

## 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.

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## 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.<sup>1-3</sup> The pathophysiology of SJIA is marked by an increased production of interleukins (IL-1, IL-6 and IL-18).<sup>4</sup> A combination of systemic features and arthritis characterizes SoJIA which has the highest morbidity among other JIA subtypes.<sup>5,6</sup>

Macrophage Activation Syndrome (MAS) is the most dreadful complication of SoJIA with an up to 8% mortality risk.<sup>7</sup> It represents acute overwhelming inflammation and uncontrolled proliferation of T-cells and macrophages leading to

extensive production of cytokines and hemophagocytosis.<sup>8,9</sup>

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.<sup>10</sup> 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 adults.<sup>11</sup> Its expression in CD4+ T cells is activated by TNF- $\alpha$ , a cytokine that is considered the key regulator of inflammation and tissue-destruction in RA.<sup>12</sup> Circulating survivin was reported in a significant portion of JIA patients in association to disease severity.<sup>13</sup>

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.<sup>14</sup> 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.<sup>15,16</sup>

#### 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.<sup>17</sup>
- 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  $\pm$  SD = 10  $\pm$  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

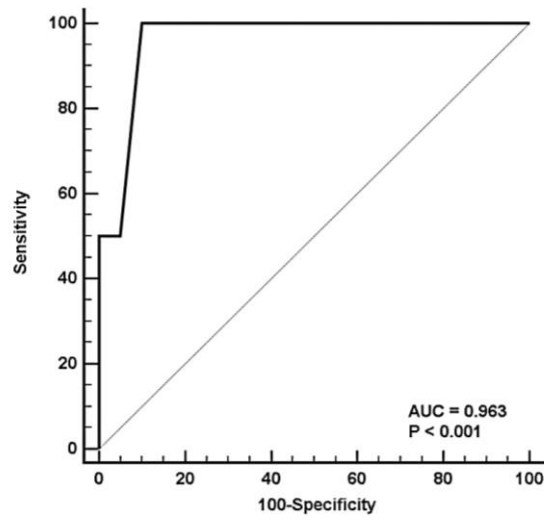
articular activity including MAS, one patient had only systemic activity, 5 patients (22.7%) had only articular activity while 6 patients (27.27%) remained in remission throughout the study. Serum survivin was comparable between cases and controls with a no significant statistical difference (37 pg/ml (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).

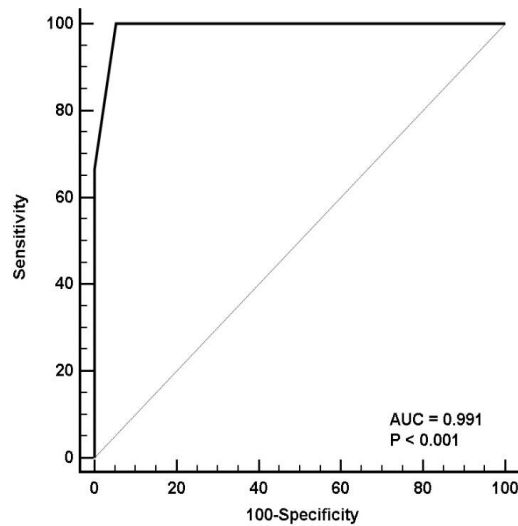
Ferritin/ESR ratio was significantly higher in the MAS group. A Ferritin/ESR ratio above three had a 100% sensitivity and 83% specificity for diagnosing of MAS (AUC=0.96). A positive correlation was found between serum survivin and ESR, ferritin and ferritin/ESR ratio during activity and during remission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

**Table 1.** Clinical data of enrolled cases

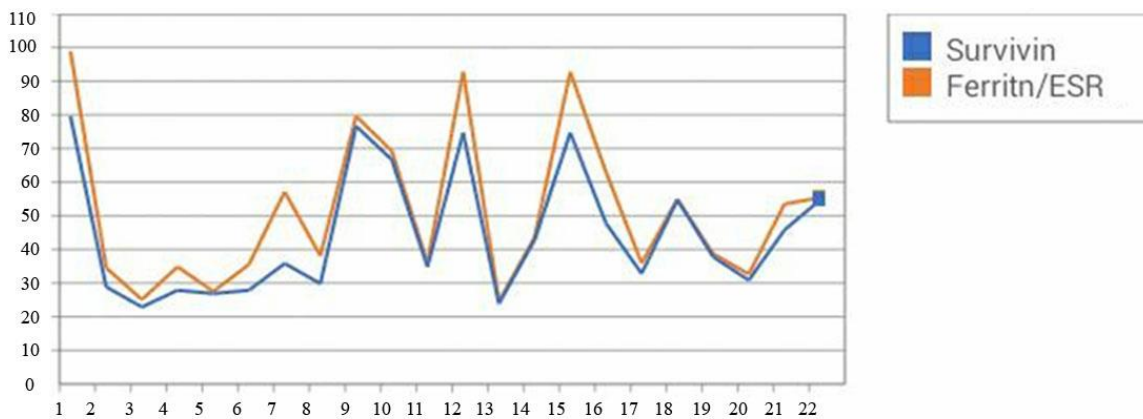
<b>Pattern of presentation</b>	<b>No.</b>	<b>%</b>
Quotidian fever	22	100.0%
Arthritis	22	100.0%
Rash	20	90.9%
Back/TMJ affection	3	13.6%
Systemic affection	10	45.5%
<b>System affected</b>	<b>No.</b>	<b>%</b>
Renal	2	9.1%
Cardiac	8	36.4%
Lungs	5	22.7%
Eyes	5	22.7%
CNS	0	0.0%
Hepatomegaly	8	36.4%
Splenomegaly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

Childhood asthma remains a heterogeneous condition, and verification of its various presentations, risk factors, and outcomes is important because of its therapeutic and prognostic relevance. Further investigation into the immunopathology and genetic basis underlying childhood phenotypes is important so therapy can be tailored accordingly.<sup>10</sup>

The current study aimed to delineate serum levels of INF- $\beta$  and analyze NOD2 gene polymorphism in a group of children with well-characterized asthma phenotypes (wheezy and cough phenotypes).

The IFN family represents a group of cytokines that play a central role in the protection against exacerbation of various infections and pathologies, including asthma. They play an indispensable role in the host immune system to fight off pathogens, which seems to be altered in both pediatric and adult asthmatics.<sup>11</sup>

Our results revealed that serum INF- $\beta$  was significantly lower in asthmatic children compared to healthy controls with lack of significant difference between patients with wheezy and cough phenotypes. Several studies, *both in vivo and in vitro*, have investigated the levels of serum INF- $\beta$  in asthma both in children and adults with contradictory results. However, none of them investigated serum INF- $\beta$  in different asthma phenotypes.

A study by Zhu et al., compared bronchial mucosal INF- $\beta$  expression before rhinovirus infection and after rhinovirus infection in ten atopic asthmatic patients with mean age  $23 \pm 1.4$  and fifteen healthy controls with mean age  $27 \pm 2.3$ , recruited from Imperial College London Healthcare NHS Trust (St Mary's Hospital). They observed INF- $\beta$  deficiency in the bronchial epithelium after viral infection in asthmatic patients *in vivo* and this was related to greater viral load, worse airway symptoms, airway hyper responsiveness, and reductions in lung function, together with lower frequencies of bronchial subepithelial monocytes/macrophages expressing INF- $\beta$ .<sup>12</sup> On the other hand, some studies observed that IFN response to viruses in airway epithelial cells *in vitro* was remarkably similar between subjects with and without asthma where the immune response was not deficient but rather modified by the atopic state.<sup>13,14</sup> For example, there are increased numbers of airway mucous cells in asthma and this subset of airway epithelial cells may have inherent differences in susceptibility to viral infection<sup>15,16</sup> as well as a distinct influence on innate and adaptive

immune responses that indirectly impact viral clearance.<sup>17,18</sup>

This discrepancy among studies regarding antiviral IFN response may be related to several factors. First, it may be related to the type of virus being studied and the possibility that different viruses might elicit distinct types of IFN responses from the host cell. Second, it could be related to airway epithelial cell culture conditions. Third, it could depend on the types of asthmatic subjects selected for study considering the possibility that asthma severity might influence antiviral response given the differences in immune characteristics among mild, moderate, and severe asthma subsets.<sup>13</sup> Type I INF signaling is especially important for the control of viral infections.<sup>19</sup> Several trials were carried out to evaluate the efficacy of inhaled INF- $\beta$  in combating viral induced asthma exacerbation, both in adults<sup>4,19,20</sup> and children<sup>21,22</sup> but results are yet to be validated.

Concerning NOD2 gene polymorphism (rs2066845), our study revealed that the heterozygous GC genotype and homozygous GG genotype were associated with a higher asthma risk. G allele frequency was significantly higher in total asthmatic cases when compared to healthy control group. Also, G allele showed significantly higher frequency among asthmatics with positive family history and with positive parental smoking. However, no significant difference in the genotype pattern was detected between the two included asthma phenotypes. Regarding the relation between the serum levels of INF- $\beta$  and different genotypes of the NOD2 gene (rs2066845) we observed that serum levels of INF- $\beta$  were significantly lower in patients with the homozygous GG genotype compared to controls of the same genotype. Also, in both studied asthma phenotypes (wheezy and cough) CC genotype was found to be associated with higher serum levels of INF- $\beta$  compared to both GC and GG genotypes.

Genetic polymorphisms in NOD1 and NOD2 (Caspases Activation and Recruitment Domain 15"CARD15") genes were previously found to be associated with the pathophysiology of allergic asthma.<sup>23</sup> Three functionally relevant single nucleotide polymorphisms (SNPs) in NOD2, including rs2066844 (Arg702Trp), rs2066845 (Gly908Arg) and rs869147565 (Leu1007Pro), have been studied in school children in Germany. The authors suggested that these SNPs might be responsible for the development of asthma and allergies in children.<sup>24</sup> Also, a Chinese study have found that NOD2 gene rs3135499 polymorphism genotype as a risk factor may influence the

development of asthma.<sup>7</sup> Another study performed in a Caucasian adult population have found that the rs1077861 T allele decreased the risk of asthma, whereas the rs3135500 A allele was significantly associated with an increased risk of asthma.<sup>25</sup> On the other hand, a relatively recent study including asthmatic Tunisian children has found no association between NOD2 gene polymorphism and the development of asthma.<sup>26</sup>

Airway exposure to NOD2 ligand is suggested to prevent tolerance mechanisms from developing in the lung, suppressing the induction of antigen-specific CD4+forkhead box protein 3 (FOXP3) + regulatory T (Treg) cells while at the same time promoting interleukin 4 (IL-4)-secreting effector CD4 T cells. NOD2 ligand was reported to induce selective expression of the innate cytokines thymic stromal lymphopoietin (TSLP) and IL-25 and TSLP-dependent induction of the Tumor necrosis factor (TNF) family costimulatory molecule OX40 ligand (OX40L), with subsequent susceptibility to develop asthmatic disease.<sup>27</sup>

Viral infection results in significant induction of NOD2 expression, activating downstream Nuclear Factor Kappa-B (NF-κB) and IFN pathways.<sup>28</sup> In addition, during viral infections, NOD2 can also be relocated to the mitochondria by its interaction with the mitochondrial antiviral signaling (MAVS) protein inducing the production of type I IFNs.<sup>29</sup>

In conclusion, our study demonstrated lower serum levels of INF-β in asthmatic children compared to healthy controls which could highlight the potential role of IFNs-based therapies for asthma. Further, this study provided evidence that *NOD2* gene rs2066845 polymorphism genotypes differed between asthma and healthy controls in a cohort of Egyptian children. The rs2066845 G allele as a risk factor may influence the development of asthma. Eventually, no significant differences were detected between the two included clinical phenotypes regarding serum biomarker and the genetic pattern. Nonetheless, due to the limited sample size, further studies are needed to verify our results.

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