Soluble ST2 as a marker of disease activity in systemic juvenile idiopathic arthritis

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Abbreviations:
s-JIA, systemic juvenile idiopathic arthritis; MAS, macrophage activation syndrome; sST2, solubleST2; IL, interleukin; LPS, lipopolysaccharide; RF, rheumatoid factor.

Running Head: ST2 in systemic juvenile idiopathic arthritis

Abstract
To assess the role of interleukin (IL)-33 and ST2, the receptor for IL-33, in the pathogenesis of systemic juvenile idiopathic arthritis (s-JIA), we sequentially measured the serum levels of IL-33 and soluble ST2 (sST2) in patients with s-JIA and determined their correlation with measures of disease activity and severity. Twenty-four patients with s-JIA, 5 with rheumatoid factor positive polyarticular JIA (RF + poly-JIA), and 20 age-matched healthy controls (HCs) were analyzed. IL-33 and sST2 levels were quantified in serum by enzyme-linked immunosorbent assays. Serum IL-33 levels in most patients with active s-JIA were below the lowest detection limit. Serum IL-33 levels in patients with RF + poly-JIA were significantly higher than those in patients with s-JIA and HC. Serum sST2 levels in patients during the active phase of s-JIA were much higher than those in patients with poly-JIA and HC. Serum sST2 levels in patients with s-JIA were significantly elevated even in the inactive phase, when other clinical parameters were normalized. Serum sST2 levels correlated positively with the clinical parameters of disease activity. These findings indicate that ST2 may be an important mediator in s-JIA. Serum sST2 levels in patients with s-JIA correlated with disease activity, suggesting a potential role as a promising indicator of disease activity.

1. Introduction

Juvenile idiopathic arthritis (JIA) is a heterogeneous and multi- factorial autoimmune disease characterized by chronic joint inflammation in children with onset ages younger than 16 years. It is the most common chronic rheumatic disease of childhood and an important cause of short-term and long-term disability. JIA has different subtypes that are defined based on the number of joints involved in the first 6 months of disease and the extra- articular involvement [1]. The subtypes include oligoarticular JIA (<5 joints), polyarticular JIA (poly-JIA) (P5 joints) and systemic JIA (s-JIA). s-JIA is defined by arthritis with spiking fever persisting for more than 2 weeks and at least one of the following clinical features of systemic inflammation: skin rash, lymphadenopathy, hepatosplenomegaly or serositis (pleuritis or pericarditis) [1]. Markedly distinct clinical and laboratory features of poly-JIA and s-JIA indicate their distinct pathogenesis and immunologic abnormality. The pathogenesis of JIA is not fully understood but likely includes genetic and environmental factors which show some commonality to various adult rheumatic diseases e.g. rheumatoid factor positive (RF+)poly-JIA and adult RA, s-JIA and adult Still's disease [2,3]. High numbers of autoreactive T cells within the joint of poly-JIA patients indicate an antigen-driven activation of the adaptive immune system [4]. However, the typical clinical signs of s-JIA are rather associated with granulocytosis, thrombocytosis and increase of acute-phase reactants in the peripheral blood, indicating an uncontrolled activation of the innate immune system. Recent studies have shown that inflammatory cytokines, including interleukin (IL)-1, IL-6, and IL-18, play pathogenic roles in the dis- ease processes of s-JIA [5]. Furthermore, the treatment with bio- logic therapies to block these cytokines has a dramatic effect for s-JIA patients [5]. These findings indicate that autoinflammatory mechanisms seem to play a major role in the pathogenesis of s-JIA.

Macrophage activation syndrome (MAS) is a severe, potentially life-threatening complication of s-JIA. It is clinically characterized by fever, hepatosplenomegaly, lymphadenopathy, profound depression of all three blood cell lines, deranged liver function, intravascular coagulation, and central nervous system dysfunction [6]. The excessive activation and proliferation of T lymphocytes and macrophages are observed in MAS. Massive hypercytokinemia is strongly associated with the pathogenesis of MAS [6].

Interleukin-33 (IL-33) is a novel IL-1 family cytokine that plays a major role in inflammatory, infectious, and autoimmune diseases [7,8]. IL-33 was identified as the ligand for the orphan receptor, ST2 [9]. ST2 molecule is a member of the IL-1 receptor family that exists in two forms: a transmembrane full-length form and a soluble, secreted form (sST2) [10]. sST2 acts as a decoyreceptor for IL-33 [11].

IL-33 affects the function of cells that express ST2 molecule. IL- 33 polarizes naive T cells to produce Th2-associated cytokines IL-4, IL-5 and IL-13 [7] and functions as a chemoattractant for Th2 cells in vitro and in vivo [12], but also induces secretion of proinflammatory cytokines and chemokines by mast cells [13], basophils [14] and Th1 type cytokines from NK and NKT cells [14,15]. Also, IL-33 amplifies polarization of alternatively activated M2 macro- phages [16], induces maturation of dendritic cells [17] and may promote Th1-type response

[18].

Besides the regulation of disease outcome through the modulation of Th1/Th2 bias, there is some evidence to suggest that ST2 may also be involved in inflammatory responses. A previous report revealed that the sST2-Fc fusion protein suppressed inflammatory responses that were induced by lipopolysaccharide (LPS) both in vitro and in vivo [19]. In normal conditions, the serum concentration of sST2 is below the detectable level, but elevated level of sST2 has been reported in patients with autoimmune diseases [20], asthma [11], idiopathic pulmonaryfibrosis [23]. The sST2 levels were found to correlate with the activity and severity of these conditions [21–24].

In this way, IL-33 and ST2 have important functions in host defense, immune regulation, and inflammation. However, its role in the pathogenesis of s-JIA and a causal relationship with disease activity are still unclear. To assess the role of IL-33 and ST2, in the pathogenesis of s-JIA, we sequentially measured serum levels of IL-33 and sST2 in patients with s-JIA and determined their correlation with measures of disease activity and severity.

2. Methods

2.1. Patients and samples

Serum samples were obtained from 24 patients with s-JIA, 5 patients with RF + poly-JIA, and 20 age- and sex-matched healthy controls (HC) [age, s-JIA: 8.9 ± 6.5 years and HC: 10.5 ± 7.4 years]. Eleven patients were evaluated longitudinally on a second occasion when their disease was in an inactive phase. Four patients with MAS were evaluated serially from the phase of MAS to remission. The clinical characteristics of the patients with s-JIA are shown in Table 1.

Diagnoses of s-JIA and RF + poly-JIA were based on the International League of Associations for Rheumatology criteria [1]. MAS was diagnosed based on the combination of cytopenias affecting at least two cell lines, coagulopathy, and liver dysfunction, according to the guidelines proposed by Ravelli et al. [24]. The criteria defining the active phase of s-JIA were active arthritis, fever, rash, hepatosplenomegaly, generalized lymphadenopathy, and serositis as well as increased erythrocyte sedimentation rates and C- reactive protein (CRP) levels. The criteria for the inactive phase of s-JIA on medication were as follows: the first time after the recovery from MAS with no clinical symptoms that were observed in the active phase, as well as normal erythrocyte sedimentation rates (<5 mm/h) and CRP levels (<0.1 mg/dL). The criterion for remission of patients with s-JIA on medication was six continuous months of inactive disease while on medication [25].

Serum was separated from cells, divided into aliquots, frozen, and stored at 80 °C until use. This study was approved by the Institutional Review Board at Kanazawa University, and all specimens were used after informed consent was obtained.

2.2. Quantification of serum cytokines

Levels of IL-33 were evaluated by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Human IL-33 DuoSet® ELISA Development System, R&D Systems, Inc., Minneapolis, MN, USA). Levels of sST2 were evaluated by ELISA according to the manufacturer's instructions (Human ST2/ IL-1R4DuoSet® ELISA Development System, R&D Systems, Inc., Minneapolis, MN, USA). The range of ELISA for IL-33 was from 3.6 to 1500 pg/mL. The range of ELISA for sST2 was from 31.5 to 2000 pg/mL. RF positivity did not interfere with the ELISA assay.

2.3. Statistical analysis

Within-group comparisons were analyzed by the Mann–Whitney test. Correlations were expressed using the Spearman rank correlation coefficient. For the analyzed measures, $P < 0.05$ was considered significant.

3. Results

3.1. Serum levels of IL-33 and sST2

We determined the serum levels of IL-33 and sST2 in patients with s-JIA and compared them with the levels in patients with RF + poly-JIA and HC. Serum IL-33 levels in most patients with active s-JIA were found to lie below the lowest detection limit of the assay (Fig. 1A). IL-33 was detected in 4 out of 24 s-JIA patients (17%) 9 out of 20 control subjects (45%). The differences in the serum IL-33 levels among these s-JIA patients (median, 68; range, 4–702 ng/mL), and HC (median, 50; range, 4–169 ng/mL) were not statistically significant. On the other hand, serum IL-33 levels in RF + poly-JIA patients (median, 155; range, 61–533 ng/mL) were significantly elevated compared with those in active s-JIA patients and HC ($P < 0.01$). These patients did not demonstrate symptoms that were suggestive of allergic diseases or atopy at the time of the study.

In contrast, serum sST2 levels in patients with MAS (median, 19,500; range, 3680–61,000 pg/mL) and in patients in the active phase of s-JIA (median, 2205; range, 496–43,000 pg/mL) were much higher than those in patients with RF + poly-JIA (577, 338– 1120 pg/mL) and HC (354, 102–1052 pg/mL)(Fig. 1B). Serum sST2 levels in patients with s-JIA were significantly elevated even in the inactive phase (839, 360–4550 pg/mL), and they normalized in the remission phase (402, 267–512 pg/mL). The serum sST2 levels in RF + patients were not significantly different compared to HC.

3.2. Markedly elevated concentrations of serum sST2 in patients in the active phase of s-JIA and MAS

To investigate the relevance of sST2 to the pathogenesis of s-JIA and MAS, serum sST2 levels were serially monitored in four cases of s-JIA (Fig. 2). The serum sST2 levels were both rapidly and markedly elevated when the

complication of MAS occurred, but gradually reduced after such manifestations disappeared with corticosteroid and cyclosporine therapy. Even a few weeks after normalization of the indicators of an inflammatory reaction, such as lactate dehydrogenase (LDH) levels, the sST2 levels were still well above the values of HCs. Disease relapse of s-JIA with MAS was complicated in 1 case (Fig. 2A) during this phase of high serum sST2 levels.

3.3. Correlation between serum sST2 and measures of disease activity in the clinical course of four cases of s-JIA

Because the levels of serum CRP, aspartate aminotransferase, LDH, and ferritin are clinically used as indicators of disease activity in s-JIA, their concentrations were compared with those of sST2. The serum sST2 levels correlated positively with each of these indicators (Fig. 3A–D). However, even during the clinically inactive phase after remission from MAS, the serum sST2 levels remained elevated, although other clinical parameters were normalized.

4. Discussion

In this study, we demonstrated that serum sST2 levels in patients with s-JIA were markedly elevated during the active phase of s-JIA and correlated with measures of disease activity. These findings indicate that serum sST2 levels may have a potential role as a promising indicator of disease activity. Furthermore, serum sST2 levels in patients with s-JIA were significantly elevated even during the inactive phase, and they were normalized in the remission phase. High serum sST2 levels persisted in patients for about six continuous months of inactive disease while on medication. These findings support the appropriateness of the Wallace criteria for the clinical remission of JIA (six continuous months of inactive disease while on medication) [25]. In this way, monitoring serum sST2 levels may be also useful for assessing treatment effects and the clinical remission of s-JIA.

Elevated IL-33 levels were infrequently observed in s-JIA patients, and their levels were found to be comparable to those of controls. Serum IL-33 levels were not found to be related to sST2 levels or the disease activity of s-JIA. It is possible that IL-33 may have formed immune complexes with sST2 or was downregulated by sST2 through negative regulatory mechanisms because sST2 functions as a negative regulator and is an antagonistic decoy receptor for IL-33 [11].

Other possibility is that biological action of increased sST2 in serum might not be mediated via IL-33 neutralization. There is some evidence to suggest that sST2 may also be involved in inflammatory responses. A previous report revealed that sST2 suppressed the expression of toll-like receptor (TLR) 1 and 4 [19] and that this may contribute to the anti-inflammatory effects of sST2 in macro- phages. The role of TLRs in s-JIA has been consistently demonstrated [26]. For example, signaling through the IL-1 and IL-18 receptors shares the downstream portion of the TLR4 signaling pathway, and IL-1 and IL-18 provide positive feedback loops that further contribute to the perpetuation of the inflammatory responses in s-JIA [26]. These findings indicate that sST2 may have a role in resolving inflammatory responses by regulating macro- phage activation in the pathogenesis of s-JIA.

Furthermore, sST2 may provide a novel approach for treating s-JIA by inhibiting the release of proinflammatory cytokines. In fact, a previous study revealed the therapeutic effects of sST2-Fc in a murine model of collagen-induced arthritis [27].

The alternative activation of monocytes and macrophages may play a role in resolving inflammatory responses in the pathogenesis of s-JIA. It has been well described that IL-33 and ST2 are modulators of inflammation and mediate Th2 immune responses [12]. IL-33 has been shown to induce the production of Th2 cytokines [7] and to possess a chemoattractant effect for human Th2 cells [12]. In this study, serum sST2 levels in patients with s-JIA were elevated not only in the active phase but also in the inactive phase. These findings indicate that sST2 may have a role in resolving inflammatory responses by promoting monocyte differentiation into M2 macrophages through Th2 immune responses in the pathogenesis of s-JIA, as previously reported [16,28].

In this study, serum IL-33 levels were significantly elevated in RF + poly-JIA patients. A previous study revealed that serum IL- 33 levels were elevated in sera and synovial fluid samples from patients with rheumatoid arthritis (RA), and the levels correlated with disease activity [29–37]. IL-33 is produced mainly in inflamed joints [29]. A recent study showed that the serum IL-33 levels were correlated with the production of IgM and RA-related autoantibodies, including RF and anticitrullinated protein antibodies [30]. Our results were consistent with these findings. Although the number of RF + poly-JIA patients in this study was small and a larger study may help define the true diagnostic value of these markers, our study indicates that IL-33 may play an important role in the joint inflammation of human RF + poly-JIA.

We have not determined the source of IL-33 and sST2 in patients with s-JIA in this study. A previous study showed that various cell types, including smooth muscle cells, epithelial cells, fibroblasts, keratinocytes, dendritic cells, and activated macro- phages, express IL-33 mRNA [7]. IL-33 mRNA was induced in fibro- blasts and keratinocytes by stimulation with tumor necrosis factor (TNF)-α and IL-1 [7]. However, IL-33 mRNA was only modestly induced in dendritic cells and macrophages by stimulation with LPS [7]. Furthermore, the expression of IL-33 mRNA has been found in endothelial cells from chronically inflamed tissues from patients with Crohn's disease and RA [38]. sST2 was induced in fibroblasts, macrophages, and monocytes by stimulation with LPS, TNF-α, or IL-1 [39]. Furthermore, previous studies showed that epithelial and endothelial cells, and cardiac myocytes can secrete sST2 [40,41]. These findings indicate that sST2 and/or IL-33 may be re- leased from activated macrophages, dendritic cells, or endothelial cells from inflamed tissues in patients with s-JIA.

sST2 levels obtained in HC were again 10-fold higher than many other papers measuring sST2 in serum. Some reasons of this discrepancy might be due to the difference of kit and/or operation difference.

The limitation of the present study was the small number of s-JIA patients. A larger study may help to define the true diagnostic value of these markers. Despite this limitation, our results indicate that ST2 may be an important mediator in s-JIA. Serum sST2 levels in s-JIA patients correlated with disease activity, suggesting a potential role of ST2 as a promising indicator of disease activity.

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Table 1
Clinical characteristics of patients with systemic juvenile idiopathic arthritis during the active phase.
CRP, C-reactive protein; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; PSL, prednisolone; MTX, methotrexate.

Figure legends

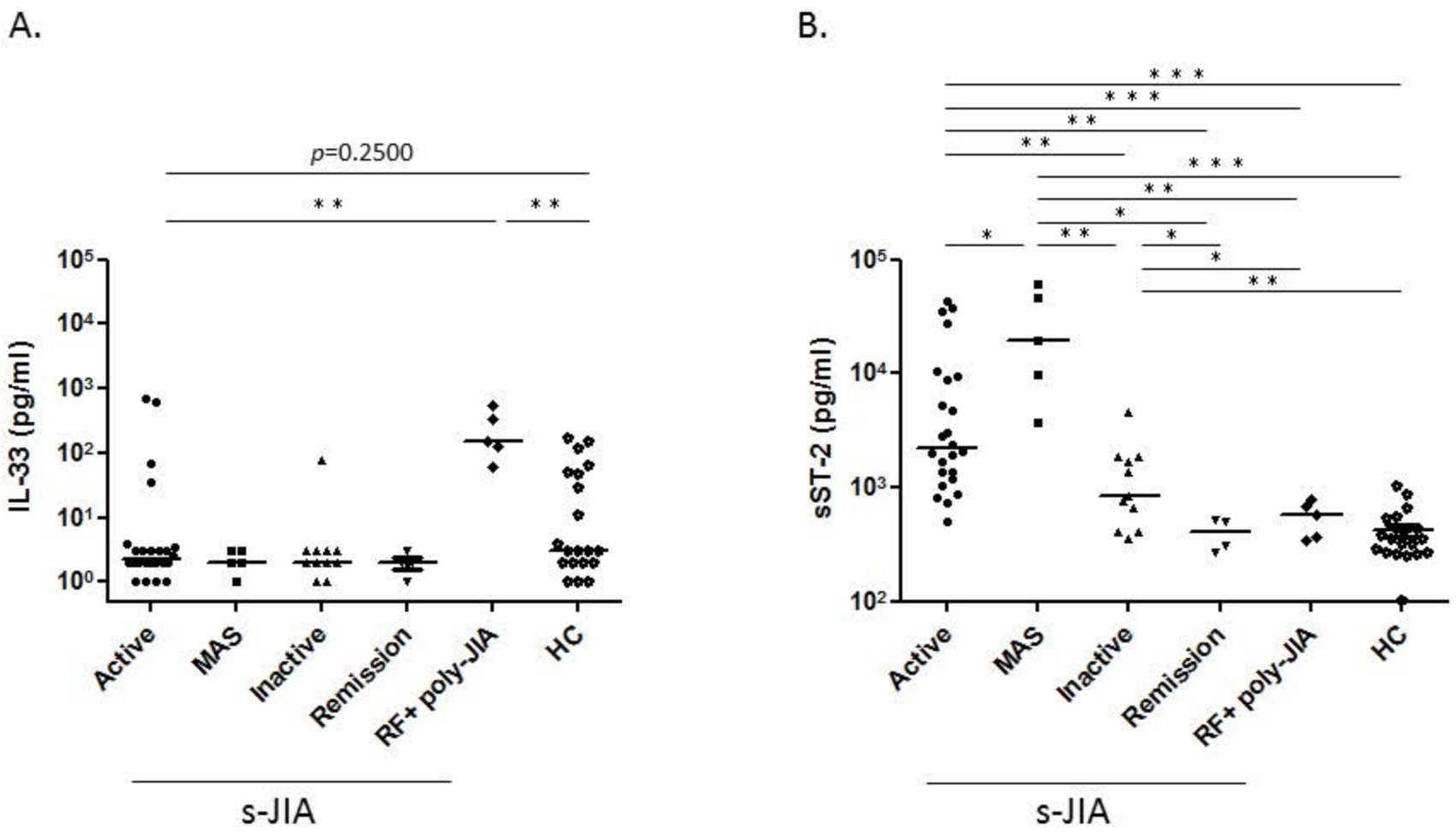
Fig. 1.
Serum levels of IL-33 (A) and sST2 (B) in patients with s-JIA.
Bars represent median values. Statistically significant differences between each patient group are shown as *P < 0.05, **P < 0.01, and ***P < 0.001.
IL, interleukin; sST2, soluble ST2; s-JIA, systemic juvenile idiopathic arthritis; RF + poly-JIA, rheumatoid factor + polyarticular juvenile idiopathic arthritis; HC, healthy control.

Fig. 2.
Longitudinal assessment of serum sST2 levels and LDH in four patients with MAS.
Changes in serum sST2 (solid lines) and LDH (dotted lines) levels are shown in the upper panels and the details of the therapeutic interventions are shown in the lower panels. Time points of blood draw are shown with arrows.
sST2, soluble ST2; LDH, lactate dehydrogenase; M, MAS; A, active phase; I, inactive phase; R, remission; mPSL, methylprednisolone; DEX-P, dexamethasone palmitate; PSL, prednisolone; CyA, cyclosporine.

Fig. 3.
Positive correlations between serum sST2 levels and measures of disease activity, including the other cytokines, during the clinical course of s-JIA. (A, CRP; B, AST; C, LDH; D, ferritin).
IL, interleukin; CRP, C-reactive protein; AST, aspartate aminotransferase; LDH, lactate dehydrogenase.

	S-JIA
patients	24
sex（male:female）	11：13
age（years）	10（1-26）
Disease duration (years)	0.1 (0-11)
Laboratory findings	
CRP (mg/dl) (n=24)	8.41 (1.6-25.8)
AST (IU/l) (n=24)	39 (11-136)
LDH (IU/l) (n=24)	344 (162-1359)
Ferritin (ng/ml) (n=17)	864 (250-17484)
Treatment	
PSL (mg/day) (n=9)	17.5 (5-37.5)
MTX (mg/m^2) (n=2)	10

Table 1


A.
p=0.2500
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**
IL-33 (pg/ml)
10^5
10^4
10^3
10^2
10^1
10^0
Active
MAS
Inactive
Remission
RF+ poly-JIA
HC
s-JIA
B.
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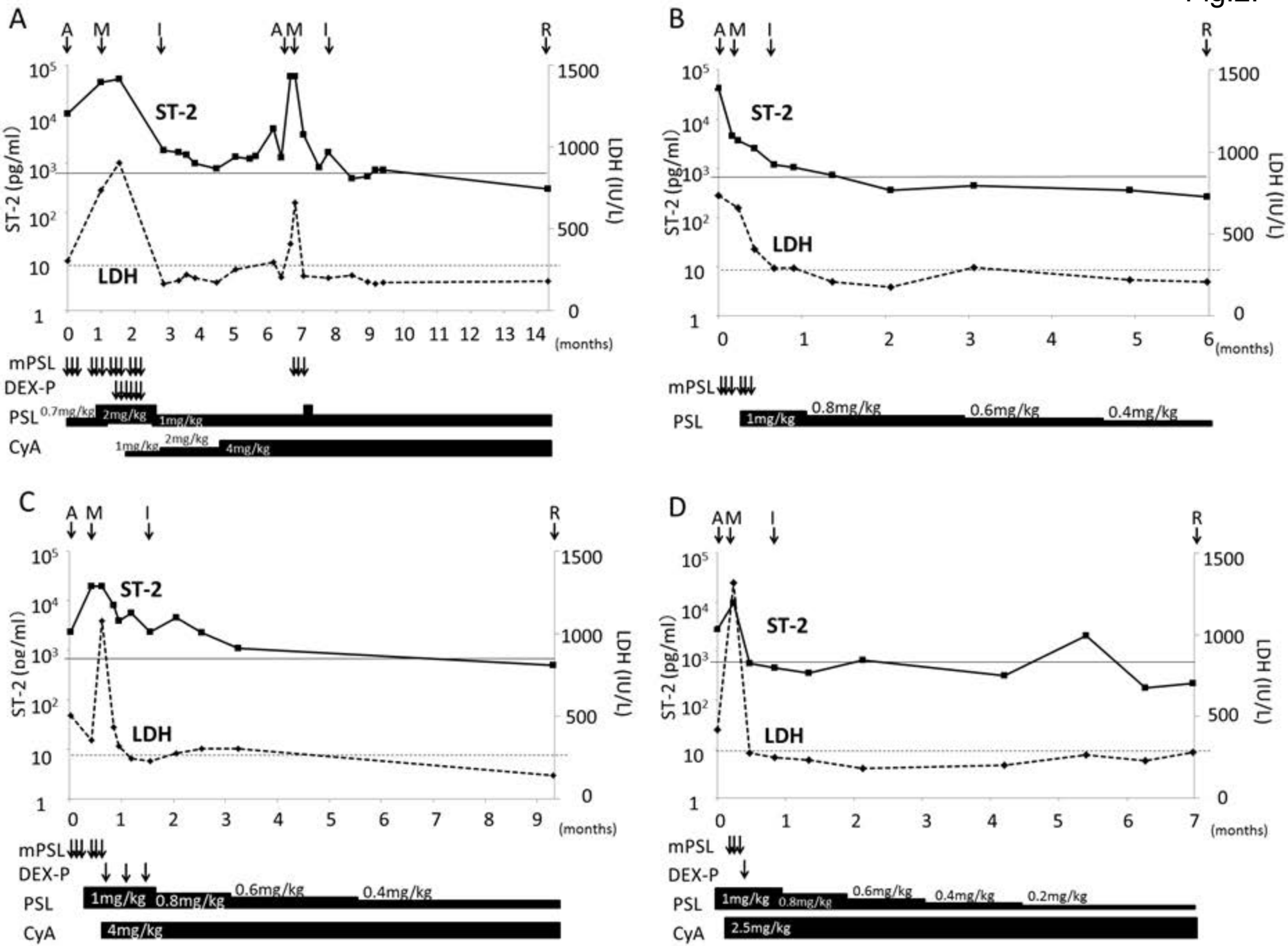
sST-2 (pg/ml)
10^5
10^4
10^3
10^2
Active
MAS
Inactive
Remission
RF+ poly-JIA
HC
s-JIA

Fig. 1.

Fig.2.


A
A M I A M I R
ST-2
LDH
ST-2 (pg/ml)
LDH (IU/L)
(months)
mPSL
DEX-P
PSL 0.7mg/kg 2mg/kg 1mg/kg
CyA 1mg/kg 2mg/kg 4mg/kg
B
A M I R
ST-2
LDH
mPSL
PSL 1mg/kg 0.8mg/kg 0.6mg/kg 0.4mg/kg
C
A M I R
ST-2
LDH
mPSL
DEX-P
PSL 1mg/kg 0.8mg/kg 0.6mg/kg 0.4mg/kg
CyA 4mg/kg
D
A M I R
ST-2
LDH
mPSL
DEX-P
PSL 1mg/kg 0.8mg/kg 0.6mg/kg 0.4mg/kg 0.2mg/kg
CyA 2.5mg/kg

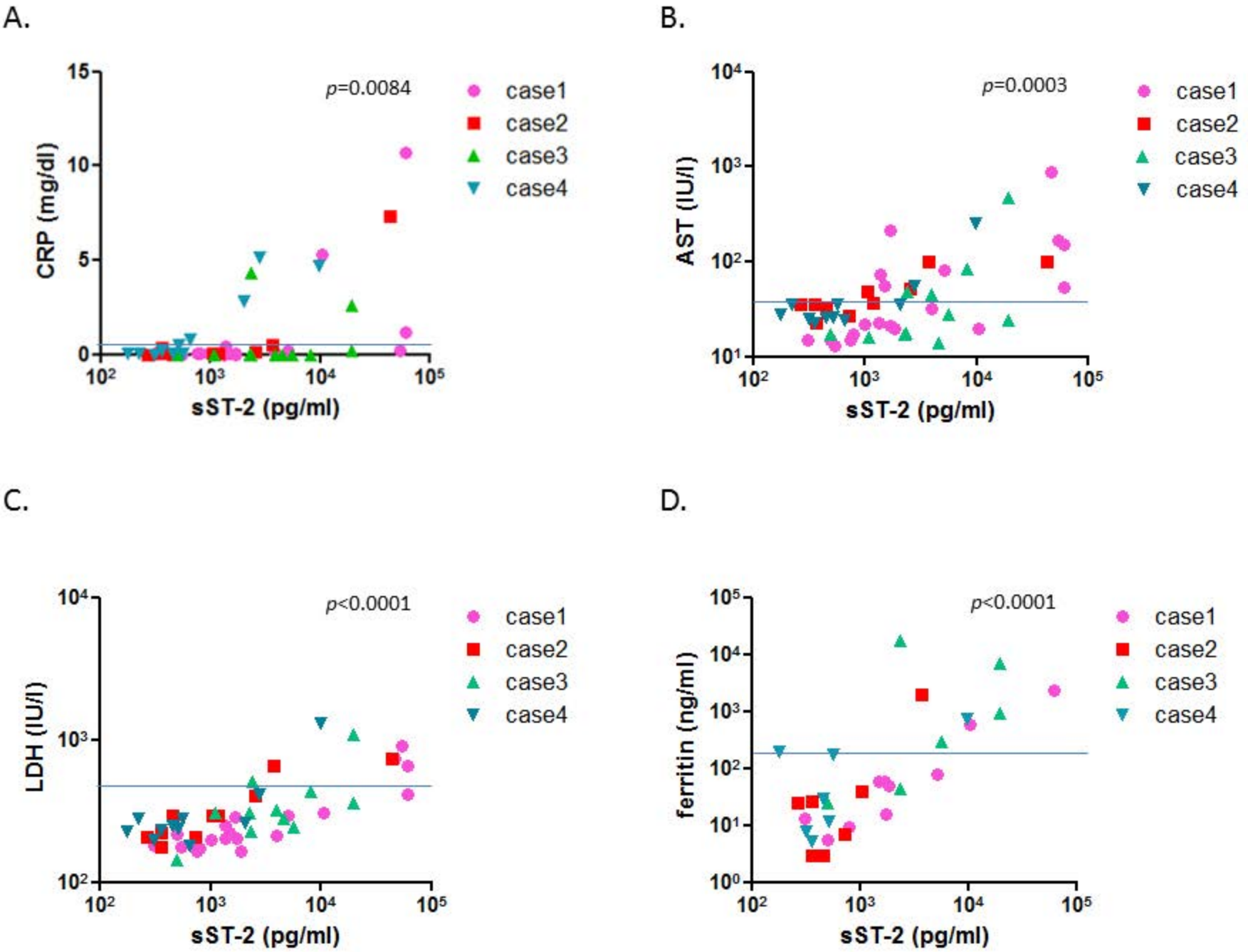

A.
B.
C.
D.
p=0.0084
p=0.0003
p<0.0001
p<0.0001
case1
case2
case3
case4
CRP (mg/dl)
AST (IU/l)
LDH (IU/l)
ferritin (ng/ml)
sST-2 (pg/ml)

Fig.3.