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doi: https://doi.org/10.1016/j.cyto.2018.05.017

Soluble CD163, a unique biomarker to evaluate the disease activity, exhibits macrophage activation in systemic juvenile idiopathic arthritis

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Running head: Serum sCD163 levels for systemic JIA

Key Words: soluble CD163, systemic juvenile idiopathic arthritis, tocilizumab, macrophage activation syndrome

Abstract

This study aims to investigate the clinical significance of serum soluble CD163 (sCD163) levels as a predictor of the disease activity of systemic juvenile idiopathic arthritis (s-JIA). In this study, we examined 63 patients with s-JIA, four with Epstein–Barr virus-induced hemophagocytic lymphohistiocytosis (EBV-HLH), and seven with Kawasaki disease (KD), along with 14 healthy controls. We quantified serum cytokine levels (sCD163, neopterin, IL-18, IL-6) by enzyme-linked immunosorbent assay and compared the results with the clinical features of s-JIA. Serum sCD163 levels were significantly elevated in patients with s-JIA associated macrophage activation syndrome (MAS) and EBV-HLH compared to those in patients with acute-phase s-JIA and KD. In addition, serum sCD163 levels profoundly increased with the progress of MAS and correlated positively with the disease activity of s-JIA, even in patients receiving tocilizumab. Furthermore, serum sCD163 levels significantly decreased in the inactive phase compared to those in the active phase and normalized in remission. The correlation between macrophage activation and serum sCD163 levels might be a unique indicator of the disease activity and a potential diagnostic laboratory criterion for clinical remission in patients with s-JIA, including those receiving tocilizumab.

1. Introduction

Systemic juvenile idiopathic arthritis (s-JIA) is characterized by chronic arthritis accompanied by high spiking fever and other systemic symptoms, including salmon-pink evanescent rash, hepatosplenomegaly, lymphadenopathy, and serositis [1]. A recent study investigating the pathophysiology of s-JIA revealed that s-JIA is an auto-inflammatory condition [2]. It is possible that the aberrant induction of proinflammatory cytokines such as IL-6, IL-1β, and IL-18 could be involved in the pathogenesis of s-JIA and might also correlate with the disease activity and secondary complications [2]. Furthermore, inadequate downregulation of the immune activation could be another crucial pathological mechanism of s-JIA [2–4].

The macrophage lineage is categorized into two different subsets: the classical M1 macrophages and the alternatively activated M2 macrophages. While M1 macrophages comprise the proinflammatory subset, M2 macrophages resolve inflammatory responses, perform scavenger functions, and promote tissue remodeling and repair [5]. In addition, M2 macrophages play a role as hemophagocytic macrophages in macrophage activation syndrome (MAS) [6], and resolve inflammatory responses in the pathogenesis of s-JIA [3].

The hemoglobin–haptoglobin scavenger receptor CD163 is a monocyte/macrophage-restricted 130-kDa transmembrane protein of the cysteine-rich

scavenger receptor family [7, 8]. The CD163 expression identifies M2 macrophages undergoing differentiation through the alternative pathway related to enhanced phagocytic activity [9]. Upon appropriate activation of the cells, the extracellular portion of CD163 is shed from the cell surface in the form of soluble CD163 (sCD163). Previously, extensive expansion of $CD163^+$ macrophages has been reported in the bone marrow of a patient with MAS [6].

Reportedly, the serum sCD163 levels are a valuable diagnostic marker in hemophagocytic syndromes and MAS [10, 11]. We have previously reported that serum sCD163 levels remained elevated even in the inactive phase of s-JIA [4]. However, the kinetics of the serum sCD163 levels from the active phase to remission in s-JIA remains unclear. Furthermore, recent research has revealed that the expression of inflammatory proteins such as C-reactive protein (CRP) is modified by the treatment of IL-6 blocking in s-JIA [12]; however, whether sCD163 expression is modified by IL-6 blocking warrants further investigation.

To assess the value of the serum sCD163 levels as a biomarker to elucidate the disease activity of s-JIA even during IL-6 blocking, we measured the levels of serum sCD163 levels in patients with s-JIA including those receiving tocilizumab (TCZ), a humanized anti-IL-6 receptor monoclonal antibody and compared them with the levels

in patients with hemophagocytic lymphohistiocytosis due to Epstein-Barr virus infection (EBV-HLH) and Kawasaki disease (KD), which are both characterized by prominent and systemic inflammation in children.

2. Materials and methods

2.1 Patients and samples

In this study, we obtained serum samples from 63 patients with s-JIA, four with EBV-HLH, seven with KD, and 14 age- and sex-matched healthy controls (HCs). Of 63 patients with s-JIA, 19 presented with MAS, three of whom presented with MAS during the study referral, and 16 developed complicated MAS during the acute disease phase and after starting steroid therapy. Two patients with s-JIA relapsed after starting TCZ. Overall, we evaluated 62 active episodes and 19 MAS episodes.

While the diagnosis of s-JIA was based on the International League of Associations for Rheumatology criteria [13], MAS was diagnosed per the 2016 European League against Rheumatism/American College of Rheumatology/Paediatric Rheumatology International Trials Organization classification criteria [14, 15]. The criteria for acute-phase s-JIA were the following:

active arthritis, fever, rash, hepatosplenomegaly, generalized lymphadenopathy, serositis, increased erythrocyte sedimentation rate, and increased CRP levels. We excluded patients with sepsis or severe bacterial infection from this study. Some patients in this study had minimal joint disease at s-JIA onset, and the presence of arthritis was confirmed later. The criteria for inactive-phase s-JIA on medication were as follows: no clinical symptoms that were otherwise observed in the active phase as well as normal erythrocyte sedimentation rate and CRP levels. The criteria for remission on medication were defined by the Wallace criteria, with a minimum of 6 consecutive months of inactive disease while receiving medication [16].

Table 1 summarizes the clinical characteristics of patients with acute-phase s-JIA. Of 62 active episodes in 60 patients, 40 received no treatment, whereas 21 were treated with prednisolone (PSL). One patient was treated with cyclosporine. Besides PSL, five patients received cyclosporine, one received methotrexate, and six received TCZ. The supplementary Table 1 summarizes the clinical characteristics of patients with MAS. In 19 patients with MAS, six received no treatment, 13 were treated with PSL, four with cyclosporine, and five with TCZ. The supplementary Table 2 summarizes the clinical characteristics of s-JIA patients in inactive and remission phase. EBV-HLH was diagnosed on the basis of the diagnostic criteria for EBV-HLH [17] as follows: positivity for EBV genome in the blood, bone marrow, and other tissues

(determined by PCR, Southern blot, and/or *in situ* hybridization for EBV-encoded RNA) and presence of antiviral capsid antigen-specific-IgG. Furthermore, KD was diagnosed on the basis of the classic clinical criteria [18]. All serum samples obtained in this study were separated from blood, divided into aliquots, frozen, and stored at −80 °C until analysis. This study was approved by the Institutional Review Board of the Kanazawa University (The ethics committee approval number 1403), and we obtained informed consents from all participants.

2.2 Measurement of serum cytokine levels

We measured serum sCD163, IL-6, and IL-18 levels using commercial enzyme-linked immunosorbent assay kits according to the manufacturer's instructions (sCD163, IL-6: R&D Systems, Inc., Minneapolis, MN; IL-18: MBL, Nagoya, Japan).

2.3 Statistical analysis

We performed multiple comparisons between groups using Tukey's test. In addition, the comparison of serum sCD163 levels in s-JIA between acute-phase and MAS, acute phase and inactive phase, and acute phase and remission was performed using the paired *t*-test. Furthermore, all correlations were expressed using the Spearman's rank correlation coefficient. We considered $P < 0.05$ as statistically significant.

3. Results

3.1 Elevation in serum sCD163 levels in active-phase s-JIA and MAS

We assessed the serum sCD163 levels in patients with s-JIA and compared them with those observed in patients with EBV-HLH or KD. Serum sCD163 levels were significantly elevated during the acute phase in patients with s-JIA (median, 1,350 [range: 290–3,798] ng/mL; $P < 0.0001$), MAS (median, 2,443 [range: 938–7,400] ng/mL; $P < 0.0001$), and EBV-HLH (median, 2,322 [range: 1,631–2,679] ng/mL; $P < 0.01$), compared to those in HCs (median, 469 [range: 195–690] ng/mL; Fig. 1). In addition, the serum sCD163 levels were significantly elevated in patients with MAS compared to those in patients with active-phase s-JIA ($P < 0.0001$), inactive-phase s-JIA (median, 517 [range: 148–1,548] ng/mL; $P < 0.0001$), remission-phase s-JIA (median, 510 [range: 142–884] ng/mL; $P < 0.0001$), and KD (median, 791 [range: 519–1,349] ng/mL; $P < 0.0001$). The serum sCD163 levels were significantly elevated in patients with active-phase s-JIA compared to those in patients with inactive-phase s-JIA ($P < 0.0001$), and remission ($P < 0.0001$). Furthermore, the serum sCD163 levels were significantly elevated in patients with EBV-HLH compared to those in patients with inactive-phase s-JIA ($P < 0.01$), and remission ($P < 0.01$).

3.2 Comparison of serum sCD163 levels in patients with s-JIA receiving and not receiving TCZ

In this study, we compared the serum sCD163 levels in patients with s-JIA receiving and not receiving TCZ. There were no differences of sCD163 levels in each phase of s-JIA between the patients not receiving TCZ and those receiving TCZ (Fig. 2). Serum sCD163 levels in patients receiving TCZ were also significantly elevated in MAS phase compared to those in inactive phase and remission phase.

3.3 Serum sCD163 levels as a biomarker of the disease activity in MAS

We serially monitored the serum sCD163 levels in three patients with s-JIA complicated with MAS (Fig. 3) to investigate their relevance in the pathogenesis of s-JIA complicated with MAS, revealing a significant increase with the progression of MAS. Furthermore, the serum sCD163 levels remained elevated until MAS was controlled. A paired analysis between active-phase s-JIA and MAS in patients after starting steroid therapy revealed that the serum sCD163 levels significantly increased after MAS developed in patients not receiving TCZ (Supplementary Fig. S1A) as well as in those receiving TCZ (Supplementary Fig. S1B).

3.4 Longitudinal analysis of serum sCD163 levels from the active phase to remission in s-JIA

A longitudinal analysis in 22 patients with s-JIA whose serum samples were serially obtained from the active phase to remission revealed that the serum sCD163 levels significantly decreased in the inactive phase compared to those in the active phase, and normalized in remission (Fig. 4). In addition, some patients exhibited elevated serum sCD163 levels even in the inactive phase.

3.5 Correlation between serum sCD163 levels and measures of the disease activity in patients with s-JIA

Serum ferritin, aspartate aminotransferase (AST), lactate dehydrogenase (LDH), CRP, IL-6, and IL-18 levels are clinically used as indicators of the disease activity in s-JIA. An assessment of the correlation between the serum sCD163 levels and these indicators in active phase including MAS phase revealed that sCD163 levels positively correlated with ferritin, AST, LDH, and IL-18 levels (Fig. 5A–C, F), but negatively correlated with CRP and IL-6 levels (Fig. 5D and E).

4. Discussion

Despite the unclear underlying etiology of s-JIA, recent studies have revealed that s-JIA is characterized by prominent innate immune activation and is classified as an auto-inflammatory disease [2]. Reportedly, the number of circulating innate immune cells, including monocytes and neutrophils, is expanded in the active phase of s-JIA [19–21]. The microarray analyses of gene expression in peripheral blood mononuclear cells from patients with s-JIA demonstrated the upregulation of genes associated with the activation of monocytes/macrophages, innate immune receptors, such as the Toll-like receptor, and signaling pathways, including TLR-IL-1, IL-6, and PPAR-γ signaling [19, 22–26]. Furthermore, most clinical manifestations of s-JIA can be described by the effects of innate proinflammatory cytokines, including IL-1, IL-6, IL-18, and TNF-α [2]. Notably, S100 proteins are also secreted by activated neutrophils and monocytes and exhibit proinflammatory, cytokine-like action [27, 28].

The pathology of various inflammatory diseases is often correlated with dynamic changes in macrophage activation. A study reported that both plasticity and flexibility are key features of macrophages and their activation states, and that the phenotype of polarized M1–M2 macrophages can be reversed [29]. Another previous study demonstrated that macrophages with a mixed M1/M2 phenotype were detected in s-JIA patients, and that both phenotypic macrophages were activated not only in the active phase of s-JIA but also in MAS [3]. These findings reveal that the macrophage

activation could be integral to the pathogenesis of s-JIA.

sCD163 reflects the extent of activation and expansion of phagocytic macrophages. Previous reports showed serum sCD163 level could indicate macrophage activation and might be a useful indicator of the disease activity [11, 30]. On the other hand, another report showed serum sCD163 level was not correlated with disease activity [31, 32]. Reportedly, serum sCD163 levels are valuable diagnostic markers for MAS and facilitate the identification of patients with occult forms [11]. By evaluating serum sCD163 levels in seven patients with s-JIA-associated MAS, Bleesing et al. reported that these levels were significantly higher than those in 25 patients with active-phase s-JIA, and that the serum sCD163 levels in patients with MAS were comparable to those in patients with primary and secondary forms of HLH [11]. Furthermore, five patients with active-phase s-JIA revealed high serum sCD163 levels compared to patients with MAS; these patients also exhibited low platelet counts and high serum ferritin levels, and two of the five patients developed MAS later [11]. In contrast, Reddy et al. assessed the serum sCD163 levels in two patients with s-JIA-associated MAS and demonstrated that these levels increased in only one patient [32]. In addition, four patients with active-phase s-JIA with high serum sCD163 levels (>1,800 ng/mL) revealed some laboratory abnormalities in MAS, including hypofibrinogenemia [32].

In this study, the serum sCD163 levels in s-JIA patients with MAS were significantly higher than those in patients with active-phase s-JIA; of note, these levels in patients with MAS were comparable to the levels in patients with EBV-HLH. A serial assessment of the serum sCD163 levels in patients with MAS revealed that these levels significantly increase with the progression of MAS and remained elevated until the complication was controlled. These results were comparable to those of previous studies, and suggest the utility of serum sCD163 levels as a disease marker of MAS.

In this study, the serum sCD163 levels positively correlated with ferritin, AST, LDH, and IL-18 levels, whereas negatively correlated with CRP and IL-6 levels. Furthermore, the serum sCD163 levels demonstrated the strongest correlation with serum IL-18 levels. Previously, we reported two subsets of patients with s-JIA comprising certain distinct clinical features based on IL-6 and IL-18 levels [33]. Patients in the IL-6–dominant subset (IL-18/IL-6 < 1,000) tended to have more severe joint disease, whereas those in the IL-18–dominant subset (IL-18/IL-6 > 1,000) were more likely to develop MAS. Furthermore, a previous study reported that TNF-α, IFN-γ, and IL-18 become dominant instead of IL-6 during the development of MAS from the acute phase of s-JIA [34]. It is possible that IL-18 plays a central role in the pathogenesis of MAS rather than IL-6, although IL-6 is a key cytokine in the pathogenesis of s-JIA. Based on these findings, it can be assumed that the serum

sCD163 levels might reflect systemic inflammation based on the activation and expansion of macrophages. Another possibility is that serum IL-6 levels might be affected by treatments such as steroids and cyclosporine given that several patients with s-JIA in this study were on medication.

Reportedly, CD163 is characterized as a scavenger receptor for hemoglobin, mediating endocytosis of hemoglobin–haptoglobin (Hb:Hp) complexes [35]. During intravascular hemolysis, free Hb binds to the plasma protein Hp to form Hb:Hp complexes. After endocytosis of Hb:Hp, the heme subunit of Hb is degraded by the induced rate-limiting enzyme heme oxygenase (HO)-1, yielding biliverdin, free iron, and carbon monoxide (CO), which has anti-inflammatory and cytoprotective effects [36, 37]. In M2 macrophages, CO reduces proinflammatory and increases anti-inflammatory cytokine secretion along with an increase in the synthesis of ferritin [38]. Ferritin binds free iron to prevent oxidative damage. Thus, HO-1-mediated anti-inflammatory effects might be closely linked to anti-inflammatory mechanisms such as suppression of immune and inflammatory responses in macrophages [39–41]. In our previous study, we reported the patient with HO-1 deficiency, who exhibited a significant increase in circulating heme and subsequent oxidative vascular and tissue injury, anemia, and chronic inflammation, resembling the phenotype of s-JIA [42, 43].

Reportedly, serum levels of HO-1 are highly elevated in patients with s-JIA and adult-onset Still's disease [44, 45].

Inadequate downregulation of immune activation is considered to be another key mechanism of s-JIA [2–4]. In this study, serum sCD163 levels significantly decreased in the inactive phase compared to those in the active phase of s-JIA; however, some patients showed elevated serum sCD163 levels even in the inactive phase. Of note, four patients with >1000 ng/mL of serum sCD163 in the inactive phase of s-JIA exhibited massively elevated levels of IL-18 (>50,000 pg/mL) in the active phase. In addition, the disease course of these patients presented a polycyclic pattern, and two of the four patients were complicated with MAS. These findings suggest that alternative macrophage activation plays an essential role not only in the active but also the inactive phase of s-JIA, and that inactive-phase s-JIA represents a state of compensated inflammation rather than absence of immune activity. However, during the remission phase, serum sCD163 levels significantly decreased to normal levels in this study. These findings validate the accuracy of the Wallace criteria for clinical remission and suggest that serum sCD163 levels might be a useful diagnostic laboratory criterion for clinical remission in s-JIA.

A study has reported that clinical symptoms and laboratory abnormalities in patients with s-JIA receiving TCZ were milder than in those not receiving TCZ therapy [12]; in particular, serum CRP concentrations did not increase during TCZ therapy, even in MAS. Hence, exploring new promising biomarkers that can detect and monitor the disease activity during the IL-6 blockade treatment is a pressing issue. In this study, the serum sCD163 levels in active-phase s-JIA were significantly elevated in patients not receiving TCZ compared to those receiving TCZ; this might be because TCZ was added to the steroid therapy with/without immunosuppressive drugs, and these treatments might affect serum sCD163 levels. In contrast, the serum sCD163 levels in MAS were similar in patients receiving and not receiving TCZ. Furthermore, these levels reflected the disease activity of s-JIA not only in patients not receiving TCZ but also in those receiving. Based on these findings, serum sCD163 levels could be considered a useful indicator of the disease activity even in patients receiving TCZ.

This study has some limitations. First, the small number of patients with s-JIA who were evaluated. Second, some patients with s-JIA were on medication which might affect serum sCD163 levels. Hence, further extensive studies are warranted to validate our preliminary data and draw firm conclusions.

In conclusion, this study suggests that serum sCD163 levels reflect the biological activities of the immune system in s-JIA and MAS. In addition, serum sCD163 levels indicating macrophage activation might be a unique indicator of the disease activity and a diagnostic laboratory criterion for clinical remission in patients with s-JIA, including patients receiving TCZ.

Acknowledgments

This work was supported by JSPS KAKENHI (Grant Number 26461571).

We thank Harumi Matsukawa for the technical assistance.

Disclosure Statement

The authors have no conflicts of interest to disclose. The authors have no financial relationship to this article to disclose.

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Figure Legends

Figure 1. Serum sCD163 levels in different patient groups.

Median value and range are shown. Statistically significant differences among the patient groups are shown as *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001. Active, patients with acute-phase systemic juvenile idiopathic arthritis; MAS, macrophage activation syndrome; EBV-HLH, Epstein–Barr virus-associated hemophagocytic syndrome; KD, Kawasaki disease; HC, healthy control.

Figure 2. Serum sCD163 levels in patients with s-JIA undergoing TCZ therapy.

Serum sCD163 levels in each s-JIA phase in patients receiving/not receiving TCZ therapy are shown. Median value and range are shown. Statistically significant differences between each patient group are shown as **P < 0.01, ***P < 0.01, and ****P < 0.0001. TCZ^-, patients not receiving tocilizumab; TCZ^+, patients receiving tocilizumab.

MAS, macrophage activation syndrome; TCZ, tocilizumab

Figure 3. A longitudinal follow-up of serum sCD163 levels in three patients with MAS.

Changes in the serum sCD163 levels (solid lines) are shown in the upper panels, and

those in LDH are shown in the lower panels. PSL, prednisolone; mPSL, methylprednisolone; DEX-P, dexamethasone palmitate; CyA, cyclosporine A; TCZ, tocilizumab; MAS, macrophage activation syndrome; LDH, lactate dehydrogenase.

Figure 4. Changes in serum sCD163 levels in each s-JIA phase.

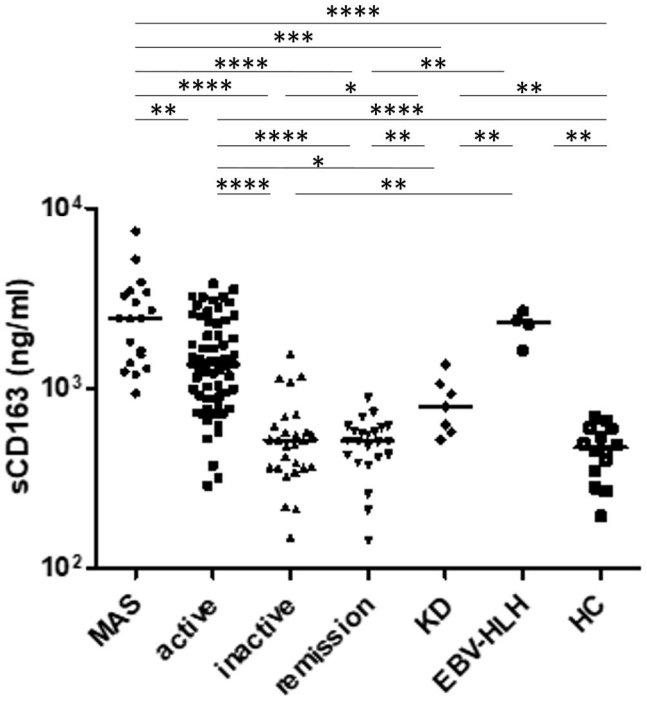
Statistically significant differences between each patient group are shown as ****P < 0.0001.

Figure 5. Correlations between serum sCD163 levels and other measures of the disease activity.

(A) AST, (B) LDH, (C) ferritin, (D) CRP, (E) IL-6, and (F) IL-18. Black cicles, active s-JIA patients, Red circles, patients complicated with MAS. AST, aspartate aminotransferase; LDH, lactate dehydrogenase; IL, interleukin; MAS, macrophage activation syndrome.

Supplementary Figure 1. Changes in serum sCD163 levels in patients with MAS.

Serum sCD163 levels in patients with MAS were significantly elevated during the MAS phase compared to those in the acute phase of s-JIA. A, results in patients not receiving tocilizumab; B, results in patients receiving tocilizumab; TCZ^-, patients not receiving tocilizumab; TCZ^+, patients receiving tocilizumab.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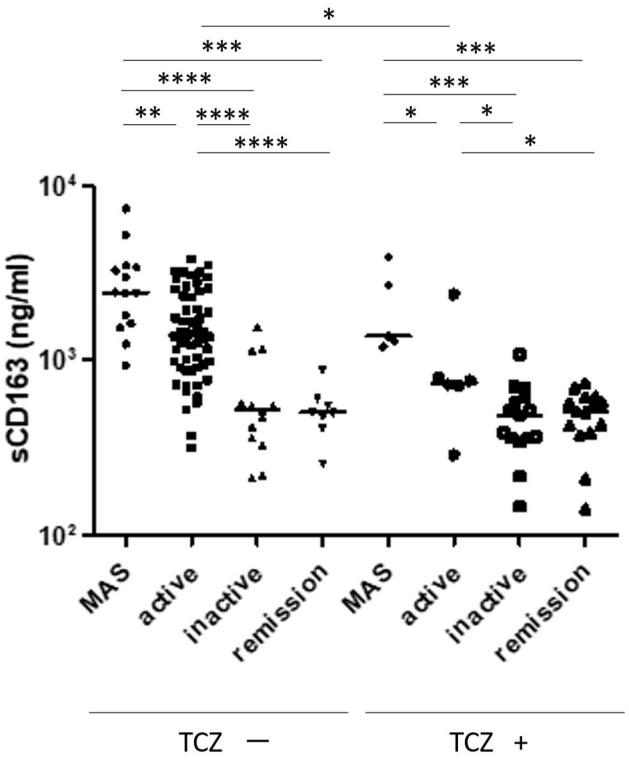
sCD163 (ng/ml)
10^4
10^3
10^2
MAS
active
inactive
remission
KD
EBV-HLH
HC

Figure 1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sCD163 (ng/ml)
10⁴
10³
10²
MAS
active
inactive
remission
MAS
active
inactive
remission
TCZ −
TCZ +

Figure 2

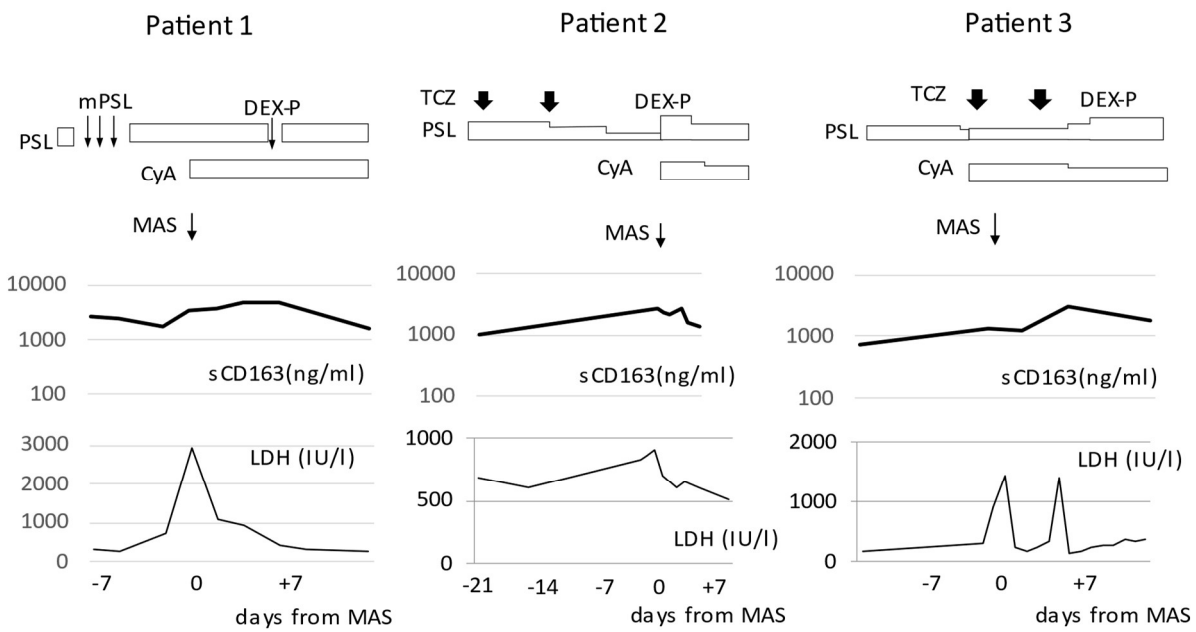

Patient 1
mPSL
DEX-P
PSL
CyA
MAS
10000
1000
100
sCD163(ng/ml)
3000
2000
1000
0
LDH (IU/l)
-7
0
+7
days from MAS
Patient 2
TCZ
DEX-P
PSL
CyA
MAS
10000
1000
100
sCD163(ng/ml)
1000
500
0
LDH (IU/l)
-21
-14
-7
0
+7
days from MAS
Patient 3
TCZ
DEX-P
PSL
CyA
MAS
10000
1000
100
sCD163(ng/ml)
2000
1000
0
LDH (IU/l)
-7
0
+7
days from MAS

Figure 3

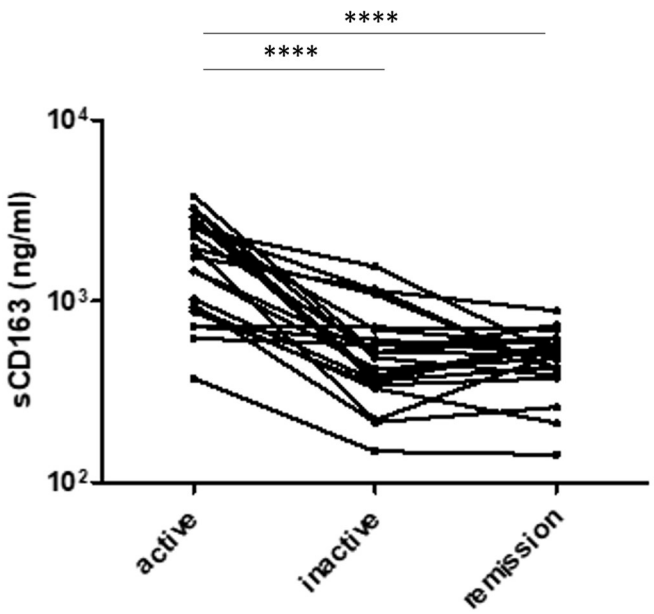


Figure 4

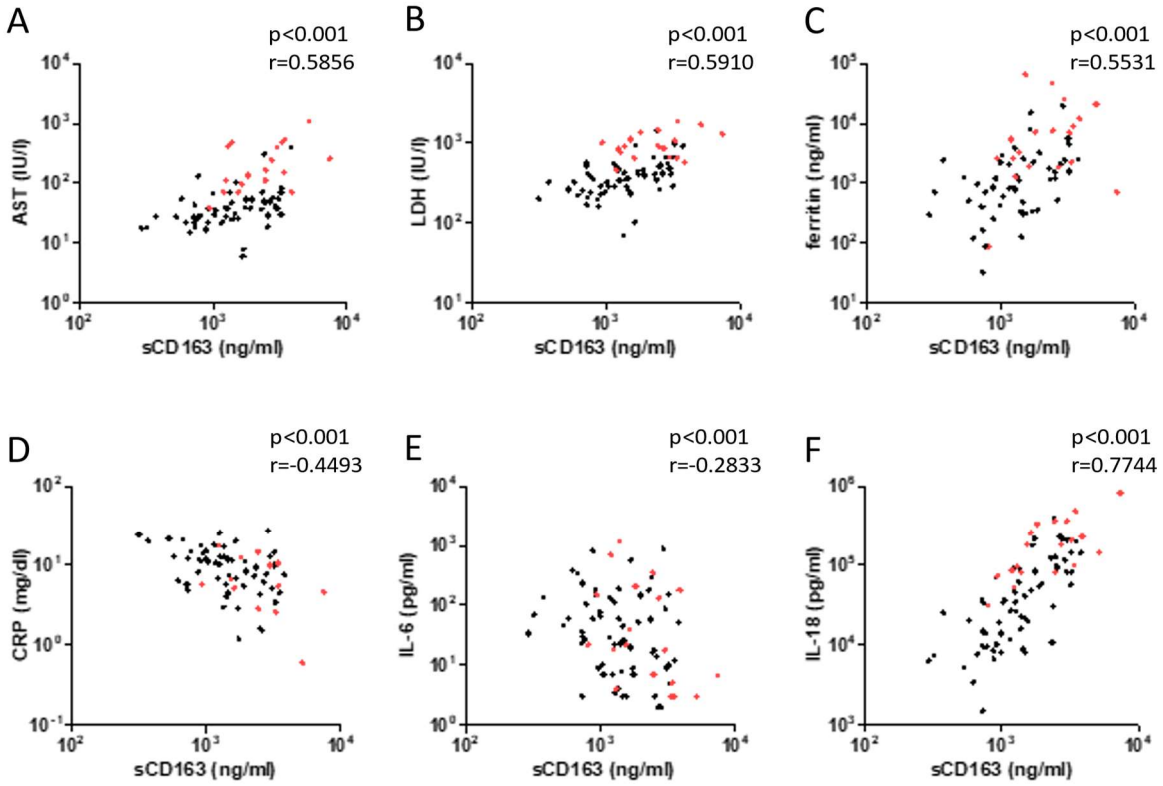
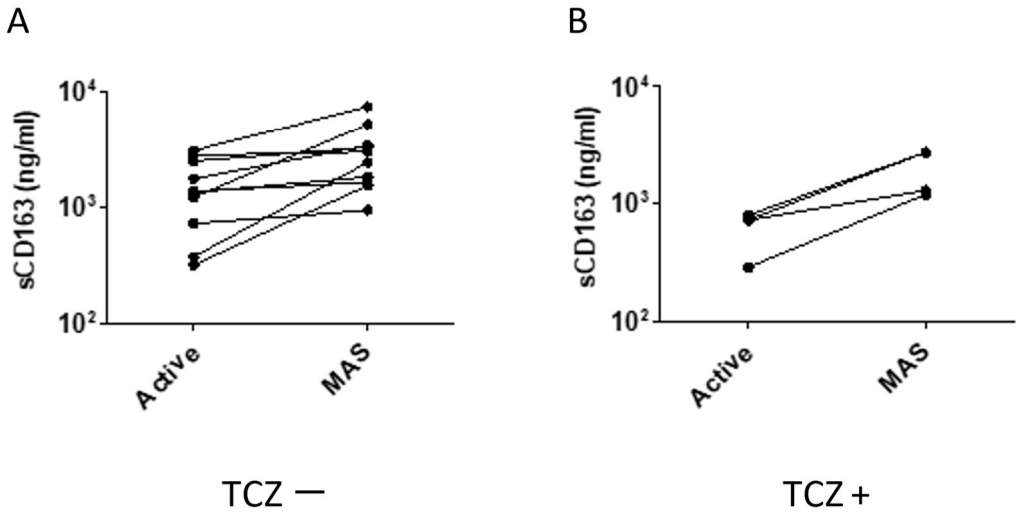

A
p<0.001
r=0.5856
AST (IU/l)
sCD163 (ng/ml)
B
p<0.001
r=0.5910
LDH (IU/l)
sCD163 (ng/ml)
C
p<0.001
r=0.5531
ferritin (ng/ml)
sCD163 (ng/ml)
D
p<0.001
r=-0.4493
CRP (mg/dl)
sCD163 (ng/ml)
E
p<0.001
r=-0.2833
IL-6 (pg/ml)
sCD163 (ng/ml)
F
p<0.001
r=0.7744
IL-18 (pg/ml)
sCD163 (ng/ml)

Figure 5



Supplement Figure 1

Table 1

Clinical characteristics of patients with acute-phase s-JIA

Patients	60 (62 episodes)
Age	6.5±4.7
Sex (male ; female)	29 ; 31
Disease duration (months)	9.3±17.1
Clinical symptoms (n=62)	
Fever	57
Rash	42
Hepatomegaly	7
Splenomegaly	3
Lymphadenopathy	16
Pleuritis	3
Pericarditis	4
Affected joint counts	1.8±2.5
Laboratory findings	
CRP (mg/dl)	10.6±6.5
AST (IU/l)	45±53
LDH (IU/l)	395±195
Ferritin (ng/ml)	2016±3436
Treatments	
prednisolone	18 (0.7±0.6mg/kg/day)
cyclosporine	6
metrexate	1
tocilizumab	6

Supplement Table 1

Clinical characteristics of patients with MAS complicating s-JIA

patients	19
Laboratory findings	
ferritin (ng/ml)	14247±18833
platelets (/mm^3)	12.8±6.0
AST (IU/l)	276±255
trigyceride (mg/dl)	250±157
fibrinogen (mg/dl)	215±102
LDH (IU/l)	1043±399
Treatments (n)	
None	6
Prednisolone	5
Prednisolone+Cyclosporine	3
Prednisolone+Tocilizumab	4
Prednisolone+Cyclosporine+Tocilizumab	1
Dose of prednisolone (mg/kg/day)	1.3±0.6

Supplement Table 2

Clinical characteristics of s-JIA patients during inactive and remission phase

Phase	inactive	remission
Patients	27	22
Age	6.6±5.8	7.0±6.1
Sex (male ; female)	8 ; 19	6 ; 16
Disease duration from active phase (months)	3.9±3.0	9.5±2.5
Clinical symptoms (n)		
Fever	0	0
Rash	0	0
Hepatomegaly	0	0
Splenomegaly	0	0
Lymphadenopathy	0	0
Pleuritis	0	0
Pericarditis	0	0
Affected joint counts	0	0
Laboratory findings		
CRP (mg/dl)	0.06±0.16	0.04±0.08
AST (IU/l)	25±5	24±6
LDH (IU/l)	283±90	250±61
Ferritin (ng/ml)	20.4±13.9	15.9±14.2
Treatments (n)		
None	3	4
Prednisolone	2	1
Cyclosporine	1	1
Tocilizumab	5	6
Prednisolone+Cyclospoine	5	2
Prednisolone+Tocilizumab	4	5
Prednisolone+Cyclosporine+Tocilizumab	7	3
Prednisolone dose (mg/kg/day)	0.69±0.57	0.28±0.22