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ORIGINAL ARTICLE

S100A12 and vascular endothelial growth factor can differentiate Blau syndrome and familial Mediterranean fever from systemic juvenile idiopathic arthritis

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Abstract

Objectives Systemic juvenile idiopathic arthritis (sJIA) has recently become regarded as one of the autoinflammatory syndromes (AIS). However, other AIS, such as familial Mediterranean fever (FMF) and Blau syndrome, have been initially misdiagnosed as sJIA because of the clinical similarities. Making the correct diagnosis in the early stage of these AIS is desirable. Therefore, we evaluated serum S100A12 and vascular endothelial growth factor (VEGF) levels to determine if they could be biomarkers for differentiating these AIS.

Method Serum S100A12 and VEGF levels were examined in patients with Blau syndrome ($n = 4$), FMF ($n = 4$), and sJIA ($n = 11$) in the active and inactive phases.

Results In the active phase, S100A12 levels were significantly higher in patients with sJIA and FMF compared with those with Blau syndrome ($p < 0.001$). VEGF levels of patients with sJIA were significantly higher than those of patients with others ($p = 0.001$). In the inactive phase, there was no significant difference in VEGF levels. However, colchicine-resistant patients or patients without treatment with FMF showed high levels of S100A12 compared with others.

Conclusions Measuring both serum S100A12 and VEGF levels may be useful for differentiating patients with Blau syndrome and FMF from those with sJIA at the early stage.

Keywords Blau syndrome · Familial Mediterranean fever · S100A12 protein · Systemic juvenile idiopathic arthritis · Vascular endothelial growth factor

Introduction

During the last decades, the description of autoinflammatory syndromes (AIS) has led to great interest worldwide. AIS are rare, childhood-onset disorders of innate immunity. In AIS, recurrent flares of fever and inflammation affecting the skin, joints, gastrointestinal tract, or serous membranes are the most striking signs, without any evidence of autoantibody production or underlying infections [1]. Recently, some reports have supported the classification of systemic juvenile idiopathic arthritis (sJIA) as one of the AIS [2–5]. Important differential diseases from sJIA are systemic infection, leukemia, and other AIS. However, clinical mimicry of symptoms, such as a high fever, skin rash, joint pain, high C-reactive protein (CRP) levels, and leukocytosis, further makes the diagnosis difficult. In fact, 9 of 13 patients with AIS who were diagnosed by a genetic examination in our clinic were initially diagnosed as sJIA [6]. Arostequi et al. reported that the initial diagnosis was sJIA in six of 12 patients with Blau syndrome [7]. Actually, fever is mentioned as a clinical symptom of Blau syndrome [8]. Moreover, there are no specific biomarkers that distinguish these diseases from sJIA.

S100A12 (extracellular newly identified receptor for advanced glycation end product-binding protein [EN-RAGE]; calgranulin C), a marker of granulocyte activation, is a useful marker protein for monitoring disease activity in several inflammatory diseases [9]. S100A12 levels in patients with familial Mediterranean fever (FMF) and sJIA are significantly different compared with those in patients with severe

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infections, leukemia, and other AIS [10]. However, the relations of S100A12 and other AIS are unclear.

Vascular endothelial growth factor (VEGF) is an angiogenic factor. Inflammation in the synovium increases VEGF production by vascularization. VEGF receptor is also present in synovial cells and chondrocytes of the joint. In patients with JIA, serum VEGF concentrations are significantly correlated with disease activity [11], especially in the polyarticular subgroup [12]. Higher VEGF concentrations in synovial fluid in patients with JIA compared with serum VEGF concentrations suggest that VEGF may have a major role at the site of tissue inflammation [11]. FMF and Blau syndrome also have joint pain as one of the main symptoms, but they show less joint destruction than sJIA. Furthermore, the relation between VEGF and disease activity of AIS has not been evaluated.

This study aimed to evaluate whether VEGF and S100A12 are useful biomarkers for differentiating Blau syndrome and FMF from sJIA.

Methods and materials

Patients

A total of 19 patients, including 4 patients with Blau syndrome, 4 patients with FMF, and 11 patients with sJIA, were studied. Diagnosis of Blau syndrome and FMF was made by confirming genetic mutation of *NOD2* for Blau syndrome, and *MEFV* for FMF in each patient (Table 1). All 4 patients with FMF in this study

Table 1 Characteristics of patients and similarity with Blau syndrome, FMF, and sJIA

	Sex	Age of onset (years)	Gene mutation	Initial diagnosis	Symptoms			Therapy	
					Fever	Skin rash	Arthritis	Active	Inactive
Blau $n = 4$	M	1	R334Q	sJIA	+	+	+	PSL	PSL, MTX, IFX
	M	1	R334Q	sJIA	+	+	+	PSL	PSL, MTX, IFX
	F	3	R334Q	pJIA	–	–	+	MTX	MTX, ETN
	F	3	R587C	pJIA	–	–	+	MTX	MTX, IFX
FMF $n = 4$	F	4	E148Q/M694I	sJIA	+	+	+	None	Colchicine
	F	7	E148Q/M694I	sJIA	+	–	+	None	Colchicine
	F	10	E148Q/M694I	–	+	–	–	None	Colchicine
	F	15	E148Q/M694I	–	+	–	–	None	None
sJIA $n = 11$	M	2	NT		+	+	+	None	None (DFR)
	M	2	NT		+	+	+	None	TCZ
	M	2	NT		+	+	+	PSL	MTX, TCZ
	M	3	NT		+	+	+	None	PSL, TCZ
	M	5	NT		+	+	+	None	PSL, TCZ
	M	5	NT		+	+	+	None	PSL, CYA
	M	6	NT		+	+	+	None	PSL
	F	3	NT		+	+	+	None	TCZ
	F	3	NT		+	+	+	None	PSL
	F	3	NT		+	+	+	None	PSL, CYA, TCZ
	F	4	NT		+	+	+	None	MTX

FMF familial Mediterranean fever

sJIA systemic juvenile idiopathic arthritis

pJIA (rheumatoid factor negative) polyarticular type juvenile idiopathic arthritis

NT not tested

PSL prednisolone

MTX methotrexate

IFX infliximab

ETN etanercept

TCZ tocilizumab

CYA cyclosporine

DFR drug-free remission

were family members with the E148Q/M694I mutation. All patients with sJIA were diagnosed according to the International League Against Rheumatism Edmonton criteria [13]. Six of 11 patients with sJIA received tocilizumab (a humanized anti-IL-6 receptor monoclonal antibody) concomitant with or without steroid therapy in the inactive phase. All of the patients with Blau syndrome received anti-tumor necrosis factor (TNF)-α treatment (3 patients used infliximab, 1 patient used etanercept) concomitant with methotrexate in the inactive phase. In the active phase of Blau syndrome, treatment of prednisolone or methotrexate had already started in all specimens. Two of the patients with Blau syndrome were complicated by uveitis. Three of 4 patients with FMF were treated with colchicine, but it was effective for only one of them (Table 1).

Serum samples

Serum samples of patients with Blau syndrome, FMF, and sJIA were obtained in the active and inactive phases. Patients with arthritis (joint pain or swelling, hotness) and/or fever and/or serositis (abdominal pain or chest pain) and with CRP levels ≥ 1 mg/dl were defined as being in the active phase; otherwise, they were defined as being in the inactive phase. Samples were obtained randomly at one point during the period of the disease active phase and inactive phase.

Patients who showed infectious symptoms, such as influenza, cold, enterocolitis, and sinusitis, were excluded from the study. Serum was separated from cells, divided into aliquots, frozen, and stored at − 40 °C until later use.

Analysis of biomarkers

Serum S100A12 and VEGF concentrations were evaluated using an ELISA, according to the manufacturer's instructions (S100A12: CycLex Co., Nagano, Japan; VEGF: GE Healthcare Bio-Sciences, Tokyo, Japan). Clinical findings and routine laboratory data, CRP levels (mg/dl), were also examined using the same serum samples.

Statistical analysis

SPSS version 19.0 for Windows (International Business Machines Corporation, Tokyo, Japan) was used for statistical analysis. The Shapiro–Wilk test was applied to parametric or nonparametric distribution. Within-group comparisons were analyzed by Tukey's honestly significant difference test for parametric variables, and by the Kruskal–Wallis test and the Mann–Whitney test for nonparametric variables. For the analyzed measures, p values less than 0.05 were considered significant.

Ethical considerations

This study was performed in accordance with the ethical standards laid down in an appropriate version of the 1964 Declaration of Helsinki. This study was approved by the Institutional Review Board at the Kagoshima University Hospital (approval number 27-110). All specimens were used after receiving informed consent from the patients or their parents.

Results

Inflammatory markers in the active phase

CRP levels of patients with sJIA (mean, 12.7 mg/dl; range, 4.5–29 mg/dl) and FMF (mean, 7.1 mg/dl; range, 1.7–15.9 mg/dl) were significantly higher than those of patients with Blau syndrome (mean, 3.1 mg/dl; range, 1.1–7.1 mg/dl) ($p = 0.004$).

S100A12 and VEGF in the active phase

Serum S100A12 levels of patients with sJIA and FMF were significantly higher than those of patients with Blau syndrome ($p < 0.001$) (Fig. 1a). The mean S100A12 concentration was 397.4 ng/ml and the median was 432.7 ng/ml (range, 255.1–468.9 ng/ml) in patients with Blau syndrome. The mean S100A12 concentration was 2569.1 ng/ml and the median was 2752.4 ng/ml (range, 1638.8–3132.9 ng/ml) in patients with FMF. The mean S100A12 concentration was 2571.1 ng/ml and the median was 2507.6 ng/ml (range, 1422.8–3795.4 ng/ml) in patients with sJIA. In contrast to S100A12, greatly elevated VEGF levels were observed only in patients with sJIA ($p = 0.001$) (Fig. 1b). The mean VEGF concentration was 2026.2 pg/ml and the median was 1996.4 pg/ml (range, 685.5–3972.9 pg/ml) in patients with sJIA. Mean VEGF concentrations in patients with Blau syndrome (497.3 pg/ml; median, 506.6 pg/ml; range, 227.9–747.9 pg/ml) and FMF (286.3 pg/ml; median, 260.0 pg/ml; range, 39.7–585.5 pg/ml) were low.

S100A12 and VEGF in the inactive phase

In the inactive phase, high S100A12 concentrations were observed only in patients with FMF. In FMF, serum S100A12 concentrations in one patient were treated effectively with colchicine, and were low. One patient with FMF who was untreated and two patients who were nonresponders to colchicine had high S100A12 concentrations, despite being in the inactive phase (Fig. 1c). In patients with Blau syndrome, the mean S100A12

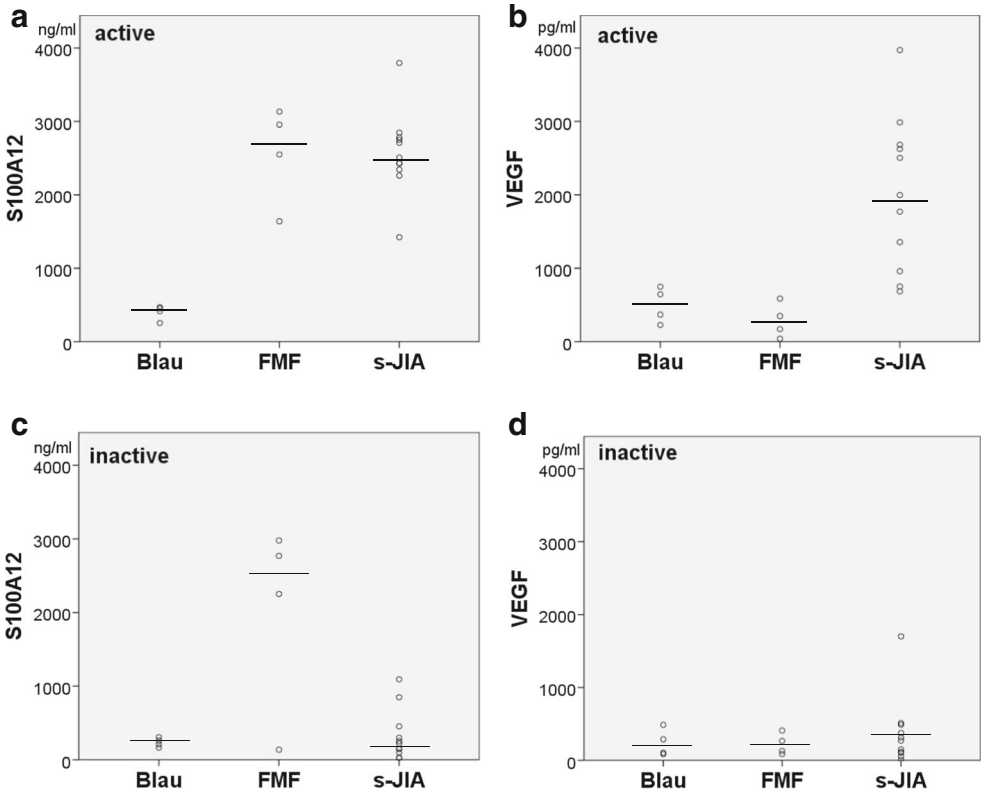

Fig. 1 Serum S100A12 (ng/ml) and VEGF (pg/ml) concentrations in the active phase and inactive phase. **a** There was no significant difference in S100A12 concentrations between FMF and sJIA, but those were significantly higher than Blau syndrome (sJIA vs Blau, $p < 0.001$; FMF vs Blau, $p < 0.001$; sJIA vs FMF, $p = 0.276$). **b** VEGF concentrations in patients with sJIA were significantly higher than those in the other groups ($p < 0.001$). **c** S100A12 concentrations in the inactive phase. S100A12 concentrations in patients with FMF were significantly higher than those in patients with Blau syndrome and sJIA. **d** VEGF concentrations in the inactive phase. There was no significant difference in VEGF concentrations among the disease groups. The bars show the median

concentration was 236.4 ng/ml (median, 236.2 ng/ml; range, 165.8–307.2 ng/ml). In patients with FMF, the mean S100A12 concentration was 2034.8 ng/ml (median, 2511.7 ng/ml; range, 138.2–2977.7 ng/ml). In patients with sJIA, the mean S100A12 concentration was 329.3 ng/ml (median, 220.5 ng/ml; range, 26.3–1092.8 ng/ml). There was no significant difference in serum VEGF concentrations among these three groups (Fig. 1d). In patients with Blau syndrome, the mean VEGF concentration was 243.4 pg/ml (median, 198.3 pg/ml; range, 88.4–488.7 pg/ml). In patients with FMF, the mean VEGF concentration was 225.0 pg/ml (median, 200.1 pg/ml; range, 89.3–410.6 pg/ml). In patients with sJIA, the mean VEGF concentration was 373.6 pg/ml (median, 272.0 pg/ml; range, 7.2–1701.4 pg/ml).

Distribution map of S100A12 and VEGF

In the active phase, in patients with Blau syndrome, S100A12 and VEGF levels were mildly elevated. In patients with FMF, S100A12 levels were high and VEGF levels were mildly elevated. In patients with sJIA, S100A12 and VEGF levels were high. In the inactive phase, only patients with FMF showed high S100A12 levels (Fig. 2). However, this finding was limited to untreated or colchicine-resistant cases.

Discussion

Diagnosis of AIS is difficult due to the presence of atypical cases, the similarity of clinical symptoms, and generally unknown. Blau syndrome is an AIS characterized by a triad of skin rash, symmetrical polyarthritis, and uveitis. Uveitis can develop severe visual impairment that affected the quality of life. The past reports showed the efficacy of anti-TNF-α therapy (infliximab and adalimumab) for childhood uveitis [14, 15] and that of infliximab for Blau syndrome [16]. SJIA has risk of moving to a fatal macrophage activation syndrome, and the basis of treatment is not anti-TNF therapy but glucocorticoid. Because complications especially prognosis of visual and treatments are different, there are clinical implications of differentiating early stage of each diseases.

The term AIS was coined by McDermott et al. who reported the discovery of mutations in the TNF receptor superfamily1A (*TNFRSF1A*) gene in a series of families with a dominant inherited periodic fever syndrome, named TRAPS [17]. Therefore, AIS originally referred to a group of inflammatory diseases with a strong genetic basis defined by a Mendelian inheritance. AIS was initially defined as hereditary recurrent fever, but has expanded to mean many disorders. AIS are thought to include, FMF, TRAPS, cryopyrin-associated periodic syndrome (CAPS), hyper-IgD with

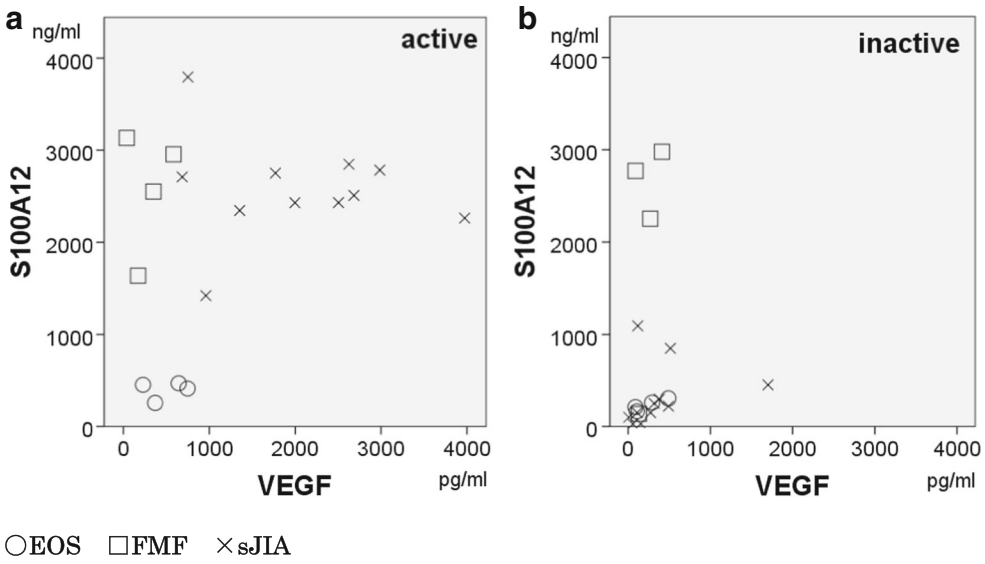

Fig. 2 Distribution map of S100A12 and VEGF. **a** In the active phase, the distribution of serum S100A12 and VEGF concentrations were different in each disease. **b** In the inactive phase, patients with FMF (non-responders to colchicine or non-treatment) are shown in the top left and colchicine responders are shown in the lower left of the panel

periodic fever syndrome, periodic fever with aphtous pharingitis and adenitis (PFAPA), Majeed syndrome, Blau syndrome, Behcet disease, gout, and sJIA [1, 4, 18]. The diagnosis of AIS is essentially based on clinical features complementing by genetic testing, which should be clinically oriented. However, genetic mutation caused by sJIA has not been found. These circumstances have led to Blau syndrome being misdiagnosed as sJIA [8, 19].

According to Foell et al. [20] and Maeno et al. [12], S100A12 is < 120 ng/ml and VEGF is < 150 pg/ml in the healthy condition. In our study, measurement of serum S100A12 and VEGF levels showed the possibility of differentiating Blau syndrome and FMF from sJIA (Fig. 2). Additionally, the clinical manifestation, family history, and genetic testing could help to rule out these rare diseases.

In sJIA, all data, S100A12, VEGF, white blood cell, hemoglobin, platelet, and CRP, were related to its activity, but this was not found in Blau syndrome and FMF (data not shown). Serum S100A8 (also known as myeloid-related protein [MRP]-8) and S100A9 (MRP-14) concentrations in sJIA are as high as those of S100A12. S100A12 is expressed and secreted by granulocytes specifically [20]. In contrast, S100A8 and S100A9 are not restricted to granulocytes. A previous study showed that the difference between healthy controls and patients with strongly active sJIA was more pronounced for S100A12 than for S100A8 and S100A9 [20]. This finding indicates a prominent role for the neutrophil-derived S100A12 during the systemic inflammatory processes that are involved in sJIA.

A relation between FMF and S100A12 has already been reported as follows. S100A12 was shown to be associated with active FMF, and colchicine treatment led to a dramatic decrease in serum S100A12 concentrations [21]. In our study, in the inactive phase, high S100A12 levels were observed only in patients with FMF, but not in those with sJIA and Blau syndrome. In the inactive phase, two patients with FMF were unable to control the attack with colchicine and one who was untreated had high S100A12 levels. Only one patient who was well controlled with colchicine had normal S100A12 levels. This result indicates that S100A12 may be a useful marker for evaluating FMF, even in the inactive period. More studies are required to confirm this finding or the effect of colchicine.

VEGF levels were increased over 1000 pg/ml in patients with sJIA, and increased less 1000 pg/ml in those with Blau syndrome and FMF in our study. In JIA, serum VEGF levels are increased and correlate with disease activity [11, 12]. Innate immune pathways that are activated in sJIA are normally triggered by the recognition of pathogen-associated molecular patterns by Toll-like receptors that are expressed on innate immune cells. However, these pathways can also be triggered by endogenous ligands in inflammatory conditions. These intracellular pathways lead to activation of the transcription factor nuclear factor-kappa B, which upregulates expression of genes encoding several proinflammatory cytokines (interleukin-6, TNF, interleukin-1, interleukin-18). VEGF is produced by platelets, macrophages, endothelial cells, and fibroblasts upon stimulation by these cytokines.

The features of symptoms should help diagnose autoinflammatory diseases in spite of uncertain conventional laboratory examinations. The typical rash that is observed in sJIA is transient, and shows pink maculae. However, eruptions that are observed in Blau syndrome are quite varied, such as erythema, papules, and plaques. With regard to eye involvement, anterior uveitis manifested by keratic precipitates, iris nodules, and focal posterior synechiae is a common finding in sarcoidosis. However, uveitis in JIA rarely involves the posterior globe [19]. Additionally, joint symptoms in Blau syndrome are

atypical. Joint pain without destruction or swelling of the joints, such as cystoma at the back of the wrist or outside of the foot joint, was observed in our patients. In FMF, erythema like erysipelas might be confirmed around the foot joint (3–46%), and eye involvement is a rare complication. We believe that S100A12 and VEGF levels are useful markers in differential diagnosis of autoinflammatory diseases when patients have suspected symptoms.

This study has some limitations. We evaluated biomarkers in the active and inactive phases at only one point with a small number of patients. However, our results are important because there have been no reports regarding biomarkers for differentiating AIS. We are planning a study with an increased number of patients with multiple sampling in the active and inactive phases. Additionally, further studies of other AIS including CAPS, TRAPS, and PFAPA in the future are also needed.

Conclusion

Measuring both serum S100A12 and VEGF levels may be useful in differentiating patients with Blau syndrome and FMF from those with sJIA. In active patients with sJIA, serum S100A12 protein and VEGF levels are over 2000 ng/ml and 1000 pg/ml but not patients with Blau syndrome. Patients with the active phase FMF, S100A12 shows over 2000 ng/ml but VEGF does not exceed 1000 pg/ml.

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Compliance with ethical standards

This study was performed in accordance with the ethical standards laid down in an appropriate version of the 1964 Declaration of Helsinki. This study was approved by the Institutional Review Board at the Kagoshima University Hospital (approval number 27-110). All specimens were used after receiving informed consent from the patients or their parents.

Disclosures None.

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