Effect of IL6 -174 G/C Gene Polymorphism on Response to Interleukin-6 Blocking Therapy in Systemic Juvenile Idiopathic Arthritis.

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Research article

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Abstract

Objective: The main objective of this study is to analyze and evaluate the association of IL6-174 G/C gene polymorphisms with response to interleukin-6 blockade in Egyptian patients with systemic juvenile idiopathic arthritis, and to assess the effect of other factors on treatment response as well as long-term efficacy of treatment with Tocilizumab.

Methods: Sixty systemic JIA patients (37 males and 23 females with median age at onset 5.2 years) who received IL-6 blockade (Tocilizumab) were recruited. The overall response to treatment with IL-6 blockade was assessed according to: 1) Clinical response, 2) switch (or no switch) to another biological DMARD therapy following IL-6 blockade, 3) achievement of clinically inactive disease within 6 months of IL-6 blockade, 4) improvement in disease activity measured using the modified JADAS-10, and 5) achievement of a glucocorticoid-free state. In addition, basic demographic, laboratory data, characteristics of the disease course and IL6 polymorphisms were assessed.

Results: Three IL6 -174 genotypes, including, GG, GC, and CC were found with higher frequencies of GC genotype. These genotypes had non-significant association with the response of sJIA patients to IL-6 blockade in this cohort study. Moreover, a longer time frame from disease onset to diagnosis was associated with poorer long-term treatment response.

Conclusion. No significant impact of IL6 -174 G/C gene polymorphisms on treatment response to IL6 blockade therapy in sJIA Egyptian patients, providing evidence of a “window of opportunity” for improved long-term treatment response with shorter time from the disease onset to diagnosis.

Key Messages:
1- No significant impact of IL6 gene polymorphisms on response to IL6 blockade in sJIA patients .
2- Higher frequencies of GC genotype among responders to IL6 blockade therapy .
3- A longer disease onset to diagnosis was associated with poorer long-term treatment response.

Introduction:

Juvenile Idiopathic arthritis (JIA) is one of the commonest forms of chronic childhood disability. Approximately 11% of patients with JCA suffer from the systemic-onset form (s-JIA), which is the subgroup most likely to be associated with severe, debilitating, extra-articular features and occasionally fatal complications [1, 2]. To control their disease, young children with s-JIA are often exposed to potentially toxic therapies for many years. However, many children still experience early joint destruction, necessitating surgical replacement. Moreover, up to 48% of these patients will still have active disease after 10 years [3].

S-JIA is a clinically homogeneous and quite unique illness. When the disease is active, patients display a typical quotidian spiking fever, an evanescent macular rash, lymphadenopathy, hepatosplenomegaly, serositis, myalgia, and arthritis. They are frequently anemic with markedly raised neutrophil and platelet counts; they have a high erythrocyte sedimentation rate, C-reactive protein, and serum fibrinogen. Patients also have a polyclonal hypergammaglobulinaemia and in severe cases raised liver enzymes and a coagulopathy [4].
Additionally, inflammatory cytokines, such as tumor necrosis factor (TNF-a), interleukin-1 (IL-1), and interleukin-6 (IL-6) have been shown to be elevated in the serum and synovial fluid of inflamed joints [5–8].

Interleukin-6 (IL-6), a broad acting cytokine, although it has the ability to maintain survival, proliferation, and differentiation of inflammatory cells in particular T and B cells, it can lead to pro-inflammatory or anti-inflammatory outcomes in infection and autoimmunity [9, 10]. Its proinflammatory role is of paramount significance to the generation and progression of the disease. In this regard, IL-6 can drive various innate and adaptive immune responses, which control leucocyte recruitment and as a consequence determine the activity and maintenance of the inflammatory infiltrate [11]. In addition, IL-6 can elicit a number of hormonal functions, including control of vascular function and normal homeostasis, insulin resistance, lipid metabolism and iron transport; it also has an impact on neuroendocrinial and neuropsychological behaviour [12].

Recently, cytokine-directed therapies targeting interleukins, mainly TNF-a, IL-1 and IL6 are approved [13]. Among these pro-inflammatory cytokines, IL-6 plays a significant role in mediation of inflammation. IL-6 receptor consists of a hexameric structure involving two molecules for IL-6, two molecules of IL-6R, and two molecules of gp130. This has led to a pharmacological design of IL-6 directed mAbs therapies that prevent IL-6 binding to IL-6R. For instance, Tocilizumab and Sarilumab mAbs target IL-6R; Sirukumab and Clazakizumab, mAbs target IL-6 engagement with gp130, and Olokizumab and EBI-029 mAbs target IL-6 itself [13].

Tocilizumab; a humanized anti–IL-6R mAb of the immunoglobulin G1 (IgG1), in sJIA, its dose was increased from 2 mg/kg to 4 mg/kg to 8 mg/kg given twice a month. At 2 mg/kg there was a good response, as measured by the JIA core set of improvement criteria, with 60% of patients achieving 30% and 50% responses. When all the doses were combined, more than 60% of patients achieved a 70% response, which is indicative of the ability of tocilizumab to offer a significant benefit in patients with sJIA without serious adverse effects [14].

The heterogenous response to IL-6 blockade may be attributed to a single nucleotide polymorphism (SNP) in IL-6, where haplotype-tagging SNPs (htSNPs) may explain patients association with sJIA risk and the response to treatment [15]. Therefore, the aim of the present study is to analyze and evaluate the association of IL6-174 G/C gene polymorphisms with response to the IL-6 blockade TCZ in patients with systemic JIA, and to assess the effect of other related factors on treatment response.

**Patients And Methods:**

**Study design and patients:**

We conducted a cohort study for the clinical and laboratory features of 60 patients (37 males and 23 females) with sJIA who were treated with Tocilizumab (TCZ) in the inpatient Clinic, Department of Physical Medicine, Rheumatology & Rehabilitation, Tanta University Hospitals, Egypt, between June 2020 and January 2021. We confirm none of the present study's procedures had violated the principles stated by the latest version of declaration of Helsinki. We followed the recommendations of STROBE guidelines during the preparation of this manuscript. The research study was approved by the ethical committee, Faculty of Medicine, Tanta University, Egypt, and the informed consent was obtained from children's parents before participation in this study. Patients were diagnosed as having sJIA if they met the ILAR classification criteria for this JIA category [16]. Patients with other rheumatic or autoinflammatory diseases were excluded from the study.
**Methods:**

A detailed questionnaire on patient demographics, disease characteristics, as well as all current and past anti-rheumatic therapies besides the details of dose escalation or reduction & switching or discontinuing those drugs were also taken.

**Assessment of treatment response:**

Our registry includes detailed longitudinal records of individual patients concerning disease activity and laboratory findings. As the main outcome of the study was the treatment response, hence it was assessed according to 2 different parameters: 1) formal assessment of the clinical response, and 2) patterns of treatment with biologic agents.

**Scoring Assessment of the clinical symptoms in JIA patients;**

The clinical symptoms of JIA disease was scored according to the following the response categories: 1) Good response, if the signs and symptoms of active disease (fever, rash, adenopathy, hepatosplenomegaly, serositis, and arthritis) had resolved and if the levels of inflammation markers (C-reactive protein [CRP] and erythrocyte sedimentation rate [ESR]) had improved by at least 50% following treatment with Tocilizumab, and if this response was maintained for at least 6 months. 2) Transient response, if there was an initial response characterized according to the parameters of a good response for at least 2 months but with later recurrence of disease. 3) Poor response, if the parameters for improvement were not met [17].

**Assessment of clinical response of sJIA patients to TCZ treatment**

sJIA patients who initiated treatment with TNF blockades (Infliximab 6-10 mg/kg IV infusion at 0, 2, 6 wk then every 6 wk as maintenance, Adalimumab 10-20 mg SC q 2 wk or Etanercept 0.8 mg /kg SC weekly) following Tocilizumab therapy were classified into one treatment pattern group, and patients who did not receive TNF blockades following TCZ therapy were classified into the other treatment pattern group. This approach estimates the drug survival that was supposed to reflect the long-term efficacy of treatment with TCZ.

**Secondary analysis of sJIA:**

For secondary analyses, we used the following additional improvement criteria: 1) any response, i.e., improvement of fever (if present) and/or arthritis (if present), defined according to the criteria used by Arthur et al. [18], 2) improvement in the physician global assessment of disease activity score by at least 30%, 50%, 70%, or 90%, 3) improvement in the modified Juvenile Arthritis Disease Activity Score in 10 joints (JADAS-10), consisting of the sum of the physician global assessment of disease activity score (scale 0–10), the count of joints with active arthritis (scale 0–10), and normalized CRP level (scale 0–10; calculated as [CRP (in mg/liter) − 10]/10, with a CRP level of <10 mg/liter representing a score of 0), 4) development of clinically inactive disease (CID) within 6 months of initiation of IL-6 blockade, defined according to the CID criteria of a physician global assessment of disease activity score of 0 (scale of 0–10), a CRP level or ESR within the normal range, and no documentation of active arthritis, fever, rash, adenopathy, hepatosplenomegaly due to systemic JIA, uveitis, or morning stiffness, and 5) Achievement of a glucocorticoid-free state within 6 months of initiation of IL-6 blockade. [19,20]
DNA extraction:

Genomic DNA was extracted from the whole peripheral blood sample with EDTA using the GeneJET Whole Blood Genomic DNA Purification Mini Kit supplied by (Thermo Scientific, Waltham, Massachusetts, USA). DNA purity and concentration were determined spectrophotometrically at 260 and 280 nm. The extracted DNA was stored at -20°C until analysis.

Genotyping for IL6 - 174 G/C polymorphism:

Restriction fragment length polymerease chain reaction (PCR-RFL) was performed to determine the different genotypes of IL6 - 174 G/C polymorphism. This polymorphism was analyzed by amplification of a 611-bp sequence using oligonucleotide primer sequences using the forward 5′-TGA CTT CAG CTT TAC TCT TGT-3′ and reverse 5′-CTG ATT GGA AAC CTT ATT AAG-3′. The protocol consisting of an initial denaturation at 94°C for 4 min, followed by 30 cycles of annealing at 54 °C for 30 s, extension at 72 °C for 30 s and denaturation at 94 °C for 30 s, and a final extension at 72 °C for 4 min. The constituents of the reaction consisted of: 1.2 µM of each primer, 10mM of dNTPs, 2mM of MgCl2, 1 U of Taq DNA polymerase enzyme and 1× PCR buffer, along with 40–50 ng of DNA. All reactions were done using the thermal cycler Applied Biosystems 9600 (Per- kin Elmer, Singapore). The amplified fragment was digested with the restriction enzyme Nla III (NEW ENGLAND BioLabsw) at 37 C for 4 hours and the products were then electrophoresed on 2% agarose gel, stained with ethidium bromide, and visualized by an ultraviolet transilluminator. DNA molecular weight marker (Qiagen Gel Pilot 100bp plus Ladder) (#Cat 239 045) was used to assess the size of the PCR-RFLP products. The amplified fragment (611bp) after digestion with Nla III restriction enzyme can either give rise to three fragments at 611, 367 and 244 bp, which indicates the presence of the heterozygous genotype (GC), or two fragments at 367 and 244 bp, which indicates the presence of the homozygous minor genotype (CC), or remains undigested as one fragment at 611 bp for the wild genotype (GG) [21].

Laboratory assessment

Laboratory variables, including complete blood count (CBC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and serum ferritin level as well as the serum level of S100A12 that shows a strong correlation with disease activity in systemic JIA were measured [19,20]. In order to achieve standardization for each patient, the serum sample with the highest S100A12 level and the serum sample with lowest S100A12 level, reflecting the highest and lowest overall inflammatory activity, respectively, for that individual patient were considered.

Statistical analysis

Descriptive statistics concerning the relationship between the various clinical parameters and treatment response were calculated. The relationship between the different IL-6 polymorphisms and treatment response was assessed using the Chi-square test. Receiver Operating Characteristic (ROC) curve analysis was performed to assess the accuracy of the continuous variables regarding outcomes.

Results

Patient characteristics and treatment responses:
This cohort consisted of 60 sJIA patients who were treated with Tocilizumab (TCZ) during the course of their disease; Of the 60 patients, 42 (70%) showed at least a transient clinical response and 15 (25%) had a poor clinical response, while for 3 patients (5 %), an assessment of the clinical response was not available. Twenty patients had received / switched to TNF blockades including 7 patients who switched following prior therapy with Tocilizumab, and 13 patients who switched prior to receiving Tocilizumab, meaning that 42 patients had not switched to TNF blockades following the initiation of IL-6 blockade (Table 1, Fig.1). The treatment responses in the responders and non-responders’ groups overlapped to some degree, as indicated by the distribution of treatment responses (Table 2). Analysis of the data indicated that patients who did not experience a switch to TNF blockades showed a lower duration from disease onset to diagnosis than those who switched to TNF blockades (median 0.07 years as compared to 0.29 years; \( P = 0.02 \)). ROC curve analysis of the duration from disease onset to diagnosis and switching to TNF blockades showed 0.67 (95% confidence interval 0.56–0.82). Concerning the total duration of follow-up between these 2 response groups, there were differences between them where the median follow-up was 3.7 years in those who did not switch to TNF blockades as compared to 7.8 years in those who switched to TNF blockades, \( P = < 0.01 \).

In addition, the maximum recorded ESR and CRP values were higher in patients who switched later to TNF blockades than in those who did not (median ESR reached 99 mm/hour versus 77 mm/hour; median CRP reached 143.3 mg/liter versus 74 mg/liter). Other parameters, including sex, age at onset, age at diagnosis, proportion of patients treated with glucocorticoids, and the median dose of glucocorticoids at initiation of IL-6 blockade, maximum recorded white blood cell count, minimum recorded hemoglobin level, and maximum recorded S100A12 protein levels were not different between the two groups.

**IL-6 gene polymorphism is not linked to the treatment response**

We then genotyped IL-6-174G/C in sJIA patients. This polymorphism was analyzed by amplification of a 611-bp sequence using oligonucleotide primer sequences as described in the materials and Methods. using the forward 5’-TGA CTT CAG CTT TAC TCT TGT-3’ and reverse 5’-CTG ATT GGA AAC CTT ATT AAG- (Move this part to Materials and Methods). Among the response group, we observed no significant difference in the distribution of the homozygous wild genotype (GG) (n = 22 [36.7%]), heterozygous (GC) (n = 32 [53.3%]), or homozygous minor genotype (CC) (n = 7 [11.7%]) (Table 3). We then compared the frequencies of the different genotypes across different outcomes, including 1) clinical response, 2) subsequent switch or not to TNF blockades, 3) improvement in the physician global assessment of disease activity score by at least 30%, 50%, 70%, and 90%, 4) achievement of a modified JADAS-10 score of 5 or better within 6 months of TCZ therapy, 5) achievement of CID within 6 months of TCZ, and 6) achievement of a glucocorticoid-free state (Table 3). None of these outcomes was significantly associated with the different genotypes of IL6 -174.

**Serum biomarkers and treatment responses**

Serum levels of S100A12 was different between the two response groups. These levels were analyzed and with regard to the transient clinical response or to switching to TNF blockades. We found that responders (TNF non-switchers) had higher serum S100A12 levels than non-responder (the TNF switchers). We have to mention that because most of the patients did not receive Tocilizumab at the time of blood sampling, we could not analyze serum level of IL-6. Patients with the highest and lowest serum levels of S100A12 did not show significant correlation with the different genotypes of IL-6.
Discussion:

Association of IL-6/IL-6R with sJIA has made IL-6 as a central player in the pathology of this disease and led to the clinical trial of an IL-6 receptor (IL-6R) monoclonal antibody to block IL-6 classical and trans-signaling in an orphan disease such as sJIA. In this cohort study of patients with systemic JIA who were treated with TCZ therapy, we could not find a significant association of IL-6 -174 G/C gene polymorphism, including rs55663133, we did not observe any association in our cohort with various definitions of an adequate treatment response. Association of IL-6 polymorphism and treatment response, however, was previously reported in French rheumatoid arthritis (RA) patients. In their study which included for 21 candidate genes, they found that the clinical response to treatment was associated with SNP genotype in the gene IL6R, where patients with the homozygous AA-genotype for rs12083537 (IL6R) showed significant better response than homozygous or heterozygous patients with the G allele [22]. It has also been found in RA patients that AA genotype for rs12083537 and CC for rs11265618 polymorphisms may act as predictors of good response to TCZ, where the patients treated with TCZ showed better EULAR response, remission, low disease activity (LDA) and DAS28 improvement rates [23].

Interestingly, whereas an association of the different IL6R SNPs with the risk of systemic JIA was seen in several of the systemic JIA cohorts (from the US, UK, Italy, Brazil, Canada, Spain and Argentina) reported by Arthur et al [18]. Therefore, it is possible that genetic risk factors for systemic JIA in particular for non-responders vary in different populations. Among the response group in our study, the homozygous minor genotype (CC) (n = 7 [11.7%]) showed the lower frequencies. In fact, this finding had come in agreement with Ogilvie et al [24] who reported significantly reduced frequency of the -174 CC genotypes in systemic JIA patients with age at onset of ≤ 5 years. Therefore, the reduction in the frequency of the CC genotype in JIA patients suggested that this genotype confers a protective influence against the development of the disease. As well as IL-6 response to a stressful stimulus [24]. On the other hand, Ciccarelli et al [25] found that IL-6 gene (-174 G/C) has been associated with bone erosive damage in RA patients especially those with CC genotype. The low frequency of the C allele in Indians and the reduction in CC genotype frequency in children aged ≤ 5 years are very interesting [26, 27] and actually similar to our findings. In addition, IL-6 plays a central role in contributing to the development of the disease in which it is considered as a pro-inflammatory cytokine that had different pleiotropic activities including induction of an acute phase proteins and stimulation of T as well as B cells. These reactions result in cartilage and bone damage as well as other systemic manifestations [28]. A polymorphism in -174G/ C of the IL-6 gene promoter region was reportedly associated with systemic onset juvenile idiopathic arthritis and susceptibility to RA in Europeans [18]. In the cohort study by Arthur or Claas Hinze et al, the patients are not well characterized, making its comparisons with our cohort less feasible. Arthur et al [18], included 38 patients in their study, and demonstrated that high expression alleles of systemic JIA-associated IL1RN SNPs were strongly associated with non-responsiveness to anakinra therapy.

The lack of association between these SNPs and non-responsiveness to tocilizumab treatment suggests that these SNPs are specifically associated with anakinra non-responsiveness, as opposed to being associated with more global treatment resistance. However, Claas Hinze et al [29] included larger population size of 61 patients and could not confirm an association of IL1RN haplotypes and SNPs with response to IL-1 blockade in their cohort of patients with systemic JIA. Also, patients who received tocilizumab following IL-1 blockade had a longer duration from disease onset to diagnosis than those who did not receive tocilizumab. Regardless this
controversy in the genetic association of IL-6 and sJIA, the IL-6 blockade (TCZ) has proven to have remarkable efficacy in this disease in a proof-of-principle phase I/II study with a single dose and in a separate dose-escalation study [30, 31]. Moreover, the results of our cohort study indicate that non-responder JIA patients, showed a longer duration from the onset of symptoms to diagnosis (median 0.29 years) when compared to those with responders (median 0.07 years). Hence, this observation supports the “window of opportunity” hypothesis, i.e., early diagnosis and treatment may positively influence the long-term outcome of the disease [32]. However, we did not find significant association between the duration from the onset to diagnosis with different measures of clinical responses to treatment. Furthermore, we could not find a correlation between serum IL-6 Ra levels with different factors, including the different IL6 SNPs and treatment response.

The higher serum levels of IL-6Ra are most likely attributable to the ongoing treatment of patients with TCZ, since the assay used does not distinguish between the endogenous IL-1Ra and those produced in response to TCZ treatment. Moreover, assessment of serum cytokine levels, including IL-6Ra, is presumably affected by multiple factors, such as degree of systemic inflammation and current medications, for which we did not control; therefore, based on our current data, it would not be possible to conclude on the effect of IL6 -174 different genotypes on IL-6 Ra expression [33].

**Conclusion:**

We found a non-significant impact of IL6 -174 G/C gene polymorphisms on treatment response to IL6 blockade therapy (Tocilizumab). However, this study provides evidence of a “window of opportunity”; improved long-term treatment response with shorter time from disease onset to diagnosis and hence treatment, presumably.

**Declarations**

**Conflict of Interest:**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Author Contributions:**

All Authors contributed to the manuscript and approved the final version.

**Ethics approval:**

The protocol of the present study was registered by the local ethics committee of Tanta University Hospital with approval code 34571/5/20.

**Consent to participate:** written informed consents were collected from eligible patients or parents.

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Data Availability Statement:

All data relevant to the study are included in the article or uploaded as supplementary information.

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Tables

Table (1): Demographic & laboratory characteristics and demographic data of 60 patients with systemic JIA
| Characteristics                  | Clinical response pattern |                             | Response based on treatment |                             | P-value |                             | P-value |
|----------------------------------|--------------------------|------------------------------|-----------------------------|------------------------------|---------|------------------------------|---------|
|                                 | All patients (n=60)      | Transient response (n=42)   | Poor response (n=15)        | No subsequent switch (n=42)  | Subsequent switch (n=18) |              |         |
|                                 |                          |                              |                             |                              |                      |              |         |
| Female/male No (%)               | 23 (38.3) / 37 (61.7)    | 17 (40.5) / 25 (59.5)        | 6 (40) / 10 (66.7)          | 17 (40.5) / 23 (54.8)        | 9 (50) / 11 (61.1)      | 0.67       | 0.92    |
| Age at disease onset, years      | 5.2 (0.5-17.3)           | 5.6 (0.5-17.4)               | 5.2 (0.6-13.0)              | 5.6 (0.6-17.4)               | 4.9 (0.5-12.9)         | 0.35       | 0.11    |
| Age at diagnosis, years          | 5.4 (0.5-17.5)           | 7.1 (0.5-17.5)               | 5.3 (0.0-10.7)              | 7.0 (0.7-17.3)               | 4.9 (0.5-16.7)         | 0.48       | 0.22    |
| Onset to diagnosis, years        | 0.09 (0.0-10.5)          | 0.09 (0.0-3.3)               | 0.12 (0.01-10.6)            | 0.08 (0.03-3.0)              | 0.27 (0.0-10.5)        | 0.35       | 0.02*   |
| Onset to final follow up, years  | 5.6 (0.7-27.5)           | 4.4 (0.7-17.7)               | 8.6 (0.9-24)                | 3.5 (0.7-27.4)               | 7.6 (0.9-23.9)         | 0.08       | <0.01*  |
| Pattern of joint involvement, no (%) |                          |                              |                             | 0.08**                       | 0.76**               |                      |         |
| None                             | 7 (11.7)                 | 7 (16.7)                     | 0 (0.0)                     | 4 (9.5)                      | 2 (11.1)              |           |         |
| Arthralgia                       | 14 (23.3)                | 10 (23.8)                    | 1 (6.7)                     | 9 (21.4)                     | 3 (16.7)              |           |         |
| Oligoarthritis                   | 7 (11.7)                 | 5 (11.9)                     | 2 (13.3)                    | 5 (11.9)                     | 3 (16.7)              |           |         |
| Polyarthritis                    | 35 (58.3)                | 19 (45.2)                    | 13 (86.7)                   | 23 (54.8)                    | 11 (61.1)             |           |         |
| WBC count, x10^9/liter           | 17.5 (5.1-53.5)          | 25.0 (9.8-48.8)              | 23.5 (10.0-53.5)            | 25.6 (9.8-48.9)              | 26.3 (10.0-53.5)       | 0.19       | 0.16    |
| HB level, gm/dl                  | 11.9 (5.1-14.5)          | 11.9 (9.2-14.9)              | 12 (9.7-15.6)               | 11.9 (8.2-15.0)              | 12.1 (9.6-15.3)        | 0.17       | 0.86    |
| CRP level, mg/liter              | 138.5 (1.0-930)          | 150.6 (9.5-930.0)            | 163.6 (130.9-302.7)         | 74.0 (1.0-930)               | 143.3 (15.0-302.8)     | 0.11       | 0.03*   |
| ESR, mm/hour                     | 80 (4-142)               | 77 (6-142)                   | 117 (4-135)                 | 77 (5-125)                   | 99 (4-143)            | 0.10       | 0.05*   |
| Serum Calprotectin level, ng/ml   | 450 (14-22.730)          | 2,160 (47-22.730)            | 340 (76-1470)               | 2,160 (47-22.730)            | 450 (76-17.850)        | 0.75       | 0.66    |
| Modified JADAS-10 score at initiation of tocilizumab therapy | 17.0 (5.0-33.0)          | 15.4 (10.0-28.0)             | 20.0 (13.0-30.0)            | 14.8 (5-29)                  | 20.0 (13-30)          | 0.07       | 0.06    |
| On                               | 50 (83.3)                | 35 (83.3)                    | 13 (86.7)                   | 32 (80)                      | 17 (85)               | 0.88       | 0.52    |

*Significant at p < 0.05
glucocorticoid therapy at initiation of tocilizumab therapy. No/total (%)

| Prednisone dose at time of initiation of tocilizumab therapy, mg/kg | 0.17 (0-15) | 0.20 (0-15) | 0.18 (0-10) | 0.77 | 0.15 (0-15) | 0.22 (0-2) | 0.44 |
|---|---|---|---|---|---|---|---|
| Off glucocorticoid therapy within 6 months of tocilizumab therapy. No / total (%) | 25/50 (50) | 19/35 | 5/13 | 0.29 | 20/32 | 5/17 | 0.05* |
| Prednisone dose at time of discontinuation of glucocorticoid therapy, mg/kg | 0.0 (0.0-0.7) | 0.0 (0.00-0.40) | 0.13 (0.0-0.78) | 0.06 | 0.0 (0.0-0.20) | 0.15 (0.0-0.78) | <0.01* |

Biologic therapy, no (%)

| TNF blockades after TCZ | 18 (33.3) | 5 (11.9) | 13 (86.7) | ND | 0 (0.0) | 18 (100.0) | ND |
|---|---|---|---|---|---|---|---|
| Tocilizumab | 57 (95) | 39 (92.9) | 15 (100.0) | 39 (92.9) | 18 (100.0) |
| Infliximab | 23 (38.3) | 10 (23.8) | 4 (26.7) | 9 (21.4) | 6 (33.3) |
| adalimumab | 11 (18.3) | 7 (16.7) | 4 (26.7) | 5 (11.9) | 6 (33.3) |
| Etanrecept | 15 (25) | 9 (21.4) | 13 (86.7) | 0 (0.0) | 18 (100.0) |

Except where indicated otherwise, values are the median (range). p-value were determined by Mann-Whitney U-test for continuous variables and by chi-square test for categorical variables. * significant p-value, ** p for trend, ND = not determined, JADAS-10 = the juvenile arthritis disease activity score in 10 joints which is the sum of the physician global assessment of disease activity score (scale 0-10), count of joints with active arthritis (scale 0-10), and normalized C-reactive protein (CRP) level (scale 0-10; calculated as [CRP(in mg/liter)-10]/10, with a CRP level < 10 mg/liter representing a score of zero), ESR = erythrocyte sedimentation rate, TCZ = tocilizumab, HB = hemoglobin, WBC = white blood cells.

**Table (2): Response to Tocilizumab therapy among sixty systemic JIA patients.**
| Treatment response | No. of responders based on lack of subsequent TNF blockades therapy | No. of non responders based on subsequent TNF blockades therapy | Total no. |
|--------------------|-------------------------------------------------------------------|-----------------------------------------------------------------|----------|
| Clinical response *|                                                                   |                                                                    |          |
| Good               | 32                                                                | 5                                                               | 37       |
| Transient          | 1                                                                 | 3                                                               | 4        |
| Poor/absent        | 3                                                                 | 13                                                              | 16       |
| Not classifiable    | 3                                                                 | 0                                                               | 3        |
| Any response **     |                                                                   |                                                                    |          |
| Yes                | 39                                                                | 10                                                              | 49       |
| No                 | 1                                                                 | 9                                                               | 10       |
| Not classifiable    | 1                                                                 | 0                                                               | 1        |
| PGA30 within 6 months |                                                                |                                                                    |          |
| Yes                | 26                                                                | 13                                                              | 39       |
| No                 | 4                                                                 | 6                                                               | 10       |
| Not classifiable    | 10                                                                | 1                                                               | 11       |
| PGA 50 within 6 months |                                                                |                                                                    |          |
| Yes                | 26                                                                | 7                                                               | 33       |
| No                 | 4                                                                 | 12                                                              | 16       |
| Not classifiable    | 10                                                                | 1                                                               | 11       |
| PGA70 within 6 months |                                                                |                                                                    |          |
| Yes                | 21                                                                | 6                                                               | 27       |
| No                 | 10                                                                | 12                                                              | 22       |
| Not classifiable    | 10                                                                | 1                                                               | 11       |
| PGA90 within 6 months |                                                                |                                                                    |          |
| Yes                | 14                                                                | 3                                                               | 17       |
| No                 | 17                                                                | 15                                                              | 32       |
| Not classifiable    | 10                                                                | 1                                                               | 11       |
| Modified JADAS-10 score <5 within 6 months |                  |                                                                  |          |
| Yes | 14 | 1 | 15 |
| --- | --- | --- | --- |
| No | 6 | 14 | 20 |
| Not classifiable | 20 | 5 | 25 |

**CID within 6 months**

| Yes | 17 | 2 | 19 |
| --- | --- | --- | --- |
| No | 23 | 15 | 38 |
| Not classifiable | 2 | 1 | 3 |

**Off glucocorticoid therapy within 6 months**

| Yes | 20 | 4 | 24 |
| --- | --- | --- | --- |
| No | 13 | 11 | 24 |
| Not classifiable | 8 | 4 | 12 |

PGA30 = 30% improvement in the physician global assessment of disease activity score; JADAS-10 = Juvenile Arthritis Disease Activity Score in 10 joints; CID = clinically inactive disease. * A good response was defined as the resolution of signs and symptoms of active disease (fever, rash, adenopathy, hepatosplenomegaly, serositis, and arthritis) and improvement by at least 50% in the levels of inflammation markers (C-reactive protein and erythrocyte sedimentation rate). ** Defined as improvement in fever (if present) and/or arthritis (if present).

Table (3): Frequency of IL6 haplotypes in patients with systemic juvenile idiopathic arthritis and their relation to clinical treatment responses.
| Patients with available data, no. | Haplotype, no /total (%) | p-value |
|----------------------------------|---------------------------|---------|
|                                 | Homozygous genotype (GG) | Homozygous genotype (GC) | Homozygous minor genotype (CC) |
| Clinical response               |                           |                           |                                 | 0.93 |
| At least transient.             | 57                        | 15/21                     | 22/31                           | 4/5  |
| Good                            | 57                        | 13/21                     | 20/31                           | 4/5  |
| No switch to TNF blockades      | 60                        | 13/22                     | 22/32                           | 6/7  |
| Any response *                  | 59                        | 5/22                      | 7/30                            | 5/7  |
| PGA score within 6 months of TCZ therapy. |                           |                           |                                 | 0.83 |
| Improved at least 30%           | 50                        | 11/16                     | 26/30                           | 2/4  |
| Improved at least 50%           | 50                        | 9/16                      | 24/30                           | 2/4  |
| Improved at least 70%           | 50                        | 8/16                      | 17/30                           | 2/4  |
| Improved at least 90%           | 50                        | 6/16                      | 11/30                           | 0/4  |
| Best modified JADAS -10 score <5 within 6 months of TCZ therapy. | 35                        | 5/11                      | 7/20                            | 3/4  | 0.76 |
| CID within 6 months of TCZ therapy. | 57                        | 9/20                      | 9/31                            | 1/6  | 0.53 |
| Off glucocorticoid therapy within 6 months of TCZ therapy. | 48                        | 11/18                     | 11/26                           | 3/4  | 0.84 |

Differences between the haplotypes (P values by chi-square test) were not significant for any response group, IL-6= interleukin-6; CID = clinically inactive disease. * Defined as improvement in fever (if present) and/or arthritis (if present). **The Juvenile Arthritis Disease Activity Score in 10 joints (JADAS-10) is the sum of physician global assessment of disease activity score (scale 0–10), count of joints with active arthritis (scale 0–10), and normalized C-reactive protein level (scale 0–10; calculated as [CRP (in mg/liter) – 10]/10, with a CRP level <10 mg/liter representing a score of 0)., PGA= physician global assessment of disease activity score.

**Figures**
Figure 1

Flow Chart of 60 sJIA patients included in this study.