

This thesis does not settle on a right or wrong approach in defining RA in register-based studies, nor does it provide an error-free solution. However, it does give reasons for methodological considerations that should be made in consideration of data availability before a cohort study with patients with RA is initiated.

## 4.6. STRENGTHS AND LIMITATIONS

In general, large population-based cohort studies using administrative health registers are subject to systematic errors from selection bias, information bias, and confounding. The errors can occur from a myriad of directions (120). In this section, the most essential limitations of this thesis are discussed. All studies relied on existing records and the limitations within them (83–86,92,93,102,104).

*Selection bias:* In Denmark, most patients with RA are treated in departments of rheumatology at hospitals, but patients with RA may also be followed by private practising rheumatologists. Historically, private practising rheumatologists have not been obligated to report diagnosis codes to the DNPR. However, since the study periods of the respective studies ended, this has changed and it is now required. This can influence the rates of RA in the future as more patients with RA will be captured through the DNPR. However, the studies in this thesis may have been affected by selection bias, as patients with RA treated by private practising rheumatologists were only included in the RA population if they have been transferred to or came in contact with a hospital because of RA or other reasons (1). If that was the case, some private practising rheumatologists reported to DANBIO, why some of the autoantibody information used in Study II could originate from this health sector. A small proportion of the patients with RA were presumably missing due to this, but we had no data to quantify it. The assumption was based on reports showing nearly half of the Danish population is in contact with a hospital within one year, a considerably larger proportion of patients with inflammatory arthritis have been in contact with hospital departments of rheumatology than private practising rheumatologists, and that 9% of incident and prevalent RA patients in DANBIO in 2018 originated from private practising rheumatologists though this indicated increasing tendency over time (124–127). Consequently, a minor underestimation of incidence and prevalence was likely present in Study I and II. The presumed incomplete information from the DNPR in 2017 and 2018 originating from the Capital and Zealand Regions of Denmark and in autoantibodies from the Autoimmune Laboratory at SSI and the Register of Laboratory Results for Research could be reflected in the rates of RA (Appendix B) (1,128).

*Information bias:* As described earlier, all studies were prone to a small risk of misclassification. By increasing the validity of the case definitions of RA with time-dependent eligibility criteria an immortal period of up to one year was introduced for the RA population in Study III. However, due to the format of the study design, starting follow-up and matching on date of fulfilling RA criteria, no immortal time bias occurred (129). Data on migration was incomplete as only the first and last date of immigration and emigration was available. Therefore, if individuals migrated multiple times this was not visible. With sick individuals less likely to emigrate than healthy individuals (as applicable in study III), this would introduce bias. Stratification was used to reduce this bias (Appendix C).

*Confounding:* In Study III matching was conducted as an attempt to reduce confounding due to age and sex which are associated with both RA disease and death. Controlling for age by year of birth and sex by matching within the index calendar year allowed for potential temporal changes in age and sex. As no method prevent confounding completely, this will reduce but not remove the risk of confounding (120).

*Generalizability:* The randomly selected matched controls in Study III were expected to be comparable to the rest of the general population and thus can be used to represent the Danish population without RA disease. The findings in mortality may not be generalisable to patients with mild RA disease, as patients with RA only in contact with private practising rheumatologists or the primary health sector were not captured and with the assumption that patients with mild disease activity were less likely to encounter hospitals than moderate to high disease activity patients. The validity of the RA-specific ICD-10 codes may vary between countries and their respective national registers, which may have affected the external validity of the findings of this thesis.

In general, the Danish health registers are of high quality, and though they have limitations, they still provide a strong foundation of information for the investigation of the aims of this dissertation (83–86). The main strength was the nearly complete follow-up of the large populations with highly valid individual-level-based information. Study IV was a closed cohort, whereas Study I, II and III were designed as open or dynamic cohorts accounting for demographic changes over time (Appendix A to D) (1,2).

# CHAPTER 5. CONCLUSION AND FUTURE PERSPECTIVE

## 5.1. CONCLUSIONS

Results from the studies included in this thesis shed light on the epidemiology and burden of RA and provided contemporary estimates of the disease in Denmark.

Conclusions from Study I highlighted, a twofold higher IR and PP of RA for women than men. Temporal trends of IR were relatively stable over time, though with a peak in 2010 and a decrease in 2017 and 2018, and the PP increased significantly from 2000 to 2018. LR of RA reflected changes in the age- and sex-specific IR with higher risk for women than for men in all ages and with increasing risk with older age up to age 80 years. The choice of RA case definition altered the level of IR estimates by 29%, but the temporal pattern was similar for all four case definitions of RA. The methodological approach to defined RA cases had a larger influence on the estimates than the choice of denominator, as IPs were found only slightly lower than their corresponding IRs (1).

Conclusions drawn from study II included an increasing temporal trend in IR of seropositive RA and a decreasing trend for seronegative RA. This led to a higher IR of seropositive than seronegative RA from 2009 and onwards, widening in the difference until 2016, ending with an approximately twofold higher incidence of seropositive RA than seronegative RA. The underlying temporal patterns depended on the definition used to distinguish the serological subtypes of RA and to identify patients with RA (Appendix B).

Study III illuminated a significant improvement in 5-year all-cause mortality for both patients with RA and matched controls over time. There were indications of sex differences in the positive development of mortality over time, with a closing of the mortality gap in men with RA and continuing excess mortality in women with RA. Therefore, continued focus on preventing RA disease progression and comorbidities is needed (Appendix C).

No conclusions could be drawn from Study IV as it was a protocol paper discussing considerations and justifications for methodological choices to investigate the association between intake of marine n-3 PUFA in relation to risk of RA (2).

Overall, the epidemiologic measurement obtained from this dissertation reflected the improved life expectancy and prognosis for patients with RA during the calendar periods of the large population-based cohort studies included in the thesis. This

dissertation demonstrated that over time, when accounting for calendar periods to reflect changes in both RA classification criteria and treatment, the prevalence increased significantly whereas the IR remained relatively stable. Temporal changes in IR for overall RA were largely driven by changes in IR for seropositive RA. Five-year all-cause mortality risk has decreased significantly. Sex-specific differences in incidence, prevalence, lifetime risk, and mortality were exposed.

## 5.2. FUTURE PERSPECTIVES

This thesis and the studies included are subject to methodological considerations that should be made when conducting register-based studies including patients with RA. However, they do not cover all aspects of relevant methodological considerations but tried to demonstrate and provide solutions to potential problems in register-based case definitions of RA, definition of seropositivity in patients with RA, and an alternative statistical method to the conventional used in time-to-event analysis. The studies have laid the groundwork for methodological decision-making in future epidemiologic studies. It is my belief that the pseudo-observation approach will, in time, gain more traction in the field of rheumatology due to it being more flexible and approachable from a public point of view than the commonly used Cox proportional hazard model.

The IR of seropositive RA increased in a period where the availability of autoantibody testing grew and where autoantibodies received greater weight in the classification criteria of RA. However, whether the observed tendencies in IRs of seropositive and seronegative RA were due to a true change in the temporal trend of serological subtypes of RA, a more frequent testing and/or improved registration practice of autoantibodies, or a combination thereof, needs further exploration. Therefore, a future study using a more advanced weighting of the data including the probability of seropositivity will be needed to investigate if the observed temporal trends in IRs of seropositive and seronegative RA in Study II were driven solely by autoantibody testing becoming more frequent over time.

This thesis investigated the mortality in overall RA. However, as stated previously RA might be a heterogeneous group of diseases with overlapping syndromes with different disease mechanisms, and therefore the 5-year all-cause mortality risk could be different in seropositive and seronegative RA. A study investigating this is under preparation. The dissertation only explored all-cause mortality, while cause-specific mortality will be investigated separately taking competing risks into account. Further, repeating study III as new calendar years becomes available is relevant to track temporal changes, and to increase the follow-up time to e.g. 10 years to investigate the mortality risk in patients with RA with longer disease exposure as disease duration is of great interest. Study III detected a sex difference in the mortality risk, which was not explored further in this dissertation, thus this needs to be investigated further to

clarify the causes of the continuing excess mortality only in women with RA. Prediction models to establish which patients with RA are at risk of premature mortality could become a tool in RA management in newly diagnosed patients.

The dissertation investigated the occurrence and severity of the RA disease and going forward a focus will also be to investigate how to prevent the development of RA. Study IV described the first step in this research area. First, investigation of separate lifestyle risk factors such as n-3 and n-6 PUFA in diet and adipose tissue, and physical activity of which the current literature is sparse, and afterwards investigation of the association of non-adherence to established dietary and lifestyle guidelines and risk of RA will be conducted. With international collaborators some of these studies can be performed not only in a Danish population but also in a UK population with different calendar time for data collection, potentially strengthening findings and helping to prevent RA.

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# APPENDICES

# Appendix A. Paper I

# Appendix B. Paper II

# Appendix C. Paper III

# Appendix D. Paper IV

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