Original article

Serum survivin expression in systemic onset juvenile idiopathic arthritis in relation to disease activity and macrophage activation

Background: *Systemic onset Juvenile Idiopathic Arthritis (SoJIA) is an auto-inflammatory disease that might be complicated by the life-threatening macrophage activation syndrome (MAS). Survivin, antiapoptotic protein, is associated with significant tissue damage and/or poor response to treatment. We sought to investigate its potential role as indicator of disease activity and predictor of MAS in SoJIA.* ***Methods:*** *We conducted a prospective controlled study that comprised 22 physician-diagnosed SoJIA patients and 20 healthy age and sex matched children as a control group. Patients were subjected to clinical and laboratory assessment every 2 months for one year to detect disease relapse or MAS. Serum survivin was measured at enrollment and in case of activity or MAS development. Other inflammatory markers of activity and MAS were also assayed including CRP, ESR, serum ferritin, ferritin/ESR ratio and triglycerides.* ***Results:*** *Over one year of follow up, ten Patients (45.5%) developed both systemic and articular activity with or without MAS, one patient (4.5%) developed systemic activity only, 5 patients (22.7%) had only articular activity and six patients (27.3%) remained in remission. Serum survivin, ferritin, ESR and the ferritin/ESR ratio were high during activity and even higher in the patients who developed MAS. Ferritin/ESR ratio above three had a 100% sensitivity and 83% specificity in the diagnosis of MAS [Area under the curve (AUC) = 0.96]. Serum survivin level above 25 pg/ml had 100% sensitivity and 90% specificity in detection of disease activity [AUC = 0.96] and a serum level above 67 pg/ml had 100% sensitivity and 94.7% specificity in the prediction of MAS [AUC = 0.99].* ***Conclusion:*** *Survivin might be a potential marker of SoJIA disease activity with special value in the prediction of MAS. Our conclusions are limited by the sample size.*

Keywords: systemic onset juvenile idiopathic arthritis, macrophage activation syndrome, survivin.

Hanan M. Abd El Lateef, Aisha G. Yehia*, Sahar M. Abd Elmaksoud, Elham M. Hossny**

Pediatric Allergy Immunology and Rheumatology Unit, Ain Shams University
**Ministry of Public Health Hospitals ,*
***Department of Clinical Pathology, Ain Shams University, Cairo, Egypt.*

Correspondence:
Hanan M. Abd El Lateef
Lecturer of Pediatrics ,
Pediatric Allergy Immunology & Rheumatology Unit ,
Children's hospital, Ain Shams University, Cairo 11566 ,Egypt .
Email: h.a.lateef @ hotmail.com

INTRODUCTION

Systemic-onset juvenile idiopathic arthritis (SoJIA) is a systemic inflammatory disease which is classified as a category of juvenile idiopathic arthritis (JIA). However, the pathogenesis of SoJIA is associated with dysregulation of the innate immune system, suggesting that it may rather be an autoinflammatory disorder.[1-3] The pathophysiology of SJIA is marked by an increased production of interleukins (IL-1, IL-6 and IL-18).[4] A combination of systemic features and arthritis characterizes SoJIA which has the highest morbidity among other JIA subtypes.[5,6]

Macrophage Activation Syndrome (MAS) is the most dreadful complication of SoJIA with an up to 8% mortality risk.[7] It represents acute overwhelming inflammation and uncontrolled proliferation of T-cells and macrophages leading to extensive production of cytokines and hemophagocytosis.[8,9]

Survivin is the smallest member of the inhibitor of apoptosis protein (IAP) family. It has a key role in regulating cell cycle division and cytokinesis and participates in a variety of signaling pathways.[10] Apoptosis dysregulation is involved in the process of autoimmunity and autoinflammation. Survivin has been identified as a marker of severe rheumatoid arthritis (RA) associated with progressive joint damage and poor response to antirheumatic treatment in aduls.[11] Its expression in CD4+ T cells is activated by TNF-α, a cytokine that is considered the key regulator of inflammation and tissue-destruction in RA.[12] Circulating survivin was reported in a significant portion of JIA patients in association to disease severity.[13]

We sought to evaluate the serum levels of survivin in relation to activity, joint morbidity, and

evolution of macrophage activation syndrome (MAS) in patients with systemic onset juvenile idiopathic arthritis (SoJIA). Our ultimate objective was to assess the prognostic gain from adding this marker to the work up of this disease.

METHODS

Study design:

This was a controlled prospective study that was conducted in the Pediatric Allergy, Immunology and Rheumatology Unit, Children's Hospital, Ain Shams University during the period from August 2015 to August 2016.

Patients' group (Group I): It comprised 22 pediatric patients with physician-diagnosed SoJIA patients who were diagnosed on basis of the American College of Rheumatology (ACR) criteria, as well as the International League of Associations for Rheumatology (ILAR) classification of JIA.[14] They were followed up for one year to assess activity or development of MAS.

Control group (Group II): This group comprised 20 age and sex matched healthy children enrolled from the Outpatient Clinic Children's Hospital, Ain Shams University after exclusion of personal or family history of possible rheumatological illness.

Ethical consideration: An informed consent was obtained from the legal guardian of each subject before enrollment in the study. The study was approved by the local research ethics' Committee of the pediatric department, Ain Shams University.

Study Measurements:

1. Clinical evaluation

- Detailed medical history was recorded concerning age, gender, parental consanguinity, family history of rheumatologic illnesses, age of disease onset, disease duration, disease activity, and medication history including corticosteroids, non-steroidal anti-inflammatory drugs (NSAIDS), disease modifying anti-rheumatic drugs (DMARDS) and biological therapy such as anti-TNF monoclonal antibody and IL-1 receptor antagonist. Co-morbid illness including diabetes, hypertension and obesity were assessed.
- Clinical examination was performed for arthritis including joint swelling, limitation of movement and deformities as well as systemic activity including quotidian fever and rash. The macrophage activation syndrome (MAS) was assessed by looking for pallor, bleeding, non-remitting fever, lymph node enlargement, hepatosplenomegaly, rash, and neurological deficit.
- Patients were followed up over a one-year period by clinical and laboratory evaluation every 2 months and earlier in case of evolution of a new relapse. Simplified Disease Activity Index (SDAI) score was used to assess JIA disease activity. Secondary MAS was diagnosed according to ACR, EULAR and Pediatric Rheumatology International Trials Organization (PRINTO) diagnostic criteria.[15,16]

2- Laboratory investigations

The patients underwent the following tests:

- Complete blood count (CBC) using Beckman Coulter-Gen. system 2, USA. Peripheral blood was smeared and stained by Lishman's stain for white cell differential count. Results were compared to age related reference range.[17]
- Erythrocyte sedimentation rate (ESR) by Westergren method
- Serum alanine transaminase (ALT), aspartate transaminase (AST)
- Serum levels of C- reactive protein (CRP)
- Serum lactate dehydrogenase (LDH) and triglycerides (after fasting for 10 hours) using NADH, Kinetic UV, IFCC rec, Spinreact Kit using Cobas (Roche, Germany)
- Serum concentration of fibrinogen (g/L) by p-Nitrophenyl phosphate, Kinetic, DGKC, Spinreact Kit using a coagulometer (DADE Behring, USA)
- Serum ferritin (ng/mL) using a coagulometer (Au680, Beckman Coulter, USA) with clinical suspension of MAS.
- Serum survivin by enzyme-linked immunosorbent assay (ELISA) at enrollment and was repeated in case of disease activity or development of secondary MAS. It was also measured for the healthy subjects at enrollment.

Statistical Methods:

Data were analyzed using IBM SPSS Statistics version 23 (IBM Corp., Armonk, NY, USA), MedCalc version 15 (MedCalc Software BVBA, Ostend, Belgium). Categorical variables were presented as number and percentage or ratio, and numerical data as mean and SD, range, and percentiles. Normality of numerical data distribution was examined using the Shapiro-Wilk test. Non-normally distributed numerical variables were presented as median and interquartile range and intergroup differences were compared using the Mann-Whitney test (for two-group comparison) or the Jonckheere-Terpstra trend test (for comparison of multiple ranked groups). Correlations were tested using the Spearman rank correlation. Receiver operating characteristic (ROC) curve analysis was

used to examine the value of survivin for discrimination between cases and controls, value of survivin in cases in remission, in relapse & those with MAS and for prediction of disease severity among cases. p values <0.05 were considered significant.

RESULTS

Ages of the patients at enrollment ranged from 3 to 15 years [mean ± SD = 10 ± 3 years]. They were 12 males (55%) and 10 females (45%). The mean age at disease onset was 3.5 years. Clinical data of enrolled children are shown in table 1. Recorded co-morbidities were hypertension in 6 patients (27.3%) and diabetes in five (22.7%). A single patient was maintained on NSAID therapy, with intermittent short courses of corticosteroid therapy upon activity, 18 patients were controlled on corticosteroids and methotrexate, while ten were receiving biological therapy as part of a triple therapy (corticosteroids, DMARDS, biologicals).

At enrollment, 12 patients were in remission, 8 in relapse and 2 had MAS. Throughout the study, 10 patients (45.45%) developed both systemic and articular activity including MAS, one patient had only systemic activity, 5 patients (22.7%) had only articular activity while 6 patients (27.27%) remained in remission throughout the study. Serum survivin was comparable between cases and controls with a no significant statistical difference (37 pg/ml (IQR 29–55) versus 44 pg/ml (IQR 26–61); p=0.837. However, a higher mean serum survivin concentration was observed among patients with MAS (p<0.001).

According to the ROC analysis, serum survivin level above 25 pg/ml had 100% sensitivity and 90% specificity in detecting disease activity (AUC = 0.96); (figure 1) and a serum level above 67 pg/ml had 100% sensitivity and 94.74% specificity in diagnosing MAS (AUC = 0.99); (figure 2).

Ferritin/ESR ratio was significantly higher in the MAS group. A Ferritin/ESR ratio above three had a 100% sensitivity and 83% specificity for diagnosing of MAS (AUC = 0.96). A positive correlation was found between serum survivin and ESR, ferritin and ferritin/ESR ratio during activity and during remission (figure 3).

Table 1. Clinical data of enrolled cases

Pattern of presentation	**No.**	**%**
Quotidian fever	22	100.0%
Arthritis	22	100.0%
Rash	20	90.9%
Back/TMJ affection	3	13.6%
Systemic affection	10	45.5%
System affected	**No.**	**%**
Renal	2	9.1%
Cardiac	8	36.4%
Lungs	5	22.7%
Eyes	5	22.7%
CNS	0	0.0%
Hepatomegaly	8	36.4%
Splenomegaly	5	22.7%
Joint deformity	8	38.1%
Back / TMJ affection	8	36.4%

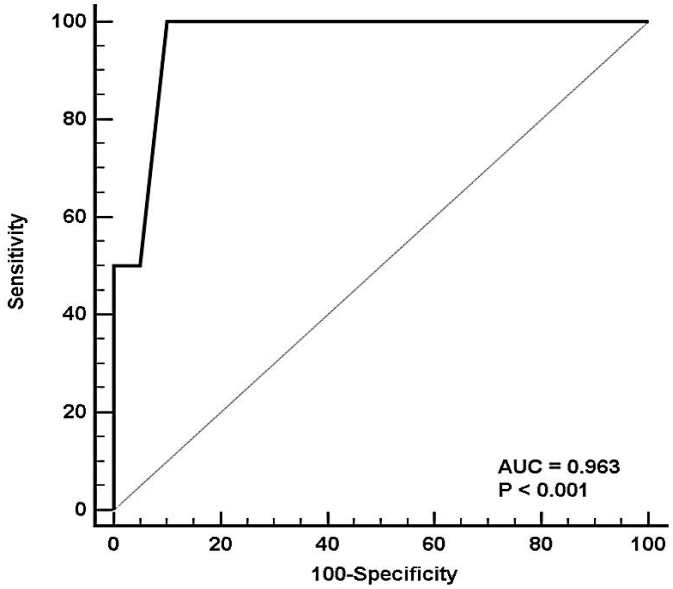

Figure 1. Receiver-operating characteristic (ROC) curve for prediction of relapse at 12 months using serum survivin measured at 6 months.

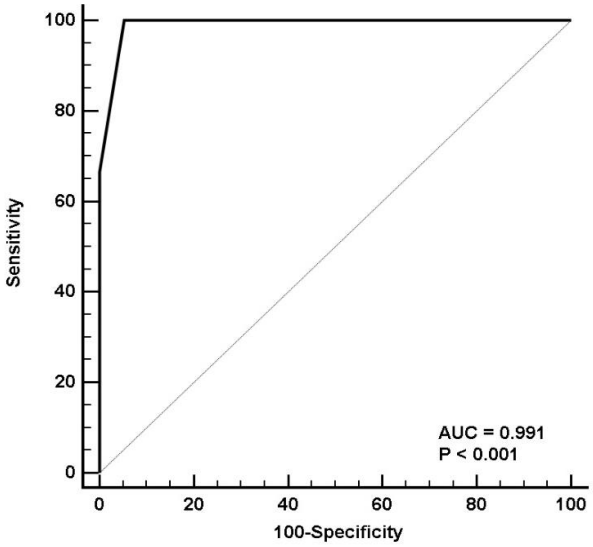

Figure 2. Receiver-operating characteristic (ROC) curve for prediction of MAS at 6 months using serum survivin measured at baseline.

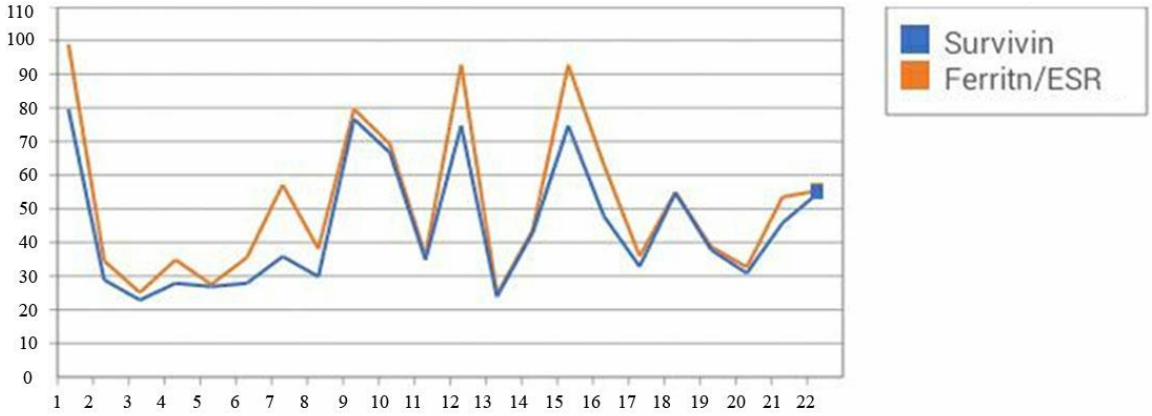

Figure 3. Correlation between baseline serum survivin and baseline ferritin/ESR ratio in patients.

DISCUSSION

At enrollment, survivin was comparable between patients and controls (p=0.84). As a diagnostic marker, it had a sensitivity of 86% and specificity of 35% It therefore cannot be considered reliable as far as the diagnosis of SoJIA is concerned. On the other hand, its levels in patients with active SJIA were significantly higher than those in remission which might point to a value in recognizing disease activity and/or chronicity. A longer follow up period is needed to validate this observation. A relevant study conducted on 70 JIA patients and 29 age and sex matched controls reported higher serum survivin expression in children with JIA compared to the control group.[18] Survivin over-expression was significantly associated with a polyarticular onset in the active phase of the disease. It was found useful in detecting patients with unfavorable prognosis who need extensive pharmacological therapy.[13] Our series comprised SoJIA rather than polyarticular JIA.

We attempted to assess the potential predictive and/or prognostic value of serum survivin in terms of SoJIA activity and MAS development. Serum survivin seems to be a dependable predictor of MAS secondary to SoJIA. Serum survivin level above 67 pg/ml had 100% sensitivity and 94.74% specificity for the diagnosis of MAS (AUC = 0.99).

We compared serum survivin levels in patients with active SoJIA and frequent relapses to patients in disease remission, we noticed that serum survivin could be a predictor of SoJIA activity and may anticipate the resistance to treatment. Serum survivin level above 25 pg/ml had a 100% sensitivity and 90% specificity as far as the detection of disease activity is concerned (AUC = 0.96). In concordance with our findings, Bokarewa and coworkers[19] demonstrated that serum survivin levels were nearly the same presymptomatic adult rheumatoid arthritis patients and controls being an insignificant diagnostic marker. Serum survivin expression then increased significantly with disease progression. They concluded that serum survivin can predict the shift of pre-rheumatoid into rheumatoid disease with 88.3% specificity.

Similarly, another study conducted on adult rheumatoid arthritis reported that serum survivin may be a significant marker of disease progression, joint destruction and resistance to treatment. Survivin positivity at baseline and after 24 months was reported to offer prediction of RA disease progression after 60 months thus being a good prognostic marker of 75% specificity (p=0.001).[20] Moreover, John Hopkins Rheumatology Center stated that serum survivin is significantly higher in rheumatoid arthritis patients with erosive disease manifested by joint destruction as compared to non-erosive rheumatoid arthritis (95% specificity; p=0.002).[21]

A relevant study that tried to set a cut off value for serum survivin in the assessment of rheumatoid activity in adults revealed a level of 330 pg/ml versus 121 pg/ml in healthy controls (p=0.002) whereas cutoff value of serum survivin 430 pg/ml indicated erosive disease as compared to non-erosive rheumatoid arthritis (127 pg/ml; p=0.002). The authors noticed no value of measuring synovial fluid survivin.[22]

The concentration of survivin was reported to be significantly higher among JIA patients positive for anti-cyclic citrullinated peptide autoantibodies, survivin can thus act as a unique biomarker of disease severity even in the early stage of the disease. In all synovial fluid samples, the concentration of survivin was higher than its matched level in the serum (p = 0.003).[18] On the contrary, a study on 123 adult rheumatoid arthritis patients revealed that survivin plasma levels were detectable in only 13.8% of patients. Survivin positivity did not correlate with RF (p = 0.180) or anti-CCP antibody levels (p = 0.926). Moreover, only 17.8% of patients with joint erosions had positive survivin levels (p = 0.35).[23]

Our patients with active SoJIA activity showed significantly elevated ESR than those in remission (mean= 42.6 mm/h; p=0.028), while the elevation of other inflammatory markers including serum ferritin, CRP, and platelet count did not reach statistical significance at enrollment. Grom and coworkers[24]reported increased ESR, platelet count and serum ferritin at time of SoJIA activity while Blessing et al.[25] who studied 16 newly diagnosed SoJIA patients reported anemia, leukocytosis, and thrombocytosis, but normal serum ferritin level.

We also assayed ferritin, ferritin/ESR ratio and serum survivin as markers of MAS. They were significantly increased in our SoJIA patients with MAS compared to the rest of the patients. Ferritin and ferritin/ESR ratio are inexpensive markers of MAS development in active SoJIA. The potential ability of serum survivin, serum ferritin, and ferritin/ESR ratio to predict MAS and assess SoJIA activity was studied using ROC curves and evaluating the area under the curve (AUC). Ferritin/ESR ratio was found to be a very good predictor of MAS with high specificity. A ratio above three had 100% sensitivity and 83% specificity for the diagnosis of MAS (AUC = 0.96).

Gorelik et al.[26] recommended ferritin/ESR ratio >80 with (100% sensitivity and 100% specificity) for early MAS prediction in SJIA, that almost agrees with our results. On the contrary, a relevant study concluded that no single laboratory value including ferritin/ESR ratio is associated with MAS development; they studied 30 SoJIA patients 8 of whom had MAS.[27] Among our series, a serum ferritin value above 287 ng/ml had 100% sensitivity and 84 % specificity (AUC = 0.98) for diagnosing MAS. The international guidelines indicate that serum ferritin level above 500 ng/ml (86% sensitivity, 95% specificity and AUC = 0.76) is one of the diagnostic criteria of MAS whereas the PRINTO diagnostic criteria (2005) for secondary MAS considered serum ferritin level above 684 ng/ml as diagnostic with 72% sensitivity and 97% specificity.[28] Kostik et al.[29] assumed that serum ferritin above 400 ng/ml is among the diagnostic criteria of MAS in SoJIA (100% sensitivity and 100% specificity). On the contrary, McClain and Eckstein[30] recommended ferritin level above 3000 ng/ml for MAS prediction in pediatric SoJIA and above 50000 ng/ml in adults. Our demonstrated cutoff value of serum ferritin for MAS prediction was much lower. Our observation is indeed limited by the sample size.

We observed a strong positive correlation between serum survivin and inflammatory markers that are mentioned in the ACR diagnostic criteria of SoJIA and in Jordan's et al.[31] diagnostic criteria of MAS as well. Serum survivin increased with the increase of ESR, ferritin and ferritin / ESR ratio in patients with active symptoms or suffering from MAS. This was assessed at baseline and after 6 months. Such observation points to the value of serum survivin in predicting the disease progression and MAS development.

Our study has several limitations; the sample size and consecutive manner of case recruitment hindered the interpretation of the results especially that the MAS group comprised only 4 patients. Also, the relatively short follow up period (one year) did not allow for accurate assessment of medication influence such as biologics on the survivin expression.

In conclusion, serum survivin might be a useful marker for the cytokine storm that underlies MAS and might be also indicative of SoJIA activity and disease progression. Our observations are supported by positive correlations between serum survivin and other markers of SoJIA activity and MAS induction. Further prospective wider scale studies are needed to validate our observations.

REFERENCES

1. **Ravelli A, Martini A.** Juvenile idiopathic arthritis. Lancet 2007;369(9563):767-78.
2. **Woerner A, von Scheven-Gête A, Cimaz R, Hofer M.** Complications of systemic juvenile idiopathic arthritis: risk factors and management recommendations. Expert Rev Clin Immunol 2015;11(5):575-88.
3. **Cimaz R.** Systemic-onset juvenile idiopathic arthritis. Autoimmun Rev 2016;15(9):931-4.
4. **Silva JR, Brito I.** Systemic juvenile idiopathic arthritis versus adult-onset Still´s disease: the pertinence of changing the current classification criteria. Acta Rheumatol Port 2020;45(2):150-1.
5. **Gurion R, Lehman T, Moorthy L.** Systemic arthritis in children: A review of clinical presentation and treatment. Int J Inflam 2012;2012:271569.
6. **Macaubas C, Nguyen KD, Peck A, Buckingham J, Deshpande C, Wong E, et al.** Alternative activation in systemic juvenile idiopathic arthritis monocytes. Clin Immunol 2012;142(3):362–72.
7. **Minoia F, Davi S, Horne AC, Demirkaya E, Bovis F, Li C, et al.** Clinical features, treatment, and outcome of macrophage activation syndrome complicating systemic juvenile idiopathic arthritis: A multinational, multicenter study of 362 patients. Arthritis Rheumatol 2014;66(11):3160-9.
8. **Crayne CB, Albeituni S, Nichols KE, Cron RQ.** The immunology of macrophage activation syndrome. Front Immunol 2019;10:119.
9. **Boom V, Anton J, Lahdenne P, Quartier P, Ravelli A, Wulffraat NM, et al.** Evidence-based diagnosis and treatment of macrophage activation syndrome in systemic juvenile idiopathic arthritis. Pediatr Rheumatol Online J 2015;13:55
10. **Xun C, Ning D, Caiguo Z, Wentao Z.** Survivin and tumorigenesis: Molecular mechanisms and therapeutic strategies. J Cancer 2016;7(3):314-23.
11. **Bokarewa M, Turkkila M, Bratt S, Erlandsson M, Pullerits R.** Utility of survivin measurements for early recognition of clinically suspect arthralgia and diagnosis of rheumatoid arthritis in west Sweden. Ann Rheum Dis 2016;75:692.
12. **Aterido A, Palacio C, Marsal S, Ávila G, Julià A.** Novel insights into the regulatory architecture of CD4+ T cells in Rheumatoid Arthritis. PLoS One 2014;9(6):e100690.
13. **Galeotti L, Adrian K, Berg S, Tarkowski A, Bokarewa M.** Circulating survivin indicates severe course of juvenile idiopathic arthritis. Clin Exp Rheumatol 2008;26(2):373-8.

14. **Petty R, Southwood T, Manners P, Baum J, Glass D, Goldenberg J, et al.** International League of Associations for Rheumatology classification of juvenile idiopathic arthritis: second revision, Edmonton, 2001. J Rheumatol 2004;31(2):390-2.
15. **Anderson J, Caplan L, Yazdany J, Robbins M, Neogi T, Michaud K, et al.** Rheumatoid Arthritis Disease Activity Measures: American College of Rheumatology Recommendations for use in clinical practice. Arthritis Care Res 2012;64(5):640-7.
16. **Ravelli A, Minoia F, Davì S, Jordan M, Horne A, Bovis F, Pistorio A, et al.** 2016 Classification criteria for macrophage activation syndrome complicating systemic juvenile idiopathic arthritis: A European League Against Rheumatism/American College of Rheumatology/ Paediatric Rheumatology International Trials Organisation Collaborative Initiative. Arthritis Rheumatol Dis 2016;68(3):566-76.
17. **Buttarello M, Plebani M.** Automated blood cell counts: state of the art. Am J Clin Pathol 2008;130(1):104-16.
18. **Lipińska J, Kaszkowiak M, Małachowska B, Świdrowska-Jaros J, Smolewska E.** Concentration of survivin in children with oligo- and polyarticular juvenile idiopathic arthritis (JIA): diagnostic and prognostic value—a single-center study. Arthritis Res Ther 2021;23(1): 40.
19. **Bokarewa M, Mikael B, Erlandsson M, Dahlqvist S.** Survivin but not Fms-like tyrosine kinase 3 ligand is up-regulated before the onset of rheumatoid arthritis: a pilot study. Arthritis Res Ther 2014;16(1):R45.
20. **Svensson B, Hafström I, Erlandsson MC, Forslind K, Bokarewa M.** Smoking in combination with antibodies to cyclic citrullinated peptides is associated with persistently high levels of survivin in early rheumatoid arthritis: a prospective cohort study. Arthritis Res Ther 2014;16(1):R12.
21. Johns Hopkins Arthritis center. Does Survivin and Survivin Antibodies Play a Role in Rheumatoid Arthritis. 2005. Available from: www.hopkinsarthritis.org. Accessed on: October 21, 2020.
22. **Bokarewa M, Lindblad S, Bokarew D, Tarkowski A.** Balance between survivin, a key member of the apoptosis inhibitor family, and its specific antibodies determines erosivity in rheumatoid arthritis. Arthritis Res Ther 2005;7(2):R349-58.
23. **Barbara J, Sonja P, Saša Č, Žiga R, Matija T.** Survivin polymorphism is associated with disease activity in rheumatoid arthritis patients. Pharmacogenomics 2016;17(1):45-9.
24. **Grom AA, Ilowite NT, Pascual V, Brunner HI, Martini A, Lovell D, et al.** Rate and clinical presentation of macrophage activation syndrome in patients with systemic juvenile idiopathic arthritis treated with canakinumab. Arthritis Rheumatol 2016;68(1);218–28.
25. **Bleesing J, Prada A, Siegel DM, Villanueva J, Olson J, Ilowite NT, et al.** The diagnostic significance of soluble CD163 and soluble interleukin-2 receptor alpha-chain in macrophage activation syndrome and untreated new onset systemic juvenile idiopathic arthritis. Arthritis Rheum 2007;56(3):965-71.
26. **Gorelik M, Fall N, Altaye M, Barenes MG, Thompson SD, Grom AA, et al.** Follistatin-like protein 1, and the ferritin / erythrocyte sedimentation rate ratio are potential biomarkers for dysregulated gene expression and macrophage activation syndrome in systemic juvenile idiopathic arthritis. J Rheumatol 2013;40(7):1191-9.
27. **Gillispie M, DeGuzman M, Gorelik M, Vogel T.** Ferritin: ESR, A Predictor of MAS? ACR 2017 Pediatric Rheumatology Symposium. Arthritis Rheumatol 2017;69 (4). Available from: https://acrabstracts.org/abstract/ferritinesr-a-predictor-of-mas/. Accessed on December 11, 2020.
28. **Minoia F, Bovis F, Davi S, Horne AC, Fischbach M, Frosch M, et al.** Development and initial validation of the MS score for diagnosis of macrophage activation syndrome in systemic juvenile idiopathic arthritis. Ann Rheum Dis 2019;78(10):1357-62.
29. **Kostik MM, Dubko MF, Masalova VV, Snegireva LS, Kornishina TL, Irina A Chikova IA, et al.** Identification of the best cutoff points and clinical signs specific for early recognition of Macrophage activation syndrome in systemic juvenile idiopathic arthritis. Semin Arthritis Rheum 2015;44(4):417-22.
30. **McClain KL, Eckstein O.** Clinical features and diagnosis of Hemophagocytic lymphohistiocytosis. UpToDate 2017. Available from: https://www.uptodate. com/contents/clinical-features-and-diagnosis-of-hemophagocytic–lymphohistiocytosis. Accessed on: October 21, 2020
31. **Jordan MB, Allen CE, Weitzman S, Filipovich AH, McClain KL.** How I treat hemophagocytic lymphohistiocytosis. Blood 2011;118(15):4041–52.