Predictors of response to methotrexate in juvenile idiopathic arthritis patients

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Abkürzungsverzeichnis

ACR	American College of Rheumatology
ADA	Adalimumab
ANA	Antinuclear Antibody
AUROC	Area Under Receiver Operating Curve
BSA	Body Surface Area
CHAQ	Childhood Health Assessment Questionnaire
CRP	C- reactive Protein
DMARD	Disease Modifying Antirheumatic Drugs
ERA	Enthesitis Related Arthritis
ESR	Erythrocyte Sedimintation Rate
HLA	Human Leucocyte Antigen
Ig	Immunoglobulin
IL	Interleukin
ILAR	International League of Associations for Rheumatology
JIA	Juvenile Idiopathic Arthritis
JPsA	Juvenile Psoriatic Arthritis
LOM	Limitation of Motion
MS	Morning Stiffness
MTX	Methotrexate
RF	Rheumatoid Factor
S.C	Subcutaneous
SD	Standard Deviation
SoJIA	Systemic onset Juvenile Idiopathic Arthritis
TNF	Tumor Necrosis Factor
VAS	Visual Analogue Scale

Deutsche Zusammenfassung

1.1 Einleitung und Zielsetzung

Juvenile Idiopathische Arthritis ist ein Begriff, der verwendet wird, um eine heterogene Gruppe von Erkrankungen unbekannter Ätiologie, gekennzeichnet durch eine chronische Arthritis bei Kindern unter 16 Jahren zu beschreiben (Petty et al., 2004). JIA ist die häufigste chronisch entzündlich rheumatische Erkrankung im Kindesalter (Weis und Ilowite, 2007).

Die Diagnose Juvenile Idiopathische Arthritis steht für eine chronische, mindestens 6 Wochen persistierende Arthritis mit einem Erkrankungsbeginn im Alter von unter 16 Jahren bei Ausschluss anderer Ursachen. Betroffene leiden unter schmerzhaften Schwellungen, Ergüssen, Druckempfindlichkeit und Bewegungseinschränkungen der Gelenke.

Um eine irreversible Schädigung der Gelenke zu vermeiden, ist deshalb eine frühzeitige und effektive Therapie wichtig. Methotrexat ist derzeit am häufigsten eingesetzte Langzeitantirheumatikum. Methotrexat wird als „first choice secondline agent“ für die JIA bezeichnet (Miller und Cassidy, 2007).

Ziel der Behandlung der JIA ist die Remission, um den Kindern eine altersgerechte Entwicklung ohne Schmerzen und Funktionseinschränkung zu ermöglichen.

Einige Patienten brechen die Therapie mit MTX wegen Unverträglichkeiten ab. Eine Vorhersage des Ansprechen auf MTX könnte helfen, eine Therapie mit Biologika sobald wie möglich zu starten, und irreversible Komplikationen zu verhindern.

Das Ziel der Studie ist es, festzustellen, ob demographische, klinische und labormedizinische Variablen das Ansprechen auf MTX vorhersagen können.

1.2 Material und Methoden

Das Patientenkollektiv wurde dem deutsche BIKER-Register entnommen. Gegründet im Jahre 2001, enthielt es zum Stichtag der Untersuchung, dem

31.12.2010, 1063 Patienten, die nach einem bestimmten Rhythmus dokumentiert wurden. 915 Patienten erfüllten alle Ein- und Ausschlusskriterien.
Einschlusskriterien waren:

- die Diagnose einer JIA nach den ILAR-Kriterien
- eine begonnene Therapie mit MTX
- Therapiedauer (mit MTX) von mindestens 3 Monaten

Ausschlusskriterium war eine Therapie mit einem Biologikum.

Um das Ausmaß der Verbesserung abzuschätzen, wurde der PedACR- Score verwendet. Nach diesen Kriterien müssen die Kinder eine Verbesserung von mindestens 30 % (PedACR 30), oder 70% (PedACR 70)in mindestens drei der sechs folgenden Variablen haben: Anzahl bewegungseingeschränkter Gelenke, Anzahl aktiver Gelenke, Patienten-VAS zur Globalen Beeinträchtigung, Arzt-VAS zur Globalen Krankheitsaktivität, CHAQ und BSG. Eine Verschlechterung von bis zu 30 % darf in nicht mehr als einem Parameter auftreten. Die Bewertung erfolgte 3, 6 und 12 Monaten nach dem Therapiestart.

Bei den verwendeten Daten handelt es sich um Daten aus einem laufenden Register, welches in einer Microsoft Access Datenbank vorliegt. Die Auswertung wurde mit SPSS (Version 21) durchgeführt. Als Verfahren der deskriptiven Statistik wurden bei kontinuierlichen Daten Mittelwerte, Median, Standardabweichung, Minimal und Maximalwert und bei Kategorien Daten (Prozente) Häufigkeiten verwendet. Statistische Tests beinhalteten den Korrelationstest nach Pearson oder Spearman, den Odds Ratios (OR) mit (confidence Interval) und AUC (Area Under the Reciever Operating Curve).

1.3 Ergebnisse

Das Ansprechen auf MTX nach PedACR 70- Kriterien zeigte in der multivariablen Analyse der logistischen Regressionsanalyse eine signifikante Assoziation mit der folgenden Parametern:

- Nach 3 Monaten: eine größere Anzahl von Gelenken mit einem eingeschränkten Bewegungsumfang (p=0.004) und eine höhere Punktzahl im Arzt-

VAS (p=0.004).

- nach 6 Monaten: Krankheitsdauer < 1 Jahr (p< 0.001), ein niedriges Alter bei Beginn der Therapie mit MTX (p= 0.018), eine höhere Punktzahl im Arzt-VAS (p= 0.004) und eine größere Anzahl von geschwollenen Gelenken (p= 0.001).

- nach 12 Monaten: Krankheitsdauer< 1 Jahr (p= 0.001), ein niedriges Alter bei Beginn der Therapie mit MTX (p< 0.001), eine größere Anzahl von aktiven Gelenken (p< 0.001), eine geringere Anzahl von Gelenken mit Schmerzen (p= 0.004), ein erhöhtes CRP (p= 0.018), eine höhere Punktzahl bei der Schmerzbeurteilung durch die Eltern (p= 0.037) und die Präsenz der Morgensteifigkeit (p= 0.016).

1.4 Diskussion

Das Ansprechen auf MTX nach PedACR 70- Kriterien war negativ assoziiert mit einer geringeren Anzahl von Gelenken mit einem eingeschränkten Bewegungsumfang, einer niedrigeren Punktzahl von Arzt-VAS, Krankheitsdauer >1 Jahr, einem höheren Alter bei Anfang der Therapie mit MTX, einer geringeren Anzahl von aktiven Gelenken, einer größeren Anzahl von Gelenken mit Schmerzen, niedrigeren CRP Werten, einer niedrigeren Punktzahl bei der Schmerzbeurteilung durch die Eltern und die Abwesenheit einer Morgensteifigkeit.

Die diagnostische Zuverlässigkeit wurde anhand der ROC Kurve (receiver characterstics curve) bestimmt. Die beste diagnostische Genauigkeit wurde gemäß PedACR 30 nach 3 Monaten (AUROC= 0.73 , Sensitivität= 98.9 % , Spezifität= 10.7 %) und laut PedACR 70 nach 12 Monaten (AUROC= 0.69 , Sensitivität= 91.5 % , Spezifität= 33.2 %) erreicht.

Die Ergebnisse dieser Studie können als Empfehlung zum Einsatz von MTX bei JIA angesehen werden, da die hier gefunden Faktoren negative Prädiktoren für ein Ansprechen auf MTX, auch nach Therapie von bis zu 12 Monaten, darstellen und dadurch die Entscheidung, eine alternative Therapie zu beginnen erleichtern.

2. Introduction and objectives

2.1 Introduction

Juvenile idiopathic arthritis is an umbrella term used to describe a heterogeneous group of disorders of unknown etiology, characterized by chronic arthritis affecting children below 16 years (Petty et al., 2004). Juvenile idiopathic arthritis is the most chronic rheumatic illness in children and it is responsible for short and long-term disability (Weis and Ilowite, 2007).

In the recent years an increased number of disease-modifying anti-rheumatic drugs (DMARDs) have been developed for treatment of juvenile idiopathic arthritis, but methotrexate still the most common second line therapeutic agent used in treatment of juvenile idiopathic arthritis worldwide (Miller and Cassidy, 2007), however, there is variation in the clinical response to methotrexate among the patients. Prediction of response can prevent further exposing of patients to side effects of methotrexate and also saving the time by progressing to the treatment with biologics as soon as possible to prevent irreversible complications.

This is a retrospective study of 915 patients with juvenile idiopathic arthritis. The cohort of patients was collected from the German Methotrexate registry. The analysis of demographic, clinical, articular and laboratory characteristics of the patients was made at baseline and during follow up at month 3, 6 and 12. The improvement was assessed according to the paediatric criteria of American College of Rheumatology by using PedACR 30 for minimal improvement analysis and PedACR 70 for strong improvement analysis.

2.2 Juvenile Idiopathic Arthritis

Traditionally, this term was used to describe chronic arthritis persisting for at least 6 weeks in an individual 16 years of age or younger after exclusion of other reasons of arthritis (Miller and Cassidy, 2007).

Juvenile idiopathic arthritis is the most common chronic rheumatic illness in children and is a significant cause of short and long term disability (Weis and Ilowite, 2007).

2.2.1 Epidemiology

There are differences in estimates for the prevalence and incidence of JIA. These differences occurred due to many factors, which include development of new diagnostic criteria, in addition to the differing of definitions of clinical cases and to the factors occurring with the passage of time, for examples; standards of living, health care resources and increasing knowledge.

In one study by Manners and Bower in 2002 a review of 34 epidemiological studies of JIA since 1966 was undertaken, the results shows that the prevalence of JIA is reported as 7-401 per 100.000 children, and the annual incidence is reported as 0.8-22.6 per 100.000 children (Manners and Bower, 2002).

2.2.2 Classification of Juvenile idiopathic arthritis

The classification of juvenile idiopathic arthritis according specific criteria in many subtypes allows better understanding of their pathogenesis and clinical course. Many of the recent studies on pathogenesis have simply divided patients in three main categories: oligoarthritis, polyarthritis and systemic arthritis.

In the fact there are different classifications for juvenile idiopathic arthritis, but in our study we used the classification according ILAR (International League of Association for Rheumatology) congress 2001, in which juvenile idiopathic arthritis divided in seven categories:

Type 1 systemic onset juvenile arthritis (formerly called Still's disease)

Type 2 seronegative polyarthritis (at least 5 joints affected during first 6 months and RF is negative)

Type 3 seropositive polyarthritis (at least 5 joints affected during first 6 months and RF is positive)

Type 4a persistent oligoarthritis (less than 5 joints affected)

Type 4b extended oligoarthritis (5 or more joints become affected after 6 months)

Type 5 Enthesitis related arthritis (ERA)

Type 6 Psoriasis related arthritis (JPsA)

Type 7 unclassified arthritis (matching no or more than 2 categories)

According the frequency of each subtype, the oligoarticular JIA is the most common (50-60 %), then polyarticular JIA (30-35 %), SoJIA (10-20 %), JPsA (2-15 %), and ERA (1-7 %).

The categories are recognised based on the clinical features during the first 6 months of the disease (Table 1). Important clinical features that assist in classifying patients include the presence of enthesitis (inflammation at the sites of attachment of ligament, tendon, or fascia to bone), dactylitis, inflammatory lumbosacral pain, nail pitting, sacroiliitis, fever, rash, and serositis.

Juvenile idiopathic arthritis is diagnosed when arthritis occurs before sixteenth birthday and has been presented for at least 6 weeks with no other apparent diagnosis. The arthritis may be unclassified between 6 weeks and 6 months, after which time it can be categorized in one of seven subtypes.

ACR classification	ILAR classification	Systemic feature
Systemic	systemic arthritis	Fever, rash, pericarditis, hemophagocytic syndrome
Pauciarticular (early onset)	Oligoarthritis (persistent)	Uveitis
	Extened oligoarthritis	Uveitis
Polyarticular, seronegative	Polyarthritis, RF-ve	Low grade fever, uveitis
Polyarticular, seropositive	Polyarthritis, RF+ve	Nodules, Felty syndrome, Sjögren syndrome
Pauciarticular (late onset)	Enthesitis related	Spondylitis, acute iritis and inflammatory bowel disease
Psoriatic arthritis*	Psoriatic arthritis	Psoriasis and uveitis

*Separate diagnostic disease, ACR= American College of Rheumatology, ILAR= International League of Associations for Rheumatology, RF= Rheumatoid Factor (Passo and Rosen, 2005)

Table 1: Comparison of the ILAR and ACR, the criteria and the associated systemic manifestations

Systemic arthritis

Systemic arthritis is the less common subtype, and usually begins with high, spiking fevers to greater than 39.4 ^{o}C, the fever frequently is accompanied by rash that comes and goes with temperature elevations. Joint involvement may occur either at onset or later in the course of the disease.

Oligoarthritis

Oligoarthritis category of JIA affects four or fewer joints in the first six months of disease. If more than four joints become involved after six months, it is defined as extended oligoarthritis, otherwise it is classified as persisting oligoarthritis. Oligoarthritis is the most common form of JIA, and preferentially afflicts 1 to 3 years old caucasian girls.

Although all races can be affected, the prevalence is much reduced in non caucasians. Girls outnumber boys 4:1, clinically about half of oligoarticular JIA patient will have a single joint involved at onset, mainly the knee. The next most commonly affected joints is the ankle, and the small joints of the hand are the third most commonly affected. Wrist involvement is rare and may indicate the progression to extended oligoarthritis. Up to 20 % of patients can develop uveitis, which usually asymptomatic and it is more prevalent in the children, who are ANA positive. (Woo et al., 2007).

Polyarthritis

In both polyarthritis categories there are five or more joints involved in the first six months, girls outnumber boys in both categories, seronegative subtype is more common than seropositive.

Polyarthritis subtypes characteristically involves the small joints of the hands and feet, but large joints involvement is also common.

Approximately one fourth of the seronegative polyarthritis patients have positive test results for ANA, and few of the patients have associated chronic uveitis. The

onset of illness usually occurs when the child is 10 years old or younger.

In general, seronegative JIA patients respond better to treatment with NSAIDs than do seropositive patients. Only about 5-8 % of all children with chronic arthritis are seropositive for rheumatoid factor. The onset of this illness usually occurs when the child is 9-16 years of age (Miller and Cassidy, 2007).

Enthesitis Related Arthritis

Enthesitis related arthritis is defined as arthritis and/or enthesitis (inflammation of tendons or ligaments where they attach to bone) with at least two of the following features: (1) Sacroiliac joint tenderness, or inflammatory lumbosacral pain (2) HLA-B27 positive (3) First degree relative with medically confirmed HLA-B27 associated disease (4) Uveitis (5) Onset of arthritis in a boy six years or older. Enthesitis related arthritis is more frequent in boys (Burgos-varagas et al., 1997). The onset typically occurs in children over six and there is a familial predilection.

Psoriatic Arthritis

Juvenile psoriatic arthritis is chronic inflammatory arthritis with a peak age of onset in mid childhood. Juvenile psoriatic arthritis is difficult to be diagnosed, because the arthritis may develop many years before the skin manifestation. JPsA is an asymmetric arthritis that often affects the knees, and the ankles, and the small joints of the hands and feet. Proximal interphalangeal joints, and tendon sheath are often inflamed, resulting in the diffuse swelling of the digit known as sausage digit (Shore, 1982). Extra-articular manifestations include rash, nail changes (including pitting, onycholysis, oil drop sign), and uveitis. One third of patients with JPsA develop the psoriasis by 15 years of age (Petty and Malleson, 1986).

All children with JPsA should have a slit-lamp examination at least every 6 months, because asymptomatic anterior uveitis may be found in up 17 % of patients.

Laboratory data show elevated acute phase reactants, anemia of chronic disease, and thrombocytosis. Also ANA may be positive (Southwood and Petty, 1989)

2.2.3 Etiology and Pathology

The causes of juvenile idiopathic arthritis remain unclear, but it seems to be a complex genetic trait involving the effects of multiple genes related to immunity and inflammation. Some hypothesize that arthritis may be triggered in a genetically predisposed individual by psychologic stress, abnormal hormone levels, trauma to a joint, or bacterial or viral infection. Several studies have implicated rubella and parvovirus B19 as possible causes of JIA because rubella virus persists in lymphocytes and establishes a focus of persistent infection in the synovium resulting in chronic inflammation (Lang and Shore, 1990).

Certain HLA class 1 and class 2 alleles are associated with an increased risk of JIA. The class 1 antigen HLA-A2 is associated with early onset oligoarthritis in girls (Murray KJ, et al, 1998). The class 2 antigens HLA-DRB1*08 and *11, DQA1*04 and *05, and DQB1*04 are associated with persistent oligoarticular and extended oligoarticular JIA. HLA-DRB1*08 confers an increased risk of rheumatoid factor (RF) negative polyarthritis, and HLA-DRB1*11 confers an increased risk of systemic onset JIA (SOJIA). HLAB1*04, which associated with adult rheumatoid arthritis, is associated with an increased risk of RF positive polyarticular arthritis. The class 1 antigen HLA-B27 and class 2 antigens HLA-DRB1*01 and DQA1*0101 are associated with enthesitis related arthritis (ERA) and JPsA. Other genes conferring risk include cytokine production regulating genes. Anti-nuclear antibodies (ANA) are found in approximately 40 % of patients with JIA, especially in young girls with oligoarticular disease (Petty RE, Cassidy JT, Sullivan DB, 1973). Approximately 5 % to 8 % of patients with JIA are rheumatoid factor positive (Lang BA, Shore A, 1990). The T-lymphocytes mediated immune response is involved in chronic inflammation, and T cells are the predominant mononuclear cells in synovial fluid.

Patients with JIA have elevated serum levels of interleukin (IL) -1,-2,-6, and IL-2

receptor (R), and elevated synovial fluid levels of IL-1B, IL-6 and IL-2R, suggesting a Th1 profile. Elevated serum levels of IL-6, IL-2R, and soluble tumor necrosis factor (TNF) receptor correlate with inflammatory parameters, such as C-reactive protein, in JIA patients indicate active disease. Serum levels of IL-6 are increased in SoJIA and rise before each fever spike, correlating with active disease and elevation of acute phase reactants (Weis and Ilowite, 2007).

2.2.4 Prognosis

The prognosis of JIA in an individual child is so far unpredictable. Studies from United States indicate that despite the current management, approximately 45 % of JIA patients have active disease persisting into early adulthood, often with severe limitation of physical function (Miller and Cassidy, 2007).

Most children with oligoarthritis do well, in about 20-30 % of these children oligoarthritis becomes extended and the outcome is poor until methotrexate is introduced as the treatment of choice. Remission is induced in 60-70 % while on methotrexate. Anti-TNF agents are effective if patients fail to respond to methotrexate, especially if given in combination with methotrexate (Woo P et al., 2007).

The child with oligoarticular disease, particularly a girl with early onset of arthritis at < 6 years of age is at risk to develop chronic uveitis. There is usually no association in the course of the arthritis and chronic uveitis. Uveitis can result in posterior synechiae, untreated or refractory disease can result in blindness, which can be decreased by frequent monitoring with slit-lamp examination to exclude asymptomatic uveitis (Miller and Cassidy, 2007).

Other sequelae include leg length discrepancy, especially in those with knee arthritis. Muscle atrophy can occur due to persistent swelling and pain. The child with polyarticular disease often has a more prolonged course. Poor prognosis associated with older age of onset, the presence of rheumatoid factor seropositivity or rheumatoid nodules, or the early involvement of cervical spine or hips (Miller and Cassidy, 2007).

The child with systemic JIA onset is often the most difficult to manage in terms of both articular and systemic manifestations. However systemic manifestations are usually present only during the first few years after onset. The prognosis after that time dependent on the number of the joints involved and severity of arthritis (Miller and Cassidy, 2007).

The long term outcome of enthesitis related arthritis is poorly described, enthesitis is more symptomatic in teens and young adults, and it improves with age. Boys with HLA-B27 and hip arthritis, or tarsitis are at high risk of developing progressive spinal involvement. Children with psoriatic arthritis tend to have longer lasting disease, and small but significant percentage (up to 10 %) may be disabled (Woo et al., 2007).

2.2.5 Treatment of Juvenile Idiopathic Arthritis

The objectives of treatment include controlling pain and inflammation, preserving function, and promoting normal growth, overall development, and wellbeing as well as to achieve these goals with minimal risk of side effects. The long term treatment of children with JIA is initiated and subsequently modified according to disease sub-type, severity of the disease, specific manifestation of the illness and the response to therapy (Miller and Cassidy, 2007).

Therapy is directed toward the underlying inflammation of JIA (e.g. joint damage). Medication include first line NSAIDs and second line drugs that include immunomodulators, biologic agents, corticosteroids and gold. Initial drug therapy for children with polyarticular juvenile idiopathic arthritis should be aggressive in order to control the inflammatory process and relieve symptoms as quickly as possible while minimizing drug side effects (Lachman, 2007). Treatment usually started with the least toxic medication usually NSAID, and proceeding through methotrexate to modulatory biologics. Medications that place the child 's present and future health most at risk, such as azathioprine, and cyclophosphamide, are reserved for the very few children who do not respond to less aggressive therapy.

NSAIDs traditionally have been the preferred first line drugs, these medications reach full efficacy within two to three months, but usually start to relieve symptoms within a few days. The improvement will be clear in most of the patients within the first three months (Ruperto et al., 2005).

Systemic corticosteroids are very powerful anti-inflammatory medications, but their use is limited, because the risk of side effects, which include Cushing's syndrome, hyperglycemia, immunosuppression, cataract, glaucoma, peptic ulcer, osteopenia, and growth retardation. Although glucocorticosteroids are the mainstay of treatment for controlling serious systemic manifestations of systemic JIA, use in polyarthritis patients should be limited as bridging agent to patients with extreme pain, disabling morning stiffness and functional limitation while waiting for a second – line agent to show some effect (Klein and Horneff, 2009). However short term use of low dose corticosteroids (less than o.25 mg /kg per day of prednisolone or it 's equivalent) may provide substantial benefits without complication, so we can say that the use of glucocorticosteroids like bridge between the NSAIDs and other DMARDs.

Intra- articular injections of corticosteroids is an effective therapy for patients with JIA, and majority of patients having complete and long lasting response (Srini--vasan et al., 2012). Triamcinolone hexacetonide (10-40 mg/joint or 1-2 mg/ kg/ joint) is commonly used and has been shown to result in improvement of signs and symptoms of arthritis. The side effects may include infection, atrophic skin changes at the site of injection, and asymptomatic calcifications on radiographs.

Disease modifying antirheumatic agents

DMARDs are a group of drugs which effective in treatment of JIA e.g. sulfasalaz-ine, methotrexate and leflunomide.

Methotrexate

MTX is the most common used as a second line treatment of JIA and it will be discussed in details in section 1.3 .

Leflunomide

Leflunomide is an immunomodulator that inhibits pyrimidine synthesis, it has been shown to be safe and effective in adults with rheumatoid arthritis, and has also been studied for use in JIA. Preliminary results show efficacy similar to that of methotrexate, but has not been shown to be superior to methotrexate (Silverman et al., 2005a, 2005b).

Side effects of leflunomide include diarrhea, elevated liver enzymes, mucocu--taneous abnormalities and teratogenicity (Weiss and Ilowite, 2007).

Sulfasalazine

Sulfasalazine has been shown to be beneficial for many children with enthesitis related arthritis, families must be warned of the possible development of rare severe reactions seen with sulfa drugs e.g Stevens-Johnson syndrome. Sulfasalazine does not prevent chronic changes and therefore should not be relied upon in erosive disease (Lachman, 2007).

Other immunomodulators like azathioprine and cyclosporine have been used with varying success in the past. Currently they are not preferred for treatment because of their significant potential toxicity, and they are less effective than the newer biologic agents (Klein and Horneff, 2009).

Biologic agents

They are group of agents, include the TNF inhibitors etanercept, infliximab, adalimumab, golimumab and certolizumab, the IL-1 inhibitor anakinra, rilonacept and canacinumab, the IL-6 receptor blocker tocilizumab and the B-cell depleter rituximab, they are currently being used in patients with RA and JIA resistant to methotrexate.

All biologic agents carry a risk of immunosuppression, infection, and possibly malignancies. Live virus vaccines are relatively contraindicated. Cases of

reactivated tuberculosis have been reported in JIA patients using the TNF inhibitors (Weiss and Ilowite, 2007).

Etanercept

Etanercept is a fusion protein containing tumor necrosis factor (TNF) receptor and FC fragment from human IgG. It is effective in many children with resistant polyarthritis disease (Horneff et al., 2004; Lovell et al., 2000).

Patients treated with etanercept show dramatic improvements within weeks of starting therapy, with benefits persisting for years. Etanercept was proven effective in controlling pain and swelling and in improving laboratory parameters (Lovell et al., 2003, 2006, 2008).

Approximately three fourths of patients who do not respond adequately to methotrexate will have a good response to etanercept. Etanercept 0.4 mg /kg (maximum 25 mg) given subcutaneously twice weekly has a dramatic response, and is highly recommended for patients with extended oligoarthritis or polyarthritis who have not responded to NSAIDs and methotrexate.

In addition there is increasing evidence from the studies that the combination of etanercept and methotrexate in synergistic was well tolerated and highly effective especially in treatment of juvenile polyarthritis but not in patients with systemic arthritis (Schmeling et al., 2001).

Infliximab

Infliximab is a chimeric mouse-human monoclonal anti-TNF-alpha antibody. Infliximab may have similar efficacy to that seen with the use of etanercept, but the incidence of adverse effects was higher and more serious in the infliximab group than in etanercept group. A short term head to head placebo controlled study in juvenile idiopathic arthritis patients has shown that infliximab failed to reach the primary end point (Ruperto et al., 2007).

Adalimumab

Adalimumab is a fully humanized monoclonal anti-TNF antibody, which is admi--nistered either weekly or every other week as a single s.c. injection. Preliminary experience has shown that adalimumab has been effective in many children who had not responded adequately to etanercept (Lovell et al. , 2004).

Rituximab

Rituximab is a chimeric anti-CD20 monoclonal antibody, which has been approved for treatment of adult onset rheumatoid arthritis (Lachman, 2007).

Anakinra

As the first line therapy for systemic JIA was associated with rapid solution of systemic symptoms and prevention of refractory arthritis, it shows superiority in short term placebo controlled study (Quartier et al., 2011).

Humanized anti-interleukin 6 receptor antibody

There is evidence that systemic onset JIA is in part an IL-6 mediated disease , and the use of humanized anti-interleukin 6 such as tocilizumab had signific-ant improvement in the ACR improvement criteria and disease activity indices , an addition to decrease in acute-phase reactant. It is effective in treatment of systemic –onset JIA and may be useful in patients with intractable disease (Yokota et al., 2008).

Autologus Stem Cell Transplantation

Autologus Stem cell transplantation has been considered in recalcitrant cases of SoJIA, and because the procedure carries a significant mortality risk (usually from macrophage activation syndrome). Stem transplantation should be

performed only in experienced centers after all other treatment options have failed (Weiss and Ilowite , 2007).

2.2.6 Treatment of complications

The treatment of JIA should be directed towards not only the inflammation of JIA but also for specific complications of JIA flexion contractures, weakness and difficulty with ambulation are not rare. The children with later complications should be referred for physical therapy. In rare cases joint replacement is indicated. In patients with temporomandibular joint involvement especially with significant micrognathia, surgical correction may be required, but this operation should not be done until the facial bones are fully developed.

Uveitis is initially treated with topical corticosteroids, if there is no improvement systemic corticosteroids and/ or MTX may be helpful, and in especially severe disease cyclosporine, adalimumab or infliximab have been used successfully.

Osteoporosis and growth retardation are associated with severe polyarthritis subtypes of JIA. Preliminary studies of growth hormone therapy have shown some improvement in bone mineral contents and accelerated linear growth (Bechtold et al., 2004; Rooney et al., 2000; Simon et al., 2003). However, the use of growth hormone therapy also has been associated with an increase incidence of deformities, such as scoliosis (Lachman, 2007).

2.3 Methotrexate

Methotrexate which formerly known as a methopterin, is an immunomodulator, by acting as antimetabolite and antifolate drug. It is used for in treatment of cancer, autoimmune disease, ectopic pregnancy, and for induction of medical abortion.

The beginning of use of MTX was in 1950 for treatment of leukemia. MTX when used at dose of 10-15 mg/ m^2 body surface area (BSA) per week acts as an anti- inflammatory agent rather than as a cytotoxic drug. MTX has been the

standard therapy for children with JIA especially with polyarticular course, and considered as the drug of choice in the second line treatment for patients with JIA who did not respond to NSAIDs (Ruperto et al., 2004; Ravelli and Martini, 2000; Wallace, 1998).

The short and long term data suggest that MTX is a safe drug in the pediatric population with rheumatic disease. And not surprisingly MTX is the DMARD of choice in JIA either as monotherapeutic drug or in combination with biologic agents (Gutierrez-Suarez and Burgos-Vargas, 2010).

Usually, the starting dose of MTX in children with JIA is 10-15 mg/m^2 and is administered weekly, either orally or parenterally (subcutaneously or intramuscular), at these standard doses, the oral route is preferred by most pediatric rheumatologists because of it 's easer administration and greater child comfort, furthermore there does not appear to be any advantages related to efficacy or safety with either the oral or parenteral method of administration (Klein et al., 2012).

Some food such as milk-rich food may decrease MTX absorption, so it is better when drug is given without food. Although MTX is effective for many patients, it does not work quickly, usually taking four to eight weeks before demonstrating it's benefits. Some physicians will initiate therapy with a low dose (0.2-0.35 mg/kg per day) of prednisone, to be taken until MTX has begun to take effect. The maximum therapeutic effect usually becomes apparent 4 to 6 months after the beginning of treatment and sometimes even after 12 months. A higher dose up to 25-30 mg/m^2/week may be considered in children who had only a partial response to the drug or have a more severe disease. Doses greater than 15-20 mg/m^2 are administered parenterally because of the decreased oral bioavailability of the drug at higher dose (Ravelli and Martini, 2000).

Interactions and Contraindications

Salicylates may delay MTX 's clearance. Sulfonamides, salicylates, phenytoin displace MTX from protein binding sites. Live virus vaccines may result in

vaccine infection. Pyrimethamine, fluorouracil, NSAIDs increase toxicity of MTX by elevating serum MTX concentration (do not use NSAIDs with high dose MTX therapy), penicillins may decrease renal clearance of MTX, probencide decrease the renal elimination of MTX. Also MTX may decrease theophylline clearance.

Contraindication for MTX use

Hypersensitivity to MTX, severe renal or hepatic impairment, lung fibrosis and pre-existing profound bone marrow suppression.

2.4 Health assessment in patients with JIA

Assessment for children with JIA should be regularly done. The historical focus in assessment of children has been on hard outcomes such as persistent disease activity, disease remission, joint damage, and organ system damage. A number of the measures of these outcomes have been grouped as a core set and used to define improvement (Giannini et al., 1997). Juvenile idiopathic arthritis like most other chronic diseases of childhood, influences all aspects of the child ‘s life, including physical, social, emotional, intellectual and economic aspects, and affects the entire family with ultimate effects on the child’s overall outcome (Allaire et al., 1992; Miller, 1993).

2.4.1 Instrument used to assess juvenile idiopathic arthritis

A- Measures of physical function

-Childhood Arthritis Impact Measurement Scales (CHAIMS)

-Juvenile Arthritis Assessment Scale (JAFAS) and Report (JAFAR)

-Childhood Health Assessment Questionnaire (CHAQ)

-Juvenile Arthritis Self-Report Index (JASI)

B- Measures of quality of life

Disease specific

-Juvenile Arthritis Quality of life Questionnaire (JAQQ)

-Childhood Arthritis Health Profile (CHAP)

Generic

-Childhood Health Questionnaire (CHQ)

-Pediatric Quality of Life Inventory Scales (Peds QL)

-Quality of My Life Questionnaire (QOMLQ)

2.4.2 CHAQ

CHAQ is a disease- specific measure of functional status that comprises two indices focus on physical function. The disability index assesses function in eight areas that include dressing, grooming, eating and general physical activities distributed among a total of 30 items. Each question is rated on difficulty in performance and is scored from 0 to 3. The disability index is calculated as the mean of eight functional areas. Discomfort is determined by the presence of pain measured by a 100-mm visual analogue scale. The CHAQ has been shown to be a useful instrument for evaluating outcome in longitudinal studies (Minden et al., 2000; Oen, et al, 2003), it has been shown to have reasonable responsiveness in clinical drug trials (Lovell et al., 2000) and in the evaluation of rehabilitative interventions (Fan et al., 1998).

The CHAQ has excellent reliability and validity, and responsiveness, it also has good discriminative properties and can be administered to children of all ages and in several languages, and because it is short and easy to use, it is used with increasing frequency in the clinical settings (Duffy CM, 2007).

2.5 Definition of improvement and remission

2.5.1 Measurement of response in JIA

Until the mid-1990's, the assessment of clinical response in JIA was not standardized, there had been no single uniform definition of improvement for use

in the clinical trials of JIA and multiple outcome measures were utilized and various trials used different endpoints.

Previous response criteria focused on single outcome measures, including the percentage improvement in number of active joints, physician preference, and overall improvement in physician global assessment, which led to difficulty in comparing study outcomes.

In 1997 the pediatric core set and the American college of Rheumatology (ACR) Pediatric 30 response criteria has been developed. The ACR Pediatric 30 was initially designed to distinguish between active treatment and placebo, it is well studied and validated but more commonly used in research versus practice.

ACR Pediatric 30 is now used as the primary outcome measure for trials of biologic agents and second line therapies and remains the only prospective validated measure of disease activity in JIA (Ringold and Wallace, 2007).

The components of ACR Pediatric criteria include the following:

-Physician's global assessment of overall disease activity (measured on a 10-cm visual analogue scale {VAS}). .

-Parents (or, if appropriate in age, patient) global assessment of overall well-being measured on a 10-cm VAS.

-Functional ability (usually assessed with the CHAQ).

-Number of joints with active arthritis.

-Number of joints with limited range of motion.

-Erythrocyte Sedimentation Rate (ESR).

ACR Pediatric 30 means a minimum of 30 % improvement from baseline in a minimum of 3 out the above 6 components with no more than one component worsening more than 30 %. Also PedACR 50% and PedACR 70% defined as 50 and 70 percent improvement in a minimum of 3 out the above 6 components with no more than one component worsening more than 30 %.

2.5.2 Definition of remission

The primary goal in management of JIA is the achievement and maintenance remission. Until recently , reaching remission has been difficult to achieve in most forms of JIA, but with the development of new therapeutic agents and combination treatment strategies, more children with arthritis can experience protracted periods of low levels of disease activity and in limited number of cases, a complete clinical remission, but it is difficult to define precisely with a single disease activity measure, although many definitions for remission hasbeen developed, until recently no uniform and widely accepted criteria for defining remission in JIA.

According to the preliminary criteria of clinical remission the patients divided in three groups:- patients with inactive disease, clinical remission with medication and clinical remission without medication (Table 2).

Inactive disease

1. No joints with active arthritis*
2. No fever, rash, serositis, splenomegaly, or generalized lymphoadenopathy attributable to JIA
3. No active uveitis (to be defined)
4. Normal ESR or CRP (if both are tested, both must be normal)
5. Physician's global assessment of disease activity indicates no disease activity (i.e., best score attainable on the scale used)

Clinical remission

Two types of clinical remission are proposed:

1. Clinical remission with medication. The criteria for inactive disease must be met for a minimum of 6 consecutive months while the patient is taking medication.

2. Clinical remission without medication. The criteria for inactive disease must be met for a minimum of 12 consecutive months while the patient is off all anti-arthritis and anti-uveitis medication

*As defined by the American College of Rheumatology: A joint with swelling not due to bony enlargement or, if no swelling is present, limitation of motion accompanied either by pain on motion and/or tenderness. Isolated finding of pain on motion, tenderness, or limitation of motion on joint examination may be present only if explained by either prior damage attributable to arthritis that is now considered inactive, or non rheumatologic reasons such as trauma (*Ravelli and Martini, 2006)*

Table 2: Preliminary criteria for inactive disease and clinical remission of JIA

2.6 Objectives

Methotrexate is the most common second line therapeutic agent used in treatment of JIA worldwide, however, there is variation in the clinical response and toxicity observable between the patients who used MTX. Although serious toxicity in patients using MTX is uncommon, a prevalence of adverse effects as high as 42 % has been reported (Ravelli et al, 1998).

The main goal of JIA treatment is the achievement of wellbeing with minimal risk of side effects. Identification of predictors of response is helpful to develop recommendations for MTX use, especially starting of MTX as well as further continuation or early discontinuation and starting use of biological drugs.

The aim of this study is to determine whether demographic, clinical, articular and laboratory variables at baseline and during follow up at month 3, 6 and 12 predict MTX response in patients with juvenile idiopathic arthritis.

3. Patients and Methods

3.1 Patient selection

Patient's data were taken from the German BIKER Registry founded in 2001. The registry is a non-interventional long term study and has been approved by the ethics committee of the Aerztekammer Nordrhein, Duesseldorf, Germany. Since 2005 patients newly started with methotrexate were included into the registry. Data of patients admitted to the registry until December 31, 2010 were used for this analysis.

The inclusion criteria used were :

1-Diagnosis of JIA according to the modified ILAR criteria (Edmonton 2001), all categories.

2- Treatment with MTX just started.

3- Pretreatment data set available

4- Duration of MTX treatment of at least 3 months.

Patients who currently receive or have been received biologics (Etanercept, Adalimumab, Infliximab, Tocilizumab……) were excluded.

Until 31.12.2010 the total number of patients included in the registry was 1063. The total number of patients fulfilling all inclusion and exclusion criteria was 915 patients who belong to all categories of JIA.

Reasons of non selection	Number of patients
Pretretment with biologics	27 patients
Wrong diagnosis (Sharp syndrom, uveitis without JIA or vasculitis)	4 patients
Treatment duration < 3 months	18 patients
Pretreatment data set incomplete	99 patients

Table 3: Reason for exclusion of patients registered before 31.12.2010

3.2 Evaluation of response to treatment

In this study the response to treatment was analysed at 3 months, 6 months, and at 12 months. At each time the patients were divided into responders and non-responders according the American Collage of Rheumatology Paediatric (PedACR) 30 or 70 improvement criteria, this means 30% or 70% improvement from baseline in at least three of the following six variables:

-Physician's global assessment of overall disease activity.

-Parent's (or patient) global assessment of well-being.

-Number of joints with active arthritis.

-Number of joints with limited range of motion.

-Childhood Health Assessment Questionnaire (CHAQ)

-Erythrocyte sedimentation rate (ESR)

As well as there is no more than one of the remaining variables worsened by more than 30 %.

The predefined parameters with a potential influence on response which included in the study listed in table 4.

Demographic parameters	Gender, age at onset of disease, age at start of treatment with MTX, disease duration until start of treatment.
Clinical parameters	JIA category, previous unresponsiveness to other DMARD, global disease activity, pain assessment and child disability assessed by CHAQ.
Articular parameters	No. of active joints, No. of joints with LOM No. of tender joints and morning stiffness.
Laboratory parameters	ESR, CRP, RF, ANA and HLA B27

Table 4: Parameters of interest with a potential influence on response

3.3 Statistics

Populations for analysis in our study are patients with a treatment duration for at least 3 months. The time of analysis of response was 3 months, 6 months, 12 months, at these times the patients divided in the following groups :

Group 1a (PedACR30 non-Responders) at month 3

Group 1b (PedACR30 non-Responders) at month 6

Group 1c (PedACR30 non-Responders) at month 12

Group 2a (PedACR30 Responders) at month 3

Group 2b (PedACR30 Responders) at month 6

Group 2c (PedACR30 Responders) at month 12

Group 3a (PedACR70 non-Responders) at month 3

Group 3b (PedACR70 non-Responders) at month 6

Group 3c (PedACR70 non-Responders) at month 12

Group 4a (PedACR70 Responders) at month 3

Group 4b (PedACR70 Responders) at month 6

Group 4c (PedACR70 Responders) at month 12

Parameters are described as medians with first and third quartiles or mean ± standard deviation (SD) for quantitative variables and as absolute frequencies and percentages for qualitative variables. To detect relations between PedACR response and the potentially influencing parameters, Pearson or Spearman correlation coefficients were calculated, depending on parameter types. Multivariate logistic regression analyses were performed to evaluate the role of all factors that were significantly correlated with the response parameters (PedACR 30 or 70 response at months 3, 6, or 12). For the final model of each response parameter the adjusted odds ratios (OR) with confidence interval for the significant factors and the area under the receiver operating curve (AUC) were calculated. Analysis was performed using IBM® SPSS® Statistics version 21. The level of significance was set at 5%.

4. Results

4.1 Characters of the study sample

4.1.1 Gender of the patients

The patient population consisted of 915 Patients in whom the diagnosis of juvenile idiopathic arthritis has been given according to the ILAR criteria. In our study the females represent 68.5 % of the sample (627 patients), while the males represent 31.5 % of the sample (288 patients).

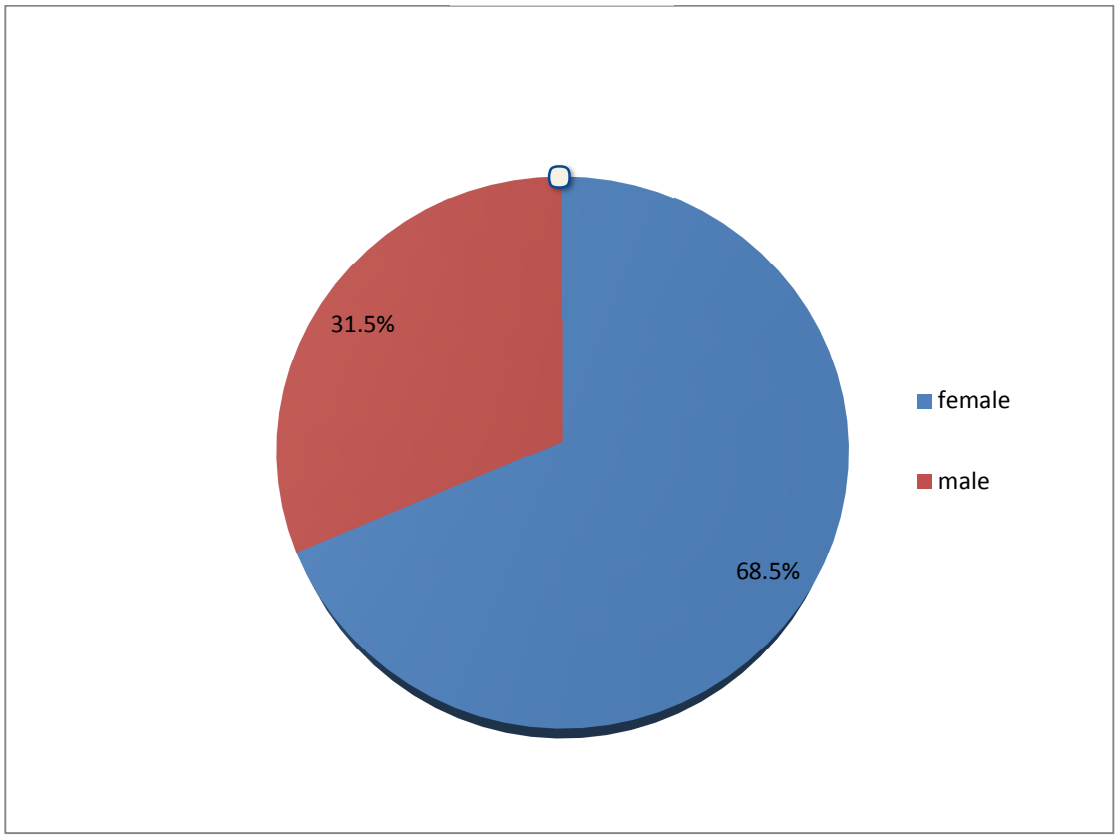

Figure 1: Distribution of patients according to gender

4.1.2 JIA categories

As seen in figures No. 2, 3 and table No. 5, persistent oligoarthritis is the most common JIA category in our study sample, 271 patients (29.6 %) have this subtype, 247 patients (26.9 %) had RF negative polyarthritis and only 23 patients (2.5 %) had unclassified arthritis.

JIA category	No. of patients	Gender		Age at start of treatment with MTX years (Mean+/-SD)
		male	female	
Systemic onset JIA	26 (2.8 %)	14 (53.8 %)	12 (46.2 %)	6.9 +/-4.66
Seronegative polyarthritis	247 (26.9 %)	50 (20.2 %)	197 (79.8 %)	9.4 +/-4.64
Seropositive polyarthritis	29 (3.2 %)	3 (10.4 %)	26 (89.6 %)	12.4 +/-4.65
Persistent oligoarthritis	271 (29.6 %)	90 (33.2 %)	181 (66.8 %)	8.3 +/-4.65
Extended oligoarthritis	118 (12.9 %)	37 (31.4 %)	81 (68.6 %)	8.6 +/-4.5
Enthesitis-related arthritis	120 (13.2 %)	66 (55 %)	54 (45 %)	12.9 +/-4.65
Psoriatic arthritis	81 (8.8 %)	21 (25.9 %)	60 (74.1 %)	10.4 +/-4.65
Unclassified JIA	23 (2.5 %)	7 (30.4 %)	16 (69.6 %)	9.1 +/-4.66

Table 5: Distribution of patients according to categories of JIA, gender and age at start of treatment with MTX

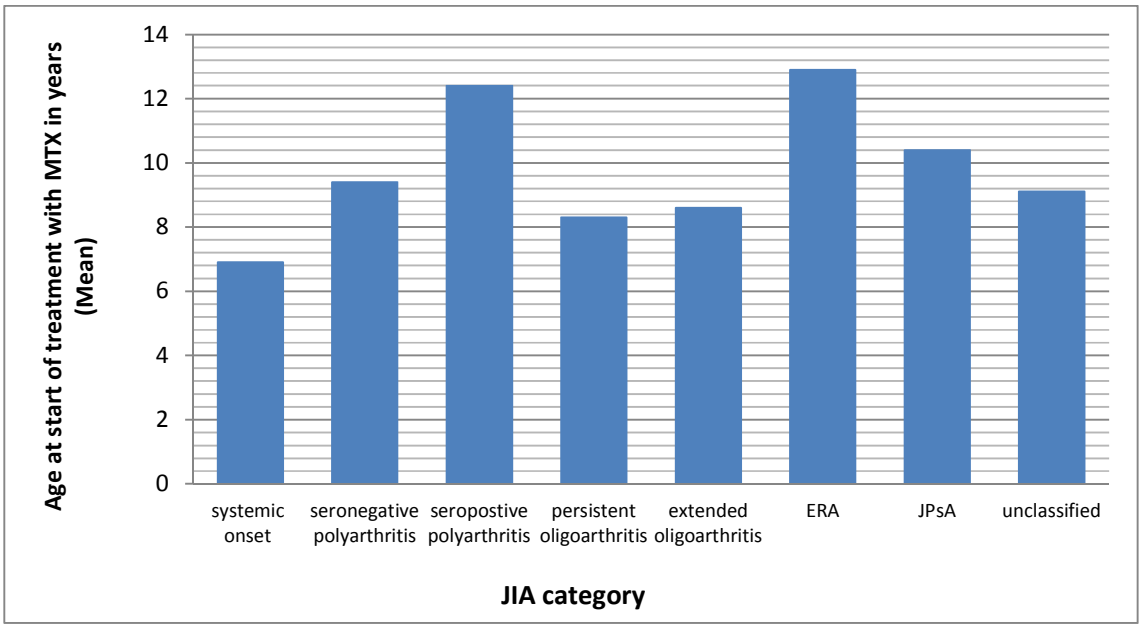

Figure 2: Mean age at start of MTX in each JIA category

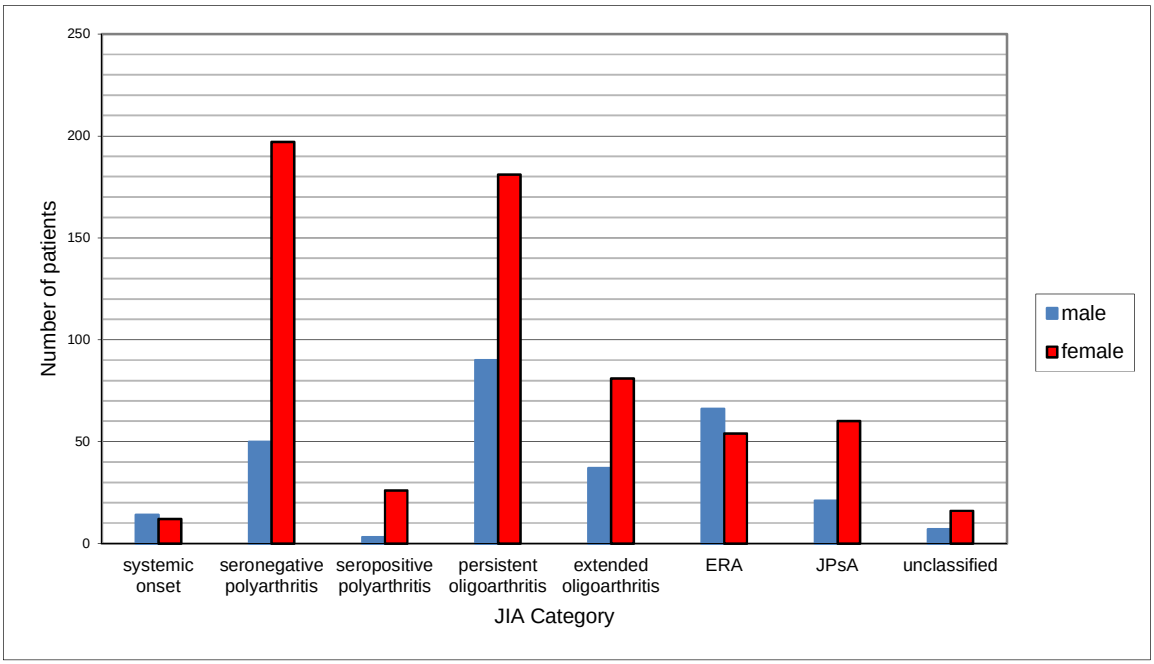

Figure 3: Distribution of patients according to the gender and JIA categories

4.1.3 Age at onset of the disease

The mean age (+/- SD) at onset of the disease was 7.4+/-4.4 years. The median age was 6.9 years, the range (0,3–15.9) years. Patients below the age of 4 contributed to the largest group with 267 (29.1 %) of all patients. The detailed data are given in table No. 6 .

Age group	Number	Percent
< 4 years	267	29.1 %
4-8 years	239	26.1 %
8-12 years	222	24.3 %
12-16 years	187	20.4 %

Table 6: Distribution of patients according to the age at onset of disease

4.1.4 Age at start of treatment with methotrexate

The mean age (+/-SD) at which the treatment with methotrexate has been started was 9.5+/-4.7 years. The median age was 9.9 years (range 1.1-18.1 years).

Age group	Number	Percent
< 4 years	140	15.3 %
4-8 years	232	25.4 %
8-12 years	202	22.1 %
12-16 years	341	37.3 %

Table 7: Distribution of patients according to the age at onset of treatment.

4.1.5 Disease duration until start of treatment with MTX

The mean disease duration until start of treatment with MTX was (+/-SD) 2.2 +/-2.7, while the median was 0.99 year (range 0.02-16.3) years. The disease duration in about 50.3 % of patients (460 patients) was less than 1 year (Table No. 8).

Disease duration	Number	Percent
<1 year	460	50.3 %
>1 year	455	49.7 %

Table 8: Distribution of patients according to disease duration before start of treatment with methotrexate

4.1.6 Medical treatment before start of MTX

About 91.1 % of patients have been treated with NSAIDs (834 patients), and 21.2 % of patients were treated with oral corticosteroids (194 patients) in addition to 34.6% of patients (317 patients) have been treated with intraarticular cortic-osteroids, 87 (9.5 %) patients have received other DMARD before starting MTX (Tables No 9 and 10).

Type of drug	No. of patients	percent
NSAID	834	91.2 %
Oral corticosteroids	194	21.2 %
Intra-articular corticosteroids	317	34.6 %

Table 9: Medical treatment before starting MTX

Type of DMARD	No. of patients
Azathioprine	6
(Hydroxy)chloroquine	38
Cyclosporine A	5
Leflunomide	3
Sulfasalazin	34
Immunoglobulins	1

Table 10: Distribution of DMARDs used before starting MTX

4.1.7 Articular characters of the patients

As outlined in table No. 11 the mean of No. of active joints at baseline was 5.9+/-7.5 and the median was 3, the mean of swollen joints was 4.9+/-6.8 and the median 3, the mean of tender joints 5.8+/-7.2 and the median 3, while the mean of joints with limitation of movement was 5.7+/-7.5 and the median was 3. 542 (59.2 %) patients had morning stiffness, the mean duration of morning stiffness was 55.7+/-72.2 minutes and the median was 30 minutes.

	Active joints	Swollen joints	Tender joints	LOM joints	Duration of MS (min.)
Mean	5.89	4.92	5.58	5.72	55.67
Median	3	3	3	3	30
SD	7.53	6.8	7.23	7.47	72.15
Minimum	0.00	0.00	0.00	0.00	1
Maximum	56	54	54	58	720

Table 11: Articular characters of the patients

4.2 Minimal Response (PedACR30)

4.2.1 PedACR30 at month 3

At month 3, 566 (77.4 %) patients were responders according to the PedACR 30 criteria, 165 (22.5 %) were non responders. Among 165 patients who were non responders, 116 patients were females (70.3 %). Their median age (first to third quartile) at onset of disease was 6.4 (3.8-10.5) years, the median age at start of MTX was 9.5 (6-13.3) years, and the median of disease duration was 1.1 (0.4-3.2) years, 54.5 % of non responders (90 patients) had disease duration more than 1 year. The most common JIA category was persistent oligoarthritis in 48 (23.2 %), followed by polyarticular RF negative JIA in 38 (19 %) patients. ANA were detected in 76 (48.4 %) patients, while HLA B27 were positive in 35 (22.9 %) patients. 28 (16.9 %) patients used steroids and 149 (90.3 %) used NSAIDs. At month 3 the PedACR30 non-responder patients had a median of 2 active joints (1-3), and a median of 2 (1-4) joints with limitation of movement, 1 (0-3) with swollen joints as well as median of 2 (1-4) with tender joints, 68 patients (41.2 %) had morning stiffness with median of 30 joints (15-60), a physician's global assessment of overall disease activity of 29 (19-51.7), ESR of 12 mm/ h(6-23.5), CRP of 3 (1-7) mg/ dl, a parents evaluation of child's overall wellbeing of 27 (8-46) and a CHAQ of 0.25 (0.0-0.6) (Tables 12,13, 14 and 15).

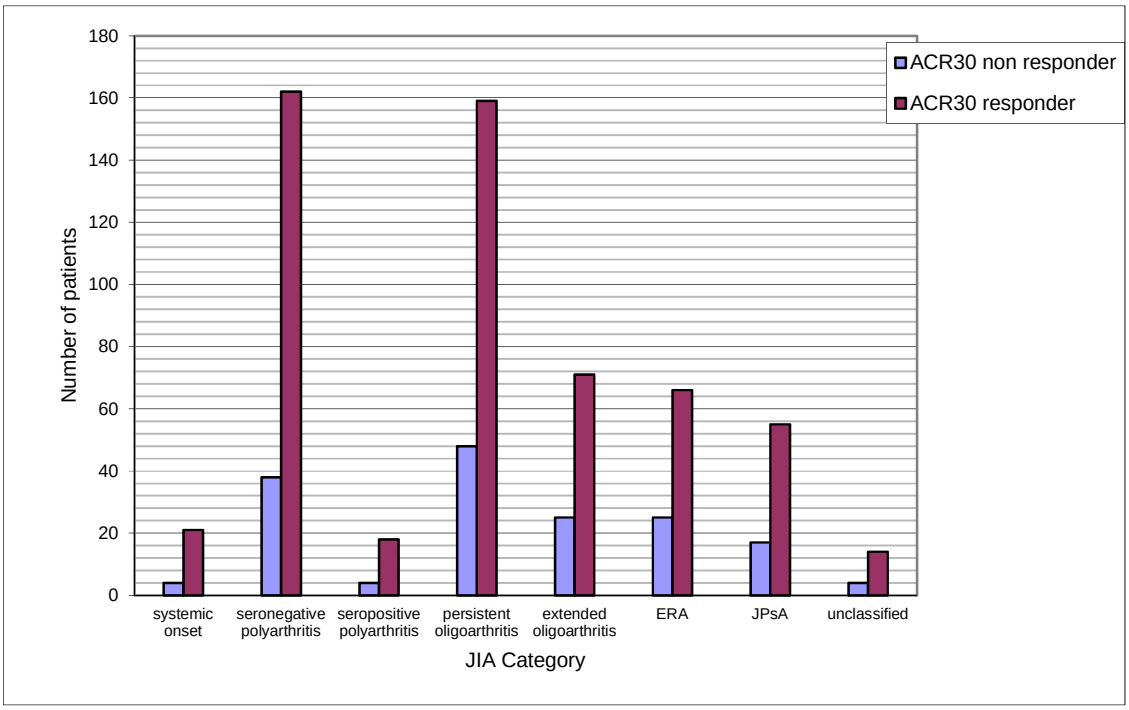

Figure 4: PedACR 30 at 3 months in different JIA categories

4.2.2 PedACR30 at month 6

At month 6, 528 (81.3 %) patients were responders, 121 (18.6 %) patients were non responders. Among 121 patients who were non responders, 85 (70.2 %) patients were females, their median age (first to third quartile) at onset of disease was 9.1 (4.2-12) years, median age at start of MTX was 12.2 ((7.8-12.4) years and the disease duration 1.8 (0.7-3.9) years, 72 % of non responders (87 patients) had disease duration more than 1 year. According to JIA categories 19.1 % of non-responders were persistent oligoarthritis category (36 patients), enthesitis related arthritis in 27 (31.4 %) and polyarticular RF negative in 23 (13.7 %) patients. ANA were detected in 47 (39.8 %) patients, HLA B27 were positive in 26 (23 %) patients, 27 (22.3 %) patients used steroids and 107 (88.4 %) patients used NSAIDs. The median of active joints was 2 (0-4), of joints with limitation of movement was 2 (1-4), of swollen joints was 1 (0-3), of tender joints was 2 (0-6), number of patients with morning stiffness was 56 (46.3 %) and median of duration of morning stiffness was 30 minutes (10-60), a physician's evaluation of disease activity was 25 (17-36.5), ESR was 10 mm/h (6-19), CRP

was 3 (1-6.6) mg/ dl, a parent evaluation of child's overall well-being was 27.5 (10-55) and a CHAQ was 0.25 (0-0.75) (Tables 12,13, 14 and 15).

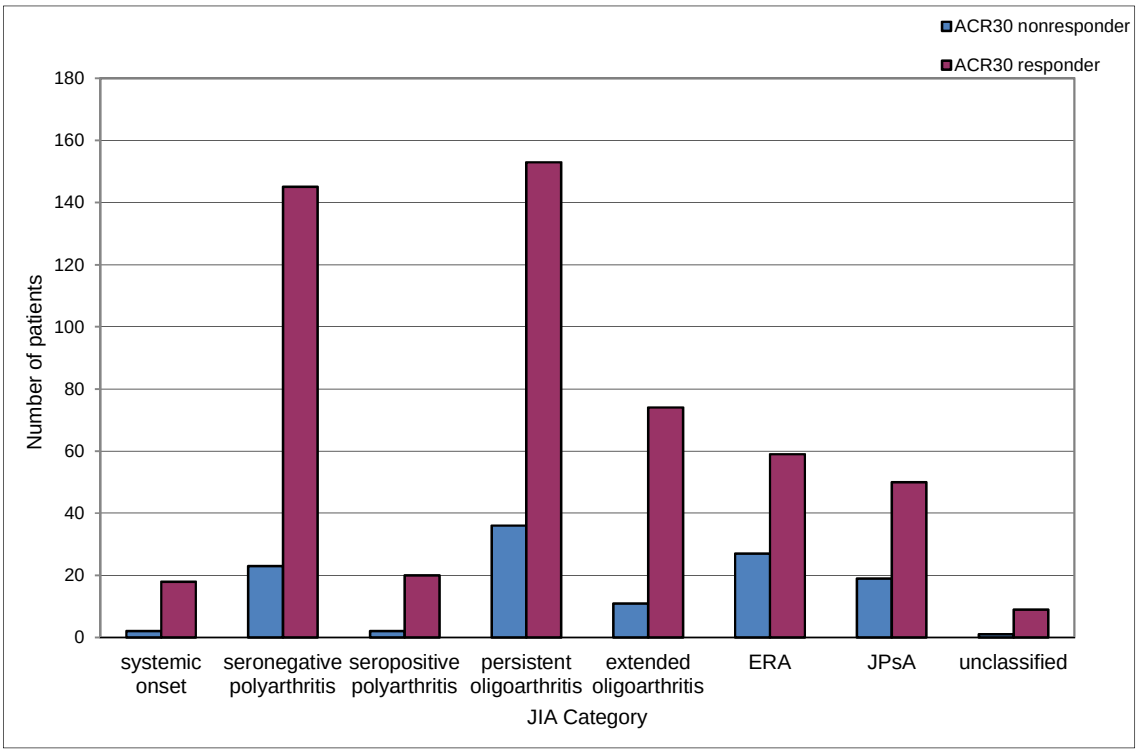

Figure 5: PedACR 30 at 6 months in different JIA categories

4.2.3 PedACR30 at month 12

At month 12, 587 (83.1 %) patients were responders, 120 (16.9 %) patients were non responders. Among 120 patients who were non responders, 82 patients are females (68.3 %). Their median age (first to third quartile) at onset of disease was 9 (4.5-12.5) years, the median age at start of MTX was 12.3 (7.8-14.7) and disease duration 1.3 (0.5-3.4) years, 63 % of non responders (76 patients) had disease duration more than 1 year. The JIA category was persistent oligoarhritis in 36 patients (16.7 %), polyarticular RF negative in 26 patients (13.5 %) and enthesitis related arthritis in 19 patients (22.6 %). ANA were detected in 51 (42.9%) patients, while HLA B27 were positive in 25 (22.7%) patients. At month 12 the PedACR30 non-responder patients had a median of 2 active joints (0-4), and also a median of 2 (0.75-4) joints with limitation of movement, of swollen joints was 1 (0-4), of tender joints was 2 (0-5), number of patients with morning

stiffness was 54 (45%) and median of duration of morning stiffness was 1 (1-2), a physician's evaluation of disease activity of 31 (20-55), ESR of 10 mm/ h (5-21), CRP of 2.9 (1-7) mg/ dl, a parent evaluation of child's overall well-being of 30 (9-54.5) and a CHAQ of 0.12 (0-0.6) (Tables 12, 13, 14 and 15).

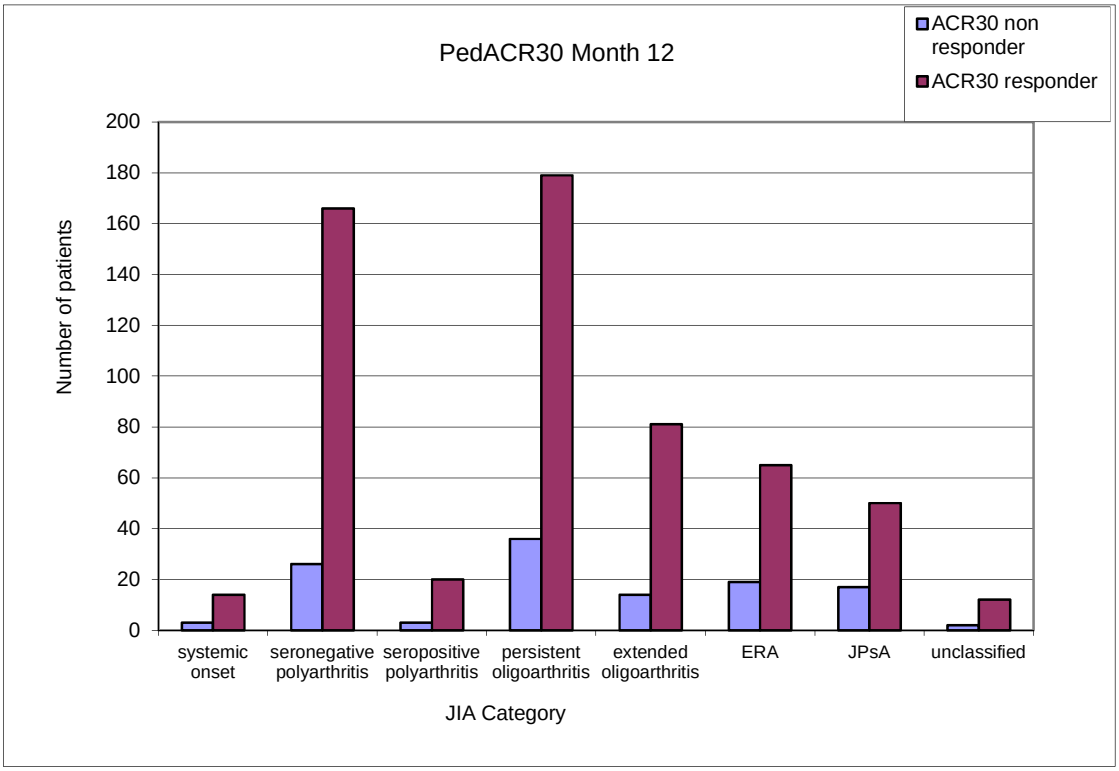

Figure 6: PedACR 30 at 12 months in different JIA categories

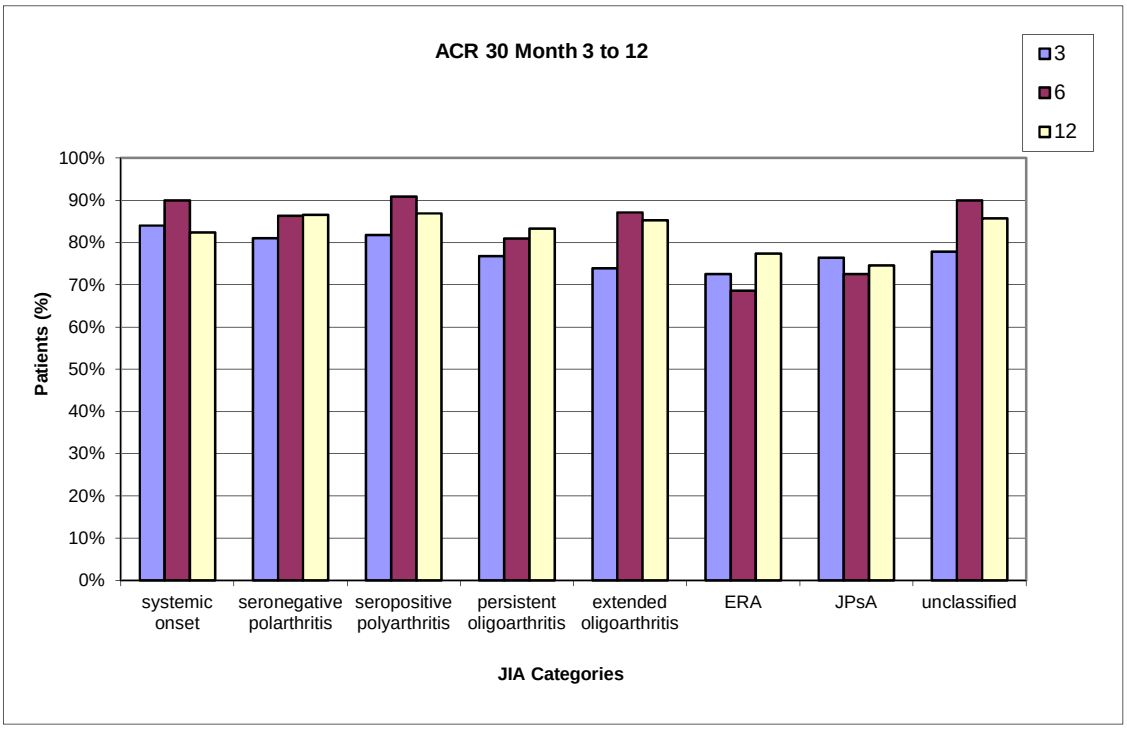

Figure 7: PedACR30 in different JIA categories at month 3, 6 and 12

Character	PedACR 30 At 3 months No. of patient 731		PedACR 30 At 6 months No. of patient 649		PedACR 30 At 12 months No. of patient 707	
	Responder 566	Non responder 165	Responder 528	Non responder 121	Responder 587	Non responder 120
ANA positive	271 (49.2 %)	76 (48.4 %)	265 (52.1 %)	47 (39.8 %)	306 (54 %)	51 (42.9 %)
ANA negative	280 (50.8 %)	81 (51.6 %)	244 (47.9 %)	71 (60.2 %)	261 (46 %)	68 (57.1 %)
HLA B27 positive	94 (19.2 %)	35 (22.9 %)	84 (18.5 %)	26 (23 %)	94 (18.5 %)	25 (22.7 %)
HLA B27 negative	395 (80.8 %)	118 (77.1 %)	370 (81.5 %)	87 (77 %)	415 (81.5 %)	85 (77.3 %)
ESR (mm/ h)	18 (10.0-31)	12 (6.0-23.5)	17 (10-31.7)	10 (6-19)	18 (10-33)	10 (5-21)
CRP (mg / dl)	4.2 (1.1-12)	3 (1-7)	4.5 (1.5-13)	3 (1-6.6)	4.9 (1.5-14)	2.9 (1-7)

Table 12: Laboratory characteristics at start of treatment in PedACR30 responders and PedACR30 non-responders. Data are in numbers (%) or median (first to third quartile)

Character	PedACR 30 At 3 months No of patient 731		PedACR 30 At 6 months No of patient 649		PedACR 30 At 12 months No. of patient 707	
	Responder 566 (77.4 %)	Non responder 165 (22.6 %)	Responder 528 (81.4 %)	Non responder 121 (18.6 %)	Responder 587 (83.1 %)	Non responder 120 (16.9 %)
Female	386(68.2 %)	116 (70.3 %)	365 (69.1 %)	85 (70.2 %)	408 (69.5%)	82 (68.3%)
Male	180 (31.8 %)	49 (29.6 %)	163 (30.8 %)	36 (29.7 %)	179 (30.5%)	38(31.6%)
Concomitant use of NSAID	514 (90.8 %)	149 (90.3 %)	483 (91.5 %)	107 (88.4 %)	546 (93 %)	105 (87.5%)
Concomitant use of Corticosteroid	125 (22.1 %)	28 (16.9 %)	113 (21.4 %)	27 (22.3 %)	127 (21.6 %)	23 (19.2 %)
Age at onset of disease	6.8 (3.3-11)	6.4 (3.8-10.5)	6.1 (2.9-10.9)	9.1 (4.2-12)	6.3 (3-10.9)	9 (4.5-12.5)
Age at MTX start	9.7 (5.3-13.5)	9.5 (6-13.3)	9.3 (5.2-13.2)	12.2 (7.8-12.4)	9 (4.9-13.2)	12.3 (7.8-14.7)
Disease duration before MTX start	0.9 (0.3-2.9)	1.1 (0.4-3.2)	0.8 (0.3-3)	1.8 (0.7-3.9)	0.7 (0.3-2.7)	1.3 (0.5-3.4)
Physician global assessment of disease activity	40 (25-65)	29 (19-51.7)	38 (24-60)	25 (17-36)	43.5 (25-66)	31 (20-55)
Parents evaluation of overall well-being	44 (19-61)	27 (8-46)	40 (18-60)	27.5 (10-55)	41 (18-59)	30 (9-54.5)
Parents evaluation of child´s pain	40 (15-60.2)	26.5 (4.2-53)	37 (15-58)	32 (8-59)	38 (15-57)	32 (4.5-59.5)
CHAQ	0.5 (0.12-0.87)	0.25 (0-0.6)	0.37 (0.12-0.8)	0.25 (0-0.75)	0.5 (0.12-0.8)	0.12 (0-0.6)

Table 13: Demographic and clinical characteristics at start of treatment in PedACR30 responders and PedACR30 non-responders. Data are numbers (%) or median (first to third quartile)

Character	PedACR 30 At 3 months No of patient 731		PedACR 30 At 6 months No of patient 649		PedACR 30 At 12 months No. of patient 707	
	Responder 566	Non responder 165	Responder 528	Non responder 121	Responder 587	Non responder 120
No. of active joints	4 (2-8)	2 (1-3)	4 (2-7.25)	2 (0-4)	4 (2-8)	2 (0-4)
No of tender joints	3 (2-7)	2 (1-4)	3 (2-7)	2 (0-6)	3 (2-7)	2 (0-5)
No. of swollen joints	3 (2-7)	1 (0-3)	3 (1.75-6)	1 (0-3)	3 (1-6)	1 (0-4)
No. of joints with LOM	4 (2-8)	2 (1-4)	4 (2-7)	2 (1-4)	4 (2-7.5)	2 (0.75-4)
Duration of MS	30 (20-60)	30 (15-60)	30 (20-60)	30 (10-60)	30 (16-60)	42.5 (30-60)

Table 14: Articular characteristics at start of treatment in PedACR30 responders and ACR30 non-responders. Data are in median (first to third quartile)

JIA categories	PedACR 30 at 3 months		PedACR 30 at 6 months		PedACR 30 at 12 months	
	Responder 566 (77.4 %)	Non responder 165(22.6 %)	Responder 528 (81.4 %)	Non responder 121(18.6 %)	Responder 587 (83.1 %)	Non responder 120(16.9 %)
Systemic onset JIA	21 (84%)	4 (16%)	18 (90%)	2 (10 %)	14 (82.4 %)	3 (17.6 %)
Seronegative Polyarthitis	162 (81 %)	38 (19%)	145 (86.3%)	23 (13.7 %)	166 (86.5 %)	26 (13.5 %)
Seropositive Polyarthritis	18 (81.8 %)	4 (18.2%)	20 (90.9%)	2 (9.1 %)	20 (86.9 %)	3 (13.1 %)
Persistent Oligoarthitis	159 (76.8 %)	48 (23.2%)	153 (80.9%)	36 (19.1 %)	179 (83.3 %)	36 (16.7 %)
Extended Oligoarthritis	71 (73.9%)	25 (26.1%)	74 (87.1%)	11 (12.9 %)	81 (85.3 %)	14 (14.7 %)
ERA	66 (72.5 %)	25 (27.5 %)	59 (68.6 %)	27 (31.4 %)	65 (77.4 %)	19 (22.6 %)
JPsA	55 (76.4 %)	17 (23.6 %)	50 (72.5 %)	19 (27.5 %)	50 (74.6 %)	17 (25.4 %)
unclassified JIA	14 (77.8 %)	4 (22.2 %)	9 (90 %)	1 (10 %)	12 (85.7 %)	2 (14.3 %)

Table 15: Distribution of patients according to JIA categories divided into PedACR30 responders and non-responders at month 3, 6 and 12

4.3 Strong Response (PedACR70-Response)

4.3.1 PedACR70 at month 3

The table No.17 shows that 416 (56.9 %) patients are PedACR70 non responder at month 3, among them there are 297(71.4 %) patients, who were females. Their median age (first to third quartile) at onset of disease was 6.6 (3.6-11.1) years, age at start of MTX was 9.7 (5.9-13.8) and disease duration 1 (0.4-3.2) years, 53 % of non responders (221 patients) had disease duration more than 1 year. The JIA category was persistent oligoarhritis in 123 (59.4 %), RF negative polyarthritis in 118 (59 %) and enthesitis related arthritis in 56 (61.5 %) patients (Table 19). ANA were detected in 200 (49.7%) patients, while HLA B27 were positive in 76 (20.5 %) patients. 75 (18 %) patients used corticosteroids and 380 (91.3 %) used NSAIDs. At month 3 the PedACR70 non-responder patients had a median of 3 active joints (1-5), and also a median of 3 (1-5) joints with limitation of movement, number of patients with morning stiffness was 232 (55.8 %) and median of duration was 30 (15-60) minutes, a physician’s evaluation of disease activity of 33 (22-55), ESR of 15 mm/ h (8-25), CRP of 4 (1-9.2) mg/dl, a parent evaluation of child's overall well-being of 35 (15-55) and a CHAQ of 0.3 (0-0.7) (Tables 16,17, and 18).

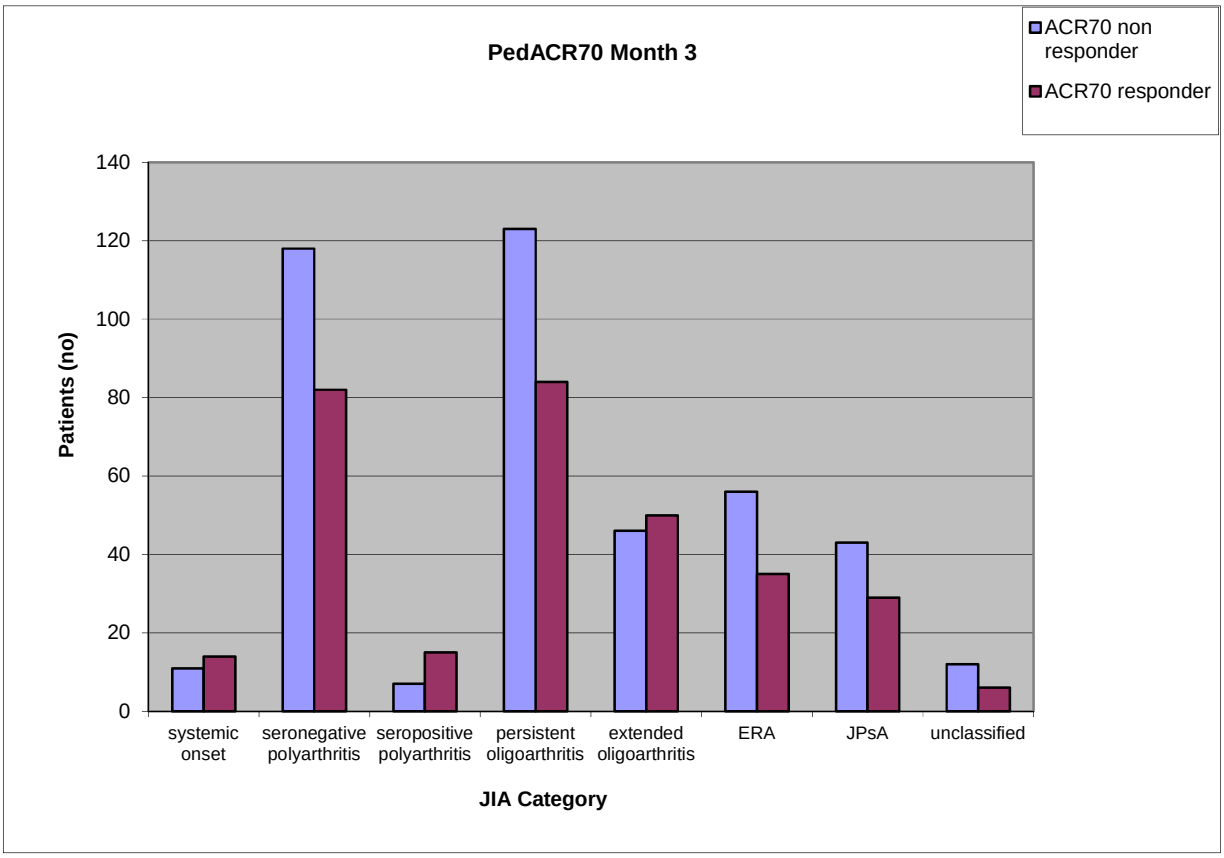

Figure 8: PedACR 70 at 3 months in different JIA categories.

4.3.2 PedACR70 at month 6

The table No. 17 shows that 281 (43.3 %) patients are PedACR70 non responder at month 6, among them there are 199 (70.8 %) patients, who were females. Their median age (first to third quartile) at onset of disease was 6.7 (3.8-11.3) years, age at start of MTX was 10.4 (6.3-14) and disease duration 1.4 (0.5-3.3) years, 63 % of non- responders (177 patients) had disease duration more than 1 year. The JIA categories were persistent oligoarhritis in 76 (40.2 %), RF negative polyarthritis in 65 (38.7 %) and enthesitis related arthritis in 47 (54.7 %) patients (Table 19). ANA were detected in 127 (46.3 %) patients, while HLA B27 were positive in 51 (19.8 %) patients. 58 (20.6 %) patients used corticosteroids And 254 (90.4 %) used NSAIDs. At month 6 the PedACR70 non- responder patients had a median of 3 active joints (1-5), and also a median of 3 (1-5) joints

with limitation of movement, the number of patients with morning stiffness was 155 (55.2 %) and the median duration was 30 (10-60) minutes, while a physician's evaluation of disease activity of 29 (20-52.7), ESR of 12 (7-24) mm/h, CRP of 3 (1-7) mg/dl, a parent evaluation of child's overall well-being of 31 (11-58) and a CHAQ of 0.25 (0.0-0.8) (Tables 16,17, and 18).

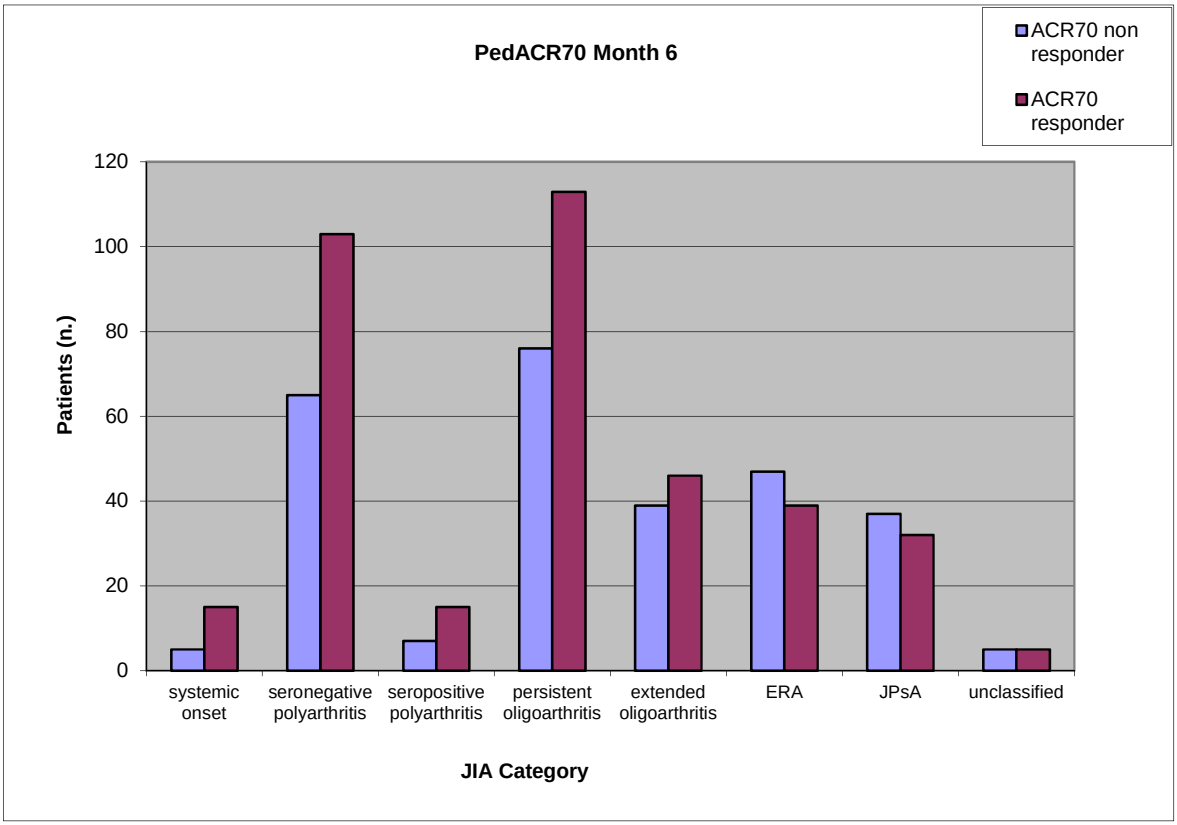

Figure 9: PedACR 70 at 6 months in different JIA categories

4.3.3 PedACR70 at month 12

As seen in table No. 16 there are 241 (34.1 %) patients are PedACR70 non responder at month 12, among them there are 172 (71.4 %) patients, who were females. Their median age (first to third quartile) at onset of disease was 8.6 (3.9-12.1) years, age at start of MTX was 11.4 (6.8-14.5) years, and disease duration 1.3 (0.5-3.4) years, 60 % of non responders (144 patients) had disease duration more than 1 year. The JIA category was persistent oligoarhritis in 70 (32.6 %), RF polyarthritis negative in 57 (29.7 %) and Psoriasis arthritis in 33

(49.3 %) patients (Table 19). ANA were detected in 117 (48.9 %) patients, while HLA B27 were positive in 42 (18.8 %) patients. 43 (17.8 %) patients used corticosteroids and 221 (91.7 %) used NSAIDs. At month 12 the PedACR70 non-responder patients had a median of 2 active joints (1-5), and also a median of 2 (1-5) joints with limitation of movement, the number of patients with morning stiffness was 120 (49.8 %) and the median duration was 30 (15-60) minutes, while a physician's evaluation of disease activity of 30 (20-55), ESR of 12 (6.5-24) mm/h, CRP of 3 (1-7) mg/dl, a parent evaluation of child's overall well-being of 31.5 (10-57) and a CHAQ of 0.25 (0-0.75) (Tables 16,17, and 18).

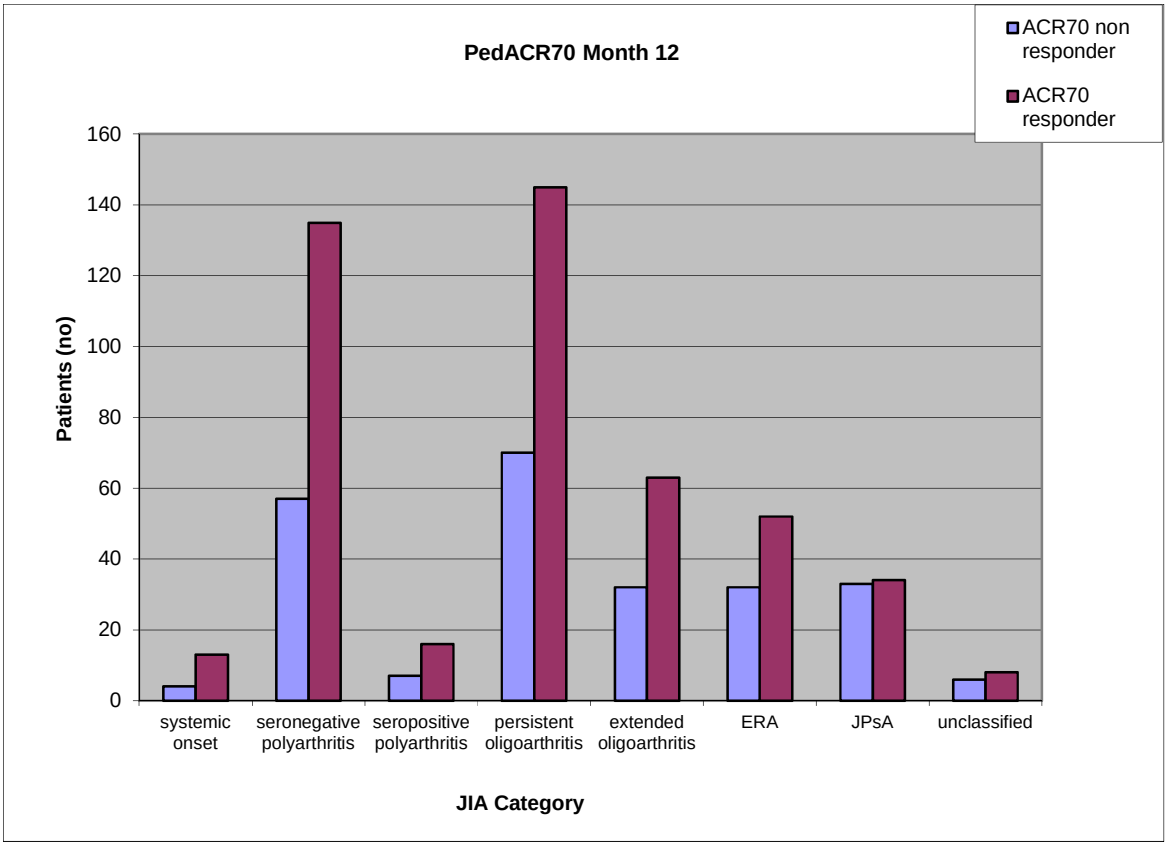

Figure 10: PedACR 70 at 12 months in different JIA categories

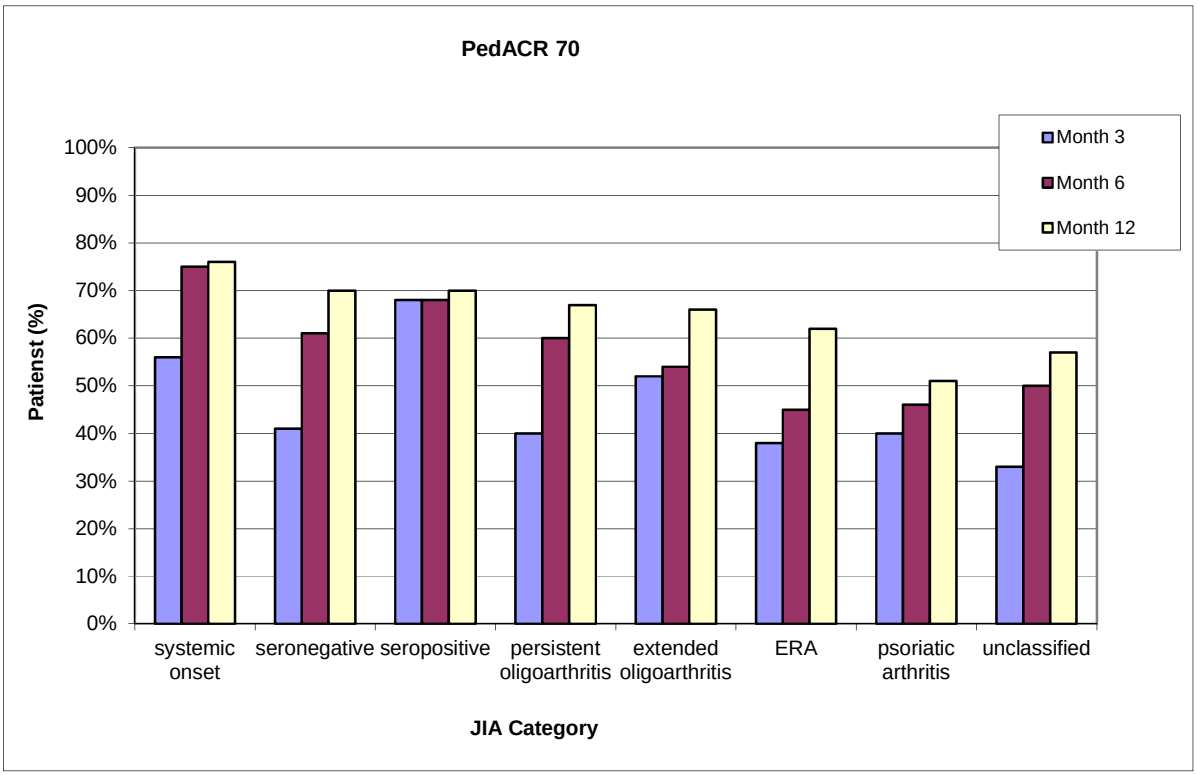

Figure 11: PedACR70 in different JIA categories at month 3, 6 and 12

Character	PedACR 70 At 3 months No. of patient 731		PedACR 70 At 6 months No. of patient 649		PedACR 70 At 12 months No. of patient 707	
	Responder 315 (43.1 %)	Non responder 416 (56.9 %)	Responder 368 (56.7 %)	Non responder 281(43.3 %)	Responder 466 (65.9 %)	Non responder 241(34.1 %)
ANA positive	147 (48%)	200 (49.7 %)	185 (52.4 %)	127(46.3 %)	240 (53.7%)	117(48.9 %)
ANA negative	159 (52 %)	202 (50.3 %)	168 (47.6 %)	147 (53.7%)	207 (46.3%)	122(51.1 %)
HLA B27 positive	53 (19.6%)	76 (20.5 %)	59 (19 %)	51 (19.8 %)	77 (19.4 %)	42 (18.8 %)
HLA B27 negative	218 (80.4 %)	295 (79.5 %)	251 (81%)	206(80.2 %)	319 (80.6 %)	181(81.2 %)
ESR (mm/ h)	18 (9.0-35)	15 (8.0-25)	19.5 (10-36.5)	12 (7-24)	19 (10-36)	12 (6.5-24)
CRP (mg/ dl)	4.4 (1.2-13.9)	4 (1-9.2)	5 (2-15)	3 (1-7)	5 (2-15.35)	3 (1-7)

Table 16: Laboratory characteristics at start of treatment in PedACR70 responders and ACR70 non-responders. Data are in numbers (%) or median (first to third quartile)

Character	PedACR 70 At 3 months No. of patient 731		PedACR 70 At 6 months No. of patient 649		PedACR 70 At 12 months No. of patient 707	
	Responder 315 (43.1 %)	Non responder 416(56.9 %)	Responder 368 (56.7 %)	Non responder 281(43.3 %)	Responder 466 (65.9 %)	Non responder 241(34.1 %)
Female	205 (65.1 %)	297 (71.4 %)	251 (68.2 %)	199 (70.8 %)	318 (68.2 %)	172 (71.4 %)
Male	110 (34.9 %)	119 (28.6 %)	117 (31.8 %)	82 (29.2 %)	148 (31.8 %)	69 (28.6 %)
Concomitant use of NSAID	283 (89.8 %)	380 (91.3 %)	336 (91.3 %)	254 (90.4 %)	430 (92.3 %)	221 (91.7 %)
Concomitant use of Corticosteroid	78 (24.7 %)	75 (18 %)	82 (22.3 %)	58 (20.6 %)	107 (22.9 %)	43 (17.8 %)
Age at onset of disease	6.6 (3.1-10.8)	6.6 (3.6-11.1)	6.2 (2.8-11)	6.7 (3.8-11.3)	6.1 (2.9-10.4)	8.6 (3.9-12.1)
Age at MTX start	9.6 (4.8-13.9)	9.7 (5.9-13.8)	9.3 (4.1-13.3)	10.4 (6.3-14)	8.5 (4.8-13)	11.4 (6.8-14.5)
Disease duration before MTX start	0.8 (0.3-2.7)	1 (0.4-3.2)	0.7 (0.3-2.9)	1.4 (0.5-3.3)	0.6 (0.3-2.3)	1.3 (0.5-3.4)
Physician's global assessment of disease activity	45.5 (25-67)	33 (22-55)	40 (25-65)	29 (20-52.7)	45 (26-67)	30 (20-55)
Parents evaluation of overall well-being	44 (20-62)	35 (15-55)	42 (19-60.25)	31 (11-58)	42 (20-59)	31.5 (10-57)
Parents evaluation of Child's pain	40 (15-62)	33.5 (10-57)	39.5 (15-58.7)	32 (13-55)	40 (17.2-58.7)	29.5 (5-56.25)
CHAQ	0.5 (0.12-0.87)	0.3 (0-0.7)	0.5 (0.12-0.8)	0.25 (0-0.8)	0.5 (0.12-0.9)	0.25 (0-0.75)

Table 17: Demographic and clinical characteristics at start of treatment in PedACR70 responders and PedACR70 non-responders. Data are in numbers (%) or median (first to third Quartile)

Character	PedACR 70 At 3 months No of patient 731		PedACR 70 At 6 months No of patient 649		PedACR 70 At 12 months No. of patient 707	
	Responder 315(43.1 %)	Non responder 416(56.9 %)	Responder 368 (56.7 %)	Non responder 281(43.3 %)	Responder 466(65.9 %)	Non responder 241(34.1 %)
No. of active joints	4 (2-8)	3 (1-5)	4 (2-7.25)	3 (1-5)	4 (2-8)	2 (1-5)
No of tender joints	3 (2-7.5)	3 (1-6)	3 (2-7)	2 (1-6)	3 (2-7)	2 (1-6)
No. of swollen joints	3 (1-6.5)	2 (1-5)	3 (2-7)	2 (0-4)	3 (2-7)	2 (0-5)
No. of joints with LOM	4 (2-8)	3 (1-5)	4 (2-7.25)	3 (1-5)	4 (2-8)	2 (1-5)
Duration of MS	30 (20-60)	30 (15-60)	30 (20-60)	30 (10-60)	30 (20-60)	30 (15-60)

Table 18: Articular characteristics at start of treatment in ACR70 responders and ACR70 non-responders. Data are in median (first to third quartile)

JIA categories	PedACR 70 at 3 months No. of patients 731		PedACR 70 at 6 months No. of patients 649		PedACR 70 at 12 months No. of patients 707	
	Responder 315 (43.1 %)	Non responder 416(56.9 %)	Responder 368 (56.7 %)	Non responder 281(43.3 %)	Responder 466 (65.9 %)	Non responder 241(34.1 %)
Systemic onset JIA	14 (56 %)	11 (44 %)	15 (75 %)	5 (25 %)	13 (76.5 %)	4 (23.5 %)
Seronegative Polyarthitis	82 (41 %)	118 (59 %)	103 (61.3 %)	65 (38.7 %)	135 (70.3 %)	57 (29.7 %)
Seropositive Polyarthritis	15 (68.2 %)	7 (31.8 %)	15 (68.2 %)	7 (31.8 %)	16 (69.6 %)	7 (30.4 %)
Persistent Oligoarthitis	84 (40.6 %)	123 (59.4 %)	113 (59.8 %)	76 (40.2 %)	145 (67.4 %)	70 (32.6 %)
Extended Oligoarthritis	50 (52.1 %)	46 (47.9 %)	46 (54.1 %)	39 (45.9 %)	63 (66.3 %)	32 (33.7 %)
ERA	35 (38.5 %)	56 (61.5 %)	39 (45.3 %)	47 (54.7 %)	52 (61.9 %)	32 (38.1 %)
JPsA	29 (40.3 %)	43 (59.7 %)	32 (46.4 %)	37 (53.6 %)	34 (50.7 %)	33 (49.3 %)
unclassified JIA	6 (33.3 %)	12 (66.7 %)	5 (50 %)	5 (50 %)	8 (57.1 %)	6 (42.9 %)

Table 19: JIA categories in patients divided into PedACR70 responders and non-responders at month 3, 6 and 12

4.4 Bivariate analysis

4.4.1 Minimal response (PedACR 30)

Tables No. 20, 21 and 22 show the demographic, laboratory, clinical and articular parameters of the patients at month 3, 6 and 12. The comparative analysis has been done to evaluate the determinants of minimal response (PedACR 30) by using correlation analysis.

Determinants of response	PedACR 30 at 3 months		PedACR 30 at 6 months		PedACR 30 at 12 months	
	Pearson's correlation	Significance	Pearson's correlation	Significance	Pearson's correlation	Significance
Female gender	-0.019	0.609	-0.009	0.81	0.01	0.8
ANA positive	0.006	0.864	0.096	0.017	0.084	0..027
HLA B27 positive	-0.039	0.326	-0.046	0.279	-0.041	0.305
Concomitant use of NSAID	0.123	0.001	0.089	0.024	0.092	0.014
Concomitant use of corticosteroid	0.044	0.234	-0.008	0.837	0.027	0.467
Presence of morning stiffness	0.168	0.000	0.101	0.01	0.128	0.001

Table 20: Evaluation of the determinants of minimal response (PedACR30) to MTX by Using Pearson's correlation

Determinants of response	PedACR 30 at 3 months		PedACR 30 at 6 months		PedACR 30 at 12 months	
	correlation coefficient	significance	correlation coefficient	significance	correlation coefficient	Significance
Age at onset of disease	0.003	0.94	-0.102	0.009	-0.111	0.003
Age at MTX start	-0.02	0.588	-0.161	0.000	-0.155	0.000
Disease duration before MTX start	-0.072	0.053	-0.162	0.000	-0.118	0.002
Diagnosis (JIA category))	-0.054	0.148	-0.013	0.741	-0.01	0.791
No. of Tender joints	0.196	0.000	0.139	0.000	0.138	0.000
No. of swollen joints	0.295	0.000	0.263	0.000	0.223	0.000
No. of active joints	0.325	0.000	0.285	0.000	0.238	0.000
No. of joints with LOM	0.256	0.000	0.195	0.000	0.239	0.000
Duration of morning stiffness	0.101	0.041	0.042	0.416	-0.042	0.4
CHAQ	0.145	0.000	0.087	0.026	0.166	0.000
Parent's evaluation of overall wellbeing	0.186	0.000	0.098	0.016	0.103	0.008
Physician's global assessment of disease activity	0.163	0.000	0.204	0.000	0.133	0.000
Parent's evaluation of child's pain	0.116	0.003	0.048	0.24	0.064	0.101
ESR	0.147	0.000	0.182	0.000	0.201	0.000
CRP	0.074	0.051	0.108	0.007	0.154	0.000

Table 21: Evaluation of the determinants of minimal response (PedACR30) to MTX by Using Spearman-correlation.

JIA categories	PedACR 30 at 3 months		PedACR 30 at 6 months		PedACR 30 at 12 months	
	Pearson's correlation	Significance	Pearson's correlation	Significance	Pearson's correlation	Significance
Systemic onset JIA	0.03	0.425	0.04	0.314	-0.003	0.94
Seronegative Polyarthitis	0.052	0.157	0.075	0.056	0.056	0.138
Seropositive Polyarthritis	0.018	0.618	0.046	0.242	0.019	0.61
Persistent Oligoarthitis	-0.009	0.802	-0.007	0.866	0.004	0.915
Extended Oligoarthritis	-0.032	0.384	0.057	0.148	0.023	0.533
ERA	-0.044	0.233	-0.128	0.001	-0.055	0.142
JPsA	-0.008	0.824	-0.079	0.045	-0.072	0.054
unclassified JIA	0,001	0.971	0.028	0.48	0.01	0.787

Table 22: Evaluation of the determinants of minimal response (PedACR30) to MTX according to different JIA categories by using Pearson's correlation.

Female gender is in tendency of value, but statistically not significant. Females are as likely as males to reach a minimal response of PedACR 30. The lack of ANA tends to be a marker for poor response. HLA B27 is not indicative for a poor response. Treatment with corticosteroid statistically is not of value, while use of NSAID is a positive predictor for minimal response. A higher number of active joints as well as a higher number of joints with limitation of movement, swollen and tender joints as well as presence of morning stiffness are positive predictors for PedACR 30 response, also a higher CHAQ score was a positive predictor to reach minimal response. A higher age at onset of disease, higher age at start of MTX, longer disease duration before starting MTX and a lower levels of ESR and CRP, as well as low score of physician's global assessment of disease

activity are negative predictors to reach minimal response. Most of the last predictors show a strong significance, while the presence of ANA and concomitant use of NSAID show a weak significance. JIA categories in general has no effect on reaching minimal response, the exception was for enthesitis related arthritis and psoriasis associated arthritis categories, both of them were at month 6 negative predictors to reach PedACR 30. While duration of morning stiffness and the parents evaluation of child's pain have no effect on reaching minimal response after month 3.

4.4.2 Strong response (PedACR 70)

Tables No. 23, 24 and 25 show the demographic, laboratory, clinical and articular parameters of the patients at month 3, 6 and 12. The comparative analysis has been done to evaluate the determinants of strong response (PedACR 70) by using correlation analysis.

Determinants of response	PedACR 70 at 3 months		PedACR 70 at 6 months		PedACR 70 at 12 months	
	Pearson's correlation	Significance	Pearson's correlation	Significance	Pearson's correlation	Significance
Female gender	-0.067	0.068	-0.028	0.475	-0.032	0.393
ANA positive	-0.017	0.652	0.06	0.133	0.045	0.237
HLA B27 positive	-0.011	0.772	-0.01	0.808	0.007	0.854
Concomitant use of NSAID	0.072	0.051	0.037	0.347	0.07	0.062
Concomitant use of corticosteroid	0.07	0.057	0.019	0.633	0.06	0.113
Presence of morning stiffness	0.035	0.341	0.057	0.145	0.14	0.000

Table 23: Evaluation of the determinants of strong response (PedACR70) to MTX by using Pearson's correlation.

JIA categories	PedACR 70 at 3 months		PedACR 70 at 6 months		PedACR 70 at 12 months	
	Pearson's correlation	Significance	Pearson's correlation	Significance	Pearson's correlation	Significance
Systemic onset JIA	0.049	0.185	0.066	0.094	0.035	0.353
Seronegative Polyarthitis	-0.026	0.484	0.055	0.162	0.057	0.132
Seropositive Polyarthritis	0.089	0.016	0.043	0.27	0.014	0.708
Persistent Oligoarthitis	-0.032	0.389	0.04	0.31	0.021	0.571
Extended Oligoarthritis	0.071	0.056	-0.02	0.607	0.003	0.929
ERA	-0.035	0.341	-0.09	0.023	-0.031	0.41
JPsA	-0.019	0.612	-0.072	0.067	-0.104	0.006
unclassified JIA	-0.031	0.398	-0.017	0.667	-0.026	0.485

Table 24: Evaluation of the determinants of strong response (PedACR70) to MTX according to different JIA categories by using Pearson's correlation.

Determinants of response	PedACR 70 at 3 months		PedACR 70 at 6 months		PedACR 70 at 12 months	
	correlation coefficient	significance	correlation coefficient	significance	correlation coefficient	Significance
Age at onset of disease	-0,022	0,551	-0,061	0,12	-0,114	0,003
Age at MTX start	-0,072	0,051	-0,108	0,006	-0,165	0,000
Disease duration before MTX start	-0,094	0,011	-0,177	0,000	-0,164	0,000
Diagnosis (JIA category)	0,016	0,657	-0,04	0,315	-0,028	0,452
No. of Tender joints	0,106	0,004	0,141	0,000	0,113	0,003
No. of swollen joints	0,116	0,002	0,229	0,000	0,223	0,000
No. of active joints	0,146	0,000	0,223	0,000	0,221	0,000
No. of joints with LOM	0,158	0,000	0,188	0,000	0,208	0,000
Duration of morning stiffness	0,042	0,399	0,089	0,088	0,045	0,367
CHAQ	0,074	0,047	0,098	0,013	0,16	0,000
Parent's evaluation of overall wellbeing	0,116	0,002	0,111	0,006	0,109	0,005
Physician's global assessment of disease activity	0,154	0,000	0,187	0,000	0,184	0,000
Parent's evaluation of child's pain	0,066	0,086	0,06	0,141	0,117	0,003
ESR	0,107	0,005	0,199	0,000	0,213	0,000
CRP	0,057	0,131	0,177	0,000	0,209	0,000

Table 25: Evaluation of the determinants of strong response (PedACR70) to MTX by Using Spearman-correlation.

At month 3, the number of strong responders (according to PedACR70 criteria) was 315 patients (43.1 %), while at month 6, the number of strong responders was 368 patients (56.7 %), and at month 12, the number of strong responders was 466 patients (65.9 %). This increase in the number of responders after month three, suggests that the three months data may be affected by the delay of clinical response achieved by MTX treatment. Statistically female gender has no effect on reaching a strong response (PedACR70), as well as lack of ANA, HLA B27, concomitant use of NSAID and corticosteroid have no significant effect on reaching strong response (PedACR70) to MTX. Poor response according to PedACR 70 criteria was associated with a longer disease duration before starting MTX, a lower score of physician's global assessment of disease activity, a lower score of CHAQ, a lower score of parent's evaluation of child's overall well-being as well as lower values of ESR and CRP. The significance of these predictors was very strong at month 12 (p value < 0,001). A higher age at start of MTX after 6 months as well as a higher age at onset of disease after 12 months from the beginning of MTX therapy considerable also as poor predictors (p value= 0,006 and 0,003 respectively). Enthesitis related arthritis at month 6 and psoriasis associated arthritis at month 12 were negative predictors to reach strong response (p value=0,023 and 0,006 respectively), while seropositive polyarthritis at month 3 (p value= 0,016) was positive predictor to reach strong response (PedACR 70), but in general the diagnosis of different JIA categories was not significant. Strong response according to PedACR 70 criteria was associated with a higher number of active joints, tender joints, swollen joints and joints with limitation of movement and presence of morning stiffness after month 12, while duration of morning stiffness has no effect on reaching strong response (PedACR70) to MTX.

4.5 Multivariate Analysis

Multivariate logistic regression analysis has been done with all variables that were significantly associated with PedACR 30 or 70 poor response at months 3, 6 and 12. Tables No. 26, 27 and 28 shows Logistic regression model obtained from the evaluations of the determinants of response to MTX according PedACR 30 at month 3, 6 and 12.

The predictors accuracy were evaluated by ROC (receiver operating characteristic) curve analysis. The best area under the ROC curve (AUC) for PedACR 30 was observed at month 3 (AUC= 0.734 , specificity= 3.9 % and sensitivity= 99.8 %), while the best area under the ROC curve (AUC) for the PedACR 70 was observed at month12 (AUC= 0.694, specificity= 29 % and sensitivity= 90 %).

Predictors of poor response according to PedACR 30 criteria at month 3 were a higher number of tender joints (OR=0.92), a lower number of active joints (OR=1.26) and a lower score of parent's evaluation of over well-being (OR = 1.06).

Determinants of response	OR (95% CI)	P Value
Number of tender joints	0.925 (0.878-0.974)	0.003
Number of active joints	1.262 (1.164-1.368)	0.000
Parents evaluation of overall well-being	1.069 (1.008-1.025)	0.000
Area under ROC curve of the model	0.734	

Table 26: Determinants of response according to PedACR 30 at month 3

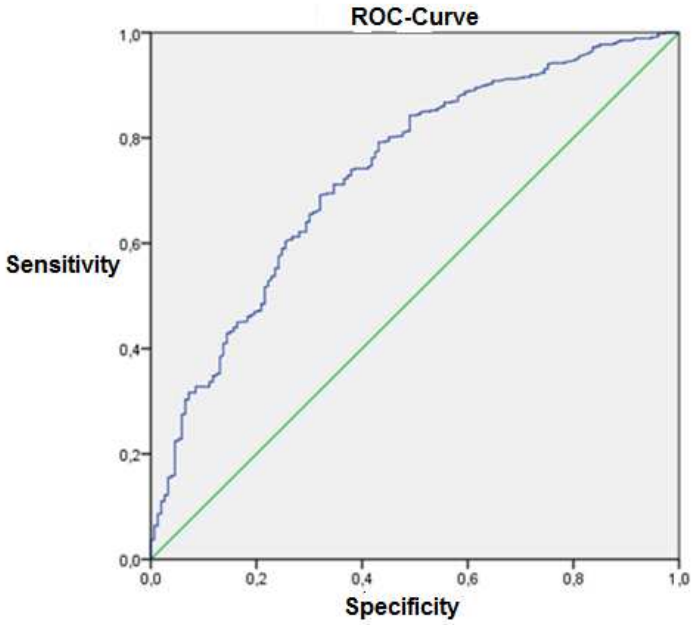

Figure 12: ROC- Curve for PedACR30 at month 3, AUC= 0.734, specificity=10.7 %, sensitivity= 98.9 %, accuracy= 84 %

At month 6 the predictors of poor response according to PedACR 30 were a disease duration more than 1 year (OR= 0.48) a higher age at MTX start (OR=0.91), a lower number of active joints, a lower score of Physician's global assessment of disease activity (OR=1.01), while concomitant use of NSAID was a positive predictor to reach PedACR 30 (OR=1.85).

Determinants of response	OR (95% CI)	P Value
Disease duration > 1 year	0.481 (0.304-0.762)	0.002
Age at MTX start	0.91 (0.87-0.96)	0.000
Number of active joints	1.11 (1.05-1.18)	0.001
Physician's global assessment of disease activity	1.02 (1.01-1.03)	0.004
Concomitant use of NSAID	1.85 (1.01-3.39)	0.048
Area under ROC curve of the model	0.726	

Table 27: Determinants of response according to PedACR 30 at month 6

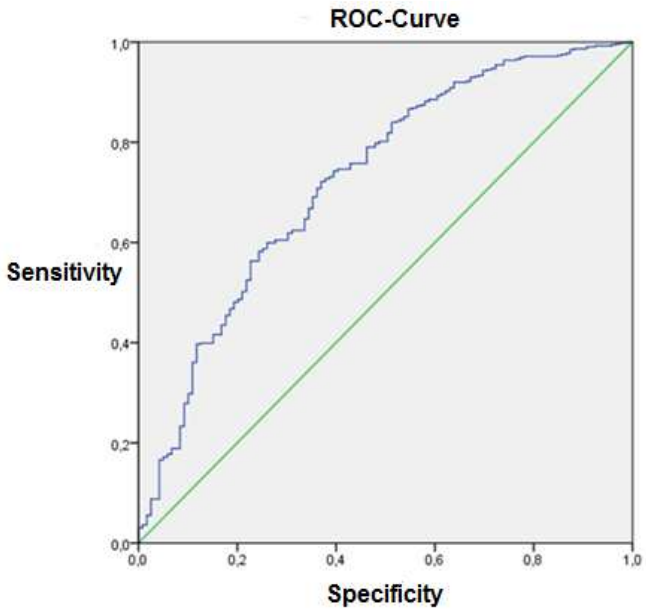

Figure 13: ROC-Curve for PedACR 30 at month 6, AUC= 0.726, specificity= 19.1 %, sensitivity= 97.5 %, accuracy= 83.6 %

At month 12 predictors of poor response according to PedACR 30 were a disease duration more than 1 year (OR= 0.64), a higher age at MTX start (OR=0.9), a lower number of joints with limitation of motion (OR=1.11), a lower ESR values (OR=1.01), while concomitant use of NSAIDs was a good predictor to reach PedACR 30 (OR=2.1).

Determinants of response	OR (95% CI)	P Value
Disease duration > 1 year	0.643 (0.416-0.994)	0.047
Age at MTX start	0.91 (0.86-0.95)	0.000
Number of joints with LOM	1.11 (1.05-1.18)	0.000
ESR	1.02 (1.002-1.029)	0.028
Concomitant use of NSAID	2.1 (1.12-3.97)	0.021
Area under ROC curve of the model	0.716	

Table 28: Determinants of response according to PedACR 30 at month12

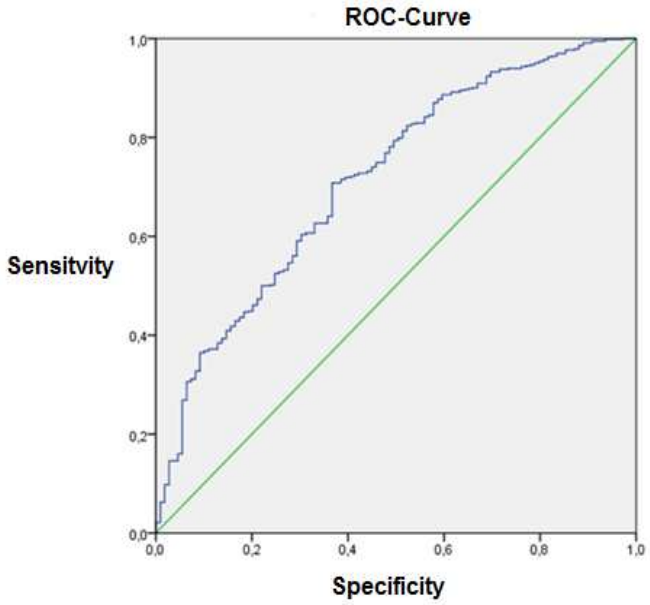

Figure 14: ROC-Curve for PedACR 30 at month 12, AUC= 0.716, specificity= 3.6 %, sensitivity= 99.6 %, accuracy= 84.4 %

Tables No. 29, 30 and 31 shows Logistic regression model obtained from the evaluations of the determinants of response to MTX according PedACR 70 at month 3, 6 and 12.

According to the PedACR 70 criteria, the baseline determinants for poor MTX response at month 3 were a lower number of joints with limitation of motion (OR=1.03) and a lower score of Physician's global assessment of disease activity (OR=1.01).

Determinants of response	OR (95% CI)	P Value
Number of joints with limitation of motion	1.038 (1.012-1.064)	0.004
Physician's global assessment of disease activity	1.01 (1.003-1.016)	0.004
Area under ROC curve of the model	0.606	

Table 29: Determinants of response according to PedACR 70 at month 3

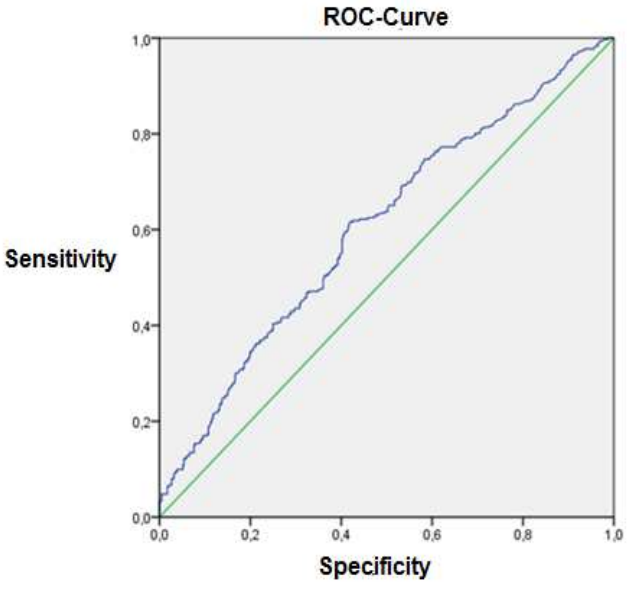

Figure 15: ROC-Curve for PedACR 70 at month 3, AUC=0.606, specificity=77.4 %, sensitivity=35 %, accuracy=58.7 %

At 6 months the determinants of poor response according PedACR 70 were a disease duration more than 1 year (OR=0.54), a higher age at MTX start (OR=0.95), a lower number of swollen joints (OR=1.06) and a lower score of a Physician's global assessment of disease activity (OR=1.01).

Determinants of response	OR (95% CI)	P Value
Disease duration > 1 year	0.546 (0.419-0.711)	0.000
Age at MTX start	0.959 (0.926-0.993)	0.018
Number of swollen joints	1.06 (1.025-1.095)	0.001
Physician's global assessment of disease activity	1.011 (1.003-1.018)	0.004
Area under ROC curve of the model	0.646	

Table 30: Determinants of response according to PedACR 70 at month 6

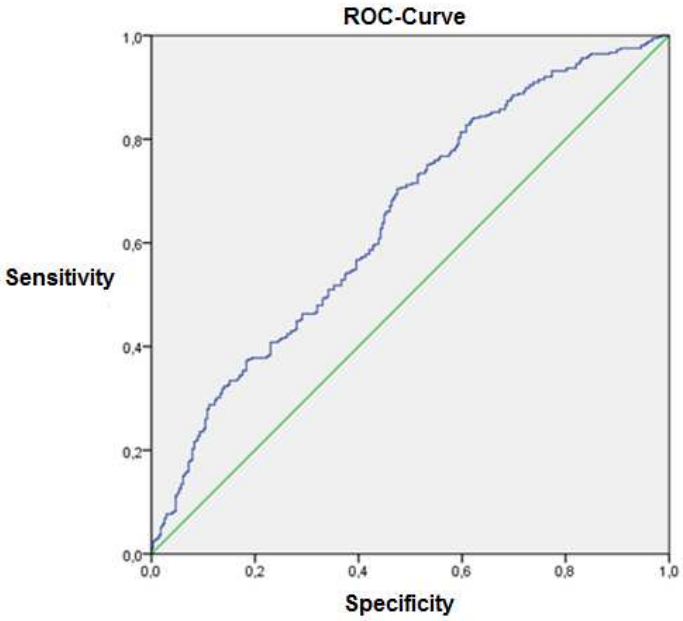

Figure 16: ROC-Curve for PedACR70 at month 6, AUC= 0.646, specificity= 46 %, sensitivity= 78.2 %, accuracy= 64.3 %

At 12 months the determinants of poor response according PedACR 70 were in addition to a higher age at MTX start (OR= 0.92), also a disease duration more than 1 year (OR= 0.54) a higher number of tender joints (OR= 0.93), a lower number of active joints (OR= 1.09), a lower score of a Parent's evaluation of child's pain (OR= 1.01), a lower value of CRP (OR = 1.01) , while a presence of morning stiffness was a good predictor to reach strong response (PedACR 70) at month 12 (OR= 1.59).

Determinants of response	OR (95% CI)	P Value
Disease duration > 1 year	0.544 (0.385-0.77)	0.001
Age at MTX start	0.926 (0.89-0.964)	0.000
Number of tender joints	0.936 (0.895-0.98)	0.004
Number of active joints	1.096 (1.042-1.153)	0.000
Parent's evaluation of child's pain	1.008 (1.000-0.015)	0.037
CRP	1.011 (1.002-1.02)	0.018
Presence of morning stiffness	1.59 (1.1-2.33)	0.016
Area under ROC curve of the model	0.694	

Table 31: Determinants of response according to PedACR 70 at month 12

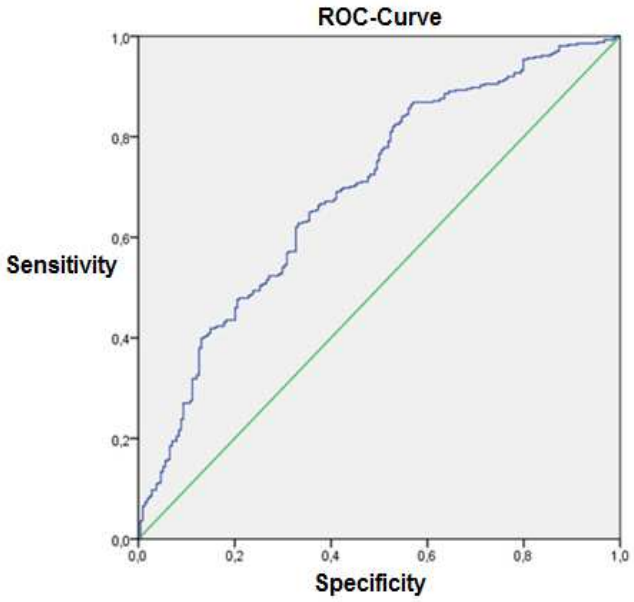

Figure 17: ROC-Curve for PedACR70 at month 12, AUC=0.694, specificity= 33.2 %, sensitivity= 91.5 %, accuracy= 71.8 %

5. Discussion

The major findings of this study were that a higher number of joints with limitation of motion and a higher score of physician's global assessment of disease activity at baseline were predictors for reaching a strong response (PedACR 70) after 3 months of treatment with MTX.

At month 6, a shorter disease duration, a lower age at start of MTX, a higher number of swollen joints and a higher score of physician's global assessment of diseases activity were associated with a higher likelihood of marked improvement (PedACR 70).

At month 12 the likelihood of a marked improvement according to the PedACR70 criteria was associated with a shorter disease duration, a lower age at start of MTX, a lower number of tender joints, a higher CRP value, a higher number of active joints, a higher score of parent's evaluation of pain and the presence of morning stiffness.

The aim of modern treatment for juvenile idiopathic arthritis is the rapid induction of disease control, to prevent joint damage, to maximise physical function, and promoting normal growth and normal lifestyle for the patients, as well as to achieve these goals with minimal risk of side effects, the recent concept of juvenile idiopathic arthritis management suggests that the early aggressive intervention may buy long term disease suppression (Albers et al., 2009; Vilca et al., 2010).

The aim of this retrospective study on 915 patients with juvenile idiopathic arthritis, who were treated with MTX for at least 3 months, was to determine the predictors of response to MTX.

Despite the presence of considerable variation in the clinical response to MTX among patients, and the availability of several new agents for the treatment of JIA, methotrexate remains the most common second line therapeutic agent used in treatment of JIA worldwide because of both cost and experience (Miller and Cassidy, 2007).

The identification of predictors of response is helpful to develop recommendations for MTX use, especially starting of MTX as well as further continuation or early discontinuation and starting use of biological drugs, and the aim of this study is to determine whether demographic, clinical, articular and laboratory variables at baseline and during follow up at months 3, 6 and 12 predict MTX response in patients with juvenile idiopathic arthritis.

In this study, the cohort of patients was collected from the German BIKER Registry, founded in 2001. Since 2005, patients newly started with MTX, were included into the registry, data of patients admitted to the registry until December 31, 2010 were used for this analysis.

The total number of patients in this retrospective study was 915 patients, who belong to all categories of JIA, and treated with MTX at least for 3 months, while the patients who currently receive or have been received biologics were excluded, the females represent 68.5 % of the sample (627 patients), while the males represent 31.5 % of the sample (288 patients).

The analysis of demographic, clinical, articular and laboratory characteristics of patients was made at baseline and during follow up at months 3, 6 and 12.

The improvement was assessed according to the paediatric criteria of American College of Rheumatology by using PedACR 30 for minimal improvement analysis and PedACR 70 for strong improvement analysis.

The PedACR 30 was initially designed to distinguish between active treatment and placebo, it was a significant step towards creating standardized outcome measures in paediatric rheumatology, these measures primarily assess relative efficacy within the context of clinical trials.

These measures are of less utility in quantifying response, tracking patient progress longitudinally and describing an individual's disease state at specific moment (Ringold and Wallace, 2007).

At month 3, 165 patients (22.6 %) were non responders according to PedACR 30, poor response to MTX according to PedACR 30 criteria at month 3 was

associated with a higher number of tender joints (OR=0.92 and p=0.003), a lower number of active joints (OR=1.26 and p<0.001) und a lower score of parent´s evaluation of overall well-being (OR=1.06 and p<0.001).

While at month 6, 121 of patients (18.6 %) were PedACR 30 non responders, the poor response was associated with disease duration > 1 year (OR= 0.48 and p= 0.002), a higher age at MTX start (OR=0.91 and P<0.001), a lower number of active joints (OR=1.11 and p=0.001), in addition to a lower score of physician's global assessment of disease activity (OR=1.01 and p=0.004), while concomitant use of NSAID was a positive predictor to reach PedACR 30 (OR=1.85 and p=0.048).

The number of patients who were PedACR 30 non responders at month 12 was 120 (16.9 %), the poor response according to PedACR 30 criteria at month 12 was associated with a disease duration < 1 year (OR= 0.64 and p= 0.047), a higher age at MTX start (OR=0.9 and p<0.001), a lower number of joints with limitation of motion (OR=1.11 and p<0.001) and a lower values of ESR (OR=1.02 and p=0.028), while concomitant use of NSAIDs was a positive predictor (OR=2.1 and p=0.021).

Although female gender was in tendency of value to reach minimal response (PedACR 30), but statistically not significant, females were as likely as males to reach minimal response. The lack of ANA, as well as enthesitis related arthritis and psoriatic associated arthritis subtypes, tend to be a poor response markers to reach PedACR 30, but statistically were not significant.

However, there is argument that only improvement in disease activity above Ped-ACR 70 predicts a more favourable long term outcome and reflect a major clinical response to treatment (Pincus et al., 2004; Vilca et al., 2010).

Previous studies have shown conflicting results regarding predictors of response to MTX, in three previous studies, the results indicate a different effect of MTX according to the type of JIA, Halle and Prieur found that the systemic form seemed less responsiveness than ANA positive form with polyarticular course (Halle and Prieur, 1991), while Woo, et al, found that MTX

is an effective treatment for both extended oligoarthritis and systemic JIA (Woo et al., 2000), as well as Ravelli, et al, concluded that the extended oligoarthicular subtype is the best predictor of methotrexate efficacy (Ravelli et al., 1999).

However the analysis of the PRINTO methotrexate trial has been found that the frequency of JIA categories was comparable between responders and non-responders (Vilca et al., 2010).

The present study confirm the observations of PRINTO study regarding JIA categories, in multivariate analysis we found that, the different JIA categories not statistically significant to predict the response to MTX (according to PedACR 70 criteria), although the bivariate analysis (using Pearson's correlation method) suggested that seropositive polyarthritis category at month 3, was associated with greater likelihood to reach early response (p=0.016), while enthesitis related arthritis at month 6, was associated with poor response (p=0.023), as well as psoriatic associated arthritis subtype at month 12, was also associated with greater likelihood of poor response (p=0.006).

The present study shows that according to PedACR 70 criteria, the number of responders at month 3 was 315 patients (43.1 %), while at month 6, the number of responders increased to 368 patients (56.7 %), and at month 12 increased to 466 patients (65.9 %).

Theses increasing in the number of responders through the progressing in course of treatment, suggest that the three months data may be affected by the delay of clinical response achieved by MTX treatment, these findings are in line with common view that the maximum therapeutic effect usually becomes apparent 4 to 6 months after the beginning of treatment (Ravelli and Martini, 2000).

In this study, at month 3, a significant relationship was found between a higher number of joints with limitation of motion (OR=1,03 p=0,004), as well as a higher score of Physician's global assessment of overall disease activity (OR=1,01 p=0,004) and strong response to MTX according to PedACR 70 criteria.

The presence of both predictors as parts of ACR pediatric criteria of response could only hardly explain the relationship, since the improvement of these criteria participates to the PedACR score, a higher values at initiation of therapy are associated with a higher likelihood of improvement.

Furthermore this study shows that the Physician's global assessment of overall disease activity as predictor of response is significant at month 3 and 6, but at month 12 not significant, this suggest that patients with lower score of Physician's global assessment of overall disease activity need longer duration to reach PedACR 70 response.

At month 6, the patients with a higher number of swollen joints at baseline, were associated with a greater likelihood of strong response to MTX (OR=1.06 p= 0.001).

We had not expected, nor can we explained the relationship between age at MTX start and the response to MTX. During this study we found that a higher age at MTX start was associated with a poorer response, with significant values at month 6 (OR=0.95 p=0.018) and month 12 (OR=0.92 p<0.001).

Previously an association between the weight of patient and the response has been observed. In a multicenter, randomized, controlled trial, Silverman et al found that weight was significantly associated with a response and influenced treatment effect, and the patients weighing less than 20 kg had the greatest improvement (Silverman et al., 2005).

Because the mean age at MTX start in seropositive polyarthritis and enthesitis related arthritis categories (12.4 and 12.9 years respectively) were higher than in other categories (for systemic onset JIA 6.9 years, in persistent oligoarthritis 8.3 years, in extended oligoarthritis 8.6 years, and in seronegative polyarthritis category was 9.4 years), to confirm our finding, the analysis has been repeated for age at MTX start without seropositive polyarthritis and enthesitis related arthritis patients, the analysis shown again a significant values at month 6 (OR=0.921 p=0.001), and at month 12 (OR=0.906 p<0.001).

These findings confirm that, a higher age at MTX start is a poor predictor for reaching PedACR 70 in different JIA categories, as well as suggest that patients with a higher age at MTX start, and who are PedACR 70 non responder at month 6, are highly recommended candidates for treatment with biologics.

At month 12, we found that, the presence of morning stiffness at baseline is a positive predictor to reach PedACR 70 (OR=1.59 p=0.016), furthermore our study shows that patients with a higher number of active joints (OR=1.09 p< 0.001), a higher score of parent's evaluation of child's pain (OR=1.01 p= 0.037) and a higher CRP level at baseline (OR=1.01 p=0.018) were associated with greater likelihood to reach PedACR 70.

The presence of the number of active joints as a part of PedACR set of improvement could hardly explain the relationship, which most likely is due to the anti-inflammatory mechanism of action of MTX. This may also explain the relationship between reaching of strong response and the number of activejoints as well as the other activity parameters such as CRP level and the pain, which considered as one of the symptoms of inflammation, which was in the present study assessed by the patient or parents global assessment of pain.

In contrast, a higher number of tender joints was a predictor of poor response to MTX at month 12, (OR=0.93 p=0.004). The significant relationship between the number of tender joints and reaching a PedACR 70 response, can be explained by the indirect effect of presence of tenderness or pain on the components of PedACR criteria, not only on physician's global assessment score and Parent's evaluation of overall well-being, but also on CHAQ. The CHAQ comprises two indices, the first is the disability index, and the other is discomfort index which is determined by the presence of pain measured by a 100 mm analogue scale (Duffy, 2007).

This study shows that, female gender statistically had no effect on reaching a strong response (PedACR 70). Disease duration before MTX start was

significantly associated with PedACR 70 response in bivariate analysis, but in general the disease duration as a variable was not significant in multivariate analysis, may be because it was highly collinear with the age at MTX start. After repeating multivariate analysis without age at MTX start, the disease duration subclases (less or more than 1 year) had a significant effect on reaching PEDACR 30 and 70, we found that a shorter disease duration (< 1 year) was significantly associated with reaching a strong response (PedACR 70) at months 6 and 12, while disease duration (> 1 year) was a poor predictor, this finding supports the results of PRINTO study and the previous clinical experience which suggests that the early treatment is more effective.

Concomitant use of NSAID was not significant in both bivariate and multivariate analysis for reaching PedACR 70, although it was significant for reaching minimal response (PedACR 30), ESR and CHAQ score were significant in the bivariate analysis but no longer significant in multivariate analysis.

In contrast to PRINTO study of predictors of response to MTX the lack of ANA, as well as disease duration and CHAQ score were not significantly predictors for a strong response(PedACR 70) (Vilca et al., 2010). These variations may be due to many differences between the present study and the PRINTO study, although the general design of PRINTO study was similar to the present study. Bivariate and logistic regression analysis was used to identify baseline predictors of poor response. Also the improvement was assessed according to the American College of Rheumatology criteria for pediatrics, by using PedACR 30 for minimal improvement analysis and PedACR 70 for strong improvement analysis.

In PRINTO study however, the patients with seropositive polyarthritis, psoriatic arthritis and enthesitis related arthritis categories were excluded from the study sample, while in the present study all JIA categories were included.

An important difference between the two studies is the time of evaluation of improvement in both studies, while in the PRINTO study the evaluation of improvement was done at month 6 only, in our study the evaluation was done at

month 3, 6 and 12. Which gives advantages to the present study and allows for better assessment of early response as well as the response after moderate and long duration of treatment and determination which variables could predict the response at these times. Another advantage of the present study is that, the study sample is larger than that of the PRINTO study. Our sample included 915 patients, while in PRINTO study the sample included 563 patients.

This large difference give advantage to the present study for better assessment of relationships between different baseline characteristics and reaching of improvement, especially in the multivariate analysis. The previous differences may explain the differences in the results between the present study and PRINTO study.

The diagnostic accuracy was evaluated using receiver operating curve, the area under the receiver characteristics curve (AUROC) at month 3, for PedACR 30 was 0.73 (sensitivity=98.9 %, specificity=10.7 %, accuracy=84 %), and for PedACR 70 was 0.61 (sensitivity=35 %, specificity=77.4 %, accuracy =58.7 %).

At month 6, AUROC for PedACR 30 was 0.73 (sensitivity= 97.5 %, specificity= 19.1 %, accuracy= 83.6 %), and for PedACR 70 was 0.65 (sensitivity = 78.2 %, specificity= 46 %, accuracy= 64.3 %).

While at month 12, AUROC for PedACR 30 was 0.72 (sensitivity= 99.6 %, specificity= 3.6 %, accuracy= 84.4 %), and the AUROC for PedACR70 at month 12 was 0.69 (sensitivity= 91.5 %, specificity= 33.2 %, accuracy= 71.8 %).

These results can be accepted especially when compared with results of other studies. These values are better than the results of PRINTO study, in which AUROC for PedACR 30 was 0.65, and for PedACR 70 was 0.66 (Vilca et al., 2010).

In conclusion, we have found that patients with a longer disease duration a higher age at MTX start, a lower number of score of physician's global assessment of disease activity, a lower score of parent's evaluation of child's pain, a lower CRP, a lower number of active joints, a higher number of tender

joints, a lower number of swollen joint and a lower number of joints with limitation of motion at baseline were significantly associated with a greater likelihood of PedACR 70 non-response, while the presence of morning stiffness was a positive predictor to reach PedACR 70. These findings can be considered as recommendations for the use of methotrexate in patients with juvenile idiopathic arthritis, since the presence of these baseline determinants predict a worse response to MTX, even after prolongation of exposure for up to12 months, thereby prompting the physicians to start an alternative drug therapy earlier.

6. Summary

Juvenile idiopathic arthritis is an umbrella term used to describe a heterogeneous group of disorders of unknown etiology, juvenile idiopathic arthritis is the most chronic rheumatic illness in children and it is responsible for short and long-term disability. Although methotrexate still the most common second line therapeutic agent used in treatment of juvenile idiopathic arthritis worldwide and effective in the majority of patients, there is variation in the clinical response to methotrexate among the patients. Prediction of response can prevent further exposure of patients to side effects of methotrexate, also save the time and start treatment with biologics as soon as possible to prevent irreversible complications.

The aim of this study is to determine whether demographic, clinical, articular and laboratory variables at baseline and during follow up at month 3, 6 and 12 predict methotrexate response in JIA patients, patients data were taken from the German BIKER Registry, which was founded in 2001.

The total number of patients fulfilling all inclusion and exclusion criteria was 915 patients, who belong to all categories of JIA, and treated with MTX for at least 3 months. The time of response analysis was at month 3, 6 and 12. For assessment of response, the American College of Rheumatology pediatric (PedACR) criteria was used, PedACR 30 for minimal response and PedACR 70 for strong response, and we found that, the number of PedACR 70 responders, were 315 patients (43.1 %) at month 3, 368 patients (56.7 %) at month 6, and 466 patients (65.9 %) at month 12.

In multivariate analyse, we used logistic regression analysis, the baseline determinants for strong response according to PedACR 70 at 3 months were a higher number of joints with limitation of motion (OR=1.03, p=0.004) as well as a higher score of physician's global assessment of disease activity (OR=1.01, p=0.004). At month 6 the determinants of strong response according to PedACR

70 were a disease duration < 1 year (OR=1.832, p<0,001), a lower age at MTX start (OR=0.95 , p=0.018), a higher number of swollen joints (OR=1.06 , p= 0.001) and a higher score of physician's global assessment of disease activity (OR=1.01 , p=0.004).

At month 12 the determinants of strong response according to PedACR 70 were a disease duration <1 year (OR=1.838, p=0.001), a lower age at methotrexate start (OR=0.92, p< 0.001), a lower number of tender joints (OR=0.93, p=0.004), a higher number of active joints (OR=1.09, p<0.001), a higher score of the parent's evaluation of child's pain (OR=1.01, p=0.037) and a higher values of CRP (OR=1.01, p=0.018), as well as the presence of morning stiffness is also a positive predictor to reach PedACR 70 (OR=1.59, p=0.016).

In conclusion, the presence of morning stiffness is a positive predictor to reach PedACR 70, while a longer disease duration before MTX start, a higher age at MTX start, a lower score of physician's global assessment of disease activity, a lower score of parent's evaluation of child's pain, a lower CRP, a lower number of active joints, a higher number of tender joints, a lower number of swollen joints and a lower number of joints with limitation of motion at baseline were significantly associated with a greater likelihood of PedACR 70 non-response. The presence of these baseline determinants predict a worse response to MTX and recommend to start an alternative therapy.

7. References

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