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CASE REPORT

Magnetic resonance imaging can detect thoracic inflammation due to familial Mediterranean fever

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Abstract A 32-year-old man presented to our hospital complaining of periodic fever and unilateral chest pain. We suspected that he had familial Mediterranean fever because of his symptoms. Magnetic resonance imaging (MRI) showed an increased intensity within the anterior chest wall, which was consistent with the site of his pain. Genomic analysis showed the patient to be heterozygous for the *E148Q/M694I* mutation in the *MEFV* gene, and we diagnosed familial Mediterranean fever. The ability of MRI to detect inflammatory changes could provide useful additional information for evaluating thoracic symptoms in FMF patients, and the detection of inflammatory changes using MRI may aid in early diagnosis, thus contributing to early and adequate treatment.

Keywords Familial Mediterranean fever · Magnetic resonance imaging · Chest pain · Pleuritis

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Introduction

Familial Mediterranean fever (FMF) is an inherited disease characterized by recurrent episodes of fever and serosal inflammation leading to abdominal, chest, or articular pain. Radiological findings of multi-organ involvement have been reported; however, radiological abnormalities of the chest include only a small amount of pleural effusion or elevation of the diaphragm [1]. In the case of FMF reported here, magnetic resonance imaging (MRI) was useful in detecting pleural inflammation as the cause of the patient's chest pain.

Case report

A 32-year-old Japanese man presented to our hospital complaining of periodic fever, dyspnea from unilateral chest pain, and joint pain in his large joints, mainly those of his legs. These symptoms initially developed at eight years of age, had occurred every six months since he was 20 years old, and then began to occur once a month at the age of 28, lasting for two days. His symptoms had disappeared by the time he reached our hospital. Although chest X-ray and computed tomography (CT) showed no abnormal findings, we suspected him of having FMF because of his recurrent and periodic fever with chest pain. We told him to return to our hospital again when the attack recurred. In June 2010, the chest pain and fever recurred, and he was readmitted for further investigation.

Physical examination was normal except for a body temperature of 38.3 °C. White blood cell count was increased to 12,000/mm^3 with 70.4 % neutrophils. C-reactive protein was elevated to 2.1 mg/dL. Chest X-ray showed no abnormal findings. However, chest CT showed

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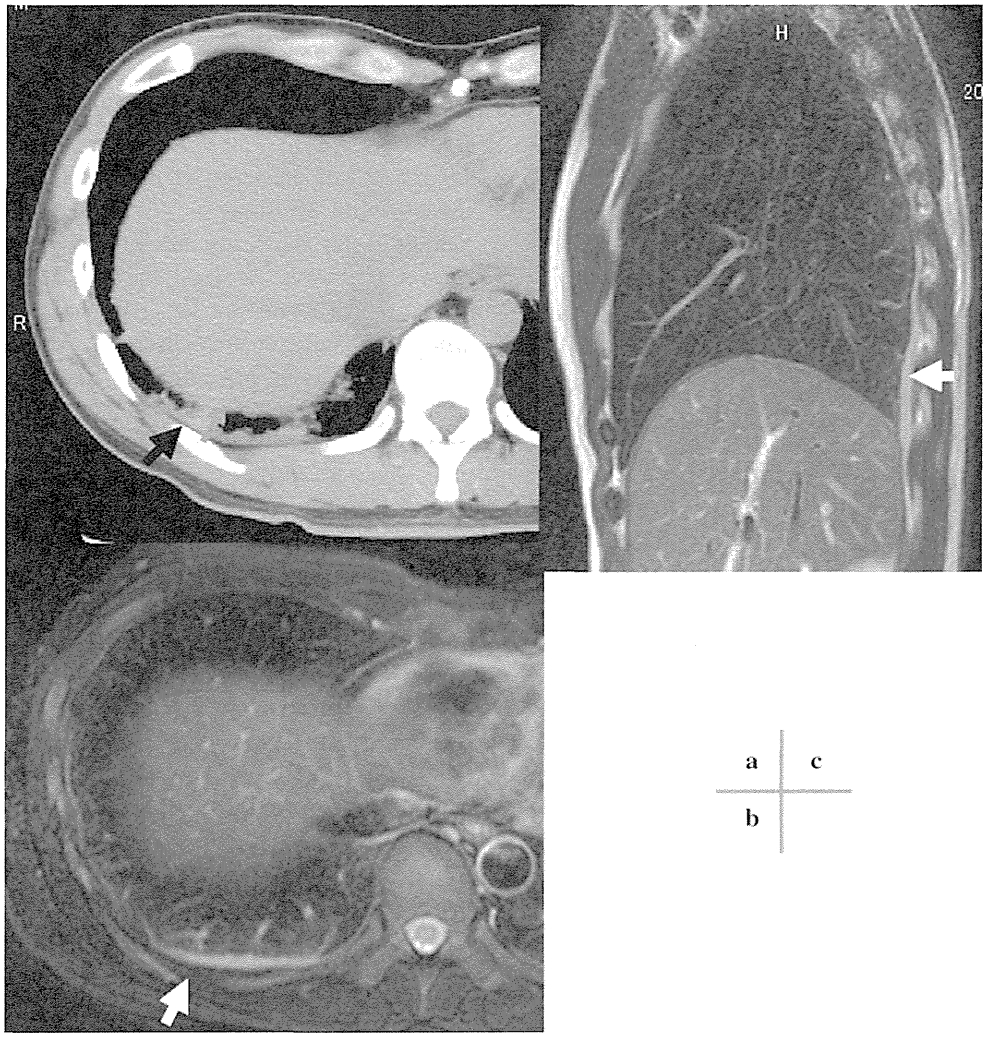

Fig. 1 Chest imaging at the level of the liver. **a** Chest computed tomography showed an irregular density along the pleura (*black arrow*). Chest magnetic resonance imaging showed pleural effusion (*white arrow*) (**b** spectral presaturation with inversion-recovery fat-suppressed T2-weighted image, **c** T2-weighted images)

an irregular density along the pleura (Fig. 1a) that could not be differentiated from atelectasis because the patient could not breathe deeply due to his striking pain. We then performed MRI, which showed that the irregular density along the dorsal pleura detected by CT was compatible with the location of the increased intensity along the pleura on T2-weighted MRI images (Fig. 1b, c), indicating pleural effusion. In addition, MRI showed an increased intensity within the anterior chest wall (Fig. 2a), which was consistent with the site of his pain, and for which CT showed no abnormality (Fig. 2b). There were no areas of increased intensity in pain-free areas. These findings suggested thoracic inflammatory changes. Fever and chest pain disappeared spontaneously in two days without antibiotics. Genomic analysis using polymerase chain reaction was performed to investigate exons 1, 2, 3, and 10 of the *MEFV* gene, where most previously reported mutations relating to FMF have been located. Genomic DNA was extracted from peripheral leukocytes using standard procedures. The patient was then found to be heterozygous for *E148Q/M694I*, the mutations of which are the most frequently detected substitutions in Japan [2]. We diagnosed the patient with FMF based on diagnostic criteria [3] and started him on colchicine at a dose of 1.0 mg daily. His symptoms subsided after one month of continuous treatment. Presently, at seven months after the start of the colchicine, the patient remains in a stable condition without further episodes of fever or chest pain. The area of increased intensity found on MRI during his attack has disappeared (Fig. 3).

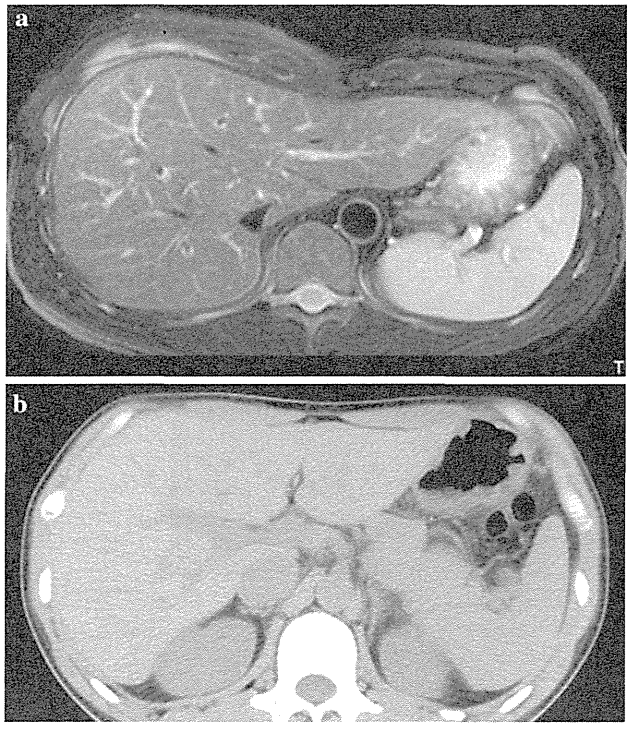

Fig. 2 Chest imaging at the level of the lung base. Magnetic resonance imaging showed increased intensity within the anterior chest wall (*white arrow*) (**a** spectral presaturation with inversion-recovery fat-suppressed T2-weighted image), whereas chest computed tomography showed no abnormal findings in the anterior chest wall (**b**)

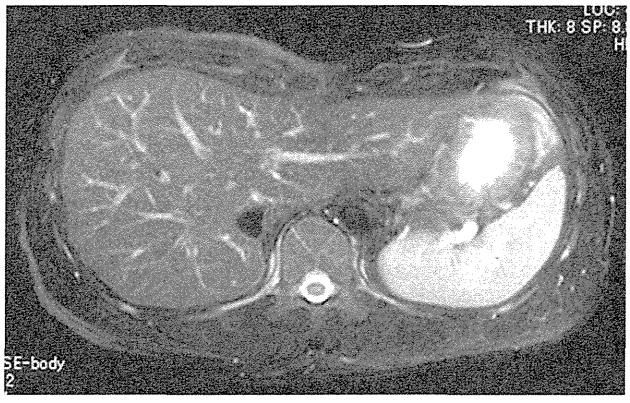

Fig. 3 Chest magnetic resonance imaging at the level of the liver seven months after the start of the colchicine. The area of increased intensity found on MRI during his attack has disappeared (spectral presaturation with inversion-recovery fat-suppressed T2-weighted image)

Discussion

FMF is a hereditary inflammatory disease characterized by recurrent attacks of fever and serositis, and is caused by mutations in the *MEFV* gene that encodes pyrin or marenostrin. Abdominal pain, the most frequent initial manifestation of FMF, occurs in 95 % of patients. Pleuritic chest pain and fever as initial manifestations are observed in less than 10 % of patients, but approximately 40 % experience an episode of febrile pleurisy during their disease course [4, 5]. Chest X-ray during the acute pleuritic attacks reveals elevation of the ipsilateral diaphragm and small pleural effusions [1, 6]. In the present case, chest X-ray did not show any abnormal findings, and although chest CT showed an irregular density along the pleura (Fig. 1a), we could not be confident that it was pleural effusion. In inflammatory disorders, MRI has the capacity to show soft tissue inflammatory changes, and in patients with FMF, edematous and inflammatory changes of the intermuscular septa and median gastrocnemius muscle can be detected by MRI [7]. Thus, we thought that MRI could also detect inflammatory changes in this patient. We therefore performed MRI, which showed pleural effusion and increased intensity within the anterior chest wall that was consistent with the site of pain. The area of increased intensity was present only at the site of the patient's pain, and it was not present during a pain-free interval. These findings indicate the importance of the increased intensity seen on MRI. To our knowledge, there have been no reports that MRI can show abnormal findings consistent with the site of pain in the detection of any abnormality related to the chest pain occurring with FMF. Although histological analysis was not performed in our patient, we thought that the increased intensity seen on MRI indicated the extension of pleural inflammation to the chest wall. The ability of MRI to detect inflammatory changes could add useful information for evaluating thoracic symptoms in FMF patients. Detection of inflammatory changes with MRI may aid in early diagnosis, which contributes to early and adequate treatment.

In conclusion, we have presented a patient with FMF in whom MRI was useful for detecting thoracic inflammation and for estimating pleural effusion. The use of MRI is worthy of consideration in cases of suspected FMF.

Acknowledgments We thank our colleagues at Saitama Cardiovascular and Respiratory Center for their detailed comments regarding this case.

Conflict of interest None.

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CASE REPORT

Coexistence of polymyositis and familial Mediterranean fever

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Abstract Familial Mediterranean fever (FMF) is an autosomal recessive disease affecting populations surrounding the Mediterranean area. In this case report, we report a Japanese female patient with polymyositis (PM) who presented with periodic fever. Genetic analysis revealed that she had compound heterozygous mutations in exon 2 of the *MEFV* gene (L110P/E148Q/R202Q). Treatment with colchicines (1.0 mg/day) successfully eliminated febrile attack and normalized the elevated levels of neutrophil CD64 expression, leading to the diagnosis of FMF. The association of FMF and PM has not previously been reported, so we discuss this rare association.

Keywords Familial Mediterranean fever · CD64 · *MEFV* gene · Polymyositis

Introduction

Familial Mediterranean fever (FMF) is a genetic autoinflammatory disease characterized by frequently relapsing fever and polyserositis. It particularly affects Jewish, Armenian, Arab, and Turkish communities in the Mediterranean region [1]. FMF is caused by mutations in the *MEFV* gene encoding pyrin, and various *MEFV* mutations have been reported in Japanese FMF patients [2]. Polymyositis is an inflammatory muscle disease of unknown etiology characterized by proximal muscle weakness [3]. Some clinical features of FMF mimic those of autoimmune diseases. Due to increasing knowledge regarding FMF myalgia [4], leg pain on exertion was defined to be one of the minor diagnostic criteria for FMF by Liveh et al. [5]. In this report, we describe a PM patient with periodic fever who had compound heterozygous mutations in the *MEFV* gene (L110P/E148Q/R202Q). Oral colchicines successfully silenced her periodic fever. This is the first case report in the literature of a patient with PM presenting with periodic fever due to FMF, and we discuss this rare association below.

Case report

A 37-year-old Japanese woman visited our hospital for treatment of polymyositis (PM) in April 2010. She presented with easy fatigability and muscle weakness of the proximal muscles of upper and lower extremities, and was diagnosed clinically with polymyositis (PM) at age 36 in the USA. Since the initial diagnosis, she had been treated with high-dose corticosteroid (prednisolone 40 mg/day), which was later combined with azathioprine (100 mg/day) in September 2009. In April 2010 she returned to Japan, and when she visited our hospital she was suffering from PM associated with proximal muscle weakness, despite the treatments that she was receiving; i.e., corticosteroid (prednisolone 20 mg/day) plus azathioprine (100 mg/day).

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Table 1 Laboratory findings on admission

Parameter	Value
Peripheral blood	
Red blood cells	394 × 10^4/μl
Hemoglobin	13.4 g/dl
Hematocrit	38.1 %
White blood cells	14100/μl
Neutrophil	94.5 %
Monocyte	0.5 %
Lymphocyte	4.5 %
Platelet	44.3 × 10^4/μl
Blood chemistry	
Total protein	7.0 g/dl
Total bilirubin	0.5 mg/dl
Glutamic-oxaloacetic transaminase	34 IU/l (7–33)
Glutamic-pyruvic transaminase	23 IU/l (5–30)
Lactate dehydrogenase	266 IU/l (119–229)
Alkaline phosphatase	210 IU/l (80–250)
Gamma-glutamyl transpeptidase	14 IU/l (5–55)
Creatinine kinase	478 IU/l (60–160)
Aldorase	16.1 IU/l
Total cholesterol	212 mg/dl
Blood urea nitrogen	10.4 mg/dl
Creatinine	0.5 mg/dl
Alb	4.1 g/dl
Na	139 mEq/l
K	4.7 mEq/l
Cl	104 mEq/l
Serological tests	
C-reactive protein	2.45 mg/dl (<0.30)
Erythrocyte sedimentation rate	53.0 mm/h
IgG	1344 mg/dl (900–2000)
C3	123 mg/dl
C4	24 mg/dl
Anti-nuclear Ab	(−)
Anti-ds DNA Ab	<10 U/ml (<10)
Anti-Jo-1 Ab	64 U/ml
KL-6	95 IU/ml
Virological test	
HCV-Ab	(−)
HBsAg	(−)
CMV-antigenemia	(−)
Urinalysis	Normal

CMV cytomegalovirus, *HBsAg* hepatitis B surface antigen, *HCV* hepatitic C virus, *RF* rheumatoid factor

She had been good health until the onset of PM, without any remarkable findings according to her family or her occupational environmental exposure history. There was no history of FMF in her first-degree relatives. Physical examination revealed muscle weakness and myalgia of the proximal muscles of the upper or lower extremities. The rest of the clinical examination was unremarkable.

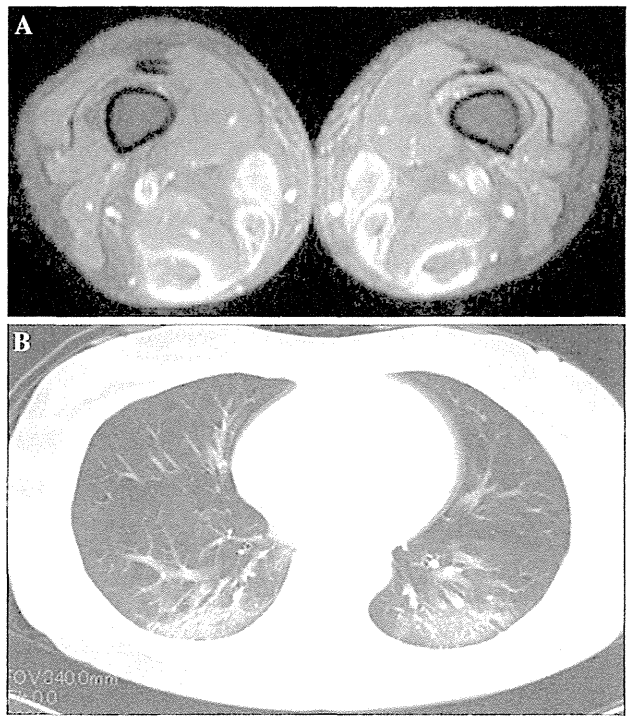

Fig. 1 **a** Magnetic resonance imaging (short tau inversion recovery, STIR) of the thigh. Note the symmetric inflammation in the affected muscle and fascia, seen as bright areas. **b** Chest CT imaging. Note the bilateral ground-glass attenuation with reticular opacity

Laboratory examination findings (Table 1) showed elevated aldolase (16.1 U/l), creatinine kinase (CK, 476 U/l), C-reactive protein (2.45 mg/dl), and KL-6 (951 IU/ml). Magnetic resonance imaging (MRI) analysis showed inflammation of the quadriceps femoris muscle (Fig. 1a), and computed tomography (CT) scan of the lungs indicated the usual findings of interstitial pneumonia (Fig. 1b). Azathioprine was switched to 3 mg/day of tacrolimus on 15 May 2010. Serum levels of CK were slightly decreased by these treatments, but still did not normalize. The efficacy of intravenous gammaglobulins (IV-IG) has been demonstrated [6], so, in view of the resistance to treatment observed, simultaneous treatment with IV-IG was added to the corticosteroid/tacrolimus treatment. IV-IG infusion (0.4 g/kg body weight/day) was performed on five consecutive days in September 2010. After this IV-IG treatment, her proximal muscle weakness recovered and her serum CK levels normalized (Fig. 2). Her muscle symptoms resolved, but she presented with recurrent episodes of high fever, along with elevated CRP and WBC. Her condition regressed spontaneously within 2 days. A diagnosis of FMF was made based on the presence of periodic fever, so a genetic analysis to check for FMF was considered. Based on the results of the

MEFV gene analysis, which showed compound heterozygous mutations (L110P/E148Q/R202Q) in exon 2 (Fig. 3), we decided to use colchicines. After starting this drug, the periodic fever disappeared, and the acute-phase reactants declined to normal levels (CRP $\leq$ 0.30mg/dl, ESR 11 mm/h). As shown in Fig. 4, CD64 expression on the patient's leukocytes was markedly elevated (MFI: 4.1) compared with that seen in healthy subjects (MFI: 1.8), as described previously [7]. Two months after starting the colchicines, this increased CD64 expression on leukocytes was downregulated (MFI: 1.9). She has since been in good health and is receiving a dose of 1 mg/day of colchicines, although she has presented fluctuations leading to mild elevations in muscle enzyme levels.

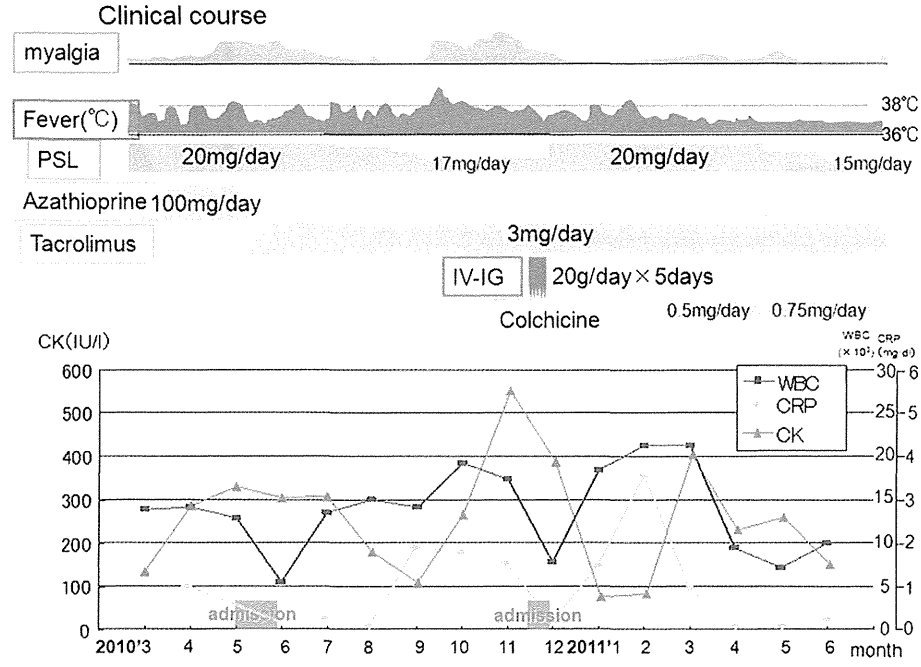

Fig. 2 Clinical course of the present case

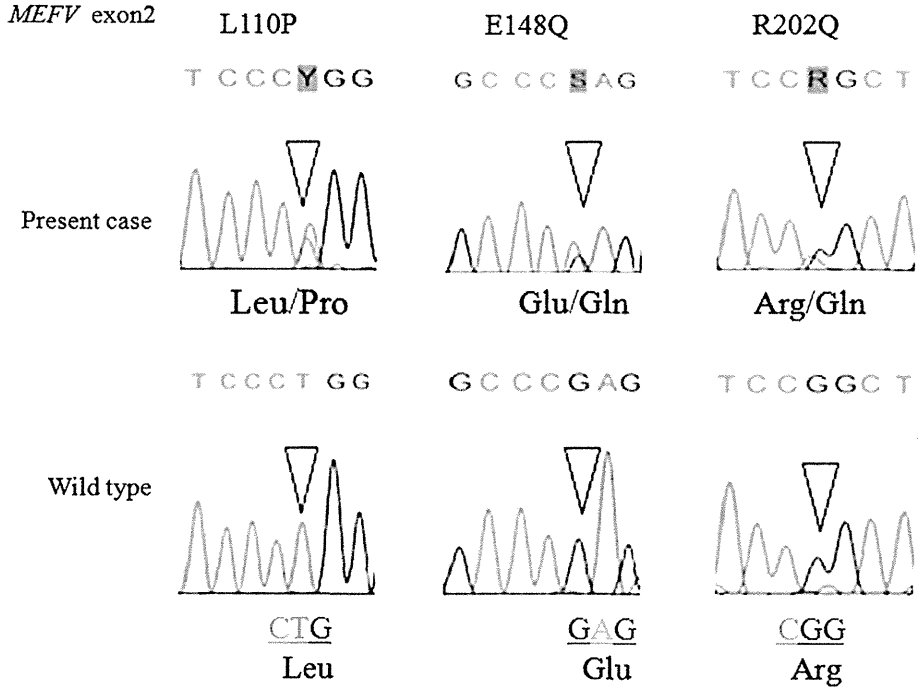

Fig. 3 *MEFV* gene analysis in a healthy control (wild type) and in the present case. In the patient, the T to C transition in codon 110 converted a leucine (L) to proline (P), the G–C transition in codon 148 converted a glutamic acid (E) to glutamine (Q), and the G–A transition in codon 202 converted an arginine (R) to glutamine (Q)

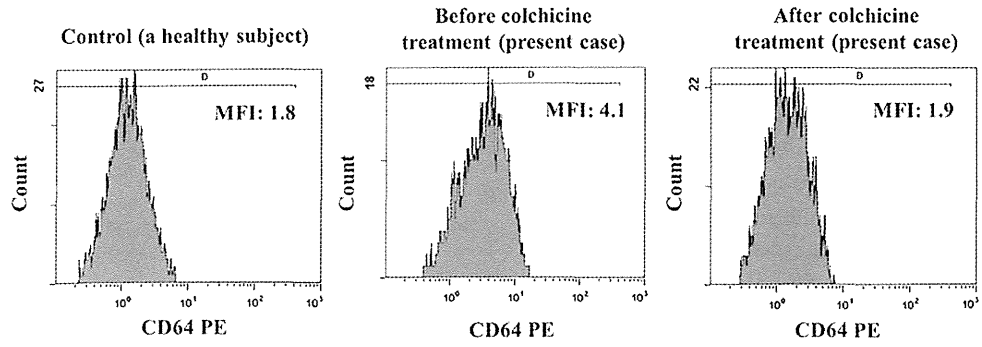

Fig. 4 Changes in CD64 expression on leukocytes after the colchicine treatment. Peripheral blood from the present case and a healthy subject were double-stained using PreCP-conjugated anti-CD45 and PE-conjugated anti-CD64 antibodies (BD Biosciences). The expression of CD64 was measured by flow cytometry using a Coulter Epics XL flow cytometer (Beckman Coulter) using Expo32 ADC analysis software (Beckman Coulter). After delineating the leukocytes by staining with the pan-leukocyte marker CD45, CD64 expression on leukocytes was determined and expressed as the mean fluorescence intensity (*MFI*)

Discussion

In this case report, we describe our experiences of a patient with PM, who carried the compound heterozygous missense mutations L110P/E148Q/R202Q in exon 2 of the *MEFV* gene. The *MEFV* gene encodes the protein pyrin, which is expressed predominantly in neutrophils, and mutations of *MEFV* are presumed to lead to abnormalities in neutrophil function, one of the pathogenetic mechanisms of FMF [8]. Indeed, we demonstrated increased expression levels of neutrophil CD64, an activation marker for neutrophils [9], in circulating neutrophils isolated from the present case. The diagnosis of FMF was made based on typical clinical manifestations. Clinical diagnosis is complex if there are only febrile episodes without serositis [10]. Based on the Tel Hashomer criteria, if 2 major criteria or 1 major criterion plus 2 minor ones are satisfied, a diagnosis of typical FMF can be made; if only 1 major and 1 minor criteria are satisfied, the diagnosis is of incomplete FMF [5]. Recently, it was also suggested that there are probably additional clinical presentations corresponding to patients who do not fully meet the typical FMF criteria [11]. Although the present case was not accompanied by serositis or synovitis, periodic fever was observed, and the patient fulfilled the diagnostic criteria for incomplete FMF [5]. It remains controversial as to whether the E148Q mutation is a disease-causing mutation or a simple polymorphism considering the high allele frequency in healthy controls [12]. Indeed, the allele frequencies of the L110P (8.7 %), E148Q (23.3 %), and R202Q (3.3 %) mutations were high in healthy Japanese subjects in our study [13]. Booth et al. [14] reported that the allele frequency of E148Q is significant among patients with AA amyloidosis. However, our study demonstrated that E148Q and L110P mutations are not associated with RA or RA-related AA amyloidosis in a Japanese population [15]. Similarly, R202Q in exon 2 of the *MEFV* gene was first identified as a common polymorphism. R202Q has no effect in the heterozygous state [16]. However, when carrying R202Q among compound heterozygous mutations, the clinical spectrum seems to appear [17]. We speculate that the compound heterozygous mutations L110P/E148Q/R202Q in the *MEFV* gene may be relevant to the occurrence of FMF manifestations during the clinical course of PM in this patient.

Myalgia is considered to be one of the clinical features of FMF [18]. In 1994, Langevitz et al. [4] demonstrated protracted febrile myalgia syndrome (PFMS) in patients with FMF. Because of an increase in knowledge regarding FMF myalgia [19], leg pain on exertion (exertion-induced myalgia) was one of the diagnostic criteria proposed by Livneh et al. [5]. Majeed et al. [20] reported that three clinical patterns of myalgia were identifiable in FMF patients: the spontaneous pattern, the exertion-induced pattern, and PFMS, as seen in 8, 81, and 11 % of patients. The diagnostic power of the response to colchicines is still very important while following atypical FMF cases [21]. The present patient suffered from recurrent fever in addition to the myositis-related symptoms. Administration of colchicines proved effective as a way to improve her recurrent fever. A diagnosis of PFMS was considered based on the presence of persistent febrile paralyzing myalgia with normal CPK [18, 19]. Since the patient fulfilled the diagnostic criteria for PM [22], judging from the clinical findings seen in the present case, we presumed that these mutations contributed to the periodic fever rather than FMF-related myopathy. Alternatively, these mutations in the MEFV gene may have some effects on the clinical manifestations of PM, such as disease activity.

Some mutations display a true dominant inheritance under certain environmental backgrounds [23]. A plausible explanation may be that a subject who has a heterozygous MEFV mutation (similar to the present case) and who carries a combination of other genetic factors that would

favor more inflammation is exposed to the wrong environment, which causes the threshold for manifesting an FMF phenotype to be exceeded [24]. To date, the constancy of PM and FMF have not been reported for the adult age group. The novel association between FMF and PM in this patient raises two questions: is this a chance association, and if not, is the occurrence of PM linked with the dysregulated inflammatory process seen in FMF patients? The possibility that MEFV mutations may help to modify the clinical phenotypes of PM cannot be ruled out completely. Future reports of individuals with FMF and PM will be required to substantiate our assumption.

In summary, FMF is occasionally associated with autoimmune diseases. When patients with rheumatic diseases present with atypical symptoms including periodic inflammation, clinicians should consider the concurrence of FMF as a possible cause.

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Conflict of interest None.

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Compensated inflammation in systemic juvenile idiopathic arthritis: Role of alternatively activated macrophages

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ABSTRACT

To investigate the role of alternative activation of macrophages, in particular, the role of the CD163/HO-1 axis in systemic juvenile idiopathic arthritis (s-JIA), we serially examined the concentrations of HO-1, sCD163 and pro-inflammatory cytokines (IL-10, IL-18, IL-6, neopterin, soluble TNF-α receptor types I and II) in patients with s-JIA complicated by macrophage activation syndrome (MAS/s-JIA). Serum concentrations of HO-1, sCD163 and IL-10 in s-JIA patients were markedly elevated in the active phase including MAS and correlated positively with indicators for s-JIA disease activity. Serum concentrations of HO-1, sCD163 and IL-10, as well as IL-18, remained elevated in s-JIA patients even in the inactive phase of disease, whereas clinical parameters and other pro-inflammatory cytokines normalized. These findings indicate that alternative macrophage activation plays an important role not only in the active phase but also in the inactive phase of s-JIA. These findings suggest that the inactive phase of s-JIA represents a state of compensated inflammation rather than absence of immune activity.

1. Introduction

Systemic juvenile idiopathic arthritis (s-JIA) is characterized by clinical features of remitting fever, typical skin rash and arthritis. Recent investigations into the pathophysiology of s-JIA have focused on mediators of the innate immune system. In particular, IL-1β, IL-6 and IL-18 correlate with disease activity and secondary complications [1]. Furthermore, phagocyte-specific S100-proteins (S100A8, S100A9 and S100A12) synergize with cytokines to perpetuate inflammation [1,2]. These findings support the hypothesis that s-JIA is an auto-inflammatory condition.

Another hypothesis is that inadequate downregulation of immune activation is a key mechanism of s-JIA [2]. Different subsets of the monocyte/macrophage lineage differentiate in response to environmental stimuli. Classically activated M1 macrophages comprise the pro-inflammatory subset, whereas alternatively activated M2 macrophages resolve inflammatory responses, perform scavenger functions and promote tissue remodelling and repair [3]. Interferon (IFN)-γ is the key cytokine driving the M1 pathway, whereas IL-4, IL-10 or steroids promote monocyte differentiation into M2 macrophages [3]. M2 phenotype monocytes are observed in s-JIA patients [4], indicating that alternative activation of monocytes and macrophages might play a role in resolving inflammatory responses in the pathogenesis of s-JIA. Furthermore, immune phenotype responses persist during clinically inactive disease, which suggests that this stage might represent compensated inflammation [2].

The hemoglobin–haptoglobin scavenger receptor (CD163) is a monocyte/macrophage-restricted 130-kDa transmembrane protein of the cysteine-rich scavenger receptor family [5,6]. CD163 expression identifies macrophages undergoing differentiation via the alternative pathway associated with enhanced phagocytic activity [7]. The extracellular portion of CD163 is shed from the cell surface in the form of sCD163 when cells are appropriately activated. Extensive expansion of CD163 + macrophages has been demonstrated in the bone marrow of a patient with macrophage activation syndrome (MAS) [8], and sCD163 appears to be a valuable diagnostic marker in hemophagocytic syndromes [9].

CD163 has been characterized as a scavenger receptor for hemoglobin, mediating endocytosis of hemoglobin:haptoglobin (Hb:Hp) complexes [6]. During intravascular hemolysis, free Hb binds to the plasma protein Hp and Hb:Hp complexes are formed. After endocytosis of Hb:Hp, the heme subunit of Hb is degraded by the rate-limiting heme oxygenase (HO) enzymes. Two main isoforms of HO have been characterized, with HO-2 being constitutively present under physiological conditions and HO-1 being inducible [10]. The breakdown of heme yields biliverdin, free iron and carbon monoxide (CO), which has anti-inflammatory and

Abbreviations: MAS, macrophage activation syndrome; s-JIA, systemic juvenile idiopathic arthritis; HO-1, heme oxygenase-1; EBV-HLH, hemophagocytic lymphohistiocytosis due to Epstein-Barr virus infection; KD, Kawasaki disease.

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cytoprotective effects [11,12]. In macrophages, CO reduces pro-inflammatory and increases anti-inflammatory cytokine secretion in response to lipopolysaccharides [13]. HO-1-mediated anti-inflammatory effects may therefore be closely linked to anti-inflammatory mechanisms, such as the suppression of immune and inflammatory responses in macrophages via diminished antigen-presenting capacity and cytokine synthesis [14–16]. Indeed, we previously reported the first patient with HO-1 deficiency, who showed a marked rise in circulating heme and subsequent oxidative vascular and tissue injury, anemia and chronic inflammation [17,18]. Recently, some reports have shown that serum levels of HO-1 are highly elevated in patients with s-JIA and adult-onset Still's disease (AOSD) [19,20].

In this study, to assess the role of alternative macrophage activation in s-JIA and in particular, the role of the CD163/HO-1 pathway, we serially measured the concentrations of serum HO-1, sCD163 and IL-10 in patients with s-JIA complicated by MAS. Furthermore, we analyzed the correlation between these measurements and other disease activity markers including ferritin.

2. Methods

2.1. Patients and samples

Serum samples were obtained from four patients with MAS as a complication of s-JIA (MAS/s-JIA), 10 with hemophagocytic lymphohistiocytosis due to Epstein-Barr virus infection (EBV-HLH), 10 with Kawasaki disease (KD), and 10 age- and sex-matched healthy controls (HCs) [age (MAS/s-JIA: 7.3 ± 6.8 years, control: 8.9 ± 5.9 years)]. Samples from MAS/s-JIA patients were also obtained during both the active and inactive phases of s-JIA, but when MAS was not present. Diagnosis of s-JIA was based on the criteria of the International League of Associations for Rheumatology [21]. 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. [22]. The clinical characteristics of patients with MAS/s-JIA are shown in Table 1.

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 rate 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 observed in the active phase, as well as normal erythrocyte sedimentation rate (<5 mm/h) and C-reactive protein (CRP) levels (<0.1 mg/dl).

Table 1
Clinical characteristics of patients with s-JIA at the time diagnosed as MAS.

Case	1	2	3	4
Age (year)	12	1	15	2
Sex	F	M	F	M
Disease duration (months)	16	1	2	1
Fever	+	+	+	+
Systemic JIA rash	+	+	+	+
Arthritis	+	+	+	+
Hepatosplenomegaly	+	+	+	+
Lymphoadenopathy	+	+	+	+
WBC/μl	8100	25,300	15,950	8000
Hemoglobin g/dl	11.5	9.1	13.8	9.3
Platelets/μl	81,000	192,000	117,000	129,000
CRP mg/dl	10.7	10.3	2.6	4.69
AST IU/l	153	241	1382	296
ALT IU/l	493	145	2437	195
LDH IU/l	659	988	2925	1318
Ferritin ng/ml	2296	1912	7000	730

F: female; M: male; +: positive; −: negative; WBC: white blood cells; AST: aspartate aminotransferase; ALT: alanine aminotransferase; LDH: lactate dehydrogenase.

All patients with EBV-HLH fulfilled the diagnostic criteria for EBV-HLH [23]; positivity for the EBV genome in the blood/bone marrow and other tissues (determined by polymerase chain reaction, southern blot and/or *in situ* hybridization for EBER) and positive anti-viral capsid antigen-specific-IgG. Diagnosis of KD was based on the classic clinical criteria [24]. 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 the receipt of informed consent.

2.2. Quantification of serum cytokines

Serum concentrations of HO-1, sCD163, IL-10, IL-18, IL-6, neopterin and soluble TNF receptor type I and II were evaluated using a commercial enzyme-linked immunosorbent assay according to the manufacturer's instructions (HO-1: Stressgen, Victoria, Canada; sCD163, IL-6, TNF-α receptor types I and II: R&D Systems, Inc., Minneapolis, MN; IL-18: MBL, Nagoya, Japan; neopterin: IBL, Hamburg, Germany, IL-10: eBioscience, Inc., San Diego, CA).

2.3. Statistical analysis

Within-group comparisons were analyzed by the Mann–Whitney U test. Correlations were expressed using the Spearman rank correlation coefficient. For the analyzed measures, p values less than 0.05 were considered significant.

3. Results

3.1. Cytokine kinetics and profile in patients with s-JIA

Serum concentrations of HO-1, sCD163 and cytokines were determined in patients with MAS as a complication of s-JIA (MAS/s-JIA). As shown in Fig. 1, serum HO-1 (A), sCD163 (B) and IL-18 (D) concentrations were all significantly elevated in MAS/s-JIA patients during both the active and MAS phase. Interestingly, these concentrations were markedly elevated in patients with s-JIA even in the inactive phase. On the other hand, serum concentrations of pro-inflammatory cytokines, including neopterin (E), IL-6 (F), sTNFRI (G) and sTNFRII (H) were elevated during the active and MAS phases; however, concentrations normalized during the inactive phase. Because many cytokines are associated with the pathogenesis of MAS and HLH, we believe that monitoring the cytokine profile of the combination of these cytokines, rather than the level of any individual cytokine might be more useful for evaluating disease activity. Consequently, we tried to represent the cytokine profile by a radar chart (Fig. 2). Serum concentrations of anti-inflammatory factors, including HO-1 and sCD163 were markedly elevated in active (A) and MAS (B) phases and remained elevated in the inactive phase of disease (C) as did IL-18. In contrast, the profile of pro-inflammatory cytokines, including neopterin, sTNFRI and sTNFRII shows that serum concentrations of these cytokines were markedly elevated in active (A) and MAS (B) phases but normalized in the inactive phase (C).

3.2. Markedly elevated concentrations of serum HO-1 and sCD163 in the clinical course of patients with MAS/s-JIA

To investigate the relevance of HO-1and sCD163 to the pathogenesis of s-JIA, serum concentrations were serially monitored in all four cases of s-JIA (Fig. 3A–D). The concentrations of serum HO-1 and sCD163 markedly increased with the development of the complication of MAS, but gradually reduced after this manifestation resolved with immunosuppressive therapy, including corti-

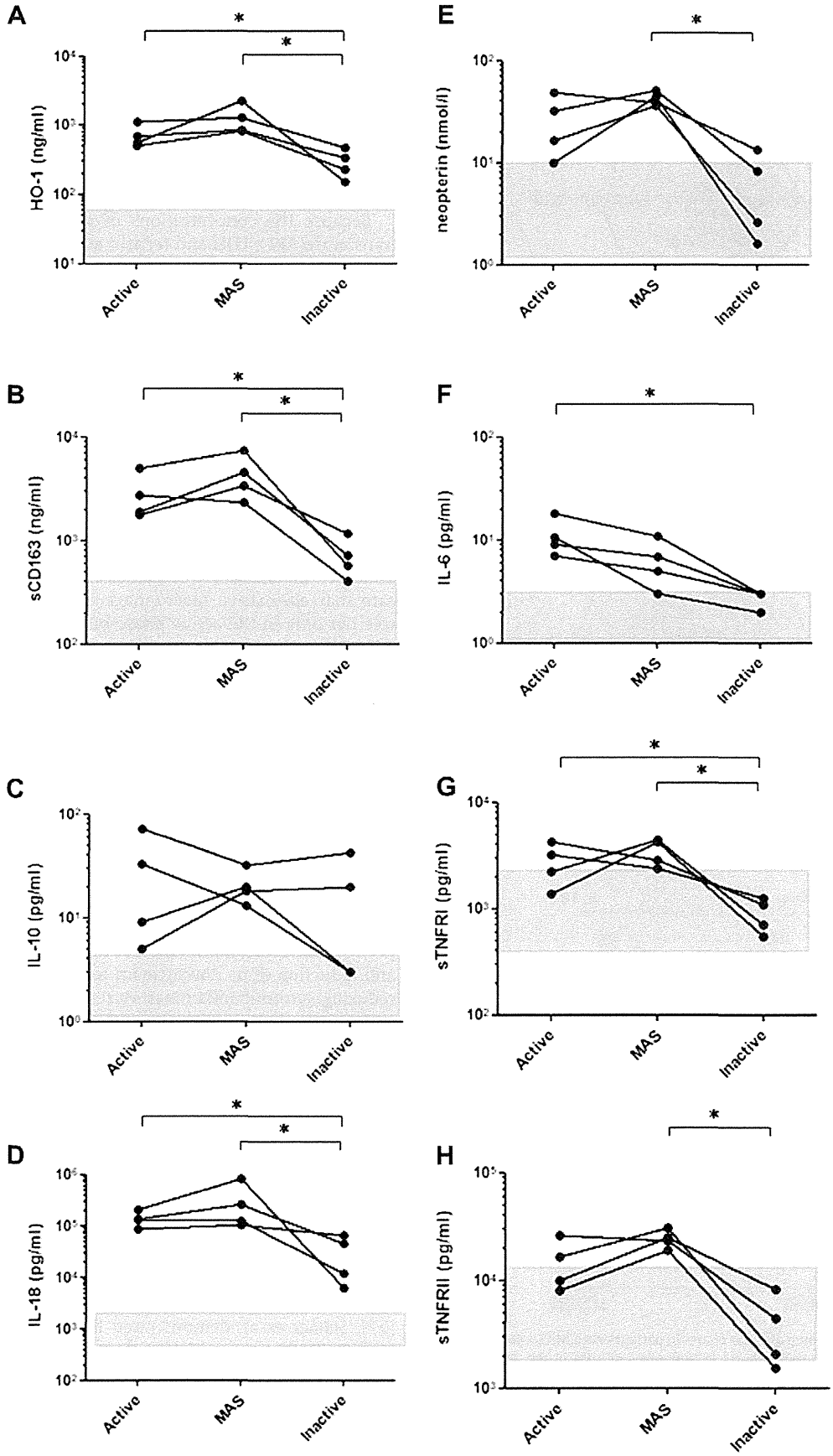

Fig. 1. Cytokine kinetics in patients with MAS/s-JIA. Longitudinal changes of serum cytokine concentrations are shown. Serum HO-1 (A), sCD163 (B) and IL-18 (D) concentrations were markedly elevated in active and MAS phases and remained elevated in the inactive phase. Blue squares indicate the normal range of each cytokine. A. HO-1, B. sCD163, C. IL-10, D. IL-18, E. neopterin, F. IL-6, G. sTNFRI, H. sTNFRII. $* = p < 0.05$.

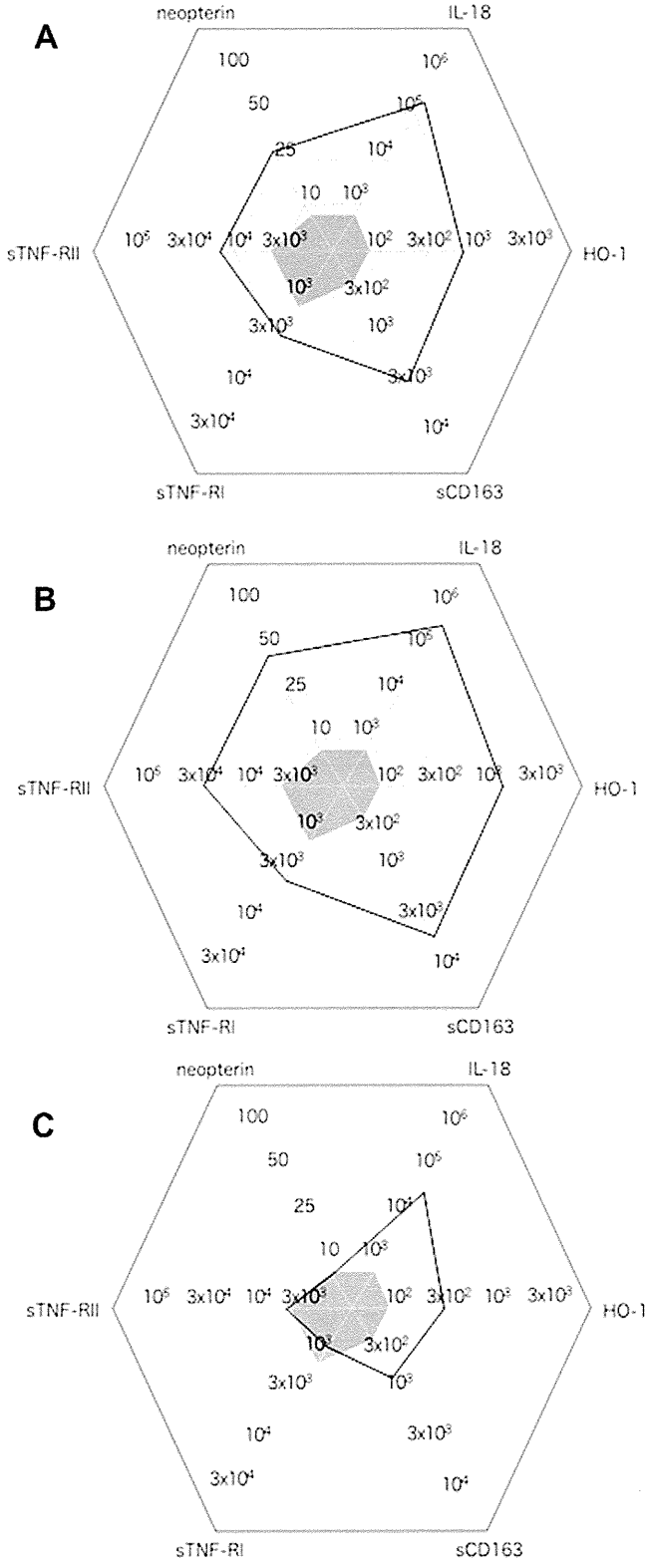

Fig. 2. Cytokine profiles shown as radar charts in patients with MAS/s-JIA. Cytokine profiles are represented by radar charts in four cases of s-JIA in the active phase (upper panels), MAS (middle panels) and inactive (lower panels) phase. Serum concentrations of anti-inflammatory factors were markedly elevated in active (A) and MAS (B) phases and remained elevated in the inactive phase of disease (C). In contrast, the profile of pro-inflammatory cytokines shows that serum concentrations of these cytokines were markedly elevated in active (A) and MAS (B) phases but normalized in the inactive phase (C). The overlaid inner dark blue areas show the mean values of healthy controls.

costeroids and/or cyclosporine. However, even a few weeks after normalization of other inflammatory indicators, such as lactate dehydrogenase (LDH), serum HO-1 and sCD163, concentrations remained well above those of HC. The kinetics of these changes were similar to those of serum IL-18.

3.3. Correlation between serum HO-1, sCD163 and measures of disease activity in the clinical course of four cases of s-JIA

Because the concentrations of serum CRP, aspartate aminotransferase(ALT), LDH and ferritin are used clinically as indicators of s-JIA disease activity, their concentrations were compared with those of HO-1 and sCD163 (Fig. 4). Even during the clinically inactive phase after remission from MAS, concentrations of serum HO-1 remained markedly elevated although other clinical parameters including CRP, AST, LDH, ferritin normalized as well as the concentrations of sCD163.

4. Discussion

In this study, we have demonstrated that serum concentrations of HO-1, sCD163 and IL-10, as well as IL-18, in s-JIA patients are elevated not only in MAS and the active phase of s-JIA, but also even in the inactive phase of s-JIA, when clinical parameters and other pro-inflammatory cytokines are normal. These findings indicate that alternative macrophage activation plays an important role not only in the active phase but also in the inactive phase of s-JIA. We also demonstrated that serum HO-1 concentrations in MAS/s-JIA patients were significantly higher than those in EBV-HLH or KD patients. The marked simultaneous elevation of serum HO-1 and IL-18 is characteristic of s-JIA, and the measurement of their concentrations is useful for the differentiation of MAS/HLH.

HO is a potent anti-inflammatory and anti-oxidative enzyme, which is involved primarily in the degradation of heme into biliverdin, CO and free iron. [25]HO-1 has been suggested to act as a potent cytoprotective enzyme through the anti-oxidative activity of biliverdin and its metabolite bilirubin, as well as the anti-inflammatory effects of CO [13,25]. Indeed, deficiency of HO-1 in humans leads to a marked increase in circulating heme and subsequent oxidative vascular and tissue injury, anemia and chronic inflammation [17,18]. Therefore, elimination of hemoglobin from the circulation and induction of its downstream metabolite pathway is crucial to reducing systemic inflammatory responses.

Recently, some reports have shown that serum levels of HO-1 are highly elevated in patients with s-JIA and adult-onset Still's disease (AOSD), an adult form of s-JIA [19,20]. However, the sources of circulating serum HO-1 in patients with s-JIA remain undetermined. We previously reported that HO-1 mRNA levels were elevated in peripheral blood mononuclear cells of children with KD [26]. Interestingly, serum concentrations of HO-1 were not increased in patients with KD in this study. These findings indicate that the sources of serum HO-1 are not derived from circulating peripheral blood mononuclear cells including monocytes.

In macrophages, HO-1 is induced by CD163-mediated Hb-Hp complex uptake. *In vitro* models have revealed that erythrophagocytosis is a potent stimulus for upregulation of HO-1 expression [27]. Schaer et al. demonstrated that HO-1 expression observed within CD163 + macrophages increases with the excessive hemophagocytic activity of these cells in patients with HLH associated with sepsis [28]. In this study, we demonstrated that serum HO-1 concentrations correlate closely with serum sCD163 concentrations, and these are extremely high in the MAS phase (Supplementary Fig. 1). These findings indicate that serum HO-1 might be derived from CD163 + alternatively activated macrophages, and

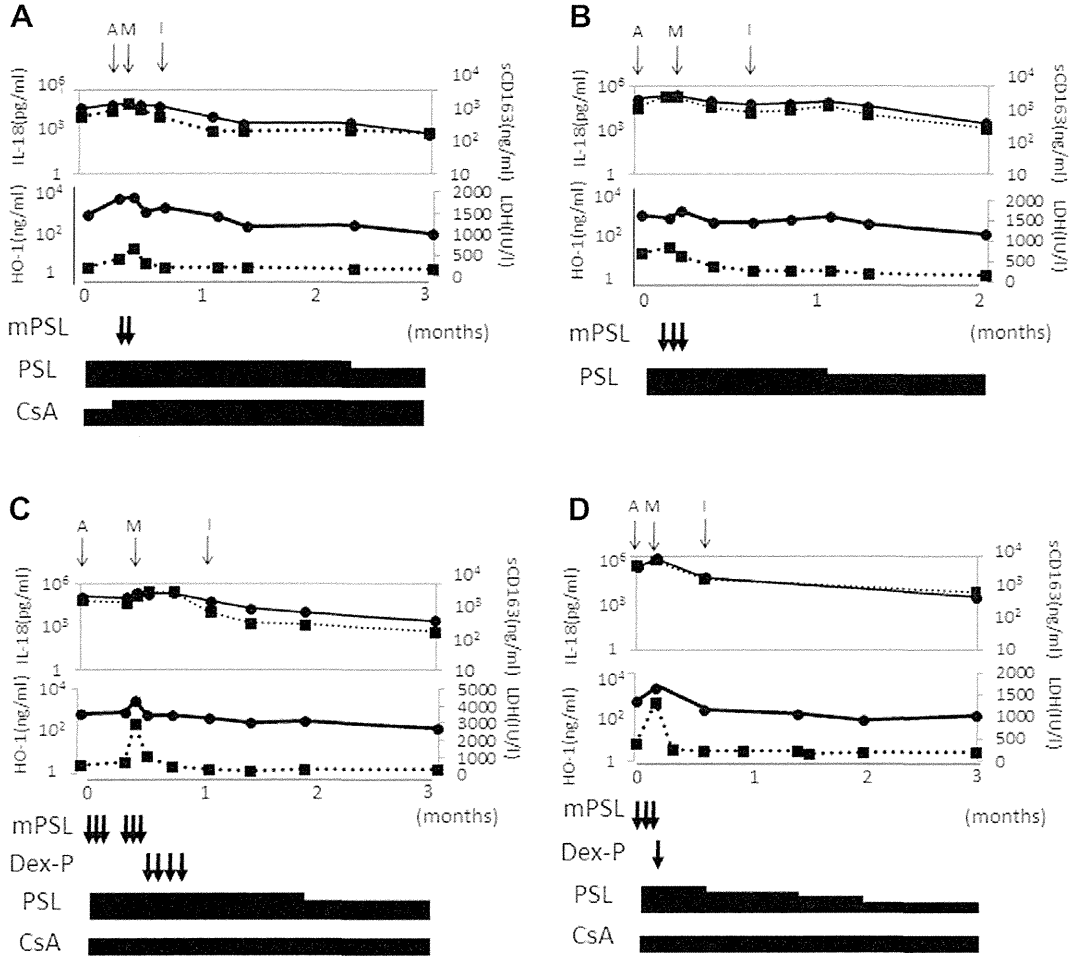

Fig. 3. Longitudinal course of serum HO-1 in five cases with MAS/s-JIA. (A–D) show the longitudinal course of case 1–4, respectively, of four patients with MAS/s-JIA. Changes in serum IL-18 (*solid lines*) and sCD163 (*dotted lines*) are shown in the upper panels. Changes in serum HO-1 (*solid lines*) and LDH (*dotted lines*) are shown in the middle panels and details of therapeutic interventions are shown in the lower panels. The concentrations of serum HO-1 and sCD163 markedly increased with the development of the complication of MAS, but gradually reduced after this manifestation resolved with immunosuppressive therapy. Even a few weeks after normalization of other inflammatory indicators, these concentrations remained well above those of HC. Time-points of blood draw are indicated by arrows; M: MAS, A: Active phase, I: Inactive, PSL: prednisolone, mPSL: methylprednisolone, Dex-P: dexamethasone palmitate, CsA: cyclosporine.

in particular, hemophagocytic macrophages may be a major source of HO-1 in s-JIA. Serum HO-1, as well as CD163, might indicate alternative activation of macrophages in s-JIA.

We previously reported that serum IL-18 concentrations are a promising indicator of s-JIA disease activity [29]. IL-18 is a major promoter of M1 macrophage activation. Serum IL-18 concentrations remained elevated even when other markers of disease activity normalized, indicating that IL-18-driven activation of M1 macrophages might persist in the inactive phase. Interestingly, patients' serum concentrations of HO-1 and sCD163, as well as IL-18, remained elevated in s-JIA even in the inactive phase of disease. Indeed, it has been reported that HO-1 expression is upregulated in macrophages during the resolution phase of inflammation [30,31]. These findings indicate that alternative activation of macrophages plays an important role not only in the active phase but also in the clinically inactive phase of s-JIA. It is possible that the inactive phase of s-JIA represents a state of compensated inflammation by M2 macrophages to suppress the IL-18-driven M1 macrophage activation, rather than the absence of immune activity.

Plasticity and flexibility are key features of macrophages and of their activation states [32]. The phenotype of polarized M1-M2 macrophages can be reversed [32]. Furthermore, pathology is frequently associated with dynamic changes in macrophage activation. Indeed, cells with a mixed M1/M2 phenotype have been described in patients with s-JIA, especially during flare [33]. Children with active s-JIA share a number of clinical features that are similar to the presentation of MAS. Occult MAS appears to be common in patients with s-JIA [34]. These findings indicate that the symptoms of active s-JIA may be part of a spectrum of the disease process of MAS in terms of their severity. Our results indicate that M1/M2 macrophages are activated not only in active phase of s-JIA but also in MAS. This suggests that macrophage activation may be integral to the pathogenesis of s-JIA.

The limitation of the present study was the small number of s-JIA patients. A larger study may help define the true diagnostic value of these markers. In spite of the limitations, our results indicate that alternative activation of macrophages plays an important role not only in the active phase but also in the inactive phase of s-JIA. It is possible that the inactive phase of s-JIA represents a state of compensated inflammation rather than absence of immune activity. The pathogenesis of MAS/s-JIA remains obscure, however, inadequate downregulation of immune activation might be central to s-JIA. Induction of alternative macrophage activation, and in particular, induction of HO-1, might form a new strategy for the treatment of s-JIA.

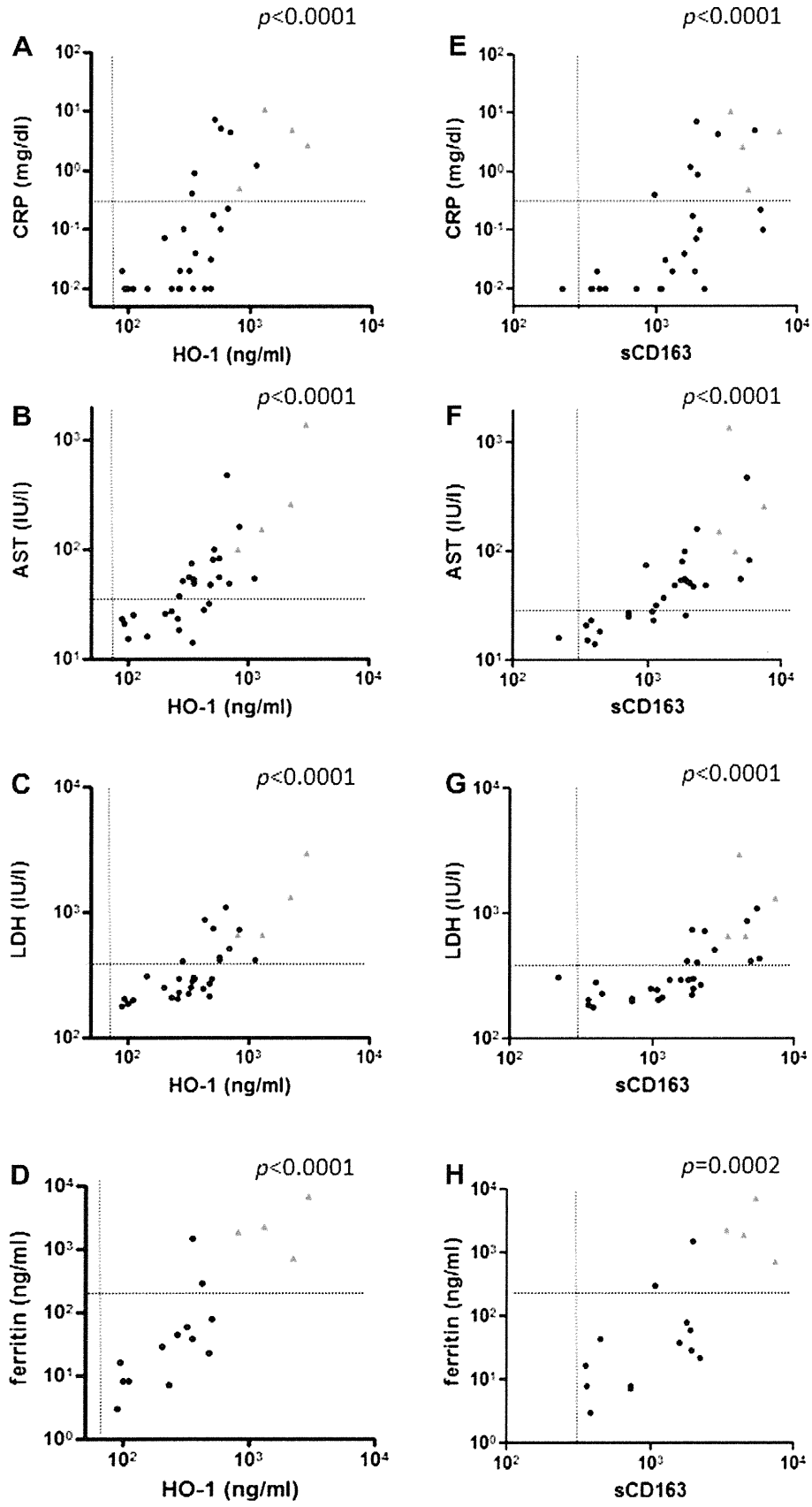

Fig. 4. The concentrations of serum HO-1 and sCD163 remained markedly elevated even in inactive phase after the recovery from MAS. Serum concentrations of HO-1 and sCD163 were compared with other serum markers in four cases of s-JIA. Even during the clinically inactive phase after remission from MAS, concentrations of serum HO-1 remained markedly elevated although other clinical parameters normalized as well as the concentrations of sCD163. The values in the MAS phase are shown as red triangles. (A and E) CRP; (B and F) AST; (C and G) LDH; (D and H) ferritin.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cyto.2012.05.003.

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CASE REPORT

38-year-old woman with recurrent abdominal pain, but no fever

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Abstract: A 38-year-old woman presented with 2 days history of left-flank pain. She had similar episodes of abdominal pain as well as chest pain several times, but symptoms disappeared spontaneously. Each time she developed pain, there was no fever. After ruling out common causes of recurrent abdominal pain, familial Mediterranean fever (FMF) was considered as a potential diagnosis. Genetic tests revealed multiple heterozygote mutations, which may be associated with FMF. Patients with Mediterranean fever mutations may present with atypical presentations without fever, like in this case. Astute clinical suspicion is required to make an accurate diagnosis.

Keywords: familial Mediterranean fever, MEFV mutation, afebrile

Introduction

Abdominal pain in premenopausal women is often a diagnostic challenge. We describe a case of 38-year-old woman who presented with recurrent abdominal pain. A conventional work-up failed to reveal a diagnosis. A detailed history suggested recurrent serositis syndrome. Although the patient denied fever, a diagnosis of FMF was considered. We discuss the rationale for the diagnosis and interpretation of genetic tests.

Case report

A 38-year-old woman presented reporting 2 days of left-sided abdominal pain. The pain was intermittent in nature. She denied any fever, gastrointestinal symptoms, respiratory symptoms and genitourinary symptoms. Her last menstrual period was a few weeks ago and she denied pregnancy. Eight years prior to the current presentation, she developed left-side chest pain worsened by deep inspiration. A chest radiograph reportedly revealed atelectasis and she was diagnosed with "pleuritis with middle lobe lingual syndrome". She did not recall whether she had a fever since then. Three years (44 months to be precise) prior to the current presentation, she developed acute onset left-flank pain without fever. She visited a urology clinic but the urinalysis was normal. An urologist who saw the patient considered her condition was unlikely to be urolithiasis but was not able to find an alternative explanation. Her pain disappeared without specific treatment several days later. Thirty-five months prior to the current presentation, she developed left-side chest and back pain without fever. Blood tests showed a white blood cell (WBC) count of 7,000/mm^3 and C-reactive protein (CRP) of 1.69 mg/dL. There was an elevated liver function test (aspartate aminotransferase

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[AST] 48 U/L, alanine aminotransferase [ALT] 66 U/L). A physician caring for her diagnosed it as pleuritis and an oral antibiotic was prescribed. At a follow-up visit 3 days later, the patient became asymptomatic and laboratory abnormalities were all normalized. Twenty-six months prior to the current presentation, she developed another left-side chest pain without fever. A laboratory exam revealed WBC 7600/mm^3 and CRP was <0.24 mg/dL. She was again diagnosed with pleuritis, and symptoms improved several days later (treatment detail unknown). Twenty-five months prior to the current presentation, she developed another abdominal pain. Pain was associated with her position and movement. WBC was 8600/mm^3, with neutrophil 67% (4% band form), lymphocyte 27%, CRP 1.32 mg/dL, and erythrocyte sedimentation rate (ESR) 36 mm/hour. The abdominal ultrasound was normal and a computed tomography scan without contrast of abdomen revealed incidental findings of spina bifida at sacrum and fatty liver but no abnormality at urinary tract or elsewhere. She had had no episode of pain since then until this current presentation.

Her past medical history was significant only for childhood inguinal hernia, which was repaired surgically, and freckles, which were treated with topical agents. She denied allergy to medications, took no alcohol or illicit drugs, and denied tobacco smoking. She became pregnant twice and was the mother of 12- and 14-year-old children. There was no significant family history. She only took over-the-counter vitamins.

Upon presentation, the patient appeared well but suddenly started to suffer abdominal pain during the interview. She was not able to hold herself upright and had to lie down on a bed. On physical examination, she appeared ill. Her blood pressure was 125/76 mmHg, pulse rate 84/minute, respiratory rate 22/minute, and body temperature taken at axilla was 36.8°C. Her pain worsened upon deep inspiration. However, her physical examination was otherwise unremarkable, and there was no tenderness on her abdomen or on her back. Laboratory tests showed a CRP of 1.45 mg/dL (reference range < 0.24 mg/dL) and an ESR of 25 mm at 1 hour. The WBC differential was neutrophil 65.0% (5% band form), lymphocyte 22.0%, monocyte 13.0%, and eosinophil 0%. Due to a technical error, CBC was not performed, but later in the day it turned out to be normal. Electrolytes, liver function tests, and kidney function tests were all normal. Urinalysis was negative for protein and sugar, but there was 10–19 RBC/HPF, 30–49 WBC/HPF, with trace bacteria. Serum thyroid-stimulating hormone (TSH) level was normal.

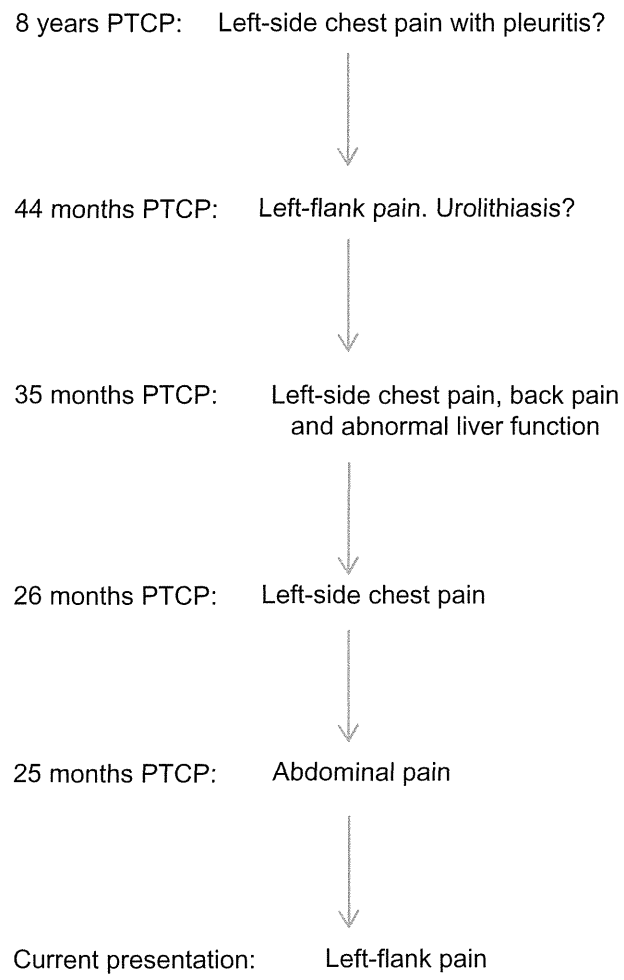

Figure 1 Patient's history of recurrent pain, prior to current presentation (PTCP).

Her cause of abdominal pain was judged to be serositis, based on history and physical examination. After resting, her pain gradually improved and she was sent home with prescription of non-steroidal anti-inflammatory medicine as needed. On the follow-up visit a week later, she was completely asymptomatic. Rheumatoid factor, anti-nuclear antibody (ANA), complement level, C3, C4 level, and serum ferritin level measured at the first visit all turned out normal. Urinary lead and porphobilinogen were negative. Given the recurrent nature of multiorgan serositis, FMF was considered as a diagnosis despite the lack of fever. A genetic blood test was performed at the Department of Pediatrics, Graduate School of Medical Science and School of Medicine, Kanazawa University, and it revealed *MEFV* mutations at exon 3 (P369S and R408Q) and intron 8 (IVS8+8 C-T). The symptom has not recurred since this interview. After a discussion with the patient, prophylactic colchicine was not prescribed, and genetic tests for family members were not performed.

Discussion

Abdominal pain in premenopausal women can be a diagnostic challenge. Its cause may range from gastrointestinal, hepatobiliary, or urological to obstetric and gynecological. Occasionally, some diseases cause abdominal pain without findings on imaging studies, such as diabetic ketoacidosis, acute intermittent porphyria, lead poisoning, or conversion disorder. However, detailed history taking and physical examination often lead to accurate diagnosis. Moreover, the list of differential diagnoses of left-side abdominal pain, as in our case, is not long, compared to right-side abdominal pain.

FMF is an autosomal recessive, inherited periodic inflammatory syndrome, characterized by self-limited recurrent attacks of fever with serositis such as peritonitis, pleuritis and arthritis.[1] It is known to occur mainly among Mediterranean and Middle Eastern populations such as non-Ashkenazi Jews, Arabs, Turks, and Armenians. Despite its name, FMF is now shown to occur worldwide. In Japan, more than 90 cases of FMF were reported.[2–4] Its onset is usually early but onset over 50 years old has been reported.[5] High fever is present in more than 90% of cases with FMF,[6] and fever was present in 98.8% of cases in a Japanese study.[3] However, this may be due to underdiagnoses and underreporting of afebrile patients. In fact, the current diagnostic criteria do not necessarily require fever for the diagnosis.[1] This patient fulfilled only one minor criterion on the simplified version of the criteria, which is now widely used. However she fulfilled two minor and five supportive criteria of the more detailed version; ie, incomplete attacks of abdomen and chest (minor criteria), and severe attacks requiring bed rest, spontaneous remission, symptom-free interval, transient inflammatory response, and episodic hematuria (supportive criteria), for which the specificity of the diagnosis is 99%.[1]

Patients with FMF at late onset tend to have a less severe form, and this might in part explain her lack of fever. In fact, the Tel Hashomer Severity Score of the patient was only[1,7] which includes age in the scoring, suggesting she had a milder form of FMF.

FMF is caused by mutations in the Mediterranean fever gene (*MEFV*) on chromosome 16p13.3, which encodes protein named pyrin. Pyrin regulates production of IL-1β and activation of NF-κB. Lack of normal pyrin activity in FMF is considered to be the cause of excess cytokines and subsequent inflammatory attack.[8]

Diagnosis of FMF is usually made clinically. Genetic tests are not necessarily mandated, and the results may be normal in patients with FMF. Likewise, a family history may not be detected, as in our case. *MEFV* mutations commonly seen in Japan are different from those of Mediterranean people, where M694V is most common. Typical mutations in Japanese patients are E148Q on exon 2, and M694I on exon 10.[3,8] Our patient did not have either, and it may partly explain the milder form of symptoms. A combination of P369S and R408Q, which was detected in our case, was found in three Japanese cases, but there was only one case without E148Q.[3] P369S and R408Q can be found even in healthy Japanese subjects. In one study, 3.9% and 3.3% of healthy Japanese had P369S and R408Q mutations, respectively.[9] According to The Registry of Hereditary Auto-Inflammatory Disorders Mutations database (http://fmf.igh.cnrs.fr/ISSAID/infevers/), the combination of P369S and R408Q was first detected in an Armenian patient with FMF in 2007. Also, IVS8+8 C-T was detected together with L110P, E148Q, P369S, R408Q, and R501R in a Vietnamese patient. However, a diagnosis of FMF was clinically not clear in the latter. The precise role of these mutations is not known, and they are seen in cases ranging from full-blown FMF to completely asymptomatic persons.[10] In addition, these genetic abnormalities may be related to inflammatory diseases other than FMF. For example, Japanese patients with rheumatoid arthritis are known to have a higher frequency of MEFV mutations.[9] Also, there is a case report of colchicine-responsive chronic recurrent multifocal osteomyelitis (CRMO) in a 14-year-old Japanese female with these mutations.[11] These mutations were also seen in higher frequency in patients with systemic onset juvenile idiopathic arthritis (SoJIA)[12] and Behçet's disease.[13–17] Further accumulation of clinical data on these mutations will help our understanding of the roles of these mutations.

In conclusion, we saw a case of recurrent serositis consisting with FMF despite the lack of fever. There were multiple MEFV gene mutations, and these may be related to the symptoms. Astute clinical suspicion is important in diagnosing FMF without fever. Further studies will be needed to delineate the precise nature of FMF in Japanese people.

Disclosure

The authors report no conflicts of interest in this work.

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