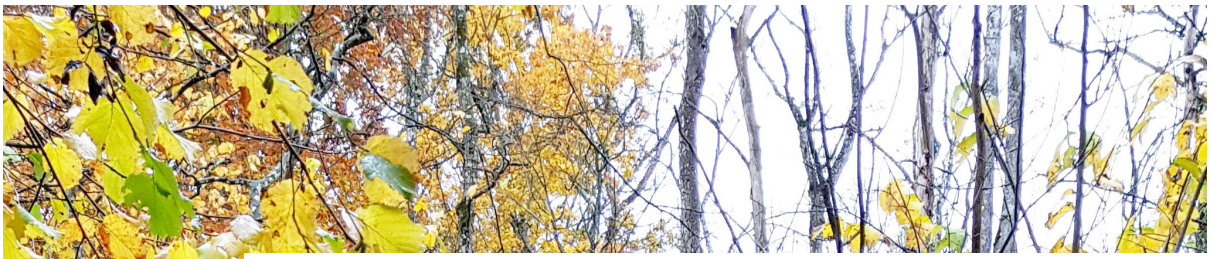
Juvenile idiopathic arthritis - from macrophage to mortality

Berthold, Elisabet

2022

Document Version:
Publisher's PDF, also known as Version of record



Juvenile idiopathic arthritis – from macrophage to mortality

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Printed by Media-Tryck, Lund 2022 NORDIC SWAN ECOLABEL 3041 0903

ELISABET BERTHOLD is working as a rheumatologist at the section for pediatric rheumatology at Skåne University Hospital in Lund. The focus of her research interest is the pediatric rheumatic disease juvenile idiopathic arthritis (JIA). The aim of her thesis is to gain new information and updated answers to the patients' questions *Why did I get this disease?*, *What will happen to me now?* and *Will I ever get well?*.

LUND UNIVERSITY

FACULTY OF MEDICINE

Department of Clinical Sciences in Lund
Rheumatology

Lund University, Faculty of Medicine
Doctoral Dissertation Series 2022:56
ISBN 978-91-8021-217-5
ISSN 1652-8220

Juvenile idiopathic arthritis – from macrophage to mortality

Elisabet Berthold

LUND
UNIVERSITY

DOCTORAL DISSERTATION
by due permission of the Faculty of Medicine, Lund University, Sweden.
To be defended at Lottasalen, Reumatologiska kliniken, Kioskgatan 5,
Lund, Friday April 29th, 2022, at 9.00 am

Faculty opponent
Professor Marite Rygg
Norwegian University of Science and Technology, Trondheim, Norway

Organization LUND UNIVERSITY Faculty of Medicine Department of Clinical Sciences in Lund	**Document name** Doctoral dissertation **Date of issue** 2022-04-29
Author: Elisabet Berthold	Sponsoring organization

Title and subtitle
Juvenile idiopathic arthritis – from macrophage to mortality

Abstract
Juvenile idiopathic arthritis (JIA) is the most common rheumatic disease in children. Studies of the immunopathogenesis in JIA has mainly focused on the adaptive immune system, while less is known of the role of the monocytes. JIA is considered a chronic disease, although only about 50% of participants in long-term follow-up studies have active disease as adults. The treatment arsenal for JIA has expanded during the last three decades, but if this has improved the long-term outcome is not known.

When a diagnosis of JIA is confirmed almost all children and parents ask the same important questions: "Why did I/my child get this disease?", "What will happen to me now?" and "Will I ever get well?". The overall aim of this thesis has been to study different aspects of these questions – from the pathophysiological role of monocytes and their function in blood and the synovial environment, to incidence, outcome measures with short- and long-term perspectives, risk of depression and anxiety, and mortality.

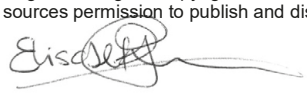
Children with active oligoarticular JIA display monocytes of a mixed pro- and anti-inflammatory polarization pattern in the synovial environment, with reduced capacity to phagocytize, unique for this JIA subtype. This supports the hypothesis that oligoarticular JIA should not be considered as a pediatric version of adult rheumatic arthritides.
The mean annual incidence rate in Skåne (the southernmost region of Sweden) 1980 – 2010 was 9.9/100,000 children < 16 years, with significantly increasing numbers during the period. The mortality was interpreted as low, fewer patients were diagnosed with JIA-associated uveitis and the need of joint corrective surgery was decreased compared to previously published data. However, children with JIA diagnosed since the introduction of biologic immunomodulatory treatment still experienced disease activity more than 50% of the follow-up years. No increased risk for diagnosis with depression or anxiety was found in JIA patients and they were also not diagnosed at an earlier age than age- and sex-matched controls.

In conclusion, the long-term outcome of JIA has improved and the risk for depression and anxiety is not found to be increased in JIA. There are however still challenges with active disease more than half of the time in spite of state-of-the-art treatment. Follow-up in adulthood is needed to answer the question of how many JIA patients that still needs healthcare as adults.

Key words
Juvenile idiopathic arthritis, Epidemiology, Outcome, Monocytes, Inflammation, Depression, Anxiety

Classification system and/or index terms (if any)

Supplementary bibliographical information	**Language** English, Swedish
ISSN and key title 1652-8220, JIA – from macrophage to mortality Lund University, Faculty of Medicine Doctoral Dissertation Series 2022:56	**ISBN** 978-91-8021-217-5
Recipient's notes	**Number of pages** 92 Security classification Price

Signature  Date 2022-03-24

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Omslagsfoto av Elisabet Berthold

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Section of Rheumatology

ISBN 978-91-8021-217-5
ISSN 1652-8220

Tryckt i Sverige av Media-Tryck, Lunds universitet
Lund 2022

"Why did I get this disease?
What will happen to me now?
Will I ever get well?"

Table of contents

List of papers

The thesis is based on studies reported in the following papers, which will be referred to in the text by their respective Roman numerals:

I. Schmidt T, **Berthold E**, Arve-Butler S, Gullstrand B, Mossberg A, Kahn F, Bengtsson AA, Månsson B, Kahn R.

Children with oligoarticular juvenile idiopathic arthritis have skewed synovial monocyte polarization pattern with functional impairment – a distinct inflammatory pattern for oligoarticular juvenile arthritis.

Arthritis research & therapy. 2020, 12;22(1):186.

II. **Berthold E**, Månsson B, Kahn R.

Outcome in juvenile idiopathic arthritis: a population-based study from Sweden.

Arthritis research & therapy. 2019, 28;21(1):218.

III. **Berthold E**, Dahlberg A, Tydén H, Månsson B, Kahn R.

Increasing incidence of juvenile idiopathic arthritis: a trend over 31 years in southern Sweden.

Manuscript.

IV. **Berthold E**, Dahlberg A, Jöud A, Månsson B, Kahn F, Kahn R.

Juvenile idiopathic arthritis does not increase the risk of depression and anxiety: results from the South-Swedish JIA cohort.

Manuscript.

Publications, published during doctoral studies, not included in the thesis

- Kahn R, **Berthold E**, Gullstrand B, Schmidt T, Kahn F, Geborek P, Saxne T, Bengtsson AA, Månsson B.

  Circulating complexes between tumour necrosis factor-alpha and etanercept predict long-term efficacy of etanercept in juvenile idiopathic arthritis.

  Acta Paediatrica. 2016;105(4):427-32.

- **Berthold E**, Månsson B, Gullstrand B, Geborek P, Saxne T, Bengtsson AA, Kahn R.

  Tumour necrosis factor-alpha/etanercept complexes in serum predict long-term efficacy of etanercept treatment in seronegative rheumatoid arthritis.

  Scandinavian Journal of Rheumatology. 2018;47(1):22-26.

- Arve-Butler S, Schmidt T, Mossberg A, **Berthold E**, Gullstrand B, Bengtsson AA, Kahn F, Kahn R.

  Synovial fluid neutrophils in oligoarticular juvenile idiopathic arthritis have an altered phenotype and impaired effector functions.

  Arthritis research & therapy. 2021 9;23(1):109.

- Kahn R, Berg S, Berntson L, **Berthold E**, Brodin P, Bäckström F, et al.

  Population-based study of multisystem inflammatory syndrome associated with COVID-19 found that 36% of children had persistent symptoms.

  Acta Paediatrica. 2021;00:1-9.

- Arve-Butler S, Mossberg A, Schmidt T, Welinder C, Yan H, **Berthold E**, Król P, Kahn R.

  Neutrophils lose the capacity to suppress T cell proliferation upon migration to inflamed joints in juvenile idiopathic arthritis.

  Frontiers in Immunology. 2022, doi.org/10.3389/fimmu.2021.795260.

Abbreviations

ACPA – Anti-citrullinated protein/peptide antibodies

ANA – Antinuclear antibodies

APC – Antigen presenting cell

bDMARD – Biological disease-modifying anti-rheumatic drug

CI – Confidence interval

CNS – Central nervous system

CRP – C-reactive protein

csDMARD – Conventional synthetic disease-modifying anti-rheumatic drug

DMARD – Disease-modifying anti-rheumatic drug

E-oligo – Extended oligoarthritis

ERA – Enthesitis related arthritis

ESR – Erythrocyte sedimentation rate

EULAR – European League Against Rheumatism

FACS – Fluorescence activated cell sorting

HR – Hazard ratio

IAC – Intra-articular corticosteroid injections

IBD – Inflammatory bowel disease

ICD – International classification of diseases

IFN – Interferon

IL – Interleukin

ILAR – International League of Associations for Rheumatology

JADAS – Juvenile arthritis disease activity score

JAK – Janus kinase

JCA – Juvenile chronic arthritis (European definition)

JIA – Juvenile idiopathic arthritis (International definition since 2001)

JPsA – Juvenile psoriatic arthritis

JRA – Juvenile rheumatoid arthritis (North American definition)

LPS – Lipopolysaccharide

MHC – Major histocompatibility complex

NBWH – National board for health and welfare

NK cells – Natural killer cells

NSAID – Non-steroid anti-inflammatory drug

PCR – Polymerase chain reaction

PMA – Phorbol-myristate-acetate

P-oligo – Persistent oligoarthritis

qPCR – Quantitative polymerase chain reaction

RA – Rheumatoid arthritis

RF – Rheumatoid factor

RF– – RF negative polyarticular JIA

RF+ – RF positive polyarticular JIA

SHR – Skåne healthcare register

SMR – Standardized mortality rate

sJIA – Systemic onset JIA

STAT – Signal transducers and activators of transcription

TNF – Tumor necrosis factor

tsDMARD – Targeted synthetic disease-modifying anti-rheumatic drug

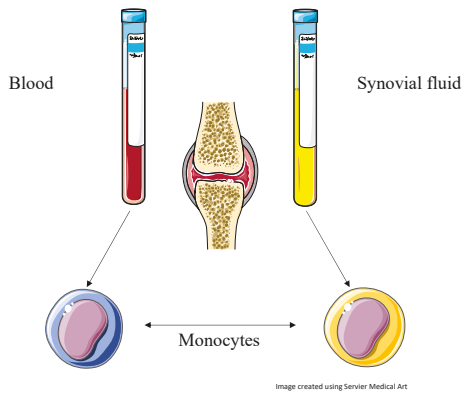
uJIA – Undifferentiated arthritis

Thesis at a glance

Paper I. *Aim:* To map the polarization state and function of monocytes in blood and the synovial environment.

Patients and methods: Paired samples of blood and synovial fluid from 13 children with untreated oligoarticular JIA and three synovial biopsies. Analysis of polarization markers using flow cytometry and qPCR, effector functions using phagocytosis assay and monocytes in biopsies using immunohistochemistry, immunofluorescence and *in situ* hybridization.

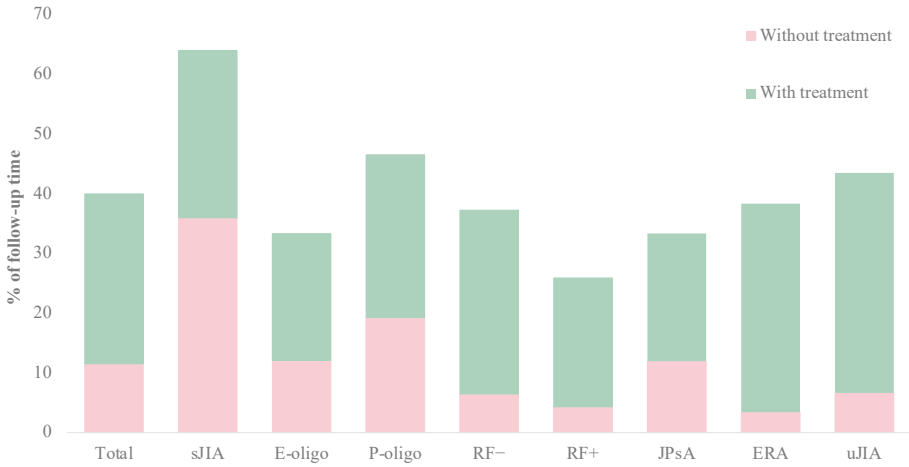
Conclusion: Children with active oligoarticular JIA display monocytes of a mixed pro- and anti-inflammatory polarization pattern in the synovial environment, with reduced capacity to phagocytize, unique for this JIA subtype.



Paper II. *Aim:* To study the epidemiology and outcome of JIA in Skåne in the era of biologic treatment.

Patients and methods: A population-based cohort of 251 individuals diagnosed with JIA 2002 – 2010 while living in Skåne. Retrospective annual registration of measures of disease activity, pharmacologic treatment, joint surgery, and diagnosis with JIA-associated uveitis until end of 2015.

Conclusion: The need for joint surgery and the presence of uveitis was diminished compared to studies with patients diagnosed in the 1980's and 90's. Children with JIA however still experienced disease activity more than 50% of the follow-up time.

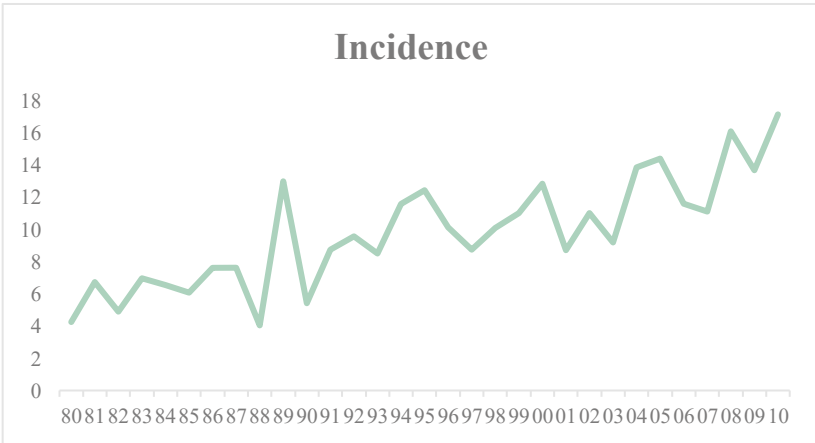


Illustrating the percentage of individual follow-up years with inactive disease defined as no uveitis or arthritis, in the total cohort as well as divided by JIA subtype.

Paper III. *Aim:* To study the incidence and mortality of JIA over 31 years.

Patients and methods: The cohort from Paper II and an additional 400 individuals diagnosed 1980 – 2001 while living in Skåne, calculation of annual incidence rate. Data on mortality from "The cause of death register".

Conclusions: There is a statistically significantly increasing incidence of JIA over the period of 1980 – 2010. Only eight mortalities (1.2%) were found, which prevented further statistical analysis.



Illustrating increasing annual incidence presented per 100,000 children between 1980 – 2010.

Paper IV. *Aim:* To compare the risk of being diagnosed with depression or anxiety for JIA patients to controls without JIA.

Patients and methods: Conditional cox proportional hazard regression analysis of registered diagnoses for depression and anxiety at the Skåne healthcare register 1998 – 2019, using 640 of the patients in the JIA cohort and 3200 controls matched for sex and year of birth.

Conclusions: No increased risk for diagnosis with depression or anxiety was found in JIA patients and they were also not diagnosed at an earlier age.

	JIA	**Controls**
Depression	14.5%	14.8%
Anxiety	17.3%	17.4%

Illustrating the percentage of patients and controls diagnosed with depression and anxiety 1998 – 2019.

Preface

I have always enjoyed working with children. My first employment was to teach children to swim and as daughter of two teachers I have been enthused to help younger children learn and develop.

During medical school I did not nourish a dream of working with children, there were so many of my student colleagues that were more passionate about that. I was more inspired by the wise and calm physicians at the rheumatology department, and to work with chronic diseases where you must look at the whole person and take all aspects in life under consideration in the treatment and not focus on a single organ. As I during my residency encountered the division of pediatric rheumatology, I remembered how much I enjoy working with children. Now I feel grateful to have the opportunity to combine these two areas of interest.

When a child is diagnosed with juvenile idiopathic arthritis, the same questions always arise. “Why did I/my child get this disease?”, “What will happen to me now?” and “Will I ever get well?”. As a doctor it is frustrating not to have answers to these questions. I have been told during my education that the best answer to the question of prognosis is “how you feel after five years of disease is how you will feel as an adult”. This assumption is based on research conducted on patients diagnosed with juvenile arthritis before immunomodulatory drugs where common and we hope, but don’t know, that children diagnosed with pediatric rheumatic diseases today feel better and develop less complications.

The aim of this PhD project has been to get better answers to the patients’ questions, and I think we at least have come some steps on the way. Working with this project, I have gained a lot of theoretical knowledge on juvenile arthritis. Now I look forward to continuing to work, in the everyday clinic as well as with research, to develop better care and treatment for children with rheumatic diseases.

Introduction to the field

Juvenile idiopathic arthritis (JIA)

Definition

JIA is the most common rheumatic disease in children. The disease is defined as "an arthritis of unknown etiology that begins before the 16th birthday and persists for at least six weeks; other known conditions (infection, trauma, malignancy) are excluded", according to the International League of Associations for Rheumatology (ILAR)(1).

Before these current international classification criteria was adapted in the late 1990's(2), there were two criteria in use and the disease had different names and slightly different definition. Juvenile rheumatoid arthritis (JRA), with six weeks arthritis duration was the American definition(3) and The European League Against Rheumatism (EULAR) termed the disease juvenile chronic arthritis (JCA) and required three months of symptoms for diagnosis. The EULAR classification system also encompassed juvenile ankylosing spondylitis, psoriatic arthritis and arthritis associated with inflammatory bowel diseases (IBD)(4). The difference in definitions complicates the comparison of results from older studies and the ILAR criteria has facilitated the international communication and possibility of research on JIA today. The goal of the current criteria has also been to define homogenous subgroups of the disease. In the last decade the question of revaluation of the current criteria has been raised and one suggestion has been to define the diagnostic subgroups by measures reflecting the immunological mechanisms rather than the number of joints and sites of inflammation involved(5-7).

Classification

The classification system used to diagnose JIA today is the revised ILAR classification of JIA from 2001(1). These are the criteria used and referred to throughout this thesis (Figure 1).

Systemic onset JIA
Arthritis in one or more joints preceded by fever for 2 weeks with a quotidian pattern for at least 3 days, and accompanied by one or more of:
- Evanescent erythematous rash
- Generalized lymphadenopathy
- Hepato- and/or splenomegaly
- Serositis

Exclusions: a, b, c, d

Oligoarthritis
Arthritis affecting 1-4 joints during the first 6 months of disease.
- Persistent: not more than 4 affected joints throughout the disease course
- Extended: affecting more than 4 joints after the first 6 months of disease

Exclusions: a, b, c, d, e

RF-negative polyarthritis
Arthritis affecting 5 or more joints during the first 6 months of disease and negative test for RF

Exclusions: a, b, c, d, e

RF-positive polyarthritis
Arthritis affecting 5 or more joints during the first 6 months of disease and at least 2 positive tests for RF at least 3 months apart during the first 6 months of disease

Exclusions: a, b, c, e

Juvenile psoriatic arthritis
Arthritis and psoriasis, or arthritis and at least 2 of:
- Dactylitis
- Nail pitting or onycholysis
- Psoriasis in a first-degree relative

Exclusions: b, c, d, e

Enthesitis related arthritis
Arthritis and enthesitis, or arthritis or enthesitis with at least 2 of:
- Sacroiliac joint tenderness and/or inflammatory lumbosacral pain in the patient history
- The presence of HLA-B27 antigen
- Onset in a male over 6 years of age
- Acute anterior uveitis
- Ankylosing spondylitis, ERA, sacroiliitis with IBD, Reiter's syndrome, or acute anterior uveitis in a first-degree relative

Exclusions: a, d, e

Undifferentiated arthritis
Arthritis that fulfill criteria in no category, or in 2 or more of the other categories

Exclusions
a) Psoriasis or a history of psoriasis in the patient or first-degree relative
b) Arthritis in an HLA-B27 positive male beginning after the 6th birthday
c) Ankylosing spondylitis, ERA, sacroiliitis with IBD, Reiter's syndrome, or acute anterior uveitis, or a history of one of these conditions in a first-degree relative
d) The presence of RF on at least 2 occasions at least 3 months apart
e) Systemic JIA in the patient

Figure 1. ILAR classification of JIA: Second revision, 2001
Abbreviations: RF – rheumatoid factor; ERA – enthesitis related arthritis; IBD – inflammatory bowel disease

Etiology

The term “idiopathic” in JIA refers to the fact that the disease origin is unknown, hence the arthritis develops without clear evidence of outer impact. It is believed to be a combination of environmental factors and genetic susceptibility that leads to clinical disease.

Genetics

JIA is a complex polygenic disease. Evidence of genetics being involved in the pathogenesis of JIA is that there is 44% concordance in monozygotic twins(8) and 32% of JIA patients have first-degree relatives with autoimmune disease(9). Also, when two siblings have JIA, they have the same subtype in the majority of cases(10).

The genetic background differs between JIA subtypes, especially for systemic onset JIA (sJIA) which is considered an autoinflammatory- rather than autoimmune disease. Genes found to be involved in JIA are both of HLA- and non-HLA type, except for sJIA that lack association to HLA genes. HLA genes of class I as well as class II, with the main function as antigen presenters to T-cells, have been found to be JIA associated(11). A strong non-HLA genetic association has been found for the T-cell regulatory protein tyrosine phosphatase nonreceptor 22, but several other non-HLA genes coding for cytokines (tumor necrosis factor alpha (TNFα), interleukin (IL)-2, IL-10, IL-6) or proteins involved in immune signaling and antigen presentation have also been suggested to be important in the susceptibility to JIA(12, 13).

Gender differences

Autoimmune disease is in general more common in females than in males, which is true for JIA as well(14). The gender difference has several possible explanations, where a combination of many most likely is the cause (Figure 2).

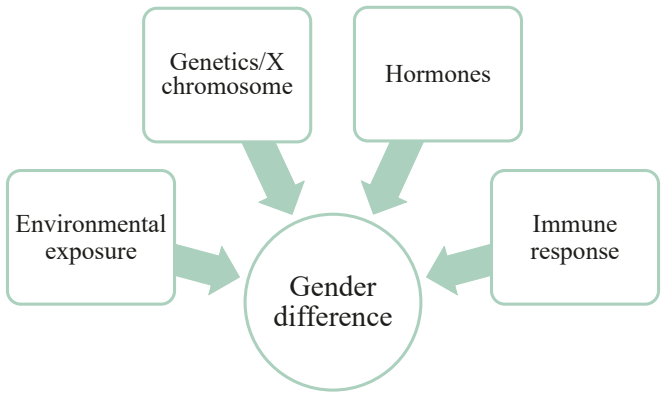


Figure 2. Factors differing between males and females contributing to gender bias in autoimmune disease.

Hypotheses to the gender difference are that there are several differences between female and male immune systems that might contribute, and that there also exist differences in exposure to environmental factors such as smoking and dietary habits. The X chromosome contains many genes that are involved in immunity. Since most X-linked genes are not sex-specific, one X chromosome must be inactivated in females to achieve gene equivalency compared to males(11). The inactivation is however incomplete, leading to an over expression of genes on the X chromosome in females. Depending on the nature of these genes, this can either promote or act protective in immune reactions(15-17). The contribution of the X chromosome to the risk of autoimmune disease is supported by a higher prevalence also among XX and XXY males(11).

Hormonal differences are also believed to contribute to the gender bias since the gender differences among patients are lower in autoimmune diseases before puberty. Estrogens, for example, are known to have an impact on the development and function of lymphocytes. The pre-pubertal gender difference that exists in JIA can however not be explained by hormonal factors since there is no difference in the levels of estrogens and androgens before puberty. Females are in all known to produce stronger humoral- and cellular immune response than males, leading to better control of infections but in turn increases the risk of autoimmune reactions(11).

Environmental factors

Various environmental factors have been explored in the study of aetiopathogenesis of JIA. Breast-feeding, for example, contributes to the imprint and programming of the infant immune system through transfer of cytokines and the mother's immunological memory, and early weaning has been shown to be associated with increased risk of JIA development(18-22). A common hypothesis today is that a

decrease of the infectious burden in industrialized countries has led to an increase of allergic and autoimmune diseases(23-25), called the "hygiene hypothesis". There are therefore many studies exploring environmental influence to the risk of JIA, with focus on how early lifestyle factors affects the imprint of the immune system (18). In line with the hygiene hypothesis, growing up with siblings (especially younger) have been shown to decrease the risk of JIA(26), whereas serious infection during the first year of life has prospectively been associated to increased risk of JIA(27). Associations between antibiotic exposure prior to JIA diagnosis have also been found, suggested to alter the fecal microbiome(28, 29). The mechanism through which infections affects the risk of developing JIA and if certain pathogens are responsible for the risk is still to be revealed(18).

Immunopathogenesis

In the following section an overview of the function of the immune system, current knowledge of the immunopathogenesis in JIA and the specific role of the monocyte will be presented, with focus on oligoarticular JIA since this is the subtype studied in Paper I.

Inflammation

Inflammation is an immune response to stimuli threatening tissue homeostasis. Factors triggering an immune reaction can be both exogenous (microorganisms and irritants) and endogenous (damaged cells and cell debris). The purpose of inflammation is to eliminate the threat and start tissue repair as fast as possible. Cardinal symptoms of inflammation are:

- redness (rubor) and increased temperature (calor), the result of increased local blood flow
- edema (tumor), caused by the leakage of fluid and plasma proteins into the tissue due to increased vessel permeability
- pain (dolor), the effect of swelling and release of mediators triggering pain receptors in the tissue(30)

Overview of the immune system

The human immune system is divided in two branches, often referred to as the innate and adaptive immune systems, with differences in function, specificity, and time of response. The innate immune system serves as our first line of defense with the functions of recognizing pathogen patterns, followed by eradicating the threat without too much harm to the host. It consists of epithelia and mucosa forming a physical and chemical barrier against microbes, various cell types (mainly phagocytes) that are always present, and plasma proteins. The adaptive immune system, consisting of lymphocytes and their products, is activated after initial

response from the innate system, hence "adapt" the response to the type of pathogenic invader. Innate immune cells are somewhat rough in their sense of recognizing structures shared by microbes and that are not present by host cells. Adaptive immune reactions are specific, since the lymphocytes are presented with detailed microbial molecules, called antigens, from antigen presenting innate immune cells and co-stimulated to create the eligible response. The cells of the different systems also differ in their life span, with phagocytes dying after fulfilling their assignment to start an immune reaction (6-24 hours), while lymphocytes lives for days and weeks and can evolve to memory cells that rest in lymphoid tissues or circulate throughout life waiting for the microbe to return(31).

The pathogenesis in JIA differs between subtypes, shown for example in studies of synovial histology and protein expression(32, 33). Due to the genetic association with HLA class II genes and the presence of autoantibodies, the main focus in studies of the pathogenesis in JIA has been on adaptive immune responses and less is known of the contribution of the innate immune cells. It is assumed that the autoimmune reaction in JIA starts with a response to a self-antigen from cells in the adaptive immune system. However, after the initial event almost all the immune system is involved(34).

Innate immunity

The cells of the innate immune system are mononuclear phagocytes (monocytes, macrophages, and dendritic cells), polymorphonuclear phagocytes (neutrophils, eosinophils, and basophils) and natural killer (NK) cells. Neutrophils are the first cells to respond to most infections and are by that also the most numerous leukocytes in the blood with several defense mechanisms and immune functions(35, 36).

The main function of macrophages and dendritic cells are, in brief, to attract other immune cells to the site of inflammation and to act as presenters of antigens to T-cells and thereby activate the adaptive immune system. The function of monocytes will be described in more detail in a separate section. Common to all the phagocytes is the ability to phagocytize (phagos = eating, cytos = cell), a process when the macrophage or neutrophil extends its plasma membrane around a pathogen, pinches off and internalizes the particle in a membrane-bound vesicle called a phagosome. The phagosome fuses with a lysosome, forming a phagolysosome that, as different enzymes are activated and form reactive oxygen intermediates and nitric oxide, eventually kills and breaks down the pathogen(37).

Innate immune reactions are however not solely cell dependent. Circulating plasma proteins, for example proteins of the complement system, antimicrobial peptides, and acute phase reactants such as C-reactive protein (CRP) also have defense functions as recognizers of pathogens and enhancers of cell reactions. Cytokines are crucial proteins responsible for communication between cells, where TNFα and IL-1 are the principal cytokines in recruiting phagocytic cells to the affected tissue(31, 37).

Memory functions of immunity have been considered to be possible only for the adaptive immune cells. In recent years, it has become clear that infections can have long-lasting effects on innate immune cells as well, with a shift to increased production of monocytes and decreased production of lymphocytes after reencounter of a pathogen. This phenomenon is called trained immunity and believed to result from epigenetic changes on hematopoietic stem cells and innate effector cells(38, 39). Trained immunity is suggested as contributor to chronic inflammation in autoimmune diseases(39).

Monocytes and macrophages and their polarization

Monocytes constitute 5-10% of circulating leukocytes in humans but can very quickly increase in numbers and mobilize to affected tissues as a response to inflammation. Monocytes are identified by their surface expression of CD14 and CD16, where the combination differentiates them into different subsets. $CD14^{+}CD16^{-}$ are named "classical" monocytes, $CD14^{+}CD16^{+}$ intermediate and $CD14^{LOW}CD16^{+}$ "non-classical" monocytes (80-90% are classical). Under healthy conditions, monocytes circulate in the blood up to 24 hours after release from the bone marrow. They can leave the circulation in response to stimulating cytokine signals from other cells and then either transmigrate to tissues to repopulate tissue-resident macrophages or convert to non-classical monocytes with a longer lifespan that might help to retain constant cell-numbers in pathological situations. The number of non-classical monocytes is strongly associated to the physiological status of the organism and is suggested being of possible diagnostic use(38). Macrophages have different appearance and functions depending on the tissue they reside in, but their overall functions are to clear dying cells and hostile particles by phagocytosis and to secrete cytokines and chemokines that activate other cells in the immune system(30). Monocytes residing in tissues adopt functions of the tissue-resident macrophages, for example, antigen-presentation and anti-inflammatory abilities such as cytokine catabolism and release of regulatory mediators(38).

Monocytes and macrophages can act pro-inflammatory or as regulators of inflammation depending on the stimuli in the local environment in which they are differentiated, a process called polarization or activation. Polarized monocytes and macrophages are *in vitro* traditionally referred to as classically activated (M1) and alternatively activated (M2), with further subdivision depending on the functions and cytokines produced(40-44). We refer to these phenotypes as M1(IFNγ), M2(IL-4) and M2(IL-10), reflecting by which cytokines they are induced (Figure 3). Polarization is believed to influence disease progression by affecting effector functions(45), but polarization is difficult to study *in vivo* since the immune cells are under the constant influence of exposure to cytokines specific for both M1 and M2 types, compared to the cells in the strict laboratory milieu. There is evidence of imbalance in M1/M2 pattern in adult rheumatic diseases(46-48). In RA, monocytes have been found in synovial fluid and tissue where they differed in CD16 expression

compared to circulating monocytes(49). Increased frequency of circulating and synovial intermediate $CD14^{+}CD16^{+}$ monocytes and a M2(IL-10)-like pattern in synovial monocytes have been demonstrated in enthesitis related arthritis (ERA)(50, 51) and monocytes in sJIA are also shown to polarize depending on environmental stimuli(52).

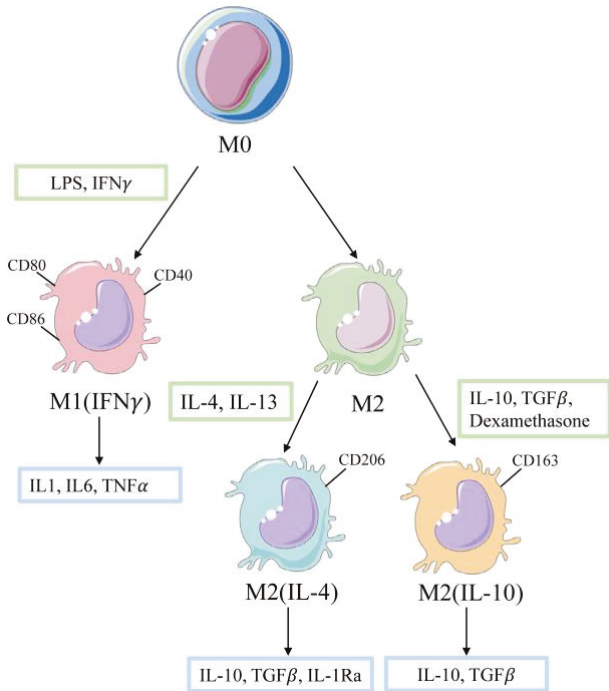


Figure 3. Schematic summary of monocyte polarization. Stimulating cytokines in green boxes and secreted cytokines in blue boxes. Image created using Servier Medical Art (smart.servier.com).
Abbreviations: LPS – lipopolysaccharide, IFN – interferon, IL – interleukin, TGF – transforming growth factor, TNF – tumor necrosis factor

Adaptive immunity

The cells of the adaptive immune system are the lymphocytes, known as T-cells and B-cells. The main features of adaptive immunity are the detailed specificity of the lymphocyte receptors enabling fast recognition and response to antigens, the creation of an immunologic memory and the regulation of immune attacks to protect us from damage to self-structures.

T-cells are activated through presentation of an antigen displayed by major histocompatibility complex (MHC) on antigen-presenting cells (APCs), which are mainly innate immune cells, combined with additional co-stimulatory signals either from receptors on the APC's surface or secreted cytokines. Class I MHC molecules are recognized by $CD8^{+}$ cells that develop into cytolytic cells that destruct cells

harboring intracellular pathogens. The most common T-cells are $CD4^+$ that recognize MHC II molecules and develop into helper cells that produces cytokines that activate effector functions of other cells or stimulate B-cells to antibody production. A subset of $CD4^+$ cells are regulatory and can inhibit response of other lymphocytes(31, 53). T-cells develop in the thymus where they undergo a control process in order to create immunologic tolerance. Peptides from endogenous proteins are presented on MHC molecules and T-cells not recognizing the self-MHC, as well as the T-cells that bind with very high avidity are eliminated (negative selection)(54).

B-cells arise in the bone marrow and the principle of tolerance are similar to that of T-cells. Depending on the cell contact and cytokine stimulation, B-cells develop into either memory cells that can be activated on future encounter with the same antigen, or plasma cells with the purpose of producing antibodies. Antibodies are antigen-recognizing immunoglobulins that opsonize (coats) microbes and thereby promote phagocytosis, activate the complement system, or stimulate cytotoxic $CD8^+$ cells(31, 53).

Autoimmunity

Autoimmunity is a failure of an organism to tolerate its own cells and extra cellular matrix, resulting in an immune response by lymphocytes and/or antibodies. When this immune reaction leads to dysfunction of an organ, an autoimmune disease is manifest(11).

Autoreactive T-cells should be eliminated already in the thymus, but since not all self-antigens occur in the thymus some T-cells escape negative selection(54). For this reason, there is also need for peripheral mechanisms to induce immunologic tolerance. Autoreactive cells can either be suppressed by regulatory T-cell or die by apoptosis as they repeatedly encounter antigens without costimulatory signals or use inhibitory receptors to recognize co-stimulators(15, 31, 54). As previously described, there are genetic associations to autoimmunity and infection can contribute to development of autoimmunity. There can be structural similarities between microbial and self-antigens, inaccurate co-stimulation from APCs due to an elevated initial immune response and an infection or trauma can cause release of antigens normally hidden from the immune system (for example in a “closed organ” such as the eye)(31, 54). In recent years, evidence of disturbances in the microbiota (microorganisms in the mucosal surfaces and skin) leading to autoimmune diseases such as RA and spondyloarthritis are emerging(15).

Autoantibodies

Evidence of JIA being an autoimmune disease is the presence of tissue infiltrates of immune cells causing organ damage, and the finding of autoantibodies. However, the subtype of sJIA is considered an autoinflammatory disease associated solely with defects in innate immune mechanisms instead of disturbances in adaptive

immunity(13, 55). The lack of autoantibodies in sJIA further supports this hypothesis(55).

Autoantibodies of clinical interest in JIA are antinuclear antibodies (ANA), rheumatoid factor (RF), and anti-citrullinated protein/peptide antibodies (ACPA). ANA are present in approximately 50% of JIA patients but are not specific for the diagnosis. The presence of ANA should be used as a prognostic factor of the risk for uveitis(55), the most common extra-articular manifestation affecting 2-20% of JIA patients(56) that will be more detailly described in a separate section. The target antigens of ANA are not specified, but there are several suggestions including nucleic acids, nucleosomes, phospholipids, and nuclear and nucleolar proteins(55). RF are antibodies with antigen binding sites specific to the Fc portion of IgG molecules, found in about 5% of JIA patients and are associated with worse prognosis and risk of bone erosions. ACPA are also present in the same extent as RF, found in most patients with RF+ polyarticular JIA, and correlates to poor prognosis(13, 55, 57). ACPA are directed against citrullinated peptides from dying cells and are more known as antibodies with high specificity for RA. *In vitro* studies suggest that RF and ACPA contributes to the pathogenesis of arthritis, but there are no current evidence connecting autoantibodies to the pathophysiology of JIA(55).

The synovial environment and pathogenesis of JIA

The synovial membrane (synovium) lines the inner surface of the joint capsule that encloses a joint and is the source of synovial fluid that protects, nourishes, and lubricates the joint cartilage. The synovium consists of an inner (intima) and outer (subintima) layer. The intima is composed of one to three layers of cells, macrophage- or fibroblast like synoviocytes, while the subintima is relatively acellular and consists of nerves, lymphatic and vascular vessels, fat cells, fibroblasts, a few lymphocytes or macrophages, and collagen fibrils(58, 59). An adult knee contains < 3.5 mL synovial fluid under healthy conditions, but during inflammation angiogenesis and blood flow to the synovium is increased and more synovial fluid is produced. Due to insufficient increase of the vasculature compared to the synovial tissue expansion, this eventually leads to intraarticular hypoxia that initiates pro-inflammatory processes(58).

Neutrophils are the most common immune cells in inflamed joints(60). In oligoarticular JIA, synovial neutrophils are found to have altered phenotype with reduced capacity to phagocytize(61) and suppress T-cells than circulating neutrophils(62). This together with findings of high levels of cytokines typical of innate immunity reactions in joint fluid(44, 63, 64) emphasizes a contributing role of the innate immune system to the pathogenesis of JIA. Monocytes and macrophages are crucial to the resolution of inflammation and if the process of tissue restorsion is disturbed, chronic inflammatory disease can develop(38).

The synovial tissue is in JIA infiltrated by T- and B-lymphocytes, plasma cells, NK-cells, macrophages, dendritic cells, and neutrophils(13). T-, B- and plasma cells are found to be aggregated and clustered around antigen-presenting dendritic cells in lymphoid follicular structures in the synovia(65). In oligoarticular JIA, imbalance between pro-inflammatory Th1/Th17 and anti-inflammatory regulatory T-cells is suggested as the origin of inflammation. The T-cells produce pro-inflammatory cytokines (TNFα, IL-17, interferon (IFN) γ, and granulocyte-macrophage colony-stimulating factor), leading to recruitment of additional inflammatory cells and activation of synoviocytes, synovial monocytes and tissue macrophages. In turn, these activated cells start production of catabolic proteases causing damage to cartilage tissue, and pro-inflammatory cytokines that activates osteoclasts to cause bone erosions and eventually loss of joint function(13, 65).

Epidemiology

Reports of incidence and prevalence of JIA differ depending on study design and geographic region. The international consensus on the ILAR criteria for JIA has facilitated comparison of epidemiological studies, since the different definitions historically used for juvenile arthritis was another factor that needed to be considered when interpreting results. There have been reports on a north-south geographic gradient in the incidence of JIA, possibly explaining the combined impact of genetics and environmental risk factors for developing the disease(66). In a population-based cohort from southwestern Sweden the reported mean annual incidence of JCA in 1992 was 10.9/100,000 children and the prevalence was 86.3/100,000 children(67).

Incidence

A pooled worldwide incidence rate of 7.8/100,000, using a combination of juvenile arthritis definitions, was reported in 2014 in a systematic review by Thierry et al(68). To this date, there are several published incidence rates of JIA, reflecting the varying numbers across countries. In a prospective population-based study from the Nordic countries (Nordic study group), the incidence of JIA was 15 per 100,000 children/year, with varying numbers across countries, reporting 15 from Sweden(69). We report an incidence of 12.8/100,000 children in our south-Swedish cohort in Paper II, which will be more detailly described in the result section. Other reported incidence rates from our neighboring countries are 14/100,000 in Norway(70), 23 in Finland(71), and 24.1 in Denmark(72). Numbers from the rest of Europe vary from 3.1 in Alsace (France) to 21.7 in Estonia(73-76), with similar numbers of 10.3 in Minnesota (USA)(77), 11.9 in California (USA)(78) and 8.5 in Manitoba (Canada)(79). A trend of increasing incidence of chronic immune diseases, such as juvenile arthritis, was observed in Denmark over 35 years(24).

Prevalence

Reported prevalence of JIA range from 15 – 150/100,000 children(80) and the pooled world-wide prevalence rate of the ILAR definition of JIA was 30.0 in the review by Thierry et al., reflecting a lower prevalence in Asian and African countries(68, 81, 82).

Clinical features

JIA is an umbrella term that encompasses seven subtypes of the disease (Figure 4). The different subtypes represent versions of JIA with, to some extent, individual pathogenesis, clinical presentation, organ involvement and prognosis(14, 34). Arthritis is the cardinal feature, causing swelling of one or several joints leading to limited range of motion/mobility and various degrees of pain. The children can also experience fatigue, general malaise and fever depending on the extent of systemic inflammation (14).

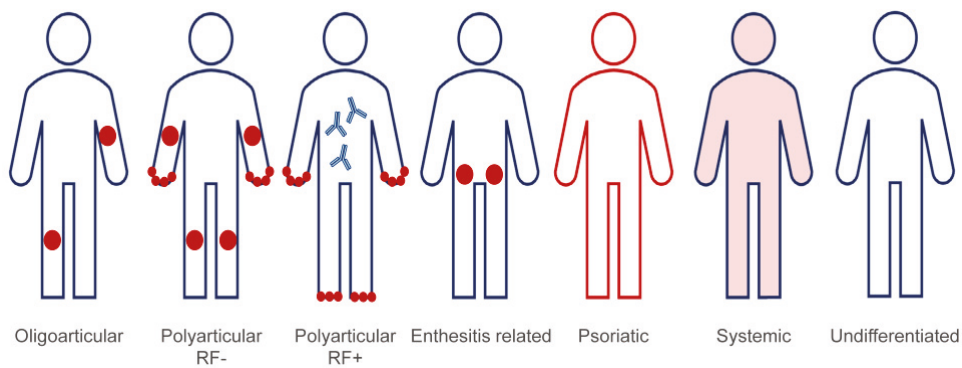


Figure 4. The subcategories of JIA(1). Picture by Arve-Butler, S(83). Red areas represents site of inflammation. Abbreviations: RF – rheumatoid factor

Systemic onset JIA

The subtype of sJIA differs distinctly from the others in the pathophysiological processes as well as in the clinical presentation. It is reported in 5-15% of JIA patients in northern Europe and North America(14, 84) and in up to 25-50% of children with JIA in Asia(18, 82). It is as common in males as in females and about half of the patients have a monocyclic or intermittent course with periods of remission(14). sJIA is an entirely clinical diagnosis with a presentation that can resemble bacterial or viral infection, malignancy, and other inflammatory conditions(85). The diagnosis requires arthritis together, or preceded, with fever for two weeks with a documented quotidian pattern for at least three days, and at least one of the following features: a typical evanescent salmon-pink rash, hepato/splenomegaly, generalized lymphadenopathy, or serositis. The arthritis often

develops later in disease course and presents as symmetric polyarthritis. Leukocytosis with neutrophilia, thrombocytosis, and increased erythrocyte sedimentation rate (ESR) and CRP values is often found in the laboratory investigation.

5-8% of the patients in this subtype develop a life-threatening complication of severe multisystem involvement named macrophage activation syndrome(14).

Oligoarthritis

Oligoarticular JIA is the most common subtype in Europe and America, affecting approximately 50 % of the children with JIA(14, 69, 86, 87). The term "oligo" refers to arthritis in few joints, more particularly fewer than five during the disease course. If not more than four joints are affected throughout the disease course, the subtype is named *persistent oligoarthritis*. However, in about 50% of the patients the arthritis extends to five or more joints after the first six months of disease and the subtype is then called *extended oligoarthritis*. Thus, if it takes more than six months from symptom onset to diagnosis it can be difficult to differentiate extended oligoarthritis from RF-negative polyarthritis(1, 87). Predictors of extended oligoarthritis are synovitis in the upper extremity and high ESR at diagnosis(84, 87).

Oligoarthritis is the only subtype specific for pediatric onset arthritis without an adult equivalent. Typical features are early disease onset before the age of six years, female predilection, high frequency of ANA (70-80%) and asymmetric arthritis in larger joints. In 30-50% only one joint is affected at disease onset(87), with the knees being the most frequently affected joints, followed by ankles(84, 88). The risk of JIA-associated uveitis is highest in this subtype, with reported numbers of 20-30%(14, 84).

Polyarthritis

When a child has five or more affected joints already during the first six months of disease, they belong to the polyarticular subtype. This group, that encompasses approximately 15-20% of JIA patients, is further divided depending on the presence of RF, into RF positive (RF+) polyarticular and RF negative (RF–) polyarticular JIA, with the latter being more common.

RF+ JIA is regarded as the pediatric variant of seropositive RA in adults. About 60% of patients have ACPA, possibly contributing to the pathogenesis of this subtype(89). RF+ JIA has female predominance with debut in adolescence and presents as symmetrical polyarthritis, often affecting the small joints of hands and feet(14, 89).

RF– JIA is a more heterogenous subtype with essentially three patterns of clinical presentation: one that resembles early-onset oligoarthritis but with an earlier extension of affected joints, one similar to adult-onset seronegative RA with symmetrical polyarthritis and negative ANA, and one known as "dry synovitis"

where stiffness, flexion contractures and arthralgia is more prominent than joint swelling(14). As approximately 50% of patients in this subtype are ANA-positive and hence share the feature of increased risk of iridocyclitis, it is argued that these patients should be considered as the same subtype as children with ANA-positive oligoarthritis(90).

Enthesitis related arthritis

ERA is the only subtype with male predominance and disease onset is usually after the age of six. ERA belongs to the group of spondyloarthropaties and accounts for about 5-10% of JIA cases in Western countries(14), even more common in parts of Asia(18). The heritability is estimated to more than 90%, the major genetic risk factor being HLA-B27(91) present in almost 90% of ERA patients(91, 92). The clinical presentation is characterized by arthritis and enthesitis. Arthritis usually affects larger joints of the lower extremity, including the hips, and the most common sites for enthesitis are the calcaneal insertion of the Achilles tendon, plantar fascia, and tarsal area. Arthritis can also progress to affect the sacroiliac and spinal joints and by that, resemble ankylosing spondylitis in adults. Acute iridocyclitis is the most common extra-articular manifestation and ERA can also occur with IBD(14).

Juvenile psoriatic arthritis

The frequency of juvenile psoriatic arthritis (JPsA) is reported as 2-11%. In JPsA there is a simultaneous presence of arthritis and psoriasis. However, since about 50% of the children lack the typical skin rash(93), they can still belong to this subtype if arthritis is accompanied with at least two of the features: psoriasis in a first-degree relative, nail pitting, or dactylitis (swelling of finger or toe)(1, 14, 94). More than 70% of children with JPsA have a family history of psoriasis(95). With two peaks in disease onset, the first before the age of six, there is a group of patients with disease course resembling early-onset oligoarthritis in this subtype as well. The other patients share features with spondyloarthritis/ERA, although more often with arthritis affecting both small and large joints(14).

Undifferentiated JIA

The subtype of undifferentiated arthritis (uJIA) encompasses patients who do not fulfill inclusion criteria for any category or who meet criteria for more than one, affecting 10-20%(14). Since patients with phenotypes from all the above-mentioned subtypes are included in the uJIA category, this is rather a subtype of academic than of clinical interest. In a study of patients from the Canadian ReACCh-Out cohort, the most common reason for inclusion in the uJIA category was family history of psoriasis (67%), followed by RF-positivity (21%)(96).

JIA-associated uveitis

The most common extra-articular complication in JIA is uveitis and JIA is one of the leading causes of uveitis in childhood. JIA-associated uveitis is a non-granulomatous, anterior inflammation affecting the iris and ciliary body (sometimes also named iridocyclitis)(56). The reported co-occurrence of uveitis in JIA varies from 2-20% depending on geographic region, being uncommon in Asian and African populations(97). The incidence of uveitis was 22.1% in the Nordic JIA cohort after 18 years of follow-up(98) and 10.8% in our south-Swedish cohort, as will be further discussed in the results section. The onset of uveitis occurs around the time of arthritis diagnosis in 50% of the patients(56, 99, 100). Most cases develop during the first five years of disease(14, 101), but uveitis can debut throughout the disease course(102).

JIA-associated uveitis can either be acute with pain and redness, or asymptomatic with insidious onset and is then often referred to as "chronic uveitis". Acute uveitis is associated with HLA-B27 positivity, hence mainly diagnosed in ERA patients. Chronic uveitis is however the most frequent form and affects both eyes in 70-80% of patients(56, 100). It is detected by slit-lamp biomicroscopy as the presence of inflammatory cells and increased protein concentration in the anterior chamber of the eye (Figure 5). Risk factors are age at JIA diagnosis (< seven years), female sex and ANA-positivity. Patients with oligoarthritis have the highest risk of chronic uveitis, while it is almost never found in sJIA or RF+ JIA. Headache, photophobia and changed vision may occur later in the disease course or if inflammation remains untreated(56). Complications such as synechiea (adhesions between the iris and anterior surface of the lens), cataracts and changes in intraocular pressure sometime leading to blindness, are common and increases with the duration of inflammation(102, 103). It is therefore important to discover the inflammation as soon as possible and many countries have screening programs with specified screening intervals based on age at diagnosis and presence of ANA(99, 104). Uveitis is primarily treated with local glucocorticoids and mydriatic agents, but disease-modifying anti-rheumatic drugs (DMARDs) can be used when failure to achieve uveitis remission. Since acute uveitis usually is diagnosed soon after onset, the prognosis of full recovery is good. The prognosis for chronic uveitis is variable and the inflammatory activity does not seem to be necessarily connected to the activity in the arthritis(56), although there are reports also showing temporal findings of arthritic and ophthalmic flares(105).

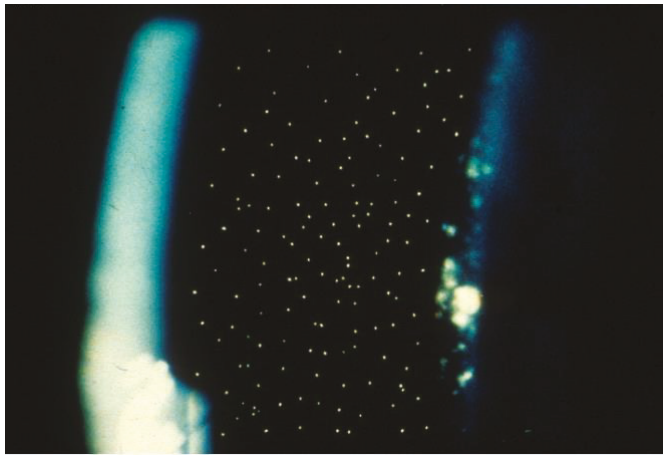


Figure 5. Chronic uveitis.
Inflammatory cells in the anterior chamber of the eye. (Photo by Elisabeth Bengtsson-Stigmar)

JIA - historically and today

Prior to the 1990s the general statement of prognosis for children with juvenile arthritis was that "80% can expect to be rid of inflammation when they reach adulthood(106)." However, there are several published studies on the subject of outcome in juvenile arthritis in the last 30 years that do not support the notion of JIA becoming less inflammatory with time. In a prospective Swedish cohort study of 133 prevalent JCA cases collected 1984 – 1988 with follow-up until the 16th birthday, approximately 50% of the patients had disease activity and needed pharmacologic treatment at follow-up. The need for orthopedic surgery during the relatively short study period was 5.6%(106, 107). In Denmark, 37% of JCA patients in a study of long-term functional outcome were reported to have active disease a mean 26.4 years after disease onset, more than 20% had undergone JCA-related surgery and at least 10% had significant functional difficulties(108). The risk of needing orthopedic joint surgery was 75% by 45 years of disease duration in a British retrospective study of 144 adult JIA patients(109). Probabilities of remission of less than 50%, 10 years after JCA onset, have been reported from Canada(110) and in a British study of 246 patients with average disease duration of 28.3 years, more than 40% had active arthritis and experienced severe disability(111).

Current treatment

The abovementioned results are based on studies of patients diagnosed in the 1970's and 80's. The pharmacologic treatment of JIA has evolved considerably since then. When first-line treatment with non-steroid anti-inflammatory drugs (NSAID) and intra-articular corticosteroid injections (IAC) are not enough to treat the symptoms of JIA, DMARDs are used as immunomodulatory treatment to reduce inflammatory

response and the damage to bone and cartilage. DMARDs are further divided into conventional synthetic (csDMARDs), targeted synthetic (tsDMARDs) and biological (bDMARDs)(14, 84, 112).

Methotrexate is the csDMARD most used for the treatment of JIA patients with persistent inflammatory activity and has been used in Sweden since the mid 1980's. It was originally developed as treatment for hematological malignancies. In rheumatic diseases it is used for its immunomodulatory qualities and is orally or subcutaneously administered in low dose regime once weekly. The molecular structure resembles that of folic acid, and it can therefore serve as a folic acid analog inhibiting different enzymes. Inhibition of tetrahydrofolate reductase blocks de novo purine synthesis eventually affecting DNA synthesis, with lymphocytes being especially sensitive to this effect since they lack an alternative pathway of purine synthesis. Inhibition of the enzyme aminoimidazole carboxamide ribonucleotide transformylase creates accumulation of extracellular adenosine that is thought to affect neutrophil adherence. Methotrexate also affects the production of different cytokines (TNF, IFNγ, IL-1, IL-6, and IL-8) and reduce the permeability of vascular endothelium(113, 114). Sulfasalazine, gold, and chloroquine phosphate are other examples of csDMARDs, mainly used in pediatric rheumatology prior to the introduction of methotrexate.

When the addition of methotrexate is still not sufficient to achieve disease control a bDMARD is used as supplement (or alone)(14, 84, 112). TNF-inhibitors were the first bDMARD introduced and has been used in Sweden since the start of the 21st century. They are either monoclonal antibodies (human or chimeric) to the TNF-receptor, or a human dimeric fusion protein of a part of the TNF-receptor linked to the Fc portion of human IgG1. TNF-inhibitors are administered as subcutaneous injection or intravenous infusion with dosing intervals from twice weekly to once every six or eight weeks depending on the mode of action for the drug(115). They have been proven to improve quality of life and reduce radiographic joint damage and complete remission can be achieved for 50% of the patients. TNF-inhibitors are still the most frequently prescribed bDMARD, but these drugs are designed to modulate a range of different aspects in the inflammatory pathway(14, 84, 112). Treatment options available for JIA today are monoclonal antibodies directed against the IL-6 receptor, monoclonal antibodies binding and neutralizing IL-17A, and a recombinant form of the IL-1-receptor inhibitor IL1-ra. There is also the option of inhibiting T-cell activation by the use of a human fusion protein comprising the extracellular domain of the receptor CTLA-4 and the Fc-portion of IgG1, inhibiting the co-stimulatory signal provided by the interaction of CTLA-4 and CD80/CD86 on the APC(113, 115). The newest addition to the treatment arsenal in JIA is the tsDMARDs. These are mainly JAK/STAT-inhibitors, that by inhibiting autophosphorylation of Janus kinase (JAK) in response to cytokine induced receptor activation, affect intracellular signal transduction (14, 84, 112, 115). However, despite all available pharmacologic treatment options, a

multidisciplinary approach with the cooperation of pediatric rheumatologists, ophthalmologists, orthopedic surgeons, nurses, physical therapists, occupational therapists, and psychologists is still needed and warranted for the care of JIA patients(14, 84).

JIA today

The prognosis of JIA is believed to have improved with the introduction of bDMARDs. A comparison of two cohorts with JIA diagnosis before and after 2000 (methotrexate vs biologic era) showed increased proportion of patients with inactive disease, as well as lower incidence of damage in the later cohort. However, more patients in the biologic era were treated with methotrexate and IACs, reflecting a more aggressive treatment approach today(116). Results from the ReACCh-out cohort, a Canadian cohort of children diagnosed with JIA 2005 – 10, showed that 72% of the children were in remission (25% with treatment) after a median follow-up of three years(117). In a German inception cohort, children with JIA were shown to experience the same health-related quality of life as healthy peers after three years of follow-up(118). However, there is a natural time-associated lack of long-term follow-up studies on children diagnosed with JIA in the 21st century and results on whether the new treatment options have contributed to adverse effects, such as malignancy, are conflicting(119-126).

Outcome

As described in the previous section, JIA continues into adulthood in around half of the patients. Although JIA is the most common rheumatic condition in childhood and effective treatment options have been available for several decades now, Beukelman et al stated in a systematic review of ongoing JIA registers and cohort studies in 2017, that "our understanding of long-term outcomes in terms of disease status, functional limitation, need for long-term immunosuppression as well as the development of comorbidities, remains relatively limited"(80). Pediatric rheumatologists today work with "treat-to-target" strategies, meaning frequent visits and treatment adjustments with the goal for the patient to achieve inactive disease as fast as possible, to avoid development of long-term consequences such as growth disturbances and damage to the growing skeleton. For evaluation of treatment effect there is a need for appropriate and validated outcome measures, combining subjective and objective health factors. However, what the state of inactive disease means for the future prognosis for the individual patient, is also still not clear and needs further research(80, 127).

Wallace criteria

Many outcome studies use the validated Wallace criteria as evaluation measure of inactive disease and clinical remission, defined by Wallace et al. in 2004(128).

Inactive disease:

- No joints with active arthritis
- No uveitis
- No systemic symptoms of inflammation (fever, rash, serositis, splenomegaly, or generalized lymphadenopathy)
- Normal ESR and/or CRP
- Physician's global assessment of disease activity indicating no disease activity

Clinical remission on medication: inactive disease for at least 6 continuous months on pharmacologic treatment.

Clinical remission off medication: inactive disease for at least 12 continuous months while off all pharmacologic treatment of the inflammatory disease.

The first study using the Wallace criteria retrospectively studied 437 patients with oligoarthritis, polyarthritis and sJIA with a follow-up time of at least four years. Inactive disease was achieved in 89% of patients with an episode lasting for a median of 12.7 months. Median time spent with inactive disease was 40% of the follow-up time. ¼ of the episodes resulted in clinical remission off medication, but only 6% of these episodes lasted longer than 5 years(129). The criteria have been further evaluated. For example, 1/3 of the patients achieved remission off medication and 75% were in sustained remission at 12 months follow-up in a study by Lurati et al (130), and 47.5±22.6% achieved inactive disease after mean follow-up of 6.5±1.5 years in a systematic review of outcome studies using the Wallace criteria(127). Patients in states of inactive disease or remission increases along with disease duration, with inactive disease being achieved in 33% of patients at 6 months and 67% at 8 years of disease in another systematic review of remission in JIA(131).

Other instruments of outcome measure

EULAR criteria of disease activity have been used for JCA, with disease status defined as:

- **Active**: increasing number of joints irrespective of drug therapy
- **Stable**: stable number of joint but requiring drug therapy
- **Inactive**: no evidence of active synovitis and/or active extra-articular features and without drugs for less than 2 years
- **Remission**: no evidence of active synovitis and/or active extra-articular features and without drugs for 2 years or more(132)

There is also a definition of remission in JIA by the American College of Rheumatology(132, 133). However, these sets of criteria are difficult to apply in the everyday clinic. Today the Juvenile Arthritis Disease Activity Score (JADAS) serves as a validated composite disease activity score often used for the estimation of disease activity and evaluation of treatment response, both in research settings and in the clinic(134-136). JADAS includes:

- Physician's global assessment of disease activity, measured on a 10-cm visual analog scale (0=no activity, 10=maximum activity)
- Parent/patient global assessment of well-being, measured as stated above
- Joint count with active disease (counted in 10, 27 or 71 joints)
- ESR

Short-term outcome

The prognosis/probability of reaching the ambition of inactive disease differs across JIA subtypes. Patients with persistent oligoarthritis and JPsA have the best prognosis of achieving remission off medication, while those with ERA and RF+ JIA have a poor prognosis of remission(86, 127, 129, 131). Factors shown to predict worse prognosis are high measures of disease activity at baseline, such as joint count, elevated ESR, symmetric arthritis and low health status on physician's and parent/patient global assessment scales(137, 138). Shorter time with active disease is a factor with positive prognostic value(139, 140).

There are few studies of short-term outcome in JIA. Inactive disease was achieved in 75% of the patients during the first 12 months of disease in a German inception cohort(140). In the Canadian ReAACh-out cohort, 91% of the patients were treated to an active joint count of 0 in a median time of 7 months. The probability of inactive disease within the first 2 years of disease ranged from 48% in patients with RF+ JIA to 91% in JPsA(86).

Long-term outcome

A study of disease progression into adulthood and predictors of long-term active disease in juvenile arthritis with a follow-up time of 30 years, wins the category of long-term outcome studies. 260 patients diagnosed 1980-85 were examined at a median of 15 years of disease and re-evaluated using questionnaires 23 years after disease onset. Patients indicating active disease at any of these time-points were invited for examination after 30 years. At this follow-up, 41% had active disease or were in remission on medication. HLA-DRB1*01, physician's global assessment of disease activity at 15-year follow-up and a short duration of total time in remission at 15 years were predictors of active disease or in need of medication after 30 years of disease(141).

Other studies with long-term follow-up report 40% of patients (JCA diagnosed 1984-88) being in remission according to EULAR after 17 years of follow-up(142) and 32.8% achieving remission off medication according to Wallace (JIA diagnosed 1997-2000) after 18 years of follow-up, 45.6% still having active disease(143).

Mortality

Mortality can also serve as a long-term outcome measure. In older studies, mortality of approximately 5% was reported(132), but today it is considerably lower. Previous results from Sweden have shown no mortality with only two deaths associated with JCA recorded in the entire country 1968 – 1986(107). There is evidence that mortality is increased in inflammatory diseases such as RA(144, 145) and IBD(146, 147). There are few studies investigating mortality in JIA, with conflicting results(148-153). A statistical measure often used for the presentation of mortality, is standardized mortality rate (SMR), representing the quota of observed and expected deaths in a cohort. The SMR for JCA was 3.4 in males and 5.1 in females in a Scottish study of cause-specific mortality in rheumatic diseases(149). SMR was also significantly elevated (2.8) for sJIA in a British follow-up study(151), confirming results from an older outcome study of the same subcategory(154).

JIA and comorbidity with other conditions

Autoimmune diseases

As previously written, since genetic factors contribute to the development of autoimmune diseases it is quite common for these diseases to cluster in certain families. Studies of co-occurrence of other autoimmune diagnoses in JIA patients have shown increased prevalence compared to patients with ADHD(155), but no difference compared to the general population(156). In diagnose specific studies, comorbid type one diabetes mellitus(157), hypothyroidism(158), and celiac disease(159, 160) has been reported as more common compared to the general population. However, data from Sweden do not motivate screening for celiac disease in children with JIA(161).

Cardiovascular diseases

There is a well-established increased risk of cardiovascular disease in RA comparable to the risk in type two diabetes mellitus. Chronic systemic inflammation and changes in physical function leading to development of other risk factors for atherosclerotic events are suggested as contributors to this risk(162, 163). There are few studies investigating the risk of cardiovascular comorbidities in JIA. In four

Norwegian studies of adult JIA patients no increased occurrence of cardiovascular disease events(164), no difference in carotid intima-media thickness(165), but increased blood pressure and altered arterial properties and haemodynamics(166, 167) was found compared to age- and sex matched controls. In an Indian study of 81 JIA patients aged 8-16 years the patients had increased lipid levels and ultrasonic arterial changes suggestive of early cardiovascular dysfunction compared to sex- and age matched controls(168). JIA patients also reported more cardiovascular events and at an earlier age compared to responders without arthritis, in a survey by the American centers for disease control(169).

Depression and anxiety

Children with a chronic disease are more likely to develop emotional comorbidities than the general population, possibly because the disease serves as a stressor as the child or adolescent must cope with managing the disease alongside the normal bodily and behavioral changes growing up(170, 171). Swedish children with the chronic, autoimmune disorders childhood-onset IBD, type one diabetes mellitus and celiac disease have significantly increased risk for mood-disorders and anxiety compared to siblings as well as the general population(172-174). Studies on the risk of psychiatric comorbidities in JIA report conflicting results, but JIA patients are shown to develop depression and anxiety at least to the same extent as in other pediatric chronic diseases(175-177). Pain and fatigue, common symptoms in an inflammatory flare, are shown to negatively affect quality of life(111, 175) and can be aggravated by co-occurrent depressive symptoms(178). In addition, in inflammatory remitting diseases such as JIA and IBD there is the constant threat of a flare even in periods of remission, adding further stress. In studies investigating predictors of depression and/or anxiety in JIA patients, inflammatory activity is not the major factor associated with symptoms of mood-disorders(111, 175, 177, 179). A hypothesis as to why depression and anxiety negatively contributes to pain and disability in JIA is that depressive symptoms such as fatigue and lack of initiative/motivation lowers compliance to medication and physiotherapy, causing increased disease activity and pain. Pain reduces the will and ability of physical activity, causing a vicious circle of reduced physical performance, more disability, and more pain, further aggravated by the social avoidant behaviour that comes with depression and anxiety. The biological processes, described in the following section, with reduced levels of monoamines in the central nervous system (CNS) also cause increased pain (Figure 6)(177, 178).

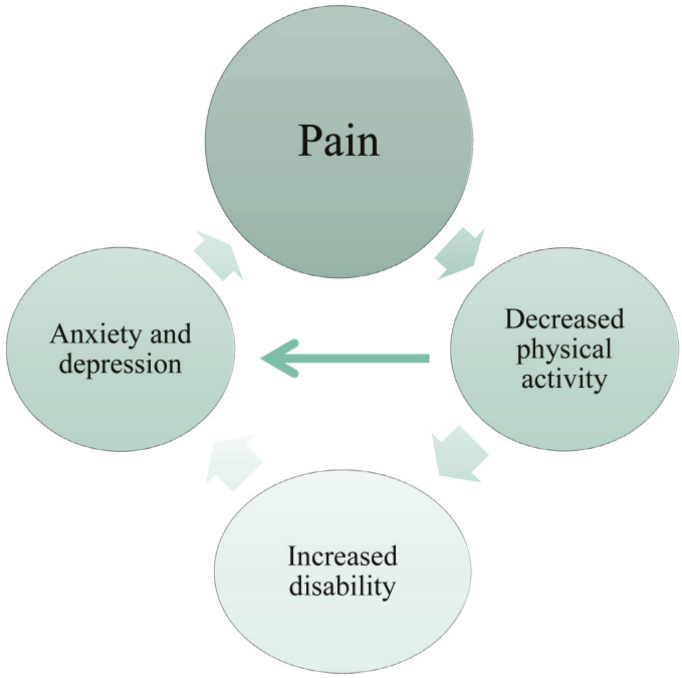


Figure 6. Visicious circle of pain and disability contributing to mental illness. Adapted from Hanns et al, 2018(177).

Inflammation in depression and anxiety

Convincing evidence from preclinical and clinical studies associating inflammation to major depressive disorders are increasing, for example do many studies show that depressed patients have increased concentrations of inflammatory markers such as CRP in blood and cerebrospinal fluid(180-182). However, inflammation is not specific for depression and not all depressed patients have measures of inflammation. There are current suggestions of inflammation being an important contributor to the development of mood disorders and that there is a subset of patients with inflammatory depression important to distinguish to personalize treatment(181, 182).

Evolutionary perspective

The causes of chronic inflammation in depression are not fully understood. In a stressful situation, the secreted stressors activate inflammatory pathways in circulating mononuclear cells, resulting in increased levels of pro-inflammatory cytokines. There is an evolutionary perspective that stress relevant neurocircuitry and immunity form a system to protect the human from environmental threats. The ancient human needed a balance in pro- and anti-inflammatory systems for the ability of wound healing and fighting infections, simultaneously with preparing for defense against enemies. This balance is believed to have been maintained by

constant exposure to tolerogenic organisms. In our sanitized environment today, we lack immunoregulatory events, suggested to lead to a dysregulation of immune responses. This together with modern environmental factors such as increased psychological stress, is believed to contribute to the high comorbidity of depression and autoimmune and inflammatory disorders(182). Evidence for this theory are that known risk alleles for depression also have pro-inflammatory and pathogen protective effects(183), environmental risk factors for depression such as obesity and psychological stress are pro-inflammatory, and stimulation with pro-inflammatory cytokines produce symptoms such as reduced appetite and irritability that overlap with symptoms of depression and anxiety. There are also results from a laboratory setting using psychological stressors showing that the subjects with the strongest inflammatory response were more probable to develop depression over the following months(182).

Pathophysiological mechanisms

The pro-inflammatory cytokines exert their biological effect on the CNS through different mechanisms, affecting for example neuroendocrine, monoaminergic, and oxidative stress systems. They can pass the blood-brain barrier and activate the hypothalamic-pituary-adrenal axis(182). The cytokines can also modify serotonergic and glutaminergic circuits through the effect on the metabolism of tryptophan (a precursor of serotonin). Tryptophane is metabolized in the kynurenine pathway in parallel with serotonin synthesis. Pro-inflammatory cytokines, such as IFNγ and TNFα, activate the enzyme indoleamine 2,3-dioxygenase (IDO) that converts tryptophane to kynurenine and, by that, decrease the synthesis of serotonin contributing to depression pathophysiology. The kynurenine pathway in microglia generates quinolonic acid that can act neurotoxic by increasing reactive oxygen species, further increase levels of chemotactic molecules, and stimulate an overproduction of glutamate, toxic to the CNS. This is believed to be the mechanism of the common depressive side-effects in IFNα treatment for diseases such as chronic hepatitis B and C, and Bechet's syndrome(181, 183). Cytokines have also been shown to decrease the release of dopamine in basal ganglia, a brain region that regulate motivation and motor activity, leading to symptoms of depression and anxiety(182). Levels of IL-6, TNFα and IL-10 significantly decrease with anti-depressive treatment of major depressive disorders(184) and there are also studies proving effect of anti-inflammatory treatment on depressive symptoms(185). However, the effects are only present in depressed patients with signs of peripheral inflammation, adding proof to inflammation being a contributing cause of depression and anxiety in a subgroup of patients, rather than the whole explanation.

Aims of the present investigation

The studies presented in this thesis aim to explore different aspects of the questions asked by every child and parent at the diagnosis of JIA: “Why did I get this disease?”, “What will happen to me now?” and “Will I ever get well?”. The specific aims, addressed in the four studies of the present investigation, are:

I. To study the pathophysiological role of monocytes, from the blood stream to the activated form in synovial fluid and tissue, by mapping their polarization states and investigate their function in oligoarticular JIA.

II. To investigate the epidemiology of JIA by creating a population-based cohort of validated cases of JIA diagnosed in Skåne 2002 – 2010 and to use this cohort for the study of outcome during a possible follow-up period of 13 years.

III. To expand the cohort from Paper II by creating an additional cohort of patients diagnosed in the same region 1980-2001 and study the incidence and mortality of JIA over a period of 31 years.

IV. To investigate if patients with JIA have an increased lifetime risk of developing depression and anxiety compared to controls without JIA.

Methods and patients

Methods used in Paper I

Paper I is an experimental study of the role of monocytes in oligoarticular JIA pathogenesis. The two major methods used in this paper will briefly be explained in the following section. The precise methodology of the study is described in the supplemented manuscript.

Flow cytometry

Flow cytometry is a method used for the analysis of cells and particles in a solution using laser. The most common application used is immunophenotyping, which provides the ability to simultaneously analyze mixed populations of cells for multiple parameters(186). The cells are labeled with fluorescent markers, usually antibodies directed against the target of interest (surface proteins, DNA, intracellular structures), and injected into the instrument where they are focused into a fluid stream passing through a series of lasers. The light scattered from the cells is measured in two directions, forward which gives a relative estimation of the cell's size, and at 90° (side) which indicates the heterogeneity or granularity of the cell. After excitation by each laser, the fluorophore-labeled cells or particles will emit light of specific wavelength where the amount of light emitted will be interpreted and reported as relative fluorescence intensity(187). Fluorescence activated cell sorting (FACS) is a type of flow cytometer used for sorting and collection of specific cell populations by the measure of physical features (186).

In Paper I, antibodies against the surface markers CD14 and CD16 were used for the detection of monocytes; CD3, CD19, and CD56 for the exclusion of other cells; and CD40, CD86, CD163 and CD206 as measures of activity and polarization state. For further investigation of polarization, *in vitro* defined surface markers were used(188) and monocytes from healthy donors were simultaneously stimulated with a cytokine cocktail as control for the induction of specific phenotypes: IFNγ and lipopolysaccharide (LPS) for M1(IFNγ), IL-4 and IL-13 for M2(IL-4), and IL-10 and Dexamethasone for M2(IL-10).

Reverse-transcription polymerase chain reaction

Polymerase chain reaction (PCR) is a method used for the amplification of a targeted DNA sequence. Quantitative PCR (qPCR) is a development of the technique, used for simultaneous determination of the amount of amplified product, especially useful for the analysis of gene expression. qPCR is more sensitive than conventional PCR but the concentration of the amplified product requires comparison with that of "housekeeping genes", genes crucial for cellular function that are not drastically changed by environmental factors(189, 190). The result of the amplification is presented as cycle time and the quantification can be measured by the use of fluorescent primers or dyes interacting with DNA, or by gel electrophoresis(189). The qPCR variant reverse-transcription PCR was used in Paper I, where mRNA was extracted and reverted to cDNA before amplification, since this method allows detection of gene expression in small numbers of specific cells(190).

Patient selection

In total, 16 patients with oligoarticular JIA undergoing a planned treatment procedure with IAC injection 2016 – 2019 at the Department of Pediatrics, Section for Pediatric Rheumatology, were included in the study. Paired samples of blood and synovial fluid, all aspirated from knees, were collected from 13 patients. Synovial biopsies were taken from three patients, where joint fluid only was used for two of them. Two patients were later excluded from the analysis due to misclassification. The patients were either newly diagnosed or had a flare after a period of remission off medication for at least six months, thus were without treatment except for NSAID.

Healthcare in the Skåne region

The Skåne region is the southernmost part of Sweden. The region has, after the Stockholm region, the highest population density in the country(191) with 1.4 million inhabitants in 2021, 13% of the Swedish population. Children 0-15 years constitutes about 1/5 of the total population(192).

Sweden has a publicly financed healthcare system and care is subsidized for all children until at least 18 years of age, with a standardized preventive healthcare program throughout childhood. The center for pediatric rheumatology in Skåne is located at the University hospital in Lund, but JIA patients can be treated at other pediatric outpatient facilities, public as well as private, in the region.

Registers at the National board for health and welfare

Swedish citizens are given a unique twelve-digit personal identification number that allows linkages between healthcare and administrative registers. The National Board of Health and Welfare (NBWH) is a government agency with a task to provide and maintain official statistics on, for example, medicine and causes of death. The agency provides registers such as "The national patient register" with information on healthcare visits, "The cause of death register" with information on deaths, and different statistical databases with one with registered International classification of diseases (ICD)-codes from inpatient and specialized outpatient care(193).

Skåne healthcare register

Since 1998, all visits at public as well as private healthcare providers in Skåne are registered with date and diagnosis code according to ICD-10 at the Skåne Healthcare Register (SHR). Registration is mandatory for reimbursement purposes. At start only inpatient care was registered, but since 2004 all outpatient visits are included and appointments with caregivers other than physicians are also available since 2004(194).

For the study of comorbidity with depression and anxiety in Paper IV we attained ICD-codes F32 (depressive disorder), F33 (recurring depression), F34.1 (dysthymia) and F41 (anxiety) according to ICD-10 registered in SHR for the patients in the cohort. Five controls with at least one registered healthcare visit (not JIA) in SHR, matched for sex, year of birth and residential region were selected for every JIA patient. A search for the above-mentioned ICD-codes was made for the control population as well. We defined one diagnosis from inpatient care or diagnosis at two separate outpatient visits as a plausible diagnosis.

Design of the south-Swedish JIA cohort

For the study of epidemiology, prognosis, and different aspects of outcome in a disease, the use of a population-based cohort is suitable since it includes all severities of the disease and an assumption of equal exposure to environmental factors can be made. In the Skåne region, we have had the advantage of the care for JIA patients having been centralized to Lund and since almost all patients 30 years ago needed hospital-based rehabilitation and inpatient care at treatment start, the local patient register at that time is believed to have had good regional coverage.

Case assessment

For the search of patients, the ICD-codes: 696.00, 712, 713.10-19 and 714.93 (ICD-8); 696A, 713B and 714 (ICD-9), and M08-M09 (ICD-10) were used. The diagnosis codes could have been registered as primary or secondary diagnosis. For as full regional coverage as possible, a search was made both in the clinical database at the local hospital register and at the diagnosis register at NBHW (Figure 7). Individuals included in the search were aged 0–18 years to secure any referred children with the diagnosis made at another healthcare facility. The registration of ICD-codes from outpatient visits in the diagnosis register was not mandatory until 2002. Thus, there is a potential risk of us having missed to include JIA patients with mild disease diagnosed before 2002 with only outpatient visits at health care facilities other than the Lund University hospital. A patient was included in the cohort if considered diagnosed correctly with JIA before the 16th birthday between 1 January 1980 and 31 December 2010 while living in Skåne.

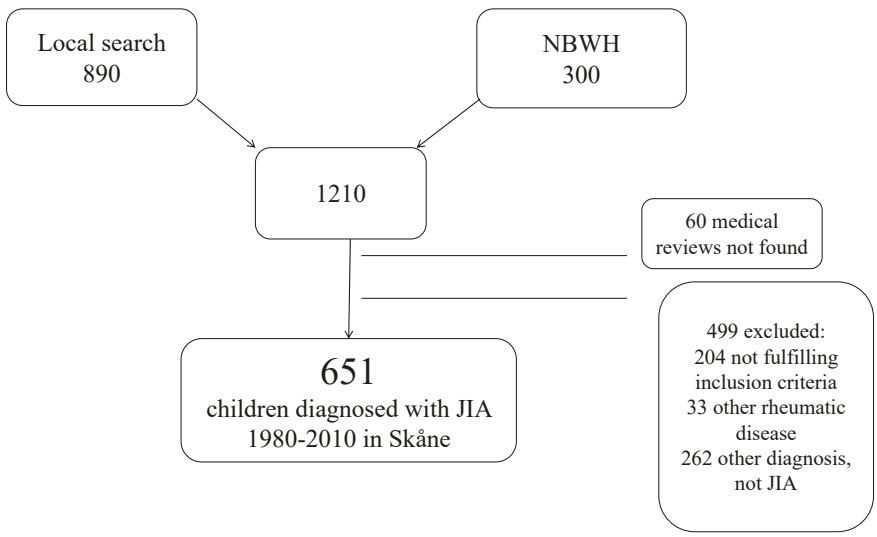


Figure 7. Case collection process with reasons for exclusion.
Abbreviations: NBWH – National Board for Health and Welfare

Validation and data collection

For the cases collected from NBHW medical records were reviewed to ensure correct diagnosis. Medical records were reviewed also for the cases from the local search, but a patient was included if diagnosed with a definite pediatric arthritic disease with symptom duration according to the criteria used at the time of diagnosis (EULAR or ILAR) by a rheumatologist. During 1980 – 2001, the majority of cases

was classified into subcategories according to the EULAR JCA definition. At study inclusion the diagnostic subgroup was adapted to the ILAR definition.

Patients were followed until migration, death, loss to follow-up or end of study period on 31 December 2015, whichever occurred first. Data collected from the medical reviews was: gender; age at diagnosis; symptom debut; diagnosis date; heredity in first-degree relative for rheumatic disease, psoriasis, IBD or non-granulomatous uveitis when clearly stated; presence of uveitis; immunological data of the presence of ANA, RF, ACPA, and HLA-B27; mean annual values of hemoglobin, white blood cell count, platelet count, ESR, and CRP; mean annual height and weight; orthopedic surgical procedures; swollen and tender joint count registered as the total number of affected joints in the 66/68 joint count that year; and annual prescribed pharmacologic treatment. (The forms used for registration are presented as supplementary material.)

Statistical analysis

Numerical data and observations in each study group were presented with descriptive statistics such as frequencies, percentages, median, and interquartile range (IQR) ($Q_1 – Q_3$). Since the data was continuous in Paper I and non-normally distributed in Paper IV Mann-Whitney U-test was used for the comparison of paired samples of blood and synovial fluid in Paper I and of median age at diagnosis in Paper IV. A p-value of < 0.05 was considered significant.

Incidence rate and mortality rate were calculated with the purpose of describing different epidemiological aspects of JIA in Skåne. Incidence rate was calculated using the number of incident cases as numerator and the pediatric population in Skåne at risk as denominator. For the comparison of incidence rates in Paper III, cases were summarized by decade due to the limited number and rate ratio was calculated with the cases diagnosed 1980 – 89 as comparator. Mortality rate was calculated using the deceased as numerator and the number in the cohort at risk the corresponding year as denominator.

For the analysis of time-to-event, Kaplan-Meier curves were generated for chronic uveitis and joint corrective surgery in Paper II and conditional cox proportional hazard regression models were used for calculation of hazard ratios (HR) in Paper IV.

Statistical analyses were performed in Prism 7 for Paper I, IBM® SPSS® version 25 and 27 for Macintosh for Papers II-IV, openepi.com(195) for the calculation of 95% confidence interval (CI) of the incidence rate, and incidence rate ratios in Paper II-IV, and in the R environment for the calculation of HR in Paper IV.

Ethical considerations

The four studies were carried out in compliance with the declaration of Helsinki and were approved by the Regional Ethical Review Board for southern Sweden (Paper I: Dnr 2016/128 and 2017/473; Paper II-IV: Dnr 2013/192 and 2015/62) and the National Ethical Review Agency (2020-02935) for Paper III and IV.

For Paper I, informed written consent was obtained from patients when deemed appropriate considering the child's age and ability to understand the information, otherwise from patient's guardians. Both blood- and synovial fluid samples were collected during planned and necessary IAC injections, thus not adding additional discomfort for the patient. Since IAC often is administered with the younger patients (< 10 years) under general anesthesia, the synovial biopsies were able to be collected using ultrasound guided technique during these procedures to reduce pain and stress.

Results and discussion

Paper I: Children with oligoarticular JIA have skewed synovial monocyte polarization pattern with functional impairment – a distinct inflammatory pattern for oligoarticular juvenile arthritis

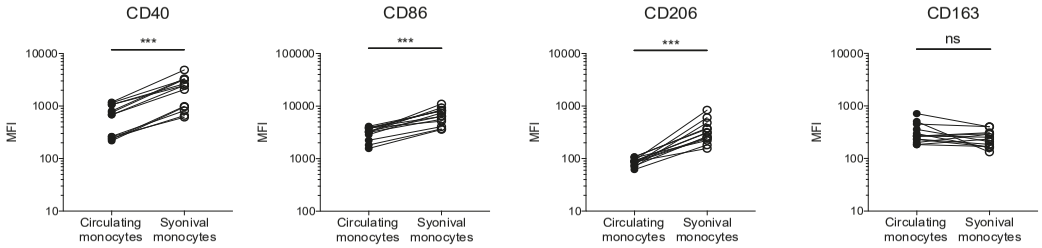
As previously described, the aim of this experimental study was to investigate the pathophysiological role of monocytes in blood and the synovial environment by mapping their polarization states and investigate their function in oligoarticular JIA. For this experiment, paired samples of blood and synovial fluid from 13 patients and three synovial biopsies were collected.

Surface and mRNA-expression

With the use of flow cytometry, a decreased frequency of classical $CD14^{+}CD16^{-}$ monocytes and an increased frequency of intermediate $CD14^{+}CD16^{+}$ monocytes were detected in synovial fluid compared to blood. The synovial fluid monocytes had increased expression of the M1 markers CD40 and CD86, as well as the M2 marker CD206, compared to circulating monocytes (Figure 8A). The polarization patterns of synovial monocytes were then further analyzed at the mRNA level for 28 polarization-related genes using qPCR and were also at this level found to express increased levels of both M1- and M2-related markers compared to circulating monocytes.

A

Surface marker expression on patient monocytes in synovial fluid and blood.



B

Surface marker expression on monocytes from healthy donors, polarized with plasma or synovial fluid.

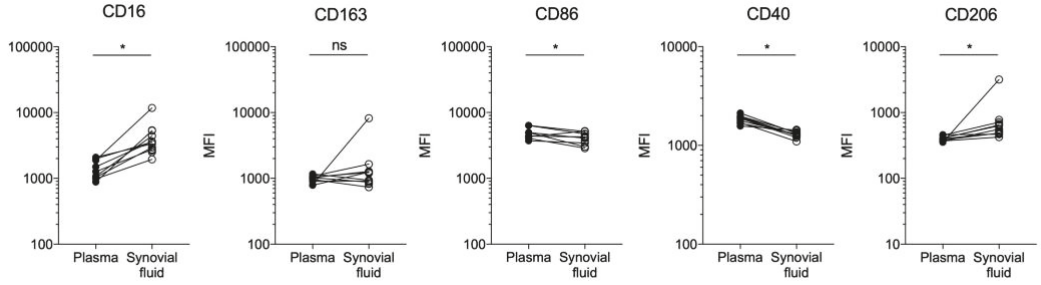


Figure 8. Surface expression of polarization markers. Asterix indicate significant difference (* - p <0.05, *** - p <0.001). A) Monocytes in synovial fluid expressed increased levels of CD40, CD86 and CD206 compared to circulating monocytes. B) Monocytes from healthy donors upregulated CD16 and CD206, but downregulated CD40 and CD86 when polarized with patient synovial fluid compared to with plasma.
Abbreviations: MFI – median fluorescense intensity

The role of the synovial fluid

The polarization pattern is believed to result from local environmental effects. *In vitro* cultured monocytes from healthy donors were polarized using synovial fluid (n=13) or plasma (n=9) from JIA patients and analyzed with flow cytometry. Monocytes polarized with synovial fluid upregulated the typical M2 surface markers CD16 and CD206 but downregulated the M1 markers CD40 and CD86 compared to plasma polarized monocytes (Figure 8B). Synovial fluid did not induce the production of TNF, IL-6, IL-8, or IL-10 in monocytes from healthy donors, compared to those stimulated with plasma. In the patients' synovial fluid, there were measurable concentrations of the cytokines IL-1β, IL-6, IL-8, and IL-10 but not of traditional cytokines used to induce M1(IFNγ) and M2(IL-4). From these experiments we concluded that the synovial fluid not alone was responsible for the specific polarization pattern.

Functional assessment

To investigate if the monocytes were affected at a functional level, the methods PhagoTest™, where phagocytosis is stimulated using opsonized *E. coli* bacteria, and PhagoBurst™, where production of reactive oxygen species is stimulated using phorbol-myristate-acetate (PMA), were used. Analyses were made with flow cytometry. The synovial fluid monocytes showed a reduced ability to phagocytize, and they had decreased ability to undergo oxidative burst compared to circulating monocytes.

Monocytes in synovial tissue

Finally, macrophages in synovial tissue from three patients were examined. Using immunohistochemistry, macrophages were found in both the lining and sub-lining region in all patients. The biopsies were stained for cytokine mRNA using *in situ* hybridization and mRNA for IL-10 (anti-inflammatory) and TNF (pro-inflammatory) was seen in the lining and sub-lining region. The biopsies were also stained for polarization markers and both CD40 (M1) and CD206 (M2) could be found using immunofluorescence.

The conclusion from these experiments is that monocytes in the synovial fluid of patients with oligoarticular JIA have a mixed M1/M2 polarization pattern unique for this JIA subtype.

Discussion

This is the first published study of monocyte polarization in oligoarticular JIA. Polarization alters the cells' capacities and how they affect their environment, believed to contribute to pathogenesis in rheumatic diseases. Stimulation of monocytes with synovial fluid from spondyloarthritis patients induced a M2-like pattern compared to RA patients that induced a M1-like pattern(46). A M2(IL-10)-like pattern in synovial monocytes have been demonstrated in ERA(51) and monocytes with mixed expression of CD206/CD163 has been found in soJIA(52). The polarization pattern found in our study of oligoarticular JIA is, at least partly, distinct from what is described in other arthritides. The fact that similar pattern was found in all children, regardless of disease duration, and that all experiments failed to show a clear M1- or M2-like pattern supports the conclusion that this is a unique polarization pattern for oligoarticular JIA.

The investigation of macrophages in synovial tissue was a result of the finding that the milieu in the synovial fluid not alone could explain the polarization. We hypothesized that monocytes might attain their polarization pattern and get activated through migration from the blood to the synovial tissue. The identification of monocytes in both layers of the synovia, the finding of both TNF and IL-10 mRNA, and the expression of CD40 as well as CD206 (single- as well as co-expression) on the macrophages support the idea that polarization can occur in the synovial tissue as well.

The main limitation to this study is the small sample size, especially of the biopsies, and larger studies are needed to elude the role of the tissue-resident macrophages in the pathogenesis of JIA.

Paper II and III: Outcome in JIA: a population-based study from Sweden, and Increasing incidence of JIA: a trend over 31 years in southern Sweden

For the rest of this thesis, a cohort of all individuals diagnosed with juvenile arthritis 1980 – 2010 while living in Skåne was established. For the study of outcome in Paper II, we wanted as complete coverage as possible and the possibility of subtype classification according to ILAR, and thus limited the patient selection to the 251 children diagnosed 2002 – 2010. For Paper III we used the total cohort of 651 individuals.

Incidence

In Paper II, the mean annual incidence rate over the study period was 12.8 (95% CI 11.3 – 14.5) per 100,000 children < 16 years. The incidence for females was 17.5 (15.0 – 20.4) and 8.3 (6.7 – 10.3) for males. There was a clear incidence peak at the age of two years, mainly driven by girls, mirroring the typical preschool age debut of oligoarthritis (Figure 9).

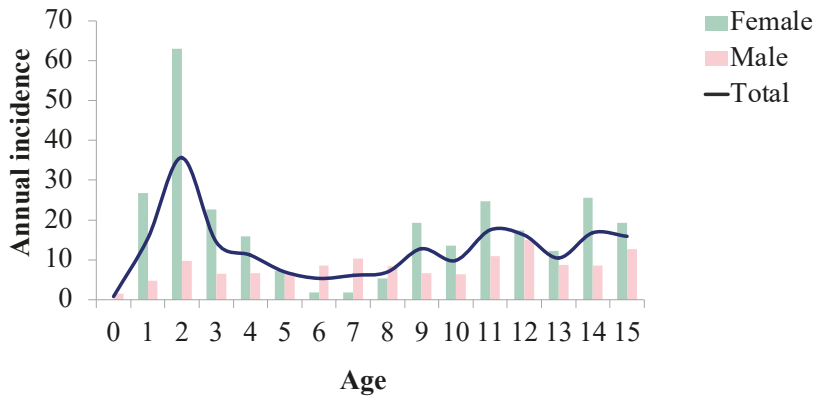


Figure 9. Mean annual incidence per 100,000 children < 16 years. The bars represents the age specific incidence divided by sex, with the line representing the incidence in the total group.

Increasing annual incidence from 4.2 (2.1 – 7.8) per 100,000 children in 1980 to 17.1 (12.3 – 23.3) in 2010 was demonstrated in Paper III (Figure 10). The mean annual incidence over this period was 9.9 (9.1 – 10.7). The incidence rate ratio was statistically significant when comparing the rate in 1980 – 89 to that in 1990 – 99 (1.4 (1.2 – 1.8)) and 2000 – 10 (1.9 (1.6 – 2.3)).

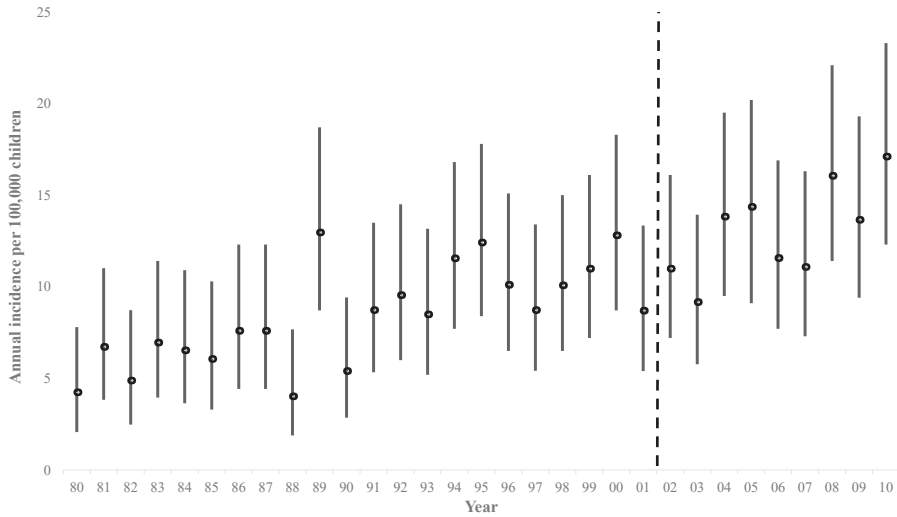


Figure 10. Annual incidence rate (dots) and 95% CI (vertical lines) 1980 – 2010. The scattered line shows the division of the cohort from Paper II (2002 – 2010) and the cases added 1980 – 2001 for Paper III.

Demographics of the south-Swedish JIA cohort

The cohort had female predominance (67.0%) except for in the ERA group where the majority of patients were males. The median age at diagnosis was 8.8 (IQR 3.7 – 12.8) years, with older ages in the ERA-, JPsA-, and RF+ groups. The median age was consistently higher when looking at results from the total cohort compared to the results in Paper II (7.3 (2.3 – 11.5)). The median follow-up time was 8.2 years (Table 1). Subtype distribution is presented in figure 11.

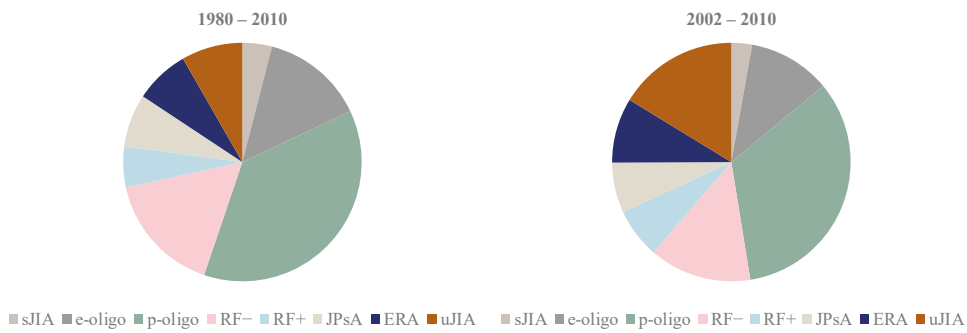


Figure 11. The percentage of patients with the different subtypes according to ILAR criteria. The left figure respresents the total cohort and the right figure shows the patients diagnosed 2002 – 2010.
Abbreviations: sJIA – systemic onset JIA, e-oligo – extended oligoarthritis, p-oligo – persistent oligoarthritis, RF– – RF negative polyarthritis, RF+ – RF positive polyarthritis, JPsA – juvenile psoriatic arthritis, ERA – enthesitis related arthritis, uJIA – undifferentiated arthritis

ANA was found in 53.9% of the patients and 11.5% were RF positive at least on one occasion. csDMARD had been prescribed to 53.8% of the cohort (64.9% in 2002 – 2010) with methotrexate as the most common option, and bDMARDs to 18.0% (23.9% in 2002 – 2010) (Table 1).

Table 1. Immunological data, treatment options, and prevalence of uveitis in the cohort of 651 JIA patients diagnosed 1980 – 2010, divided by subtype.

	Total (651)	sJIA (26)	E-oligo (91)	P-oligo (242)	RF− (107)	RF+ (36)	JPsA (47)	ERA (48)	uJIA (54)
Female (%)	67.0	53.8	70.3	67.8	74.8	77.8	72.3	37.5	63.0
Age at diagnosis (years)*	8.8 (3.7-12.7) (n 644)	4.3 (2.9-8.9) (n 26)	5.9 (2.6-11.5) (n 90)	7.0 (3.3-11.1) (n 240)	8.7 (3.7-12.8) (n 107)	14.2 (11.6-15.4) (n 36)	12.5 (8.1-14.4) (n 47)	12.2 (10.6-14.5) (n 45)	6.7 (3.0-12.1) (n 53)
Follow-up time (years)*	8.2 (5.1-12.3) (n 634)	11.2 (4.6-15.4) (n 26)	12.1 (7.4-16.2) (n 90)	6.8 (3.5-10.7) (n 235)	9.8 (5.8-13.3) (n 106)	8.5 (5.9-15.2) (n 36)	7.8 (5.0-11.0) (n 46)	6.8 (4.8-10.9) (n 44)	8.8 (6.3-12.0) (n 51)
ANA (%)	53.9 (93.7)	14.3 (80.8)	64.0 (96.7)	60.6 (94.2)	63.1 (96.3)	48.6 (97.2)	28.6 (89.4)	21.4 (87.5)	54.9 (94.4)
RF (%)	11.5 (76.3)	0 (61.5)	8.0 (82.4)	5.7 (72.7)	0 (73.8)	97.1 (97.2)	5.9 (72.3)	2.9 (72.9)	8.9 (83.3)
ACPA (%)	9.1 (33.8)	0 (15.4)	10.3 (31.9)	0 (28.9)	2.6 (35.5)	61.9 (58.3)	0 (31.9)	10.5 (39.6)	4.2 (44.4)
HLA-B27 (%)	33.3 (44.2)	0 (7.8)	21.3 (51.6)	22.1 (35.5)	24.0 (46.7)	11.1 (25.0)	15.4 (55.3)	88.9 (93.8)	43.5 (42.6)
Oral glucocorticoids	291 (44.7)	23 (88.5)	55 (60.4)	39 (16.1)	68 (63.6)	30 (83.3)	22 (46.8)	11 (50)	28 (51.9)
csDMARDs	350 (53.8)	17 (65.4)	73 (80.2)	55 (22.7)	78 (72.9)	36 (100)	24 (51.1)	17 (77.3)	36 (66.7)
Methotrexate	271 (41.6)	14 (53.8)	58 (63.7)	39 (16.1)	61 (57.0)	28 (77.8)	22 (46.8)	15 (68.1)	29 (53.7)
bDMARDs	118 (18.1)	6 (23.1)	22 (24.2)	4 (1.7)	29 (27.1)	19 (52.8)	10 (21.3)	7 (31.8)	17 (31.5)
TNF-inhibitor	117 (18.0)	6 (23.1)	22 (24.2)	4 (1.7)	29 (27.1)	19 (52.8)	10 (21.3)	4 (18.2)	16 (29.6)
Chronic uveitis (%)	54 (8.3)	0	17 (18.7)	18 (7.4)	12 (11.2)	0	0	2 (4.2)	5 (9.3)
Acute uveitis (%)	49 (7.5)	0	11 (12.1)	12 (5.0)	6 (5.6)	0	2 (4.3)	9 (18.8)	9 (16.7)

Treatment options are presented as the percentage of patients in the subgroups with at least one treatment year. * - median with IQR.

Abbreviations: sJIA – systemic onset JIA, e-oligo – extended oligoarthritis, p-oligo – persistent oligoarthritis, RF – rheumatoid factor, RF– – RF negative polyarthritis, RF+ – RF positive polyarthritis, JPsA – juvenile psoriatic arthritis, ERA – enthesitis related arthritis, uJIA – undifferentiated arthritis, ANA – antinuclear antibodies, ACPA – anti-citrullinated protein/peptide antibodies, DMARD – disease-modifying anti-rheumatic drug, csDMARD – conventional synthetic DMARD, bDMARD – biological DMARD, TNF – tumor necrosis factor

Outcome

Short-term outcome – inactive disease

Annual disease activity was studied in Paper II. Inactive disease was defined as a year with no registered arthritis or uveitis and years when no pharmacologic treatment was prescribed were also registered. The diagnosis year was excluded from the analysis. In total, 40.0% of the follow-up years were with inactive disease and the sJIA group was the only group with > 50% of the follow-up time spent with inactive disease. 28.8% of the follow-up years were without pharmacologic treatment (Figure 12).

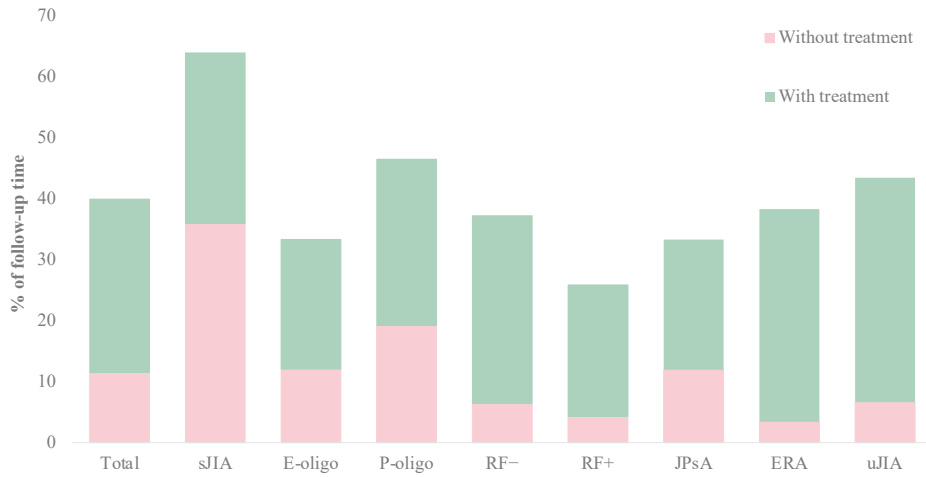


Figure 12. The percentage of follow-up time with inactive disease (no arthritis or uveitis) divided by subtype. The pink sections of the bars represent follow-up years with inactive disease without pharmacologic treatment.
Abbreviations: sJIA – systemic onset JIA, E-oligo – extended oligoarthritis, P-oligo – persistent oligoarthritis, RF– – RF negative polyarthritis, RF+ – RF positive polyarthritis, JPsA – juvenile psoriatic arthritis, ERA – enthesitis related arthritis, uJIA – undifferentiated arthritis

Long-term outcome – uveitis, orthopedic surgery, mortality

The prevalence of uveitis in the cohort is presented in detail in table 1. JIA-associated uveitis was diagnosed in 14.0 % of the total cohort (10.8% in 2002 – 2010), with 8.3% as chronic and 7.5% as acute. There were no patients with chronic uveitis in the sJIA-, RF+-, or JPsA groups. The risk for chronic uveitis was calculated in Paper II to 10.0% after 12 years of disease.

In Paper II, the need of joint corrective surgery was also registered. Serious orthopedic procedures such as arthrodesis, osteotomy and arthroplasty were performed only on 4.4% of the patients, but 9.2% had to undergo some joint corrective surgical procedure. This was needed for ¼ of the patients in the RF+ group, while no patient with sJIA needed orthopedic surgery. The risk of needing

joint corrective surgery was 17.9% after 12 years of disease (9.4% for the more serious procedures).

Mortality was studied in Paper III and information on causes of death between 1 January 1980 and 31 December 2019 was obtained. Eight patients had died during the study period, where one death was interpreted as complication related to JIA. Other causes were suicide, traumatic accidents, infections, and diabetes. Mortality rate is presented in table 2.

Table 2. Mortality rate

	JIA-cohort			General population		
Year of death (age range (years))	**Deaths (n)**	**Cohort (n)**	**Proportion (%)**	**Deaths (n)**	**General population (n)**	**Proportion (%)**
1987 (0-20)	1	276	0.004	155	266786	0.0006
1992 (0-25)	1	407	0.002	171	353198	0.0005
1999 (0-32)	1	525	0.002	164	458800	0.0004
2004 (0-35)	1	568	0.002	220	545891	0.0004
2005 (0-38)	1	570	0.002	217	564696	0.0004
2010 (2-43)	1	581	0.002	253	656610	0.0004
2015 (7-48)	1	556	0.002	320	688246	0.0005
2016 (8-49)	1	546	0.002	356	701015	0.0005

The proportion represents the quota of deaths and individuals at risk in the cohort, and the general populations in the corresponding age range, respectively.

Discussion

The south-Swedish JIA cohort is to our knowledge the only published retrospectively validated population-based cohort. It has minimal selection bias and patients of all disease severities has been included. As many as 20% of the initial cases were excluded in the case collection process, where 60% of these were patients misdiagnosed with JIA. This number was higher than expected. A similar finding was reported by Harrold, et al, where review of a random sample of medical records led to an exclusion of 31%(78).

There are limitations to our study. In Paper III we discuss the fact that we only have total coverage for inpatient visits before 2002. Since the majority of patients in the 1980's and partly also in the 90's was admitted to inpatient care at diagnosis, treatment start and/or periods of intense rehabilitation, we argued that the low number of cases during the first half of the study period only to a small part can be explained by missing cases with our search method. The increasing incidence might instead reflect the increased general awareness among colleagues of other specialties, of the importance of early treatment of pediatric rheumatic diseases to

prevent permanent joint damage. Our results are also supported by a similar trend recently reported from Denmark(24).

A retrospective study design comes with the challenge of having to work with already stated facts; you do not have the opportunity of asking for or adding complementary information. The ILAR classification system is meticulous with distinct criteria for exclusion, where for example RF positivity is defined as the presence of RF on at least two occasions at least three months apart. The work in the everyday clinic does not necessarily proceed in line with criteria partly written for research purposes, and this is even more evident when it comes to the documentation of findings in the patients' medical review. For this reason, we have used the presence of RF on one occasion as an inclusion criterion for patients who otherwise met the criteria for polyarticular disease, but not as an exclusion criterion in patients with manifestation of oligoarticular disease. Hereditary information was used as exclusion criterion when clearly stated. However, the subtype distribution in the part of the cohort diagnosed 2002 – 2010 is similar to that in the prospectively collected Canadian ReAACh-out cohort(86), as well as in the Nordic study group(69). There are differences in the subtype distribution between the total cohort and the group in Paper II, partly explained by the fact that the majority of the patients with diagnosis before 2001 were originally diagnosed using the EULAR criteria. The uJIA group is underestimated, with an assumed corresponding overestimation of the oligoarticular- and RF– group that can explain the relatively large difference in median age at diagnosis in these groups compared to the selected cohort in Paper II. There may however also be a historical perspective to the increased median age at diagnosis, where we believe that referral of a patient with synovitis to a pediatric rheumatologist early in the disease course, is more established today than it was 30 years ago.

The historical perspective is also evident in the increasing number of patients that are prescribed DMARDs throughout the study period, more clearly demonstrated in Paper IV were treatment is presented with the cohort divided by decade of diagnosis (table 1 included in the manuscript). Since the pharmacologic treatment has been registered annually over the follow-up period, even the patients diagnosed in the 1980's have had the possibility to receive bDMARDs. A decrease of the use of cDMARDs was observed in the part of the cohort diagnosed 1990 – 99, compared to the other decades. This finding might be explained by the fact that the use of other DMARDs than methotrexate became more uncommon during this period due to the superior anti-inflammatory and joint protective effect of methotrexate(14).

The retrospective nature of our study is also reflected in the need for us to define inactive disease with non-validated criteria. Blood samples are not generally collected from JIA patients, unless they are treated with DMARDs or have a flare where infection needs to be ruled out, and we therefore had a large amount of follow-up years lacking laboratory information that could indicate disease activity.

We did also not have access to estimated values of "Physician's global assessment of disease activity" needed for definition according to the Wallace criteria(128), or "Patient/parent global assessment of well-being" needed for calculation of JADAS score(134-136). The EULAR criteria do not include laboratory data(132), but are only validated for JCA.

We interpret the outcome results from Paper II, where all patients were diagnosed in the era of bDMARDs and tight disease control, as a long-term positive improvement with fewer patients diagnosed with uveitis and a decreased need for joint corrective surgery than previously published data, but that there still are short-term challenges with active disease more than half of the follow-up time. The long-term outcome of mortality is in all interpreted as low, even though the relative mortality rate indicates an increase. With only eight mortalities, and the majority having died from the same causes as in the age-corresponding general population, our results must be interpreted with great caution. We need longer follow-up time and a larger cohort to elucidate if JIA contributes to a change in mortality compared to healthy individuals.

Paper IV: JIA does not increase the risk of depression and anxiety – results from the south-Swedish JIA cohort

For the study of risk of depression and anxiety in Paper IV, ICD-codes for depression and anxiety registered between 1 January 1998 and 31 December 2019 at the SHR were obtained for 640 of the JIA patients and 3200 age- and sex matched controls from the Skåne population. Eleven of the individuals in the JIA cohort did not have a registered healthcare visit in Skåne during the study period and were excluded from the cohort for this study.

Depression and anxiety in JIA

During the study period 14.5% of the JIA individuals were diagnosed with a depressive disorder compared to 14.8% of the controls. Median age at depression was 23.5 years in the JIA cohort and 24.1 years in the controls ($p = 0.4586$ with Mann-Whitney U-test).

ICD-codes for anxiety disorder were registered for 17.3% of the JIA individuals and for 17.4% of the controls. Median age at anxiety was 23.6 years in the JIA cohort and 23.7 years in the controls ($p = 0.5413$ with Mann-Whitney U-test).

Risk of depression and anxiety in JIA

HR was calculated using conditional cox proportional hazard regression models with days from JIA diagnosis/cohort inclusion as time variable. Individuals diagnosed with depression (n=1 for JIA and n=5 for controls) and anxiety (n=2 for JIA and n=8 for controls) prior to onset of JIA were excluded from the analysis. Information on date of JIA diagnosis was missing in five JIA cases and 25 controls, why these individuals also were excluded from the analysis.

HR for depression was 1.1 (95% CI 0.9 – 1.5) for females and 0.8 (0.5 – 1.4) for males. HR for anxiety was 1.2 (0.9 – 1.5) for females and 0.6 (0.4 – 1.1) for males. Further analyses of JIA subgroups with ANA-positive disease with onset before the age of six years, treatment with csDMARDs, and treatment with any DMARD compared to matched controls, did not result in any statistically significant HR (Figure 13). The analyses were carried out for the selection of patients and controls included since 1 January 1998 and a significant decreased HR 0.4 (0.1 – 0.9) for anxiety in males with JIA was found in the analysis of the total cohort, but in none of the other analyses.

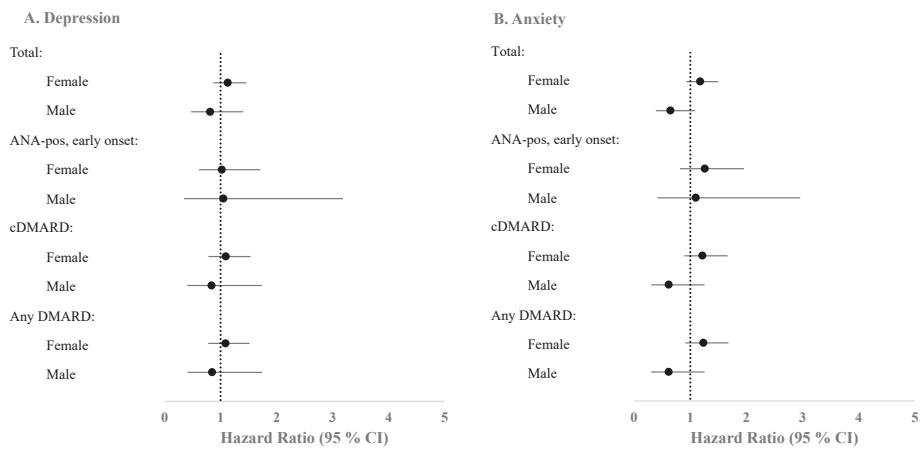


Figure 13. Hazard ratios for depression and anxiety in JIA patients compared to age- and sex matched controls without JIA. No statistical significant difference was found.

Discussion

There are previous studies of the risk of emotional comorbidities in JIA showing that the risk is similar to that in other chronic pediatric diseases(175, 176). Depression and anxiety is common in the general population with approximately 10% of Swedish citizens having been diagnosed with a depressive and/or anxiety disorder in 2019(196). The high frequency is reflected among the JIA patients in our study, but also among the controls. One could assume that, with all the possible

negative factors for mental health that come with a disease such as JIA described in the introduction, individuals with JIA would be diagnosed at an earlier age. Median age at diagnosis did however also not differ between the patients and controls. There might in fact be protective factors in the care of chronic diseases, such as frequent healthcare visits with the opportunity of early detection of symptoms indicating stress or mental malaise, and the team centered care including psychological support, that is recommended in the treatment plan for JIA patients. These factors may also result in fewer registered diagnoses of mental illness since symptoms of depression or anxiety might be handled as natural consequences of the "crisis" that a diagnosis with a chronic disease can cause.

This study is, to our knowledge, the largest study with longitudinal follow-up of psychiatric comorbidities in JIA. The main limitation is the lack of diagnosis data before 1998. This factor may result in an overestimation of incident cases since we did not exclude all individuals with comorbid diagnosis before study inclusion, but on the other hand to an underestimation due to missing information. The regression analysis with the selection of JIA cases and controls included 1998 – 2010 did however not change the results, except for decreased HR for anxiety in JIA males. The clinical relevance of this finding is uncertain, since the cases were few, the CI was wide and just below the significance level.

Conclusions

In reference to the aims of this thesis I would like to conclude that:

- Monocytes in the synovial environment of children with oligoarticular JIA express a mixed M1(IFNγ)/M2(IL-4) polarization pattern, unique for this subtype.
- Synovial fluid monocytes have decreased ability to phagocytize and respond with oxidative burst upon stimulation compared to circulating monocytes, suggestive of a specific role for the monocytes in the pathogenesis of oligoarticular JIA.
- In children diagnosed in the era of biologic treatment and strategies to diminish inflammatory activity in order to prevent damage, still more than 50% of the follow-up years includes symptoms of active disease.
- In terms of long-term outcome, fewer individuals are diagnosed with uveitis and the need for joint corrective surgery is lower than previously published results. JIA does also not seem to contribute to excess mortality.
- The annual incidence rate 1980 – 2010 of JIA in Skåne is 9.9/100,000 children < 16 years, with significantly increasing annual incidence over the study period.
- Individuals with JIA do not appear to be diagnosed with depression or anxiety at an earlier age or more often than age corresponding controls without JIA.

Future perspectives

I would like to mention some of the reflections made and questions raised during this process, that I think should be further explored in the future:

- Does the functional impairment of synovial monocytes, with decreased ability to phagocytize and respond with production of reactive oxygen species, contribute to inflammation in oligoarticular JIA?
- Tissue-resident macrophages are known to be important in the development of synovitis. The role and the polarization pattern of these cells in oligoarticular JIA should be further investigated in a larger sample size.
- The south-Swedish JIA cohort should be continuously complemented with new cases, as well as with follow-up time. Increased follow-up time is needed to study mortality and risk of other complications that usually develops at an older age, for example cardiovascular events and malignancy.
- A larger sample size is needed for the study of effect of treatment. Does treatment with bDMARDs change the risk of developing comorbidities?
- Finally, the very important question "Will I ever get well?" has not been fully answered. It is not known if just the fact of having had rheumatic inflammation during childhood has consequences in adulthood. Therefore, I rather talk about the chances of being symptom free than "getting well" when asked about prognosis. A follow-up study of adult JIA patients is needed to get an answer to the question: has the change of treatment in the last two decades improved the prognosis of having inactive disease as adults for children diagnosed with JIA today?

Populärvetenskaplig sammanfattning

Juvenil idiopatisk artrit (JIA) är den vanligaste reumatiska sjukdomen hos barn och kallas i folkmun för barnreumatism. I Sverige diagnosticeras mellan 200 - 250 barn varje år med JIA. Sjukdomen innebär att man insjuknar före 16 års ålder och har ihållande ledinflammation under minst sex veckor som det inte hittas någon annan förklaring till, som till exempel en skelettskada eller infektion. Det finns sju olika varianter av JIA som delvis skiljer sig åt gällande symtombild, prognos och uppkomstmekanism. Den vanligaste varianten är fåledsartrit, oligoartikulär JIA, vilken oftast debuterar hos flickor i förskoleåldern.

JIA är en autoimmun sjukdom, vilket innebär att immunförsvaret av någon anledning har reagerat och börjat angripa kroppens egen vävnad. Vid JIA är det främst ledhinnor som drabbas och blir inflammerade. Det kan också bli inflammation i andra organ, där ögats främre hinnor (uveit) är det vanligaste utanför lederna. I forskningen kring hur immunförsvaret fungerar vid JIA har man mest fokuserat på de vita blodceller som tillverkar antikroppar och som går till direkt attack på andra celler. Det är mindre känt vilken roll cellvarianten monocyter har. Monocyterna anses kunna påverkas av miljön de befinner sig i och profilera sig (polarisera) mot att antingen bidra till pro-inflammatoriska/drivande eller anti-inflammatoriska/skyddande processer vid inflammation.

JIA betraktas som en kronisk sjukdom, men från uppföljningsstudier gjorda på barn som fått diagnos på 1980- och 90-talet verkar det som att bara ungefär hälften fortsätter att ha aktiv sjukdom med symtom även som vuxna. Från äldre studier vet vi också att cirka 1/5 får reumatisk ögoninflammation och att det är hög risk för skador i de drabbade lederna och det lednära skelettet. Under de senaste 20 åren har behandlingen av JIA tydligt förändrats. Idag sätts läkemedel för att bromsa aktiviteten i immunförsvaret in i ett så tidigt skede som möjligt, och sedan 20 år finns så kallade biologiska läkemedel. Dessa läkemedel är specifikt inriktade på olika inflammatoriska processer vid reumatisk sjukdom och kan på så vis effektivt släcka inflammation. På kort sikt har biologiska läkemedel visats vara effektiva och den vetenskapliga hypotesen är att dagens barn med JIA bör drabbas av färre komplikationer än tidigare. Av tidsmässiga skäl saknas studier med lång uppföljningstid på barn som diagnosticerats med JIA sedan biologiska läkemedel funnits tillgängliga.

Vid andra kroniska barnsjukdomar har det visats finnas en ökad risk att också diagnosticeras med depression och ångest. Studier på samsjuklighet med psykiatriska sjukdomar vid JIA har inte visat lika tydliga resultat. Smärta och trötthet, som är vanliga symtom vid JIA, kan bidra till sämre livskvalitet. JIA kan dessutom komma och gå i skov, så även en bra dag kan det finnas oro för att det imorgon kan vara dåligt. Att ha en sjukdom som aktiviteter måste anpassas efter och att behöva ta mediciner, kan också bidra till att livet blir extra jobbigt i perioder.

När JIA konstateras hos ett barn ställs i princip alltid frågorna: ”Varför har jag/vårt barn fått denna sjukdom?”, ”Vad kommer att hända med mig nu?” och ”Kommer jag bli frisk?”. Syftet med denna avhandling har varit att belysa olika aspekter av dessa frågor – från monocytens/makrofagens aktivitet och funktion i immunförsvaret vid fåledsengagerande (oligoartikulär) JIA, till hur det ser ut med sjukdomsaktivitet, komplikationer, depression och ångest, och mortalitet (dödlighet) vid JIA.

I det första delarbetet användes vävnadsprover av ledhinna, samt blod och ledvätska från 13 barn med oligoartikulär JIA. Monocyterna i ledvätska hade en enhetlig profilering som skiljer sig från profilen vid andra reumatiska sjukdomar. Monocyterna i ledvätska hade sämre förmåga att städa undan skadliga element och döda celler än vad de hade i blodet. Resultaten ger stöd åt att oligoartikulär JIA är en egen sjukdom med unik uppkomstmekanism och inte bara en barnvariant av reumatisk sjukdom hos vuxna.

Till resterande tre delarbeten skapades en kohort (patientgrupp) av alla som i Skåne fått diagnosen JIA mellan 1980 – 2010. I gruppen som fått diagnos 2002 – 2010 hade 2/3 fått behandling med läkemedel som påverkar immunförsvaret, men trots det hade personerna i kohorten inflammation i leder eller ögon under mer än hälften av uppföljningstiden. Däremot utvecklade färre personer komplikationerna reumatisk ögoninflammation och operationskrävande ledskada än vad som tidigare publicerats. JIA verkar heller inte bidra till överdödlighet, med förbehåll för att antalet dödsfall i kohorten var så lågt att det inte kunde jämföras med dödligheten i den allmänna befolkningen. JIA har blivit vanligare i Skåne sedan 1980, vilket bekräftar rapporter om ökad förekomst av andra autoimmuna sjukdomar och allergi i dagens samhälle. Slutligen var depression och ångest vanligt i kohorten, men vi hittade ingen ökad risk för de med JIA att få dessa diagnoser jämfört med de köns- och åldersmatchade kontrollerna utan JIA och heller ingen skillnad på åldern när sådana diagnoser har ställts.

Det unika med kohorten som skapats för avhandlingen är att alla diagnoser är bekräftade och att den spänner över ett enhetligt geografiskt område där alla sjukdomsfall, oavsett allvarlighetsgrad, har inkluderats. Kohorten kommer kunna fortsätta att växa både i storlek och uppföljningstid, och användas för att undersöka olika möjliga konsekvenser av att leva med JIA, samt att svara på frågan om hur många som blir av med symtomen medan de är barn.

Acknowledgements

This thesis would not have been possible without the work and support from many people. I would especially like to express my gratitude to

My main supervisor, **Robin Kahn**. Already the first time I met you I was enthused by your passion for research. Your optimism has been invaluable to me throughout this project. Your positive spirit has convinced me to engage and find joy in experimental projects and to keep the discipline to proceed with the medical record review, even though it turned out to be somewhat of a "Sisyphos project". You have supported me, bribed me with cake and gently pushed me to become the physician and researcher I am today, and for that I am forever grateful.

My co-supervisor and clinical hero **Bengt Månsson**. Thank you for sensing in 2014 that pediatric rheumatology and this project would suit me and that you by that convinced Robin, who did not know me at all, to take me on as a PhD student. Your enormous clinical knowledge and your warm and empathic way of meeting the patients and their families inspires me. I also thank you for humorous sharing of anecdotes and "life wisdoms".

My co-supervisor and professor **Anders Bengtsson**. Thank you for initially taking me on as a PhD student, although completely unexperienced in the lab environment. I am thankful that you have encouraged me to spend time in the lab and by that widen my interest to the field of translational research.

Heads of the Rheumatology department during my time as PhD student, **Jehns Martinéus** and **Jon Einarson**. Thank you for promoting research alongside with clinical work and for creating an environment where this has been possible.

Anki Mossberg and **Gittan Gullstrand**. Thank you for your endless patience with learning me how to handle lab equipment and understand the experiments, as well as always answering my often very basic questions on immunology. You are amazing in cheering on and give praise in a way that can change any possible doubts into a feeling of being invincible.

My fellow PhD-student colleagues **Sabine Arve-Butler, Tobias Schmidt,** and **Alma Dahlberg**. Thank you for cooperation in our different projects, for generously lending me your top-class power point slides and for just being there sharing the successes and disappointments that comes with this position. A special thanks to you, Sabine and Tobias, for making immunology comprehensible during our journal

clubs, and to you, Alma, for your enthusiastic way of committing to and continuing the work with the cohort.

Fredrik Kahn, thank you for excellent guidance in the statistical field and for prioritizing finding the best solution to our risk calculations in Paper IV, even though you have been swamped with other tasks.

The rest of the colleagues in the Kahn/Bengtsson lab group, thank you for interesting discussions and fun times during our meetings and outings. I love how you all talk about/to the cells as your friends and colleagues - that has made immunology more concrete to me. A special thanks to Helena for co-authorship in paper III, I look forward to our future research cooperation.

My colleagues at the Section for Pediatric Rheumatology: **Elisabet Åstrand, Michele Compagno** and **Petra Król**. Thank you for your dedication in our patients and for covering for me during my weeks of research. I would also like to thank all the present and former wonderful co-workers in the Pediatric Rheumatology team. Thank you for welcoming me to your unit and for always cheering me on. I look forward to now increase my time with you and the patients.

My clinical supervisor, **Aladdin Mohammad**. Thank you for caring for my development as a clinician and for the advice to sometimes prioritize time for research even at times when I have been overwhelmed with clinical related responsibilities.

All other **colleagues** at the **Skåne Rheumatology Department**. Thank you for an interesting and inspiring work environment. A special thanks to my room mates Kristofer and Johanna, for inspiration, encouragement and interesting private as well as professional discussions.

My mentor **Naomi Clyne**. Thank you for listening and caring for my professional, as well as personal development, and for excellent advice on how to mount the process of writing the thesis. I have really appreciated our regular lunches.

Professor emeritus **Tore Saxne**. Thank you for raising my interest in rheumatology and for arranging my master thesis at your department. I appreciate how you have kept track of my project "from the court side" and your calm and comforting way of offering advice.

Pierre Geborek, former colleague, and the original mind behind the south-Swedish JIA cohort. Thank you for starting up and believing in the possibility and importance of this project.

Anna Jöud, thank you for helping me with the connection to SHR, for co-authorship in Paper IV, and for guiding me through the process of writing permit applications.

Minna Willim, data manager. The south-Swedish JIA cohort would not have been possible to establish or keep without your expertise. Thank you for all the thought effort and time you put in "connecting the dots" to sort out the initial cases and create functioning data files.

Jan-Åke Nilsson, statistician, thank you for having explained statistics in an understandable and pedagogic way, for teaching me SPSS, and for always knowing how to solve (or advising me to surrender to) my problems concerning statistical analyses.

Sofie Eklund, research administrator, thank you for your help with understanding the ALF system, your quick response, and good eye for layout.

All patients and parents accepting to participate in our ongoing studies.

The Pettersson family - my mother Kristin, thank you for giving me the joy of learning, for your constant support and for your mantra "du kan inte göra mer än ditt bästa" that I still turn to at nervous times – **my sisters Stina** and **Lotta**, thank you for being the true older sisters that you are, always cheering me on and providing life perspectives when I need to step out of my tunnel.

My husband **Anton**, for technical support, for googling how to help me with my Apple products, your skills in excel, for interesting questions that forces me to explain my work in understandable words, for knowing when to give me space, and for your loyal support. Thank you will never be enough!

Och slutligen **mina (omslags)pojkar, Theodor och Edvard**, tack för att ni dagligen visar mig vad som är viktigt i livet.

Supplementary material

The three forms used for data registration from the medical records are supplemented below.

1. Form for case ascertainment and general information

Id för personen (Pid)			
Personnummer			
Given name			
Sur name			
Date of birth			
Yes= male sex	0 = kvinna	1 = man	
Certain JIA diagnosis	0 = nej	1 = ja	
Uncertain JIA diagnosis	0 = nej	1 = ja	
Other certain diagnosis than JIA	0 = nej	1 = ja	
JIA diagnosis unclassifiable due to missing data regarding within 6 months of disease onset	0 = nej	1 = ja	
Not JIA	0 = nej	1 = ja	
Comment on diagnosis and classification			
Disease onset according to medical record			
Disease onset when established by physician			
Disease onset when established by specialist			
Physicians diagnosis (ILAR: se lista)			
Specialists diagnosis (ILAR: se lista)			
Classificators diagnosis (ILAR: se lista)			
Physicians diagnosis date			
Specialist diagnosis date			
Classificators diagnosis date			
Extended oligo Yes	0 = nej	1 = ja	999 = uppgift saknas
Date of certain extended oligoarthritis			
Classificators ID/Date of classification			
Date of death			
Comment on death			
Registrator ID/Registration date			
Last follow up date medical records			
Maximal Follow up time med records (years)			
Chronic uveitis ever (0 = no, 1 = yes, 999 = no information)	0 = nej	1 = ja	999 = uppgift saknas
Date of confirmed Chronic Uveitis diagnosis			
Acute uveitis ever (0 = no, 1 = yes, 999 = no information)	0 = nej	1 = ja	999 = uppgift saknas
Amyloidosis (0 = no, 1 = yes, 999 = no information)	0 = nej	1 = ja	999 = uppgift saknas
Date of confirmed amyloidosis diagnosis			
End joint failure (spontaneous ankylosis, arthrodesis, prosthesis			
ANA (1 = yes, 0 = no, 999 = uppgift saknas)	0 = nej	1 = ja	999 = uppgift saknas
RF (1 = yes, 0 = no, 999 = uppgift saknas)	0 = nej	1 = ja	999 = uppgift saknas
Anti-CCP (1 = yes, 0 = no, 999 = uppgift saknas)	0 = nej	1 = ja	999 = uppgift saknas
HLAB27 (pos = yes, neg = no, 999 = uppgift saknas)	0 = nej	1 = ja	999 = uppgift saknas

2. Form for annual registration of clinical data

Pid	CRPmv	SRmv	CRPantal	SRantal	Hbmv	Vitamv	Trombmv	SwollenJC	Tender JC	StiffJC	Hip_affection	Shoulder_affection	TM_affection	ThLumbColumna_affe	CervColumna_affectio	SI_affection	CHAQmv	Lengthmv	Weightmv	BMI	AcuteUveitis	ChronicUveitis	Amyloidosis	Op	OpEyes	OpDental	OpCulumna	ArtrodesNr	ProtesNr	OpKnee	OpHip	OpElbow	OpShoulder	OpWrist	OpnKle	OpFinger	OpSubtalus	OpToes	OpSynovetomy	OpSoftTissue	Kommentar
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3. Form for annual registration of pharmacologic treatment

Pid	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	Kommentar
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2 MTX																																					
3 SSZ																																					
4 AMA																																					
5 AZA																																					
6 Adalimumab																																					
7 Anakinra																																					
8 CellCept																																					
9 Ciklosporin																																					
10 Cyklofosfamid																																					
11 Etanercept																																					
12 Infliximab																																					
13 Ivlg																																					
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16 Myocrisin																																					
17 Penicillamin																																					
18 Podofylotoxin																																					
19 Ridaura																																					
20 Talidomid																																					
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24 Steroidinject																																					
25 Steroidinf																																					
26 Certolizumab																																					
27 Golimumab																																					
28 Abatacept																																					
29 Tocilizumab																																					
30 Rituximab																																					
31 Kanakinumab																																					
32 Ustekinumab																																					

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