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Serum, synovial and mRNA expression of interleukin-33 in juvenile idiopathic arthritis patients: Potential role as a marker of disease activity and relation to musculoskeletal ultrasound

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ABSTRACT

Aim of the work: To measure interleukin-33 (IL-33) serum and synovial fluid (SF) levels as well as its relative expression in peripheral blood mononuclear cells (PBMC) of juvenile idiopathic arthritis (JIA) patients and to study their relation to clinical, laboratory and musculoskeletal ultrasound characteristics, disease activity and functional status.

Patients and methods: The study included 60 JIA patients and 60 healthy controls and SF levels were measured in 20. Juvenile arthritis disease activity score (JADAS27) and Juvenile Arthritis Multidimensional Assessment Report (JAMAR) were assessed; Ten-joint grey scale (GS) and power Doppler (PD) MSUS score was performed. Rheumatoid factor (RF) titer and C-reactive protein (CRP) levels were measured.

Results: In JIA patients, serum IL-33 levels (median 12.6; 7.4–23.8 ng/l) and its relative mRNA expression (median 3.3; 2.5–3.7) were significantly higher than their levels in the controls (median 1.7; 0.8–2.4 ng/l and median 1 ng/ml; $p < 0.001$). Polyarticular subtype ($n = 20$) had higher IL-33 serum levels compared to oligoarticular ($n = 28$, $p < 0.001$) and systemic-onset ($n = 12$, $p = 0.006$) subtypes. In JIA patients, the serum and SF levels of IL-33 significantly correlated with JADAS27 ($p < 0.001$ and 0.002 respectively), CRP ($p < 0.001$ and 0.007 respectively), GS ($p < 0.001$ and 0.001 respectively) and PD ($p < 0.001$ and 0.005 respectively). Serum IL-33 correlated with RF ($p = 0.039$) while, SF IL-33 correlated with physical function ($p = 0.02$).

Conclusions: JIA patients have significantly elevated IL-33 serum concentrations and mRNA expression that considerably correlated with different inflammatory parameters, RF and physical function suggesting that it could be a valuable marker of JIA disease activity and implies a possible prognostic role.

1. Introduction

Juvenile idiopathic arthritis (JIA) refers to a series of unexplained etiology inflammatory arthritides that occur before 16 years and with a minimum of six weeks duration [1]. Although, the exact etiology of JIA is still not fully clear, recent advances in molecular biology in the last decade led to better understanding of the disease [2]. Current insight into the immunopathogenesis of JIA in Egyptian patients emphasis a key role of cytokines and other immune mediators such as tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1 [3,4], markers of apoptosis [5,6] and gene polymorphism [7] as well as in other studies implicating IL-6 [8], chemokines [9], calgranulins and toll-like receptors [10] in the initiation and propagation of inflammatory process and many of them have been considered as possible therapeutic targets [11].

Expression of IL-33, a cytokine that belongs to the IL-1 family, is induced by damage of the epithelial and endothelial cells [12] and it acts as an alarm signal to maintain homeostasis through binding with its specific receptor known as suppression of tumorigenicity 2 (ST2) [13]. The IL33/ST2 axis plays an important immunoregulatory role and it is implicated in the pathogenesis of cancer, asthma, type 2 diabetes mellitus (DM) and many infectious and inflammatory diseases [14]. Elevated serum IL-33 and soluble ST2 concentrations have been established in several rheumatic auto-immune diseases, such as rheumatoid arthritis (RA) [15],

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ankylosing spondylitis (AS) [16], adult-onset stills disease [17] systemic lupus erythematosus [18] and inflammatory bowel disease (IBD) [19]. Moreover, Li et al. [20] found individuals with single nucleotide polymorphism with CC genotype of rs7044343 gene to have lower IL-33 serum concentrations and less susceptibility to develop RA. However, to date, only a few reports reported the possible association between IL-33 serum concentrations and JIA [21,22] and with no available data about its synovial fluid (SF) concentrations.

Musculoskeletal ultrasound (MSUS) has emerged as an essential and attractive instrument for pediatric rheumatologists. In Egyptian JIA patients, power Doppler US (PDUS) was helpful in detecting subclinical synovitis and in indicating the degree of disease severity. It should be used in standard clinical practice for JIA patients' assessment for better monitoring and disease management [23].

The aim of this study was to measure serum, SF levels and relative expression in peripheral blood mononuclear cells (PBMC) of IL-33 in JIA patients and to study their relation to clinical, laboratory and MSUS characteristics, disease activity and functional status.

2. Patients and methods

Sixty JIA patients fulfilling the International League of Associations for Rheumatology (ILAR) classification criteria for JIA [24] were enrolled in this study from the Rheumatology and Pediatrics Departments, outpatient clinics, Benha University Hospital and 60 apparently healthy children with comparable age and gender were selected as a control group. Patients were excluded if they had a recent infection, malignancy, other autoimmune disease, DM, IBD or received anti-TNF in the past 3 months. SF samples from patients with a history of local steroid injection in the previous 6 months were excluded. Written consent was given by the legal guardian of both JIA patients and controls before being enrolled. The study was registered ClinicalTrials.gov ID: NCT03835624; Feb.2019 and was approved by Benha Faculty of Medicine ethics committee.

All JIA patients were subjected to detailed history taking, full clinical examination with emphasis on pattern and distribution of articular involvement, presence of uveitis and other extra-articular findings and current medications. Juvenile arthritis disease activity score (JADAS27) [25] was assessed in oligoarticular and polyarticular patients as its performance in systemic-onset is unclear [26]. Disease activity was graded to high, moderate and low according to Beukelman et al. [27] and inactive disease was identified using Wallace criteria [28]. Juvenile Arthritis Multidimensional Assessment Report (JAMAR) [29] was used to assess functional status and quality of life (QoL) of JIA patients. It is a composite scale which consists of 15 items and can be reported by the patient or his parents. The evaluation involved physical function (PF) scale (0–45), pain visual analogue scale (VAS) (0–10), physician assessment of disease activity (0–10), health related (HRQoL) (0–30) and self-assessment of patient's overall well-being (0–10).

Venous blood (10 ml) was collected and separated into two tubes: one on EDTA for Complete blood count (CBC) and IL-33 relative gene expression and the other left to clot for 10–15 min. Serum was centrifuged (2000 rpm for 10 min.) and used for clinical chemistry tests: aminotransferases (AST, ALT), ferritin, C-reactive protein (CRP), rheumatoid factor (RF) and serum IL-33. Another blood sample on citrate was collected to assay the erythrocyte sedimentation rate (ESR). 20 synovial fluid samples were aspirated in concordance with blood sampling for assay of IL-33. Serum and SF IL-33 level was assessed by enzyme-linked immunosorbent assay (ELISA) sandwich technique (Cat No: 201-12-0045, Sunred bio Co., Shanghai, China).

IL-33 mRNA relative expression on PBMCs: RNA was extracted from 0.5 ml EDTA-anticoagulated blood samples according to the manufacturer's instructions (GeneJET Whole RNA purification Mini kit, Thermo Scientific, USA). Reverse transcription (RT) was accomplished using *Maxime*RT PreMix Kit, (Intron biotechnology, Korea). The RT reactions were performed in an Applied Biosystems Veriti™ 96-well thermal cycler (Singapore), with the thermal conditions; for 2 min at 95 °C; 15 s at 95 °C; 15 s at 60 °C, and 45 s at 72 °C. cDNA level was determined using pre-designed primers and Taqman probes (Applied Biosystems) over 40 cycles. β-actin gene was used as a control for cDNA. The data were normalized against β-actin expression [30].

MSUS examination was performed using linear high-frequency (8–13 MHz) transducer (Logiq e, GE Medical Systems, Wisconsin, USA) according to the EULAR guideline [31] on the same day of clinical examination. Ten joints score [32] was used to assess synovitis using grayscale (GS) and power Doppler (PD) according to 4-points semi-quantitative grades through a bilateral examination of 2nd metacarpophalangeal (MCP) joints, wrists, elbows, knees and ankles. Image analysis was executed by two experienced observers (WH; rheumatologist and SA; radiologist), one of them (SA) blinded to patients' clinical and laboratory data, with a significant inter-observer agreement regarding both GS ($k = 0.92$, $P < 0.001$) and PD ($k = 0.96$, $P < 0.001$) synovitis scores.

Statistical analysis: It was carried out using the SPSS version 20 software (Inc, Chicago, ILL Company). Normally distributed data were presented as mean and standard deviation (SD), while, non-parametric data were shown as median and interquartile range (IQR). Student's *t*-test and one way analysis of variance (ANOVA) were used to compare variables of normally distributed data. Mann-Whitney and Kruskal-Wallis tests were used for non-parametric variables. We used Fisher's exact test and Chi-square test (X^2) to compare categorical variables. Spearman's correlation coefficient was used to test linear associations between variables. The diagnostic performance of serum IL33 levels in comparison to ESR and CRP for disease activity measured using JADAS27 and PD scales was calculated using receiver Operating Curve (ROC). The area under the Curve (AUC), specificity, sensitivity and the best cut-off value were calculated.

3. Results

The mean age of the 60 patients was 7.3 ± 2.8 years; 42 females and 18 males (F:M 2.3:1). 28 (46.7%) were oligoarticular, 20 (33.3%) polyarticular and 12 (20%) were systemic-onset JIA. None of the patients had psoriatic or enthesitis-related arthritis. Characteristics of the JIA patients according to the subtypes are presented in Table 1. The mean age (7.5 ± 2.58 years) and sex (47 females and 13 males; 3.6:1) of the control were comparable ($p = 0.7$ and $p = 0.4$ respectively). However, the BMI was significantly lower in the JIA patients (15.9 ± 1.8) compared to the control (16.8 ± 1.6; $p = 0.006$). RF was positive in 9 polyarticular JIA patients. Twelve (20%) patients were newly diagnosed and received no treatment, 25 (41.7%) were on nonsteroidal anti-inflammatory drugs (NSAIDs), 43 (71.7%) were receiving methotrexate, 3 (5%) were on leflunomide, 18 (30%) on prednisone and 9 (15%) were receiving tocilizumab.

The JIA patients had a significantly higher serum IL-33 levels (median 12.6; 7.4–23.8 ng/l; $p < 0.001$) and its relative mRNA expression in PBMC (median 3.3; 2.5–3.7, $p < 0.001$) compared to IL-33 serum levels (median 1.7; 0.8–2.4 ng/l) and its relative mRNA expression (median 1 ng/l in all) in the control. There was a significant increase in SF IL-33 (median, 36.1; 24.4–80.1 ng/l, $p < 0.001$)

Table 1
Characteristics of juvenile idiopathic arthritis (JIA) patients.

Parameter	Juvenile idiopathic arthritis (JIA) patients				p
	Total (n = 60)	Oligo (n = 28)	Poly (n = 20)	Systemic (n = 12)	
Age (years)	7.3 ± 2.8	6.9 ± 2.2	8.5 ± 2.5	6.3 ± 2.1	**0.048**
Sex F:M	42:18	21:7	13:7	8:4	0.76
BMI	15.9 ± 1.8	15.6 ± 1.8	16.6 ± 1.8	15.5 ± 1.3	0.13
Dis. Dur. (mo)	12 (7–24)	12 (8.5–24)	12 (8.5–24)	3.5 (2–6.5)	**0.001**
TJC	1 (0–3)	1 (0–2)	2 (0–4)	0 (0–2.5)	0.44
SJC	1 (0–2)	1 (0.5–1.5)	1 (0–3)	0.5 (0–1.5)	0.58
Uveitis	5 (8.33)	4 (14.28)	1 (5)	–	0.58
SC nodules	2 (3.33)	–	2 (10)	–	–
ESR (mm/1sth)	21 (12–33.5)	19 (12–26.5)	21 (15–41.5)	23 (11–35)	0.65
CRP (mg/l)	7.3 (3.4–12.5)	5.3 (2.3–11.6)	7.8 (3.7–20.5)	8.8 (4.7–12.2)	0.26
Hb (g/dL)	10.52 ± 1.04	10.8 ± 0.9	10.2 ± 0.9	10.4 ± 1.4	0.09
WBC ($\times 10^3$/ul^3)	9.16 ± 3.78	7.9 ± 2.3	10.3 ± 3.1	10.1 ± 3.4	0.06
Pl ($\times 10^3$/ul^3)	359.1 ± 127.3	330.3 ± 102.3	405.2 ± 119.2	349.5 ± 103.7	0.13
Ferritin (ng/mL)	90 (50–167.5)	57.5 (32.5–93)	112 (60–157.5)	355 (185–673.5)	**<0.001**
RF titer (IU/mL)	11.4 (9.5–13.5)	10.6 (9.4–12)	20.2 (11.1–39.4)	11.6 (8.4–13.1)	**0.003**
sIL-33 (ng/l)	12.6 (7.4–23.8)	7.7 (4.8–18.1)	30.5 (15.8–48.3)	11.4 (9.6–16.5)	**0.006**
Syn IL-33 (ng/l)	36.1 (24.4–80.1)	32.2 (23.7–48.2)	80.3 (37.3–105.1)	–	0.08
IL-33 mRNA	3.3 (2.5–3.7)	3.1 (2.5–3.7)	3.4 (2.4–4)	2.6 (2.5–3.5)	0.48
JADAS 27	3.45 (1.5–14.05)	3 (1–11)	5.2 (2.65–18.8)	–	0.052
MSUS					
Greyscale	2 (0–5)	2 (0–3)	3.5 (1–6.5)	2 (0–5.5)	0.16
Power Doppler	1 (0–2)	0 (0–1)	1 (0–2.5)	1 (0–3)	0.26
JAMAR					
PF score	7 (4–12)	5.5 (3–10)	10 (6.5–12.5)	6.5 (3–12)	**0.03**
HRQoL	4 (2–8)	3.5 (1–7)	5 (2–8.5)	4 (2.5–6.5)	0.64
pain VAS	7 (3–9)	5.5 (3–8.5)	7 (5–10)	7.5 (4.5–8.5)	0.6
PW VAS	3 (1–7)	1 (0–4.5)	5 (2.5–7)	5 (3–9.5)	**0.008**
Ph activity	2 (1–4)	1 (0–3)	3.5 (0–7)	3 (1–5)	0.07

BMI: body mass index; TJC: tender joint count, SJC: swollen joint count, SC: subcutaneous, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, Hb: hemoglobin, WBC: white blood cells, Pl: platelets, RF; rheumatoid factor; IL: interleukin, s: serum, Syn: synovial, mRNA: messenger ribonucleic acid, JADAS: juvenile disease activity score; MSUS: musculoskeletal ultrasound; JAMAR: Juvenile Arthritis Multidimensional Assessment Report; PF: physical function, HRQoL: health related quality of life; VAS: visual analogue scale, Patients wellbeing, Ph: physicians assessment of activity. Results are presented as mean ± SD, median (IQR) or n (%). Bold values are significant at p < 0.05.

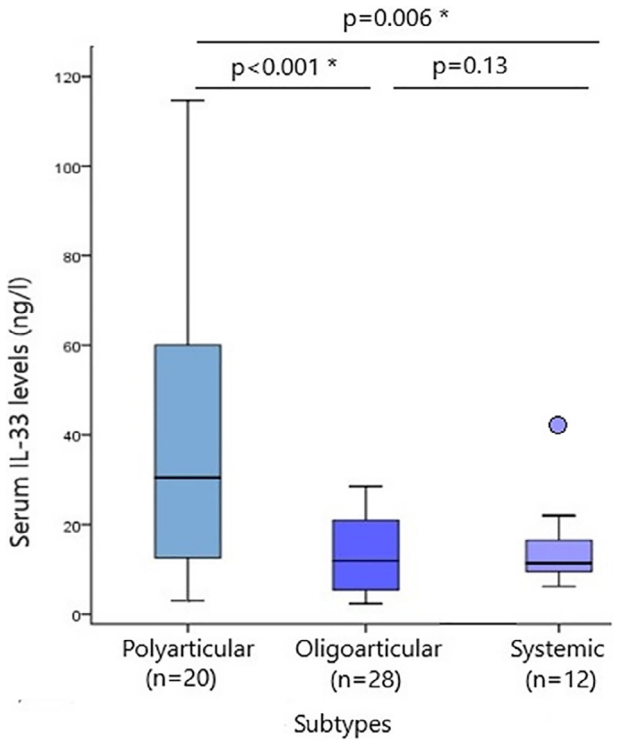

Fig. 1. Comparison of interleukin-33 serum levels between, polyarticular, oligoarticular and systemic-onset subtypes in juvenile idiopathic arthritis (JIA) patients. * = significant with p < 0.05.

than in serum samples obtained from JIA patients. Serum IL-33 levels tended to be higher in patients who did not receive medication (median 16.5; 11.5–22.3 ng/l) compared to those receiving (median 11.4;6.4–25 ng/l)(p = 0.24). Serum IL-33 was significantly higher in polyarticular compared to systemic (p = 0.006) and to oligoarticular (p < 0.001) (Fig. 1). Serum level and mRNA expression was significantly higher in those with high activity (n = 15) compared to inactive (n = 19, p = 0.008 and p = 0.002, respectively) or low (n = 8, p = 0.04 and p = 0.003, respectively). Those with moderate activity (n = 18) had significantly elevated serum IL-33 than inactive (p = 0.03) (Fig. 2). PDUS activity is shown in Fig. 3.

Table 2 shows the correlation between serum and SF IL-33 concentrations as well as its mRNA relative expression with patients' characteristics. Fig. 4 shows predictive performance of serum IL-33, ESR and CRP for disease activity using JADAS27 and PD synovitis score. Regarding JADAS27: IL-33 (AUC 0.76; cutoff 11.5 ng/l, sensitivity 76.7% and specificity 72.2%), ESR (AUC 0.7; cutoff 18.5 mm/1st h, sensitivity 73.3% and specificity 72.2%), CRP (AUC 0.91; cutoff 4.7 mg/l, sensitivity 83.3%, specificity 94.4%. Regarding PD score: IL-33 (AUC 0.81; cutoff 7.9 ng/l, sensitivity 93.9% and specificity 59.3%), ESR (AUC 0.7; cutoff 23.5 mm/1st h, sensitivity 57.6%, specificity 85.2%), CRP (AUC 0.86; cutoff 8.81 mg/l, sensitivity 72.7% and specificity 96.3%.

4. Discussion

Interleukin-33 has many regulatory functions in both physiological and pathological conditions as it is involved in the maintenance of tissue homeostasis besides its role in the immune reaction against various infectious and inflammatory diseases [14]. It is

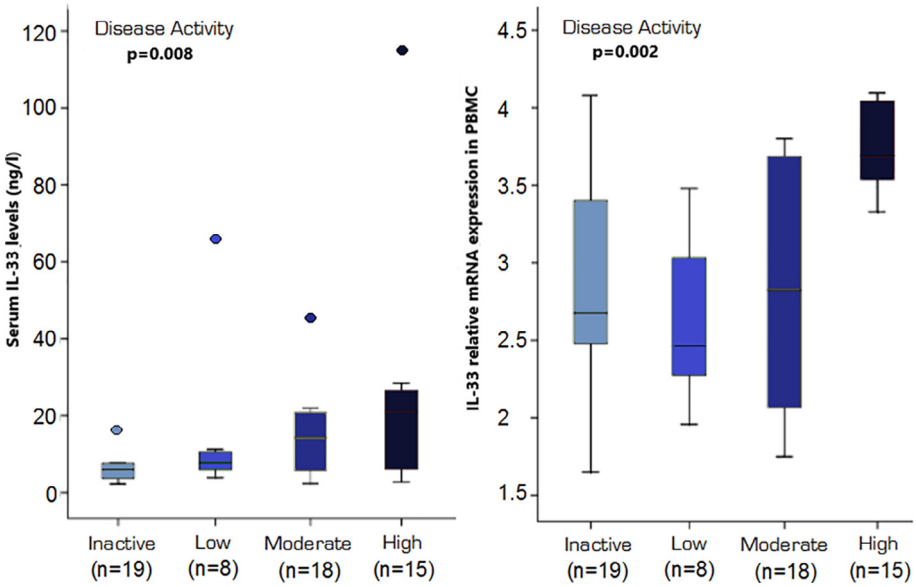

Fig. 2. Comparison of interleukin-33 serum levels (left) and mRNA relative expression on peripheral blood mononuclear cells (PBMC) (right) between juvenile idiopathic arthritis (JIA) patients with inactive disease, low, moderate and high disease activity.

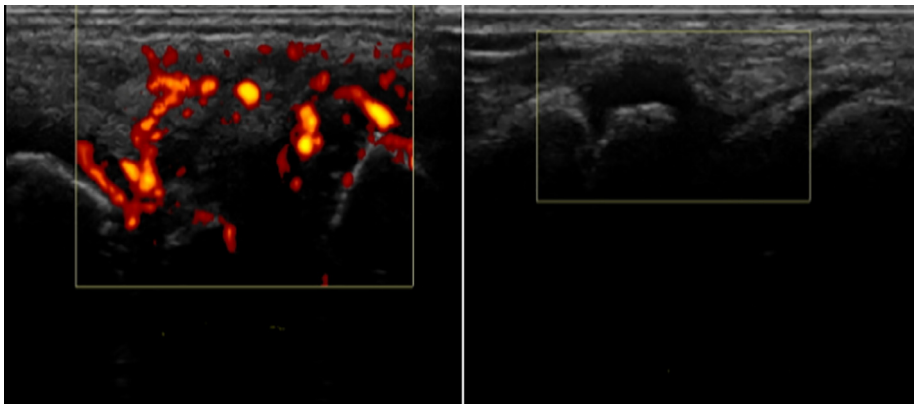

Fig. 3. Power Doppler ultrasound activity in the wrist joints of juvenile idiopathic arthritis (JIA) patients with high activity (left) and inactivity (right).

released following tissue injury and act as "alarmin" to activate many immune cells that express ST2 receptor such as T helper 2 (Th2) cells, regulatory T cells (Tregs), mast cells, eosinophils and macrophages [33].

In this study, IL-33 serum levels and its relative mRNA expression in PBMC in JIA patients were significantly higher than their levels in controls. IL-33 serum levels were significantly higher in polyarticular patients compared to oligoarticular and systemic-onset. Matsuyama et al. [34] suggested that increased IL-33 levels represent an enhanced inflammatory process at the joint level rather than systemic inflammation.

Ishikawa et al. [21] reported higher serum IL-33 in seropositive polyarticular subtype than JIA patients with systemic-onset. IL-33 serum level was higher in seropositive polyarticular JIA patients compared to its serum concentrations in seronegative polyarticular and oligoarticular JIA patients [22]. SF IL-33 was significantly higher than in the serum concentrations and both significantly correlated. This can be attributed to local IL-33 synthesis in the synovium reported by others [33–35]. Carriere et al. [34] considered inflamed synovium as a main source of IL-33 as they reported increased expression of IL-33 in endothelial cells and synovial tissues. Furthermore, Palmer et al. [35] described increased IL-33 expression by cultured synovial fibroblasts following stimulation with TNF-α.

In this study, IL-33 serum and SF levels had a significant positive correlation with different disease activity parameters such as tender and swollen joint counts, ESR, CRP, JADAS27 as well as ultrasonographic synovitis activity score. Binding of IL-33 to ST2 receptor leads to activation of many intracellular pathways [36] that enhance inflammation through many mechanisms as degranulation of mast cells, the release of several pro-inflammatory cytokines and enhancement of neutrophils migration to the synovial tissue [12]. Ishikawa1 et al. [21] found soluble ST2 receptors serum levels to be correlated with clinical disease activity parameters in systemic-onset JIA patients and observed normalizations of these levels during the remission stage.

Serum IL-33 had a higher sensitivity in predicting PD synovitis activity than JDAS27. This can be explained by the superiority of MSUS to clinical evaluation in synovitis detection [37] and its ability to identify subclinical synovial inflammation has been established in Egyptian JIA patients [38].

Ishikawa1 et al. [22] found a significant correlation between serum IL-33 and the production of RF and they suggested that IL-33 is involved in B-cell mediated pathology in JIA patient, this

Table 2
Correlations between serum and synovial fluid interleukin-33 levels and its relative mRNA expression with different variables in juvenile idiopathic arthritis (JIA) patients.

Variable r (p)	Interleukin-33 in JIA patients (n = 60)					
	Serum		Synovial fluid		mRNA expression	
Age	0.02	(0.9)	0.4	(0.09)	−0.08	(0.55)
BMI	0.1	(0.43)	0.07	(0.77)	0.04	(0.77)
Dis. Dur.	−0.07	(0.59)	0.25	(0.28)	−0.02	(0.89)
TJC	0.31	**(0.02)**	0.63	**(0.005)**	0.12	(0.35)
SJC	0.31	**(0.02)**	0.67	**(0.002)**	0.13	(0.32)
ESR	0.44	**(<0.001)**	0.42	(0.07)	0.34	**(0.009)**
CRP	0.73	**(<0.001)**	0.58	**(0.007)**	0.54	**(<0.001)**
Hb	−0.34	**(0.008)**	−0.085	(0.72)	−0.241	(0.06)
WBCs	0.13	(0.26)	0.09	(0.71)	0.24	(0.06)
Platelets	0.25	(0.054)	0.03	(0.89)	0.17	(0.2)
Ferritin	0.22	(0.08)	−0.08	(0.72)	0.14	(0.28)
RF titer	0.27	**(0.039)**	0.24	(0.32)	0.04	(0.76)
IL-33						
Serum	–	–	0.63	**(0.003)**	0.62	**(<0.001)**
Synovial	0.63	**(0.003)**	–	–	0.06	(0.82)
mRNA	0.62	**(<0.001)**	0.06	(0.82)	–	–
JADAS 27	0.5	**(<0.001)**	0.64	**(0.002)**	0.34	**(0.02)**
MSUS						
Greyscale	0.55	**(<0.001)**	0.67	**(0.001)**	0.25	(0.057)
PD	0.59	**(<0.001)**	0.6	**(0.005)**	0.38	**(0.002)**
JAMAR						
PF score	0.13	(0.34)	0.53	**(0.02)**	0.12	(0.38)
HRQoL	0.09	(0.49)	0.3	(0.19)	0.1	(0.44)
Pain VAS	0.19	(0.15)	0.19	(0.43)	0.04	(0.79)
PW VAS	0.27	**(0.04)**	0.34	(0.14)	0.03	(0.8)
Ph activity	0.48	**(<0.001)**	0.49	**(0.03)**	0.25	(0.057)

BMI: body mass index; TJC: tender joint count, SJC: swollen joint count, SC: subcutaneous, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, Hb: hemoglobin, WBC: white blood cells, Pl: platelets, RF; rheumatoid factor; IL: interleukin, mRNA: messenger ribonucleic acid, JADAS: juvenile disease activity score; MSUS: musculoskeletal ultrasound; PD: power Doppler, JAMAR: Juvenile Arthritis Multidimensional Assessment Report; PF: physical function, HRQoL: health related quality of life; VAS: visual analogue scale, Patients wellbeing, Ph: physicians assessment of activity. Bold values are significant at $p < 0.05$.

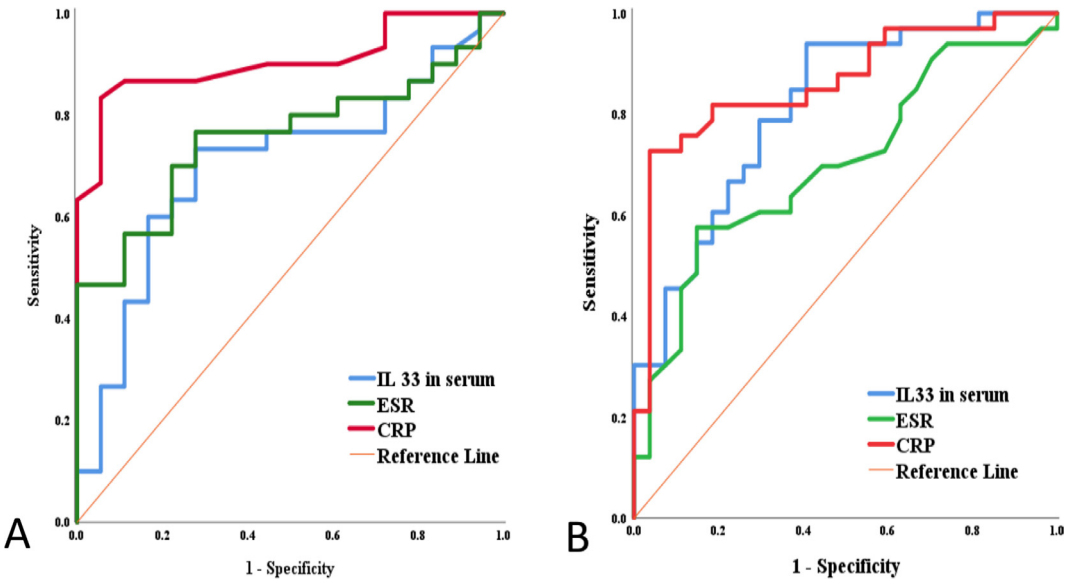

Fig. 4. Receiver operating characteristic (ROC) curve for performance of serum interleukin-33 (IL-33), erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) in predicting disease activity (A) and power Doppler synovitis score (B) in juvenile idiopathic arthritis (JIA) patients.

was established in the present work as serum IL-33 was significantly associated with RF titer, a known poor prognostic factor, raising questions about the possible prognostic role of IL-33. Schmitz et al. [39] suggested that IL-33 can enhance the production of autoantibodies by B cells through enhancement of the secretion of IL-5 and IL-13 which are known Th2 cytokines.

To best of our knowledge, only 2 studies [21,22] investigated IL-33 serum concentrations in JIA patients. However, none of them measured IL-33 mRNA relative expression or measured its level in the SF. Also, the relation between IL-33 and JIA disease activity was evaluated using MSUS in addition to the clinical and laboratory parameter.

A limitation of this work lies in the relative low number JIA patients and the cross-sectional study design. Many of the patients were on medications thus the exact effect of medications on IL-33 was not assessed.

In a conclusion, JIA patients have significantly elevated IL-33 serum concentrations and mRNA expression that considerably correlated with clinical, laboratory and MSUS parameters of inflammations suggesting that it could be a valuable marker of JIA disease activity. The considerable relation between IL-33 and physical functioning and rheumatoid factor production implies a possible prognostic role in JIA patients.

Conflict of interest

Declared none.

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