Juvenile Idiopathic Arthritis: Towards Improving Clinical Care

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Juvenile Idiopathic Arthritis: Towards Improving Clinical Care

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Voor Carmen, Louise, Joost en mijn ouders

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General introduction

JUVENILE IDIOPATHIC ARTHRITIS

Juvenile idiopathic arthritis (JIA) is the collective name of diseases characterized by chronic arthritis commencing in the pediatric age. The first descriptions of the disease dates back to as far as 1864-1900[1]. Initial organized attempts to classify the disease gave rise to the 1973 juvenile rheumatoid arthritis (JRA) criteria[2], hampered by difficulty of establishing criteria for a condition including more than one entity. In the years to follow important landmark meetings were held both in Europe and the United States: a new pediatric specialty was born. A new classification of Juvenile Chronic arthritis (JCA) by the European League against Rheumatism[3] was adopted in 1978. The two sets of criteria differed, hindering to compare research data overseas. A new consensus classification was introduced[4] which is currently still in use(see table 1). The umbrella term JIA is defined as any arthritis of unknown etiology with onset before the age of 16 years old and lasts for at least 6 weeks. Each category is mutually exclusive. In recent years systemic JIA has been recognized as an auto-inflammatory disease. This thesis focusses on patients with oligo-arthritis, polyarthritis and JIA with psoriasis.

The treatment of JIA consists of medical interventions and supportive care. In this thesis we focus on the medical treatment, supportive care is addressed in chapter 2.

Table 1 | International League of Associations for Rheumatology classification criteria for juvenile idiopathic arthritis in childhood

ILAR category	Characteristics	% of total
Systemic JIA	Arthritis and daily fevers ≥3 days, accompanied by at least one of the following: evanescent erythematous rash, generalised lymph node enlargement, hepato-/splenomegaly, serositis.	4-17
Oligoarticular Persistent Extended	Arthritis in 1-4 joints during the first 6 months of disease Arthritis in 1-4 joints through the entire disease course Arthritis in 5 of more joints after 6 months of disease	27-60 40 20
Polyarticular JIA RF+	Arthritis in 5 or more joints in the first 6 months of disease, at least two positive tests for rheumatoid factor at least 3 months apart.	2-7
Polyarticulair JIA RF-	Arthritis in 5 or more joints in the first 6 months of disease, negative tests for rheumatoid factor.	11-30
Psoriatic JIA	Arthritis and psoriasis, or arthritis and at least 2 of the following: dactylitis, nailpitting or onycholysis, psoriasis in a first degree relative.	2-11
Enthesitis related Arthritis	Arthritis and enthesitis, or arthritis or enthesitis with at least 2 of the following: sacroiliac joint tenderness or inflammatory lumbosacral pain, HLA B27 antigen positive, onset in a boy> 8 years old, HLA B27 associated disease* in a first degree relative.	1-11
Undifferentiated JIA	Arthritis that does not fulfil criteria of one of the categories or meets criteria of more than 1 category.	11-21

*Ankylosing spondylitis, enthesitis related arthritis, sacro-iliitis with inflammatory bowel disease, Reiter's syndrome, or acute uveitis.

Antirheumatic drugs

Several drugs have been introduced for the medical treatment of patients with juvenile idiopathic arthritis. An overview of the most commonly used antirheumatic drugs is given here.

Medical treatment

Medical treatment for juvenile idiopathic arthritis was historically aiming at symptom relief and consisted after aspirin of non-steroidal anti-inflammatory drugs (NSAIDs) which became available in the seventees[5]. The first slow-acting antirheumatic drugs like penicillamine and hydroxycholoroquine appeared in the eighties for use in children and proved not to be effective in JIA.[6] Methotrexate (MTX) and to a lesser extent sulphasalazine (SSZ), were more successful and were registered for JIA in early nineties.[7,8] Methotrexate is still considered corner stone treatment for several categories of JIA[9].

Glucocorticoids are a therapeutic option for patients with non-systemic JIA in case of severe disease, as bridging therapy, while waiting for the response of systemic therapy, although evidence on its effectiveness is lacking[9]. Intra-articular steroids in the form of triamcinolonehexacetonide are effective and safe for the treatment of arthritis in children[28,29]. Recently it was suggested in retrospective studies that multiple injections were able to induce remission in a proportion of patients[30,31].

Sulfasalazine is one of the first antirheumatic drugs especially designed for the treatment of rheumatoid arthritis (RA) after its proven effectiveness for inflammatory bowel disease. It is a well-established DMARD for patients with RA either as monotherapy or in combination[10]. Its effectiveness in oligo- and polyarticular JIA was amongst others demonstrated in a multicenter randomized placebo-controlled trial in the Netherlands in 1992-1999[11]. A longterm follow-up study showed that the beneficial effects persist for many years[8].

Methotrexate (MTX) is an effective disease-modifying anti rheumatic drug in children with JIA. At a dose of 10 mg/m^2/week MTX has been shown to have a significant therapeutic advantage over placebo and an acceptable safety profile[7]. A plateau of efficacy is reached with parental administration of 15 mg/ m^2/week[12]. The response to MTX may be inadequate in some patients, even at higher dosages, and lack of tolerability limits its usefulness sometimes[13,14]. Due to suspected increased bioavailability a subcutaneous dosing regime is advised at higher dosages[15,16]. An initial robust response to MTX is predictive for a better long term outcome[17]. Remission rates vary greatly from 7-45%[18-20]. Relapse rates before the year 2000 were quite high, over 50% although reported in small series[21,22]. Foell et al studied the optimal moment to stop MTX in JIA patients in a retrospective analysis and did not see a difference in terms of flare frequency in case of longer MTX therapy (mean

3.8 vs mean 12.6 months)[23]. This result was confirmed in a multicenter, 12 vs 6 months withdrawal MTX study. In both groups flare rate was almost 40% within 1 year despite longer treatment with MTX in the 12months group[24].

Hydroxychloroquine, an antimalarial, is known since the 1950s in the treatment of rheumatoid arthritis. It was first applied in a study in JIA patients in the eighties and proved ineffective[6]. It is one of the least potent antirheumatic drugs, but still in use as an adjunct to combination therapy in rheumatoid arthritis[25]. Although the role of hydroxychloroquine as adjunct therapy has been subject of study[26], convincing evidence is still lacking and this remains subject of further study.

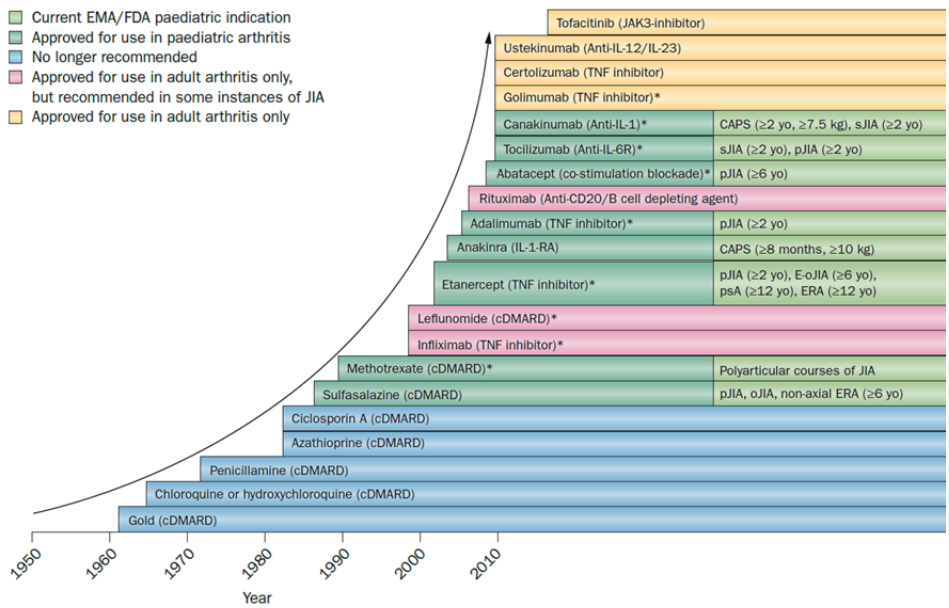

Figure 1 | The armamentarium of antirheumatic drugs available for the treatment of JIA. The evolution of biologic DMARDs in past decades revolutionized the therapeutic management of arthritis. However, although the number of variety of therapies available for use in paediatric rheumatology (excluding glucocorticoids and NSAIDs) is greater than ever, deciding on a treatment strategy has correspondingly increased in complexity. Whereas some therapies have been tested in high-quality paediatric studies, only some have received specific approval for use in children with rheumatology diseases, and such approval may also be limited to certain JIA categories. Additionally, soms drugs approved in adult rheumatology have been recommended for use in JIA without EMA or FDA approval. *Therapies tested in high-quality paediatric studies. Abbreviations: CAPS, cryopyrin-associated autoinflammatory syndromes; cDMARD, conventional disease-modifying drug; EMA, European Medicines Agency; E-oJIA, extended oligoarticular juvenile idiopathic, arthritis; ERA, enthesitis-related arthritis; IL-1-RA, IL-1 receptor antagonist; JIA, juvenile idiopathic arthritis: oJIA, oligoarticular juvenile idiopathic arthritis; pJIA, polyarticular juvenile idiopathic arthritis; psA, psoriatic arthritis; sJIA, systemic juvenile idiopathic arthritis; TNFi, tumour necrosis factor inhibitor; yo, years old.

Figure from: Management of juvenile idiopathic arthritis: hitting the target
Nature Reviews Rheumatology 2015;11(5): 290-300 (With permission of Prof Dr D Foell)

Leflunomide, a reversible inhibitor of de novo pyrimidine synthesis, was studied in polyarticular JIA only in one open-label trial[27]. ACR30 response criteria were met in the majority in the short and long term.
By the end of the last century a new category of medication appeared with the development of the biologicals.

Bioogic treatment in JIA: what do we know up to now?
Technical developments and better understanding of the disease process led to the development of monoclonal antibodies against an important cytokine in juvenile idiopathic arthritis, tumor necrosis factor-alpha, TNF-α.
Since the development of biologicals and the first use in juvenile idiopathic arthritis almost 2 decades have past. Initially the effectiveness of etanercept was established in severe treatment refractory JIA[32 33].
Dedicated observational studies have taught us about the effectiveness and improved quality of life in JIA patients treated with anti-TNF[34-36]. The safety profile, both in the short and long term is good[37-40]. Prolonged use up to 5 or even 10 years is described[34,38,41,42], underscoring its effectiveness and tolerability. Growth in JIA patients seems to be restored in treatment with etanercept[43-45]. The efficacy does not change when etanercept is given in a once weekly dose[46].

Secondly adalimumab[47], infliximab[48] and biologicals with other modes of action were studied[49-51] and often subsequently approved in JIA treatment.
As the years are passing, biologicals are used more often and earlier in the disease course[52]. Their place in the treatment of patients with recently diagnosed JIA and their effectiveness compared to other aggressive treatment strategies has yet to be determined[53,54].

Bottom up or top down?
Although the medical treatment in JIA depends to a large extent on the category of JIA, improved disease outcome has been achieved by early and aggressive immunosuppressive treatment, instead of by a gradual add-on approach of medication in case of ongoing disease activity[55]. The ultimate outcome is irrespective of the JIA category: destruction of joints resulting in severe incapacity can occur in children with oligoarticular JIA, while some children with severe polyarthritis at onset can easily reach inactive disease within weeks when responding to medication[55,56]. Despite advances in treatment options and modalities during the last decade, a considerable number of children with JIA remain refractory to therapy[57]. The overall outcome of JIA needs to be improved while children are still developing joint damage and/or limitations in daily functioning[58]. Many patients with JIA continued to have active disease 10 years after onset, with persistence of active

disease into adulthood as well[8,59]. In a longitudinal study by Wallace et al. evaluating 437 patients with JIA over a median follow-up of 7 years showed that during the entire course of the disease, the patients spent less than 35% of the time in a state of clinical remission off medication, regardless of the JIA subcategory[60]. Ringold described in 2009 a cohort of patients who spent the majority of their follow up time in active disease[61]. These data highlight the urgent need for improved treatments in JIA that are capable of inducing extended periods of clinical remission off medication. It is unclear how to position the available drugs in JIA in daily practice. The traditional (bottom up) approach was to 'go low, go slow', aiming to avoid in part unknown side effects of the available drugs. This approach to drug treatment emphasizes the stepped use of one disease modifying antirheumatic drug (DMARD) at a time. When first-line agents such as nonsteroidal anti-inflammatory drugs (NSAID) failed after months "second-line", "slow-acting" or "DMARDs" such as antimalarial agents, sulfasalazine (SSZ), methotrexate (MTX) were considered. In the past years a shift to an earlier introduction and more aggressive combination (top down) therapy was seen although comparative trials of different combinations lacked in JIA at the start of this thesis. A too cautious approach can lead to undertreatment and permanent disability.

The current approach in 2009 to the treatment of children with oligoarticular JIA was to start with a NSAID and/or intra-articular glucocorticoids. When that is not effective, to add SSZ and/or MTX, Patients with extended oligoarticular JIA, Rf negative polyarticular JIA and psoriatic arthritis also usually start with a NSAID. When that is not effective SSZ and/or MTX are added. MTX first in a relatively low dose, next a higher dose, sometimes with prednisone bridging. Only for patients with polyarthritis who have shown to be MTX resistant or for those who do not tolerate MTX the TNF-blocking agents are available. Studies have shown this to be a very effective drug, both on clinical outcome measures and on radiological damage progression[37,62]. In 2011 ACR recommendations were published to guide treatment for JIA[9], discerning three levels of disease activity, low, moderate and high, by recognizing features of poor prognosis, like radiographic damage at presentation or involvement of hip or cervical spine.

Combination therapy

At the start of this thesis (2009) combination therapies were not commonly administered to JIA patients. In several trials, patients already treated with MTX receiving new biological therapies were treated with MTX as co-medication in varying percentages of patients[50,63,64]. After the success of combination therapies in rheumatoid arthritis, studies on initial combination therapies were first described in 2011 and onwards, although in the recommendations by Beukelman[9] no advice is formulated on non-biological DMARD combinations due to a lack of evidence.

The TREAT study, a randomized blinded placebo controlled trial and the ACUTE JIA trial, with a randomized open-label design, both used the combination of methotrexate and antiTNF: (etanercept in TREAT, infliximab in ACUTE). Both trials demonstrated that early use of a biological agent resulted in higher frequencies of inactive disease.

Window of opportunity

As shown in the BeSt study in rheumatoid arthritis patients[65,66] it is likely that also in JIA a window of opportunity exists where the disease is most responsive to treatment and susceptible for permanent suppression. In several papers the existence of this window of opportunity in children with JIA was illustrated[8,67]. When inactive disease can be achieved during an early phase, the opportunity arises to discontinue the treatment, thus shortening the time of exposure to possible side effects of drugs.

Discontinuation of anti-rheumatic drugs

What is known from literature about stopping treatments in JIA? As mentioned previously stopping methotrexate is feasible with the risk of flare approximately 40% irrespective of treatment continuation for 6 or 12 months[24]. Tapering or stopping TNF inhibitors has been evaluated in several retrospective studies[68-73] which were all published after the onset of the study serving as the basis of this thesis. Flare rates are overall high varying from 50-69%. In the large recent prospective cohort study from Guzman et al[74], flares occurred in over 50% of patients, although significant flares requiring additional treatment occurred within the first year after stopping therapy in 25%.

Monitoring of clinical response

By the end of the nineties the ACR Pedi improvement scores were published[75] to aid in standardization of the conduct and reporting of clinical trials and additionally to facilitate the physician to determine whether a patient responds adequately to therapy.
The JIA Core Outcome Variables consist of:

1. Physician Global Assessment of Disease Activity (10 cm Visual Analogue Scale (VAS))
2. Parent/patient global assessment of overall well-being (10 cm VAS)
3. Functional Ability (Childhood Health Assessment Questionnaire)
4. Number of Joints with Active Arthritis
5. Number of Joints with Limitation of Movement
6. Erythrocyte Sedimentation Rate ESR.

For efficacy assessment, patients will be evaluated as “improved” or “not improved” by comparing the values of core outcome variables at the post-dose assessment time points with baseline values.

Definition of Improvement in Juvenile Idiopathic Arthritis

JIA ACRPedi30/50/70/90 improvement is defined as 3 of any 6 core outcome variables improved by at least 30/50/70/90% from the baseline assessments, with no more than 1 of the remaining variables worsened by more than 30%.

The advantage of reporting outcome measures uniformly proofed beneficial in comparing clinical data. However the relative improvements does not reflect actual disease status and prevents comparison of disease activity between patients. Additionally it is not easy to use in daily practice.

JADAS score

Therefore more recently the Juvenile arthritis disease activity score (JADAS-71,-27, or-10) was developed and validated[76].

The scores of 4 domains are added:

1. Physician Global Assessment of Disease Activity (0-10 cm Visual Analogue Scale (VAS))
2. Parent/patient global assessment of overall well-being (0-10 cm VAS)
3. Number of Joints with Active Arthritis (0-10 count of any involved joint, irrespective of its type, up to a maximum of 10 joints.
4. Normalized ESR; (ESR-20)/10; all values ESR above 120 are converted to 120, all values <20 are converted to 0.

To a maximum of 0-40 (JADAS-10) 0-57 (JADAS-27) and 0-101 (JADAS-71).

Since then adjustments were developed to a 3-point score, which facilitates use in a daily clinic because it is not necessary to wait for the sedimentation rate to determine the disease activity score[77]. Cut-off values for various disease states are available for use in clinical trials[78,79].

Definition of inactive disease in Juvenile Idiopathic arthritis

Wallace defined inactive disease in 2004[80] which was adopted in the study design of the BeSt for Kids study: Inactive disease is characterized by criteria: no clinical symptoms of active synovitis; no fever, rash, serositis, splenomegaly, or generalized lymphadenopathy attributable to JIA; no active uveitis; normal ESR and/or CRP; Physician´s global assessment of disease activity indicates no active disease. In addition to this definition the duration of morning stiffness of ≤15 minutes was added as criterion for inactive disease in 2011.[81]

Child Health Assessment Questionnaire

The Child Health Assessment Questionnaire is a measure of functional status, which is disease specific for JIA[82,83]. Both disability and discomfort are tested focused on physical functioning. Eight domains are tested including dressing, grooming, eating and different general physical abilities divided over 30 items questioning difficulty in performance. The CHAQ score is calculated as the mean of the 8 functional areas. The final score is 0-3. Values of 0.13, 0.63, and 1.75 have previously been described to represent respectively mild, mild to moderate, and moderate disability[84]. A change in score of 0.13 represents an important change in clinical status[84,85]. The test is easy to use and short, therefore increasingly used in clinical trials as well as longitudinal studies. The test is characterized by good reliability and validity, as well as good discriminative properties and reasonable responsiveness[86].

Monitoring of radiographic damage

Over the years several scoring systems for assessing joint damage and progression have been developed[87]. Since in children growing joints change anatomically over time, it is not easy to determine cartilage loss and erosions. This unique feature of growth in children limits the use of the most well-known scoring system from adult rheumatology: the Sharp-van der Heijde score in its original form[88]. A pediatric adaptation (adding 5 areas in the wrist and omitting to score the feet, since foot joints are rarely involved in JIA) to the Sharp-van der Heide score was developed and validated[89] and has been used, although few, eversince[90,91]. The older and more easy to perform Poznanski score[92] is a measure of the ratio between carpo: metacarpal length and reflects the amount of radiographic damage in the wrist. Poznanski scores that are more negative represent more severe radiographic damage[62,93-96].

Which target are we aiming at?

Classically the ACRpedi30% was regarded as the clinical target in studies[75], reflecting 30% improvement in at least 3 out of 6 core set variables, with no more than one of the remaining variables worsening by more than 30%. As such this target was used in several trials.[32,97,98] Against the background of all improvements due to increasing therapeutic options, mainly the introduction of etanercept, this target was gradually regarded as too low. In trials as well as observational studies ACRpedi50 and 70% became alternative targets[26,38]. In clinical care inactive disease was gradually recognized and mentioned as the target to aim at[99]. It took some time however before inactive disease was chosen as the primary outcome measure in a JIA clinical trial, as was proven to be a realistic target in RA[65].

The BeSt for Kids study

Despite new therapeutic options, it is still unclear how and when a drug or combination of drugs should be introduced over time in patients with juvenile idiopathic arthritis. Previous studies focused on the comparison of one drug or the combination with another drug or combination. More relevant for clinical practice is what consecutive therapeutic steps should be taken when disease activity is insufficiently suppressed. Additionally with inactive disease as a therapeutic target, which strategy results in the best long term outcomes, with the least side effects? Should all patients be treated initially with a combination of antihreumatic drugs or biologic agents, or can they be reserved for patients who fail on initial monotherapy? There are concerns about the long-term safety of more aggressive approaches, especially about infections and malignancies although results up to today are reassuring. Financial restrictions preclude treatment of newly diagnose JIA patients with expensive TNF-antagonists in many countries.

Against this background, and after the success of the BeSt study in RA[65], the BeSt for Kids study was developed by a group of (pediatric) rheumatologists. In this study three treatment strategies are compared (figure 1 page 99).

1 Sequential monotherapy, starting with methotrexate or Sulphasalazine, thereafter increasing MTX dose or switching to MTX, thereafter adding etanercept (anti-TNF).
2 Initial combination therapy with methotrexate and prednisolone bridging therapy, thereafter increasing MTX dose, thereafter adding etanercept (anti-TNF).
3 Initial combination therapy with methotrexate and etanercept (anti-TNF).

For patients failing on their medication, the treatment protocol prescribed a number of subsequent treatment steps. The decision whether or not to adjust medication was made every three months based on ACRPedi50% after initial 3 months, and based on inactive disease from 6 months and onwards. To avoid bias, the joint examination was performed by a physiotherapist or research nurse who remained blinded for the allocated treatment group during the entire study period. If the target (ACRpedi50% or inactive disease) was not met, the treating physician immediately adjusted therapy by proceeding to the next step in the allocated treatment group. In the target was met for at least 3 (oligoarticular disease) or 6 (polyarticular disease) months, medication was tapered and stopped according to a predetermined protocol. To avoid overtreatment of patients with oligoarticular JIA especially in the arm with initially etanercept and methotrexate the required duration of inactive disease is chosen to be three months for those patients. This study combined three study questions in one study: 1 Can we achieve inactive disease, 2 can we taper and stop DMARD therapy and if we can, 3 what is the flare frequency?

JIA categories under study

Three categories of JIA will be studied: oligoarticular JIA (both with persistent and extended phenotype), RF-negative polyarticular JIA and juvenile psoriatic arthritis. Inclusion of children with extended oligoarticular JIA, RF-negative polyarticular JIA and juvenile psoriatic arthritis when they need a DMARD probably warrants no further discussion. We have chosen to also include children with persistent oligoarticular JIA that continue to have active synovitis despite treatment with NSAID's and/or intra-articular glucocorticoids, considering the fact that in these patients outcome is highly variable. Albers et al[100] in a cohort of patients with JIA reveal that the percentage of time with active disease in the first two years after diagnosis is an objective parameter of the course of disease and is highly variable in all JIA categories. Within each category of JIA, patients can have a wide range of duration of disease activity varying from a short duration of time with active disease to ongoing disease activity.[100] The incidence of the other categories of JIA, for instance RF-positive polyarticular JIA, is too low to allow stratification.

Outline of this Thesis

This thesis consists of two parts. The first part describes three studies related to pathogenesis of JIA and the second part describes the results of a treatment strategy study in JIA, the BeSt for Kids study.

Part One Aspects of Pathogenesis of JIA

First the pathogenesis of JIA is reviewed against the latest research novelties in **chapter 2**. One of our objectives was to discover the role of the newly recognized antibodies in rheumatoid arthritis, the anticarbamylated proteins. In **Chapter 3** anticarbamylated proteins were studied in a large cohort of JIA patients, combined from the ABC-register and the BeSt for kids cohort.

Novel in the pathogenesis of auto-immune diseases in general and JIA in particular is the possible role of the intestinal microbiome. One of the first (pilot)studies worldwide between the onset of JIA and the possible role of the composition of the microbiota was initiated in JIA patients generated from the BeSt for Kids cohort and will be described in **chapter 4**.

Part Two Treatment Strategies in JIA

The BeSt for Kids study serves as the basis for this thesis. In this single-blinded multicenter randomized 3-armed treatment strategy study sequential monotherapy with either Sulphasalazine or Methotrexate (arm 1) is compared to initial combination therapy with Methotrexate and prednisolone (arm 2), and with initial combination therapy with etanercept and MTX (arm 3). In **chapter 5.1** we describe the 3-months clinical results of the

three treatment strategies in early onset JIA. The long term (24 months) clinical outcome is set out in **chapter 5.2**. **Chapter 6** deals with the results of the radiological outcome of the BeSt for Kids study. An interview study was conducted to decipher aspects of clinical equipoise in the BeSt for Kids study, set out in **chapter 7**. As the study proceeded one patient was wrongly diagnosed and included in the BeSt for Kids study due to a JIA mimic. **Chapter 8** describes the disease course as the patient had to be excluded from the BeSt for Kids study, secondarily diagnosed as systemic polyarteritis nodosa (PAN). The general discussion is presented in **chapter 9** and the summary is given in **chapter 10.**

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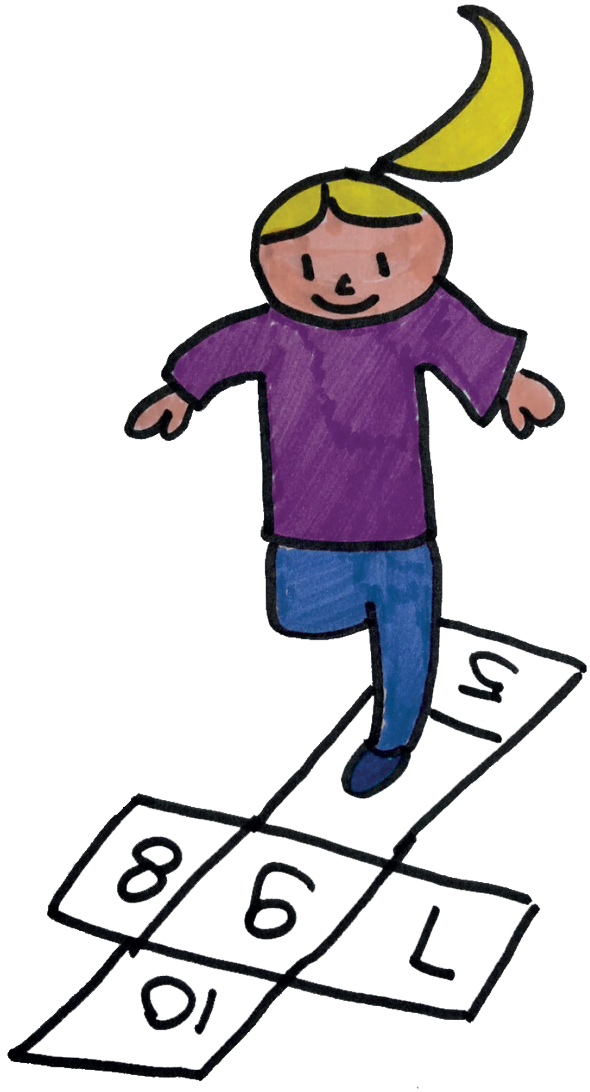
PART ONE

PATHOGENESIS of Juvenile Idiopathic Arthritis



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Oligoarticular and Polyarticular Juvenile Idiopathic Arthritis

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INTRODUCTION

When a child under the age of 16 years has arthritis with a duration exceeding 6 weeks the diagnosis of juvenile idiopathic arthritis (JIA) is probable. Other diseases need to be excluded, especially in the absence of commonly found serological factors such as antinuclear antibodies (ANAs). JIA is a heterogeneous group of diseases and only the category with immunoglobulin M (IgM) rheumatoid factor (RF) is thought to be equivalent to adult rheumatoid arthritis (RA).

In the past, several names have been given to chronic arthritis in childhood such as juvenile RA and juvenile chronic arthritis. Since an International League of Associations for Rheumatology work force (last revised in 2007) proposed the name juvenile idiopathic arthritis, this name has been adopted, both in Europe and the United States. Seven categories of JIA are recognized, as shown in Table 1.1[1].

Table 2.1 | Categories of Juvenile Idiopathic Arthritis (ILAR Classification)

Systemic
Polyarticular RF-negative
Polyarticular RF-positive
Oligoarticular
- Persistent
- Extended
Psoriatic arthritis
Enteritis-related arthritis
Undifferentiated arthritis
- Fits no other category
- Fits more than one category

This classification is currently being questioned. New classification schemes are suggested to incorporate ANA status, age of onset, and symmetry of arthritis[2] but cytokines, genetics, and gene expression profiles might be promising fields to include in future classification[3-8].

Usually children with JIA are first seen by an orthopedic surgeon or a pediatrician and referred to a pediatric rheumatologist in a later stage[9].

As damage to the joints can be prevented by early and adequate medical therapy, permanent postacademic education on the subject of rheumatic diseases in childhood is mandatory in order to obtain early referral of children with a suspicion of inflammatory arthritis.

Having (a child with) JIA creates a considerable burden for the child, the family, friends, and school[10] and interventions have been developed to reduce the impact of the disease[11]. The importance of adequate patient and parent information and education is emphasized[12].

Definitions

Oligoarticular JIA is defined as arthritis in one to four joints during the first 6 months with exclusion of

- psoriasis, diagnosed by a dermatologist in at least one first or second grade family member;
- Human leukocyte antigen-B27 (HLA-B27) associated disease in at least one first or second grade family member;
- Presence of RF;
- Arthritis in a boy older than 8 years and HLA-B27 positive;
- Systemic JIA.

In children with persistent oligoarticular JIA the number of joints involved remains four or less throughout the course of the disease. In extended oligoarticular JIA the arthritis shows extension to polyarthritis after the first 6 months.

IgM RF negative polyarticular JIA is defined as arthritis in five or more joints during the first 6 months with negative tests for IgM RF and exclusion of systemic JIA.

IgM RF positive polyarticular JIA is defined as arthritis in five or more joints during the first 6 months and presence of the IgM RF on two occasions with an interval of 3 months with the exclusion of systemic JIA.

PREVALENCE/EPIDEMIOLOGY

Joint pain, joint swelling, and morning stiffness are not uncommon in childhood. However, only a minority of children with these symptoms, suggestive of arthritis, are diagnosed with JIA by objective criteria and thorough physical examination performed by an experienced (pediatric) rheumatologist. The exact incidence and prevalence of JIA is unknown. Studies on these topics have shown different results, influenced by the different populations that have been studied as well as ethnicity and environmental factors[13, 14]. Also seasonal variation has been described, even in the month of birth[15]. The incidence probably varies from 11 to 35 per 100,000 in the population[16].

Using the American College of Rheumatology criteria, the prevalence of JIA also shows a considerable variability ranging from 15 to 150 per 100,000[13]. Several studies on the prevalence of JIA have suggested that these figures might underestimate the true prevalence. Close examination of the children under study and restriction to those who are at risk will probably lead to a considerably increase in the prevalence of JIA. A trend toward increasing incidence of JIA is observed, unclear whether due to greater awareness or a real increase in patients[14].

Although JIA can be divided into different categories with different peak incidences of age, it is obvious that despite these differences, generally more girls than boys develop JIA except for the systemic onset type.

ETIOLOGY AND PATHOGENESIS

JIA probably has a multifactorial etiology[17]. Genetic predisposition, environmental influences, provoking infections, hormonal factors, and vulnerability in childhood are involved in the development of JIA[18]. The genetic predisposition includes multiple genes that are related to immunity and inflammation.

Genetic Predisposition

HLA class I and class II alleles are both associated with an increased risk to develop JIA. The last decade's research in this field has expanded. It is estimated that genetical factors account for 13% of the risk in JIA[19]. Early-onset oligoarticular JIA in girls is related to the class I antigen HLA-A2. Persistent and extended oligoarticular JIA are associated with class II antigens HLA-DRB108 and HLA-DRB111, DQA104, DQA105, and DQB104. Enthesitis-related JIA is associated with HLA-B27 (class I) and the class II antigens HLA-DRB101 and HLA-DQA10101. Systemic-onset JIA is related to HLA-DRB111. HLA DR4 is associated with RF-positive disease, like in adult disease[20].The genetical associations of JIA were recently reviewed by Cobb[21]. Non-HLA immune regulatory genes are also involved, and the list has expanded in the last years to include PTPN22[22] STAT4[23], TRAF-C5[24], TNF308a, 4q27[25], DNAM-1[26], and VTCN1[27], although not all of them have been independently replicated yet.

The genetic predisposition also includes genes that are related to cytokine production[28]. Nowadays, JIA is thought to be triggered by an initial adaptive immune response toward an unknown autoantigen. Right after this event, almost all cells of the immune system start to be involved. T-lymphocytes, cytokine production, immune complexes (ICs), and immunodysregulation all lead to inflammation of the joint.

Cytokines that are involved in the pathogenesis of nonsystemic JIA are tumor necrosis factor (TNF)-a, IL-1, IL-2, IL-4, IL-6, IL-7, IL-18, and IL2R[29, 30]. Concentrations of these cytokines are increased, both in plasma and in the synovial fluid. IL-1a and IL-1ß are both particularly involved in oligoarticular and polyarticular JIA and related to disease activity. Increase of IL-1a has been detected in plasma and increase of IL-ß in synovial fluid. Soluble TNF-a is also increased in plasma as well as synovial fluid in the more restricted disease types of oligoarticular disease. IL-4 is more prominent in the synovium[31]. IL-17 can induce production of IL-6, MMP-1 and -3, and IL-8 at the synovium, which all can lead to joint damage[32].

During phases of clinical remission, cytokines do not return to a normal healthy-control situation but reflect a condition of compensated inflammation[33].

Bacterial infections not only can cause reactive arthritis, but are also involved in the development of JIA. In children with JIA humoral as well as cellular immune responses against bacterial heat shock proteins (HSPs) have been described. These HSPs are highly conserved proteins of bacterial origin and have been demonstrated in plasma and synovial fluid of JIA patients. T-lymphocyte responses to HSP-60 were demonstrated before remission of JIA and it thus has been speculated that induction of immunotolerance to specific T-cells might be beneficial for JIA patients, and nasal administration of HSP-60 might be used as future immunotherapy[34, 35]. In 2011 HSP-60 serum concentrations were found to be predictive for disease flare[36].

Complement activation is also involved in the pathogenesis of JIA. Complement components of both the classical pathway (C4) and the alternative pathway (Bb) showed increased levels and correlated with disease activity[37, 38]. Mannose-binding lectin (MBL) is a major component of the lectin route of complement activation. An increased frequency of mutations in exon 1 of the MBL2 gene has been demonstrated in RA and JIA patients, indicating a possible role of MBL deficiency in the pathogenesis of JIA[39, 40]. The possible role of infection in JIA has also been demonstrated by increased incidence of chronic arthritis in patients with hypogammaglobulinemia, IgA deficiency, and C2 deficiency[41].

Furthermore, MBL deficiency might lead to defective clearance of ICs and apoptotic cells, as seen in individuals with C1q deficiency. Partial C4 deficiency has also been linked to JIA[42]. Recently the alternative pathway was suspected to be involved in oligoarticular JIA[43].

Circulating immune complexes (CICs) have been demonstrated in JIA. These CICs have been detected in plasma and synovial fluid and revealed complement activation as well as

cytokine secretion potential. The CICs correlated with disease activity and systemic features of JIA[37]. The activating capacity of CICs is related to their size. Although often undetectable in plasma of JIA patients, IgM RF is bound to the ICs and concentration of this RF is related to disease activity[44]. Membrane-attack complex bound to CICs correlated significantly with erythrocyte sedimentation rate (ESR), further supporting the notion of complement-mediated tissue injury that is triggered by IC-mediated classical pathway activation[45].

The T-lymphocyte-mediated immune response is important in chronic inflammation. T-lymphocytes are the most prominent mononuclear cells in synovial fluid. The T-lymphocytes can be differentiated in CD4 (helper/inducer) and CD8 (suppressor/cytotoxic) cells with different functional abilities.

An impaired thymic function, reflected by a decrease in CD4+ T-cells, was described in oligoarticular and polyarticular JIA[46]. Recently the same phenomenon of premature aging was observed for CD8+ T-cells in JIA patients[47].

The results of different studies on the possible pathogenetic role of CD4+ and CD8+ T-lymphocytes have been inconsistent[18]. Increased CD8+ T-lymphocytes have been demonstrated in systemic and polyarticular JIA; however, other studies showed decreased CD8+ T-lymphocytes, especially in systemic disease[48].

Regulatory T-cells (T-regs) first described by Sakaguchi[49] are increasingly thought to play a role in the pathogenesis of (the remitting course of) JIA[50]. These cells are characterized by expression of FOXP3, a transcriptional factor, necessary for the control of inflammation. Recently a review on the emerging role of the T-regs was published[51].

Co-expression of CD25 and FOXP3 in combination with a hypomethylated region within the FOXP3 gene, called the Treg-specific demethylated region, is considered the hallmark of stable T-regs. Recently it was discovered that environment-specific breakdown in FOXP3 stability may threaten the abrogation of inflammation in JIA[51].

Natural (thymic derived) and adaptive T-regs exist, but cannot be discerned as of this writing[51]. It seems that T-regs are heterogeneous and can differ in function. T-regs can be divided by HLA expression (DR+ and DR-)and by cellular markers (naive or memory). In oligoarticular JIA, at the site of inflammation T-regs of the activated memory phenotype are present.

Th17 cells, a subset of the CD4+ effector T-cells producing IL-17, are also present at the site of inflammation[52] and have a reciprocal relation to T-regs[53]. Both need TGF-beta for induction and the T-regs and Th17 cells need to be balanced in a healthy-state situation. T-regs even seem to be able to convert into Th17 cells under certain circumstances[54].

In 2004 it was discovered that in JIA the numbers of T-regs are in fact paradoxically increased in the inflamed joint[55]. Serum Treg numbers, on the other hand, are normal or decreased in O-JIA. In SF of persistent oligoarticular JIA patients the numbers of T-regs are higher than in SF of extended to-be JIA patients. It is hypothesized that the balance between T-regs and Th17 cells is crucial in JIA and that they behave in a reciprocal relationship at the site of inflammation[56].

Possibly T-regs are dysfunctional in JIA? Studies suggest that the T-regs function well (are potent suppressors of inflammation) when taken out of the synovium, suggesting a role for the microenvironment affecting the T-regs in their function.

Dendritic Cells

Dendritic cells (DCs) are antigen-presenting cells, necessary for T-cell activation. Evidence for their suspected role in the initiation and perpetuation of inflammation is scarce. Increased numbers of DC in synovial fluid have been described in oligoarticular and polyarticular JIA[57]. B-cell concentration is normal in oligoarticular and polyarticular JIA, however generally increased in patients with systemic JIA. Total levels of IgG might be elevated and a diversity of autoantibodies can be detected in sera of JIA patients.

Like IgM rheumatoid-factor JIA, anticyclic citrullinated peptide (CCP) is associated with erosive disease[58]. The pathogenesis of JIA is also influenced by psychological factors. Dysregulation of the autonomic nervous system is related to impaired immunologic response and possible development of autoimmune disease[59].

Gene Expression Profiling

Gene expression profiling, also known as transcriptomics, measures the expression level of mRNAs (transcripts) in a cell population at a certain time. In oligoarticular JIA, gene expression profiling on synovial fluid could help predict patients with an extended disease phenotype[60]. In polyarticular JIA, recently it was shown that gene expression was linked to therapeutic outcome at 6 months[61]. Furthermore, in polyarticular JIA, remission could be genomically characterized and differed markedly between methotrexate (MTX) and MTX/etanercept-induced remission. At the therapeutic level, gene expression profiles were studied before and after MTX administration to analyze differentially expressed

genes. A gene was identified that could contribute to genetic variability in MTX response[6, 62, 63]. Clinical remission on medication and clinical remission reflect states of balanced homeostasis between pro-and anti-inflammatory since gene expression profiling differed between healthy controls and the aforementioned categories[33].

Myeloid-Related Protein 8/14 (S100A8/A9), S100A12

These calcium-binding proteins produced by activated neutrophils and monocytes are present in the serum of patients with both oligoarticular and polyarticular JIA, next to their extreme elevation in active systemic JIA. These danger signals increase before clinical flare is obvious and therefore have predictive value. The serum concentrations of S100A8/A9 and S100A12 are related to the amount of inflammation[64, 65]. Levels to predict disease flares have been determined and an ELISA is commercially available[66].

Clinical Manifestations

When taking a history of (parents of) children with JIA pain is usually not a major symptom at onset[67], but the parents of young children may have noticed a regression in the motor phase of their child[68]. An asymmetric pattern is especially alarming. Swelling of the knee or ankle is often noticed by chance when parents are undressing or bathing the child. Other signs at onset can be behavioural problems, limping, or refusal to walk. In older children, especially in those with (IgM RF positive) polyarticular JIA, pain can be a presenting symptom. General malaise, low-grade fever, and fatigue can be present in severely affected children, mostly in those with polyarticular JIA. Morning stiffness and stiffness after spending prolonged time in the same position are common. The onset of JIA may be acute but usually is insidious.

At physical examination the general condition of the child should be noticed. They may look anemic and, when suffering from systemic features, ill and in pain. Length and weight should be measured regularly as general growth impairment points at active disease and this may be aggravated by prolonged use of glucocorticoids, which is, in the biological era, becoming less common. In children with IgM RF-positive polyarticular JIA rheumatoid nodules at the extensor surface of the elbows or at the lateral sides of the feet can be found.

Asymmetrical diffuse edema of hands or lower leg and ankle in a sock-like form can be found in some children with polyarticular JIA[69]. This lymphedema is usually non-pitting and not painful.

In children with JIA who develop chronic anterior uveitis (CAU) before the onset of arthritis secondary changes in the eyes can be noticed, irregular pupils that do not respond properly

to light may reflect the presence of synechia. Calcifications of the cornea may be present in the form of band keratopathy.

Signs of arthritis are local swelling, increased temperature, pain elicited by movement, and limitation of motion. Local discoloration is very unusual except over the small joints in the hands and feet[70], and when present over a knee or ankle should be a reason for reconsideration of the JIA diagnosis.

Establishing arthritis can be difficult, especially in young children with baby fat. An observation by an experienced child physiotherapist and/or the use of ultrasound or MRI with gadolinium enhancement can be helpful[71]. In children with oligoarticular JIA asymmetric swelling of large joints like the knee, ankle, and elbow are most frequent, while in children with polyarticular JIA symmetric involvement of the small joints of the hands and feet is more common. In children with IgM RF swelling around the styloid processus of the ulnar can be prominent. Several complications may develop in children with oligoarticular and polyarticular JIA.

Impaired growth and delay of puberty may be the result of disease activity[72] and the (currently outdated) chronic use of glucocorticoids aggravates the impairment of linear growth. Muscle atrophy and leg length discrepancy by accelerated local growth are findings in longstanding asymmetric arthritis[73]. Decreased bone mineral content can be observed in a quarter of children with early onset JIA[74]. Osteopenia can be detected in adolescents with early onset JIA[75].

Cardiac manifestations are rare but may be the cause of significant morbidity, especially valvular disease in RF-positive polyarticular JIA[76]. Parenchymal lung disease is an infrequent finding, but pulmonary function is impaired in some children with JIA[77].

Temporomandibular involvement is common in children with oligoarticular and polyarticular JIA[78, 79]. Because of the high prevalence and discrepancy between clinical signs and presence of arthritis in the temporomandibular joint, regular orthodontic evaluation and orthopantomograms are recommended to enable early intervention[78]. Involvement of the temporomandibular joints may lead to impaired opening of the mouth and retrognatia.

Chronic Anterior Uveitis (CAU) is reported in up to 10-20%[80, 81] of patients with JIA, and is the main secondary disease in JIA. It is associated with the presence of ANA. All children with JIA need to be screened with regular intervals (see Table 1.2) as this type of uveitis is asymptomatic until complications develop. The classic presentation of CAU is an anterior,

nongranulomatous, uni-or bilateral uveitis. Slit-lamp examination is necessary to detect the inflammatory cells in the anterior chamber of the eye. It is therefore essential that all children with JIA are seen by an ophthalmologist at regular intervals.

Early detection and treatment of CAU is of major importance to avoid sight-threatening complications including band keratopathy, synechia, cataract, glaucoma, macular edema, decreased vision, phthisis bulbi, and blindness[82]. Young age of onset (arthritis and uveitis), active uveitis at the time of onset of arthritis, and high uveitis activity at the time of diagnosis is associated with a higher risk of sight-threatening complications. The recommended frequency is listed in Table 1.2.

Generally patients with JIA are divided into high-and low-risk groups depending on known risk factors for uveitis. Risk factors are young age of onset, female gender, and oligoarticular onset of JIA. Onset of arthritis usually precedes the onset of uveitis, but uveitis may also start first. The risk of developing uveitis is the highest shortly after onset of arthritis and decreases gradually after the first year[83].

DIAGNOSTIC INVESTIGATIONS

In all children with chronic arthritis a full blood count is indicated. In most children with oligoarticular JIA normal hemoglobin levels are found. In some children with oligoarticular JIA with high disease activity and in children with polyarthritis moderate normocytic, hypochromic anemia can be present characteristic of the chronic anemia of inflammation[84]. Anemia and raised platelet count are associated with a less favorable prognosis.

In a child with other systemic features like fever, skin rash, and lymph node enlargement other diagnosis like systemic JIA, other autoimmune diseases (such as systemic lupus erythematosus, SLE), or malignancy should be considered.

The leukocyte count usually is normal. In children with active disease leukocytosis may be present. During treatment with sulfasalazine or MTX a low leukocyte count may represent drug-induced bone marrow suppression. In a child with possible JIA a low leukocyte count could be the key to another diagnosis; for example, SLE or leukemia. Platelets are within the normal range in most children with oligoarticular and polyarticular JIA, but may be raised in children with high disease activity.

Table 2.2 | Recommended frequency of Ophthalmological Investigation in Children With Persistent or Extended Oligoarticular Juvenile Idiopathic Arthritis According to the American Academy of Paediatrics (1993)

Subtype of Arthritis	Onset of Arthritis (Years of Age)	
	< 7 years[a]	≥ 7 years[b]
Persistent oligoarticular		
+ANA	H[c]	M
– ANA	M	M
Extended oligoarticular		
+ANA	H[c]	M
– ANA	M	M

H: high risk = every 3-4 months ophthalmological investigation.
M: medium risk = every 6 months ophthalmological investigation.
L: Low risk = every 12 months ophthalmological investigation (all other patients with JIA).
[a] All patients are regarded low risk 7 years from onset of arthritis; ophthalmological investigation yearly.
[b] All patients are regarded low risk 4 years from onset of arthritis; ophthalmological investigation yearly.
[c] All patients are regarded medium risk 4 years from onset of arthritis.

The acute phase reactants (ESR, C-reactive protein (CRP) levels) can be normal in children with JIA, but may be raised at onset of the disease and during exacerbations[85]. Blood chemistry is usually not abnormal at onset of JIA. The level of serum urea can increase during the use of nonsteroidal anti-inflammatory drugs (NSAIDs). Liver function tests need to be carefully followed during the use of SSZ and MTX. In the diagnostic phase, broad screening for infectious causes of arthritis is indicated as a variety of microorganisms may induce arthritis (see differential diagnosis).The onset of JIA and its exacerbations are frequently preceded by infections[86]. In children with active (poly)arthritis, a raised IgG can be present. In children with oligoarticular JIA the IgA may be low or even absent. During treatment with sulfasalazine the level of IgA may decrease[87].

ANAs are found in about 75% of children with oligoarticular JIA and in 50% of children with polyarticular JIA[8, 88]. Their presence is strongly associated with the risk of developing CAU but does not seem to be associated with the severity of the uveitis[81]. A positive ANA is rare in children, but can be a temporary false-positive finding in infections (streptococci, viral). Antibodies to dsDNA are usually not found. When they are detected the child could have SLE. Antibodies to extractable nuclear antigens (anti-ENA) are rarely present; anti-ENA may indicate other autoimmune diseases such as mixed connective tissue disease (MCTD).

Tests for the IgM RF are less frequently positive in children with JIA than in adults with RA. In 5-10% of children with JIA, IgM RF can be detected during the course of the disease.

Their presence is associated with onset of disease in girls around 13 years of age with clinical signs as in RA, progressive disease, and early erosions. Anti-CCP antibodies can be detected in the sera of patients with JIA but almost exclusively in the subset of children with IgM RF-positive disease[58].

Serum complement levels usually are within the normal ranges, but may be elevated at onset and during exacerbations of the illness.
Analysis of the synovial fluid may provide valuable information in the diagnostic phase of a child with monoarthritis. The number of leukocytes reflects the severity of inflammation. A low leukocyte count (<2x 10^9) is unlikely in infectious arthritis and suggests a mechanical disorder of the joint. Gram preparation and culture are indispensable to rule out infection. Polymerase chain reaction (PCR) for Borrelia burgdorferi and Mycobacterium tuberculosis are available.

In children with oligoarticular and polyarticular JIA the synovial fluid is yellow with decreased viscosity. The white cell count is usually around 20,000 x 10^9/L with predominantly polymorphonuclear neutrophils and mononuclear cells.
In a child with a chronic monoarthritis without circulating ANA, a synovial biopsy can be necessary to exclude local abnormalities of the synovial membrane when MRI findings are insufficient to make a diagnosis. In children with JIA the histological finding is an aspecific chronic inflammation. For the follow-up of radiological damage by JIA plain X-rays can be used. But in the changing era with early initiation of highly effective therapies there is a need for more sophisticated imaging modalities, more sensitive in detecting pre-erosive changes. Ultrasound and MRI are suitable to serve that purpose, but have not been validated yet[88-90]. The growing skeleton gives rise to numerous physiological changes over time which need to be taken into account during ultrasound and MRI examinations. There is an urgent need for normal values regarding ultrasound and MRI in healthy children. Up to the time of this writing, the exact value of MRI and ultrasound in detecting disease activity in JIA needs to be determined.

Ultrasound can be a useful tool, but is not validated yet for detection of synovitis[89]. Subclinical synovitis on ultrasound is present in 3% of JIA patients in clinical remission. Subclinical synovial abnormalities are not related with early flare, in contrast with RA patients[90].

Pediatric scoring systems for MRI have been developed and are validated[91-93]. Recently a study showed that MRI is a promising biomarker in measuring therapeutic response[94]. Evidence-based points regarding the role of imaging in JIA are summarized and have been published[71].

At plain X-rays, at onset of the disease, usually only soft tissue swelling and periarticular osteoporosis can be detected. During the course of disease various radiological abnormalities may develop[95]. Ossification centers can develop earlier by increase of blood flow in the involved extremity, resulting in overgrowth but eventually premature closing of the epiphysis may lead to stunting of bone growth. Loss of cartilage can be reflected by narrowing of the joint space. Development of erosions in an early stage can be present in children with IgM RF polyarticular JIA. A radiological scoring system for children with JIA has been developed[96].

DIFFERENTIAL DIAGNOSIS

Joint complaints are relatively frequent in childhood, but usually self-limiting and seldom require referral to a hospital. For a correct differential diagnosis, a clear difference must be made between myalgia, arthralgia, arthritis, and possible involvement of the bones.

Infectious arthritis as well as reactive arthritis often have an acute onset and will recover with or without medication within approximately 6 weeks. Duration of arthritis of more than 6 weeks is called chronic arthritis. The most frequent cause of chronic arthritis in childhood is JIA. Not only infection or inflammatory diseases can cause complaints of the joints; other possibilities include traumatic, metabolic, hematological, malignant, and even psychogenic causes[97].

Infectious or bacterial arthritis is an acute illness, also called septic arthritis[98]. The child with septic arthritis is often very ill with high fever, and refuses to use the involved joint. One of the most important characteristics is extreme pain. Bacteria can enter the joint either by hematological spread or directly by penetration of the skin. Physical examination should include inspection of the skin to detect a porte d'entrée, which might lead to identification of the microorganism. Most frequently involved microorganisms are Staphylococci and Streptococci. When bacterial arthritis is suspected, puncture of the joint to obtain synovial fluid should be performed for analysis on leukocyte count and bacterial culture. In recent years the occurrence of Kingella kingae as a pathogenic microorganism is increasingly recognized in cases of septic arthritis. This Gram-negative bacterium is the number one causative organism of septic arthritis in the age group 6-36 months[99, 100]. It is recommended to have a high index of suspicion in young children presenting with joint inflammation, especially in cases of mildly elevated inflammatory markers[101]. PCR techniques for detection of K. kingae are therefore advocated. K. kingae is generally susceptible to the main antibiotics used in children with osteoarticular infections. The clinical course after

treatment initiation is benign. It should be taken into account that osteomyelitis in the vicinity of a joint may give a clinical picture similar to that of infectious arthritis without yielding a positive culture of the synovial fluid[102]. Another microorganism that can cause arthritis is B. burgdorferi[103]. Lyme arthritis is often preceded by erythema migrans, myalgia, and arthralgia, which can be followed by recurrent as well as chronic arthritis[104]. Only in the minority of the patients is a tick bite remembered. After the erythema migrans, arthritis can develop even after months, often starting in one or both knees in episodes. Chronic arthritis occurs in approximately 20% of patients with Lyme arthritis, most frequent as monoarthritis of the knee. Diagnostic tests include PCR of synovial fluid and serology. The presence of anti-Borrelia IgG antibodies is not definite proof of Lyme arthritis because 5% of the adult population have positive IgG antibodies due to asymptomatic infection in the past. Anti-Borrelia IgM antibodies turn positive after approximately 6 weeks and are present for months, whereas IgG antibodies can be detected for years. Lyme arthritis eventually has a relatively good prognosis with complete recovery but a substantial proportion of patients need more than one course of antibiotics and/or additional treatment with NSAIDs, disease-modifying antirheumatic drugs (DMARDs), intra-articular steroids, or even synovectomy[105].

Arthritis can also develop after viral infections such as parvovirus, rubella, and hepatitis B, as well as viruses of the herpes group, adenoviruses, and para-myxoviruses[106]. Most of these viral infections will lead to reactive arthritis, and detection of the virus in the synovial fluid is often negative. Viral arthritis generally completely resolves within 6 weeks although chronic duration is possible. Arthritis can develop even after vaccination. Clinical presentation, viral exanthema, and duration of the arthritis are helpful in the diagnosis of possible viral arthritis.

Mycoplasma pneumonia is another microorganism that can cause arthritis as well as spondylarthropathy in children[107]. Important symptoms include fever, cough, headache, and myalgia. Approximately 30% of the children with M. pneumonia infection will develop arthritis.

Reactive arthritis can develop after viral infections, Neisseria meningococci infection, as well as gastroenteritis by Salmonella, Shigellla, Campylobacter, or Yersinia[108]. Reiter's syndrome is an example of reactive arthritis, associated with HLA-B27, characterized by the triad of arthritis, conjunctivitis, and urethritis[109]. Acute rheumatic fever (ARF) is caused by Group A beta-hemolytic Streptococci (GABHS)[110]. It develops approximately 3 weeks after the GABHS infection, characterized by angina, high fever, illness, and headache. A sore throat is not always present. Arthritis is present in 80% of the patients, often with acute onset, painful polyarticular, migrating from one joint to another, and preferentially involving

the large joints of the lower extremities[111]. The diagnosis of ARF is made according to the Jones criteria[112]. Carditis with possible heart failure is an important complication of ARF that occurs in 50% of patients. Mitralis valve and aorta valve stenosis can develop[113]. Other complications include Sydenham's chorea, subcutaneous noduli, erythema marginatum, and (cutaneous) vasculitis[114]. When carditis is present, prophylactic antibiotic therapy (penicillin) is indicated according to the American Heart Association[115].

Reactive arthritis can develop after an infection with Streptococci without fulfilling the Jones criteria[116, 117]. This arthritis is often less severe and not migrating, but with a longer duration. Other Streptococci species in addition to GABHS can cause this form of arthritis[118]. Diagnostic tests include bacterial culture of the throat, nose, and/or ears, as well as antistreptolysin-O-antibody titer and anti-Dnase-B[119, 120]. Polyarthritis can be the first clinical sign of other autoimmune diseases like SLE, juvenile dermatomyositis, scleroderma, Sjögren syndrome, MCTD, and systemic vasculitis. Apart from arthralgia, arthritis, and myalgia, these autoimmune diseases are characterized by organ involvement and positive autoantibodies. Hemophilia and sickle cell disease are both hematological disorders with possible involvement of the joint. Vaso-occlusive episodes can be very painful and difficult to distinguish from arthritis[121]. Malignancies like leukemia, lymphoma, Hodgkin disease, osteosarcoma, Ewing sarcoma, as well as neuroblastoma can lead to bone pain, arthralgia, and/or swelling of joints. Nocturnal bone and joint pain is one of the most characteristic clinical signs for malignancy. Differentiation between systemic JIA and malignancy can be difficult when general malaise, fever, anemia, and leukocytosis are present[67, 102, 122-126].

Another group of diseases that can present with arthritis, as well as arthralgia or myalgia, is the rare group of periodic fever syndromes like familial Mediterranean fever; hyper IgD syndrome; TNF receptor-1 associated periodic syndrome; Muckle-Wells syndrome; and periodic fever, aphtous stomatitis, pharyngitis, and adenitis[127]. These genetic diseases are also known as auto-inflammatory disorders. Other possible causes of arthritis and arthralgia in childhood include coxitis fugax, Perthes disease, epiphysiolysis, Osgood-Schlatter disease, chondromalacia, hypermobility syndromes, and trauma[128].

TREATMENT

The treatment of children with JIA is mostly medical but physiotherapy and other non-pharmacological therapies are also of importance in some patients. Nowadays, children are less frequently referred to a rehabilitation center where therapies can be organized in a multidisciplinary way. In the last two decades an expanding spectrum of effective drugs has

become available[129]. The arrival of biologicals has made a significant difference initially to children who were refractory to previously used anti-rheumatic drugs. But as time progressed biologicals were prescribed earlier to JIA patients[130]. Nowadays the biggest challenges are to select the right JIA patients for treatment with biologicals[131] and to choose the optimal moment to apply them. Furthermore, a concern is how to deal with biosimilars in the treatment of patients with JIA. Long-term safety is guarded by the use of international databases.

Outcome Measures

To measure efficacy of medical treatment a definition of improvement[132] has been developed using a core set of outcome variables and a definition of remission (clinically inactive disease, CID) is available[133]. Definitions have followed for clinical remission on (CID on 6 months therapy) and off medication (CID for more than 12 months off therapy)[134]. More recently the juvenile arthritis disease activity score (JADAS) was developed[135], including cut-off values[136] summarized in table 1.3.

Increasing evidence[137, 138] advocates the early initiation of therapy depending on mild, moderate, or severe disease clinical characteristics. Several guidelines for children with oligoarticular and polyarticular JIA are available and summarized in the paper by Hinze et al[88,87]. Illustrative algorithms for children with oligoarticular and polyarticular JIA can be found in the paper by Beukelman[139].

Drugs Used in Children With JIA

NSAIDs are effective in suppressing fever and signs of inflammation such as pain and stiffness. Naproxen, ibuprofen, and indomethacine are frequently used. Pseudoporhyria can occur with the propionacid derivatives like naproxen and ibuprofen and induce scarring of sun-exposed areas. Indomethacine can cause headache and malaise in some children.

As DMARDs hydroxychloroquine, sulfasalazine, MTX, and leflunomide are used. Regular blood checks are advised, especially liver function tests. Retinopathy can occur as an adverse reaction to hydroxychloroquine accumulation. Ophthalmological follow-up is advised every 6 months. Sulfasalazine is effective and safe in children with oligoarticular and polyarticular JIA, but not well tolerated in about one-third of children[140]. Sulfasalazine may induce or worsen low levels of IgA[87]. Nowadays sulfasalazine is mainly reserved for the category of JIA with enthesitis[139]. MTX has gained the position of gold standard for RA and is also very effective in JIA patients[141, 142] as monotherapy, but also in combination therapy with a biological[143-145]. It is administered once a week as tablets or subcutaneous injection. The use of folic acid can decrease nausea, malaise, and mucosal ulcerations[146].

Table 2.3 | Definitions of Disease Status an Juvenile Arthritis Disease Activity Score Cut-off Values

Clinical Condition	Cut-off Value
Clinical remission	1
Minimal disease activity	Oligo 2 Poly 3.8
Acceptable symptom state	Oligo 3 Poly 4.3
Low disease activity	One or no active joints, normal ESR of C-reactive protein, physician global assessment of overall disease activity score < 3 (0-10), patients/parent GA of overall well-being score < 2 (0-10)

In some children anti-emetics are necessary to alleviate the weekly misery of MTX. During high-dose treatment with MTX, vaccination with live attenuated viruses should be avoided. Further advice on vaccinations in JIA patients is summarized in the European League Against Rheumatism recommendations by Heijstek et al[147]. Leflunomide is tolerated well by most children with slightly less efficacy as MTX[148]. The use of systemic glucocorticoids should be restricted to life-threatening complications. In some children low-to-medium doses glucocorticoids are used to bridge the interval between start and moment of effectiveness of DMARDs. Glucocorticoids are mainly used as intra-articular injection with good results[149] and no short-term adverse effects on the cartilage. Leakage to the system may induce secondary Cushing syndrome[150]. Triamcinolonhexacetonide has shown to be superior to triamcinolonacetonide judged by the duration of remission[151]. The biologicals have extended the therapeutic arsenal for children with therapy-resistant JIA. To hamper TNF-a both soluble receptors (etanercept) as blocking antibodies (infliximab, adalimumab) are available, but for the use in children only etanercept and adalimumab are registered. The child has to fail on sufficiently high-dose MTX or show unacceptable side effects. About 75% of children with longstanding, resistant, polyarticular course JIA respond to etanercept in a blinded randomized controlled trial[152]. The clinical improvement lasts for over 2 years in the majority of patients[153] without significant adverse events[154]. Since 2008 abatacept, a CTLA4/IgG fusion protein-inhibiting T-cell activation by co-stimulation blocking, was added to the spectrum of therapeutics. In the original withdrawal trial[155] significantly more flares occurred in the placebo group compared to the abatacept group. It has to be noted that during the initial open-label period approximately 25% did not respond to abatacept, comparable to nonresponse rates as observed in TNF-a blockers. Tocilizumab is a humanized, monoclonal, IL-6-receptor antibody, inhibiting IL-6-mediated signaling. Recently the efficacy of tocilizumab in polyarticular course JIA was noted[156]; likewise, in a withdrawal trial design with significantly more flares in the placebo group compared to the tocilizumab group. Tocilizumab for polyarticular course JIA is dosed in 4-week intervals compared to 2-week intervals in systemic JIA. The GO-KIDS trial of golimumab

(a human monoclonal antibody that binds both to soluble and transmembrane TNF-a) in polyarticular course JIA has not met its primary and secondary end-points[157], therefore previously preventing golimumab from registration for JIA.

FUTURE

In the near future the development of other anticytokine-directed strategies (certolizumab, ustekinumab, tofacitinib)[158] holds great promise for the treatment of children with resistant JIA although the concerns about infections and long-term consequences remain[159].

Concerns of Infectious Complications During (Biological) Treatment

Large cohort studies in JIA patients have not revealed increasing numbers of severe infections, requiring hospital admission due to biological treatment[160]. Having JIA without DMARD treatment increased the changes on these complications twofold, which remained twofold with the use of MTX or biologicals irrespective of MTX. Swart et al. reported that additional biologicals increased the chances to severe adverse events and infections compared to MTX only[161].

Concerns of Opportunistic Infections During (Biological) Treatment

Opportunistic infections are rare in JIA patients. In almost 14,000 person-years of follow-up a few cases of coccidioides (occurring while not on biological treatment) and Salmonella were mentioned, as well as 32 cases of herpes zoster[164]. An increased incidence rate of herpes zoster in JIA patients on etanercept was confirmed by Nimmrich et al[162].

Concerns of Development of Other Autoimmune Diseases

Under the use of diverse biologicals, cases of other autoimmune diseases like demyelinating disease, inflammatory bowel disease, or the new development of autoantibodies have been documented, which is summarized in the manuscript by Swart et al[159].

Concerns on Malignancies During Biological Treatment

In 2008 a black-box warning on the use of biologicals in children with JIA was warranted by the FDA. To know the background rate of malignancy in JIA, it is essential to address this issue. Studies on this topic are scarce and difficult due to relative small sample sizes[163, 164]. Beukelman demonstrated no increase in malignancies due to use of TNF inhibitors, but in general a small increased rate of incident malignancy in JIA was noted[165]. Large registries are highly needed to gather information on this topic and initiatives to do so are evident both in the United States and Europe[159, 166]. Autologous stem cell transplantation was

used as an experimental treatment in children with therapy refractory polyarticular and systemic JIA[167] with significant mortality. In recent years less children were candidates for this procedure as a consequence of high effectiveness of biologicals. Complications are less common due to better treatment strategies, therefore diminishing the need for treatment of complications. The experimental use of recombinant human growth hormone restores linear growth and improves body composition in children with glucocorticoid-induced impaired growth and severe osteoporosis[168, 169].

The treatment of uveitis consists of topical and systemic drugs

Treatment recommendations for JIA-associated uveitis are summarized in a recent guideline[170]. Topical glucocorticoids are the first choice of treatment and sometimes are combined with mydriatic agents. Subtenon injections of steroids can be used. A large group of patients with uveitis need systemic treatment to achieve adequate disease control. Systemic glucocorticoids are effective, but the use should be minimized because of the harmful effects on bone and growth. Systemic glucocorticoids may also contribute to cataract formation and glaucoma. Intravenous pulses of glucocorticoids (30 mg/kg per dose with a maximum of 1g) may be effective at lower risk of side effects. MTX and cyclosporin A can be effective and glucocorticoid sparing, but reports about the use of immunosuppressive drugs are scarce[171]. Other agents that are reported to have some effect in smaller series are mycophenol mofetil, intravenous immunoglobulins, and anti-TNF-a drugs, especially adalimumab. Immunomodulatory therapy started early in the course of uveitis is associated with better visual acuity[172]. Several cases of a flare of uveitis or de novo development of uveitis are reported during the use of etanercept[154, 173, 174].

The use of abatacept in severe therapy refractory uveitis did not lead to sustained benefit in 21 patients[175].

PROGNOSIS

Literature on the outcome of JIA in the prebiological era described ongoing arthritis in almost half of the patients with polyarticular JIA after 10 years[176]. In addition, oligoarticular-onset JIA was shown to be a severe disease with frequent complications[177]. In 2003 Fantini et al. reported that 75% of their patients with a minimum follow-up of 10 years never reached remission[178]. The course of the disease and outcome depend on the category of JIA and the presence of IgM RF or ANA[179, 180]. Patients with ANA-positive JIA seem to constitute a homogeneous subgroup[181] and ocular complications determine visual prognosis in this subgroup[82, 172]. The prognosis of IgM-RF polyarticular JIA can be compared with that of RA in

adults. After introduction of the biologicals, outcome has improved due to earlier initiation of therapy and efficacy of TNF blockers and other biologicals reflected by almost 80% of JIA patients reaching an active joint count of 0 after 2 years in the ReACCh-Out cohort[182], although flares still frequently occur[183]. At this moment JIA remains a chronic disease with considerable psychosocial impact that often extends into adulthood[184], although outcome has improved over the years[185-187].

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Anticarbamylated protein (anti-CarP) antibodies are present in sera of Juvenile Idiopathic Arthritis (JIA) patients

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Key words: Juvenile Idiopathic Arthritis, anti-CarP antibodies, anti-citrullinated protein antibodies.

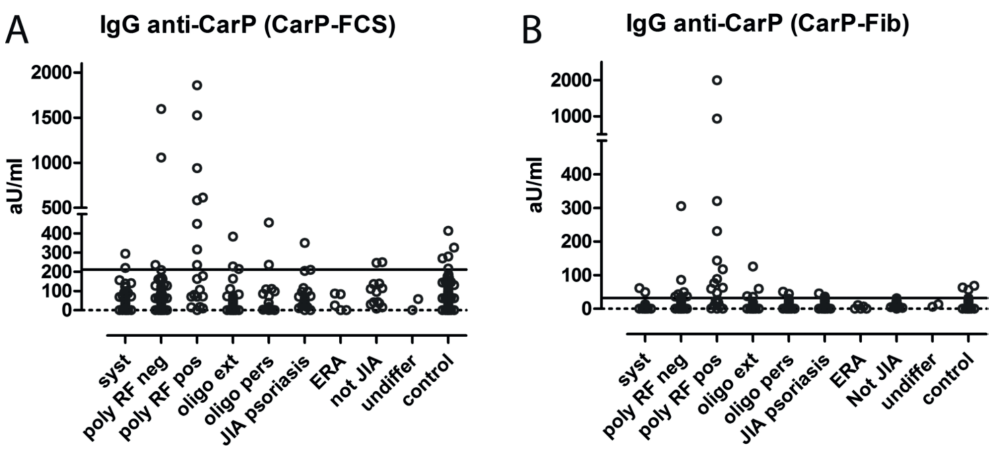
In Juvenile Idiopathic Arthritis (JIA) patients there is a lack of markers that predict severe disease. Although anti-citrullinated protein antibodies (ACPA) have contributed substantially to the understanding of Rheumatoid Arthritis (RA)[1], their detection in JIA has not been equally useful as incidence rates in JIA patients are low[2] and merely confined to the polyarticular IgM-RF positive category resembling RA. Recently, anti-carbamylated protein antibodies (anti-CarP) were detected in 45% of RA patients and importantly also in 16-20% ACPA-negative patients[3-5]. Within the ACPA-negative patients, anti-CarP antibodies were associated with more severe radiographic progression[3]. Since most JIA patients are ACPA-negative we investigated whether anti-CarP antibodies are present in sera of JIA patients and how they are related to ACPA and IgM-RF.

JIA patients from three Dutch sources were included: the *BeSt for Kids trial* (NTR 1574, a treatment strategy study) (n=33), a previously described cohort[6] (n=48) and the Arthritis and Biologicals in Children (ABC) Register[7] (n=153). Healthy controls (n=107) (mean age/ range 11/(2-20)) are stem-cell graft donors. Written informed consent was obtained from all patients and controls. Blood collection and storage are comparable among different cohorts. Cross-sectionally obtained sera from 234 JIA patients at variable time points in disease course were analyzed. All ILAR JIA categories were included[8] with polyarticular JIA overrepresented. Median disease duration at the time of serum collection was 2.3 years (IQR 0.7-6.8) (Table 1). Patients' disease characteristics were collected from patient files. Anti-CarP and ACPA antibodies were measured by ELISA as described previously.[3]

Table 1 | Disease characteristics of 234 JIA patients

Characteristics	**Number**
Gender m/f (%f)	76/158 (67,5%)
Median age (years) (IQR)	12.1 (8.4-16.2)
Median disease duration (IQR)	2.3 (0.7-6.8)
Median age at JIA onset (IQR)	8.8 (3.4-12.4)
ANA positive at disease onset	64 (27,4%)
Systemic JIA	35 (15,0%)
Poly-articular JIA RF negative	90 (38.5%)
Poly-articular JIA RF positive	19 (8,1%)
Oligo-articular JIA extended	41 (17,5%)
Oligo-articular JIA persistent	18 (7.7%)
Juvenile Psoriatic Arthritis	24 (10,3%)
Enthesitis Related Arthritis	5 (2.1%)
Undifferentiated	2 (0.8%)

We observed that 8.1% (19/234) of the JIA patients were positive for anti-Ca-FCS antibodies versus 4.7% (5/107) of controls (p=0.20); 13.2% (31/234) of patients vs 2.8% (3/107) of controls were positive for anti-Ca-Fib antibodies (p=0.003); 16.7% (39/234) of patients vs 8/107 (7,5%) of controls were positive for at least one anti-CarP antibody (p=0.028); 11/234 (4,7%) vs 0 of controls (p=0,017) were positive for both anti-CarP reactivities. Both anti-Ca-FCS and anti-Ca-Fib antibodies were predominantly present in polyarticular IgM-RF positive patients compared to other JIA categories (p<0.0001) (Figure 1). Additionally 53% (8/15) of ACPA-positive patients and 42.1% (8/19) of IgM-RF-positive patients were also



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	anti-CarP-FCS		anti-CarP-Fib	
	n/total (%)		n/total (%)	
All JIA in total	19/234 (8.1%)	NS	31/234(13.2%)	**
Systemic onset JIA	2/35 (5.7%)	NS	2/35(5.7%)	NS
Polyarticular RF negative	3/90 (3.3%)	NS	9/90(10%)	*
Polyarticular RF positive	8/19 (42.1%)	**	11/19(57.9%)	**
Oligo articular extended	3/41 (7.3%)	NS	4/41(9.8%)	NS
Oligo articular persistent	2/18 (11.1%)	NS	2/18(11.1%)	NS
Juvenile Psoriatic Arthritis	1/24 (4.2%)	NS	3/24(12.5%)	NS
Enthesitis related Arthritis	0	NS	0	NS
Undifferentiated	0	NS	0	NS
Controls	5/107 (4.7%)		3/107 (2.8%)	

Figure 1 | IgG anticarbamylated protein (anti-CarP) antibodies are present in juvenile idiopathic arthritis (JIA) sera. A cut-off for positivity(horizontal line) was determined using the mean plus two times the SD of the healthy controls. Antibodies against Ca-FCS (A) and Ca-Fib (B) in thesera of JIA patients and healthy controls are depicted in aU/mL. (C) Results of anti-CarP antibodies: positivity above cut-off per JIA category inabsolute number, percentage and significance (NS, not significant, *p<0.05, **p<0.01). FCS, fetal calfs serum; RF, rheumatoid factor.

positive for anti-CarP antibodies. Importantly, anti-CarP antibodies were also found in ACPA and IgM-RF-negative patients as 57,9% (11/19) of anti-CarP positive patients were negative for ACPA and 27,3% (3/11) were negative for IgM-RF. In total 9 JIA patients were positive for IgM-RF, ACPA and anti-CarP (Ca-FCS and/or Ca-Fib) antibodies. All triple positive patients were part of the ABC-register.

Disease duration at sample collection, ANA status or age were not associated with the presence of anti-CarP antibodies. In the second cohort[6] we did not find an association of anti-CarP positivity with disease activity measured by time-in-active-disease at the time of sampling. Within the ABC-register cohort no association was found between the presence of anti-CarP antibodies and ACR-Pedi 30 response[9] or reaching inactive disease[10] at 15 months after start of anti-TNF treatment. The cross-sectional nature of these three cohorts did not allow a more in depth analysis on association with clinical outcome.

This is the first study showing the presence of anti-CarP antibodies in JIA stimulating future studies on the diagnostic and prognostic value of anti-CarP antibodies in JIA.

Competing Interest: None declared.

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Disturbance of microbial core species in new onset Juvenile Idiopathic Arthritis

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ABSTRACT

Over the past decades, the intestinal microbiota has increasingly gained attention in studies addressing pathophysiology of (paediatric) autoimmune diseases, like inflammatory joint diseases, inflammatory bowel disease (IBD) and type 1 diabetes. In this study, we have analysed composition of gut microbiota of newly diagnosed juvenile idiopathic arthritis (JIA) patients, prior to initiation of disease-modifying antirheumatic drugs (DMARD). Faecal microbiota profiles of 8 JIA patients (median age 11,1 years, 6 girls) were compared to 22 healthy age-matched controls using IS-pro, a 16S-23S interspacer (IS) region-based, eubacterial molecular detection technique. By partial least squares discriminant analysis (PLS-DA), microbiota profiles of JIA and controls could significantly be discriminated based on a limited set of species belonging to the phylum *Bacteroidetes* (Fig B), with sensitivity of 88% and specificity of 73% (Fig C), Area Under the Curve (AUC) 0.87 (95% CI: 0.73-0.87), but not within other phyla. These discriminative species have been considered to be part of the microbial core in healthy children.

Conclusion: Our findings add to the increasing notion that the gut microbiota may be involved in the pathophysiology of JIA. Involved species in the discrimination between JIA and controls are members of the microbial core in health state. Expanding knowledge on JIA-specific microbial signatures and host-interactions may open avenues to explore options to develop individualized, microbiota-based preventive and therapeutic interventions in JIA.

Key words: Autoimmunity, juvenile idiopathic arthritis, intestinal microbiota, gut, S-pro
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INTRODUCTION

In the pathogenesis of Juvenile Idiopathic Arthritis (JIA) environmental triggers are considered to provoke the onset of symptoms in genetically susceptible hosts[1]. Auto-reactive T-cell responses to yet unrecognized antigens have been described to trigger an inflammatory response. In adults the role of the intestinal microbiota in the aetiology of rheumatoid arthritis and other auto-immune diseases has been increasingly recognized[2]. Exemplary are Reactive arthritis after a gastro-intestinal infection and the association between arthritis and jejuno-ileal-bypass surgery[3,4]. In the latter, disruption of gastrointestinal anatomy provokes microbial disturbance by small intestinal bacterial overgrowth, leading to antibody production and synovial inflammation. Multiple animal studies have established a link between the intestinal microbiota in the onset or reduction of arthritis. Illustrative is the occurrence of adjuvant-induced arthritis exclusively in rats grown in germ-free conditions. The potential immunomodulatory effect of the microbiome is also reflected in the protective role of some enterobacteria in arthritis-susceptible rats [2]. In humans with new-onset rheumatoid arthritis (RA) one recently described example is the higher abundance of *Prevotella copri* in combination with less *Bacteroides* compared to controls[2].

In RA, disturbance of bacterial homeostasis (dysbiosis) is considered to provoke an increased mucosal permeability, loss of immune tolerance to microbial components and trafficking of immune cells and antigenic material to joints, provoking an inflammatory cascade leading to RA[5].

Analogue to RA, an aetiological role for the gut microbiota has been suggested in JIA.[6-9] Comparable observations on alterations in microbiota are currently rapidly accumulating for other paediatric auto immune diseases, like type 1 diabetes [10] and IBD [11]. However, despite recognition of disease-specific microbial signatures for different diseases, it remains largely unclear whether microbial changes precede or are rather a consequence of these diseases. Aim of this prospective, case-control study was to describe composition and diversity of intestinal gut microbiota of children with new-onset, DMARD-naive JIA, compared to age-matched, healthy controls.

MATERIALS AND METHODS

JIA patients, enrolled in the BeSt for Kids study (dutch trial register 1574), were eligible to participate in this prospective pilot-study. The BeSt for Kids study is a multi-center

clinical trial which included patients with newly diagnosed JIA and aimed to investigate treatment strategies. (1) initial methotrexate or sulphasalazine monotherapy, (2) initial therapy with methotrexate and prednisolone, (3) initial therapy with methotrexate and etanercept in certain categories (rheumatoid factor negative oligo- or polyarticular JIA, JIA with psoriasis) of DMARD-naive JIA patients[12]. From September 2011 to May 2012, eight consecutive patients were included in this add-on study and instructed to collect and store a faecal sample in regular freezers until centrally stored at -20˚C. Controls were 22 age-matched children, selected from a cohort consisting of 61 healthy children, aged 2-18 years, who participated in a previous study on microbiota composition and microbial dynamics in healthy state [13]. Similar exclusion criteria were applied to both groups: an episode of infectious gastroenteritis within 3 months prior to inclusion, use of antibiotics or immune-modulating agents both within 3 months prior to inclusion, history of major surgery of gastrointestinal tract and an established diagnosis of chronic gastro-intestinal disease (celiac disease, short bowel syndrome, IBD).

Ethical considerations

This study was conducted according to the principles expressed in the Declaration of Helsinki. The protocol was approved by the Medical Ethical Committee from the Leiden University Medical Center. JIA patients and parents signed a written informed consent.

IS-Pro

We used IS-pro, an eubacterial molecular detection technique to characterize the microbiota[13,14] . IS-pro makes use of phylum-specific fluorescently labelled PCR primers and differentiates bacterial species by the length of the 16S–23S rDNA IS region. For a detailed description of the used protocol on DNA isolation and sample preparation we refer to previous reports[13,14]. The procedure consists of two separate multiplex PCRs: the first PCR contains two different fluorescently labeled primers. One amplifying the phyla *Firmicutes, Actinobacteria, Fusobacteria* and *Verrucomicrobia* (FAFV) and the other labeled primer for the phylum *Bacteroidetes*. A separate PCR with a third labeled primer is performed for the phylum *Proteobacteria*. The resulting polymerase chain reaction (PCR) products were subsequently amplified by means of IS-pro [10].

DATA ANALYSIS

DNA fragment analysis was performed on an ABI Prism 3130XL Genetic Analyzer (Applied Biosystems Carlsbad, California, USA). Data were further analyzed with the BioNumerics (Applied Maths, Sint-Martens-Latem, Belgium) and Spotfire (TIBCO, Palo Alto, CA, USA)

software packages. Correlation between profiles were calculated with Pearson's product-moment correlation coefficient on log2 transformed data. Fragments were assigned a taxonomic classification based on fragment length. Lengths were compared to a database consisting of IS lengths of known bacterial species. This classification was confirmed by separating fragments on an agarose gel and excising and sequencing these fragments. Sequences were compared to the GenBank database with the BLAST algorithm. Taxonomic classification to species level was based on >97% sequence identity[14].

Within-sample microbial diversity was calculated as the Shannon diversity index based on the resulting profiles using the R 2.15.2 software package. Diversity was calculated both per phylum and for overall microbial composition (by pooling the phyla *FAFV, Bacteroidetes* and *Proteobacteria* together). A p-value of < 0.05 was considered statistically significant.

Partial least squares discriminant analysis (PLS-DA) regression, a supervised classification technique, was used for the prediction of the clinical status of faecal samples, JIA or healthy[15]. This statistical model was performed for each phylum and for all phyla together to predict case-control classification. Validation of this PLS-DA model was carried out by a 10-fold cross-validation procedure[16]. In practice, the dataset was split into 90 % of samples for model construction (i.e., training set), with the aim to predict the other 10 % (i.e., test set). This procedure was repeated for ten iterations, where each sample served as a test sample exactly once. Accuracy rates, specificity, and sensitivity were computed for the samples that were used as a test set in every iteration, and the model predictive power was further assessed using a receiver operating characteristic (ROC) curve with calculation of the Area Under the Curve (AUC) and 95% CI values.

RESULTS

All included patients suffered from polyarticular rheumatoid factor negative JIA. Other patient characteristics are shown in table 1. No significant differences in microbial diversity were observed between JIA and controls; median Shannon diversity index for all phyla together was 3.92 and 3.88, for *Bacteroidetes* 2.95 and 2.87, for *Proteobacteria* 2.75 and 2.75 and for *FAFV* 2.41 and 2.75, respectively . A heatmap consisting of all microbial data from all JIA patients and healthy controls is shown in Figure A. By partial least squares discriminant analysis (PLS-DA), profiles of JIA and controls could significantly be discriminated on the level of the phylum *Bacteroidetes* (Fig B), with sensitivity of 88% and specificity of 73%, AUC 0.87 (95% CI 0.73-0.87) but not within other phyla (Fig C). Most discriminative species between the two subgroups were *Alistipes finegoldii* and *Prevotella multisacharivorax* (decreased in JIA) and *Bacteroides fragilis* (increased in JIA).

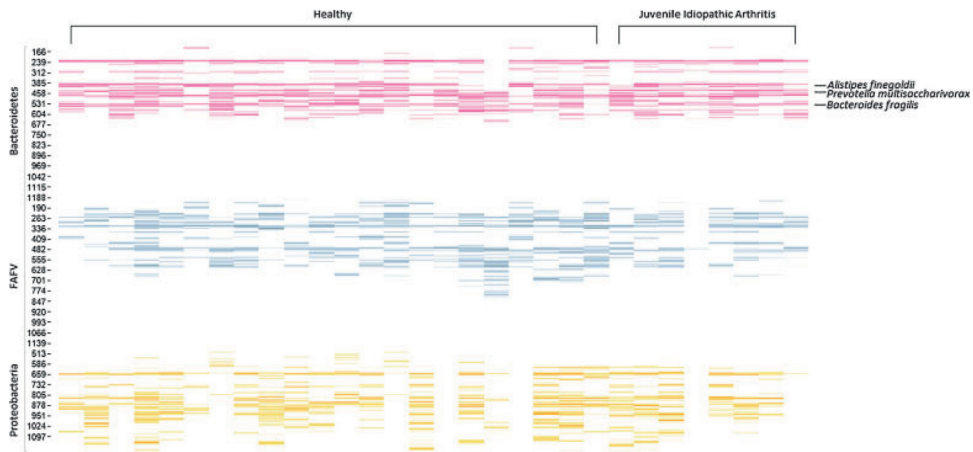

Figure 1 | Heat map displaying IS profiles of 8 children with JIA and 22 healthy controls. Individual subjects are shown on the X axis; children with JIA in red, healthy controls in green. On the Y axis, IS fragment lengths are expressed in number of nucleotides, corresponding with bacterial strain type (OTU). Blue peaks represent OUT belonging to the phyla *Firmicutes, Actinobacteria, Fusobacteria, Verrucomicrobia* (FAFV), red peaks represent *Bacteroidetes* and yellow peaks represent *Proteobacteria*. Intensity of the colors reflect the relative abundance of each indicated OTU, grey signals represent less prevalent IS fragment lengths. The most discriminative OTUs between both study groups, as calculated by PLS-DA, were *Alistipes finegoldii* (peaks 231/396/400/406), *Prevotella multisacharivorax* (peak 437) and *Bacteroides fragilis* (peak 537), all within the phylum *Bacteriodetes*.

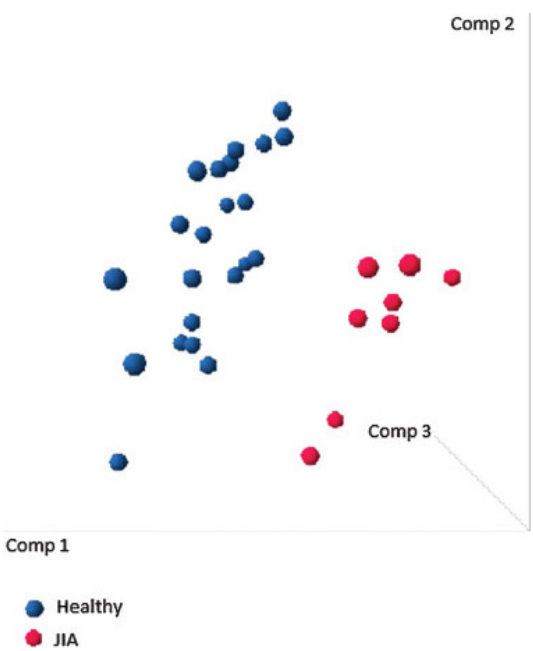

Figure 2 | The PLS-DA scores plot for the phylum *Bacteroidetes* shows a clear differentiation between JIA cases and controls

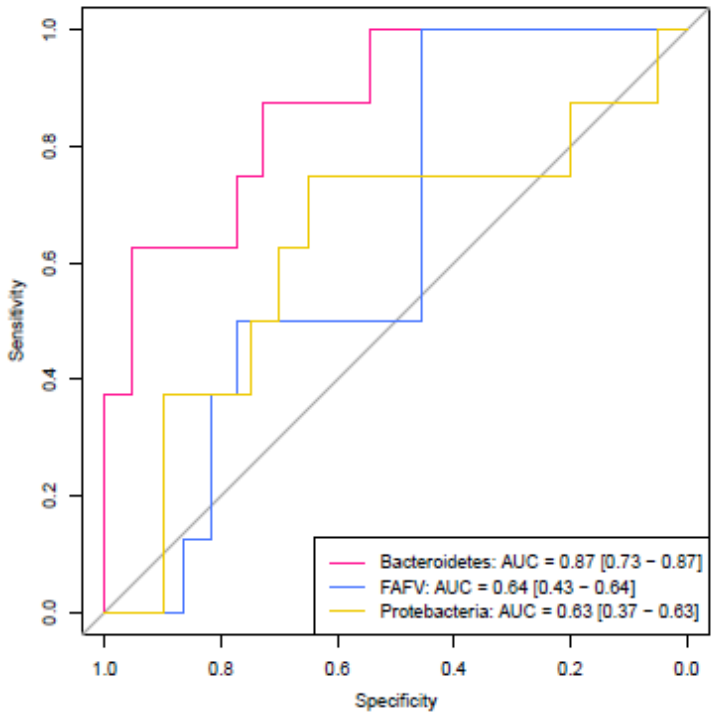

Figure 3 | Receiver operating characteristic (ROC) curves summarizing the predictive power of the PLS-DA model for clinical status (JIA or control) per phylum, including 95% confidence intervals

DISCUSSION

In this study we have shown that gut microbiota in JIA can be discriminated from matched controls with high accuracy, based on a limited set of species belonging to of the phylum *Bacteroidetes*. In a recent study by Tejesvi et al[6], faecal microbiota analysis of 30 patients with newly onset JIA revealed an increased abundance of species belonging to the phylum *Bacteroidetes,* in particular *Bacteroides spp.,* and a low abundance of bacteria within the phylum of *Firmicutes* compared to controls[6]. Secondly, Stoll et al[7] observed a reduced abundance of faecal *F. prausnitzii* in juvenile enthesitis related arthritis (ERA) patients compared to controls. Additionally, a non-statistically significant increased abundance in *Bacteroides spp.* and *Akkermansia municiphila* was observed in subsets of ERA patients, suggesting a role for a humoral response to these specific species in the pathophysiology[7]. An increased abundance of *Bacteroides spp.* was observed in paediatric ERA patients, with decreased abundance of *Prevotella spp*[8]. Di Paola et al. described different microbial profiles between JIA-ERA patients and non-ERA patients versus healthy controls and, notably,

Table 1 | Patient and control characteristics

	JIA patients n=8	Controls n=22
Female (%)	6 (75)	11 (50)
Median Age (years)	11.1 (7.3-13.1)	8.7 (7.5-11.5) (p=0.33)
ANA positive (%)	3 (38)	NA
Median clinical symptoms (months)	7.1 (4.4-13.2)	NA
VAS physician (mm)	47 (32-58)	NA
VAS patient well-being (mm)	32(27-52)	NA
ESR (mm)	8 (2-9)	NA
Active Joint Count	10 (7-14)	NA
Limited Joint Count	2 (0-4)	NA
CHAQ score (0-3)	1.2 (0.4-1.7)	NA

ANA, Antinuclear Antibodies; VAS, Visual Analogue Scale; ESR, erythrocyte sedimentation rate; CHAQ, Child Health Assessment Questionnaire; NA, not applicable. Characteristics are presented in medians (Interquartile Range).

also between active disease and remission state.[9] Similar alterations in microbiome were previously reported in paediatric type 1 diabetes and IBD[10,11]. This study adds as novelty the increased knowledge on the composition of the microbiota of a large control group of healthy children[13]. In that study, species within the phylum *Bacteroidetes* were described to be dominant members of a shared microbial core in healthy state [13]. Notably, we found in the present study that JIA could be differentiated from controls based on alterations in the abundance of species belonging to this healthy core. This suggests that onset of JIA may be associated with disruption of this microbial core and that JIA-related dysbiosis is rather reflected by a loss of healthy microbial state than by the introduction of pathogens. The high accuracy to discriminate new-onset JIA from healthy state based on a limited number of bacterial species, as observed in this study, may have several implications. Firstly, a microbiota-based test with high sensitivity may have the potential to serve as a diagnostic tool in clinical practice. However, to reliable assess the potential of microbiota analysis as a diagnostic instrument, comparison with an intention-to-diagnose cohort, including children with suspected JIA, is preferable, and has to be tested in future studies. Secondly, the presence of a JIA-specific microbial signature, which can robustly discriminate diseased state from controls could possibly allow for the development of microbiota targeted therapeutic or even preventive interventions in JIA treatment.

Future studies are needed to externally validate our findings, to address the significance of the observed disturbance of core species and to assess whether clinical remission of JIA merges with restoration of this core. In a recent review, an overview was given of

current knowledge on the role of gut microbiota in JIA aetiology, including factors possibly predisposing to dysbiosis, and mechanisms by which altered microbiota might predispose to arthritis[17]. Important factors influencing the composition of microbiota are mode of delivery[18], feeding habits in early life[19] and exposure to (multiple) medication, in particular antibiotics[17]. Sex related differences and body morphometrics have also been described to affect microbiota composition, however, in the control group of this study, microbial communities did not differ between both sexes[20-23]. Limitation of this study is that the cohort was too small to take different environmental factors possibly affecting microbiota composition into account, increasing the risk for type I error.

In conclusion, our observation of intestinal dysbiosis in new-onset JIA confirms the increasing notion that aberrant microbiota composition may play a role in the aetiology of JIA. In particular, we found compositional alterations in species within the phylum *Bacteriodetes*, which have been described dominant members of a microbial core in healthy state.

Expanding knowledge on JIA-specific microbial signatures and host-interactions may open opportunities to explore the options to develop individualized, microbiota-based preventive and therapeutic interventions in JIA.

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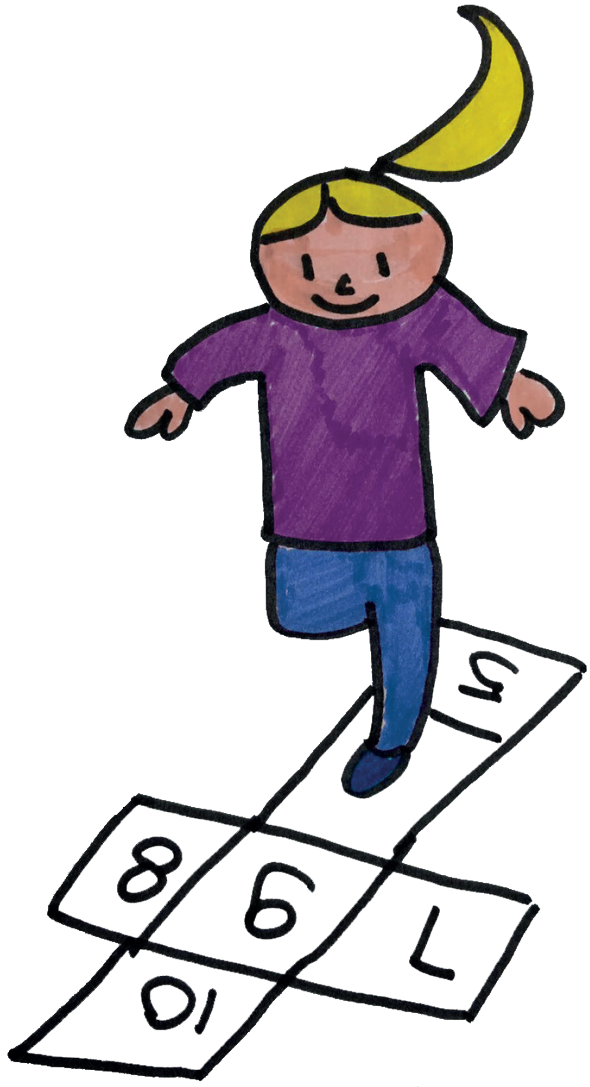
PART TWO

CLINICAL ASPECTS
Treatment Strategies



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A comparison of three treatment strategies in recent onset DMARD naive non-systemic Juvenile Idiopathic Arthritis: initial 3-months results of the Best for Kids-study

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ABSTRACT

Background: Combination therapy with prednisone or etanercept may induce earlier and/or more improvement in disease activity in Disease Modifying Anti Rheumatic Drug (DMARD) naive non-systemic Juvenile Idiopathic Arthritis (JIA) patients. Here we present three months clinical outcome of initial treatments of the BeSt-for-Kids study**.**

Methods: Included patients were randomized to either: 1. initial DMARD-monotherapy (sulfasalazine (SSZ) or methotrexate (MTX)), 2. Initial MTX / prednisolone-bridging, 3. Initial combination MTX/etanercept. Percentage inactive disease, adjusted (a) ACR Pedi30, 50 and 70 and JADAS after 6 and 12 weeks of treatment (intention to treat analysis) and side effects are reported.

Results: 94 patients (67% girls, 32 (arm 1), 32 (arm 2 and 30 arm 3) with median (InterQuartileRange) age of 9.1 (4.7-12.9) years were included. 38% were ANA positive, 12 had oligo-articular disease, 68 polyarticular JIA and 15 psoriatic arthritis. Baseline median (IQR) ACRpedi-scores: VAS physician 49 (40-58) mm, VAS patient 54 (37-70) mm, ESR 6.5 (2-14.8)mm/hr, active joint count 8 (5-12), limited joint count 3 (1-5), CHAQ score 0.88 (0.63-1.5). In arm 1, 17 started with MTX, 15 with SSZ.
After 3 months, aACR Pedi 50 was reached by 10/32 (31%), 12/32(38%) and 16/30 (53%) (p=0.19) and aACR Pedi 70 was reached by 8/32 (25%), 6/32(19%) and 14/30(47%) in arms 1-3 (p=0.04). Toxicity was similar. Few serious adverse events were reported.

Conclusion: After 3 months of treatment in a randomized trial, patients with recent-onset JIA achieved significantly more clinical improvement (aACRPedi70) on initial combination therapy with MTX / etanercept than on initial MTX or SSZ monotherapy.
Trial registration: NTR 1574. Registered 3 december 2008.

Key words: Juvenile idiopathic arthritis, treat to target, window of opportunity, treatment strategy study, biologicals, inactive disease

INTRODUCTION

Juvenile Idiopathic Arthritis (JIA) is the most common auto-immune disease in children[1] except for systemic JIA which is nowadays viewed as an auto-inflammatory disease[2]. Many children suffer from chronic functional disability and damage due to prolonged inflammation[3]. The ILAR-criteria divide the heterogeneous disease in 7 categories[4]. Prognosis is difficult to predict and even oligoarticular disease can have a debilitating course[5]. Nowadays an expanding repertoire of disease modifying antirheumatic drugs (DMARD) including biologicals is available for treatment[6]. Evidence-based information is available on the efficacy of individual products[7-15] but knowledge on therapeutic strategies in children is still scarce[16, 17]. As shown in the BeSt study in rheumatoid arthritis patients[18], it is likely and was illustrated previously that also in JIA a window-of-opportunity exists where the disease is most responsive to treatment and susceptible for permanent suppression[11, 16, 17, 19]. Additionally we know that an early response to therapy is related to a better outcome[20, 21].

In the current study we investigate which of 3 treatment strategies is most effective, fast-acting and safe in a randomized clinical trial comparing three initial therapies: arm 1 initial monotherapy with MTX or SSZ; arm 2 initial combination therapy with MTX and prednisolone and arm 3 initial combination therapy with etancercept and MTX. We hypothesized that compared to initial monotherapy (arm 1) with SSZ or MTX or initial combination therapy with MTX/prednisone (arm 2) early treatment with etanercept and methotrexate (arm 3) would lead to significantly more and earlier clinical inactive disease.

METHODS

Patients

Patients diagnosed as DMARD-naive JIA, either rheumatoid factor negative polyarticular, oligoarticular JIA, or juvenile psoriatic arthritis, in need of systemic DMARD therapy according to treating physician, with less than 18 months of complaints, aged between 2-16, were eligible at 5 participating sites in the Netherlands. Patients suffering from rheumatoid factor-positive JIA are preferably treated with combination therapy from the start and were excluded[17] as well as systemic JIA and Enthesitis Related JIA since they comprise of JIA patients with different clinical features potentially increasing heterogeneity. Patients with JIA related uveitis were excluded due to possible exposure to etanercept which is known to be less effective in uveitis treatment[22-26].

Study design

Data are collected through the BeSt for Kids study, an investigator-initiated multicentre randomised single blinded clinical trial which will have 2 years follow-up in three treatment arms in a treat-to-target setting. The study was approved by the Medical Ethical Committee of the Leiden University Medical Center and local Ethical Committees prior to start at each study site. Written Informed consent was obtained from patients above 12 years of age and parents of all participating patients. Patients were enrolled and randomly assigned to one of three treatment arms by variable block randomization, stratified per center, as oligo or polyarticular disease.

Initial treatments

Patients assigned to arm 1 started with Sulphasalazine 50mg/kg up to 2000mg/day or MTX10mg/m2/wk orally or subcutaneous (sc)(max 25mg/wk).

Patients assigned to arm 2 started with MTX 10mg/m2/wk orally or sc (max 25mg/wk) in combination with prednisolone orally 0,5mg/kg for four weeks, tapering by 1 week 0,25mg/kg and 1 week 0,125mg/kg, then stop.

Patients assigned to arm 3 started with a combination of etanercept 0,8mg/kg/wk sc and MTX 10mg/m2/wk orally or sc (max25mg).

Prior to etanercept treatment, all children were screened for tuberculosis by a purified protein derivative skin test and a chest radiograph. All tested negative. Concomitant treatment with non-steroidal anti-inflammatory drugs (NSAIDs) and intra-articular glucocorticoid injections were permitted without a maximum and registered per strategy. Other parenteral glucocorticoids were not allowed. The use of DMARD or oral glucocorticoids was only permitted as dictated by the treatment protocol. All protocol deviations were recorded. All patients received folic acid during MTX treatment.

Assessment of disease activity: definition of improvement and inactive disease

The core set criteria[30] were scored at 6 weeks and 3 months by a research nurse, physical therapist or pediatric rheumatologist who remained blinded to the allocated treatment group during study period. Since the protocol was written in 2008 inactive disease on medication was defined based on the modified Wallace 2004 definition[27] instead of the current definition[28]. Based on previous results[29] we stated that a doctor's overall assessment score below 10 mm (instead of 0 mm) on the VAS indicated no disease activity provided that all other parameters as defined[27] indicated inactive disease. We defined ESR values under 16 mm/h as normal.

Definition of improvement was based on ACRPedi30/50/70%[30]. Changes in outcomes that remained within normal limits (ESR≤16mm/h and VAS physician<10mm) were not taken into account in ACRPedi calculations and were corrected for, resulting in adjusted (aACRPedi30/50/70%) scores.

Juvenile Arthritis Disease Activity Score (JADAS)-10 score were calculated as described previously[31]. Delta JADAS10 was defined as the difference between JADAS10 score at subsequent visits with baseline score.

Toxicity

At each visit (baseline, 6 weeks, 12 weeks), laboratory tests were performed as clinically indicated: complete blood count, liver and kidney function. The treating physician recorded all adverse events (AEs), serious adverse events (SAEs), and if necessary, made treatment adjustments in accordance to the protocol. SAEs were defined as any adverse reaction resulting in any of the following outcomes: a life threatening condition or death, a significant or permanent disability, a malignancy, and (prolonged) hospitalization.

Sample size calculations

Expected percentages of time to inactive disease were extrapolated from available literature in 2008(6, 11-13) and based on estimation. For the comparison of arm 1 versus arm 3, with power > 90% a difference of 10% in arm 1 versus 60% in arm 3 can be detected with two groups of 30 patients assuming a hazard ratio of 8.70, a drop-out rate 20%, a percentage that switched groups 20%, an alpha 0.05, by two-sided log rank test. Based on analogous calculations (PASS2008) two groups of 45 and 54 patients were needed to detect differences between arm 2 versus arm 3 and between arm 1 versus arm 2. Initially 60 patients per arm was aimed for. Due to slow inclusion rate, the study protocol was amended in 2012 to include 3 groups of 30 patients, leaving enough power to compare arm 1 versus arm 3.

Statistical methods

Missing data in core set variables were scarce (<1%). All available data were included for intention-to-treat analysis. Last observation carried forward was used to deal with few missing values (N=5). Student's t-test was used to compare continuous normally distributed variables between groups. Non-parametric Mann Whitney U tests were used otherwise. For dichotomous variables, Pearson's chi-square test was used. A two-tailed probability value of P<0.05 was considered statistically significant. P-values were not adjusted for multiple statistical tests.

The Trial was registered in the Dutch Trial Register number 1574.

RESULTS

Baseline characteristics

Baseline demographics and disease characteristics of the three groups showed no statistically significant differences and are summarized in Table 1.

Table 1 | Baseline demographics and disease characteristics

	arm 1 MTX or SSZ monotherapy (n=32)	arm 2 Combo MTX+ 6 wks prednisone (n=32)	arm 3 Combo MTX+ etanercept (n=30)
Age (years)	8.8 (4.8-12.7)	10.2 (6.6-13.9)	8.6 (4.2-12.4)
Symptom duration* (month)	7.8 (5.3-11.6)	5.9 (4.4-13.3)	8.5 (5.0-13.1)
ANA positive (%)	15 (47)	11 (34)	9 (30)
Female (%)	24 (75)	19 (59)	20 (67)
JIA category:			
Oligo (persistent)	5 (3)	3 (1)	2 (2)
Polyarticular	22	22	24
Psoriatic (poly)	5	7	4
VAS physician (mm)	48 (40-55)	50 (39-58)	51 (37-61)
VAS patient/parent (mm)	48 (31-58)	59 (35-74)	58 (39-71)
CHAQ (0-3)	0.88 (0.28-1.50)	0.94 (0.63-1.69)	0.88 (0.75-1.53)
No. active joints	7.5 (5.0-12.5)	7.5 (6.0-11.8)	8.5 (5.8-13.0)
No. limited joints	2 (0-4.5)	2 (1.0-3.8)	3 (1.8-5.0)
ESR (mm/hour)	6.5 (2-11)	6.0 (2-24)	9.0 (4-25)
JADAS-10 (0-40)	15.7 (13.5-20.2)	17.9 (15.2-21.9)	19.1 (13.8-23.2)

All results in medians (InterQuartile Range) unless stated otherwise;*time from first presenting symptoms to inclusion in the study

Outcome

Figure 1 shows the flow diagram of the study. 94 patients with early JIA, with a median duration between diagnosis and inclusion of 6 weeks (IQR 3-14) and a median duration of symptoms of 7.5 months (IQR 5-12,5), were randomized to one of three treatment groups: 32 patients assigned to monotherapy (arm 1), 32 patients assigned to combination with methotrexate and prednisone-bridging (arm2) and 30 patients were assigned to combination of etanercept and methotrexate (arm 3).

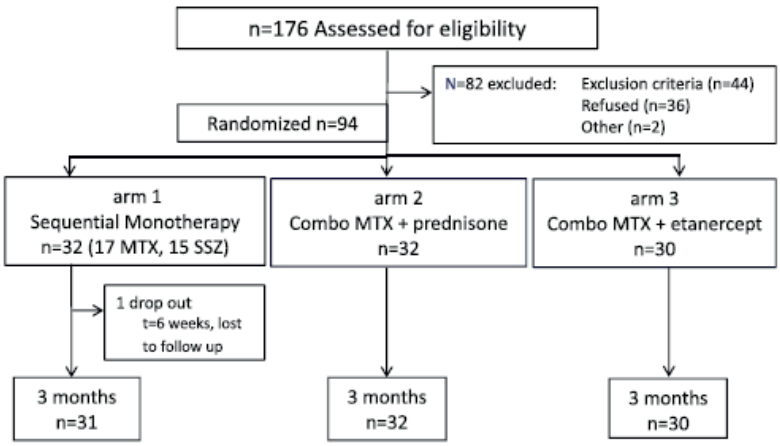

Figure 1 | Study profile of the BeSt for Kids study

Adjusted ACRPedi30/50/70 and early inactive disease

Results are summarized in Table 2. From the patients in inactive disease according to our definition: 11/21(52%) had a VAS physician of 0mm, while 10/21(48%) had a VAS that was scored >0mm, the average was 3.8mm.

Table 2 | Outcome after 6 weeks and 3 months in BeSt for Kids study

		arm 1 Sequential monotherapy n=32	arm 2 Combo MTX+6 wks prednisone n=32	arm 3 Combo MTX+ etanercept n=30	p
Inactive disease (%)*	**6wks**	0 (0)	4 (13)	1 (3)	
	3mths	8 (25)	3 (9)	5 (17)	0.25
aACR Pedi 30 (%)	**6wks**	15 (47)	18 (56)	17 (57)	0.68
	3mths	16 (50)	17 (53)	22 (73)	0.13
aACR Pedi 50 (%)	**6wks**	9 (28)	14 (44)	11 (37)	0.56
	3mths	10 (31)	12 (38)	16 (53)	0.19
aACR Pedi 70 (%)	**6wks**	3 (9)	8(25)	6(20)	0.25
	3mths	8 (25)	6 (19)	14 (47)	0.04
JADAS-10 (median)	**6wks**	13.9	9.6	12.4	0.12
	3mths	9.0	11.5	8.2	0.25
Δ JADAS-10 (median)	**6wks**	3.2	6.6	5.0	0.012
	3mths	6.9	5.7	10.2	0.22

*according to our definition of inactive disease modified to Wallace 2004 definition: no active synovitis, no fever, rash, serositis, splenomegaly or generalized lymphadenopathy attributable to JIA. No active uveitis, ESR≤16mm/h and physician's VAS <10mm.

Medication changes and protocol violations

Medication changes and protocol violations are summarized in table 3. In arm 1 and arm 2 more medication changes occurred compared to arm 3 in the first three months of therapy due to adverse events (n=5). Use of prednisone outside of protocol occurred 3 times in arm 1. Of the 15 patients who started on SSZ, 3 switched to MTX after 6 weeks due to nausea, malaise, headache.

Table 3 | Medication changes and protocol violations in first 3 months. SSZ=sulphasalazine, MTX=methotrexate, sc=subcutaneous, IM=intramuscular, NA=not applicable

	arm 1 MTX or SSZ monotherapy n=32	**arm 2 Combo MTX+ 6 wks prednisone n=32**	**arm 3 Combo MTX+ etanercept n=30**
MTX dose reduction/switch to SC	2	1	2
Switch SSZ to MTX	3/15	NA	NA
Corticosteroids outside of protocol	3	0	0
- kenacort intramuscular	2	0	0
- prednisone orally 4-6wk	1	0	0
Intra articular corticosteroid injections	0	0	0

Adverse events

A summary of toxicity is given in table 4. A total of 28% (26/94) of all patients experienced ≥one AEs: 7/32(22%), 9/32 (28%) and 10/30(33%). Gastro-intestinal symptoms were most frequently reported and were observed 7/32 (22%), 14/32 (44%) and 9/30(28%) in arm 1, 2 and 3. Second mostly reported were mild infectious complications (8/32 (25%)in arm 1, 6/32 (19%) in arm 2 and 13/30 (43%) in arm 3) with 8 upper respiratory tract infections documented in arm 3. Hospital admissions accounted for 3 SAEs in the first three months. One SAE due to viral pneumonia with mild oxygen demand (on SSZ, arm 1), one patient (on MTX, arm 1) suffered from prolonged vomiting which resolved after admission and stopping of MTX. One patient (on MTX, arm 2) had fever of unknown origin while on MTX and was observed shortly without additional therapy.

DISCUSSION

In the BeSt for Kids study, early clinical improvement in patients with early JIA was the aim of the three initial therapies: initial monotherapy with MTX or SSZ, MTX with initial bridging with prednisone, and MTX with etanercept. We found comparable outcomes in

all three arms, with the exception that initial combination therapy with etanercept /MTX resulted in a significantly higher percentage of children that had reached aACRPedi70 after three months of treatment. All three groups already after 6 weeks showed improvement, and there was a trend for further improvement in arms 1 and 3, possibly related to discontinuation of bridging therapy with prednisone in arm 2. The effect of prednisone bridging is visible in high aACRPedi 30/50/70% responses after six weeks but improvements diminished after tapering and stopping of prednisone.
Medication changes had occurred more often in arm 1 and arm 2 as compared to arm 3. Toxicity was comparable and acceptable. A subgroup of arm 1 patients performed better than expected by reaching inactive disease after only three months of monotherapy: 4 of them on SSZ and 4 on MTX (25% of all patients in arm 1). Inactive disease after 3 months was rare in arm 2 (9%), and occurred in 17% of patients in arm 3. Outside-of-protocol use of corticosteroids in arm 1 occurred three times in the first three months, these patients did not reach an ACRPedi50 or inactive disease after three months. Apparently for today's physicians it was hard to hold on to the protocol dictating no additional use of steroids in the current era of impatient doctors and demanding patients, but in this study it helped little to achieve inactive disease.

To minimize the risk of bias of the open design, all outcome measurements were assessed by trained research nurses/physiotherapists/physicians who were blinded to the allocated treatment strategy during entire study period.

Limitations of our study are the relatively small sample size because of slow inclusion rate. These results are promising, but follow up is too short to advocate as yet a primary start with etanercept in DMARD naive new onset JIA patients. The BeSt for Kids study will continue with a treat-to-target design, with medication adjustments aiming to achieve and maintain inactive disease, including after tapering strategies in all three arms. Prospective data on follow-up to 24 months in the BeSt for Kids study will include assessment of possible radiographic joint damage and level of physical functioning.

In conclusion, during the first 3 months of the BeSt for Kids study patients with newly diagnosed JIA who received initial combination therapy with methotrexate and etanercept had significantly more aACRpedi70% responses, comparable side effects and fewer medication changes as compared to methotrexate or sulfasalazine alone or methotrexate and 6 weeks prednisone bridging therapy. Long term follow up data on the extension of initial treatments aiming at inactive disease by a treat to target regime, are needed to relate to these initial positive results.

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Treat to target (drug-free) inactive disease in DMARD naive Juvenile Idiopathic Arthritis: 24-months clinical outcomes of a three-armed randomised trial

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ABSTRACT

Question: Which is the best strategy to achieve (drug-free) inactive disease in Juvenile Idiopathic Arthritis (JIA)?

Methods: In a randomized, single-blinded, study in disease-modifying-anti-rheumatic-drug(DMARD)-naive JIA patients three treatment-strategies were compared 1: Sequential DMARD-monotherapy (sulfasalazine (SSZ) or methotrexate (MTX)), 2: Combination therapy MTX+ 6 weeks prednisolone, 3: Combination therapy MTX+ etanercept. Treatment-to-target entailed three-monthly DMARD/biologic adjustments in case of persistent disease activity, with drug-tapering to nil in case of inactive disease.
After 24 months, primary outcomes were time-to-inactive-disease and time-to-flare after DMARD discontinuation. Secondary outcomes were adapted ACRPedi30/50/70/90scores, functional ability and adverse events.

Results: 94 children (67% girls) aged median (InterQuartileRange) 9.1 (4.6-12.9)years were enrolled: 32 in arms 1 and 2, 30 in arm 3. At baseline VASphysician was mean 49 (SD 16) mm, VASpatient 53 (22) mm, ESR 12.8(14.7), active joints median 8(5-12), limited joints 2.5(1-4.8), and CHAQ score mean 1.0 (0.6).
After 24 months 71% (arm 1), 70% (arm 2) and 72% (arm 3) of patients had inactive disease and 45% (arm 1), 31% (arm 2) and 41% (arm 3) had drug free inactive disease. Time-to-inactive-disease was median 9.0 (5.3-15.0)months in arm 1, 9.0(6.0-12.8)months in arm 2 and 9.0(6.0-12.0)months in arm 3 (p=0.30). Time-to-flare was not significantly different (overall 3.0(3.0-6.8)months, p=0.7). Adapted ACRpedi-scores were comparably high between arms. Adverse events were similar.

Conclusion: Regardless of initial specific treatments, after 24 months of treatment-to-target aimed at drug-free inactive disease, 71% of recent-onset JIA patients had inactive disease (median onset 9 months), and 39% were drug free. Tightly-controlled treatment-to-target is feasible.
Dutch Trial Register 1574

Key words: Juvenile idiopathic arthritis, treatment-to-target, treatment strategy study, inactive disease

INTRODUCTION

Juvenile Idiopathic Arthritis (JIA) is the most common auto-immune disease in children[1]. In recent years, earlier introduction of conventional synthetic disease modifying antirheumatic drugs (csDMARDs) and the development of biologic (b)DMARDs have improved the outcome for JIA patients[2-4], but ongoing inflammation in JIA may still cause functional disability and joint damage[5]. Early inactive disease may be the optimal therapeutic target[6-10]. Studies in JIA support the window of opportunity hypothesis when the disease is optimally responding to treatment and chronicity may be prevented[10-14].

Once inactive disease is achieved, discontinuation of treatment might be possible[15-19]. Comparative drug studies have shown that initial treatment with csDMARD results in less rapid response than initial treatment including glucocorticoids or a bDMARD[10 20], but the latter two have not been directly compared. If the initial treatment is not effective, subsequent treatment adjustments should still aim at achieving the treatment target. In adults with rheumatoid arthritis, such targeted therapy has been proven effective in long term prevention of damage progression and maintaining functional ability, even irrespective of initial treatment success[21-23]. In JIA, continuous treatment-to-target therapy in a tight-control setting, with treatment adjustments based on frequent evaluations of disease activity, has not yet been studied. Recent recommendations agree that treatment-to-target should be implemented in daily practice[24].

The aim of the BeSt (acronym for Dutch 'treatment strategies') for Kids study was to investigate which of three treatment-to-target strategies, using treatment-to-target aimed at inactive disease, is most effective and safe. Here, we report the results of one of the first treat-to-target study in patients with recent-onset JIA.

METHODS

Patients

Patients, 2-16 years old, with new-onset (oligoarticular, juvenile psoriatic arthritis or rheumatoid factor (RF) negative polyarticular) JIA, without previous DMARD-therapy and symptom duration less than 18 months were eligible. RF-positive JIA patients were excluded because monotherapy might be inappropriate for this severe category. Also the number was too low to stratify. Uveitis at enrolment was an exclusion criterion. Rest of exclusion criteria are summarized in supplementary file 1.

Study design and medical intervention

The BeSt for Kids study is an investigator-initiated multicenter randomised study with 2 years of follow-up. To minimize the risk of bias of the open design, all outcome measurements were assessed by trained research nurses, physiotherapists and physicians who remained unaware of the allocated treatment strategy during entire study-period (single-blind design). Medical Ethics Committees of all 5 participating hospitals approved the protocol, and all parents and patients older than 12 years of age gave informed consent. The trial was registered in the Dutch Trial Register, number 1574.

Patients were enrolled starting October 2009 to April 2014 by diagnosing paediatric rheumatologists. Randomization was by variable block, stratified per centre and per oligo- or polyarticular disease, into three strategy-arms: 1. initial treatment with csDMARD monotherapy (methotrexate or sulfasalazine if preferred by treating physician); 2. Initial treatment with MTX and 6 weeks of tapered prednisolone ('bridging therapy'); 3. Initial treatment with MTX and etanercept. For all arms, the treatment protocol described a number of subsequent treatment steps in case patients failed to fulfil treatment targets (figure 1 and supplementary file 2).

In case of side effects, the responsible drug was reduced to the lowest tolerated dose, but if it wasn't tolerated at all or contraindicated, patients on monotherapy proceeded to the next step in the allocated treatment group, and patients on combination therapy continued with the other drug of the combination. Additional treatment with non-steroidal anti-inflammatory drugs (NSAIDs) and intra-articular injections with glucocorticoids were permitted without a maximum and registered per strategy. All patients on MTX received folic acid 5mg/week. The use of DMARD or oral glucocorticoids was only permitted as dictated by the protocol. All protocol violations were recorded.

After 3 months of treatment, the initial target was an adjusted ACRPedi50%, calculated as described previously[25] (supplementary file 3) and scored by a research nurse or physiotherapist who remained blinded to the allocated treatment group during the entire study period. Treatment was continued if this target was met, escalated according to protocol if not.

After 6 months of treatment, the treatment target was inactive disease, defined according to Wallace 2004 criteria[26] (supplementary file 3) modified by Physicians Global Assessment (PGA) <10 mm indicating no disease activity.

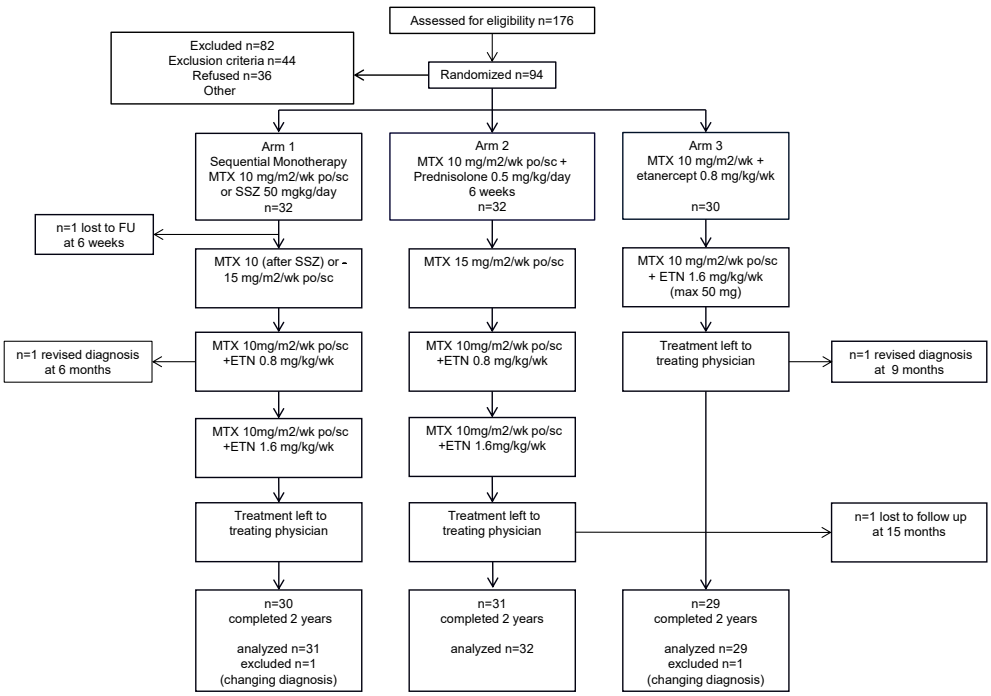

Figure 1 | Flow diagram of the three treatment strategies compared in the BeSt for Kids study;
Revised diagnosis were localized scleroderma with arthritis (arm 1) and polyarteritis nodosa (arm3)[32]. See patients and methods section for description of treatment groups. SSZ= sulfasalazine, MTX= methotrexate, ETN= etanercept, po= orally, sc= subcutaneous. n=21 patients had ≥18months of complaints' duration at first consultation, n=7 had comorbidities considered (relative) contra-indication for the DMARD therapy by either the pediatric rheumatologist or reason for (parents of) patients to refuse participation. These were morphea (1 patient), morbid obesity (n=1), hashimoto thyreoiditis (n=1), type 1 diabetes (n=1), previous uveitis (n=3).

In all three arms, in case of inactive disease for at least 3 (oligoarticular disease) or 6 (polyarticular disease) consecutive months, DMARD(s) were tapered and stopped. In case of combination therapy, first etanercept was tapered to once per 2 weeks, only once, directly followed by 50% dose reduction, then stopped. On the same requirements, methotrexate or sulfasalazine dose was reduced with 25% per week to zero. Following tapering strategies (supplementary file 4) , in case of a disease flare, defined by recurrence of arthritis (supplementary file 5), the last discontinued drug and/or the last effective dose was reintroduced. By protocol, prednisolone could not be restarted, and etanercept could be restarted but not discontinued for a second time.

Outcomes and analyses

Primary outcome measures are time-to-inactive-disease and time-to-flare after tapering and stopping all DMARD therapy. Time-to-flare was defined as the time between first moment of drug-free inactive disease (DFID) and the first arthritis judged as flare by the treating physician (supplementary file 5). Secondary outcome measures were adjusted ACRPedi30/50/70/90 scores, adverse events, functional ability. The Juvenile Disease Activity Score (JADAS)-10 score, JADAS-minimal disease activity (JADAS-MDA) and JADAS-inactive disease (JADAS-ID) were calculated as described previously (supplementary file 6)[27 28]. Functional ability was determined by the Childhood Health Assessment Questionnaire (CHAQ)[29]. Side effects were registered through open-end interviewing at each study visit combined with incidental reports in the intervals, and routine safety laboratory tests at each study visit (complete blood count, serum liver transaminases and creatinine). Severe Adverse Events (SAE) were defined as any adverse reaction resulting in any of the following outcomes: a life threatening condition or death, significant or permanent disability, malignancy, and (prolonged) hospitalization.

Sample size calculations

Percentages of time-to-inactive-disease were estimated since literature in 2008 [13 30 31] reported only on non-DMARD-naive JIA patients.

After three months of therapy an estimated difference of 10% inactive disease in arm 1 versus 60% in arm 3 could be detected with two groups of 30 patients by two-sided log rank test (α=0.05) with power > 90% assuming a hazard ratio of 8.70, a drop-out rate of 20% and 20% not treated according to initial treatment protocol. For an assumed hazard ratio of 4.11, with follow-up two years, a drop-out rate of 20%, a percentage not treated according to initial protocol of 20%, an alpha 0.05, a two-sided log rank test, two groups of 45 patients would be needed. The differences between arm 1 and arm 2 could be detected with two groups of 54, with a power of 80%, assuming a drop-out rate of 10% and no patients not treated according to initial protocol (HR = 2.12). Initially 60 patients per arm was aimed for. Due to slow inclusion rate, the study protocol was amended in 2012 to include 3 groups of 30 patients, leaving potentially enough power to compare arm 1 versus arm 3.

Statistical methods

Multiple imputation using package *mice* in software package R (version 3.4.0, http://r-project.org) was used to deal with missing values with n=10 imputed data-sets. Imputation variables were gender, age at inclusion, duration of symptoms, ANA positivity, diagnosis, number of affected joints, and all outcome variables. In case of drug free clinically inactive

disease often intentionally no blood was drawn causing non-random missing ESR, and here '0' was imputed for analysis of inactive disease.

Where measured repeatedly, measurements were treated as separate variables (wide format). Student's t-test was used to compare continuous normally distributed variables between groups. Non-parametric Kruskal-Wallis tests were used otherwise. For dichotomous variables, Pearson's chi-square test was used. A two-tailed probability value of P<0.05 was considered statistically significant. P-values were not adjusted for multiple statistical tests. Time-to-inactive-disease and time-to-flare was evaluated using log-rank test. The comparison of the groups over time in reaching aACRPedi 30/50/70/90, JADAS-10 and CHAQ-score was analyzed by generalized estimation equation models for continuous outcomes with time-by-strategy interaction as variable of interest. The third arm was treated as reference arm, since we hypothesized that arm 3 would be superior compared to arm 1 or arm 2, based on previous results[12 21].

Table 1 | Baseline demographic and disease characteristics*

	Arm 1 Sequential monotherapy (n=31)	**Arm 2 MTX + 6wks Prednisolone (n=32)**	**Arm 3 MTX+ Etanercept (n=29)**
Age (years), median (IQR)	9.0 (4.7-12.9)	10.2 (6.6-13.9)	8.6 (4.2-12.4)
Symptom duration (mo.), median (IQR)	8.1 (5.5-11.9)	5.9 (4.6-13.3)	8.6 (5.2-13.4)
ANA pos, n (%)	14 (45.2)	11 (34.4)	9 (31.0)
Female, n (%)	23 (74.2)	19 (59.4)	19 (65.5)
JIA Category:			
Oligo, n (%)	5 (16.1)	3 (9.4)	3 (10.3)
Oligoarticular <6 months	1	1	3
Oligoarticular >=6 months	4 (12.9)	2 (6.3)	0
Poly*, n (%)	24 (77.4)	25 (78.1)	24 (82.8)
Psoriatic, n (%)	2 (6.4)	4 (12.5)	2 (6.9)
VAS physician, mean (SD) in mm	46.4 ± 15.4	49.7 ± 16.1	51.2 ± 16.6
VAS patient/parent, mean (SD) in mm	48.9 ± 21.9	56.3 ± 21.4	54.6 ± 22.6
CHAQ, mean (SD)	0.9 ± 0.7	1.1 ± 0.6	1.1 ± 0.5
No. active joints, median (IQR)	7.0 (5.0-13.0)	7.5 (6.0-11.8)	8.0 (5.5-13.0)
No. limited joints, median (IQR)	2.0 (0-3.0)	2.0 (1.0-3.8)	3.0 (1.5-5.0)
ESR, median (IQR)	6.0 (2.0-11.0)	6.0 (2.0-23.5)	9.0 (3.5-26.0)
JADAS-10, mean (SD)	16.5 ± 4.2	18.8 ± 4.4	18.8 ± 5.4

MTX=methotrexate, oligo=oligoarticular JIA, poly=polyarticular RF-negative JIA, IQR=InterQuartile Range ANA=antinuclear antibodies, pos=positive, psoriatic=JIA with psoriasis, VAS=visual analogue scale. CHAQ=child Health Assessment Questionnaire, No.=number, ESR=erythrocyte sedimentation rate. JADAS-10=juvenile arthritis disease activity score in up to maximum 10 joints. Missing follow-up data occurred in 4% for active joint count, in 4% for limited joint count and physician VAS, 7% for parent/patient VAS, 7% for CHAQ score and 16% for ESR.

RESULTS

Patient characteristics

Baseline demographics and disease characteristics are summarized in Table 1. Figure 1 summarizes the study in a flow diagram. Ninety-four patients were randomized to one of three treatment groups: 32 patients were assigned to initial monotherapy (arm 1), 32 patients to initial combination of MTX with 6 weeks prednisolone-bridging therapy (arm2) and 30 patients to arm 3, initial combination of MTX/etanercept. Median symptom duration was 7.5 (IQR 5-12.5) months and median duration between diagnosis and inclusion was 6 (IQR 3-14) weeks. During follow-up 2 patients left the study because of revised diagnosis, one patient with localized scleroderma (in arm 1) and one (arm 3) with polyarteritis nodosa[32]. They were left out of further analyses. Two patients who were lost-to-follow-up, one in arm 1 after inclusion and one in arm 2 after 15 months, were included in the intention to treat (ITT) analysis.

Time-to-inactive-disease and time-to-flare

Median time-to-inactive-disease was 9.0(5.3-15.0)months in arm 1, 9.0(6.0-12.8)months in arm 2 and 9.0(6.0-12.0)months in arm 3 (Overall 9.0(6.0-12.0)months (log rank test p=0.3)). After one year 54% of patients in arm 1, 47% in arm 2 and 62% in arm 3 were in inactive disease (Figure 2).

During 24 months 59% (19 (3 oligo)/31 (61%) of patients in arm 1, 16 (1 oligo)/32 (50%) in arm 2 and 19(1 oligo)/29 (65%) in arm 3) had tapered and stopped all DMARDs (drug free inactive disease (DFID)), after median 15.0 (IQR 12.0-18.0) months (arm 1), 19.5 (12.0-24.0) months (arm 2) and 18.0 (12.0-21.0) months (arm 3) of therapy. However, 26% (6 (1 oligo) patients in arm 1, 3 in arm 2 and 5 in arm 3) subsequently had to restart treatment before the end of the study, in arm 1 median after 4.5(3.0-9.0) months, in arm 2 after 3.0(3.0-3.0) months and in arm 3 after 3.0(3.0-7.5) months (overall 3.0(3.0-6.8)months (p=0.7)). Three months later, inactive disease was regained by 10/14 (71%) (6 in arm 1, 1 in arm 2 and 3 in arm 3). After 24 months 71% (arm 1), 70% (arm 2) and 72% (arm 3) of patients had inactive disease and 45% (arm 1), 31% (arm 2) and 41% (arm 3) had DFID.

Adjusted ACRPedi30/50/70/90, JADAS-10 and CHAQ-score

Adjusted ACRPedi-scores were reached in similar high percentages over time in all three arms (figure 2 and supplementary table S1). JADAS-10 scores after 24 months improved comparably (figure 2), JADAS MDA and ID-criteria are in supplementary table S3. Overall, flares were characterised by a JADAS-10 of 9.7 (8.1-11.3), which improved 3 months after restart of treatment to JADAS-10 of 3.9(1.8-6.0). In all three arms CHAQ values improved from mean 1.0 (SD 0.6) to 0.5 (0.6).

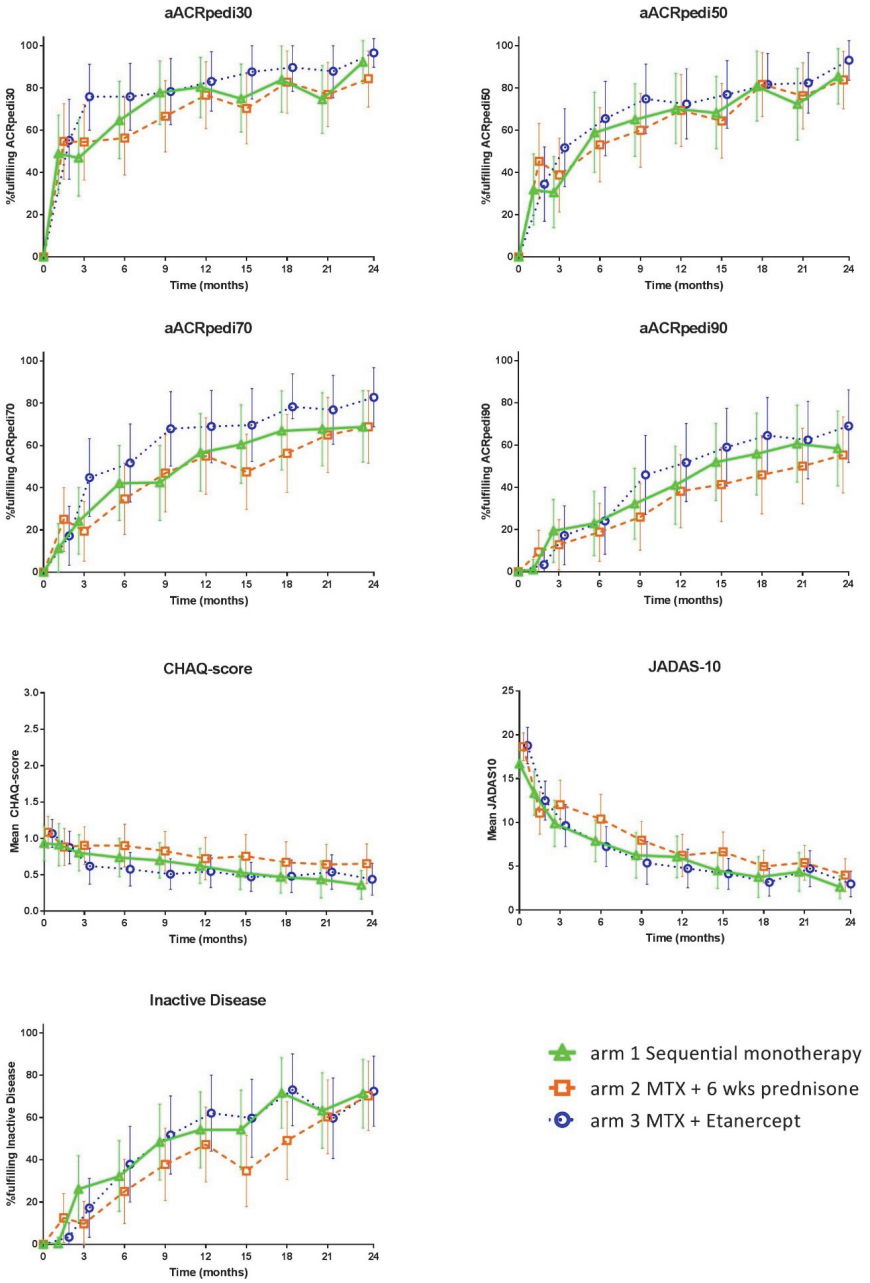

Figure 2 | Clinical outcomes after 24 months: adjusted ACRPedi30/50/70/90, inactive disease, CHAQ and JADAS-10 score, based on Generalised Estimating Equations (GEE)-analyses on imputed data. Error bars indicate 95% confidence intervals. Adjusted ACRPedi30/50/70/90= 30/50/70/90% improvement according to adjusted American College of Rheumatology Pediatric response criteria. CHAQ= Dutch version of the Child Health Assessment Questionnaire; JADAS-10=Juvenile Arthritis Disease Activity Score up to maximum of 10 joints.

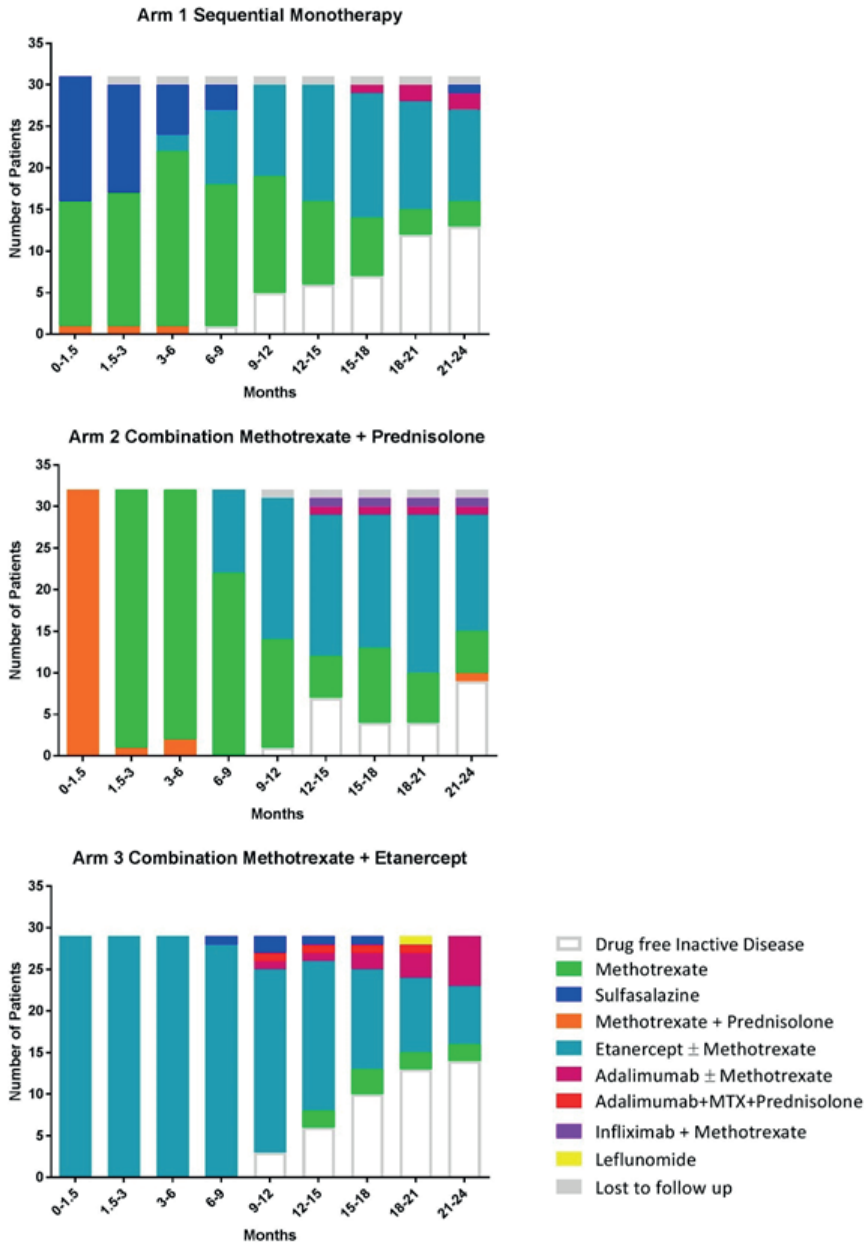

Figure 3 | Treatment of patients during two years of follow-up
Treatment was started and when necessary adapted to reach inactive disease. Within the first year of therapy more treatment changes occurred in arms 1 and 2 compared to arm 3. When inactive disease was reached for a consecutive period of 3 months in case of oligoarticular disease, and 6 months for polyarticular disease, all DMARDs where tapered and stopped according to protocol within approximately 2 months.

Medication changes and protocol violations

Figure 3 shows all medication actually used in the study per arm (i.e. including protocol violations). In arm 1 treating physicians prescribed SSZ (n=15) almost as often as MTX (n=17). By t=3 months 10/15 patients had switched from SSZ to MTX, 2 due to side effects, 8 because of insufficient response. After three months, patients who remained on SSZ had similar ACRPpedi50% scores as patients who started on MTX (data not shown). During 24 months in arm 1, 9 patients in arm 1 reached inactive disease while still on monotherapy, 4 on initial SSZ (one flared later) and 5 on initial MTX (3 flared later). In arm 2 (17/32) 53% of patients who started on MTX plus 6 weeks of prednisolone switched to MTX with ETN before end of year 1. Overall 17 patients (55%) in arm 1 and 23 patients (72%) in arm 2, progressed to a biological, at various time-points, according to protocol. Treatment was left to treating physician due to end of protocol in 4 patients in arm 1, versus 15 and 18 in arms 2 and 3. In arm 3 significantly less treatment adjustments were needed to achieve firstinactive disease: 0.6 (0.3-1.0) treatment steps compared to 1.4 (0.9-1.8) steps in arm 1 and 1.5 (1.0-1.9) steps in arm 2 (p=0.011). Across all arms, 10 (2 in arm 1, 2 in arm 2, 6 in arm 3) patients failed to achieve inactive disease on ETN and switched to adalimumab (9) or infliximab (1). After 24 months, five of these 10 patients gained inactive disease on the second anti-TNF.

Supplemental table S2 summarizes protocol violations including outside of protocol glucocorticoid-use across the 3 arms. Incorrect glucocorticoid treatments were given in the first months in arm 1 (3 times) and in arm 2 (4 times) compared to none in arm 3. Overall, treatment was not escalated according to protocol in all three arms for refusal to start or increase the dose of MTX or etanercept (table 3).

Adverse events

Adverse events (AE) were similar across the arms. AEs are summarized in Table 2. AEs were mild in general and involved mostly gastro-intestinal complaints, upper respiratory tract and other infections and general malaise. One patient in arm 1 while on MTX developed de-novo uveitis anterior after 6 months of treatment. No patients had permanent sequelae.

DISCUSSION

This is one of the first treatment-to-target studies, tightly-controlled and single-blinded, in newly diagnosed DMARD-naive JIA patients, aiming at inactive disease. Efficacy and safety of three treatment strategies were compared that are frequently used and comparable with the Childhood Arthritis and Rheumatology Research Alliance American Consensus

Table 2 | Adverse events in 92 patients with JIA in three treatment arms: sequential monotherapy, combination therapy MTX/prednisolone and combination therapy MTX/etanercept

	Arm 1 Sequential monotherapy n=31	**Arm 2 Combination MTX + 6 wks Prednisolone n=32**	**Arm 3 Combination MTX + Etanercept n=29**
	No. of events (No. pts)		
Common adverse events			
Nausea or abdominal pain	18 (12)	26 (16)	28 (13)
URTI	9 (9)	20 (13)	23 (14)
Gastro-enteritis	4 (4)	4 (4)	6 (6)
Other infections	8 (7)	12 (9)	12(8)
General malaise	11 (8)	12 (8)	7 (7)
New onset CAU*	1 (1)	0	0
Liver enzyme abnormalities	9 (5)	11 (8)	4 (3)
Other adverse events			
Headache and psychosomatic complaints**	10 (9)	12 (8)	4 (3)
Anemia	1 (1)	2 (2)	0
Leucopenia	8 (6)	2 (2)	1 (1)
Other	25	31	30
Severe adverse events			
Hospital admissions***	4 (3)	3 (3)	5 (5)

URTI=Upper Respiratory Tract Infections; No=number; pts=patients;
*CAU= Chronic Anterior Uveitis, treated additionally with local therapy.
**Psychosomatic complaints comprise: sleep disturbances, mood disturbances, concentration problems, temporary conversion disorder, eating disorder, dizziness.
***AE's with hospitalisation: In arm 1: 2 episodes of viral pneumonia with oxygen demand in one patient; one patient with prolonged vomiting on MTX for supportive care; one patient with varicella while on MTX; In arm 2 one case of scarlet fever; one patient with fever and confusion after to MTX, intake was observed; one case of hypovolemia in combination with skin infection while on MTX, cultures remained negative; In arm 3 one patient with pneumonia; 2 patients with gastro-enteritis who were admitted for supportive care; Campylobacter jejuni was cultured in one patient with complaints of diarrhea; one patient was observed for skin rash on SSZ which resolved spontaneously.

Treatment Plans[33]. Abrogation of inflammation by treating JIA to target has recently been recommended[24]. Our results show that after 24 months inactive disease was achieved by more than 70% of patients, irrespective of initial treatment, including tapering and stop-strategies. Fifty-nine percent achieved DFID, although early flares occurred that were successfully retreated.

After 3 months of treatment, more patients who started with methotrexate and etanercept (arm 3) had achieved rapid improvement as determined by aACRPedi70scores[34], but time-to-inactive-disease was similar across the arms. Due to treatment adjustments in case of active disease, which were needed more often in arms 1 and 2 than in arm 3, aACRpedi

improvement scores were met in similar percentages of patients over time across the arms. After 24 months of treatment-to-target JADAS-10-scores were considerably reduced and functional ability as assessed by CHAQ was lowered substantially across the arms.
Our results show higher percentages of patients achieving inactive disease than in the prospective randomised double-blinded TREAT-study[10] which included only polyarticular JIA patients (n=85) including 30-40% RF-positives. In the ACUTE-JIA study (n=59), 68% achieved inactive disease after 1 year in the infliximab arm[20]. This unblinded study allowed one treatment intensification step but did not include tapering or stop-strategies. In the daily practice-based ReACCh-out-cohort[35], polyarticular and oligoarticular JIA achieved inactive disease after 24 months in 71% and 86% mainly by additional glucocorticoid-use.
The current study also aimed at systematically tapering and discontinuing treatment when inactive disease was achieved. DFID was achieved by 54/92 (59%) of all patients, although in 14 patients (6 (1 oligo) in arm 1, 3 in arm 2 and 5 in arm 3), flares occurred, requiring restart of treatment, resulting in overall 39% of patients still in DFID at the 2 years endpoint. Time-to-flare was similar across the arms. Overall flare rates (26%) were lower than 37-60% mentioned in previous cohorts [16 17 36 37] which may also depend on our limited total follow-up period of 24 months.

Contrary to previous studies we included oligoarticular patients (n=11) because they can have substantial disease burden and adverse outcomes[38], but used a rapid drug-tapering scheme (tapering and stopping medication after 3 months of inactive disease, compared to after 6 months in polyarticular disease) as we hypothesized that DFID could be achieved earlier in patients with less inflamed joints. We could not establish this difference significantly, possibly due to low numbers. Only one oligo-articular patient out of 5 who achieved DFID, flared. These limited results suggest that oligoarticular JIA patients could benefit from a treatment-to-target strategy.

There are several limitations to our study. First, the sample size, which may obscure differences between groups that in a larger population might have become clear. This can be explained by rarity of the disease, delays in referral (21 patients had ≥18 months symptom duration at the first consultation), comorbidities preventing DMARD-use (7 patients) and reluctance of parents to enrol their children in a clinical trial. Data on the clinical course of non-participating patients, receiving 'routine care' are currently not available. Recent retrospective studies in polyarticular JIA showed that despite achieving inactive disease for some time, most patients had active disease during follow up[39-41]. Second, this study was performed in a single-blinded setting, with the clinical assessors remaining unaware of the treatment received. Third, there was a relatively high frequency of protocol violations or intra-articular injections. (Not-allowed) glucocorticoid treatments were given in the first

months in arm 1 (3 times) and 2 (4 times) compared to none in arm 3. These findings may indicate that the clinical efficacy of treatment in arm 3 was better, and that with less effective csDMARDs, additional glucocorticoid-courses are required to achieve similar results. These protocol violations suggest that physicians at least tried to follow the treatment-to-target approach. However, in a larger number of patients across the three arms the physicians did not follow protocol for various reasons, mainly reluctance to intensify therapy based on shared decision making[24].

Based on the results from our study we conclude that DFID is a feasible goal in treatment of children with JIA, as was recently recommended[24], resulting in over 70% achieving inactive disease and 39% stopping all DMARDs after 24 months. In addition, we showed that tapering and discontinuation of treatment is a realistic goal. On the other hand, treatment-to-target resulted in a relatively high use of bDMARDs, >50% of patients in all arms. The adverse events were nonetheless mostly mild, as previously reported[42]. Long term follow-up of the BeSt for Kids cohort, including radiology results, is initiated to investigate possible lasting positive results of treatment-to-target in JIA.

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SUPPLEMENTARY FILE 1

Exclusion criteria BeSt for Kids study as per protocol

- Systemic JIA
- Rheumatoid factor positive JIA
- JIA with enthesitis
- Undifferentiated JIA
- Previous treatment with DMARDs or biologicals
- Known contraindication for one of the study drugs, such as G6PD deficiency
- Bone marrow hypoplasia
- Inborn conditions characterized by a compromised immune system
- Known HIV infection or other acquired forms of immune compromise
- Any significant concurrent medical or surgical condition which would jeopardize the patient's safety or ability to complete the trial
- Sepsis or risk of sepsis
- Significant cardiac [e.g. congenital heart disease, valvular heart disease, constrictive pericarditis myocarditis] or pulmonary disease, (e.g. cystic fibrosis);
- Asthma for which the patient has required the use of oral or parenteral corticosteroids for ≥ 2 weeks within 6 months prior to the baseline visit
- History or concurrent serious gastrointestinal disorders such as ulcer or inflammatory bowel disease, Crohn's disease, ulcerative colitis or other symptomatic lower gastrointestinal conditions, including ulcer and perforation
- Current or recent infections (last three months), including chronic or localized; evidence of active CMV or EBV, infectious hepatitis, active pneumocystis carinii, drug resistant atypical mycobacterium or other bacterial infections.
- Positive PPD and/or X-thorax (PPD is left out in patients that were vaccinated with BCG)
- History of lymphoproliferative disease including lymphoma or signs suggestive of possible lymphoproliferative disease, such as lymphadenopathy of unusual size or location (such as nodes in the posterior triangle of the neck, infraclavicular, epitrochlear, or periaortic areas), or splenomegaly.
- At increased risk of malignancy; history or presence of malignancy within the last five years
- Other comorbidity that prevents treatment with oral glucocorticoids and/or sulfasalazine and/or methotrexate and/or etanercept, or other comorbidity that, in the opinion of the pediatrician, prevents participation in the trial
- Vaccination with live vaccine in last 4 weeks, or expected to require such vaccination during the course of the study

- Current or prior history of blood dyscrasias. Abnormal safety baseline blood test e.g. haemoglobin ≤ 5 mmol/l; haematocrit ≤ 27%; platelet count ≤ 125 x 10^9 /L; white blood cell count ≤ 3.5 x 10^9 /L; serum creatinine ≥ 2 times the laboratory's upper limit of normal ; aspartate aminotransferase (AST [SGOT]) and alanine aminotransferase (ALT [SGPT]) ≥ 2 times the laboratory's upper limit of normal.
- Reasonable expectation that the subject will not be able to satisfactorily complete the study.
- History of or current psychiatric illness, alcohol or drug abuse that would interfere with the subject's ability to comply with protocol requirements or give informed consent.
- Receipt of any investigational drug within 3 months of screening visit.

SUPPLEMENTARY FILE 2

Extended Description of treatment strategies

In arm 1 (sequential monotherapy) the patients started with Sulfasalazine 50mg/kg up to 2000mg/day or (MTX10mg/m2/wk orally or subcutaneous (max 25mg/wk). After three months aACRPedi50 was calculated. If the patient did not reach aACRPedi50, patients on SSZ switched to MTX 10mg/m2/wk and patients on MTX increased the dose to 15mg/m2/wk, max 25mg/wk, preferably subcutaneous. After 6 months the target was inactive disease according to adapted Wallace definition (Supplementary appendix page 9). Subsequent steps for patients with an inadequate response were adding etanercept 0,8mg/kg/wk with MTX dose reduction to 10mg/m2/wk, followed by a three month period of increased dose of etanercept (1,6mg/kg/wk, max 50mg/wk). In case of still not reaching inactive disease, the treating physician could decide how to proceed.

In arm 2 (combination therapy with MTX and prednisolone) the patients started with MTX 10mg/m2/wk (max 25mg/wk) in combination with prednisolone orally 0,5mg/kg for four weeks, tapering by halving of the dose two times in two weeks to zero. If aACRPedi50 was not reached after three months, MTX dose was increased to 15mg/m2/wk, max 25mg, preferably subcutaneous. If after 6 months or every next step no inactive disease was reached, subsequent steps are (equal to arm 1) adding etanercept 0,8mg/kg/wk with MTX dose reduction to 10mg/m2/wk, and after that a three month period of increased dose of etanercept (1,6mg/kg/wk, max 50mg/wk). In case inactive disease was not realized with this regime, the next step was left to the treating physician.

In arm 3 (combination therapy with etanercept and methotrexate) the patients started with a combination of etanercept 0,8mg/kg/wk sc and MTX 10mg/m2/wk. If after three

months aACRPedi50 was not accomplished, a three months dose increase of etanercept (1,6mg/kg/wk, max 50mg/wk) was advocated. In case of insufficient response after 6 months and onwards, the next step was left to the treating physician.
If inactive disease on medication is reached continue therapy in the same dose for 3 or 6 months (depending on type JIA: oligoarticular vs polyarticular).

SUPPLEMENTARY FILE 3

ACR Pedi calculations

The JIA Core Outcome Variables(1) consist of:

1. Physician Global Assessment of Disease Activity (10 cm Visual Analogue Scale (VAS))
2. Parent/patient global assessment of overall well-being (10 cm VAS)
3. Functional Ability (Childhood Health Assessment Questionnaire)
4. Number of Joints with Active Arthritis
5. Number of Joints with Limitation of Movement
6. ESR

For the efficacy assessment, patients will be evaluated as “improved” or “not improved” by comparing the values of core outcome variables at the post-dose assessment time points with baseline values.

Definition of Improvement in Juvenile Idiopathic Arthritis

ACRPedi30/50/70/90 improvement is defined as 3 of any 6 core outcome variables improved by at least 30/50/70/90% from the baseline assessments, with no more than 1 of the remaining variables worsened by more than 30%(1).
Changes in outcomes that remained within normal limits (ESR≤16 mm/hour and Physician Visual Analogue Scale (VAS) <1 cm (range 0-10cm) were not taken into account in ACRPedi-calculations and were corrected for, resulting in adjusted scores (aACRPedi30/50/70/90%).

Definition of inactive disease in Juvenile Idiopathic arthritis:

Criteria:
no clinical symptoms of active synovitis
no fever, rash, serositis, splenomegaly, or generalized lympadenopathy attributable to JIA
no active uveitis
normal ESR and/or CRP
Physician´s global assessment (PGA) of disease activity indicates no active disease;
Id est PGA<1cm*(0-10cm).
* adapted version of original definition by Wallace(2)

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SUPPLEMENTARY FILE 4

Tapering regime a priori defined in the protocol

If inactive disease lasts 3 months in oligoarticular (2 consecutive visits) taper and stop DMARD therapy according to protocol.
If inactive disease lasts 6 months in polyarticular JIA (3 consecutive visits) taper and stop DMARD therapy according to protocol.

- How to stop the combination of etanercept (ETN) and MTX.
  After 3 or 6 months of inactive disease first taper ETN from 50mg/week to 25mg/week, then to 25mg every other week, then stop. (or full dose/week-> half dose/week-> half dose every other week-> stop). Next MTX is tapered with ¼ of the dose per week, rounding is allowed.
  In case of flare reintroduce ETN and MTX in the last effective and maximum tolerated dose. After the first flare further decisions will be according to the treating physician.
- How to stop MTX monotherapy.
  After 3 or 6 months of inactive disease, MTX is tapered with ¼ of the dose per week, rounding is allowed.
- How to stop SSZ monotherapy.
  After 3 or 6 months of inactive disease, SSZ is tapered with ¼ of the dose per week, rounding is allowed.

SUPPLEMENTARY FILE 5

Flare definition

If, after termination of the DMARDs according to the protocol, the arthritis becomes active again and the treating paediatric rheumatologist judges it as a flare, it is a flare.

Background

In the original protocol BeSt for Kids time to flare was defined as the duration of time until a flare of the disease occurred after tapering and stopping medication, defined as a minimum of 40% worsening in a minimum of 2 out of 6 outcome variables with no more than one of the remaining components improving by ≥30% as defined by Brunner in 2002(1).

During the study we noticed that worsening in % is impossible to compute starting from 0. Therefore the current definition of flare could not be maintained.
In 2013 in literature no consensus was reached concerning flare definition. Alternative definitions included loss of criteria for inactive disease(2) or recurrence of synovitis requiring treatment(3), or VAS physician/parent worsening of 20/100mm or worsening in 2 or more active joints(4).

Later, in 2016 Guzman et al(5), defined a flare as a recurrence of manifestations of active disease or a Physician Global Assessment ≥10 mm, and a significant flare as one requiring treatment intensification, analogous to the proposed definition of flare in rheumatoid arthritis(6).

In 2013 we amended the protocol with an alternative flare definition, which was approved 05-04-2013.

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SUPPLEMENTARY FILE 6

JADAS-10 score

The JADAS-10 score is the linear sum of 4 components, which yields a global score of 0-40(1).

1 PGA 0-10cm
2 Parent/patient Global Assessment 0-10cm
3 Active joint count up to max of 10 joints, any involved joint, irrespective of its type.
4 Normalized ESR according to formula: (ESR(mm/hour)-20)/10; before the calculation, ESR values<20 are converted to 0 and ESR values>120 are converted to 120.

JADAS Minimal disease activity definition(2)

For Oligoarticular JIA: JADAS10 < 2
For Polyarticular JIA: JADAS10 < 3.8

JADAS Inactive disease definition

For oligo and polyarticular JIA: JADAS10 ≤ 1

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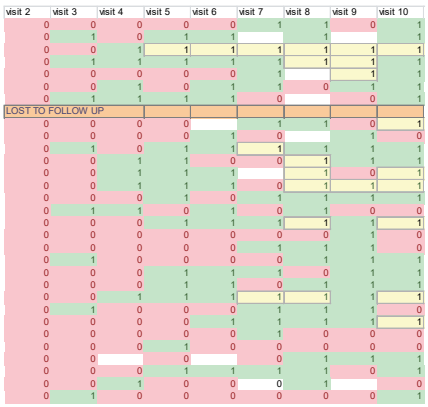
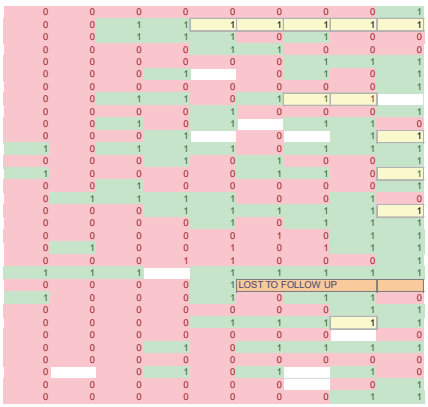
SUPPLEMENTARY FILE 7

Heat map representing active or inactive disease in arm 1, 2 and 3 for individual patients

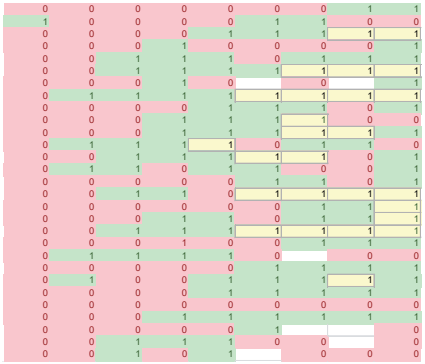
0 active disease
1 inactive disease
1 inactive disease, ESR missing due to no blood draw due to drug free inactive disease



Arm 1 initial sequential monotherapy



Arm 2 Initial MTX and prednisolone



Arm 3 Initial etanercept and MTX

Table S1 | Clinical results BeSt for Kids study after 24months per arm

	Arm 1 Sequential monotherapy n=31	Arm 2 MTX+ Prednisone n=32	Arm 3 MTX+ Etanercept n=29	Arm 1* p; OR (CI)	Arm 2* p; OR (CI)
aACRPedi30 (%) (CI)	92.2 (82,1-102.4)	84.4 (71.2-97.5)	96.6 (89.8-103.3)	0.84; 0.99 (0.93-1.09)	0.56; 0.97 (0.92-1.05)
aACRPedi50 (%) (CI)	85.5 (72.4-98.6)	83.8 (70.1-97.4)	93.1 (83.7-102.4)	0.89; 0.99 (0.92-1.07)	0.49; 0.98 (0.92-1.04)
aACRPedi70 (%) (CI)	69.0 (52.1-85.9)	68.8 (51.6-85.9)	82.8 (68.8-96.8)	0.92; 1.00 (0.93-1.07)	0.46; 0.98 (0.92-1.04)
aACRPedi90 (%) (CI)	58.4 (40.6-76.1)	55.3 (37.3-73.3)	69.0 (51.8-86.1)	0.72; 0.99 (0.93-1.05)	0.39; 0.98 (0.92-1.03)
Inactive disease (%) (CI)	71.3 (55.0-87.6)	70.3 (53.9-86.7)	72.4 (55.9-89.0)	0.99; 0.99 (0.94-1.06)	0.82; 0.99 (0.93-1.06)
VAS physician, mean (CI)	4.4 (1.2-7.7)	5.0 (1.2-8.9)	4.6 (1.2-8.0)	0.48; 0.85 (0.54-1.34)	0.61; 0.89 (0.57-1.38)
VAS patient/parent, mean (CI)	14.9 (6.9-22.9)	25.5 (16.2-34.8)	18.0 (10.4-25.8)	0.79; 0.92 (0.51-1.67)	0.48; 1.20 (0.72-2.01)
CHAQ, mean (CI)	0.4 (0.2-0.5)	0.7 (0.4-0.9)	0.4 (0.2-0.6)	0.14; 0.99 (0.98-1.00)	0.90; 1.00 (0.99-1.01)
No. active joints, mean (CI)	0.6 (0.1-1.1)	0.9 (0.1-1.7)	0.6 (0.1-1.2)	0.54; 0.96 (0.84-1.10)	0.52; 0.95 (0.83-1.10)
No. limited joints, mean (CI)	0.8 (0.1-1.4)	0.6 (0.3-1.0)	1.3 (0-2.7)	0.39; 0.96 (0.88-1.05)	0.53; 1.02 (0.95-1.10)
ESR, mm/hour mean (CI)	8 (4-12)	9 (5-12)	7 (3-11)	0.31; 0.90 (0.72-1.10)	0.29; 0.91 (0.76-1.09)
JADAS-10 mean (CI)	2.6 (1.4-3.8)	4.0 (2.2-5.8)	3.0 (1.6-4.4)	0.61; 0.96 (0.82-1.13)	0.97; 1.00 (0.87-1.16)

*Results from GEE-analysis on imputed data, arm 3 was used as reference arm; Vs=versus, OR=Odds Ratio, CI=95% confidence interval, aACRpedi30/50/70/90= adjusted ACRpedi 30/50/70/90 improvement scores; CHAQ=Child Health Assessment Questionnaire; JADAS-10= Juvenile Arthritis Disease Activity Score with maximum up to 10 active joints.

Table S2 | Protocol violations per arm

	Arm 1 Sequential monotherapy n=31	Arm 2 MTX+6 wks Prednisone n=32	Arm 3 MTX+ Etanercept n=29
Glucocorticoid treatments out of protocol 0-6 months			
IM kenacort injection*[1]	2	0	0
Oral prednisolone course*[2]	1	2	0
Intra-articular injection*[3]	0	2	0
6-24 months			
Oral prednisolone course*[4]	0	1	1
Intra-articular injection*[5]	0	6	2
0-24 months Cumulative			
Oral	60 mg/kg (n=1)	16.6mg/kg (n=32) + 36mg/kg (n=2) + 2w DU*[6]	216mg/kg (n=1)
Parenteral (IM;IA)	2 (n=2)	8 (n=5)	2 (n=2)
No change of therapy against protocol			
No MTX dose increase or restart due to:			
Preference parent/patient	5		
Preference physician	1	1	
Unknown	1		
No start etanercept, due to:			
Preference parent/patient	2	1	NA
Preference physician	4	4	NA
Unknown	2	1	
No etanercept dose increase, due to:			
Preference parent/patient	1		1
Preference physician	2	2	6
Tapering violation*[7]	1	1	1
Skipped time point(s)	10 visits in 9pts	6 visits in 4 pts	4 visits in 4 pts

IM =intramuscular, IA= intra-articular, MTX=methotrexate, NSAID=non-steroidal anti-inflammatory drugs, Pts=patients

*1 In arm 1: in 2 patients a single kenacort IM injection was administered after 6 weeks;
*2: In arm 1: 1 patient received oral prednisone : 4 months 0.5mg/kg,
in arm 2: 2 patients received 3 months of prednisone 0.4-0.5mg/kg,
*3: In arm 2 in the first 6 months one proximal interphalangeal (PIP) joint was injected with methylprednisolone in 1 patient, and one knee joint in another patient was injected with triamcinolonhexacetonide.
*4: in arm 2: one patient received a 1-2 week course of prednisone prescribed by the general practitioner due to irritation of enthesis, dose unknown.
In arm 3 one patient received 15mg/day tapering to 7.5mg/day after six months, tapering to 0 the next 6 months.
*5 In arm 2 the same PIP joint was injected in 1 patient; Five knees in 4 patients at 5 time points. In arm 3 one wrist injection and one knee injection in 2 different patients.
*6 DU=prednisone dose unknown for 2 weeks by the general physician.
*7 In arm 1 one patient did not taper MTX when appropriate according to protocol. Likewise in arm 2 one patient did not taper etanercept when in inactive disease for 6 months. In arm 3 one polyarticular patient tapered etanercept too soon, MTX was continued too long.

Table S3 | JADAS MDA and JADAS inactive disease after 1 and 2 years (based on imputed data)

JADAS MDA	Arm 1 (n=31)	Arm 2 (n=32)	Arm 3 (n=29)
Oligoarticular JIA JADAS < 2.0	1y: 2 of 5	1y: 2 of 3	1y: 0 of 3
	2y: 3 of 5	2y: 2 of 3	2y: 1 of 3
Polyarticular JIA JADAS < 3.8	1y: 12.3 of 26	1y: 13.3 of 29	1y: 17 of 26
	2y: 18.3 of 26	2y: 17.9 of 29	2y: 20.8 of 26
Total	1y: 46%	1y: 48%	1y: 59%
	2y: 69%	2y: 62%	2y: 75%
JADAS ID JADAS ≤ 1			
Total after 1 year	8.4 (27%)	9 (28%)	9 (31%)
Total after 2 years	16.2 (52%)	14.1 (44%)	12.5 (43%)

1y: after 1 year, 2y: after 2 years.

5
8
6
7
10

6

No radiographic wrist damage after treatment to target in recent-onset Juvenile Idiopathic Arthritis

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ABSTRACT

Background: To evaluate radiographic progression of patients with new-onset juvenile idiopathic arthritis (JIA) in response to an early, tightly-controlled, treatment-to-target.

Methods: Patients with JIA participating in the BeSt-for-Kids-study, randomized to 3 treatment strategy arms, were eligible if at least 1 conventional wrist-radiograph was available. Bone damage as reflected by carpal length was assessed using the Poznanski-score. The BoneXpert-method was used to determine the Bone Age (BA, >5 years) and bone mineral density (BMD) of the wrist. These scores were evaluated over time and compared between the treatment arms and mean JADAS10-score using linear mixed models corrected for age and symptom duration.

Results: In 60 patients, 252 radiographs were analysed. Baseline age and symptom duration were different between the arms. No difference in comparison to the healthy reference population was found at baseline for the Poznanski-score (IQR varying from -0,82- 0.68), nor for BA (varying from -0.88 to 0.74). Baseline BMD was statistically significantly lower in arm 3 (initial treatment with etanercept and methotrexate) (-1.48; -0.68) compared to arm 1 (-0.84;-0.04) and arm 2 (-0.93; 0.15). After treatment to target inactive disease, the Poznanski-scores and the BA remained clinically unchanged, while the BMD in arm 3 improved ($p<0.05$ vs arm 1).

Conclusions: Recent-onset JIA patients, treated-to-target aimed at inactive disease, showed no signs of radiographic wrist damage (Poznanski-score, BA or BMD) either at baseline or at follow-up, irrespective of treatment arm. A lower BMD at baseline in arm 3, initially treated with methotrexate and etanercept, improved significantly after treatment.
Trial registration: NTR, NL1504 (NTR1574). Registered 01-06-2009, https://www.trialregister.nl/trial/1504

Key words: juvenile idiopathic arthritis, treatment to target, radiographic outcome, conventional radiography

BACKGROUND

Juvenile idiopathic arthritis (JIA) is a potentially chronic disease that comprises 7 categories of childhood arthritis of unknown cause, that persists for more than 6 weeks and starts before the age of 16[1]. Osteopenia, bony deformity, erosions, and cartilage loss in carpalia, resulting in carpus shortening, can be complications of inflammation in JIA patients[2-5]. Previous studies have shown that early damage on conventional radiography is correlated with functional deterioration and radiographic progression after 5 years[2, 5, 6], and also with smaller chances to achieve clinical remission[7]. Monitoring of radiographic damage progression is therefore important to evaluate treatment effect and predict prognosis. Since joint damage is assumed to be the result of ongoing inflammation, reaching inactive disease as early as possible and thereby preventing structural joint damage and consequently limitations in physical functioning, should be the goal of treatment[8]. This is facilitated by the availability of new effective disease modifying antirheumatic drugs (DMARDs)[9]. In accordance, current JIA treatment recommendations focus on earlier introduction of DMARDs aiming to achieve remission or at least low disease activity[8, 9].

We have recently performed a randomized clinical trial using the treatment-to-target approach in recent-onset JIA patients, comparing 3 strategy-arms with different initial and subsequent treatment steps, aiming at inactive disease, including tapering and stopping DMARD therapy[10, 11]. In this population we studied radiographic wrist damage using the Poznanski-score, at baseline and evaluated whether damage occurred or recovered with the abrogation of inflammation in the 3 strategy-arms. In addition we used the BoneXpert-method to determine the Bone Age (BA) and Bone Mineral Density (BMD) as markers for joint damage[12].

METHODS

Patient selection

The Best-for-Kids-study (NTR 1574), a multicenter randomized single-blinded clinical trial, was designed to investigate the effectiveness of three different treatment-strategies in newly diagnosed patients with the following JIA categories: oligoarticular JIA, rheumatoid factor (RF) negative polyarticular JIA and juvenile psoriatic arthritis. DMARD-naive patients with a disease duration of less than 18 months were randomized to one of the three treatment arms.

Patients in arm 1 were treated with initial monotherapy with methotrexate (MTX) or sulfasalazine (SSZ); patients in arm 2 were treated with initial MTX and prednisone bridging and patients in arm 3 were initially treated with etanercept and MTX. Patients were treated to target, aimed at inactive disease, with three-monthly assessments. If predefined targets of suppression of inflammation were not met, treatment was intensified, as can be seen in figure 1, with subsequent treatment-steps, including etanercept also in arm 1 and 2. In case of at least 6 months of inactive disease, treatment was tapered. The current sub-analysis was done in all patients who had radiographs of one or both hands obtained at study inclusion (with a range of maximum 4 months before) or at any follow-up visit up to 40 months. Radiographs of hands and wrists were encouraged at baseline, year 1 and year 2. In practice, physicians were reluctant to do this if there was no local arthritis. Juvenile Arthritis Disease Activity Score (JADAS)10-scores were available from all the patients[11]. To investigate the effect of the relatively fast changing disease activity on slower changing

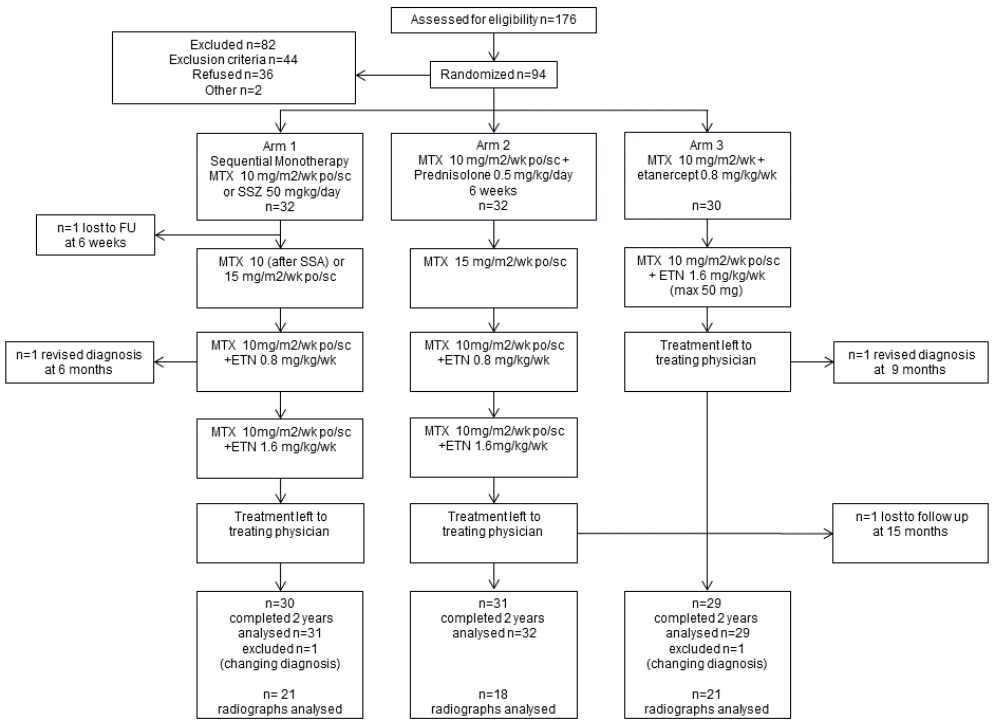

Figure 1 | The three treatment strategies compared in the BeSt for Kids study
Flow diagram of the three treatment strategies compared in the BeSt for Kids study; Revised diagnosis were localized scleroderma with arthritis (arm 1) and polyarteritis nodosa (arm 3). See patients and methods section for description of treatment groups. FU=follow-up, SSZ=sulfasalazine, MTX=methotrexate, ETN=etanercept, po=orally, sc=subcutaneous

radiological outcome parameters, we have used mean JADAS10-scores over 2 years' time as a predictor for the radiological outcomes.

The BeSt-for-Kids-study was approved by the Institutional Review Board at Leiden University Medical Center and written informed consent was obtained from all participants before enrollment.

Radiographic scoring

All radiographs were anonymized and randomized by an independent computer-technician, and then evaluated using two different scoring methods: the Poznanski-score[13] and the BoneXpert-method. When radiographs of both wrists were available, scores of both wrists were included. The Poznanski-score was used to measure carpal size, and was calculated as the mean score of 2 independent readers (DS and WB), who were unaware of clinical data. Open growth plates are necessary to determine the Poznanski-score. The radiometacarpal length (RM, defined as the line from the mid-growth plate of the radius to the center of the proximal end of the third metacarpal) and the length of the second metacarpal (M2, defined as the maximum length of the second metacarpal as defined by Garn[14]) were measured, in millimeters using RadiAnt DICOM viewer version 2.2.8, as shown in figure 2. Poznanski's gender-specific formulas were used to calculate the expected RM for the observed M2[13]. The difference between expected and measured RM was then calculated and converted into a Z-score[13], which represents the number of standard deviations that the observed RM diverges from the expected RM. A negative Z-score indicates delayed growth in the radiometacarpal bones with loss of cartilage or loss of joint space as potential causes, whereas a positive Poznanski-score may indicate growth acceleration, a phenomenon thought to be caused by early ossification of carpal bones under influence of chronic

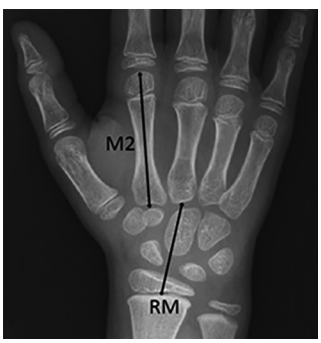

Figure 2 | Poznanski measurements used to determine the RM/M2 score
RM = radiometacarpal length; M2 = length of the second metacarpal
RM = radiometacarpal length; M2 = length of the second metacarpal

hyperemia and inflammation (15). Radiographic bone damage progression was determined by calculating the change in Z-score between the baseline and follow-up radiographs.

Next, all radiographs were imported as DICOM-files in the BoneXpert-software for automatic assessment of the BA (using the average Greulich and Pyle bone age[16]), and BMD (BoneXpert Version 2.1.0.12; Visiana, Holte, Denmark). The software generates Z-scores of the BA computed relative to provided scores of healthy children of equal gender, age (>5 years) and ethnicity. A negative Z-score for BA reflects a delayed bone maturation[12, 17] whereas a positive Z-score reflects enhanced focal maturation, also possible due to inflammation[15]. The BMD is automatically determined by measuring the amount of cortical bone in the shafts of metacarpal 2-4. The Z-score of BMD is computed compared to provided scores in healthy children of the same bone age and gender. A negative Z-score indicates a diminished BMD. For the Z-scores, the normal population has a normal (Gaussian) distribution around 0.

Statistical analyses

The single measure intra-class correlation coefficient (ICC) and Bland-Altman plots with 95% Confidence Interval (CI) were used to determine the agreement of measurements of Poznanski between the two observers and to determine the inter-reader reliability. Baseline characteristics were compared using one-way analysis of variance, Kruskal-Wallis tests or Pearson Chi-square tests, as appropriate. Linear mixed model (LMM) analyses were performed to evaluate the Z-scores of the Poznanski-score, Bone Age and BMD over time between the 3 treatment groups. LMM was also used to evaluate the different Z-scores over time for the mean JADAS-10 score over 2 years' time, since we assumed that average disease activity over 2 years' time could have an effect on slower changing variables like Poznanski score, Bone Age and BMD. We assumed a multilevel structure of measurements over time (level 1), nested within hands (left or right, level 2), nested within patients (level 3), and added a random intercept and slope to take into account correlations of measurements performed within the same hand within the same patient and differences in time periods between the different radiographs. For the Poznanski-score, the model was adjusted for the potential baseline confounders age and duration of symptoms. Since the BA and BMD account for age in itself, the models for BA and BMD were adjusted for duration of symptoms only. Multiple imputation using package mice in software package R was used to deal with missing values for symptom duration and JADAS10-score, with n=10 imputed data sets[11].

For all statistical analyses a p-value<0.05 was considered statistically significant. A deviation of >1 in Z-score, indicating a deviation > 1SD from the mean in a normal population, was

arbitrarily defined as clinically relevant[17]. Statistical analyses were performed with SPSS version 23 software (SPSS, Chicago, IL., USA) and Stata SE version 14 (StataCorp LP).

RESULTS

Patients

Baseline characteristics of the included patients are presented in table 1. Patients in arm 3 were younger and had longer symptom duration than patients in arm 1 and 2. Nine patients with radiographs initially did not have wrist arthritis, six patients never had wrist arthritis clinically.

Of the original 94 patients included in the BeSt-for-Kids cohort, 75 patients had at least 1 hand radiograph available. Overall, 268 radiographs were available. Sixteen radiographs, made outside the selected time frame, were left out, leaving 252 radiographs (n=127 of the left hand and n=125 of the right hand). Of these 92 (in 47 patients) were taken at baseline (with a window of 4 months before and 3 month after inclusion) and 160 (in 52 patients, 27 patients had more than 2 radiographs) during follow-up. Fourteen patients had closed growth plates at baseline and were left out, one patient left the study due to changing diagnosis and was not included in the current analysis[18]. A flow chart of the patient selection process is provided (supplementary file 1). Sixty patients with 252 radiographs (85 in arm 1, 79 in arm 2 and 88 in arm 3) were eligible for scoring by the Poznanski-method and BMD. For analysis of the BA 196 radiographs of 49 patients (65 in arm 1, 67 In arm 2, 64 in arm 3) were eligible, while 56 X-rays (20 in arm 1, 12 in arm 2 and 24 in arm 3) of 11 patients could not be scored because patients' age was <5 years.

The Poznanski-score

For the Poznanski-score the inter-observer correlations were 0.996 for RM and 0.999 for M2. The intra-observer correlations were ≥0.996 for all measurements. Supplementary file 2 provides the Bland-Altman-plots.

At baseline, Poznanski-scores were comparable to those in healthy children, with a median Poznanski-score of-0.45 (-0.74- 0.45). Over time, overall no significant change in Poznanski-score, unadjusted nor after adjusting for age and symptom duration, was observed, and there were no differences between the 3 arms (see figures 3 and 4 for observed and predicted changes in Poznanski-score, BA and BMD Z-scores per arm). The outlier in figure 3B with a high Poznanski-score (Z-score 3,9) is a competing mountain-biker.

Table 1 | Baseline characteristics of the patients selected from the original 3 arms

	Arm 1 Sequential monotherapy (n=21/31)	Arm 2 Combo MTX + 6wks Prednisone (n=18/32)	Arm 3 Combo MTX + etanercept (n=21/29)	P
Age (years), median (IQR)	8.2 (4.1-10.2)	7.9 (5.7-11.7)	6.2 (3.8-10.4)	<0.001
Symptom duration (months), median (IQR)	7.8 (4.2-11.3)	5.3 (2.6-6.1)	8.5 (4.2-12.1)	0.015
ANA pos, n (%)	8 (38)	6 (33)	8 (28)	0.94
Female, n (%)	14 (66.7)	9 (50)	15 (71.4)	0.36
JIA Category:				0.90
Oligo, n (%)	3 (14.3)	2 (11.1)	2 (9.5)	
Poly, n (%)	17 (81)	14 (77.8)	18 (85.7)	
Psoriatic, n (%)	1 (4.8)	1 (5.6)	1 (4.8)	
VAS physician, mean ± SD (mm)	43.6 ± 15.7	54.0 ± 17.0	52.9 **±** 17.5	0.44
VAS patient/parent, mean ± SD (mm)	53 ± 17.1	56.8 ± 23.4	55.2 ± 24.9	0.31
CHAQ, mean ± SD	0.95 ± 0.7	1.1 ± 0.6	1.0 ± 0.6	0.88
No. active joints, median (IQR)	6 (4.5-14.5)	8 (5.8-11.5)	8 (5.5-11.5)	0.56
No. limited joints, median (IQR)	2 (0.5-4)	1.5 (0.8-3.3)	3 (2.5-5.5)	0.68
ESR, median (IQR) (mm)	6 (2-12)	6 (3.5-32)	9 (6-31.5)	0.28
JADAS-10 mean ± SD (0-40)	16.7 ± 4.5	19.6 ± 5.1	19.1 ± 5.8	0.24
Z-score Poznanski median (IQR)[1]	-0.45 (-0.70; 0.56)	-0.19 (-0.57; 0.68)	-0.61 (-0.82; 0.17)	0.056
Z-score Bone Age mean (CI)[2]	-0.38 (-0.88; 0.11)	0.51 (0.28; 0.74)	-0.43 (-0.82;-0.04)	0.001
Z-score BMD mean (CI)[3]	-0.44 (-0.84; -0.04)	-0.39 (-0.93; 0.15)	-1.08 (-1.48;-0.68)	0.03
Wrist arthritis, inclusion (%)	19/21 (90)	14/18 (78)	18/21 (86)	
Wrist arthritis, follow-up (%)	17/21 (81)	14/18 (78)	18/21 (86)	
Wrist arthritis, inclusion or follow-up (%)	21/21 (100)	14/18 (78)	19/21 (90)	

JIA: juvenile idiopathic arthritis; oligo: oligoarticular JIA, poly: polyarticular JIA; IQR: interquartile range; VAS: Visual Analogue Scale; ANA: antinuclear antibody; RF: rheumatoid factor; SD: standard deviation, CI: confidence interval, CHAQ: Child Health Assessment Questionnaire; No: number; ESR: Erythrocyte Sedimentation Rate; JADAS: Juvenile Arthritis Disease Activity Score; BMD: Bone Mineral Density. BA: Bone Age (both using BoneXpert method) Z-scores were based on all available radiographs, including left and right hand radiographs. 1: n=35 in arm 1, n=25 for arm 2, n=31 for arm 3; 2: n=16 for arm 1, n=18 for arm 2 and n=18 for arm 3, 3: n=33, n=25 for arm 2 and n=32 for arm 3. n=amount of X-rays.

Bone Age

At baseline, the mean BA Z-score was 0.04 (-0.58 – 0.67) for the entire group, similar to the normal reference population. Baseline scores in arm 3 were significantly lower than in arms 1 and 2, but still within the normal range (1 SD from 0). Over time there was a decrease in BA in arm 3 (arm 3 versus arm 1 p=0.024, β=0.014 (95%CI -0.002; 0.027) which remained within the normal range (figure 4).

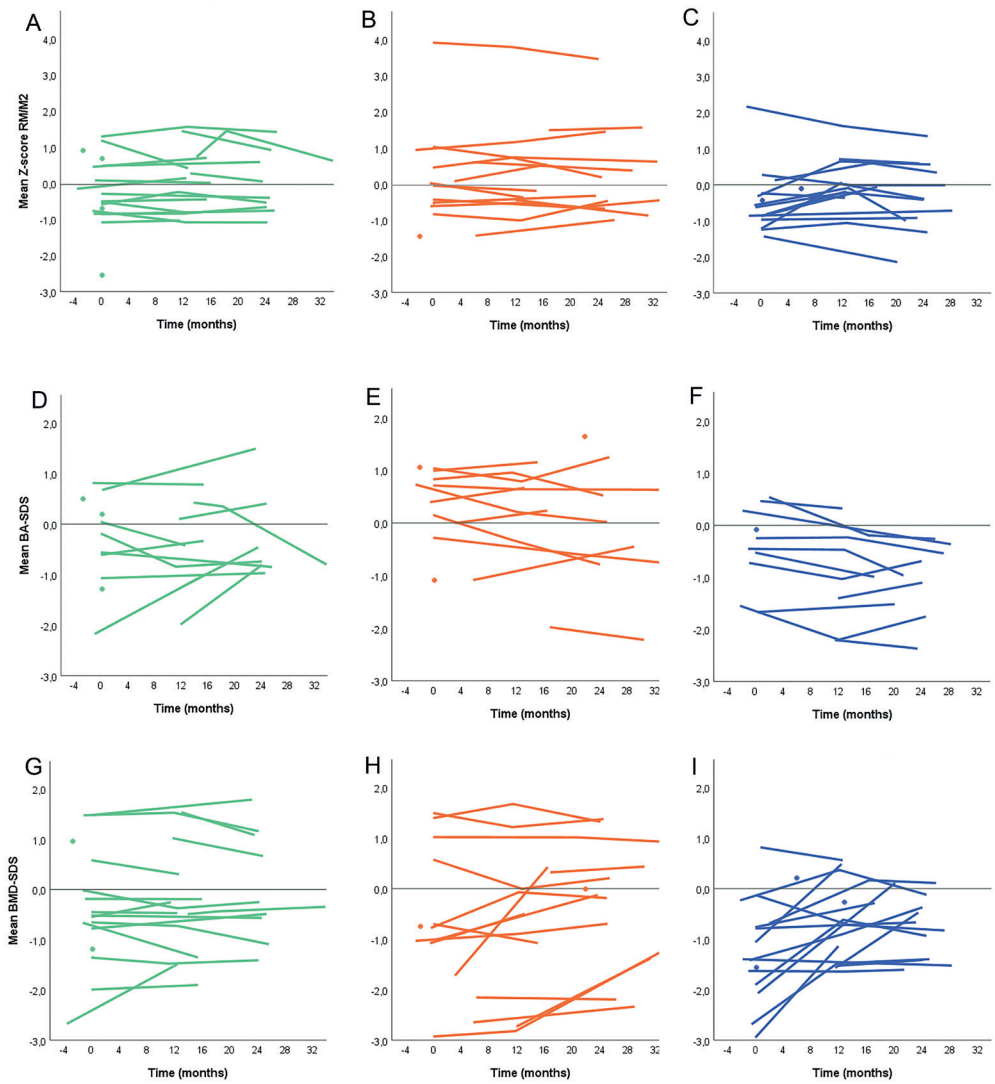

Figure 3 | A,B,C: Poznanski-score depicted in Z-scores of RM/M2 ratio. 3A represents patients in arm 1, 3B represents patients in arm 2, 3C represents patients in arm 3.
D,E,F: Bone Age depicted in Z-score. 3D represents patients in arm 1, 3E represents patients in arm 2, 3F represents patients in arm 3.
G,H,I: Bone Mineral Density depicted in Z-scores. 3G represents patients in arm 1, 3H represents patients in arm 2, 3I represents patients in arm 3.
Each graph line represents one individual patient from baseline to follow-up. Each dot represents one patient with a single radiograph available.

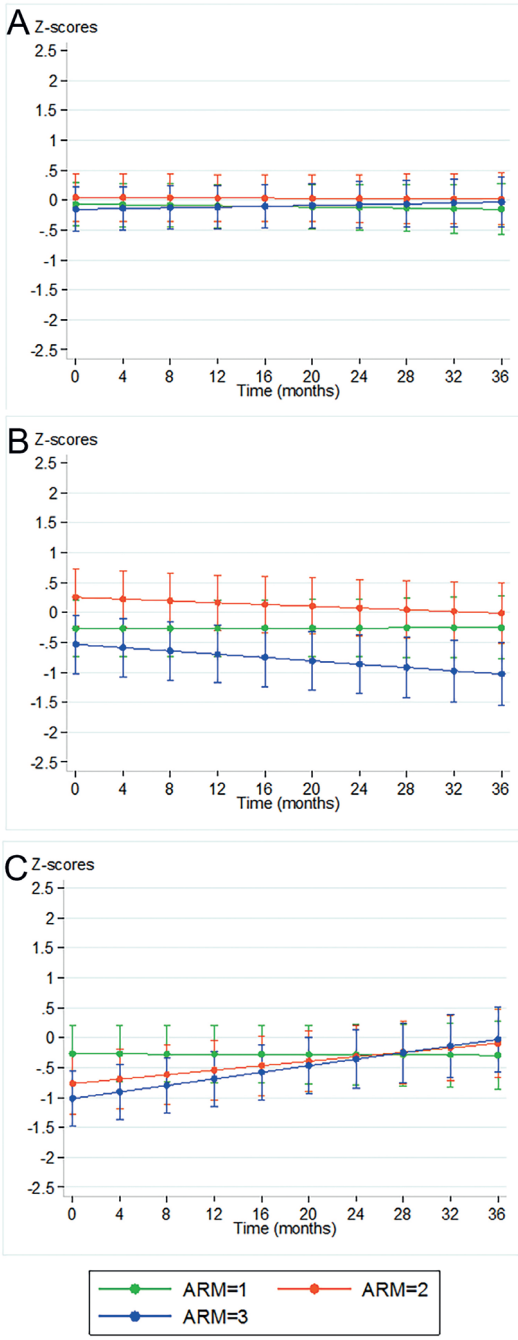

Figure 4 | **A**: Predicted Z-score RM/M2 over time, **B**: Predicted Z-score Bone Age over time, **C**: Predicted Z-score BMD over time. All predictions are from Linear Mixed Models, corrected for age and symptom duration for Poznanski score, corrected for symptom duration for BA and BMD. BA=Bone Age, BMD=Bone Mineral Density.

Bone Mineral Density

At baseline, the mean BMD Z-score was -0.65 (-0.90; -0.40) for the entire group, with statistically significantly lower baseline BMD in arm 3 compared to the normal reference population. Over time the BMD, adjusted or unadjusted for symptom duration, remained unchanged in arm 1, showed a trend for increase in arm 2 and significantly increased in arm 3 ($p<0.001$ for arm 3 versus arm 1 , β=-0.028 (95%CI-0.043;-0.013).

Tables with detailed results of the LMM of the Poznanski-score, BA and BMD are presented in supplementary file 3. Results comparing all left with all right hands, both at baseline and during follow-up, were not statistically different for Poznanski-score (p=0.809, BA (p=0.825) nor BMD (p=0.404). Six patients with radiographs never had clinical inflammation of wrists. Sensitivity analyses excluding these patients showed similar results to the main analysis (supplementary file 4a). Since we included n=7 patients with oligoarthritis and n=3 patients with psoriatic arthritis, numbers are too small to analyse these groups separately. Sensitivity analysis of the polyarticular subgroup only, showed similar results (supplementary file 4b).

Effect mean JADAS-10 score over time on Poznanski, Bone Age and BMD

To investigate whether mean JADAS10-score over time correlated with any of the radiological outcomes, we have performed separate analyses for the 3 radiological outcome measures. Mean JADAS10-score over time did not influence Poznanski score, [β (95% CI) 0.0010 (-0.00038; 0.0024), p=0.154], Bone Age [β (95% CI)-0.00017 (-0.0018; 0.0014), p=0.84] or BMD [β (95% CI) 0.00069 (-0.0012; 0.0026), p=0.48]. Tables and graphs from this analysis are reported in supplementary file 4c.

DISCUSSION

Our study is the first to describe longitudinal radiological outcomes of a tightly controlled treat-to-target approach, aimed at inactive disease during 24 months of treatment, in recent onset poly- and oligoarticular JIA patients. Despite a symptom duration of mean (SD) 7.6 (4.9) months and a JADAS-10 of 18.7 (5.6), at baseline, we found no significant differences in Poznanski-score and BA (as measured by the BoneXpert-method) of wrist radiographs compared to healthy children. Only in arm 3 BMD as measured by the BoneXpert-method was significantly lower than the normal reference population. After 24 months of treatment, there was no deterioration in any of the scores and in arm 3 BMD had statistically significantly improved. Mean JADAS10-scores over time were not associated with any of the radiological outcomes in this analysis.

Combined with rapid suppression of symptoms of active arthritis, prevention of damage is an important treatment goal in JIA. Damage has been most notably found in patients with longstanding and/or seropositive polyarticular JIA, but may also occur in seronegative polyarticular JIA and oligoarticular JIA[19-21]. As has been shown in rheumatoid arthritis, it is thought likely that, also in JIA, damage progression is driven by inflammatory processes. Assessing damage in patients who are in very different phases of joint development can be challenging. In growing children, cartilage thinning, delayed or accelerated growth and reduced bone mineral density rather than bony erosions and joint space narrowing may indicate damage. Decreased bone age often reflects delayed bony maturation in JIA[22, 23] but also increased focal bone maturation can be a result of joint inflammation[15].

Compared to older cohorts[12, 24] or recent cohorts with longer disease duration[17], we found little damage at baseline in this cohort with recent-onset disease. Since we did not include patients with RF-positive polyarticular JIA, this could be a mildly affected cohort although initial JADAS10 scores were similar to other cohorts[25]. In addition we found no significant damage progression. This is possibly due to our strategy of tightly controlled treatment-to-target aiming at inactive disease in all 3 treatment arms, resulting in rapid suppression of inflammation in most patients, without significant differences between the strategy-arms after 24 months. Only in arm 3 there was an initial greater clinical improvement[10]. We cannot rule out an additional positive effect of use of etanercept, in all patients in arm 3, and in many in arms 1 and 2 after they failed to achieve remission on initial treatment with methotrexate (with or without temporary prednisone). Previous studies suggest that treatment with methotrexate cannot prevent joint damage progression whereas use of biologic DMARDs (used as initial treatment in our arm 3) may be more successful, although data are limited[26, 27]. Apart from strategy, we did not find an effect of mean JADAS10-score over time, possibly in all patients due to rapid suppression of inflammation, therefore inhibiting the disease to have time to create damage.

To score differences in potentially little damage, we needed a sensitive scoring method. Conventional radiography has proven to be a useful modality to monitor wrist damage of JIA patients[2, 4, 5, 13, 28-31]. Several methods, like the Dijkstra-score[31], modified Sharp van der Heijde-score[5, 19] the modified Larsen-score[4, 32] and the Steinbrocker-scale[33, 34] have been developed to evaluate radiographic damage to the osteochondral structures of the wrist and hand. The Dijkstra composite-score is limited in the grading of changes for severity over time[35]. We stopped using the modified Sharp van der Heijde for pediatric assessment of joint damage[5] as it proved too difficult to uniformly score subtle changes in joint space narrowing, bony erosions and bone deformity, as was recognized previously[35]. Magnetic resonance imaging (MRI) and ultrasound (US) are suitable for monitoring disease

activity for evaluating treatment response, and may also detect damage[36]. However, interpretation of MRI findings of the osteochondral domain in JIA patients is challenging due to characteristics of the growing skeleton, in particular in hand and wrist joints. Bone marrow edema and bony depressions are also frequently seen on MRI in wrists of healthy children[37-40]. Until now, no optimal method has been found to differentiate pathological and standardized age-specific findings in healthy children on MRI and US which limits their use to accurately assess damage and damage progression in the wrist of JIA patients.

The Poznanski-score, which measures relative carpal length on radiographs of the wrist, is able to detect deviating growth in absence of distinct joint space narrowing or erosions[13]. A disadvantage of the Poznanski-score is that it requires open growth plates, which caused ineligibility in 14 of our patients, and unreliability in case of carpometacarpal erosions which hampers discriminating bony ends, which did not occur in our cohort. In addition, we used the relatively new BoneXpert method to score Bone Age and BMD, which, compared to a healthy reference population, can indicate damage due to inflammation.

The BoneXpert method, based on digital X-ray radiogrammetry (DXR), allows to determine the Bone Age and BMD compared to a normal reference population, at lower costs and with lower radiation than manually comparing the hand radiograph with images in the atlas by Greulich and Pyle[16] and than measuring BMD by Dual Energy X-ray Absorptiometry (DXA)[41]. The BMD measurement by BoneXpert is corrected for the size of the cortical bones to compensate for the high variation in stature of growing children, in contrast to DXA. Previous studies have reported on delayed bone maturation as reflected by negative Z-scores for Bone Age[12, 17]. These studies had included patients with more severe or longstanding active disease. However, Borzutzky and others have warned previously, that determining bone age can be challenging in JIA due to accelerated maturation[15, 42].

In JIA patients, BMD is often reduced[12, 17, 43-45]. BMD was significantly lower at baseline in arm 3 (-1.1 SD, (-1.48; -0.68)). This could indicate longstanding or more severe disease. Indeed symptom duration in arm 3 was slightly longer than in arms 1 and 2, although JADAS-10 scores at baseline were similar in the 3 arms. Possibly as a result of rapid and sustained suppression of inflammation, BMD improved significantly over time in arm 3. A previous study also reported improvement of BMD after therapy[45]. It is speculated that this improvement is due to the anti-inflammatory effect of DMARD treatment[46], more specifically due to etanercept[47, 48]. However, no comparison cohort is available to prove that the treatment-to-target approach is responsible for a better radiological outcome.

Future studies are needed to delineate the effect of the treatment-to-target concept on improving bone health as reflected by bone maturation and BMD in JIA.

Our study has some limitations. Although comparable with other studies ([2, 26, 49]) in children with JIA, we had a relatively small sample size (n=60), and we may have lacked power to detect small differences. In previous studies, results were based on clinically inflamed wrists only. Since we have examined also 6 patients with wrist radiographs of unaffected wrists, in this study we may have underestimated damage, although sensitivity analyses excluding patients who never had any clinical wrist arthritis over 24 months showed similar results. It remains to be determined whether joint damage is mainly due to local inflammation or (also) to systemic inflammatory processes of JIA.
Due to our choice of scoring methods, patients were excluded who had closed growth plates. Also we disregarded results of radiographs made outside the selected time frame. Follow-up time was relatively short compared to previous cohorts. However, often radiographic damage is expected to occur within the first one or two years[2]. Finally, determination of bone health by BoneXpert software needs further validation, including further comparison with existing methods for the determination of BMD in JIA patients[41, 50, 51].

Conclusions

We conclude that in our cohort of patients with recent-onset JIA who were treated-to-target aiming at inactive disease, wrist-radiographs showed neither damage according to Poznanski at baseline, nor progression after 2 years. Bone age was within normal values at baseline and after follow-up. In arm 3, BMD was lower at baseline but improved significantly towards normalization during treatment. We propose that with earlier start of treatment and treatment to target, the focus of current treatment regimens shifts to damage prevention rather than suppression of damage progression. This will likely also prevent long-term disability. Future JIA-cohorts with more patients and longer follow-up are warranted to confirm these promising results for children with JIA.

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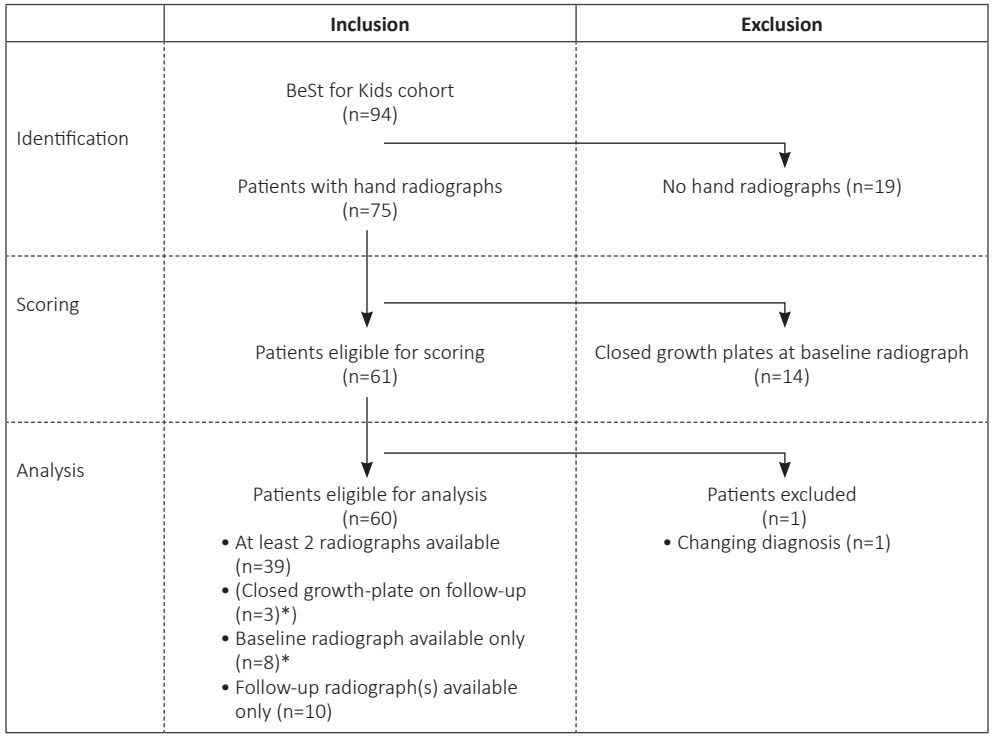
SUPPLEMENTARY FILE 1

Flow chart of patient selection process for the Poznanski-score

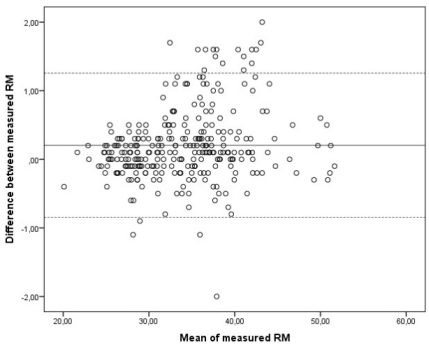


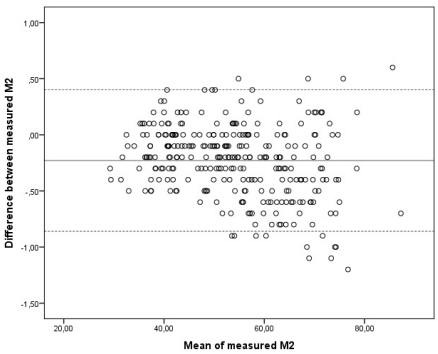
*One radiograph moment used for analysis

SUPPLEMENTARY FILE 2

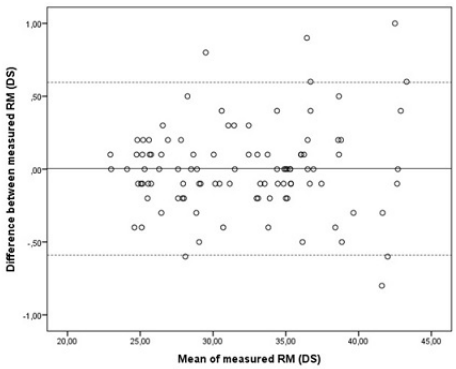
Bland-Altman plots with 95% limits of agreement

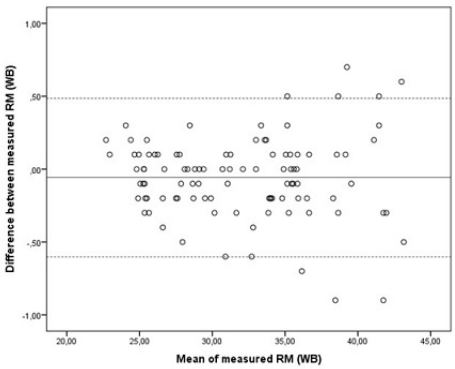
Interreader reliability RM and M2

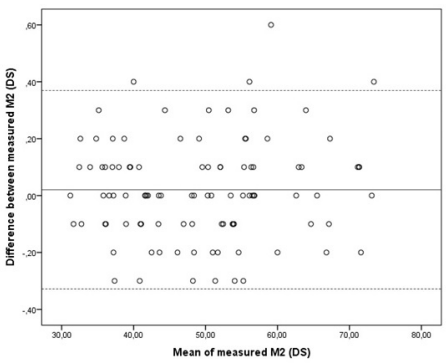


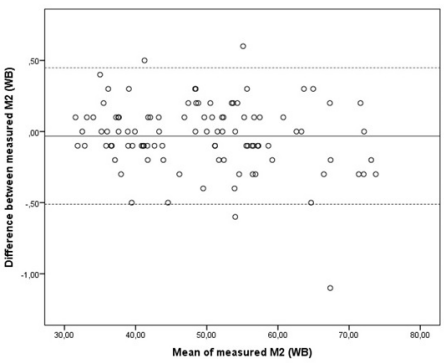


Intra-reader reliability RM and Intra-reader reliability M2









SUPPLEMENTARY FILE 3

Table 2 | LMM for Poznanski, BA and BMD adjusted for age and/or symptom duration

Patients	60
X rays	117

A Poznanski adjusted for age and symptom duration

	Adjusted		Unadjusted	
	B (95% CI)	**P-value**	**B (95% CI)**	**P-value**
Arm 3	ref	-	ref	-
Arm 2	0.20 (-0.35; 0.75)	0.47	0.36 (-0.26; 0.98)	0.26
Arm 1	0.09 (-0.43; 0.61)	0.74	0.21 (-0.39; 0.81)	0.49
Time	0.0033 (-0.004; 0.011)	0.42	0.0035 (-0.004; 0.012)	0.39
Arm 3 * Time	ref	-	ref	-
Arm 2 * Time	-0.005 (-0.015 ; 0.007)	0.47	-0.005 (-0.016; 0.007)	0.42
Arm 1 * Time	-0.006 (-0.017; 0.006)	0.34	-0.006 (-0.017; 0.006)	0.32

Bone Age

Patients	41
X rays	80

B Bone Age adjusted for symptom duration

	Adjusted		Unadjusted	
	B (95% CI)	**P-value**	**B (95% CI)**	**P-value**
Arm 3	ref	-	ref	-
Arm 2	0.78 (-0.09 ; 1.5)	0.026	0.78 (0.11; 1.46)	0.022
Arm 1	0.26 (-0.42 ; 0.94)	0.45	0.26 (-0.41 ; 0.93)	0.45
Time	-0.014 (-0.022;-0.006)	0.001	-0.014 (-0.022;-0.006)	0.001
Arm 3 * Time	ref	-	ref	-
Arm 2 * Time	-0.007 (-0.005 ; 0.018)	0.26	0.07 (-0.005 ; 0.018)	0.26
Arm 1 * Time	0.014 (-0.002; 0.027)	0.024	0.014 (0.002 ; 0.027)	0.024

Bone Mineral Density

Patients	59
X rays	116

C Bone Mineral Density adjusted for symptom duration

	Adjusted		Unadjusted	
	B (95% CI)	**P-value**	**B (95% CI)**	**P-value**
Arm 3	ref	-	ref	-
Arm 2	0.24 (-0.45 ; 0.94)	0.49	0.28 (-0.41 ; 0.98)	0.42
Arm 1	0.74 (0.077 ; 1.41)	0.030	0.74 (0.07 ; 1.41)	0.031
Time	0.027 (-0.017 ; 0.038)	<0.001	0.027	<0.001
Arm 3 * Time	Ref	-	ref	-
Arm 2 * Time	-0.009 (-0.023 ; 0.006)	0.26	-0.009 (-0.02 ; 0.006)	0.25
Arm 1 * Time	-0.028 (-0.043 ;-0.013)	<0.001	-0.028 (-0.043 ;-0.012)	<0.001

LMM: linear mixed model, arm 1: initial sequential monotherapy, arm 2 initial MTX with prednisolone bridging 6 weeks, arm 3 initial MTX with etanercept. BMD Bone Mineral Density; B= β ; 95%CI: 95% Confidence Interval.

SUPPLEMENTARY FILE 4

A Sensitivity Analysis patients with wrist arthritis, without n=6 with never wrist arthritis

Table 3 | LMM for Poznanski, BA and BMD

Poznanski adjusted for age and symptom duration		
Patients	54	
X rays	105	
	B (95% CI)	**P**
Arm 3	ref	-
Arm 2	0.076 (-0.43; 0.58)	0.771
Arm 1	0.16 (-0.30; 0.61)	0.498
Time	0.0036 (-0.0047; 0.012)	0.391
Arm 3 * Time	ref	-
Arm 2 * Time	-0.0058 (-0.018; 0.0066)	0.359
Arm 1 * Time	-0.0058 (-0.018; 0.0061)	0.337

Bone Age adjusted for symptom duration		
Patients	37	
X rays	72	
	B (95% CI)	**P**
Arm 3	ref	-
Arm 2	0.99 (0.31; 1.68)	0.005
Arm 1	0.34 (-0.31; 0.98)	0.304
Time	-0.014 (-0.022;-0.0053)	0.001
Arm 3 * Time	Ref	
Arm 2 * Time	0.0092 (-0.0029; 0.021)	0.136
Arm 1 * Time	0.014 (0.0016; 0.027)	0.027

Bone Mineral Density adjusted for symptom duration		
Patients	53	
X rays	104	
	B (95% CI)	**P**
Arm 3	ref	-
Arm 2	0.077 (-0.67; 0.82)	0.841
Arm 1	0.84 (0.16; 1.51)	0.015
Time	0.028 (0.017; 0.039)	<0.001
Arm 3 * Time	ref	-
Arm 2 * Time	-0.0054 (-0.022; 0.011)	0.524
Arm 1 * Time	-0.029 (-0.045;-0.013)	<0.001

LMM linear mixed model, arm 1: initial sequential monotherapy, arm 2 initial MTX with prednisolone bridging 6 weeks, arm 3 initial MTX with etanercept. BMD Bone Mineral Density; B: β ; 95%CI: 95% Confidence Interval

ADDITIONAL FILE 4 B

Sensitivity Analysis Polyarticular JIA patients only

Table 4 | LMM for Poznanski, BA and BMD adjusted for age and/or symptom duration

Poznanski			
Patients	50		
X rays	99		
		B (95% CI)	**P-value**
Arm 3		ref	-
Arm 2		0.227 (-0.035; 0.81)	0.444
Arm 1		0.219 (-0.34; 0.77)	0.44
Time		0.0035 (-0.0049; 0.012)	0.415
Arm 3 * Time		ref	-
Arm 2 * Time		-0.0041 (-0.016; 0.0075)	0.493
Arm 1 * Time		-0.0042 (-0.016; 0.0078)	0.491

Bone age adjusted for symptom duration			
Patients	34		
X rays	68		
		B (95% CI)	**P-value**
Arm 3		Ref	-
Arm 2		0.89 (0.18;1,61)	0.015
Arm 1		0.45 (-0.28 ; 1.17)	0.225
Time		-0.012 (-0.023;-0.0017)	0.023
Arm 3 * Time		Ref	-
Arm 2 * Time		0.0086 (-0.0056; 0.023)	0.24
Arm 1 * Time		0.015 (-0.0003; 0.03)	0.055

Bone Mineral Density adjusted for symptom duration			
Patients	50		
X rays	100		
		B (95% CI)	**P-value**
Arm 3		ref	-
Arm 2		0.37 (-0.38 ; 1.12)	0.33
Arm 1		0.64 (-0.08 ; 1.36)	0.082
Time		0.027 (0.015 ; 0.038)	0
Arm 3 * Time		Ref	-
Arm 2 * Time		-0.10 (-0.026 ; 0.0053)	0.20
Arm 1 * Time		-0.026 (-0.043 ;-0.010)	0.001

LMM linear mixed model, arm 1: initial sequential monotherapy, arm 2 initial MTX with prednisolone bridging 6 weeks, arm 3 initial MTX with etanercept. BMD Bone Mineral Density; B: β ; 95%CI: 95%Confidence Interval

ADDITIONAL FILE 4C

Mean JADAS-10 score over time

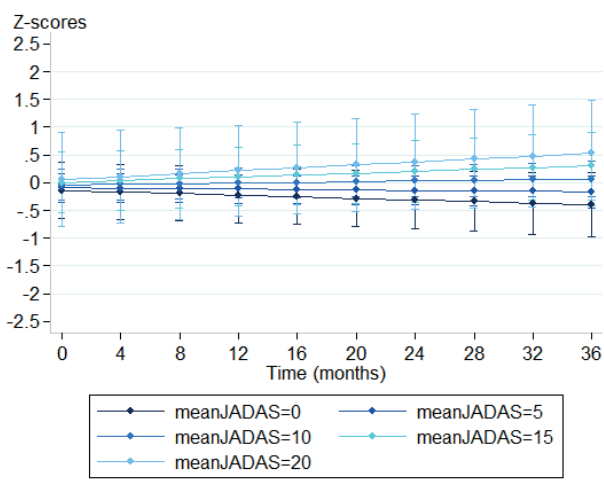
Poznanski adjusted for age and symptom duration		
Patients	60	
X rays	117	
	B (95% CI)	**P-value**
meanJADAS10 * Time	0.0010 (-0.00038-0.0024)	0.15

Bone Age adjusted for symptom duration		
Patients	41	
X rays	80	
	B (95% CI)	**P-value**
meanJADAS10 * Time	-0.00017 (-0.0018-0.0014)	0.84

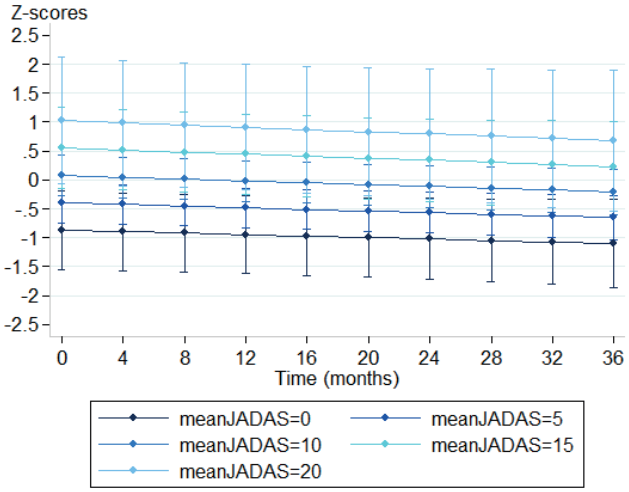
Bone Mineral Density adjusted for symptom duration		
Patients	59	
X rays	116	
	B (95% CI)	**P-value**
meanJADAS10 * Time	0.00069 (-0.0012-0.0026)	0.48

LMM linear mixed model; meanJADAS10: mean Juvenile Arthritis Disease Activity Score with up to max 10 joints, over 2 years' time; B: β ; 95%CI: 95%Confidence Interval

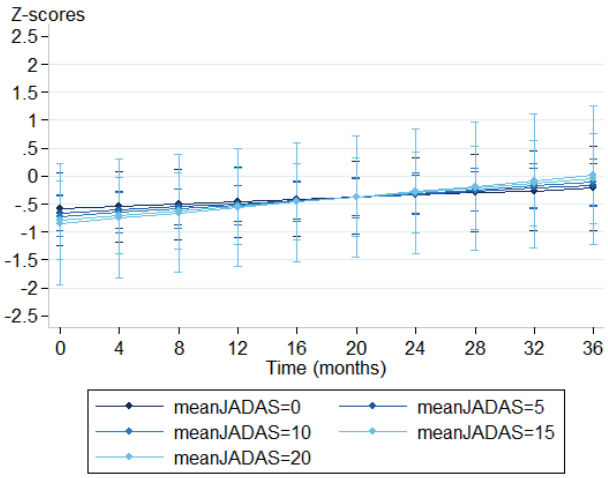
Predicted Z-scores RM/M2 (Poznanski score) over time, for different mean JADAS-10 scores



Predicted Z-scores for Bone Age over time for different mean JADAS-10 scores



Predicted Z-scores for Bone Mineral Density over time for different mean JADAS-10 scores



All predictions are from Linear Mixed Models, corrected for age and symptom duration for Poznanski score, corrected for symptom duration for Bone Age and Bone Mineral Density

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Participation in a single-blinded Pediatric Therapeutic Strategy Study for Juvenile Idiopathic Arthritis: Are Parents and Patient-participants in Equipoise?

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ABSTRACT

Background: Genuine uncertainty on superiority of one intervention over the other is called equipoise. Physician-investigators in randomized controlled trials (RCT) need equipoise at least in studies with more than minimal risks. Ideally, this equipoise is also present in patient-participants. In pediatrics, data on equipoise are lacking. We hypothesize that 1) lack of equipoise at enrolment among parents may reduce recruitment; 2) lack of equipoise during participation may reduce retention in patients assigned to a less favoured treatment-strategy.

Methods: We compared preferences of parents/patients at enrolment, documented by a questionnaire (phase 1), with preferences developed during follow-up by an interview-study (phase 2) to investigate equipoise of child-participants and parents in the BeSt-for-Kids-study (NTR 1574). This trial in new-onset Juvenile Idiopathic Arthritis-patients consists of three strategies. One strategy comprises initial treatment with a biological disease-modifying-antirheumatic-drug (DMARD), currently not standard-of-care. Semi-structured interviews were conducted with 23 parents and 7 patients, median 11 months after enrolment.

Results: Initially most parents and children were not in equipoise. Parents/patients who refused participation, regularly declined due to specific preferences. Many participating families preferred the biological-first-strategy. They participated to have a chance for this initial treatment, and would even consider stopping trial-participation when not randomized for it Their conviction of superiority of the biological-first strategy was based on knowledge from internet and close relations. According to four parents, the physician-investigator preferred the biological-first-strategy, but the majority (n = 19) stated that she had no preferred strategy. In phase 2, preferences tended to change to the treatment actually received.

Conclusions: Lack of equipoise during enrolment did not reduce study recruitment, mainly due to the fact that preferred treatment was only available within the study. Still, when developing a trial it is important to evaluate whether the physicians' research question is in line with preferences of the patient-group. By exploring so-called 'informed patient-group'-equipoise, successful recruitment may be enhanced and bias avoided.
In our study, lack of equipoise during trial-participation did not reduce retention in those assigned to a less favoured option. We observed a change for preference towards treatment actually received, possibly explained by comparable outcomes in all three arms.

Key words: randomization, equipoise, pediatric rheumatology, clinical trial, informed consent

BACKGROUND

It is an ethical requirement that physician-investigators provide research-participants the best treatment available in randomized controlled trials (RCT)[1-3]. In a clinical trial comparing different treatment strategies there should be uncertainty regarding preferred treatment option considering therapeutic efficacy and safety[1-4]. This is called equipoise. 'Individual equipoise' implies that the *individual* physician-investigator must possess this genuine uncertainty[1]. Affected by preliminary results during a trial, a physician-investigator could develop a preference which might lead to a perceived conflict whether the best treatment known is actually provided. Therefore, Freedman described 'clinical equipoise' as genuine uncertainty in the *medical expert community* instead of genuine uncertainty in the individual physician-investigator[1]. Clinical equipoise allows the physician-investigator to collect evidence to convince the expert community of either superiority. Critics on clinical equipoise argue that this concept needs to be transformed to adapt to forward modern health care: if the potential social value of a study is relevant and participants are not exposed to excessive net risks, clinical equipoise can be amended[5, 6]. Kimmelmann questions whether equipoise should be rethought as a prima facie principle rather than an absolute one[7] and we agree on that. Miller et al even question the necessity of equipoise[8].

Literature suggests that not only the medical expert community should be in equipoise but also patient-participants (and their parents, if children are concerned)[8, 9].
In pediatrics, experience with clinical equipoise is limited[10, 11]. In pediatric oncology, parental and physician equipoise has been scarcely studied[12, 13]. Difficult protocols, strong emotions and the parents' dependency on their child's physician are reasons for often lacking parental equipoise. In pediatric rheumatology, treatment preferences among physicians have been studied by Hugle et al[14] revealing that availability and funding influenced physicians' choices. A discrete choice experiment explored parents preferences in juvenile idiopathic arthritis in daily clinical care[15]. Parents have strong preferences for treatments that reduce pain and improve daily functioning, regardless of side effects. With increasing disease duration, parents preferences focused on therapeutic effectiveness. Little is known about patient-participant-equipoise or parent-equipoise[9, 16, 12] in pediatric clinical research on chronic diseases. More insight in this equipoise is particularly important when considering the inherent vulnerability of children in research[17].

METHODS

Aims

In this study we aimed to evaluate (1) the preferences of Juvenile Idiopathic Arthritis (JIA) patients aged 12 years and older, and their parents for a certain treatment strategy in the setting of a randomized clinical trial, and (2) the influence of the informed consent procedure on these preferences.

Context BeSt for Kids study

The trial concerned *the BeSt for Kids study* (Dutch Trial Register NTR 1574), a multicenter, randomized, single blinded two year follow-up clinical trial comparing time- to-inactive disease and time-to-flare in selected categories of newly diagnosed JIA patient-participants. In this study patient-participants (age between 2-16 years) with a maximum of 18 months of complaints and 1) oligoarticular JIA 2) Rheumatoid Factor (RF) negative polyarticular JIA and 3) Juvenile Psoriatic Arthritis, (6) with active disease requiring treatment with a disease modifying antirheumatic drug (DMARD) according to the treating pediatric rheumatologist were randomized in three treatment strategies. These strategies are

1. initial monotherapy with sulfasalazine or methotrexate,
2. initial combination therapy with methotrexate and prednisone bridging
3. initial combination therapy with methotrexate and etanercept.

In the study protocol subsequent steps to reach inactive disease are dictated in case of insufficient response in all three arms (supplementary file 1).

Data on efficacy of the different individual DMARD is available in literature[18-20]. No data existed before and during inclusion on the superiority of either of those strategies.
The treatment was single-blinded: the periodic assessment of disease activity was performed by a physiotherapist, unaware of the allocated treatment, but patient and physician were not blinded.

The informed consent procedure for inclusion in the BeSt for Kids study consisted of at least one visit to the outpatient clinic, with an oral explanation by the attending physician and the research nurse and complementary written information. In addition to the patient-subjects information form (PIF) (supplementary file 2) , all newly diagnosed patients with juvenile idiopathic arthritis are referred to www.printo.it for general information on juvenile idiopathic arthritis. Besides that, patients and parents had several days to week(s) between receiving written PIF and actual enrolment.

Design equipoise study

Phase 1 (questionnaire): When parents and children (aged 12 years and older) consented to participate in the trial, they were randomized. Subsequently the physician completed the Case Report Form which included a questionnaire asking parents and patients for their preferred strategy before actual allocation to the strategy(supplementary file 3). To diminish bias by only asking participating parents and patients we additionally collected the reasons for not participating in the study.

Phase 2 (interview study): We conducted an interview study with parents and patient-participants aged 12 years and older participating in the BeSt for Kids study which was designed after of the onset of the study. Parents and patient-participants were informed of the interview by a letter asking them to participate in the interview study. All patients enrolled in the study at that time point (n=29) were contacted by telephone for participation, which lead to an appointment for an interview with a short questionnaire. To facilitate families, the actual interview was held in the hospital or at home by choice. One-to-one, semi-structured interviews were conducted with the parents and patient-participants (supplementary file 4).

We choose the age of 12 years for patient interviews since in the Netherlands by law children from the age of 12 years old are actively involved in their healthcare related decisions in consultation with their parent(s) or guardian(s).

Interview Procedure and Analysis

All parents and patient-participants were interviewed by researcher B.Y. Interview topics and questions were formulated after evaluation of the relevant literature. Topics were: 1 Evaluation of the Informed consent procedure, 2 Preference for treatment strategy, 3 Comments on preference, 4 Impression of physicians' preference and 5 Main reasons for participation in BeSt for Kids study. Interviews contained closed-ended as well as open-ended questions. Using the latter, participants could elaborate on their answers on closed-ended questions. Interviews lasted between 20 and 45 minutes.

The interviews were recorded and transcribed verbatim. Data analysis of the interviews was based on the constant comparative method[21, 22]. One of the researchers encoded the full transcripts manually by identifying and labeling discrete units of texts which refer to one or more concepts relevant to the study. Through comparison across transcripts, open codes were developed into higher order themes to provide a framework for coding subsequent transcripts. P.H.M. and M.d.V. coded a random sample of the interviews to check for consistency and adequacy of the framework. When no new thematic content was found in the parent interviews, subject enrolment was stopped. This process, called

thematic saturation, is a well-described qualitative method to avoid unnecessarily large and repetitive data sets[23, 24].
Finally, representative quotations from parents and physicians were chosen to demonstrate the themes identified.

Setting
The BeSt for Kids and the current project were approved by the Institutional Review Board at Leiden University Medical Center and written informed consent was obtained from all participants before enrolment.

RESULTS

Phase 1 Questionnaire: Preference at inclusion in the BeSt for Kids study
During recruitment, we have received information on reasons for refusal in 15 out of 36 refusals (table II). In total 94 children were randomized in the BeSt for Kids study.
All parents and children aged 12 and older were asked during enrolment in the BeSt for Kids study, before randomization, whether they hoped to be assigned to a particular treatment strategy. At the start 46% of parents of all enrolled patient-participants (n=94) expressed to have no preference, 34% hoped for assignment to strategy 3 (initial etanercept with methotrexate) and 7% hoped against assignment to strategy 3. Primary aversion was highest for the second strategy (25%) due to a dislike of prednisone (data not shown). To compare, reasons for refusal to participate in the study were documented in 15/36(42%) and are summarised in table II. Multiple reasons were expressed, ranging from strong preference to dislike for a particular arm. Six out of 36 (17%) expressed explicit preferences or dislike of arm 1 or arm 3.

Table 1 | Summary of patients/parents who refused to participate in the BeSt for Kids study with reasons for refusal

Number of patients who refused to participate **Known Reason for refusal of trial participation**	**n=36** **n=15 (42%)**
Preference for arm 1	1
Preference for arm 3	1
Fear in general	1
Do not want to randomize at all	2
Do not want to receive prednisone or injections	2
Do not want to receive arm 3	2
Too busy to participate	2
Don't feel like it	2
No reason mentioned when asked	2

Phase 2: Interview study

Figure 1 shows the recruitment of parents and patient-participants in our interview study. Twenty-nine patients were approached for the interview-study, finally 23 interviews were conducted. Parents had a mean age of 40.0 years (range 32-51 years) and patient-participants 14.3 years (range 12-17 years). All participants were Dutch speaking. Characteristics of parents and patient-participants are given in Table I. Between inclusion in the BeSt for Kids study and the interview was a period of mean 12 (3-19) months.

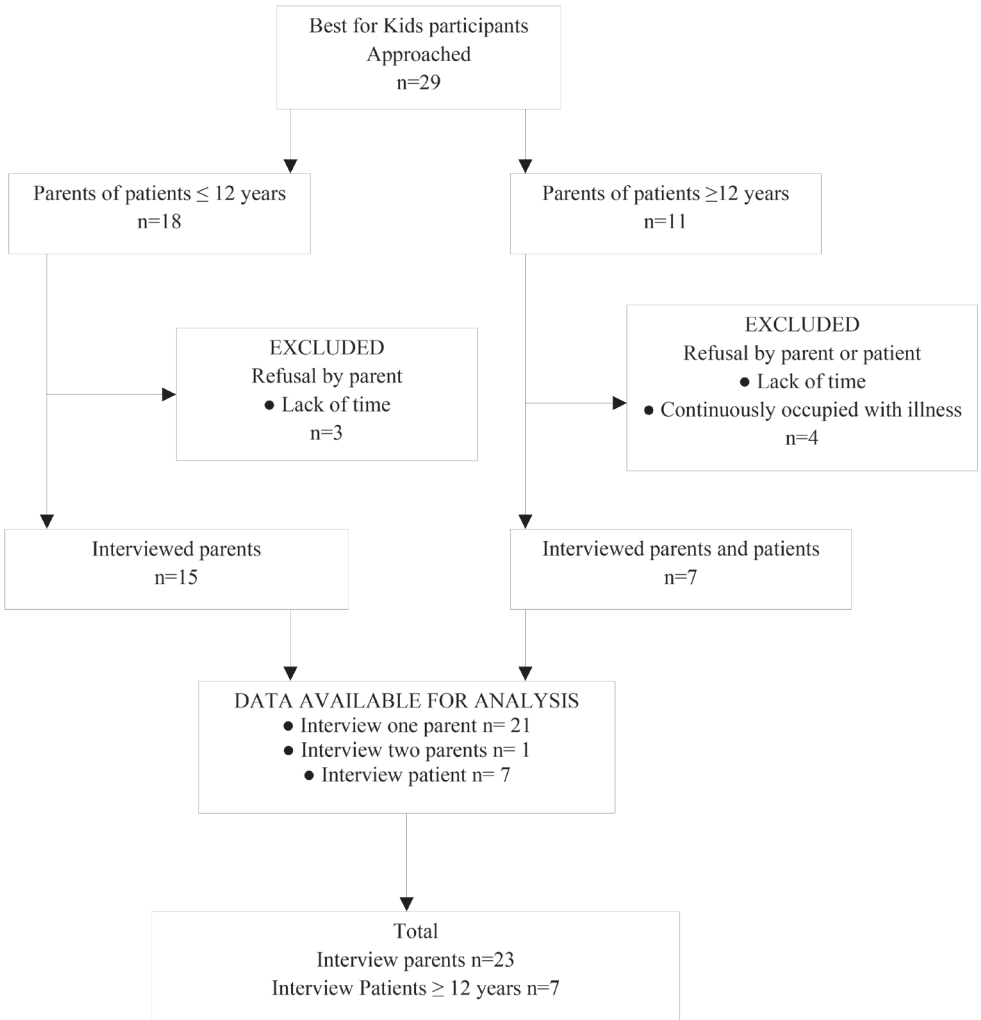

Figure 1 | Recruitment of parents and patients

The concepts that were identified in the qualitative analysis resulted in a framework that comprises the following three themes which will be discussed separately:

I Participation is not without consequences.
II The conviction of superiority of the 'experimental' strategy.
III Participation is in the best interest of the child.

Theme 1: Non-participation or withdrawal is not without consequences

The majority of parents and children was well aware of the study design. Results indicated no differences between parental and patient-participants' understanding concerning study name, duration, aim and number of treatment strategies. Although almost all participants (parents n=23, patient-participants n=6) knew that they were allowed to withdraw from the study at all times, seven parents and four patient-participants believed that stopping the trial would have consequences for the treatment in terms of less quality and quantity of the patient-participant care.

(Parent 8) Sure it has consequences. This means he is not being looked after […]
(Parent 18) Now she gets such good care. That would be less; there would be less time and less checkups.

Two parents and three patient-participants argued that it would have consequences because initial treatment with etanercept is not covered by insurance companies outside the trial.

(Parent 19) Yes I think so. If you stop then you receive no further medication.
(Patient-participant 17) It could be that the insurance company requires you to pay for the drugs.
(Patient-participant 23) Because if I stop it has consequences for my treatment. Because I could never get this treatment by the insurance.

Theme 2: The conviction of superiority of the experimental strategy

All parents (n=23) expressed that they had a preference for a particular treatment strategy. Fourteen parents (61%) preferred the third strategy (initial etanercept with methotrexate) whereas 3 (13%) preferred the first strategy (initial sulfasalazine or methotrexate), one parent preferred the second strategy (initial methotrexate and prednisone bridging), one parent preferred the first or second strategy and four (17%) preferred a non-prednisone strategy.

Five of seven patient-participants had a preferred strategy. Three of them preferred initial etanercept with methotrexate (third strategy) and two preferred a non-prednisone strategy. Two of the patient-participants had no preference.

Generally, as main explanation for their preference for the 3rd strategy (initial etanercept with methotrexate) parents and patient-participants stated that they believed it is the best treatment for JIA given the results of previous studies[25-28].

(Patient-participant 23) I also wanted that drug (etanercept), even though we did not know the side effects. Still, if you hear that it works very well and that the arthritis completely disappears from your joints, and therefore it is the best drug, then it seems obvious to me that you would want that.
(Parent 3) At one moment I read on the internet an experience by a mother who said she finally got her teenage daughter back, this was not the crucial reason but at that moment it confirmed my gut feeling.
(Parent 18) Well we have been searching quite a lot on the internet and it just gives reasonably good results as far as they presently know.

Parents and patient-participants indicated that these beliefs were mainly based on knowledge they had gained through the internet and from experiences in their environment. For both parents and patient-participants reluctance to prednisone was due to well-known side-effects, mainly gaining weight.
(Patient-participant 19) What I didn't want was prednisone, actually. That's because my mother had to use it for a long time and I have seen what it does, it has a lot of severe side effects.

The majority of parents (n=19) mentioned that the physician did not express a preferred treatment strategy. Four parents stated that the physician preferred etanercept-first strategy.
(Parent 6) The physician had no preference. She indicated that she was very curious about what the outcomes of the study will be.

(Parent 9) Yes, that new medicine. That's what she really said. She literally said that they would like to give it to us. But that simply couldn't, because of the study and because insurance companies do not want to pay.

(Parent 14a) I thought that the physician absolutely had no preference at all.

(Parent 16) Because of course, I didn't know it yet, we were confronted with a diagnosis that really was unexpected. […] So I had the impression that they were very enthusiastic about this new drug, and that the study gave us the opportunity to get it earlier.

One of the patient-participants thought that the physician preferred initial treatment with etanercept. One did not remember and five recalled that the physician did not have a preference.

Theme 3 Participation is in the best interest of the child
Although some families stated that they (also) participated in the BeSt for Kids study to help the good cause of research and therefore to support the next generations of JIA patients (n=6 parents), many families expressed personal reasons to participate.

(Parent 4) […] It was more the extra attention [….] However, also a little bit for the good cause, of course , that other patients could benefit from it as well in the long term.

Seven parents joined the study because they assumed that in participating, their child would be more closely observed. Parents also participated for the best prospect of their child (n=5), hoping that joining the study was the best they could do. Five parents stated that their reason for participation was to have the opportunity for initial treatment with etanercept (strategy 3). One mother even expressed that she would have withdrawn from the trial if her child had not randomized in their preferred strategy.

(Parent 23), Had I drawn strategy 2, I would have immediately stopped. Then I would have chosen my own direction with my child, off course in consultation with the physician In that case I would not want to participate in the study, and then you should have to work with the available resources.

(Parent 5) [….]Of course there are many benefits as a result of taking part…
Patient-participants also joined the study because of a chance for initial treatment with etanercept (strategy 3)(n=3).

(Patient-participant 23) I believe that our main goal was that we could get the really good medication.

Two patient-participants only mentioned their wish to recover (n=2) without giving another reason for participation. Two patient-participants chose to participate for the good cause of research.

Changing preferences
When comparing the results from phase 1, before randomization, to the results during the interview (phase 2), half of the parents (11/23) showed a different preference in the

interview compared to their opinion at enrolment, from which 6 out of 11 changed to preference of the actual enrolled treatment strategy, mainly increasing preference for arm 1 and arm 3. Eight out of 23 (36%) had a persistent preference for strategy 3 (Supplementary file 5 Table III and IV).

DISCUSSION

The results of our interview study demonstrate that at enrolment (phase 1) many parents and children in the BeSt for Kids study are not in equipoise, because most of them hold the conviction that strategy 3, initial combination therapy with methotrexate and etanercept, is medically superior to the other strategies, as described in theme 2. In the majority of parents this is not caused by an assumed preference of the physician-investigator but by information on the various treatment possibilities obtained from other sources.

Aversion for prednisone was based on fear for possible side-effects, whereas information on etanercept appeared to focus more on the efficacy and less on possible (future) side effects. This result is consistent with previous results in the BeSt-trial in rheumatoid arthritis patient-participants[29].

This is an evaluation of parents' preferences for treatment strategies by a questionnaire at enrolment (phase 1) as well as by interviews several months into the different treatment strategies (phase 2). When comparing results of the preferences at the two time points we conclude that parents increasingly preferred initial combination therapy with etanercept and methotrexate and disliked taking prednisone. Having a preferred strategy in general increased from 62% at enrolment to 100% of parents during the study period. This difference can be explained by the fact that the interview took place almost a year after study enrolment so that perception can be modified by experience. Initially preferences focused on fear of side effects of prednisolone and suspected superiority of the initial etanercept (arm 3). In phase 2 preferences shifted to mainly arm 1 and arm 3, often the actual strategy children received. Parents and children by then seemed to focus on effectiveness of the therapy received, as was described previously[15].

Parents have many motivations when deciding whether or not to let their child enter a randomized clinical trial. They will not easily agree on randomization because an ethics committee has approved the study[30, 31]. The primary responsibility of parents is to act in (what they think is) the best interest of the child, and the choice to enter a trial is based both on 'objective' probabilities of trial outcomes and on the value that parents

and patient-participant place on those outcomes[32]. Also in our study, the main reason for parents to participate in the BeSt for Kids study was to support the best interest of their child(theme 3). For some parents, the trial represented the prospect of receiving a new, not routinely available treatment with a potentially important direct benefit to their child as was recognized previously as an important motivator for parents and patients to participate in studies[33, 30]. Etanercept is in the Netherlands not reimbursed as first treatment option. Therefore, as initial treatment, it was only available within the trial and parents may consent to their child's entry because of the chance of receiving these assumed benefits[34, 30]. It may cause them firstly to anticipate remorse for not at least trying to obtain this new treatment through trial participation, and secondly to expect consequences when withdrawing during the study. This is understandable from their perspective as guardian of the interests of their child[12]. One could imagine a different outcome in cases where parents prefer a standard treatment which is routinely available outside of research. Additionally, a short course of Prednisolone is regularly applied in daily patient care in Juvenile idiopathic arthritis patients as bridging therapy, since methotrexate is a slow-acting DMARD.

Whatever trial strategy parents think is better for their child, their preference shows that the idea of clinical equipoise held by the expert medical community is not directly transferable to the parent setting as a proxy[35]. For parents and children the different strategies of a trial are often not in equipoise, because they hold the conviction that one strategy is medically superior[33, 34, 36]. Although in this study many parents, but not all, were prepared to enter a study (and continue participation) when they had preferences for therapy other than what they received, this lack of equipoise could be a major problem for recruitment in RCT's.

The question is whether the absence of equipoise in parents and patient-participants indicates that randomization is unethical. Some authors state that participation is not permissible when there is no patient-participant equipoise[9]. We however argue that equipoise is based on 2 obligations: 1) a clinical obligation, which contains a) a scientific and b) a therapeutic part. The therapeutic obligation (1b) means that children should not be randomized to inferior treatments at least in studies with more than minimal risks. This is an obligation of the medical community which is not influenced by parental or child preferences; the scientific obligation (1a) means there are clear epistemic reasons to conduct a randomized clinical trial to acquire robust data. This *is* influenced by parental or child preferences, especially when families withdraw their consent due to a preference and thereby introduce bias. 2) an ethical obligation i.e. patient-participants and parents should be well informed on the experimental nature of the study and should understand the aim of the study. They should also understand the scientific consequences (limited resources, inefficiency in the study, possible loss of trust in medical research) to the trial when withdrawing if not randomized in the preferred strategy.

In this study we evaluated the concept of equipoise by exploring patients' and parents' decisions about whether or not to participate in a trial for a chronic disease and their individual reasons to do so. Viewed that way, patient equipoise is concerned with a "committed personal decision"[35] and is an *individual* standard. One could however also imagine an informed *patient group* equipoise, in analogy to equipoise in the expert community (as opposed to individual equipoise of a single researcher or treating physician). What seems to be equally important is that not only the patient group should be informed, but also the researcher, by gathering information on the values and preferences patients have at the start of treatment. This will help to know the patient group as a whole and can facilitate recruitment.

Recently it was suggested by Whybrow[35] to take an epidemiological approach to the concept of equipoise situating it as a measurable characteristic of a target patient group. We argue that both types of patient equipoise (individual and group) are relevant at different time points in the clinical research setting. Individual patient equipoise is relevant when actually contemplating trial participation between physician and patient/parent. Individual values need to be discussed and exchanged to explore possible trial participation. Informed group patient equipoise is relevant, and we would say even necessary, in the phase of developing a trial to evaluate whether the research question the physician wants to answer is in line with the preferences of the patient group.

If scientists are aware of the consequences of strong patient preferences by evaluating the patient group equipoise they can anticipate to the possible lack of inclusions in a trial. Vigilance is warranted especially in studies with a placebo-arm since retention in those studies potentially is at risk. One example with a deviating clinical expert equipoise and patient group equipoise is the CONCERT study. *The CONgenital Cmv: Efficacy of antiviral treatment in a Randomized controlled Trial (*CONCERT) study aimed to evaluate the efficacy of antiviral therapy in congenital cytomegalovirus (CMV) infection in a randomized clinical trial (clinical trials.gov NCT01655212). The inclusion period was terminated prematurely due to lack of inclusions since parents did not want to randomize for placebo treatment (personal communication). The study design was changed to an efficacy study with a historical control group. 'Placebo' versus 'treatment' is different from 3 different treatment strategies. Therefore every particular study design may need a different approach in case of lacking equipoise on the level of the informed patient group. Preferably the informed patient group is involved early in the study design to prevent lack of inclusion in studies. As an example, the future research agenda in JIA will be created according to the James Lind Alliance method[37], to create research that really matters to parents/patients and caregivers and this will potentially increase enrolment in future studies.

CONCLUSION

Parental and patient equipoise is important to investigate to enhance recruitment for and retention in studies involving children. In our study, lack of equipoise during enrolment did not reduce study recruitment, due to the fact that preferred treatment was only available within the study. Still, when developing a trial it is important to evaluate whether the research question the physicians want to answer is in line with the preferences of the patient group. By exploring 'informed patient-group' equipoise successful recruitment may be enhanced and bias may be avoided.

Lack of equipoise during participation in our long term follow-up trial did not reduce retention in those who were assigned to a less favoured option. We observed a change for preference towards treatment actually received, possibly explained by favourable outcomes in all three arms[38].

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SUPPLEMENTARY FILE 1

The three treatment strategies in the BeSt for Kids study

arm 1 **Sequential Monotherapy**	**arm 2** **Combination MTX + 6 wks prednisone**	**arm 3** **Combination etanercept and MTX**
MTX 10 mg/m^2/wk po/sc Alternative: SSZ 50 mg/kg/day po	MTX 10 mg/m^2/wk po/sc + pred 0.5 mg/kg/day tapered to 0	MTX 10 mg/m^2/wk po/sc + ETN 0.8 mg/kg/wk
↓	↓	↓
MTX 10 mg/m^2/wk po/sc	MTX 15 mg/m^2/wk po/sc	MTX 10 mg/m^2/wk + ETN 1.6 mg/kg/wk
↓	↓	↓
MTX 15 mg/m^2/wk po/sc	MTX 10 mg/m^2/wk po/sc + ETN 0.8 mg/kg/wk	Treatment left to treating physician
↓	↓	
MTX 10 mg/m^2/wk po/sc + ETN 0.8 mg/kg/wk (max. 50 mg)	MTX 10 mg/m^2/wk + ETN 1.6 mg/kg/wk	
↓	↓	
MTX 10 mg/m^2/wk + ETN 1.6 mg/kg/wk (max. 50 mg)	Treatment left to treating physician	
↓		
Treatment left to treating physician		

MTX=methotrexate, SSZ=Sulphasalazine, ETN=etanercept, Po=orally, Sc=subcutaneous

SUPPLEMENTARY FILE 2

Questionnaire during enrolment BeSt for Kids study (Translated from Dutch)

This questionnaire is concerning satisfaction of patient and/or his/her parents with the treatment in the study.

Your child/you are participating in the BeSt for Kids study, a study in search for the optimal treatment strategy in patients diagnosed with juvenile idiopathic arthritis.

What we do not know yet, is, if you as patient and parents have a preference for one of the different strategies in the BeSt for Kids study. Because your preference is also very important, we would like to investigate this. Your opinion could be of significant influence on future treatment of patients with juvenile idiopathic arthritis.

Background information:
Patients recently diagnosed with juvenile idiopathic arthritis are treated with anti-rheumatic drugs.
Frequently treatment is started with one medicine, sulfasalazine or methotrexate. If this medicine is not effective enough, treatment is changed to the next anti-rheumatic drug. However, some indications show that direct treatment with a combination of anti-rheumatic drugs could be more successful. Besides that, since some time a new medicine is available administered by injection, etanercept (Enbrel), which is clearly beneficial in patients with chronic, severe juvenile arthritis not responding to traditional treatment.

What was the treatment group that you **do** hoped to be allotted to?
- o No
- o Yes we hoped to be allotted to arm 1
- o Yes we hoped to be allotted to arm 2
- o Yes we hoped to be allotted to arm 3

What was the treatment group that you hoped **not** to be allotted to?
- o No
- o Yes we hoped not te be allotted to arm 1
- o Yes we hoped not to be allotted to arm 2
- o Yes we hoped not to be allotted to arm 3

SUPPLEMENTARY FILE 3

Relevant information from parental informed consent brochure

Why this study

The goal of this study is to determine which of three treatment strategies is the best for a child with oligo or polyarticular juvenile idiopathic arthritis.

It is not the point which individual medication is superior but we want to compare if it is better to give the medication one after another or to start with a combination or directly with maximum treatment. This comparison is relevant to study if early aggressive treatment is better able to prevent joint damage in the future. At the moment we cannot predict the course of the disease in an individual patient.

We know that if a child has only a few or even one joint with arthritis (oligoarticular course) at the start of disease, problems later in life can be comparable with a child with many joints with arthritis at the start (polyarticular course). Maybe it is superior to treat the disease from the start maximally and then, if all arthritis is over, taper medication.
All medication used in this study is already in use in the treatment of children with juvenile arthritis.

What does the study entail?

If you decide to participate in the study your child will be randomized in one of the following 3 options:

Option 1: treatment with antirheumatic drugs sequentially until arthritis is over. The first medicine is named sulfasalazine or methotrexate (tablets, first low dose, later high dose if necessary) and the third medicine is etanercept (administered through an injection, first normal dose, possible higher dose later)

Option 2: the antirheumatic drug methotrexate is combined with prednisolone and if this is not sufficient or in case of (severe) side effects treatment is changed to etanercept.

Option 3: etanercept is started directly combined with methotrexate.

Risks and concerns

There are pros and cons to each of the three treatments strategies mentioned. In short, the most experience exists with the first and second option. The medication has been known for a long time, so the short and long term effects and adverse effects are known. From the third treatment strategy we know that it can quickly have a good effect on children

with juvenile arthritis, especially when many joints are involved in inflammation. The side effects of this treatment on the long-term are not well known yet, but by stopping the medication soon after joint inflammation disappears, the child is exposed relatively short.

SUPPLEMENTARY FILE 4

Questionnaire Informed Consent Evaluation BeSt for Kids

1. General questions and demographic information

1. Sex patient
   □ Male □ Female

2. Date of birth patient:

3. Date of birth parents:
   Father:
   Mother:

4. Place of residency of patient and family

5. Composition of the family
   □ Only child
   □ Brothers and sisters, that is .....

   □ Parents together
   □ Parents divorced

6. Working Activities of parents:
   Father:
   Mother:

7. Highest education level father :
   Primary school / lower level high school
   Middle level high school
   Advanced vocational / university

8. Highest education level mother:
   Primary school / lower level high school
   Middle level high school
   Advanced vocational / university

9. Nationality parents:
   □ Dutch
   □ Other, that is,.....

10. Religion of parents
    □ Christianity
    □ Jewish
    □ Islam
    □ No religion
    □ Other, that is....

2. Retainment of information concerning the trial

1. What trial is your child participating in?
   a. BeSt for Kids
   b. Reumatism for Kids
   c. JIA for Kids
   d. I don't know

2. What is the goal of the trial?
   a. To test new medication for children with certain types of Juvenile Idiopathic Arthritis
   b. To investigate the best treatment strategy from three possibilities for children with certain types of juvenile idiopathic arthritis
   c. To research what particular drug is the best treatment for children with JIA
   d. I don't know

3. How many strategies exist in the trial
   a. 5
   b. 3
   c. 4
   d. I don't know

4. Who decided which strategy your child received?
   a. The treating physician
   b. This was assigned by lot
   c. The principal investigator
   d. I don't know

5. Have you been informed about risks concerning trial participation, and if yes what risks?

6. Have you been informed about benefits of trial participation, and if yes what benefits?

7. Are there any extra procedures (like blooddraws, visits to the outpatient clinic) that your child would not receive if not participating in the trial?

8. What is the duration of the trial?
   a. 1 year
   b. 2 years
   c. 3 years
   d. I don't know

9. Are data of your child preserved?
   a. Yes
   b. Yes, as long as permission was given
   c. No
   d. I don't know

10. Is the treating physician aware of the treatment strategy of your child?
    a. No this is a blinded trial
    b. Yes the physician is informed
    c. I don't know

11. Are you allowed to withdraw at all times from the trial?
    a. Yes
    b. No
    c. I don't know

12. Does this have consequences for the treatment of your child?
    a. Yes
    b. No
    c. I don't know

13. Is it possible to discuss issues with an independent physician?
    a. Yes
    b. No
    c. I don't know

14. If yes, do you know who it is?
    a. No I don't know
    b. Yes, that is…..

15. Did you understand the provided information at that time?
    a. es it was clear
    b. Yes after extra verbal explanation
    c. No it was not clear
    d. I don't know

16. Have you been asked if everything was clear to you?
    a. Yes
    b. No
    c. I don't know

17. Did you have enough time to answer questions?
    a. Yes
    b. No

18. Did you receive information to take home?
    a. Yes
    b. No
    c. I don't know

19. Was this information sufficient?
    a. Yes
    b. Too much information
    c. Too little information

20. Did you experience enough time to think about participation in the trial?
    a. Yes, enough
    b. Yes but could have been more
    c. No, not enough

21. Who finally decided to participate in the trial?
    a. the physician
    b. the physician and parents
    c. the physician, parents and patient
    d. the parents
    e. the parents and patient

3. The next questions are to increase our perception of your considerations regarding the treatment

1. What was your preferred strategy at the moment of trial inclusion?

In other words: did you wish your child to be assigned to a particular treatment strategy?

2. Can you explain why?
3. What is your preferred strategy now?
4. Can you explain why?
5. Are you satisfied with the given treatment?
6. To your opinion, did the physician have a preferred strategy?

4. Suggestions for improvement and questions

1. What was your impression of the conversation about trial participation of your child?
2. Are you satisfied with the communication with the physician during the process towards participation in the trial?
3. What was your main reason for participation in the trial?
4. Would you like to stay informed about the results of the trial?
5. Do you have suggestions for improvement of the informed consent procedure?
6. Do you have any more questions or remarks?

SUPPLEMENTARY FILE 5

Table 2 | Parent preferences at inclusion (phase 1) and during interview (phase 2), in relation to actual enrolled treatment strategy

Parent number	Preferred strategy at inclusion (phase 1)	Actual Treatment strategy	Preferred Strategy during interview (phase 2)	Change of preference To actual treatment strategy	Change of preference to Arm 3
1	2	1	1	X	
2	No preference	2	3		X
3	3	3	3		
4	No preference	2	2	X	
5	2	2	2		
6	No preference	3	3	X	X
7	3	1	3		
8	3	1	3		
9	1	1	3		X
10	No preference	3	1		
11	3	1	3		
12	3	3	3		
13	3	1	1	X	
14	3	1	3		
15	3	1	1	X	
16	No preference	3	3	x	X
17	No preference	1	1	X	
18	3	3	3		
19	No preference	2	1		
20	3	3	3		
21	No preference	3	1		
22	3	2	3		
23	3	1	3		

Table 3 | Summary of initial preferences (phase 1), actual enrolled arm and preference during the interview (phase 2). NA = not applicable

	No preference	**Arm 1**	**Arm 2**	**Arm 3**
Initial preference (phase 1)	8	1	2	12
Actual treatment strategy	NA	10	5	8
Preference during interview (phase 2)				
Compatible with actual treatment strategy	0	7	2	14
		4	2	6

5
8
6
7
10

8

Polyarteritis Nodosa Mimicking Juvenile Idiopathic Arthritis: A Case Report

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ABSTRACT

In this case report we present the case of a 9-year-old girl who developed myalgia after she was diagnosed with Juvenile Idiopathic Arthritis (JIA), which was treated with etanercept and methotrexate. The primary diagnosis JIA was based on symmetric polyarthritis without signs of systemic involvement. Six months later myalgia, hypertension, fever and angiographic abnormalities led to the diagnosis of juvenile systemic polyarteritis nodosa (PAN). Juvenile PAN is a rare inflammatory disease affecting small to medium-sized muscular arteries. Due to a wide range of affected organs, it causes a variable clinical presentation. Diagnosis can be difficult, because disease symptoms at the onset of disease are nonspecific and often mimic other inflammatory diseases. Even though juvenile PAN is a rare disease, it should be included in any differential diagnosis in patients with undetermined systemic symptoms or inflammatory disorders.

Key words: Polyarteritis nodosa, Juvenile Idiopathic Arthritis, Myalgia, Etanercept, Biologicals

INTRODUCTION

Polyarteritis Nodosa (PAN) is a severe inflammatory disease of insidious onset and variable clinical presentation affecting small to medium-sized muscular arteries. PAN is rare in childhood. In the general population, it has an estimated prevalence of 30.7 per 1.000.000[1]. Diagnosis is made based on the EULAR/PReS/PRINTO classification criteria for childhood Polyarteritis Nodosa, including histopathology or angiographic abnormalities plus one of five of the following symptoms: skin involvement, myalgia or muscle tenderness, hypertension, peripheral neuropathy or renal involvement[2]. In this case report we present the case of a 9-year-old girl who developed myalgia after being diagnosed with Juvenile Idiopathic Arthritis (JIA) and was treated with Etanercept and Methotrexate.

CASE PRESENTATION

A healthy 8-year-old girl was sent to our paediatric rheumatology outpatient department with chronic arthritis. One year before she had started to develop joint complaints. She went to a local hospital where she was diagnosed with post streptococcal reactive arthritis of both ankles with minor elevated antistreptolysin 200-400 U/L (<200 U/L). With the use of ibuprofen her joint complaints subsided. The NSAID was gradually tapered and stopped over the next few weeks.

A few months later joint complaints returned. The patient had difficulties walking, pain in the right shoulder and morning stiffness lasting more than 30 minutes. She was admitted to our tertiary care center. Family history revealed that her brother had one episode of reactive arthritis, and her mother mentioned several episodes of uveitis of unknown origin. On physical examination we found arthritis of the right shoulder, left wrist, metacarpophalangeal joints 2 and 3, both ankles and the right knee with flexion contracture. The patient had a severely disturbed walking pattern. Laboratory evaluation showed no presence of ANA, no IgM Rheumatoid Factor and no anti-ENA. Immunoglobulin levels were normal except for a slightly elevated IgA (2,78g/L, normal range of 0.5-2.5g/L), complement and urinary analysis were unremarkable. Based on these findings our patient was diagnosed with polyarticular JIA. Both ankles and the right knee were injected with triamcinolonhexacetonide and lidocaine, with good effect on the knee and partial effect on the ankles. Due to persistent polyarthritis she was enrolled in the BeSt for Kids study (NTR 1574). After parental consent and exclusion of mycobacterial infection, she started methotrexate and etanercept. Six months later arthritis in all joints had resolved.

When the patient returned to our outpatient department 3 months later, she was unable to walk or completely extend the right leg due to a painful, swollen right calf. There was no preceding trauma or illness. On physical examination, her right calf was painful and diffusely swollen with limited extension of at least 30 degrees, but without signs of arthritis in knee or ankle. Furthermore, she had an arthritis of the right shoulder and left wrist. She was admitted. The first differential diagnostic thoughts were deep venous thrombosis, rupture of a Baker's cyst, abscess and bony fissure, which could all be ruled out by conventional X-rays and ultrasonography. Initial laboratory testing demonstrated high acute phase reactants (CRP 70mg/L (<10mg/L), ESR 46 mm/hour (3-13mm/hour), thrombocytes 554 x109 (150-400x109)) with normal CPK. MRI of the lower legs revealed bilateral diffuse edema in the muscles of the lower legs with enhancement after intravenous gadolinium, suggestive of myositis. The neurologist reported normal muscle strength, but striking pain on stretching the lower leg muscles suggestive for fasciitis. A skin/muscle/fascia biopsy was planned and etanercept and methotrexate were stopped.

Microbiological testing revealed no evidence of viral or bacterial infection: PCR for respiratory and gastrointestinal viruses (adenovirus, enterovirus and parechovirus) was negative; IgM and IgG for Borrelia, EBV and CMV were not present. Two blood cultures and a urine culture were negative. There was no peripheral eosinophilia suggestive of eosinophilic fasciitis.

In search of a systemic inflammatory disease we evaluated organ involvement. Plain radiograph of the thorax, lung function including CO-diffusion capacity, electrocardiography, cardiac ultrasound, abdominal ultrasound and urinalysis were all unremarkable. Ophthalmologic examination revealed no signs of uveitis or vasculitis. Faeces calprotectin was low (<15µg/g) and ACE 27 U/L, ANA, anti-ds DNA, anti-ENA and ANCA were all negative, as were myositis-specific antibodies. Complement levels were normal. As a systemic inflammatory process was suspected, three methylprednisolon pulses of 30 mg/kg were administered resulting in decreased pain in the calves and a decrease of all acute phase parameters (CRP from 110 to 8 mg/L).The biopsy demonstrated no signs of myositis or fasciitis. Due to the lack of a classifying diagnosis, no oral prednisolone was started and the patient was discharged in good clinical condition and able to walk normally.

Four days following discharge the patient returned to our outpatient department in general diminished condition with constitutional symptoms of fatigue and malaise, polyarthritis and an increase in the painful swelling of the calves. The blood pressure was raised on admission (150/85mm Hg, >P95) and she developed fever (39,5 ºC) the same day. Acute phase reactants had increased (CRP 133.6 mg/L). Because of previous administration of

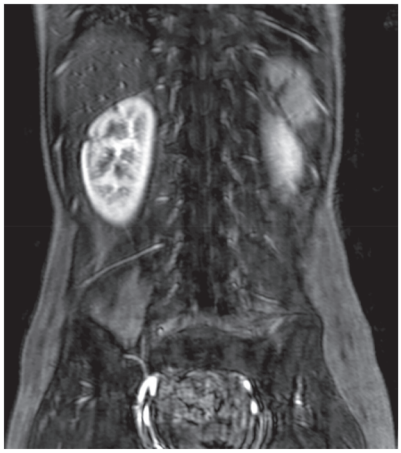

Figure 1 | MRI/MRA of the abdominal arteries showing parenchymal defects in the right kidney

immunosuppression, she was started on sepsis therapy. After 72 hours, sepsis therapy was stopped because blood and urine cultures were negative. The combination of arthritis, myalgia, fever and hypertension raised the suspicion of systemic PAN. MRI/MRA of the abdominal arteries showed no abnormalities in the large vessels but revealed parenchymal defects in the kidneys suspicious of vasculitis in smaller vessels (figure 1). A classic angiography showed subtle abnormalities in accordance with the MRI/MRA: cortical defects, irregular kidney arteries, a cut-off sign and some micro-aneurysms (figure 2).

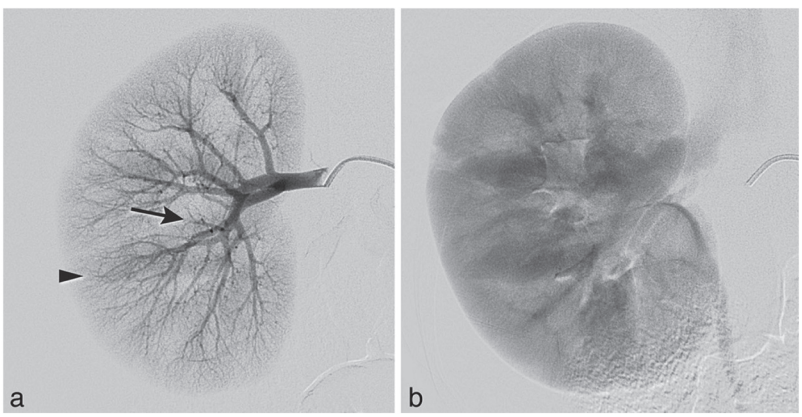

Figure 2 | Classic angiography of the kidneys. **A:** both arrows indicate cut-off signs of kidney arteries. **B:** Cortical defects in the right kidney cortex in accordance with the MRI/MRA.

According to the EULAR/Pres/PRINTO classification criteria for Polyarteritis Nodosa, the patient was diagnosed with PAN[2] with a Paediatric Vasculitis Activity Score (PVAS) of 9/64. Standard treatment for remission induction currently combines high-dose corticosteroids with cyclophosphamide[3]. If remission is achieved, treatment can be continued with azathioprine as maintenance therapy. The role of mycophenolate mofetil (MMF), instead of cyclophosphamide, in the induction of remission will be investigated in the future planned MYPAN study. Our patient received another pulse of intravenous methylprednisolone. As induction therapy oral prednisolone (2 mg/kg/day) and MMF were started ahead of the MYPAN study. The calf pain diminished, arthritis resolved and acute phase reactants dropped again. Unfortunately, after lowering prednisolone below 17,5 mg the disease flared up: acute phase reactants increased and arthritis recurred. MMF was replaced by cyclophosphamide pulse therapy (750 mg/m2 once a month IV). The prednisolone dosage was gradually tapered. To control blood pressure, amlodipine and labetolol were prescribed. Our patient returned to our tertiary care center for regular check-ups and short stay admissions for administration of the cyclophosphamide pulses. Currently in remission, she nearly finished 6 cyclophosphamide pulses combined with 12,5 mg prednisone daily and is actively involved in synchronic swimming without limitations in daily life.

DISCUSSION

This report describes a case of systemic juvenile PAN initially mimicking polyarticular JIA. JIA is the most common rheumatic disease in children and the symptoms of JIA and PAN can overlap. Early in the course of the disease both cause non-specific signs and symptoms such as myalgia, arthritis, malaise and fever. In our patient, the primary diagnosis was based on symmetric polyarthritis without any signs of systemic involvement. Later in the course of the disease myalgia, hypertension, fever and angiographic abnormalities led to the diagnosis of juvenile PAN[2].

A report from 2012 describes a similar case in which the initial diagnosis was also JIA. A few years later the patient was diagnosed with PAN because of the development of coronary artery aneurysms, fever, hypertension and myalgia[4]. Others have also pointed to the insidious onset of childhood PAN[3;5]. A recent single-center retrospective study including 69 children over 32 years concluded that many of the presenting features of PAN are non-specific and mimic other inflammatory diseases in childhood[3]. Most patients show constitutional symptoms like fatigue, weight loss, myalgia and elevated acute-phase reactants, reflecting systemic inflammation[5].

As the disease progresses and vessel damage increases, characteristic symptoms arise and PAN presents itself as a more likely cause of disease symptoms[6].

In the differential diagnosis we also considered the inflammatory process in the calf muscles as a side-effect of etanercept. Biologicals, like etanercept, antagonize immunological cytokines and receptors and might affect the quality of the immune system. This can lead to a defective immunoregulation resulting in auto-inflammation[7]. In their review, Swart et al. describe an increase in the incidence of demyelinating diseases, inflammatory bowel disease, and development of auto-immune antibodies with the use of etanercept (no cases of systemic vasculitis were described)[7]. An emerging number of autoimmune adverse events related to the use of biologics was described in another recent review article[8]. 140 cases of vasculitis were described, most commonly caused by etanercept. Ninety percent involved cases with a cutaneous form of vasculitis, glomerulonephritis or peripheral neuropathy, no systemic PAN was described. These reviews indicate that it is unlikely that our patient developed PAN because she was treated with etanercept.

CONCLUSION

This case illustrates the course of symptoms of juvenile PAN. The initial non-specific symptoms and insidious onset of disease led to the primary diagnosis of polyarticular rheumatoid factor negative JIA. During the course of disease, more specific symptoms arose and our patient was diagnosed with juvenile systemic PAN. Even though juvenile systemic PAN is a rare disease, it should be included in any differential diagnosis in patients with undetermined systemic symptoms or inflammatory disorders[9].

Consent

Informed consent was obtained from the parents of the patient for publication of this case report and any accompanying images.

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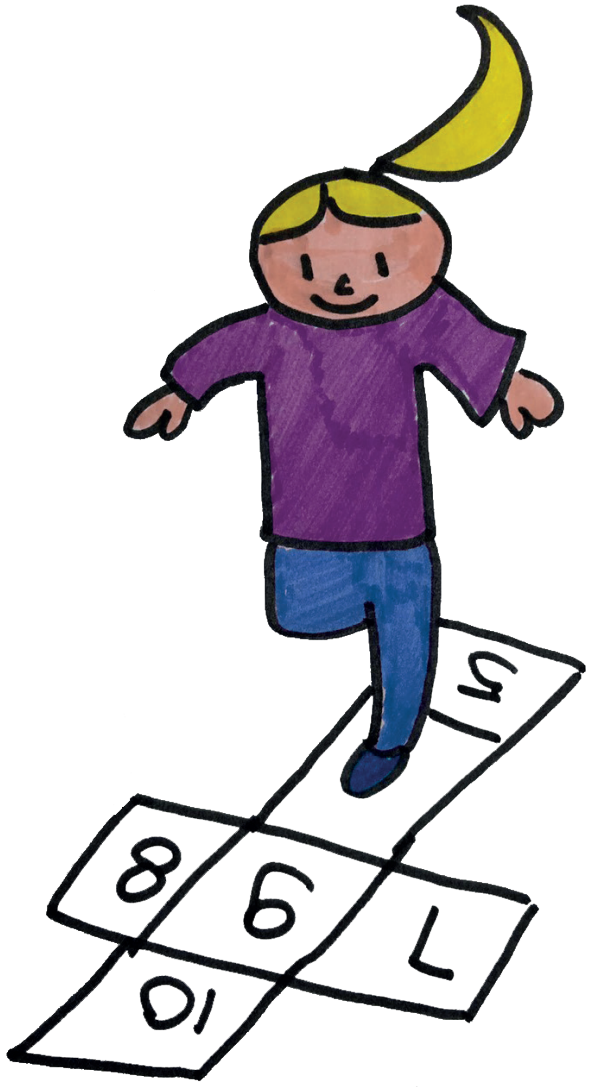
PART THREE

Discussion



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General discussion and conclusion

In the following chapter the main findings of this thesis are reviewed and discussed against the background of relevances in current clinical care.

PART 1 PATHOGENESIS OF JUVENILE IDIOPATHIC ARTHRITIS

Juvenile Idiopathic Arthritis

Juvenile Idiopathic Arthritis (JIA) is a collective name for a group of complex autoimmune diseases with a variable course and outcome. The main feature is arthritis of one or multiple joints in children. A general introduction on JIA and a brief history on therapeutic approach to JIA are given in Chapter 1. Pathogenesis has not been completely unravelled yet, but is currently viewed as a combination of genetic susceptibility and environmental triggers causing a disturbed balance between tolerance and inflammation. Due to the heterogeneous nature of JIA, biomarkers would be helpful in determining the window of opportunity and in selecting patients for particular treatments or strategies or help in determining the moment for tapering and stopping therapy. They can be used for diagnosis, response to therapy, or prediction of flare.

The role of biomarkers in the treatment of JIA

As such for example the myeloid related proteins (MRP's) have recently gained attention[1] as potential biomarker for a disease flare, although they are not incorporated routinely in clinical care yet. Other potential biomarkers could include the anti-Carp antibodies, already routinely screened in rheumatoid arthritis (RA)[2] and the composition of the collective gut flora including their genetic material, further referred to as the microbiome.

AntiCarp antibodies

To study the potential value of a new biomarker in RA for prediction of prognosis in JIA, we performed a pilot study in a group of JIA patients. The so-called anti-CarP antibodies are antibodies which are related to a poor prognosis/radiographic damage in RA, independent of the presence of RF or anti-CCP antibodies[3]. In our pilot study, anti-CarP antibodies are present in low percentages (8-13%) in JIA patients, even if (when) they are RF-negative and anti-CCP-negative. Most often however the three autoantibodies (Rheumatoid factor, anti-CarP antibodies and anti-CCP antibodies) could be demonstrated together, probably reflecting a more severe disease course. The exact role of the anti-Carp antibodies in the prognosis of JIA remains unclear. Future and ideally larger studies will have to be performed to investigate this in more detail. Probably the combination of several biomarkers will, in the future, help to predict responses and guide personalized medicine[4].

Microbiome in JIA

Over the last decade the gut microbiome has gained increased attention in the research field of inflammatory diseases, also in JIA[5]. We have performed a pilot study to investigate the diversity of microbiota in JIA as a potential biomarker, since it is easy to collect and

not invasive. We hoped to identify subsets of JIA patients, when compared with healthy controls by their gut microbiome, based on differences in the phylum Bacteroidetes, usually dominantly present in healthy children[6]. Almost simultaneously in 2016 microbiota aberrations were reported in non-systemic JIA[7] reflecting the same changes observed in type 1 diabetes, as in Enthesitis Related Arthritis (ERA)[8]. In the latter, associations with different states of disease activity were established. Intestinal dysbiosis has since long been implicated in the pathogenesis of spondylarthropathies. Therefore, research in the last years seems to focus on pediatric ERA patients[9, 10].

Indirect evidence for a role of the composition of the microbiota comes from studies linking environmental factors influencing the microbiota to the risk of developing JIA as described by Horton et al[11]. Microbiota research is complex due to complicated comparison of results when using different detection techniques, big data with complex statistical analysis and environmental differences. A recent study incorporating the use of microbiota analysis has found some clues that a lower relative abundance of a certain type of bacteria (Mogibacteriaceae) in oligoarticular JIA patients might play a role in predicting inactive disease in the first two years[12]. Further studies are needed to delineate the role of the microbiota and to appreciate its potential as therapeutic target in JIA.

PART II CLINICAL ASPECTS TREATMENT STRATEGIES

Medical treatment in JIA

The treatment of JIA has changed revolutionary over the last 2 decades. Improved understanding on pathogenesis in inflammatory diseases like RA and JIA and recognition of important mediators of inflammation have led to the development of TNF-blocking medicines on top of classical DMARDs like MTX and sulphasalazine (SSZ). The term refers to a group of therapeutic agents that specifically target a particular cell or cytokine involved in the inflammatory process of JIA. Especially with the biologicals inhibiting TNF-α (etanercept, infliximab and adalimumab)—clinical improvement is very significant. The introduction of TNF-blocking medication in the disease course has improved the outcome for JIA patients substantially. Additionally, increasing uniformity in monitoring the disease has further improved the outcome. Nowadays, treatment with Disease Modifying Antirheumatic Drugs (DMARDs) is started as soon as possible after diagnosis, resulting in more effective suppression of disease activity and substantial reduction of joint damage. Current treatment recommendations are based on national or international publications[13, 14]. The DMARD therapies recommended are all well-known. The optimal timing or combination is still a matter of debate.

Targeting Inactive disease

There are several indications that earlier or even immediate treatment with TNF-α inhibitors might even be more beneficial than reserving such treatment for patients who have failed on traditional DMARDs. Tynjala and Wallace et al both showed the beneficial effects of early aggressive treatment on outcome[15, 16]. Gradually even inactive disease has become the realistic goal that comes into view in JIA patients[17].

An important question remains when to initiate an TNF-blocking agent. Their place in the treatment of patients with recently diagnosed JIA and their effectiveness compared to other aggressive treatment strategies has yet to be determined[18].

In line with previous (RA) studies, these insights have led to the development of a practice-based study in JIA patients, comparing treatment strategies rather than individual drugs, called BeSt for Kids study.

The BeSt for Kids study

In this study three treatment strategies are compared (figure x page y).
1 Sequential monotherapy, where patients started with one DMARD: either methotrexate (MTX) or Sulphasalazine (SSZ), thereafter increasing MTX dose or switching to MTX, thereafter adding etanercept (anti-TNF) in case of insufficient response (n=31).
2 Initial combination therapy with MTX and prednisolone, where patients started directly with methotrexate and prednisolone bridging therapy (4 weeks 0.5mg/kg, tapering to 0 in 2 weeks), thereafter increasing MTX dose, thereafter adding etanercept (anti-TNF) in case of insufficient response (n=32).
*3 Initial combination therapy with etanercept and MTX (*anti-TNF) where patients started with a combination of the TNF-inhibitor etanercept and MTX (n=29).

Between October 2009 and April 2014, 94 JIA patients with recent onset active juvenile idiopathic arthritis (oligoarthritis, RF negative polyarthritis and juvenile psoriatic arthritis) were included and followed for 2 years. The initial target was to achieve an adjusted ACRpedi50% after 3 months of treatment and inactive disease from six months and onwards in all patients. To resemble the dynamics of daily practice, the patients moved through the treatment protocol and proceeded to the next step (increasing dose, switching to another drug, or adding another drug) in case of an insufficient response (no ACRpedi50% or no inactive disease). Tapering was commenced once a period of 3 (oligoarticular disease) or 6 (polyarticular disease) months of inactive disease was reached. Measurements of disease activity were performed every 3 months by a physiotherapist who was blinded for the allocated treatment strategy. The treating paediatric rheumatologists used the results of these core set criteria for the adjustment of therapy.

The primary clinical outcome was time to inactive disease and time to flare after tapering and stopping DMARD medication. Secondary outcomes were adjusted ACRPedi30/50/70/90% calculations, toxicity and physical function as measured by Child Health Assessment Questionnaire (CHAQ) every 3 months.

EARLY CLINICAL OUTCOMES

Adjusted ACRpedi improvements

After three months of therapy the target was ACRPedi50%. The patients who started with an initial combination of etanercept and MTX (arm 3) had a higher ACRpedi70% response after 3 months of 47% compared to arm 1 (25%) and arm 2 (19%) of treatment in comparison to the monotherapy strategy (arm 1, p=0.04). Less medication changes had occurred and toxicity was similar between the groups. These results are worldwide one of the first data on recent-onset DMARD-naive JIA patients treated DMARD naive with etanercept and MTX[19].

If we compare our data to the literature we find data on JIA patients with chronic severe disease course, previously treated with MTX or other DMARDs. In those patients after 3 months of etanercept treatment percentages of improvement ranging from 36% in original etanercept study[20] to 39%[21, 22] and 51%[23] depending on registry and JIA category.
This is relatively similar, although patients from both groups are not comparable in terms of disease duration before start of anti-TNF. The 3-months period is relatively short and outcome over a longer period is more important. Whether ACRpedi70% after 3 months is predictive for later outcome is subject of further study although results in literature previously reported on this[24-27].

If we focus on percentage of improvement we do not take the actual disease activity into account. Therefore, the JADAS-score was developed.

JADAS-score

The JADAS-score, first described in 2009[28] is a composite score of Physician Global Assessment (PGA), Patient/parent global assessment of well-being, ESR and number of active joints. JADAS-10 (joint count is up to a maximum of 10 active joints) scores after 3 months treatment were measured additionally, since this score was developed after finalizing the protocol for the BeSt for Kids study. As recently described[29] the difference in JADAS (ΔJADAS) compared to baseline is helpful and more easy to determine improvement. At baseline JADAS-10 scores were median 15.7 (13.5-20.2) in arm 1, 17.9 (15.2-21.9) in

arm 2 and 19.1 (13.8-23.2) in arm 3. ΔJADAS after 3 months of treatment was median 6.9 in arm 1, 5.7 in arm 2 and 10.2 in arm 3 (p=0.22). In literature JADAS-10 scores after 3 months of etanercept treatment were published[29], differentiating between baseline low, moderate or high disease activity. Cut-off values for improvement could be defined by the minimal decrease in the JADAS-10 in baseline class: low by 4, moderate by 10 and high by 17. Our patients are in the category of moderate disease activity corresponding with JADAS 15-25 at baseline. Our 3-months responses in ΔJADAS are in line with these responses in arm 3 only, underscoring the small but significant difference in ACRpedi70% responses after 3 months of therapy.

Clinical outcomes after 24 months of follow-up (see Chapter 5.2, figure 2).
Time-to-inactive-disease

Time-to-inactive-disease was not significantly different across the arms, median after 9 months. Between 12 and 24 months medication was tapered and stopped in case of prolonged inactive disease, giving rise to flares and therefore loss of inactive disease criteria explaining the bumps in the curves in the second year. After 2 years of treatment, in all three arms more than 70% was in inactive disease despite tapering strategies.

In literature, inactive disease after 54 weeks in ACUTE[16] was reached in 68% (infliximab), 40% (COMBO) and 25% (MTX). In TREAT[15] after 12 months numbers are 21% (arm1, MTX/etanercept/prednisolone) versus 7% (arm2, MTX with placebo prednisone/etanercept). The results of the extension study are in line with our results with prolonged periods of inactive disease in most patients and those not in clinical inactive disease had low levels of disease activity[30]. Differences in study design hamper the possibility to compare these results in further detail. In our study the much smaller and thus apparently non-significant differences can be explained since we used a dynamic treatment- to-target approach with a final common pathway in all three arms therefore final results tend to approach each other after 2 years. Flares in this specific study design are responsible for loss of inactive disease status.

Observational studies from the comparable time frame of our study (2009-2014) reach lower levels of inactive disease and all manuscripts discuss mainly *periods* of inactive disease instead of prolonged inactive disease, not mentioning tapering or stopping of DMARDs[31-35]. For example Ringold describes a cohort of patients with polyarticular disease, spending most of the follow up time in active disease[36]. A highly variable disease activity pattern was described by Albers et al, with, in general, a predictive course in the first two years for the course in the following 3 years[32]. Papsdorf, in 2011, has reported on 50% of patients reaching inactive disease on medication[33]. Anink has reported on the first episode

of inactive disease occurring after median 10 months in 77% of patients, but does not report on prolonged inactive disease state[34]. Minden reported on 20% inactive disease while 78% was still on therapy in the Jumbo registry in 2012[35].

Results from more recent observational studies reflecting daily current practice even with the most modern treatments earlier in the disease course, describe lower levels of inactive disease after 1 year[37-41] varying from 25-50% on average underscoring the importance of an additional treat to target (T2T) approach in JIA. For example, Mc Erlane reports that one third of patients is still in high disease activity at one year[37]. Worrisome is the fact that they lacked to find any improvement over the last 10 years possibly due to poor awareness and delays in referral. Solari et al report in 2012 that half the patients on etanercept reach inactive disease. After 24 months of continued therapy 57% was in the state of inactive disease[42].

Shoop-Worrall (2017) highlights that the majority of patients have persistent disease activity after 1 year of treatment in a large inception cohort[39]. Verrazza (2016)[40] also mentioned that half of the patients on etanercept reach complete disease quiescence. Sengler reports that the majority of patients with JIA reach the state of inactive disease within the first year of specialised care[41]. This cohort consisted mainly of oligoarticular JIA patients and therefore differed from our population.

Adjusted ACRPedi 30/50/70/90 improvements over 24 months (see Chapter 5.2 figure 2)

High percentages of improvement are reached in all three arms during 24 months, without significant differences over time between the 3 arms.

12 months outcome

Our study showed 69% ACRPedi70 in arm 3 after 1 year of treatment with etanercept and methotrexate combination from the start, compared to approximately 56% in the other 2 arms where etanercept, if needed, was initiated at a later stage. In 2004 early registries on etanercept describe in non-systemic established DMARD refractory JIA ACRpedi70 of 34% after 1 months to 64% improvement after 1 year of treatment[21]. Five years later the same authors describe 62% of ACRpedi70 improvement after one year of combination therapy with etanercept and MTX as compared to 45% for the etanercept only treatment. These registries contain the most severe JIA patients with long-lasting disease, in contrast to our study, where all children regardless of disease activity were included when they were in need of a DMARD. In the previously mentioned ACUTE study ACRpedi 75 after 54 weeks was primary end point. This high goal was reached in 100% (anti TNF), 65% (COMBO) en

50% (MTX) arm. The ACUTE study did not allow a tapering regime and was not blinded, two reasons probably contributing to the differences found in our results. Whether ACRpedi70% after 3 months is a predictor for long term outcome is subject of further study but this seems likely since more ACRPedi70% is reached in the third arm after 3 months and this result lasts over a period of 24 months.

24 months outcome

In our study even after 24 months, results continue to improve, mainly in ACRpedi30/50% in all arms without reaching a plateau phase yet. In arm 3 for ACRpedi 70 and 90 due to tapering and stopping therapy and therefore loss of improvement a plateau seems to have been reached, but not yet for arm 1 and 2. Earlier saturation of maximum clinical effect on group level might have been reached in arm 3 as an explanation to these graphs. This is special when we take tapering/stopping of DMARDs into account, mostly occurring between 12 and 24 months of follow-up as can be seen in figure chapter 5.2 figure 3. The continued improvement in all arms emphasize again the importance of a T2T-approach which can include a tapering and stopregime, but with careful monitoring and swift response in case of a flare. And again, although these percentages seem comparable, they do not allow us to compare actual disease activity.

JADAS-10 score

Since the development of the JADAS score[28] we added JADAS-10 score as secondary outcome measure after 24 months. JADAS-10 scores at baseline were calculated once more, since 2 patients were left out of the 24 months analysis due to changing diagnosis. Baseline JADAS-10 mean was 16.5 ± 4.2, in arm 2 18.8 ± 4.4, and in arm 3 18.8 ± 5.4. After treatment in this treatment-to-target regime JADAS-10 scores improved after 12 months to 6.1 (3.8-8.3) in arm 2 to 6.2 (3.8-8.6) and in arm 3 to 4.7 (2.6-6.8) (see figure Chapter 5.2 figure 2). All fulfil the criteria of improvement with ΔJADAS of at least >10 points from mediate baseline disease activity[29]. Numerically highest ΔJADAS was observed in arm 3. After 24 months of continued T2T-strategy, including tapering and stopping if predetermined criteria were met, JADAS-10 scores reached 2.6 (1.4-3.8) in arm 1, 4.0 (2.2-5.8) in arm 2 and 3.0 (1.6-4.4) in arm 3.

In accordance with previous results, JADAS-scores in all arms continue to go down and have not reached a plateau yet suggesting the on-going beneficial effect of the T2T-approach even allowing for tapering strategies.

Recently clinical (c)JADAS as adaptation of the original JADAS was developed[43]. The advantage is the lack of ESR in this score. Even more recently it was proposed that

cJADAS was able to identify patients in need of anti-TNF according to the Beukelman recommendations[13] and therefore it is a user-friendly tool easy to be used for T2T in JIA[44]. The patient VAS appeared to be a critical item in the cJADAS for the decision to escalate to anti-TNF. Since we know from previous studies[45] that patient VAS frequently overestimated disease activity when having pain and being functionally limited, these results need to be confirmed in future studies.

CHAQ

Functional ability was measured by the Dutch version of the Child Health Assessment Questionnaire (CHAQ)[46]. CHAQ levels were comparable at the start of the BeSt for Kids study on average (1.1± on a scale from 0-3) quite low, corresponding with between mild-to-moderate and moderate disability[47] yet comparable with CHAQ-levels in the TREAT (1.1±0.8 and 1.3±0.7) and ACUTE study (0.5-1.1±0.55-0.60)[15, 16]. Over time they improved in all three arms although CHAQ's in the second arm remained the highest numerically. The minimal clinically important differences (MCID) of the CHAQ both for improvement and worsening are often at or close to the level of the smallest potential difference, which is 0.13[48]. The problem in low disease activity is the fact that the CHAQ in its current form probably is too insensitive to determine important short term changes in health and disease for a given patient. If this problem was relevant in our study, it has affected all three groups in a similar way. Possibly also a so-called 'response shift' has occurred if patients, which means that although somewhat worsened or improved, the patients have become used to the altered health state and rate themselves as unchanged, even though an actual change in their health had taken place. For example in ACUTE study, baseline CHAQ in TNF arm was 0.5±0.1 and CHAQ after 54 weeks was 0.4±0.1, although 68% had reached inactive disease. Whether this response shift occurs in JIA patients is currently unknown. Interesting is that CHAQ scores in our study are low compared to for example the PRINTO-MTX study', where ACRpedi70 non-responders or even ACRpedi30 non-responders could be predicted by higher CHAQ scores (>1.0)[49]. Observational cohort studies in the TNF-era however describe low CHAQ levels (0.43-0.63) even before initiation of a biological[50] underlining the lack of sensitivity of the score in the lower ranges.

Medication changes in the BeSt for Kids study

More medication changes were needed in the first and second arm compared to arm 3.
In arm 3 all patients were treated with etanercept and methotrexate. In arm 2 after 1 year more than 50% started on etanercept and after 2 years 70% of patients used or had used it. In arm 1 50% of patients eventually needed etanercept at various time points according to protocol. Despite the different number of medication changes, comparable numbers of patients 1) reached inactive disease and 2) could taper and stop DMARD therapy, with 3) comparable numbers of flare.

It seems that the T2T approach is more important than initial treatment in terms of primary outcome measures: time-to-inactive disease and time-to-flare. Combination therapies were not superior in our study after 24months, as was previously reported in rheumatoid arthritis[51]. However, out of protocol use of glucocorticoids (see Chapter 5.2, Table S2 protocol violations) either oral, IM or intra-articular could have improved the outcomes of arm 1 and 2. More studies are needed to establish the optimal treatment strategies, although our study supplies proof-of-principle evidence that treatment-to-target is potentially equally/more important than the drug used as was previously described in RA[52, 53]. Consensus treatment plans (CTP) have been developed to study different initial treatment strategies outside of clinical trials, which are described further down.

Tapering and stopping DMARDS

In our study 59% of patients were able to taper and stop medication after on average 15-18 months of therapy. This duration of therapy is shorter in comparison to the patients previously described in literature, who were treated variably between 19 months and over 4 years[54-60] before tapering was attempted, although they had a more prolonged total disease duration and had started a biological later in the disease course.

In a large cohort of patients managed with contemporary treatments according to current standard-of-care, described by Guzman et al, probabilities of discontinuing treatment of 46% for oligoarthritis, 21% for RF negative polyarthritis and 44% for psoriatic arthritis are mentioned[61]. This large cohort contained patients with comparable patient characteristics in comparison to our study. Guzman explicitly states in the discussion that they report on *attaining* a clinical outcome, these results should not be interpreted as probabilities of *maintaining* these outcomes.

Chang et al describe a cohort of polyarticular JIA and Enthesitis related Arthritis[59]. 29% of RF negative polyarticular JIA could stop all DMARD therapy.

Recently, Minden et al describe higher chances of reaching drug-free remission is related to earlier initiation of biologicals, underscoring the concept of a window-of-opportunity[62]. Tapering and stopping therapy therefore is a logical step in the treatment of prolonged inactive JIA, especially when therapy was initiated early in the disease course. From our experiences in the BeSt for Kids study, motivation to be treated continually tends to decrease in JIA and patients/parents actively request for tapering and stopping therapy and this was observed previously[63].

At the end of our study about 39% in all arms were (still) in drug-free inactive disease. Although recent recommendations do not advice on tapering and stopping yet[64], in our experience, tapering and even stopping DMARDs was feasible in children with JIA once inactive disease had been reached for at least 6 months in polyarticular disease or 3 months in oligoarticular disease. More studies need to be done to recognize patients at risk for flare and to determine the optimal period of therapy before tapering since Klotsche et al describe less flares when inactive disease was maintained for 12 months before MTX withdrawal[65].

Time to flare

After tapering and stopping DMARDs, time-to-flare was not significantly different between the arms and occurred after on average 3.0 (3.0-6.0) months. Flares were described in 25% of cases in all three arms with the relative limited follow-up time up of 24 months.
A recent observational study by Chang et al[59] among polyarticular JIA and enthesitis related arthritis (n=335) describes a flare rate of 63% within the first year. More patients on combination therapy flared if they first stopped the TNF-blocker and continued MTX. This is not the case in our study. Long term data need to be collected to reflect on our flare percentages over longer period of time.
Data from another recent large observational study by Guzman show higher numbers of flare in up to 54.7% out of 1146 patients[60] depending on definition of flare and among all JIA categories. In this study significant flares, defined by the need to intensify therapy, occurred 26.6% (24% to 30%) within a year after achieving inactive disease and within a year after stopping treatment 25.0% (21% to 29%), respectively.

Flares in our study required restart of therapy in 4/6 patients in arm 1 (1 SSZ 3 MTX), 3/3 in arm 2 (MTX and n=1 one local injection) and 5/5 in arm 3 (MTX/etanercept). Numbers are in the same range as in the recent paper by Guzman[60] although comparability between our RCT in selected categories of JIA and this large prospective observational cohort study in all JIA categories is limited. Already known from Guzman et al is, that children with a severe disease course have higher chances for flare[60] yet there is a need for prediction of flares to determine in which patient therapy can be withdrawn safely.

Flares in our study were characterised by on average low disease activity: cJADAS 9.7 (8.1-11.3). After restart of last effective therapy (3 months later) cJADAS lowered substantially to 3.9 (1.8-6.0).

We were able to include a few oligoarticular JIA patients. In the n=11 oligoarticular patients in our study the amount of flares in oligoarticular patients (n=1 out of 5) was in proportion

with the amount of flares in polyarticular patients (n=13 out of 83), suggesting that 3 months of inactive disease in oligoarticular disease, before tapering needs further study. On the other hand, a recent study containing 40% oligoarticular JIA patients, described a lower flare rate in case of 12 months of inactive disease before MTX tapering[65].

Toxicity

Over 24 months toxicity was similar across the arms. Some severe adverse events occurred, all due to hospital admission for several reasons (see Chapter 5.2, table 2 Adverse events), in all three arms, none with permanent damage. In recent literature comparable data on toxicity can be found for patients treated with anti-TNF's or MTX/combinations[23, 66-69]. Concerns on serious infections[66, 69] exist but they seem to be mild on group-level. Long term pharmacovigilance remains of importance since the era of the use of biologicals in JIA patients is currently less than 20 years[70, 71]. Since we started and stopped biologicals early in the disease course we aim to reduce exposure to these drugs and thus diminish possible adverse events.

Protocol violations

In our study in all arms protocol violations occurred (see Chapter 5.2, table S2 protocol violations), mainly due to the wish of parent/patient or physicians wish not to increase therapy. Since this was a long term follow-up pragmatic clinical trial, we tried to mimic routine clinical care. Although parents and patients were informed and aware of the treatment protocol, we used shared decision making[64] as an important principle in the consulting room, and obviously this is complex in children when treated with TNF-blockers[72]. Protocol violations occurred in all arms in the study in comparable numbers. Previously described in the original BeSt-study, disagreement with the disease activity score (DAS) or the required treatment and dissatisfaction with the level of disease suppression were risk factors for non-adherence[73]. This is subject of further study from the results of our trial.

The use of glucocorticoids

In arm 2, 6 weeks of prednisone was administered as bridging therapy. Four weeks of 0.5mg/kg tapering in 2 weeks to 0. The six weeks results show a clear short-term benefit although the effects are short-lived and do not seem to sustain over longer time since we observed a 'rebound effect' in terms of adjusted ACRpedi improvements, inactive disease and JADAS10 score after withdrawal. The duration of administration of glucocorticoids in this trial is presumably too short for a lasting effect. Due to the inherent characteristics of glucocorticoids in children a prolonged use is not eligible. The optimal dose and duration for bridging purposes is subject of further studies although individual preferences exist among physicians[74].

In the paper by Guzman[61], the cumulative probability of attaining inactive disease after 2 years is high in patients with oligoarticular disease (86%) and RF negative polyarticular disease (70,9%), by using glucocorticoids relatively often and less biologicals compared to our cohort.
Throughout the study parenteral glucocorticoids were administered outside of protocol: in the first months in arm 1 and 2: 7 times compared to none in arm 3. These findings may indicate that the clinical efficacy of treatment in arm 3 was better, and that with less effective csDMARDs, additional glucocorticoid-courses are required to achieve similar results.

Radiographic outcome

As the time has come to include radiographic progression as outcome in JIA clinical trials[75] we evaluated radiological outcome in our cohort from recent onset active JIA patients in the BeSt for Kids study who were treated to target, early and with tight control.

Poznanski score

As determined by Poznanski[76] we found in the wrist no radiological damage, neither at baseline, nor at follow-up after 24 months of treatment. This result is remarkable since it was described previously that radiographic damage in polyarticular JIA mainly occurs in wrists[77, 78] both at baseline and with progression at follow-up, up to 10 years. Other studies describe variable degrees of damage[79, 80] and the potential of etanercept to reduce radiographic progression was recognized[81]. Patients in our study at first presentation were not as badly affected as they used to be 25 years ago, since Poznanski scores were comparable to a healthy population. Maybe patients are referred earlier although literature does not support that argument[37]. An explanation could be that current targeted treatments seem to prevent radiographic damage. Evidence for the hypothesis came from Malattia et al when they compared American College of Rheumatology paediatric (ACRpedi) response criteria and conventional radiography with MRI findings in a cohort of patients with JIA[82]. Exclusively patients reaching ACRpedi90 responses showed significant decrease in synovitis on MRI and the halting of structural damage. Those data strongly suggest that ACRpedi30 can no longer be considered a sufficient therapeutic response. Since MRI of the wrist in JIA is not yet validated for synovitis in JIA we still must view these results with caution since MRI abnormalities are sometimes seen in healthy children[83] and MRI data on healthy age matched controls are currently lacking.

Bone age and BMD

Additionally, by using BoneXpert, a validated and automated program to evaluate bone age and bone mineral density (BMD), previously it was found that a JIA population treated

with biologicals at some time in the disease course had delayed bone maturation and lower cortical BMD than healthy children[84]. In our study, bone age (BA) was comparable at start although differences occurred, that were interpreted as not clinically relevant. Changes over time also remained within 1 SD from 0, thus the normal range.
Additionally, BMD of the wrist was significantly reduced (>1SD) at baseline in arm 3 compared with arm 1 and improved significantly in subsequent post-treatment studies in arm 3. In treatment strategy studies in RA, the BeSt-study and the IMPROVED-study, BMD loss was detected and related to joint damage progression[85, 86]. In JIA, previously the relation between diminished BMD and disease activity was observed[87, 88], with exemptions[89], including the possibility for improvement of BMD after therapy[90, 91] although normalization of BMD over time was often not reached[92, 93].
The significant improvement in BMD in arm 3, the relatively preserved BMD in arm 1 and 2 and relatively preserved bone development underscore the importance of the T2T approach in the current era of early adequate treatment.

Patient perspective

Most outcome measurements of clinical studies focus on clinical and radiographic efficacy and do not take the patients' perspective into account. For successful enrolment in future studies such as the BeSt for Kids study, not only the outcomes of the study, but also the patients' willingness to participate in trials, their thoughts and ideas at study entry as well as later on, based on personal experiences are important. Are patients in equipoise at the beginning of participation in clinical trial? And how about later in the course of the study? Equipoise is genuine uncertainty on superiority of one intervention over the other. With those questions in mind we conducted an interview study with parents/patients in the study while the actual BeSt for Kids study was still ongoing so we could compare initial preferences with later ones, shaped by experiences. The results are described in chapter 6. Initial preferences of the majority of families were to be assigned to arm 3, initial treatment with etanercept/MTX combination, therefore preference as a proxy for equipoise was not present during enrolment. During the interview study preferences tended to change towards the actual treatment strategy, possibly reflecting positive experiences with the treatment strategy received. Adverse opinions towards prednisolone were strong at study enrolment. The core message of this manuscript is the importance to evaluate the so-called 'informed patient-group' equipoise in the development of future studies. The importance to include patients and families in all aspects of trial development was recently acknowledged by other groups[94]. Elaborating on this, the next research agenda for juvenile idiopathic arthritis will be made by a collaboration of the Dutch Juvenile Arthritis Association (jeugdreumavereniging) and the Dutch Society of Pediatric Rheumatology (NVKR) according

to the James Lind Alliance method in so-called Priority Setting Partnership (PSP)[95, 96] in which the author of this thesis is participating as a member of the steering group.

Clinical trials, Consensus treatment plans and treatment recommendations

Next to the observational studies with etanercept, clinical trials with a design of early aggressive treatment are scarce: two important in the last decade are the TREAT[15] and the ACUTE[97]. In the TREAT study (Trial of Early Aggressive Therapy in Polyarticular Juvenile Idiopathic Arthritis) 85 polyarticular JIA patients were treated with either the combination of methotrexate, etanercept and prednisolone (arm1) or with methotrexate, placebo prednisolone and placebo etanercept (arm2). After 4 months ACRPedi70 was reached by 71% in arm 1 versus 44% in arm 2, which was significantly different (p=0.011), although not the primary outcome measure. An important result from the TREAT-study was the predictive value of disease duration. The shorter the disease duration at baseline, the more likely it was that clinical inactive disease would be achieved at 6 months.

The Aggressive Combination Drug Therapy in Very Early Polyarticular JIA (ACUTE) study[97] compared 3 treatment strategies in a multicenter, randomized, open-label trial: 1 MTX monotherapy, 2 MTX with infliximab and 3 MTX with SSZ and hydroxychloroquine. Sixty patients had an average disease duration of 2 months. If the target of ACRpedi75 was not reached by 12 weeks or thereafter in any of the treatment arms, methotrexate was doubled to 30 mg/m^2 weekly up to 25 mg and administered parenterally. At 6 months, modified clinical inactive disease was achieved in 60% of the patients in the MTX and infliximab arm, 30% in the combination arm, and 5% in the MTX only arm. Although for both studies the study design is different, as well as the number of included patients, JIA categories and the use of additional medications, the results provide evidence of the advantage of early aggressive therapy. Novel in our study in relation to the previous 2 clinical trials are the ongoing treatment to target (T2T) approach and the tapering and stopping strategy.

Due to difficulty performing clinical trials in JIA[98], several initiatives were launched to investigate[14, 99] optimal initial treatments for polyarticular course JIA. Comparative effectiveness studies could provide information reducing variation in care by evaluation of the comparative effectiveness of treatment timing and selection and collecting large numbers of patient[100].

The first initiative came from The Childhood Arthritis and Rheumatology Research Alliance (CARRA), a North American organization of pediatric rheumatologists who have joined together to facilitate research in pediatric rheumatology diseases[101]. The first plan was a step-up plan comparable with our arm 1, the second was a combination plan, comparable

with our second arm and the third plan was a biologic only plan, comparable with our arm 3 (although we used combination therapy in arm 3). Those plans were made based on consensus opinion as compared to the treatment recommendations from 2011[13] by Beukelman which were developed by using an evidence-based method, with limited room for expert opinion and not forcing consensus. CARRA used the physician global assessment (PGA), the ability to taper/discontinue glucocorticoids and the "patient much improved" statement as criteria for treatment evaluation. First results are awaited.

Subsequently in 2016 the German initiative by Horneff et al describes the design of 4 treatment plans: 1 adding a biological to MTX in case of insufficient response; 2 initial MTX thereafter biological monotherapy, 3 initial glucocorticoid pulse therapy with MTX and 4 initial multiple glucocorticoid joint injection with MTX. Improvement is more clearly defined, based on JADAS10 or delta JADAS (amount of improvement) cut off values[29]. After 3 months the target is minimal JADAS improvement as previously described. At six months the target is JADAS "parent acceptable disease activity" (JADAS10 ≤ 5.4)[102]. From 12 months the treatment goal is "inactive disease" (JADAS ≤ 1) or at least "low disease activity" (JADAS ≤3.8) . No advice for a treatment withdrawal is given after the first year. First results are awaited for this project as well.

Which target to address and when: is inactive disease too high to aim at?

The treatment-to-target concept has made it way in pediatric rheumatology[103], although it is still in its infancy[64]. Based on the results of our study, inactive disease has proven to be a feasible goal, which is necessary to aim at in trials and clinical care, before tapering and stopping can be considered. However, current consensus treatment plans or treatment recommendations do not advice on tapering and stopping of DMARD/biologics yet due to lack of evidence and still debate the optimal initial goal. Additionally, considerable variability exists on tapering regimes[104]. A previous attempt to study tapering strategies failed as insufficient patient number could be included (personal communication: ABC Stop study). Patients who could taper according to the treating physician, were not prepared to randomize between different tapering strategies, since they wanted to stop right away, emphasizing again the need to include the 'informed patient group' in developing new trial designs.

As a target CARRA aims at ACRpedi90 at 12 months[101] although this is a difficult target to calculate in daily clinical practice. The 'Protocols on classification, monitoring and therapy in children's rheumatology' (PRO-KIND) commission from Germany aims at JADAS remission or at least JADAS low disease activity[14]. On the other hand, from long term experience in rheumatology it is recognized that a goal set too high can hamper the effectuation of a

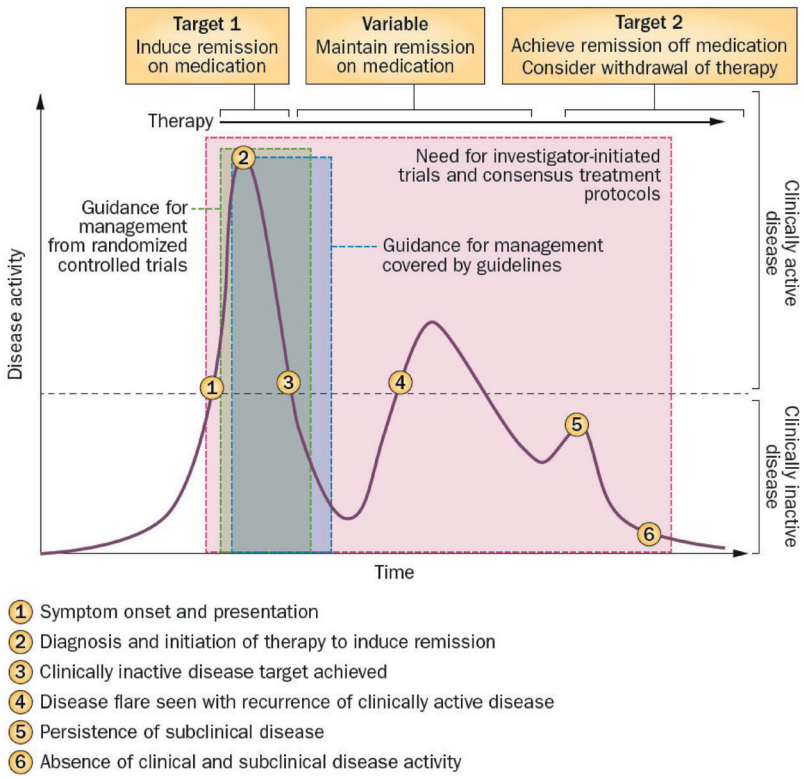

Figure 2 | Matching evidence-based guidance to individual disease courses in clinical practice. A typical disease sequence experienced by a patient with JIA begins with disease onset and diagnosis, after which disease activity (manifesting as periods of disease flare or disease remission) typically varies with time; scenarios of clinically inactive disease, in which subclinical disease persists, can also occur. Clinical practice guidelines and recommendations are founded on evidence-based statements, data from randomized controlled trials or consensus-derived definitions, but these are focused on particular aspects of the management of JIA such as initiation of therapy or achieving remission on medication. The disease activity patterns seen over time indicate other important long-term targets, including managing subclinical active disease, preventing disease flares and timing withdrawal of medication to achieve the ultimate goal of remission off medication. These targets are not yet adequately addressed by existing evidence or guidelines; thus, continued efforts to establish consensus-derived treatment strategies and undertake investigator-initiated trials are required.

Figure from: Management of juvenile idiopathic arthritis: hitting the target
Nature Reviews Rheumatology 2015;11(5): 290-300 (With permission of Prof Dr D Foell)

trial or treatment. Adherence to a DAS-steered protocol was better in the long run if the target was DAS ≤2.4 in the BeSt study, compared with the IMPROVED study aiming at a more strict DAS<1.6.[105] where protocol adherence diminished more over 5 years' time. In the latter study, protocol violations more often occurred against required treatment intensification. Perceived risks (side-effects or costs) of the required steps may reduce physicians' adherence to the protocol. Although not so dedicatedly studied, these results are in line with our experiences in the BeSt for Kids study. In all arms protocol violations occurred mainly against treatment intensifications and are important to keep in mind when developing future studies. Still, inactive disease seems is the right target. Reason to aim that high are the fact that, once treatment was started, we did not observe the development of damage, neither at the wrist, as was studied, nor in other joints. Undertreatment, with the risk of developing (permanent?) damage, is a greater risk for children with JIA than overtreatment, if you consider timely tapering and stopping regimens.

Which treatment strategy is the BeSt?

The evaluation of the three treatment strategies in early JIA as described in Chapter 5 and 6 in this thesis shows that all three arms are comparably effective after 24 months. These results may, next to early DMARD initiation, be attributed to tight control as achieved by intensive monitoring and immediate adjustment of medication.

Although the beneficial effect of tight control has just started to be recognized in JIA[106], the beneficial effect of early treatment and a few initial strategies has been previously described by Tynjala[16], Wallace[15] and Minden[62].

Heterogeneity in JIA categories as well as disease severity, additional use of glucocorticoids out of protocol and complicated estimations used for samples size calculations, have probably contributed to less than expected differences between the three arms.

In this study T2T and tight control seemed more important than the agents inducing it.

How to treat the individual JIA patient?

Reviewing the data from the BeSt for Kids study, it can be argued that, when starting a combination of drugs in all patients that present with JIA (oligo, poly or JIA with psoriasis) a considerable proportion of patients would have been 'overtreated'. Indeed after 2 years of follow up approximately 50% that started with initial monotherapy still showed good clinical response. What could argue against starting with monotherapy in (a subgroup of) JIA patient? In RA characteristics associated with poor prognosis could be overruled when starting early with a combination of drugs[107, 108]. Although diseases are not the same, treatment principles are similar. Therefore one could recommend for further study, based on these data, that JIA with features of poor prognosis as summarized by Beukelman[13]

should all start with combination therapy with MTX and anti-TNF or another biological (il-6 blockade), as nowadays occasionally is done by a pediatric rheumatologist (personal communication). Due to the well-known limitations in JIA patient numbers, we could apply this strategy as routine clinical care and observe the outcome[109].

Identification of patients with less severe disease, not in need of initial combination therapy is of major importance[44]. However, in general the risk of undertreatment seems higher than the risk of overtreatment with subsequent tapering and stopping[110] of combination therapy.

Methodological considerations of our design

The randomized clinical trial is the gold standard to provide evidence for good clinical practice. Alternative study designs have been applied in JIA patients due to several reasons. Trials in JIA patients are challenging[94, 98] mainly due to heterogeneity and rarity of juvenile idiopathic arthritis. In the BeSt for Kids study over 4 years were needed to recruite 'enough' patients. Sample size calculations were complicated since they were based on estimated percentages since actual data were lacking. The study was powered for time to inactive disease, although it tried to 1) evaluate effectiveness and safety of three initial treatment strategies, 2) evaluate the possibility to taper and stop DMARD therapy after prolonged clinical response 3) evaluate flare rates once medication was withdrawn. Although these goals were set (too?) high to answer all these questions we have given proof-of-principle that this type of study is feasible in JIA patients including all the pros and cons of this design.

Reasons for this slower than expected inclusion were delay in referral, passing the artificial 'window of opportunity' of 18 months as determined in the protocol, comorbidities prohibiting trial participation and refusal to participate, mainly at one inclusion site. Additionally, this study was not performed nationwide due to several reasons, which further hampered inclusion.

Our study is a combination between a clinical trial and a comparative effectiveness study and is performed as a large pragmatic trial. Facing the difficulties mentioned above it took a lot of time, perseverance and creativity to finalize the study. For example the inclusion of the oligoarticular patients was less than expected. Often, when a patient was referred as oligo-articular patient, during examination by our experienced physiotherapist, more joints with arthritis were recognized, changing diagnosis from oligo to polyarticular JIA. Probably oligoarticular disease was less severe in the past and by the time it remained active, the period of 18 months needed to be possibly included, had passed.

Inclusion and follow-up visits were time consuming, with single blinded joint examinations every 3 months, Clinical Record Forms (CRF) to fill in. Ideally we wanted to analyse oligoarticular JIA, psoriatic arthritis and polyarticular disease separately, although this did not seem sensible at the moment due to small numbers included of both the subgroups.

The JIA study design historically and future perspectives

In the eighties already it was recognized that performing clinical studies in JIA is challenging[111] and this is an actual issue ever since[98, 112].

Improved legislation in combination with collaboration through large research networks have improved the options for studies in JIA. Inventive study designs like utilizing an active comparator instead of placebo, adding an escape arm to minimize possible exposure to harm, and having an open-label extension for responders to assure direct benefit to research participants who respond well to the study drug. Including families in trial development as discussed in the previous chapter will enhance study appeal. Funding is needed and seems to be invested these days in comparative effectiveness studies and precision medicine (UCAN CAN DU). Several trial designs have passed in recent years, all with specific possibilities and challenges, the most important example is the randomized withdrawal trial[112]. The population under study is treated with a new drug in the first phase, secondly the responders are randomized to continue the drug or receive placebo. The outcome (time to flare) of the withdrawal trial is the efficacy of the drug to suppress a flare, instead of the true efficacy of a the drug. This study design does not support clinical equipoise since only responders are randomized. Secondly information on non-responders is lacking and carryover effect (carryover effect means that if the effect of the treatment carries on after the treatment is withdrawn, and the following response to a second treatment or placebo could be due in part to the previous treatment) will diminish changes to detect significant differences as occurred in the recent golimumab study[113].

To overcome these issues in future studies, extrapolation of efficacy data on adults is possible in diseases with similar progression and similar response to therapy, although studies for safety and drug-dosing always need to be performed in children. For biosimilars proof of similarity and extrapolation from adult studies is currently used, although immunogenicity can be different in less mature immune systems in children, advocating post-marketing studies in children.

Future perspectives: From care to cure

We realised that in our study more than 50% of patients intensified treatment with the need for anti-TNF medication, which is expensive and not equally accessible worldwide.

The value of combination therapy with relatively cheap conventional DMARDs: MTX, SSZ and plaquenil has been established in RA[114] with proven superiority of this triple therapy compared to MTX monotherapy after 1 year of therapy. Thanks to a ZOnMW grant on 'goed geneesmiddelen gebruik' number 80-83600-98-3172, we are currently investigating in a randomized multicenter single-blinded study, whether this superiority exists in JIA as well, while still applying the T2T-approach. The CHAMP study is successfully including JIA patients, over 49 patients in the last 12 months, and inclusion is ongoing. After coordinating and conducting the BeSt for Kids study, we realized how important it is to collaborate on larger scale to improve recruitment of JIA patients in studies. Therefore we are now investigating the possibility to enrol the study in countries for which this strategy would be particularly important, due to low accessibility to anti-TNF medication, like South Africa.

As mentioned previously the biggest challenge in JIA treatment is to provide the correct treatment at the right time. Since some patients can reach inactive disease with only 1 DMARD and others need many, personalized medicine is the way to proceed. Since the biological therapies are so effective, but we are still unable to predict which children need biological therapies and which can stop therapy without disease flare.

The recently launched UCAN CAN DU initiative has the goal to transform the care for JIA patients. The ultimate goal of this Canadian – Dutch Personalized Medicine Network in Childhood Arthritis and Rheumatic diseases is to address this gap in treatment approaches and support translational research in children with JIA.

The highly intruiging microbiome needs further exploration in larger scaled studies, financial support needs to be searched for. The role of the patients and parents will be increased in the research agenda to enhance the development of successful studies.

Potentially, personalized aspects should be added to treatment strategies with increasingly high and prolonged targets. This can be achieved among others by including pharmacogenomics, (will this drug be effective and not toxic in this patient?)[115-117] and by using (multiple) biomarkers that will help the clinician and patient in guiding therapy to personalize medicine.

Summary of lessons learned from this study

Treatment-to-target & tight control are feasible principles in a JIA clinical trial and give additional benefit in the short and long-term treatment of juvenile idiopathic arthritis.

Inactive disease should be the target to aim at after 6 months of treatment and onwards. Tapering strategies can be introduced in JIA studies safely since flare frequency was low and responses to restart of medication were good.

Radiographic damage did not occur on group level and BMD significantly increased after targeted therapy in the third arm.

Current research is focused on reaching inactive disease and therapy burden. Future research will focus on personalised therapies combined with treatment-to-target strategies aiming at inactive disease.

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Summary
Nederlandse samenvatting

Summary

In this thesis, the central theme is the treatment of JIA, as well as some aspects of the pathogenesis.
In part 1 on pathogenesis, two pilot studies are discussed. The first study discusses the presence of anti-carP antibodies in sera of JIA patients and the second study is on the role of the microbiome in JIA.

The results of the BeSt for Kids study are documented in part two: the clinical section.

Chapter 2 describes, in a nutshell, the latest developments in pathofyiology and treatment of oligo and polyarticular JIA. The pathogenesis is determined by a combination of factors, the most important of which are: genetic predisposition, environmental factors, triggering infections and hormonal factors. An expanding amount of treatment options has become available in the last 20 years, of which the biologicals are the most important group.

Evidence for the existence of a window of opportunity is increasing, when the disease is most susceptible for (permanent) modification. The optimal treatment strategy has not been determined yet. The conclusion is that it is the biggest challenge of this time to treat the right JIA patient at the right time with the right medicine.

Chapter 3
In chapter 3 we describe a study that determines whether anti-CarP antibodies are present in sera from JIA patients. In patients with rheumatoid arthritis (RA), the presence of anti-CarP antibodies is associated with a poor prognosis, independent of anti-CCP / ACPA (antiCyclic citrullinated protein) or rheumatoid factor (RF). Sera from 234 JIA patients from 3 different cohorts were examined for the presence of anti-CarP antibodies. Anti-CarP antibodies were more frequently detected in sera from JIA patients (8-13%) than in sera from healthy controls (3-5%).
About half of the anti-CarP positive cases were also positive whether RF or ACPA was positive. Anti-CarP antibodies were also found in ACPA and RF-negative JIA patients. The prognostic value of anti-CarP in JIA has yet to be determined but has low prevalence.

Chapter 4
In the pilot study described in chapter 4, the composition of the gut microbiome in children with JIA was compared with healthy controls. With the so-called IS-Pro technique, molecular detection can take place on the basis of the 16S rDNA region, which is characteristic per microorganism. Based on a specific analysis called PLS-DA (Partial Least Squares Discriminant

Analysis), the gut microbiota profiles of 8 JIA patients could be distinguished from 22 healthy controls, based on differences in the phylum Bacteroidetes. Phylum Bacteroidetes plays an important role in the healthy microbiome. More studies are needed to unravel the role of the microbiome in the development of JIA.

PART II

Chapter 5

In chapter 5 the results of the BeSt for Kids study are reported, which form the basis of this thesis. In this single-blinded, randomized, multicenter treat-to-target (T2T) strategy study, 3 treatment strategies are compared in terms of effectiveness and safety in children with recent-onset JIA. The treatment arms are: 1) Starting with sequential therapy, one drug at the time, starting with sulfasalazine (SSZ) or methotrexate (MTX) (depending on the choice of the physician) 2) Initial combination treatment of methotrexate with 6 weeks prednisone and 3) in the beginning combination treatment of methotrexate with etanercept. This is the most innovative arm because etanercept up to present is only available for children with JIA who have failed on MTX or are intolerant to this. The first outcome measure is the time-to-inactive-disease after starting the treatment. A second outcome measure is the time-to-flare after tapering and stopping the anti-rheumatic medication. Improvement is measured according to adjusted ACR Pedi score 30, 50, 70 or 90% and inactive disease. Furthermore, we looked at safety and quality of life.

In **5.1** the results are discussed after the first 3 months of treatment.

Ninety-four children were included, including 32 in arm 1 and in arm 2 and 30 in arm 3, median age at inclusion of 9.1 (4.7-12.9) years. ANA positive was 38%, 12 patients had oligo articular disease, 68 polyarticular and 15 JIA with psoriasis (also polyarticular). Baseline median (IQR) ACRpedi scores: VAS doctor 49 (40-58) mm, VAS patient 54 (37-70) mm, BSE 6.5 (2-14.8) mm/hr, number of active joints 8 (5-12), number of limited joints 3 (1-5), CHAQ score 0.88 (0.63-1.5). In arm 1, 17/32 started with MTX. An intention-to-treat analysis was performed. Toxicity was similar. There were few serious side effects reported. All without permanent injury.

After 3 months of treatment, the following improvement was found:

	arm 1 Sequential monotherapy n=32	arm 2 Combo MTX+6 wks prednisone n=32	arm 3 Combo MTX+ etanercept n=30	p
aACRpedi30 (%)	16 (50)	17 (53)	22 (73)	0.13
aACRpedi50 (%)	10 (31)	12 (38)	16 (53)	0.19
aACRpedi70 (%)	8 (25)	6 (19)	14(47)	0.04

In conclusion, after 3 months of treatment in a 3-arm strategy study, more clinical improvement in the form of ACRpedi70% was achieved on the initial treatment combination of etanercept with MTX than on initial monotherapy with MTX or SSZ.

In **5.2** the long-term outcome after 24 months follow-up is summarized.

In this follow-up treat to target (T2T) study the central question is what the optimal treatment strategy is over the longer term. The initial treatment in 3 arms consisted of 1) Initially sequential DMARD monotherapy, starting with SSZ or MTX (depending on choice of physician) 2) Initial combination treatment of methotrexate with 6 weeks prednisone and 3) in the beginning combination treatment of etanercept and MTX . In the case of persistent disease activity, treatment was intensified according to protocol. In case of persistent inactive disease during 3 (oligoarticular disease) or 6 (polyarticular disease) months, the treatment was phased out and stopped. After 24 months, the first outcome measures were time-to-inactive-disease and time-to-flare after stopping medication. Secondary outcome measures were adjusted ACRpedi30/ 50/70/90 scores, functioning and toxicity.

Of the 94 children who were included, 2 were lost-to-follow-up and 2 others had a revised diagnosis. As a result, baseline characteristics were determined again. At start, these were the initial values: VAS doctor 50 (39-58) mm, VAS patient 54 (37-70) mm, BSE 6 (2-14) mm/hr, number of active joints 8 (5-12), number limited joints 2.5 (1-5), CHAQ score 0.9 (0.6-1.5).

Time-to-inactive disease was median 9 (6-12)months and was not significantly different between the arms. Time-to-flare was median 3.0 (3.0-6.8) months, not different between the 3 strategies. The adjusted ACRpedi scores were similar. After 24 months, 71% (arm1), 70 % (arm2) and 72% (arm3) achieved inactive disease. Up to 45% (arm 1) 31% (arm 2) and 41% (arm 3) of the patients had stopped using all DMARD (s). Toxicity was similar. In

conclusion, more than 70% had inactive disease in each arm and up to 39% had stopped all DMARDs. No statistically significant differences were found, suggesting that treatment-to-target, aimed at inactive disease, is feasible and more important than initial treatment strategy.

Summary of results

	arm 1 Sequential monotherapy n=31	arm 2 Combo MTX+6 wks prednisone n=32	arm 3 Combo MTX+ etanercept n=29
Time to ID(m))	9.0 (5.3-15.0)	9.0 (6.0-12.8)	9.0 (6.0-12.0)
Timing of first DFID, mnths	15.0 (12.0-18.0)	19.5 (12.0-24.0)	18 (12.0-21.0)
Drug free ID	19/31, 61%	16/32, 50%	19/29, 66%
ID after 1 year (%)	54	47	62
ID after 2 yr (%)	71	70	72
DFID after 2 yr (%)	45	31	41
Time to flare (mth)	4.5 (3.0-9.0)	3.0 (3.0-3.0)	3.0 (3.0-6.8)
Flares	6 (1=oligo)	3	5
JADAS-10 1 year	6.1 (3.8-8.3)	6.2 (3.8-8.6)	4.7 (2.6-6.8)
JADAS-10 2 year	2.6 (1.4-3.8)	4.0 (2.2-5.8)	3.0 (1.6-4.4)
JADAS ID 1 year	8.4 (27%)	9 (28%)	9 (31%)
JADAS ID 2 years	16.2 (52%)	14.1 (44%)	12.5 (43%)

In Chapter 6 is reported on the radiological outcomes of the children that participated in the BeSt for Kids study. The aim of the study was to evaluate the response to early aggressive treatment using conventional X-rays of the affected wrists. An additional goal was to compare 2 methods for evaluating the presence and progression of radiological damage in the wrist. The Poznanski-score, in which the relative carpal length is measured, was used as a measure of the radiological damage in the wrist. The bone age and bone density were determined with the help of BoneXpert, an automated program that is proven to be feasible and easy to use. X-rays of 60 children were available for evaluation. With regard to the Poznanski-scores, starting and follow-up scores did not differ from each other and not from a healthy population. Bone age was also comparable with start and follow-up measurement. Bone mineral density was significantly reduced at baseline in arm 3 compared with healthy controls and improved significantly in subsequent post-treatment studies. In conclusion, we found no radiological damage in the wrist in this cohort of children with JIA who were treated early and to target.

Chapter 7 discusses the concept of equipoise in patients and / or parents against the background of the BeSt for Kids study. Equipoise means sincere uncertainty with respect to the superiority of one treatment over the other. Physician researchers need equipoise to perform randomized controlled trials (RCTs), at least in studies with more than minimal risks. Whether this equipoise is also present in patients and parents in clinical trials is unclear. Participating children and their parents were asked about their preference immediately upon inclusion in the BeSt for Kids study with the aid of a questionnaire (phase 1). During an interview study (phase 2) to evaluate the equipoise in parents/patients who participated in this study preferences were questioned again. Semi-structured interviews were held with 23 parents and 7 patients older than 12 years, 11 months on average after inclusion in the study. Most parents and patients were initially not in equipoise. Many in phase 1 preferred arm 3, initial treatment with a biological. They participated in the study because of this opportunity and would even stop participating if they had not drawn arm 3. The conviction that the strategy with initial treatment with a biological (arm 3) was superior, was based on knowledge obtained via the internet and close relations. Four parents were convinced that the physician-researcher had a preference for arm 3, while the majority (n=19) felt that the physician-researcher had no preference. In phase 2, the preferences tended to change to the actual strategy in which the patient was randomized.
In conclusion, we argue that parents of children who participate in studies have preferences in treatments. It is important to understand all concerns and values of parents of children participating in studies. Their preferences may change over time, especially in an unblinded context. Moreover, their preferences may differ from the preferences of the physicians involved.

A study that does not correspond to the concerns of the relevant patient group is unlikely to be supported by this patient group. In future studies it pays to examine the equipoise of the 'informed patient group' as a whole, to improve recruitment of patients in studies.

In chapter 8 we discuss a special case history of one of the participants in the BeSt for Kids study. Following the diagnosis of juvenile idiopathic arthritis (JIA), she was treated with arm 3 after inclusion, initially etanercept and methotrexate. The primary diagnosis JIA was based on symmetric polyarthritis with no signs of systemic involvement. Six months later she developed myalgia, hypertension and fever with elevated inflammatory parameters. The combination of symptoms along with angiographic abnormalities in the kidneys led to the diagnosis of juvenile systemic polyarteritis nodosa (PAN). Juvenile PAN is a rare inflammatory disease in which small to medium-sized muscular arteries are affected. The highly variable clinical presentation is caused by the large amount of potentially involved organs. The diagnosis can be difficult because the first symptoms are non-specific and often

can mimic other inflammatory diseases. Although juvenile PAN is a rare disease, it belongs in every differential diagnosis of undetermined systemic complaints or inflammatory diseases.

Chapter 9 reflects on the results of this thesis against the background of current clinical care. Results of our studies are compared with previous studies. Secondly our and other studies are reviewed in terms of design and treatment target. Lessons to be learned from this thesis are:

- Treatment to target combined with tight control are feasible in a JIA clinical trial and give additional benefit in the short and long term treatment of juvenile idiopathic arthritis.
- Inactive disease should be the target to aim at after 6 months of treatment and onwards.
- Tapering strategies can be introduced in JIA studies safely since flare frequency was low and responses to restart of medication were good.
- Radiographic damage did not occur in any of the study arms and bone mineral density significantly increased after treatment to target in the third arm (initial etanercept and methotrexate).

Current research is focused on reaching inactive disease and reducing disease and therapy burden. Future research will focus on personalized therapies combined with treatment-to-target strategies aiming at inactive disease.

Samenvatting Nederlands

In dit proefschrift staat naast de pathogenese met name de behandeling van JIA centraal.
In deel 1 over pathogenese worden, naast recente ontwikkelingen, 2 pilot studies besproken, te weten de aanwezigheid van anti-carP antistoffen in sera van JIA patiënten en de rol van het microbiome in JIA.
Vervolgens worden de resultaten van het BeSt for Kids onderzoek toegelicht in het 2de en klinische deel.

Hoofdstuk 2 beschrijft in vogelvlucht ontwikkelingen van oligo en polyarticulaire JIA op het gebied van oa pathogenese en behandeling. De pathogenese wordt bepaald door een samenspel van factoren, waarvan de belangrijkste lijken: genetische aanleg, omgevingsfactoren, uitlokkende infecties en hormonale factoren. Een expanderende hoeveelheid behandelopties is beschikbaar geworden de laatste 20 jaar, waarvan de biologicals de belangrijkste groep is. Conclusie is dat het de grootste uitdaging is om de juiste JIA patiënt te behandelen op het juiste moment met het juiste medicijn.

Hoofdstuk 3
In hoofdstuk 3 wordt een studie beschreven, die onderzoekt of anti-CarP antistoffen aanwezig zijn in sera van JIA patiënten. Bij patiënten met reumatoïde artritis (RA) is de aanwezigheid van anti-CarP antistoffen geassocieerd met een slechte prognose, onafhankelijk van anti-CCP/ACPA (antiCyclic citrullinated protein) of reumafactor. Serum van 234 JIA patiënten uit 3 verschillende cohorten werd onderzocht op de aanwezigheid van anti-CarP antistoffen. Anti-CarP antistoffen werden vaker aangetoond in serum van JIA patiënten (8-13%) dan in serum van gezonde controles (3-5%). Ongeveer in de helft van de anti CarP positieve gevallen was ook of RF of ACPA positief. In ACPA en RF-negatieve JIA patiënten werden ook anti-CarP antistoffen gevonden. De precieze prognostische waarde van anti-CarP in JIA moet nog nader bepaald worden.

Hoofdstuk 4
In de pilotstudie beschreven in hoofdstuk 4 is gekeken naar de samenstelling van het darm-microbiome bij kinderen met JIA vergeleken met gezonde controles. Met de zogenaamde IS-Pro techniek kan moleculaire detectie plaatsvinden op basis van de zogenaamde 16S rDNA regio, die karakteristiek is per micro-organisme. Op basis van een specifieke analyse, genaamd PLS-DA (Partial Least Squares Discriminant Analysis) konden de darm microbiota-profielen van 8 JIA patiënten onderscheiden worden van 22 gezonde controles, op basis van verschillen in het phylum Bacteroidetes. Phylum Bacteroidetes speelt een voorname rol in het gezonde microbiome. Meer studies zijn nodig om de rol van het microbiome in het ontstaan van JIA te ontrafelen.

Hoofdstuk 5

Hoofdstuk 5 behandelt de resultaten van de BeSt for Kids studie hetgeen de basis vormt van dit proefschrift. In deze enkel blinde, gerandomiseerde, multicenter treat-to-target (T2T) strategie studie worden 3 behandelstrategieën vergeleken qua effectiviteit en veiligheid bij kinderen met recent ontstane JIA. De behandelarmen zijn: 1) In het begin sequentiële therapie, één medicijn tegelijk, startend met sulfasalazine of methotrexaat (afhankelijk van keuze arts) 2) In het begin combinatie behandeling van methotrexaat met 6 weken prednison en 3) in het begin combinatie behandeling van methotrexaat met etanercept. Dit is de meest vernieuwende arm omdat etanercept tot heden alleen beschikbaar is voor kinderen met JIA die gefaald hebben op MTX of intolerant zijn hiervoor. De eerste uitkomstmaat is de duur van de tijd tot inactieve ziekte na starten van de behandeling. Een tweede uitkomstmaat is de duur van de tijd tot de gewrichtsontsteking eventueel weer opvlamt na afbouw en stop van de anti-reuma-medicatie. Verbetering wordt gemeten volgens een bepaalde score (aangepaste ACR Pedi score, 30, 50 of 70 of 90%). Verder kijken we naar veiligheid en kwaliteit van leven.

In **5.1** worden de resultaten na de eerste 3 maanden behandeling besproken.

Vier-en-negentig kinderen werden geïncludeerd, waarvan 32 in arm 1 en in arm 2 en 30 in arm 3, mediane leeftijd bij inclusie van 9.1 (4.7-12,9) jaar. ANA positief was 38%, 12 patienten hadden oligo articulaire ziekte, 68 polyarticulair en 15 JIA met psoriasis (ook polyarticulair). Baseline mediane (IQR) ACRpedi-scores: VAS arts 49 (40-58) mm, VAS patiënt 54 (37-70) mm, BSE 6,5 (2-14,8)mm/hr, aantal actieve gewrichten 8 (5-12), aantal beperkte gewrichten 3 (1-5), CHAQ score 0.88 (0.63-1.5). In arm 1, 17/32 startte met methotrexaat. Er werd een intention-to-treat analyse verricht.

Na 3 maanden van behandeling werden de volgende % verbetering gevonden:

	arm 1 Sequential monotherapy n=32	**arm 2 Combo MTX+6 wks prednisone n=32**	**arm 3 Combo MTX+ etanercept n=30**	**p**
aACRpedi30 (%)	16 (50)	17 (53)	22 (73)	0.13
aACRpedi50 (%)	10 (31)	12 (38)	16 (53)	0.19
aACRpedi70 (%)	8 (25)	6 (19)	14(47)	0.04

Toxiciteit was vergelijkbaar. Er werden weinig ernstige bijwerkingen gemeld. Allen zonder blijvend letsel.

Concluderend was na 3 maanden behandeling in een 3-armige strategie studie meer klinische verbetering in de vorm van ACRpedi70% bereikt op de initiële behandelcombinatie van etanercept met methotrexaat dan op initiële monotherapie met MTX of SSZ.

In **5.2** zijn de lange termijn uitkomsten na 24 maanden follow-up samengevat.

In dit onderzoek staat de vraag centraal wat de optimale behandelstrategie is over langere termijn. De initiële behandeling in 3 armen bestond uit 1) In het begin sequentiële DMARD-monotherapie, startend met sulfasalazine of methotrexaat (afhankelijk van keuze arts) 2) In het begin combinatie behandeling van methotrexaat met 6 weken prednison en 3) in het begin combinatie behandeling van methotrexaat met etanercept. In het geval van aanhoudende ziekteactiviteit werd de behandeling geïntensiveerd. Bij aanhoudend inactieve ziekte gedurende 3 (oligoarticulaire ziekte) of 6 (polyartculaire ziekte) maanden werd de behandeling afgebouwd en gestopt. Na 24 maanden waren de eerste uitkomstmaten tijd tot inactieve ziekte en tijd tot flare na stoppen van medicatie. Tweede uitkomstmaten waren aangepaste ACRpedi30/50/70/90 scores, functioneren en toxiciteit. Van de 94 kinderen die geïncludeerd waren, zijn er 2 uitgevallen (lost to follow-up) en 2 anderen kregen een andere diagnose. Hierdoor zijn baseline bepalingen opnieuw verricht ten opzichte van de resultaten na 3 maanden. Bij start zijn dit de uitgangs waarden: VAS arts 50 (39-58) mm, VAS patiënt 54 (37-70) mm, BSE 6(2-14) mm/hr, aantal actieve gewrichten 8 (5-12), aantal beperkte gewrichten 2.5 (1-5), CHAQ score 0.9 (0.6-1.5).

Tijd tot inactieve ziekte was mediaan 9.9 (8.6-11.3) maanden en was niet significant verschillend tussen de armen. Tijd tot flare was mediaan 22.3 (21.5-23.2) maanden, ook niet verschillend tussen de 3 strategieën. Na 24 maanden bereikte 71% (arm1), 70% (arm2) en 72% (arm3) inactieve ziekte. Tot maar liefst 45% (arm 1) 31% (arm 2) and 41% (arm 3) van de patiënten was gestopt met alle DMARD(s). De aangepaste ACRpedi-scores waren vergelijkbaar, alhoewel iets hoger in arm 3 en net significant voor ACRpedi 70% over 24 maanden. Toxiciteit was vergelijkbaar. Concluderend had meer dan 70% in elke arm inactieve ziekte en was tot 39% gestopt met alle DMARDs. Er werden geen statistisch significante verschillen gevonden, hetgeen suggereert dat behandeling gericht op inactieve ziekte haalbaar is en belangrijker dan de initiële behandeling.

Samenvatting van resultaten

	arm 1 Sequential monotherapy n=31	arm 2 Combo MTX+6 wks prednisone n=32	arm 3 Combo MTX+ etanercept n=29
Tijd tot ID(m)	9.0 (5.3-15.0)	9.0 (6.0-12.8)	9.0 (6.0-12.0)
Timing eerste DFID,	15.0 (12.0-18.0)	19.5 (12.0-24.0)	18 (12.0-21.0)
Drug free ID	19/31, 61%	16/32, 50%	19/29, 66%
ID na 1 jaar (%)	54	47	62
ID na 2 jaar (%)	71	70	72
DFID na 2 yr (%)	45	31	41
Tijd tot flare (mth)	4.5 (3.0-9.0)	3.0 (3.0-3.0)	3.0 (3.0-6.8)
Flares	6 (1=oligo)	3	5
JADAS-10 1 jaar	6.1 (3.8-8.3)	6.2 (3.8-8.6)	4.7 (2.6-6.8)
JADAS-10 2 jaar	2.6 (1.4-3.8)	4.0 (2.2-5.8)	3.0 (1.6-4.4)
JADAS ID 1 jaar	8.4 (27%)	9 (28%)	9 (31%)
JADAS ID 2 jaar	16.2 (52%)	14.1 (44%)	12.5 (43%)

In **hoofdstuk 6** worden de radiologische uitkomsten besproken van de kinderen die deelnamen aan de BeSt for Kids studie. Doel van de studie was om de respons op vroege doelgerichte behandeling met behulp van conventionele röntgenfoto's van de aangedane polsen te evalueren. Bijkomend doel was om 2 methoden voor evaluatie van aanwezigheid en progressie van radiologische schade in de pols te vergelijken. De Poznanski-score, waarbij de relatieve carpale lengte wordt gemeten, werd gebruikt als maat voor de radiologische schade in de pols. De botleeftijd en botdichtheid werden bepaald met behulp van BoneXpert, een geautomatiseerd programma dat bewezen uitvoerbaar en makkelijk in gebruik is. Röntgenfoto's van 60 kinderen waren beschikbaar voor evaluatie. Met betrekking tot de Poznanski-scores verschilden uitgangs- en vervolg- Z scores niet van elkaar en niet van een gezonde populatie. Ook botleeftijd was vergelijkbaar bij start en vervolgmeting. Botdichtheid was significant verminderd bij aanvang van de studie bij de patiënten in arm 3 (initiële behandeling met etanercept en methotrexaat) vergeleken met gezonde controles en verbeterde significant bij vervolg onderzoek na behandeling.

Concluderend vonden we geen radiologische schade in de pols in dit cohort kinderen met JIA die vroeg en doelgericht behandeld zijn.

Hoofdstuk 7 behandelt het concept van equipoise bij patiënt en/of ouders tegen de achtergrond van de BeSt for Kids studie. Onder equipoise wordt oprechte onzekerheid

verstaan ten opzichte van de superioriteit van de ene behandeling boven de andere. Artsen-onderzoekers hebben equipoise nodig om randomized controlled studies (RCT's) uit te voeren, ten minste bij studies met meer dan minimale risico's. Of deze equipoise ook bij patiënten en ouders aanwezig is in klinische studies, is onduidelijk. Deelnemende kinderen en hun ouders werd gevraagd naar hun voorkeur direct bij insluiting in de BeSt for Kids studie met behulp van een vragenlijst (fase 1). Tijdens een interview studie (fase 2) ter evaluatie van de equipoise bij patiënten die deelnamen aan deze studie en hun ouders werden voorkeuren nogmaals bevraagd. De BeSt for Kids studie omvat drie strategieën, waarvan 1 bestaat uit het direct behandelen met een biological, hetgeen op dit moment niet de standaard behandeling is. Semi-gestructureerde interviews werden gehouden met 23 ouders en 7 patiënten ouder dan 12 jaar, gemiddeld 11 maanden na inclusie in de studie. De meeste ouders en patiënten waren initieel niet in equipoise. Velen hadden in fase 1 de voorkeur voor arm 3, initiële behandeling met een biological. Zij deden mee met het onderzoek vanwege deze kans en zouden zelfs stoppen met deelname aan het onderzoek als ze geen arm 3 geloot hadden. De overtuiging van superioriteit van de strategie met initiële behandeling met een biological (arm 3) was gebaseerd op kennis verkregen via internet en nauwe contacten. Vier ouders waren van mening dat de arts-onderzoeker een voorkeur had voor arm 3, terwijl de meerderheid (n=19) vond dat de arts-onderzoeker geen voorkeur had. In fase 2 neigden de voorkeuren te veranderen naar de feitelijke strategie waarin de patiënt gerandomiseerd was.

Concluderend stellen we dat ouders van kinderen die in studies deelnemen voorkeuren hebben in behandelingen. Het is belangrijk om alle zorgen en waarden van ouders van aan studies deelnemende kinderen te begrijpen. Hun voorkeuren kunnen gedurende de tijd veranderen, met name in een niet-geblindeerde context. Bovendien kunnen hun voorkeuren verschillen van de voorkeuren van de betrokken artsen.

Een studie die niet overeenstemt met de zorgen van de desbetreffende patiëntengroep zal waarschijnlijk niet worden ondersteund door deze patiëntengroep. In toekomstige studies loont het om de equipoise van de 'geïnformeerde patiëntengroep' als geheel te onderzoeken, om insluiting van patiënten in studies te verbeteren.

In **hoofdstuk 8** bespreken we de bijzondere casus van een van de deelnemers aan de BeSt for Kids studie. Volgend op de diagnose juveniele idiopathische arthritis (JIA) werd ze na inclusie behandeld met arm 3, initieel etanercept en methotrexaat. De primaire diagnose JIA was gebaseerd op symmetrische polyartritis zonder tekenen van systemische betrokkenheid. Zes maanden later ontwikkelde zij myalgie, hypertensie en koorts met verhoogde inflammatoire parameters. De combinatie van symptomen samen met

angiografische afwijkingen in de nieren leidde tot de diagnose juveniele systemische polyarteritis nodosa (PAN). Juveniele PAN is een zeldzame inflammatoire ziekte waarbij kleine tot middelgrote musculaire arteriën aangetast worden. De sterk variabele klinische presentatie wordt veroorzaakt door de grote hoeveelheid potentieel betrokken organen. De diagnose kan lastig zijn omdat de eerste symptomen niet-specifiek zijn en vaak andere inflammatoire ziekten kunnen nabootsen. Hoewel juveniele PAN een zeldzame ziekte is, zou het in elke differentiaal diagnose van onbepaalde systemische klachten of inflammatoire ziekten thuishoren.

Hoofdstuk 9 reflecteert op de resultaten van dit proefschrift tegen de achtergrond van de huidige klinische stand van zaken. Er wordt vergeleken met resultaten van eerdere studies. Daarnaast worden de verschillende studies vergeleken qua opzet en behandeldoel. Lessen die worden getrokken uit dit proefschrift zijn:

- Doelgericht behandelen in combinatie met nauwkeurige controle zijn haalbare doelen bij de behandeling van JIA patiënten in een klinische studie. Ze geven voordeel op korte en lange termijn.
- Inactieve ziekte zou het behandeldoel moeten zijn vanaf 6 maanden behandeling en verder.
- Afbouw strategieën kunnen veilig geïntroduceerd worden in JIA studies omdat de frequentie van opvlammingen laag was en de reactie op herstart van therapie goed.
- Er werd geen radiografische schade gevonden in de verschillende armen, en de botdichtheid verbeterde na behandeling in arm 3 (initieel etanercept en methotrexaat).

De huidige studie was gericht op het bereiken van inactieve ziekte en het verminderen van ziekte en therapie last. Toekomstig onderzoek zal zich richten op gepersonaliseerde behandelingen gecombineerd met doelgerichte strategieën gericht op inactieve ziekte.

Appendix - List of Publications

Related to thesis

1) Treatment strategies aiming at inactive disease in recent onset Juvenile Idiopathic Arthritis: Clinical outcomes of a randomized trial after 24 months. **PCE Hissink Muller**, D.M.C. Brinkman, W.B. van Den Bosch, D Schonenberg, Y. Koopman-Keemink, J.M. van den Berg, P. Bekkering, M.A.J. van Rossum, L.W.A. van Suijlekom-Smit, S Böhringer, C. F. Allaart, R. ten Cate; Ann Rheum Dis. **2019** Jan;78(1):51-59. doi: 10.1136/annrheumdis-2018-213902. Epub 2018 Oct 11.

2) A comparison of three treatment strategies in recent onset DMARD Naive Juvenile Idiopathic Arthritis: 3 months results of the BeSt for Kids study. **PCE Hissink Muller**, D.M.C. Brinkman, D Schonenberg, Y. Koopman-Keemink, J.M. van den Berg, P. Bekkering, M.A.J. van Rossum, L.W.A. van Suijlekom-Smit, C. F. Allaart, R. ten Cate; Pediatr Rheumatol Online J. **2017** Feb 6;15(1):11. doi: 10.1186/s12969-017-0138-4.

3) Randomization in a Pediatric Clinical Trial: Are Parents and Patient-participants in Equipoise? **PCE Hissink Muller**, B Yildiz, CF Allaart, DMC Brinkman, MAJ van Rossum, JM van den Berg, LWA van Suijlekom-Smit, R ten Cate, MC de Vries; BMC Med Ethics. **2018** Dec 20;19(1):96. doi: 10.1186/s12910-018-0336-8.

4) Disturbance of Microbial Core Species in New-Onset Juvenile Idiopathic Arthritis. **PCE Hissink Muller**, A.E. Budding, P.M. Westedt, C.F. Allaart, D.M.C. Brinkman, T.W. Kuijpers, J.M. van den Berg, L.W.A. van Suijlekom-Smit, M.A.J. van Rossum, T.G.J. de Meij, R. ten Cate; Journal of Pediatric Infectious Diseases. **2017;** volume 12 Issue 2 p131-135.

5) No radiographic wrist damage after treatment to target in early onset JIA; **PCE Hissink Muller**, WG van Braak, D Schreurs, SA Bergstra, CM Nusman, R Hemke, D Schonenberg, JM van den Berg, TW Kuijpers, Y Koopman-Keemink, MAJ van Rossum, LWA van Suijlekom-Smit, DMC Brinkman, CF Allaart, R ten Cate, M Maas; Pediatr Rheumatol Online J. 2019 Aug. 17:62 doi.org/10.1186/s12969-019-0362-1.

6) Polyarteritis Nodosa mimicking Juvenile Idiopathic Arthritis: A case report. **PCE Hissink Muller**, S Donze, R ten Cate; Annals of Paediatric Rheumatology. Sept **2014**; 3: 141-145.

7) Anti-carbamylated protein (anti-CarP) antibodies are present in sera of Juvenile Idiopathic Arthritis (JIA) patients. **P. C. E. Hissink Muller**, J. Anink , J. Shi , E.W.N. Levarht , T. H. Reinards,

M. H. Otten , M. J. D. van Tol, C.M. Jol-van der Zijde, D.M.C. Brinkman, C. F. Allaart, E. P. Hoppenreijs, Y. Koopman-Keemink, S Kamphuis, K. Dolman, J. M. van den Berg, M. A.J. van Rossum, L. W.A. van Suijlekom-Smit, M. W. Schilham, T.W.J. Huizinga, R. E. M. Toes, R. ten Cate, L. A. Trouw; Ann Rheum Dis. **2013** Dec;72(12):2053-5. doi: 10.1136/annrheumdis-2013-203650. Epub 2013 Jul 19.

8) Dutch Juvenile Idiopathic Arthritis patients, carers and clinicians create a research agenda together following the James Lind Alliance method: a study protocol. Casper G Schoemaker; Wineke Armbrust; Joost F Swart; Sebastiaan J Vastert; Jorg van Loosdregt; Anouk Verwoerd; Caroline Whiting; Katherine Cowan; Wendy Olsder; Els Versluis; Rens van Vliet; Marlous J Fernhout; Sanne L Bookelman; Jeannette Cappon; J Merlijn van den Berg; Ellen Schatorjé; **Petra CE Hissink Muller**; Sylvia Kamphuis; Joke de Boer; Otto THM Lelieveld; Janjaap van der Net; Karin R Jongsma; Annemiek van Rensen; Christine Dedding; Nico M Wulffraat; Pediatr Rheumatol Online J. 2018 Sep 15;16(1):57. doi: 10.1186/s12969-018-0276-3.

9) Galectin-9 and CXCL10 as biomarkers for disease activity in juvenile dermatomyositis: a longitudinal cohort study and multi-cohort validation. Judith Wienke, MD; Felicitas Bellutti Enders, MD/PhD; Johan Lim, MD; Jorre S Mertens, MD; Lucas L Van Den Hoogen, MD ; Camiel A Wijngaarde, MD; Joo G Yeo, MBBS; Alain Meyer, MD/PhD; Henny G Otten, PhD; Ruth D Fritsch-Stork, MD/PhD; Sylvia S Kamphuis, MD/PhD; Esther P Hoppenreijs, MD; Wineke Armbrust, MD/PhD; Jason M Van den Berg, MD/PhD; **Petra C Hissink Muller, MD**; Janneke Tekstra, MD/PhD; Jessica E Hoogendijk, MD/PhD; Claire Deakin, PhD; Wilco De Jager, PhD; Joël A Van Roon, PhD; Willem L Van der Pol, MD/PhD; Kiran Nistala, MD/PhD; Clarissa Pilkington, MD; Marianne De Visser, MD/PhD; Thaschawee Arkachaisri, MD/PhD; Timothy R Radstake, MD/PhD; Anneke J Van der Kooi, MD/PhD; Stefan Nierkens, PhD; Lucy R Wedderburn, MD/PhD; Annet Van Royen-Kerkhof, MD/PhD; Arthritis Rheumatol. **2019** Mar 12. doi: 10.1002/art.40881.

10) Een tiener met een niet infectieuze osteomyelitis. C Deden, **P. Hissink Muller**, H Kroon, N Appelman, G Tramper-Stranders; Nederlands Tijdschrift voor Geneeskunde **2017**;161;D1475.

Not related to thesis

11) Overlap between linear scleroderma, progressive facial hemiatrophy and immune-inflammatory encephalitis in a paediatric cohort. L. De Somer, MA Morren, **PCE Hissink Muller**, K. Despontin, K. Jansen, L. Lagae, Carine Wouters; Eur Journal of Pediatrics 2015 Sep;174(9):1247-54.

12) Chronische urticaria: of is er meer aan de hand? **PCE Hissink Muller**, E Hoppenreijs, dr. M. Seyger; Praktische pediatrie, November **2014**.

13) Varicella zoster reactivation after hematopoietic stem cell transplant in children is strongly correlated with leukemia treatment and suppression of host T lymphocyte immunity. Clementien.L. Vermont, Els C.M. Jol-van der Zijde, **Petra Hissink Muller**, Lynne M. Ball, Robbert G.M. Bredius, Ann C. Vossen, Arjan C. Lankester; Transpl Infect Dis. **2014** Apr;16(2):188-94. doi: 10.1111/tid.12180.

14) A child with a necrotic finger. Van den Bruele T; **Hissink Muller, PCE**; ten Cate, R; BMJ case reports **2013**; doi:10-1136/bcr-2012-007493.

15) Low complement levels in pediatric systemic lupus erythematosus and the risk of bacteremia: a case report. Hagen J, **Hissink Muller PCE**, Bredius R, ten Cate R; BMJ case reports 2013 doi; 10.1136/bcr-2013-010378

16) Juvenile Arthritis: Current Situation. **Hissink Muller PCE**, Brinkman DBR, ten Cate R; Modern Medicine 2009, 11, 394-398.

17) Neonatal carnitine palmitoyltransferase II deficiency: failure of treatment despite prolonged survival. **Hissink Muller PCE**, Lopriore E, Boelen C, Klumper F, Duran M, Walther F; BMJ Case Reports **2009**; doi:10.1136/bcr.02.2009.1550.

18) Axillary and rectal temperature measurements poorly agree in new born infants. **Hissink Muller PCE**, van Berkel LH, de Beaufort AJ; Neonatology **2008**;94(1):31-4. DOI:10.1159/000112840.

List of Abbreviations

AE	=	Adverse event
ADA	=	adalimumab
AUC	=	Area Under the Curve
BMD	=	Bone Mineral Density
CONCERT study	=	CONgenital Cmv: Efficacy of antiviral treatment in a Randomized controlled Trial
CI	=	Confidence Interval
CMV	=	Cytomegalovirus
CR	=	Conventional Radiography
DMARD	=	Disease Modifying Antirheumatic Drug, cs = conventional synthetic; b = biologic
DFID	=	drug free inactive disease
ETN	=	etanercept
GEE	=	Generalised Estimating Equations
IBD	=	inflammatory bowel disease
ICC	=	intra-class correlation coefficient
ID	=	inactive disease
IFX	=	infliximab
IM	=	intramuscular
ITT	=	intention to treat
IQR	=	Interquartile Range
JADAS	=	Juvenile Arthritis Disease Activity Score
JIA	=	Juvenile Idiopathic Arthritis
NS	=	not significant
NSAID	=	Non-Steroidal Anti-Inflammatory Drug
NTR	=	Dutch Trial Register
M2	=	second metacarpal
Max	=	maximum
MDA	=	Minimal Disease Activity
MMF	=	mycophenolate mofetil.
MRI	=	magnetic resonance imaging
MTX	=	methotrexate
Oligo	=	Oligoarticular JIA
PAN	=	polyarteritis nodosa
PIF	=	Patient-subjects Information Form
RCT	=	Randomized Controlled Trial

RF	=	Rheumatoid Factor
RM	=	radiometacarpal length
RM	=	exp expected radiometacarpal length
RMexp	=	expected radiometacarpal length
RMobs	=	observed radiometacarpal length
PGA	=	physicians global assessment
PLS-DA	=	partial least squares discriminant analysis
Poly	=	Polyarticular JIA
RCT	=	Randomized Controlled Trial
ROC	=	receiver operating characteristic
SAE	=	Serious Adverse event
SC	=	subcutaneous
SSZ	=	Sulfasalazine
T2T	=	Treat to target
US	=	Ultrasound
VAS	=	Visual Analogue Scale

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Curriculum vitae

Petra Hissink Muller was born on May 25th, 1977 in Amsterdam. After high school (Maurick College Vught) she started to study Medicine in 1995 at the Catholic University of Leuven, Belgium. In 2000 she completed the master's degree (magna cum laude). After traveling for 8 months, she started her internships at Leiden University Medical Center and passed her medical exam in 2003 (cum laude). After a period as a physician-trainee in the Lange Land Hospital, she started training as a pediatrician (2004) at the LUMC (under the direction of Prof. Dr. J.M. Wit and later Prof. Dr. R. Sukhai) and followed her peripheral internship at the Juliana Children's Hospital (led by Dr. G. Derksen-Lubsen and later Dr. M. Reeser). During the final phase of her training at the LUMC she started to work in the pediatric rheumatology department under the supervision of Prof. Dr. Rebecca ten Cate. From 2009-2013 she followed the fellowship pediatric rheumatology-immunology. During these years, she coordinated and conducted the BeSt for Kids study in collaboration with pediatric rheumatologists in the Randstad. This study examined treatment strategies for patients with juvenile idiopathic arthritis shortly after and gave rise to the results described in this disserartation. The BeSt for Kids study was investigator-initiated and financially supported by Pfizer. June 2013, she was registered as a pediatrician rheumatology immunology. Since June 2013, she has been working as a registered pediatrician rheumatology- immunology in the LUMC. Between 2015-2019, she worked parttime at the Sophia Children's Hospital in the context of the LUMC-SKZ Alliance. Petra is married to Joost Colaris, together they have two daughters, Carmen (2009) and Louise (2011).