www.jpnim.com Open Access eISSN: 2281-0692
Journal of Pediatric and Neonatal Individualized Medicine 2021;10(1):e100140
doi: 10.7363/100140
Received: 2019 Dec 17; revised: 2020 Feb 17; rerevised: 2020 Jul 17; accepted: 2020 Jul 21; published online: 2021 Mar 20

Original article

Evaluation of serum calprotectin (MRP-8/MRP-14) levels in patients with juvenile idiopathic arthritis

Tetiana Marushko, Yuliia Holubovska, Yeva-Emiliia Kulchytska

Department of Paediatrics No. 2, Shupyk National Healthcare University of Ukraine, Kyiv, Ukraine

Abstract

Aim: To compare the levels of serum calprotectin (sCal) in patients with juvenile idiopathic arthritis (JIA) depending on the type of therapy to assess the disease activity comprehensively for further treatment correction.

Material and methods: We determined the sCal levels in 74 JIA patients who had normal C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) levels. High-sensitivity C-reactive protein was evaluated in 16 patients. All children were divided into 2 groups depending on the type of therapy. Group I consisted of 33 children on methotrexate (MTX) therapy, 11 of which were in the state of clinical pharmacologic remission. Group II included 41 children treated with biological Disease-Modifying Antirheumatic Drugs (bDMARDs), 14 of which achieved the state of clinical pharmacologic remission. A results analysis was carried out according to the Mann-Whitney U test. A Spearman rank correlation was performed to define the type of correlation between the indicators.

Results: sCal level was 5.5 times higher in patients with JIA (3,300 μg/L), compared with healthy children (600 μg/L) ($p = 0.015$). The highest sCal level observed was among the patients in Group I, who received MTX exclusively both in the active phase of the disease ($U = 71.5$, $p = 0.000006$) and in the state of clinical pharmacologic remission ($U = 11$, $p = 0.00034$). There was a moderate positive correlation of sCal level and disease activity indices, such as the 27-joint Juvenile Arthritis Disease Activity Score (JADAS27) (Spearman's rho = 0.58, $p = 0.0001$) and high-sensitivity CRP (Spearman's rho = 0.56, $p = 0.024$).

Conclusions: sCal levels should be used to monitor the subclinical inflammatory activity in patients with JIA. The use of bDMARDs in JIA treatment is effective.

Keywords

Juvenile idiopathic arthritis, serum calprotectin, methotrexate, biological DMARDs.

Corresponding author

Dr Yeva-Emiliia Kulchytska, Department of Paediatrics No. 2, Shupyk National Healthcare University of Ukraine, 9 Dorohozhytska Str., 04112 Kyiv, Ukraine; email: dr.evaemilia.kulchytska@gmail.com.

How to cite

Marushko T, Holubovska Y, Kulchytska Y-E. Evaluation of serum calprotectin (MRP-8/MRP-14) levels in patients with juvenile idiopathic arthritis. J Pediatr Neonat Individual Med. 2021;10(1):e100140. doi: 10.7363/100140.

Introduction

One of the urgent issues in modern paediatrics is to improve the treatment of chronic rheumatic diseases. Among this group of diseases, juvenile idiopathic arthritis (JIA) occupies a prominent place.

Evaluation of inflammatory activity is a crucial aspect in the management of patients with JIA. In clinical practice, a quantitative C-reactive protein (CRP) test is traditionally used for laboratory monitoring of the disease activity as well as the erythrocyte sedimentation rate (ESR), although their sensitivity and specificity in assessing the inflammation activity are rather limited, especially when the process is subclinical or localized.

It has been noted that the serum level of the S100A8/A9 complex (also known as myeloid-related peptide [MRP] 8/14 or calprotectin) strongly correlates with arthritis activity [1, 2]. This is because calprotectin protein is produced by activated cells directly in synovia and may better reflect the extent of inflammation activity in the synovial membrane than ESR and CRP [3]. The results of the meta-analysis have shown that an increased level of serum calprotectin (sCal) is a risk factor for exacerbation of the disease even in clinically inactive JIA [4, 5], and, in case of a systemic onset of JIA, sCal concentration was 20 times higher than that in patients with sepsis [6, 7].

Traditional terms in rheumatology include the achievement of clinical pharmacologic remission (the state of inactive disease continuing more than 6 months on Disease-Modifying Antirheumatic Drugs [DMARDs] therapy without the use of non-steroidal or steroidal anti-inflammatory drugs) and clinical non-pharmacologic remission (the state of inactive disease continuing more than 12 months without any treatment). In recent years, in addition to traditional terms in rheumatology, it is acceptable to use the term immunological remission when the sCal level remains within normal ranges [8]. Thus, in JIA patients with achieved clinical pharmacologic remission, the level of sCal was less than 250 μg/L before methotrexate (MTX) withdrawal and less than 505 μg/L before etanercept withdrawal. There was no worsening of the disease over the next year [4, 9]. Furthermore, there is an exacerbation in the disease when the sCal levels are more than 740 μg/L in patients with systemic JIA in a state of clinical remission [10].

Similar data were obtained in 121 patients by George: the median levels of sCal were 2 times higher in the active phase of the disease (3,954 μg/L), compared with that in the inactive phase (1,899 μg/L) and 16 times higher compared with healthy children in the control group (233 μg/L) [11]. Some authors have also suggested a weak correlation between sCal levels and Childhood Health Assessment Questionnaire score (CHAQ) results, a moderate correlation with the CRP level, and a significant correlation with the ESR level.

The importance of sCal as a marker of synovial inflammation creates the preconditions for further research to determine its concentration for monitoring the disease activity. Primarily this is needed in patients with a subclinical form of the disease. Traditional markers of inflammation such as ESR and CRP are less sensitive, less specific, and not able to reflect the extent of disease activity adequately. The study addresses several further questions on the correlation between the sCal level and the degree of response to treatment, its possibility of use as a prognostic marker tool, and in the development of new treatment tactics.

Based on the foregoing, the purpose of our study was to compare the levels of sCal in patients with JIA depending on the type of therapy to assess the disease activity comprehensively for further treatment correction.

Methods

A total of 74 patients with JIA were examined (49 [66%] females and 25 [34%] males), aged 3-17 years old, who were on the in-patient treatment in the Cardio-rheumatology department of the Kyiv City Children's Clinical Hospital No. 1. The mean age was 11.3 ± 0.4 years; the disease duration was 5.2 ± 0.4 years. 18 patients had an oligoarticular form of JIA, 39 a polyarticular form, and 17 a systemic form. The control group

included 26 healthy children (16 [61.5%] females and 10 [38.5%] males, mean age 10.7 ± 0.4 years). No children in the control group had any acute or chronic inflammatory diseases. The children were examined according to generally accepted standards.

The study was approved by the Ethical Committee of Shupyk National Healthcare University of Ukraine, and informed consent was obtained from all participants' guardians (according to the instructions of the Helsinki Declaration).

For manual evaluation of sCal levels, the commercially available enzyme-linked immunosorbent assay (ELISA) kits (EK-MRP8/14 BÜHLMANN, Switzerland) were used, and manufacturer instructions were followed. The blood samples were centrifuged for two hours to obtain serum. Concentrations of sCal were determined by the Double-Antibody Sandwich ELISA system in the immunological laboratory of the National Scientific Centre "M.D. Strazhesko Institute of Cardiology" of the National Academy of Medical Sciences of Ukraine in Kyiv. All samples were diluted to the linear range of the assay. The readers of the laboratory assays were blinded with regard to the diagnosis and the inflammatory activity of the patients. Internal control sera were included in the ELISA studies.

Statistical analysis

Serum levels were presented as a median [5^{th}; 95^{th} percentile] since the levels were not normally distributed. Data processing and analysis was performed using statistical methods using Microsoft® Excel® 2010 and IBM® STATISTICA 10.0 and evaluated with the Mann-Whitney U test. Rank correlation analyses between different parameters were performed to obtain the Spearman correlation coefficient. A p-value of ≤ 0.05 was considered significant.

Results

All patients were divided into 2 groups depending on the therapy type.

Group I consisted of 33 children treated with MTX, 11 of which were in clinical pharmacologic remission. The remaining 22 children in the active disease phase were stratified according to the JIA activity index assessed by the 27-joint Juvenile Arthritis Disease Activity Score (JADAS27): low disease activity in 14 patients, moderate disease activity in 5 patients and high disease activity in 3 patients. 27 children (81.8%) preferred the subcutaneous or intramuscular administration route, while 6 patients (18.2%) took MTX orally.

Group II consisted of 41 children treated with biological DMARDs (bDMARDs) exclusively or in combination with MTX, including 14 in clinical pharmacologic remission. There were 27 children in the active disease phase, with low disease activity in 20 children, moderate disease activity in 5 children and high disease activity in 2 children. Patients in Group II were prescribed with the following bDMARDs: adalimumab (24 children), etanercept (2 children), tocilizumab (15 children). Among the children in Group II, 15 (75.0%) were given MTX by the subcutaneous or intramuscular routes, and 5 (25.0%) by the peroral route. Inclusion criteria of Group II comprised having used a bDMARD for at least 6 months before the data collection and evaluation. All samples were collected after at least 1 year of therapy with the prerequisite that a patient had normal levels of CRP and ESR for the last 3 months. In Group II, 21 children had not received MTX for at least 3 months prior to the study (5.9 ± 0.4 months) because of adverse events (in 7 patients) or achieved clinical pharmacologic remission (in 14 patients). Thus, 8 patients were prescribed with adalimumab exclusively, and 13 with tocilizumab. We selected the therapy based on the Ukrainian unified clinical protocol for JIA patients and the *2019 American College of Rheumatology/Arthritis Foundation Guideline for the Treatment of Juvenile Idiopathic Arthritis: Therapeutic Approaches for Non-Systemic Polyarthritis, Sacroiliitis, and Enthesitis* [12, 13]. Initially, conventional synthetic DMARDs such as MTX are administered, but in case of their possible ineffectiveness, bDMARDs should be included in the treatment scheme for at least 3 months from the beginning of treatment.

Clinical data of JIA patients are depicted in **Tab. 1**.

All patients who were evaluated in the study had CRP and ESR levels within the normal ranges. The state of inactive disease continuing more than 6 months on DMARD therapy without the use of non-steroidal or steroidal anti-inflammatory drugs, and the JIA activity index by JADAS27 from 0 to 1 was considered as the clinical pharmacologic remission state. In children out of clinical pharmacologic remission state, despite the rates of CRP and ESR within the normal range, it was an

active phase of the disease on the JADAS27 scale (more than 1).

High-sensitivity CRP was evaluated in 16 patients in the state of clinical pharmacologic remission (9 from Group I and 7 from Group II), and the obtained results were within the normal ranges (they did not exceed 3 mg/L), the median level was 1.8 [0.6; 2.9] mg/L, the sCal levels of these patients were 2,100 [100; 6,000] μg/L, respectively.

Tab. 2 shows the levels of sCal in JIA patients undergoing different treatment and sCal of healthy children in the control group.

Table 1. Clinical data of juvenile idiopathic arthritis (JIA) patients.

Clinical characteristics		Groups and variables	
		Group I, all patients prescribed with MTX for at least 1 year	Group II, all patients prescribed with bDMARDs for at least 6 months
Number of patients, n (%)		33 (44.5%)	41 (55.5%)
Gender (male/female), n		10/23	15/26
Age, years		10.4 ± 0.8	11.6 ± 0.6
Age at disease onset, years		5.9 ± 0.8	6.4 ± 0.7
Time of the disease duration, years		4.5 ± 0.6	5.3 ± 0.6
Distribution of patients according to the disease form, n	Systemic	5	12
	Oligoarticular	10	8
	Oligoarticular extended	4	1
	Oligoarticular with uveitis	0	7
	Polyarticular	18	21
	Polyarticular RF-positive [a]	4	5
	Polyarticular with uveitis	0	6
The number of patients taking steroidal drugs, n (%)	In the anamnesis	15 (45.5%)	33 (80.5%)
	At the time of study	7 (21.2%)	8 (19.5%)
Disease activity index assessed by JADAS27 at the time of study	In children with active phase of disease	4.8 ± 0.7	3.1 ± 0.3
	In children with inactive phase of disease	0.7 ± 0.2	0.8 ± 0.1
Number of patients according to the disease activity state assessed by JADAS27, n (%)	Inactive disease	11 (33.3%)	14 (34.1%)
	Low disease activity	14 (42.4%)	20 (48.8%)
	Moderate disease activity	5 (15.2%)	5 (12.2%)
	High disease activity	3 (9.1%)	2 (4.9%)
Treatment duration, years	With MTX	3.5 ± 0.4	3.7 ± 0.4
	With bDMARDs	-	2.3 ± 0.3
Functional Disability Index by CHAQ		0.43 ± 0.09	0.37 ± 0.09

The values are presented as mean ± standard error of mean (SEM), if not otherwise stated.
[a] Rheumatoid factor (RF)-positive (two or more tests for RF at least 3 months apart during the first 6 months of disease were positive).
bDMARDs: biological Disease-Modifying Antirheumatic Drugs; CHAQ: Childhood Health Assessment Questionnaire; JADAS27: 27-joint Juvenile Arthritis Disease Activity Score; MTX: methotrexate; RF: rheumatoid factor.

Table 2. Serum calprotectin (sCal) levels in juvenile idiopathic arthritis (JIA) patients undergoing different treatment and sCal levels of healthy children in the control group.

Disease phase	Group, total number of examined patients, n and sCal level, μg/L		
	Group I, all patients prescribed with MTX for at least 1 year (n = 33)	Group II, all patients prescribed with bDMARDs for at least 6 months (n = 41)	Control group (n = 26)
Active	n = 22 8,750 [3,700; 17,100]	n = 27 2,900 [1,200; 24,900]	-
Clinical pharmacologic remission	n = 11 3,400 [1,200; 6,000]	n = 14 1,000 [100; 2,800]	n = 26 600 [400; 1,200]

Serum levels are presented as a median [5th; 95th percentile].
bDMARDs: biological Disease-Modifying Antirheumatic Drugs; MTX: methotrexate; sCal: serum calprotectin.

Discussion

Notably, the sCal level was significantly higher in the group of patients who were treated exclusively with MTX and did not receive bDMARDs, both in the active phase of the disease (U = 71.5, p = 0.000006) and in the state of clinical pharmacologic remission (U = 11, p = 0.00034). Among these patients, all collected sCal levels exceeded the 95th percentile in the control group (1,200 µg/L) except the sample from Group 1 patient in the clinical pharmacologic remission state, which level was exactly 1,200 µg/L. In patients undergoing treatment with bDMARDs, 9 (22%) had sCal levels which were within the range of 5th-95th percentile (400 and 1,200 µg/L, respectively) in the control group. In addition, 8 patients out of 9 (88.9%) were in the state of clinical pharmacologic remission. It is important to highlight the fact that the sCal level was 5,850 µg/L less in patients with active phase of the disease and 2,400 µg/L less in patients with clinical pharmacologic remission, both treated with bDMARDs compared with the MTX treatment results. Our results demonstrated that the level of sCal was 5.5 times higher in JIA patients (3,300 µg/L) compared with the control group (600 µg/L) (p = 0.015).

Two patients of Group II in the active disease phase showed high sCal levels (24,900 and 25,900 µg/L). Their therapy tactics have been changed, and another bDMARD has been chosen, resulting in both clinical and laboratory improvement.

There was no significant difference between sCal levels in different forms of the disease (articular or systemic), both during active and inactive disease phases. There was also no significant difference between the sCal level in children of Group II, with the concomitant use of MTX along with bDMARD or bDMARDs exclusively. Thus, the sCal level strongly depends on the degree of activity of the inflammatory process.

According to the ELISA kit manual used, the median sCal level should be 1,140 µg/L, and the 95th percentile – 2,900 µg/L. These values are significantly higher compared with the literature data given above and with the control group data that we obtained. Therefore, a novel approach is needed to standardize this indicator. This could be addressed in future studies.

Our results demonstrated that there was a moderate positive correlation between sCal level and high-sensitivity CRP (Spearman's rho = 0.56, p = 0.024), and between sCal level and JADAS27 index (Spearman's rho = 0.58, p = 0.0001) according to the Spearman's rank correlation test.

No similar works were found in the literature. In our opinion, the level of inflammation is lower when bDMARDs are used, even in the active phase of the disease. When using MTX exclusively, the level of subclinical inflammation is higher even with clinically inactive disease. However, the results may be associated with the fact that bDMARDs have a direct pathogenetic effect on the inflammation process in JIA, in contrast to MTX.

Conclusions

1. sCal level is 5.5 times higher in JIA patients (3,300 µg/L) compared with control group (600 µg/L) (p = 0.015), which indicates the high informativeness of this indicator and the expediency of its use both for monitoring the inflammatory activity of the disease and the treatment effectiveness in patients with JIA;
2. sCal level is significantly higher in the group of patients who was treated exclusively with MTX and did not receive bDMARDs, both in the active phase of disease (U = 71.5, p = 0.000006) and in the state of clinical pharmacologic remission (U = 11, p = 0.00034), which indicates a higher efficacy of complex therapy with bDMARDs in JIA treatment, compared with standard immunosuppressive therapy with MTX;
3. there is a moderate positive correlation of sCal level and disease activity indices, such as JADAS27 (Spearman's rho = 0.58, p = 0.0001) and high-sensitivity CRP (Spearman's rho = 0.56, p = 0.024), which may be useful while considering immunosuppressive therapy withdrawal.

Declaration of interest

The Authors declare no conflicts of interest. There was no funding to perform this study.

References

1. Brun JG, Jonsson R, Haga HJ. Measurement of plasma calprotectin as an indicator of arthritis and disease activity in patients with inflammatory rheumatic diseases. J Rheumatol. 1994;21(4):733-8.
2. Frosch M, Vogl T, Seeliger S, Wulffraat N, Kuis W, Viemann D, Foell D, Sorg C, Sunderkötter C, Roth J. Expression of myeloid-related proteins 8 and 14 in systemic onset juvenile rheumatoid arthritis. Arthritis Rheum. 2003;48(9):2622-6.

3. Youssef P, Roth J, Frosch M, Costello P, Fitzgerald O, Sorg C, Bresnihan B. Expression of myeloid related proteins (MRP) 8 and 14 and the MRP8/14 heterodimer in rheumatoid arthritis synovial membrane. J Rheumatol. 1999;26(12):252-8.
4. Foell D, Frosch M, Schulze zur Wiesch A, Vogl T, Sorg C, Roth J. Methotrexate treatment in juvenile idiopathic arthritis: when is the right time to stop? Ann Rheum Dis. 2004;63(2):206-8.
5. Schulze zur Wiesch A, Foell D, Frosch M, Vogl T, Sorg C, Roth J. Myeloid related proteins MRP8/MRP14 may predict disease flares in juvenile idiopathic arthritis. Clin Exp Rheumatol. 2004;22(3):368-73.
6. Wulffraat NM, Haas PJ, Frosch M, de Kleer IM, Vogl T, Brinkman DMC, Quartier P, Roth J, Kuis W. Myeloid related protein 8 and 14 secretion reflects phagocyte activation and correlates with disease activity in juvenile idiopathic arthritis treated with autologous stem cell transplantation. Ann Rheum Dis. 2003;62(3):236-41.
7. Frosch M, Ahlmann M, Vogl T, Wittkowski H, Wulffraat N, Foell D, Roth J. The myeloid-related proteins 8 and 14 complex, a novel ligand of toll-like receptor 4, and interleukin-1beta form a positive feedback mechanism in systemic onset juvenile idiopathic arthritis. Arthritis Rheum. 2009;60(3):883-91.
8. Nasonov EL, Olyunin YA, Lila AM. Rheumatoid arthritis: the problems of remission and therapy resistance. Rheumatol Sci Pract. 2018;56(3):263-71.
9. Anink J, Van Suijlekom-Smit LW, Otten MH, Prince FH, van Rossum MA, Dolman KM, Hoppenreijs EP, ten Cate R, Ursu S, Wedderburn LR, Horneff G, Frosch M, Vogl T, Gohar F, Foell D, Roth J, Holzinger D. MRP8/14 serum levels as a predictor of response to starting and stopping anti-TNF treatment in juvenile idiopathic arthritis. Arthritis Res Ther. 2015;17(1):200.
10. Holzinger D, Frosch M, Kastrup A, Prince FH, Otten MH, Van Suijlekom-Smit LW, ten Cate R, Hoppenreijs EP, Hansmann S, Moncrieffe H, Ursu S, Wedderburn LR, Roth J, Foell D, Wittkowski H. The Toll-like receptor 4 agonist MRP8/14 protein complex is a sensitive indicator for disease activity and predicts relapses in systemic-onset juvenile idiopathic arthritis. Ann Rheum Dis. 2012;71(6):974-80.
11. George AS. Serum calprotectin levels as a marker of disease activity in children with juvenile idiopathic arthritis. [Master's thesis]. Vellore (India): Christian Medical College, Vellore, 2018, p. 109.
12. https://ips.ligazakon.net/document/view/MOZ16856?an=36, last access: February 2020.
13. Ringold S, Angeles-Han ST, Beukelman T, Lovell D, Cuello CA, Becker ML, Colbert RA, Feldman BM, Ferguson PJ, Gewanter H, Guzman J, Horonjeff J, Nigrovic PA, Ombrello MJ, Passo MH, Stoll ML, Rabinovich CE, Schneider R, Halyabar O, Hays K, Shah AA, Sullivan N, Szymanski AM, Turgunbaev M, Turner A, Reston J. 2019 American College of Rheumatology/ Arthritis Foundation Guideline for the Treatment of Juvenile Idiopathic Arthritis: Therapeutic Approaches for Non-Systemic Polyarthritis, Sacroiliitis, and Enthesitis. Arthritis Care Res (Hoboken). 2019;71(6):717-34.