Patients treated for juvenile idiopathic arthritis - from immunosuppression to infections

PhD Thesis

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List of abbreviations

ACR Pedi – American College of Rheumatology, Pediatric

ANA – antinuclear antibody

AP – psoriatic arthritis

ERA – enthesitis-related arthritis

ILAR – International League of Associations for Rheumatology

JIA – juvenile idiopathic arthritis

MTX – methotrexate

OA – oligoarticular juvenile idiopathic arthritis

PA – polyarticular juvenile idiopathic arthritis

PRINTO – Pediatric Rheumatology International Trials Organization

RA – rheumatoid arthritis

RF – rheumatoid factor

SOJIA – systemic onset JIA

SSL – sulfasalazine

TNF-α – tumor necrosis factor-alpha

Overview

The juvenile idiopathic arthritis (JIA) is the most common immunological disease of childhood. The JIA does not mean a single entity, but it is the umbrella term for seven different immune-mediated diseases. According to the definition we can talk about JIA under the age of 18 when chronic inflammation is present. Its etiology and pathophysiology are still not entirely known. Beside genetical factors environmental and immunological risk factors should be mentioned in the development of the disease. The different JIA subtypes possess different pathogenesis and course of disease. The most common form is the oligoarticular JIA (OA), which in most cases affect a large joint, but less than four joints at the beginning of the illness. We talk about an extensive type if there is progression to more than four joints in less than six months. In the case of polyarticular form (PA) inflammation in more than four joints can be detected with physical examination in the first six months of the disease. Two types are known, the seropositive (rheumatoid factor positive) and the seronegative type which are considered separate JIA entities. The psoriatic arthritis (AP) comes with symptoms on the skin and the nails and varied extent of arthritis.

The aforementioned diseases are more common among the female population. The only male dominant form is enthesitis related arthritis (ERA). It is characterized by the inflammation of ligaments, enthesis' and/or axial joint involvement. Systemic-onset JIA (SOJIA) is a disease classified as an autoinflammatory disease. It affects both sexes in equal proportions. This subtype beside articular involvement is defined by sterile serositis, appearing at least for three consecutive days, fever for more than two weeks and the appearance of other signs of systemic inflammation. Multiorgan inflammation may mimic the symptoms of systemic infection or malignancy. The exclusion and treatment of these is vital. The therapy for SOJIA is different from the other

subtypes. Another form of the disease is undifferentiated arthritis, which includes cases that do not fall into the previous categories.

The terminology used in clinical practice was introduced by the International League of Associations for Rheumatology (ILAR). The diagnosis beside arhtritis is based on extra-articular involvement which are fever, skin symptoms, ophthalmic abnormalities, weight loss, liver and spleen enlargement, laboratory abnormalities. With the common criteria system created by ILAR, a well understood and adapted classification has been developed in everyday clinical practice. It has been further reworked and developed with active research ever since. The last consensus conference led by the Pediatric Rheumatology International Trials Organization (PRINTO) was in 2018. Based on disease phenotypes, genetics, and cytokine profiles, four categories have been identified: systemic-onset JIA (SOJIA), rheumatoid factor (RF) -positive JIA, early-onset antinuclear antibody (ANA) -positive JIA, and enthesitis-related JIA. An important goal was the more precise differentiation of the subtypes, creating more homogenous patient groups and the separation from rheumatoid arthritis (RA). The validation of this classification has been ongoing.

As with all diseases, a detailed medical history and physical examination are essential for JIA. For JIA, pathognomic deviation is not known in laboratory parameters. Imaging is suitable for detecting fluid, synovial processes, or destruction. In the case of joint involvement, the exclusion of bulky processes such as bone tumors and tumors infiltrating the bone marrow (leukemia or neuroblastoma) is primary. The arthritis can be the partial phenomenon of several autoimmune diseases, the differentiation may be aided by associated clinical symptoms and characteristic laboratory abnormalities. The exclusion of bacterial origin or other autoinflammatory diseases is necessary in the case of systemic form. Early treatment of early-onset and well-diagnosed arthritis is of paramount importance, and regular specialist check-ups are essential. The therapeutic goal is

to achieve remission on a lasting basis. The focus is on pain relief and inflammation reduction. Effective treatment of extra-articular symptoms is also an important part of therapy, otherwise a significant deterioration in the quality of life is expected. Structured algorithms are available for the treatment. Controlling the inflammation and the early prevention of joint destruction require aggressive treatment. During the remission phase, drugs need to be phased out in a preset order to prevent toxic side effects (step down treatment strategy). Without treatment joint pain, arthritis and the destruction can cause lasting deterioration of quality of life. At the beginning of the disease, non-steroidal anti-inflammatory drugs (NSAIDs) are used, in most cases supplementing the so-called conventional synthetic disease-modifying drugs (csDMARD). Methotrexate (MTX) and sulfasalazine (SSL) agents are the most common csDMARD therapies. The efficacy of these drugs and their side effects have been addressed in several studies. It is often used in combination with intra-articular steroids. In about 30% of cases the csDMARD drugs are not effective enough. If the process progresses further or remission is not achieved, biological therapy is introduced. Increasingly accurate understanding of the molecules that play a key role in the pathomechanism of the disease allows targeted therapy. The blocking of the inflammation cascade results in good disease-control and cessation of complaints. Macromolecular biologics produced by recombinant DNA technology cause a decrease in inflammatory activity and an improvement in erosion caused by synovitis. Long-term, sustained remission can be achieved by inhibition of tumor necrosis factor alpha (TNF-α). Anti-TNF-α drugs include infliximab, adalimumab (ADA), certolizumab pegol, golimumab, and etanercept (ETA, TNFR-IgG1 Fc fusion protein) for the treatment of a JIA subtype. Since 2006, ETA, ADA, tocilizumab (anti-IL-6 monoclonal antibody) and abatacept (CTLA-4-IgG1 Fc fusion protein) have been registered in Hungary for pediatric use in JIA therapy.

The efficacy of these new therapeutic options is also well known. However, it is important to pay attention to their immunosuppressive effects. The latter issue is one of the pillars of our research.

Aim of the study

I. Retrospective analysis of the characteristics, laboratory results of children receiving TNF-α blocking therapy for JIA at the Department of Paediatrics of the University of Pécs. Efficacy and safety analysis of the TNF-α inhibitors being used for treating these patiens.

II. The immunosuppressive effect of drug treatment was studied in three groups. Children receiving biological and MTX treatment were placed in the first group, the second received MTX monotherapy, whereas the third group consisted of healthy children. In addition to routine laboratory parameters, serum immunoglobulin isotypes, complement system analysis were performed and lymphocyte populations were analyzed by flow cytometry. Our goal was the comparison of the three groups' laboratory results.

III. In our meta-analysis, we investigated the incidence of infectious complications discussed in the literature in children with JIA receiving TNF-α inhibitor therapy. In our comprehensive study, we systematically compared high-quality articles that focus on the relationship between JIA and infections.

Methods

As a first step in our retrospective study, we performed a descriptive statistical characterization of the patients. The laboratory parameters of the children were compared to the given reference values using a one-sample t-test in order to establish whether there is a significant deviation from normal. The comparison of the efficacy of the two drugs was analyzed using an independent sample t-test.

During our prospective, single-center clinical study, we selected our JIA patients based on specific selection criteria. First, we prepared a descriptive statistical analysis of the three study groups: biological and MTX-treated, MTX monotherapy recipients and the healthy control group. The continuous variables were expressed in median and interquartile range (IQR) or mean ± standard deviation. The primary goal and result of the analysis was the quantitative comparison of immune cells and laboratory parameters in the case of the three different patient groups. Besides following the relevant routine laboratory parameters, the evolution of the composition and absolute number of T- and B-lymphocytes, activation, and the ratio of the naive and the memory cells in the peripheral blood samples of the patients at a given time of the various treatments were examined using flowcytometry. To reduce the heterogeneity of the sample, only extensive-oligoarticular and polyarticular JIA patients were included in the study.

The aim of our meta-analysis was to investigate the frequency of infection in children with JIA receiving TNF-α inhibitors. In the analysis we included publications that compared patients receiving TNF-α inhibitor treatment with a control group in terms of frequency and occurrence of infections. More precisely, during the meta-analysis we investigated prospective studies where the number of patients with infection was compared to the TNF-α inhibitor-treated and the control

groups. To calculate the odds ratio (OR) we used the frequency values of patients with infection observed in the TNF-α inhibitor group and the control group. The OR shows the risk of infection in each group; a value of ">1" indicated an increased chance of infection among patients receiving TNF-α inhibitor treatment.

Results

We conducted our retrospective study between 2010-2019 among children who appeared in the Immunology Department of our Clinic and who received TNF-α blocking therapy during their treatment. The data of a total of 73 patients were selected for statistical analysis based on specific criteria. Among the 73 patients receiving TNF-α inhibitor treatment, 23 children (31.5%) were classified as extended OA, 30 children (41.1%) as PA, 16 (21.9%) as ERA, and 4 (5.5%) as AP subtype. In terms of gender distribution, 29 (39.7%) of the children were boys and 44 (60.3%) were girls, including all subtypes. The gender differences observed in the individual subtypes were the same as those known in the literature: in the OA and PA forms the proportion of girls dominated, in ERA the proportion of boys, while in AP the proportion of the sexes was equal. The average age of the patients at the onset of symptoms was 8.62 years. No difference could be detected between girls and boys in terms of average age. For the OA form, the average age was 6.72 years (min-max: 1.00 -13.50 years), PA 8.07 years (1.00-16.00 years), for ERA 13.4 years (7.00-16.00 years), while for AP it was 4.63 years (1.50-10.50 years). The average age of patients diagnosed with ERA was significantly higher ($p < 0.001$) compared to any other JIA form examined. Our statistics revealed that an average of 8.92 months (0.5 months-4.8 years) passed from the onset of complaints to the diagnosis of JIA. Inflammation of large joints was observed in 61 patients (83.6%). Small joint involvement of the hands and feet was seen in 43 patients (58.9%),

coxitis and/or sacroiliitis in 28 (38.4%). Cervical spine involvement occurred in four (5.5%) cases while inflammation of the temporo-mandibular joint occurred in two (2.7%) cases. Symmetric distribution was diagnosed in 21 patients (28.8%), asymmetric involvement in 52 (71.2%). The main symptoms of arthritis are swelling, warmth, limited motion, and arthralgia. Joint pain (95.9%), limited mobility (98.6%) and swelling (91.8%) was present in nearly all our patients. Morning joint stiffness gradually improving during the day was reported by only one third of the patients (38.4%). Among the extra-articular involvements, chronic anterior uveitis is of particular importance. In both the extended OA and PA groups, seven (30.4% and 23.3%) patients respectively while in ERA it was detected in two (12.5%) patients. In the laboratory test results we saw increased inflammatory activity. The erythrocyte sedimentation rate was an average of 30.5 mm/h (3-120 mm/h) taking the different subgroups together. The highest average value was shown in PA (38.5 mm/h). The average value of C-reactive protein was 16.3 mg/l (0.18-119 mg/l) which was also the highest in PA (22.9 mg/l). During the analysis of immunoserologogic laboratory values, antineutral antibody (ANA) positivity was detected in a total of 25 children (34.2 %). It appeared most often in the PA subtype (n=15, 20.54 %). We saw an elevated titer in almost half of the uveitis cases (n=7, 9.6 %). A higher rheumatoid factor (RF) level was observed in 21 children (28.8 %). Biological therapy was introduced on average 16.4 months (0-108 months) after starting MTX base therapy. ADA was the first choice in 53 (72.6%) cases, and ETA in 20 (27.3%) cases. There was no significant difference between the subgroups in the duration of the introduction of the TNF-α inhibitor (p=0.839). When comparing the effectiveness of the two drugs, ADA proved to be significantly superior than ETA at all test times which was respectively determined by ACR Pedi 30, 50, 70, and 90 in the 1st 3rd, 6th and 12th months. In the ADA-treated group (n=53), 41.7%, 68.9%, 93.7% and 97.3% improvement was observed. In the ETA

group (n=20) we experienced 36.5%, 62.0%, 87.0% and 91.0% ACR responses. During the comparison of the two drugs, a significance of $p<0.001$ in the 1st month, $p=0.016$ in the 3rd month, $p=0.010$ in the 6th month, and $p=0.006$ after one year could be measured. Although better therapeutic efficiency was achieved with ADA based on the ACR Pedi point system, it can be seen that the ETA-treated group also achieved an adequate therapeutic response. During the observation period, a total of 24 children switched from the initial anti-TNF-α drug to another biological therapy. During the application, in 19 cases (26%) the reason was loss of efficacy or relapse. In the case of eight children, there was a change from ETA to ADA, and in nine from ADA to ETA. In two children, tocilizumab was introduced instead of ADA. In the case of two other children, the medication was changed due to ETA intolerance, and in three children, due to the appearance of uveitis, ADA treatment was introduced. During the further analysis of the data, we also examined the need for therapy changes broken down into subtypes. Based on these, a change of treatment was justified in three cases (13%, 18% and 75%) of the extending OA, ERA and AP patients, and in 33.3% (n=10) of the PA patients. A significant correlation ($p=0.001$) was found between the population requiring a change of therapy and the time elapsed between the start of primary treatment. While it took an average of 5.49 months from the onset of symptoms to the start of treatment in patients who did not require a change of therapy, it was 18.7 months for children having a change of therapy. Local skin irritation was one of the most common complications observed in connection with the treatments, which was seen in three children (15%) in the case of ETA, and in one case (1.9%) in the case of ADA. The differences in the peripheral blood count are of particular importance: slight neutropenia developed in three patients (4.1%) in correlation with the treatment. In terms of infectious complications, the respiratory system was affected in the highest percentage. In 16 patients (21.9%) we diagnosed an upper respiratory tract infection and

in seven children (9.6%) pneumonia, the latter was cured in all cases after the administration of oral antibiotics. Another point of investigation was the analysis of the number of relapses, which also proved the superior effectiveness of ADA: in the case of ETA, the rate of relapses was significantly higher ($p<0.001$).

In our prospective study, 26 out of 41 patients (63.5%) received ADA treatment in addition to MTX, 15 patients (36.5%) received MTX monotherapy, while 22 patients formed the healthy control group. None of the children received systemic steroid treatment at least four months before the examination. Children belonging to the PA group had a significantly longer ($p=0.005$) disease course and received therapy for a significantly longer time (average R=18.18 and 25.41, $p=0.049$). There were no differences between the three groups in laboratory abnormalities indicating acute or chronic inflammation. Regarding autoantibodies, seven patients (17%) were ANA-positive, eight (19.5%) were RF-positive. During the flow cytometric measurements, a significant difference was detected in the absolute number of CD3+ T cells between the MTX/ADA (2067.07±642.04, 95% CI: 1807.74-2326.40) and the healthy group (1628.36+/-353.42, 95% CI: 1471.66-1785.06, $p=0.017$). Further comparing the above mentioned two groups, a difference regarding the number of CD4+ T-helper cells (1108.80+/-240.30 vs. 915.09+/-251.85, $p=0.054$) and CD8+ T-cytotoxic cells (788.03+/-400.47 vs. 585.36+/-180.47, $p=0.060$) were observed, but it did not reach a level of significance. There were no differences between the groups in the number of other T-cell subtypes. The number of CD56+ NK cells was significantly lower in the group treated with biological therapy (254.70±131.25, 95% CI: 182.02-327.39) compared to the healthy control (341.50±152.48, 95% CI: 273.89-409.10, $p2=0.039$). Examining the humoral immune system, there was also a significant difference in the number of CD19+ naïve B-cells between MTX/ADA (199.60±94.95, 95% CI: 147.01-252.18) and MTX groups (291.30±126.69, 95% CI:

240.13-342.48, p3=0.042). At the same time no functional difference was observed, as no difference was detected in the age-specific immunoglobulin (IgM, G and A) levels. There was no significant difference between class-switched memory B cells and CD5+ B cells. No difference was observed in complement levels (C3, C4, CH50-total complement).

In our meta-analysis, a total of 2130 children with JIA formed the study population. 1434 patients received biological therapy and 696 belonged to the control group, receiving csDMARD or placebo. Among the TNF-α inhibitors, 20 patients received INX, 78 golimumab, 91 ADA, while the majority, 1245 patients, received ETA. As co-medication, csDMARD, NSAID or low-dose systemic glucocorticoids (equivalent to 0.2 mg/kg or less prednisolone) may also have been included in the patients' anamnesis. Regarding the control group, six studies used placebo and three csDMARD for statistical comparison. The latter was MTX and/or SSZ. It is important to highlight that in all placebo-controlled studies standard dose of csDMARD therapy was used in both patients receiving active biological treatment and those in the control group. The risk of infection was increased for patients receiving biological therapy, although it was not statistically significant (OR=1.13 95% CI: 0.76-1.69; p=0.543). A subgroup analysis was also performed regarding infectious complications localized to individual organ systems. Upper respiratory tract infections were the most common infections; a non-significantly increased infection risk could be detected in the active group (OR=1.10; 95% CI: 0.65–1.84; p=0.729). With the exception of gastrointestinal infections (OR=0.83, 95% CI: 0.29–2.36; p=0.721) the risk of infection in all other examined organ systems increased in the population receiving biological therapy, but this did not reach statistical significance in any case. The appearance of opportunistic pathogens or tuberculosis was not detected in the studied patient population. Our study also covered serious, even life-threatening infections. By definition, those pathologies are included in this category that require hospital

treatment and/or intravenous antibiotic treatment, or that end in death. There was no precedent for any of these.

Summary of findings

I. During the processing of the demographic data of the children cared for in our clinic due to JIA we noticed a high degree of similarity with international data.

II. Anti-TNF-α drug treatment has proven to be effective and safe.

III. Our study showed that the incidence of serious infections did not increase among patients receiving TNF-α inhibitor therapy.

IV. To our knowledge, our study is the first meta-analysis that examined the infectious complications of JIA patients receiving TNF-α inhibitor treatment. It pointed out that there is an increased risk of upper respiratory tract infections in these patients.

V. During the examination of immune cells, the number of CD56 NK cells was significantly lower with TNF-α inhibitor treatment, which may explain the increased number of upper respiratory tract infections.

VI. As a conclusion, it can be said that the therapeutic effect of TNF-α inhibitors in mitigating disease activity significantly exceeds their risk of possible infectious complications.

1. The original publications that form the basis of the thesis:

Nagy A, Mosdosi B, Simon D, Dergez T, Berki T. Peripheral Blood Lymphocyte Analysis in Oligo- and Polyarticular Juvenile Idiopathic Arthritis Patients Receiving Methotrexate or Adalimumab Therapy: A Cross-Sectional Study. Front Pediatr. 2020 Dec 10;8:614354.

Nagy A, Mátrai P, Hegyi P, Alizadeh H, Bajor J, Czopf L, Gyöngyi Z, Kiss Z, Márta K, Simon M, Szilágyi ÁL, Veres G, Mosdósi B. The effects of TNF-alpha inhibitor therapy on the incidence of infection in JIA children: a meta-analysis. Pediatr Rheumatol Online J. 2019 Jan 18;17(1):4.

2. Other full publications:

Wobbe B, Wagner J, Szabó DK, Rostás I, Farkas N, Garami A, Balaskó M, Hartmann P, Solymár M, Tenk J, Ottóffy M, **Nagy A**, Habon T, Hegyi P, Czopf L. Ultrafiltration is better than diuretic therapy for volume-overloaded acute heart failure patients: a meta-analysis. Heart Fail Rev. 2021 May;26(3):577-585.

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