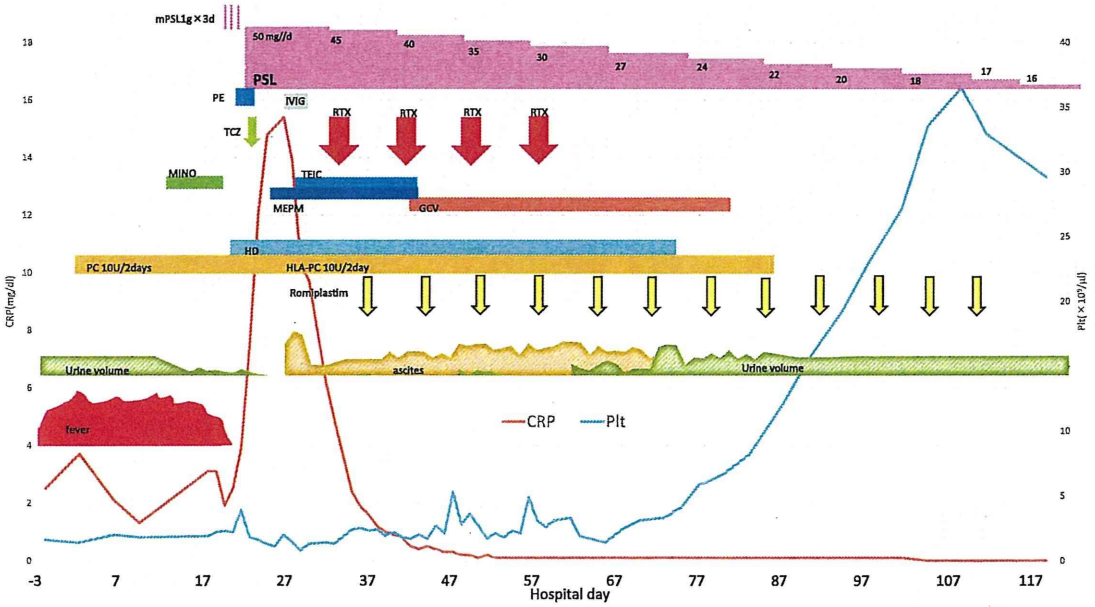

Fig. 1 Clinical course of our case

mPSL; methylprednisolone, PE; plasma exchange, PSL; prednisolone, IVIG; intravenous immunoglobulin, TCZ; tocilizumab, RTX; rituximab, MINO; minocycline, TEIC; teicoplanin, MEPM; meropenem, GCV; ganciclovir, HD; hemodialysis, PC; platelet transfusion, HLA-PC; HLA-matched platelet transfusion. Ascites are shown by the amount of ascites drained.

Cardiac ultrasonography showed no vegetation on the valves, although diffuse hypokinesis (LVEF 52%) and moderate pericardial infusion were detected (**Fig. 5**). The serum level of IL-6 was 16.8 pg/ml (reference range 0–4 pg/ml), whereas IL-6 level in pleural effusion was much higher (945 pg/ml) than that in serum. After admission, continuous fever (> 38°C) persisted, and platelet, albumin and red blood cell transfusions were required. Additionally, the patient's renal function rapidly deteriorated. Nineteen days after admission, he suddenly developed cardiogenic shock with difficulty in breathing, low blood pressure and dysuria. He was transferred to the intensive care unit and underwent non-invasive positive pressure ventilation and was administered catecholamine. Cardiac ultrasonography showed diffuse hypokinesis of the left ventricle (LV) and moderate pericardial effusion. Treatment with pulse methylprednisolone (1 g/day for three days) was effective only for fever but not for the rest of the symptoms, such as anasarca, severe thrombocytopenia and renal dysfunction with dysuria. On day 21, hemodialysis and plasma exchange were initiated because the patient's urine volume had decreased to below 50 mL/day and it was difficult to hemodyalize without infusion of a bulk of plasma. On day 23, tocilizumab (400 mg ivd) was injected. After day 26, occasional drainage of

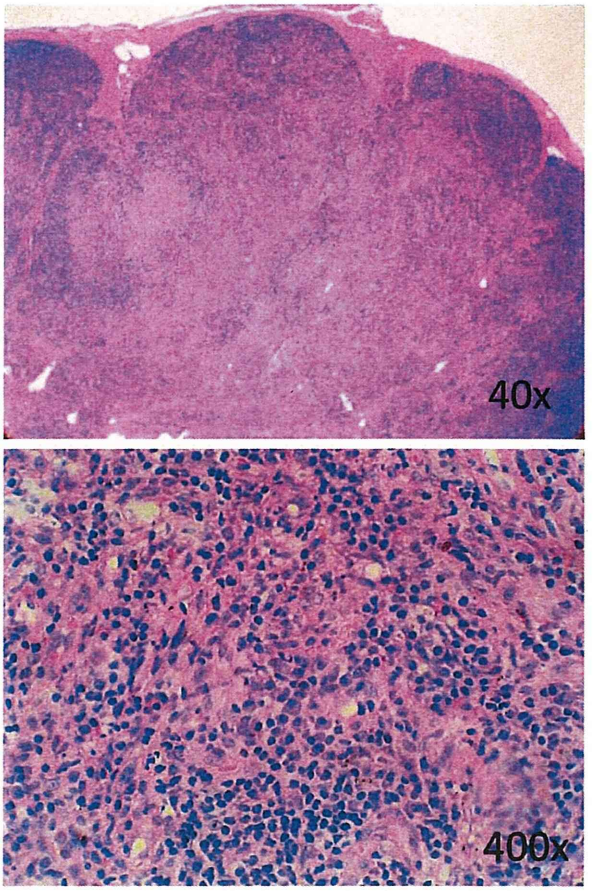

Fig. 2 Inguinal lymph node biopsy histology section by hematoxylin and eosin staining. Medullary hyperplasia with strong plasma cell infiltration was seen (upper panel: ×40, lower panel: ×400).

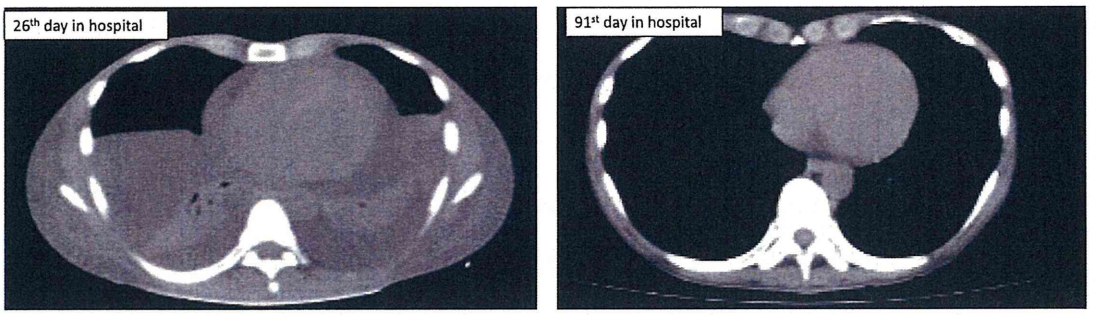

Fig. 3 Clinical course of pleural and pericardial effusion (CT scans at the heart level)
Large amount of bilateral pleural effusion and moderate amount of pericardial effusion were detected on the 26th hospital day, which disappeared on the 91st hospital day.

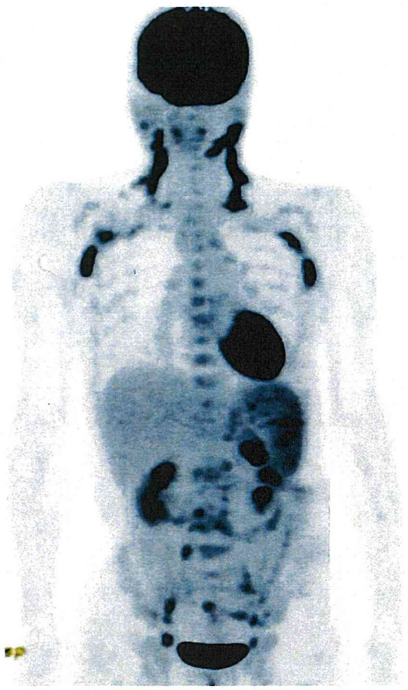

Fig. 4 18-fluorodeoxyglucose (^{18}FDG)-Positron Emission Tomography (PET) findings. Multiple lymphadenopathy and splenomegaly were detected.

the ascites and pleural effusion were performed to relieve the patient's abdominal distention and dyspnea. Although CRP gradually decreased, thrombocytopenia continued and platelet transfusion was needed every other day. On day 29, high-dose immunoglobulin therapy was started for thrombocytopenia because of positive PA-IgG, but was not effective. HLA-matched platelet transfusion was started because of elevated anti-HLA antibody, but platelet elevation was similar to that with non-HLA-matched platelet transfusion. On day 34, a total of four cycles of rituximab (600 mg/body: 375 mg/m^2, weekly) was started with written informed consent after approval of the ethical committee at our institute. After day 35, romiplostim, a TPO receptor agonist, was started for refractory thrombocytopenia. The patient's urine volume suddenly increased to over 100 mL/day on day 50, then to over 1000 mL/day on day 73, and hemodialysis was discontinued on day 76. After day 75, the platelet count finally started to elevate, so platelet transfusion was discontinued on day 87. After day 78, tachycardia continued and cardiac ultrasonography repeatedly showed persistence of diffuse hypokinesis (LVEF 43.9%). On day 85, contrast-enhanced cardiac MRI also showed diffuse hypokinesis (LVEF 36.8%), and serum troponin T was slightly elevated. Cardiomyopathy was suspected to be caused by TAFRO syndrome because no other etiologies were identified. On day 92, a CT scan revealed no ascites, pleural effusion or lymphadenopathy. On day 120, cardiac ultrasonography showed improvement of LV wall motion (LVEF 47.3%) and pericardial effusion (**Fig. 5**). He was discharged on foot on day 135.

Discussion

The pathogenesis of this syndrome is not clearly understood, but is considered to be a cytokine storm involving IL-6 and VEGF, as was MCD[3, 4]. Dysregulated and overproduced IL-6 stimulates the production of acute phase reactants in the liver, resulting in constitutional symptoms, including fever, sweats, and fatigue, and laboratory abnormalities, such as anemia and hypoalbuminemia. IL-6 also induces B cell proliferation and VEGF expression, leading to angiogenesis. IL-6 has emerged as a therapeutic target in CD based on its critical role in the pathogenesis and driving of symptomatology. The currently available treatments of CD are glucocorticoids, single-agent and combination chemotherapy, antiviral strategies, and monoclonal antibodies targeting CD20

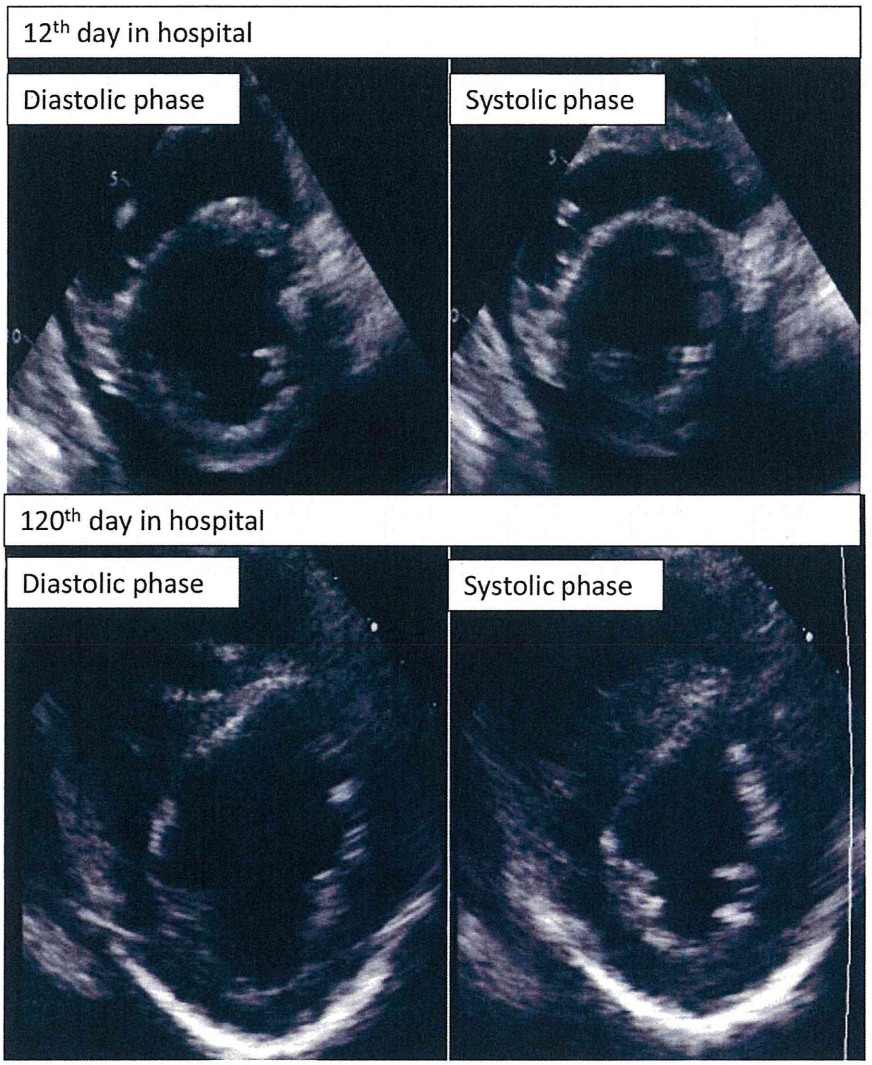

Fig. 5 Clinical course of cardiomyopathy (echocardiogram)
Mild diffuse hypokinesis (LVEF 52%) and mild pericardial effusion were detected on the 12th hospital day. On the 19th hospital day, sudden cardiogenic shock occurred. (No image stored. Severe diffuse hypokinesis described by chart description.) By the 120th hospital day, pericardial effusion had disappeared and wall motion had improved (LVEF 47.3%).

(rituximab) or IL-6 (tocilizumab)[16]. We chose rituximab because high-dose glucocorticoid had minimal effect and tocilizumab would not remain in the body due to the frequent drainage of pleural effusion and ascites to relieve the severe discomfort. In some cases, cyclosporine A (CyA) is effective (see **Table 2**), which suggests the involvement of T lymphocytes in the pathogenesis of TAFRO syndrome. Notably, our case showed various autoimmune findings, including positive tests for PA-IgG, direct Coombs, anti-SS-A (Ro) antibody, anti-TPO antibody, and anti-HLA antibody, relatively soon after platelet transfusion. Regarding the trigger of this syndrome, there was a report implicating HHV-6 infection[11], but it was negative in our case. Of note, our patient works for a meat slaughtering company and there was an incident a few weeks before the first symptoms in which he dropped a block of meat on his foot, which might have caused an infection of unknown microorganisms, leading to this syndrome.

Our case is the first report of TAFRO syndrome with cardiomyopathy. In cases of MCD, 7 cases with cardiac complications have been reported to date[17, 18]. Diffuse hypokinesis of LV wall motion was evident on echocardiograms in all these cases. Cardiac amyloidosis was diagnosed in 1 case by Congo red staining of the myocardium biopsy specimen, but the etiology of the cardiac dysfunction was not determined in the other 6 cases. Cardiac function and systemic symptoms were restored

Table 2 Previous case reports on TAFRO syndrome

Journal (author, year)	Age/Sex	Bone marrow biopsy	Thrombocytopenia		Liver failure			Heart failure	Renal failure				Treatment	Outcome
			Plt (10^3/μL)	PA-IgG (ng/10^7 cells)	T.bil (mg/dl)	ALP (IU/L)	Liver biopsy		Cre (mg/dl)	U-P	U-B	ARF/HD		
Inoue, 2013[2]	49, F	mild fibrosis	17	488	0.6	179			0.96	1+			DEX, PSL, CyA	remission
Iwaki, 2013[3]	43, F		135	98	1.0	1357			0.86	±			mPSL, PSL, RTX, TCZ	remission
Kawabata, 2013[4]	47, F	reticulin fibrosis	39	normal		660			1.4			HD	PSL, mPSL, TCZ	remission
Masaki, 2013[5]	57, F	without fibrosis	13	86.7	2.3	710			1.34	mild	microscopic	ARF	mPSL, PSL, CHOEP	died (candida infection)
	73, M	mild fibrosis	24		1.1	630			1.45	mild	microscopic		mPSL, PSL	died (MOF)
Takai, 2013[6]	47, F	reticulin fibrosis	14	> 300	1.5	1258	normal		0.7	1+	2+		CHOP, PSL	remission
	56, M	reticulin fibrosis	19	320	0.7	390	normal		1.9	3+	3+	ARF	mPSL, IVIG, CyA	remission
	49, M	reticulin fibrosis	10	300	0.7	756			0.9	−	−		mPSL, IVIG	died (CMV infection)
	53, F	reticulin fibrosis	38		1.9	1696	normal		0.7	1+	−		PSL, splenectomy CsA, IVCY	remission
	56, F	reticulin fibrosis	44	21.9	0.4	242			1.53	1+	2+	HD	mPSL, CyA	remission
Awano, 2013[7]	78, F	mild fibrosis	160	normal		459		BNP 126.0 UCG: normal	0.93				mPSL, PSL, TCZ	died (exacerbation)
Ozawa, 2014[8]	48, F	reticulin fibrosis	23	42.2	0.38	172		BNP 60.7	0.72	1+			PSL, RTX	remission
Tedesco, 2015[9]	21, F	reticulin fibrosis	7						1.6	1.96 g/d		ARF	PSL, PSL, TCZ, R-CVP	remission
Kubokawa, 2014[10]	15, M	reticulin fibrosis	thrombo-cytopenia				normal			0.4 g/gCre	−		mPSL, TCZ, IVIG PSL	remission
Jain, 2015[11]	28, M	reticulin fibrosis	75						2.5	1+	2+	HD	PSL, RTX	remission
Ishii, 2014[12]	50, M	reticulin fibrosis	65		1.4	753			3.24			ARF	TCZ, PSL	remission
Konishi, 2015[13]	77, F	no fibrosis	44	96	0.9	387			0.93				TCZ, PSL, RTX→CyA (maintenance)	remission
Tatekawa, 2015[14]	56, M	no fibrosis	76			1007			1.43				PSL+TCZ, thalidomide	remission
Kawashima, 2015[15]	39, M	no fibrosis	36	positive	0.3	1793				−	−		mPSL, PSL	remission
	38, M		202	positive	0.5	358	normal		2.59	+	+	ARF	PSL	remission
Our case	48, M	no fibrosis	16	294	0.8	1105		MRI: EF37%	1.50	3+	3+	HD	PSL, TCZ, IVIG, RTX	remission

U-P; urine protein, U-B; urine blood, DEX; dexamethasone, PSL; prednisolone, CyA; cyclosporine, mPSL; methylprednisolone, RTX; rituximab, TCZ; tocilizumab, CHOEP; cyclophosphamide, doxorubicin, etoposide, vincristine and prednisolone, CHOP; cyclophosphamide, doxorubicin, vincristine and prednisolone, IVIG; intravenous immunoglobulin, IVCY; intravenous cyclophosphamide, R-CVP; cyclophosphamide, vincristine, prednisolone, rituximab, HD; hemodialysis, ARF; acute renal failure

in 2 cases with the administration of glucocorticoid and in 2 cases with tocilizumab. An association between IL-6 and cardiovascular diseases has been implicated in both clinical and experimental settings. Hirota et al. reported that the circulating level of IL-6 is elevated in patients with congestive heart failure, implying an association of IL-6 with cardiac dysfunction[19]. Ancey et al. showed that human cardiomyocyte hypertrophy can be induced by gp130 (a counterpart of IL-6 receptor) stimulation in vitro, and this action was associated with STAT3 pathway activation[20]. Double-transgenic mice overexpressing both IL-6 and IL-6 receptor showed constitutive tyrosine phosphorylation of gp130 and STAT3 in the heart, and concentric hypertrophy and decreased LV volume were observed in these mice[21]. Thus, tocilizumab can be an effective treatment for CD-associated cardiomyopathy. Rituximab may deplete B cells that produce by IL-6.

As shown in **Table 2**, 21 cases of TAFRO syndrome have been reported. Median age was 49 years old and 11 were female (52%). Fourteen cases had myeloid fibrosis, all cases had severe thrombocytopenia, and 11 cases were positive for PA-IgG. Thirteen cases showed elevation of ALP, but liver biopsies in some cases showed no specific abnormalities. ALP isozyme analysis was liver type dominant. All cases had severe anasarca; the peritoneal biopsy of one case had no specific abnormalities. Eleven cases showed elevation of creatinine, 12 cases were positive for urinary protein or occult blood, and the renal biopsies in two cases showed mild to moderate proliferation of mesangial cells and thickened basement membrane with double contour and spike formation[15]. Treatments with glucocorticoid, tocilizumab, rituximab, CyA, and intravenous immunoglobulin were reported to be effective, but 4 cases died of infection or exacerbation of the disease. Our case had very severe generalized edema and intravascular dehydration probably because of cytokine storm, which may have been associated with the cardiac complications.

Conclusion

We reported a case of TAFRO syndrome with cardiomyopathy that was successfully treated with rituximab.

Conflict of interest

None

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RESEARCH ARTICLE

Clinicopathologic analysis of TAFRO syndrome demonstrates a distinct subtype of HHV-8-negative multicentric Castleman disease

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Multicentric Castleman disease (MCD) describes a heterogeneous group of disorders involving systemic inflammation, characteristic lymph node histopathology, and multi-organ dysfunction because of pathologic hypercytokinemia. Whereas Human Herpes Virus-8 (HHV-8) drives the hypercytokinemia in a cohort of immunocompromised patients, the etiology of HHV-8-negative MCD is idiopathic (iMCD). Recently, a limited series of iMCD cases in Japan sharing a constellation of clinical features, including thrombocytopenia (T), anasarca (A), fever (F), reticulin fibrosis (R), and organomegaly (O) has been described as TAFRO syndrome. Herein, we report clinicopathological findings on 25 patients (14 males and 11 females; 23 Japanese-born and two US-born), the largest TAFRO syndrome case series, including the first report of cases from the USA. The median age of onset was 50 years old (range: 23–72). The frequency of each feature was as follows: thrombocytopenia (21/25), anasarca (24/25), fever (21/25), organomegaly (25/25), and reticulin fibrosis (13/16). These patients frequently demonstrated abdominal pain, elevated serum alkaline phosphatase levels, and acute kidney failure. Surprisingly, none of the cases demonstrated marked hypergammoglobulinemia, which is frequently reported in iMCD. Lymph node biopsies revealed atrophic germinal centers with enlarged nuclei of endothelial cells and proliferation of endothelial venules in interfollicular zone. 23 of 25 cases were treated initially with corticosteroids; 12 patients responded poorly and required further therapy. Three patients died during the observation period (median: 9 months) because of disease progression or infections. TAFRO syndrome is a unique subtype of iMCD that demonstrates characteristic clinicopathological findings. Further study to clarify prognosis, pathophysiology, and appropriate treatment is needed.

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Introduction

Multicentric Castleman disease (MCD) is a rare disorder often characterized by episodes of systemic inflammation, reactive proliferation of morphologically benign lymphocytes, multicentric lymphadenopathy, polyclonal gammaglobulinemia, microcytic anemia, hypoalbuminemia, and elevated serum inflammatory proteins, such as C-reactive protein (CRP) [1–3]. The diagnosis of MCD is established histologically upon lymph node biopsy when characteristic hyaline vascular (HV), plasma cell (PC), or mixed type features are observed [4,5]. These histopathological and clinical abnormalities are believed to stem from pathologic hypercytokinemia, most notably of interleukin-6 (IL-6), though the cytokine responsible for initiating the inflammatory cascade may vary from patient to patient. These clinical features and lymph node changes can be seen in other diseases as well; therefore, MCD represents a diagnosis of exclusion [6–10].

Additional Supporting Information may be found in the online version of this article.

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Disclosure: D. Fajgenbaum serves on an advisory board to Janssen Pharmaceuticals. The rest of the authors have no potential conflicts of interest.
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TABLE I. TAFRO Symptoms and Histological Findings in TAFRO-iMCD

TAFRO symptoms		
Thrombocytopenia	Platelet count	<100 x10^3/μL
Anasarca	Pleural fluids and ascites	on computed tomography
Fever	Body temperature	>38.0°C (100.4F)
Reticulin fibrosis	Evaluated in bone marrow biopsy	
Organomegaly	Lymphadenopathyand/or hepatomegaly/splenomegaly	on computed tomography

*These were evaluated at time of diagnosis.

Histological findings of lymph nodes		
	TAFRO-iMCD	iMCD-NOS
Germinal centers	Atrophic germinal centers with enlarged nuclei of endothelial cells	Hyperplastic germinal centers of varying sizes
Interfollicular zone	Proliferation of endothelial venules, small numbers of mature plasma cells	Sheets of proliferating mature plasma cells
HHV-8	Negative	Negative
Light chain restrictions	None	None

HHV-8; Latency-associated nuclear antigen-1 assess to Human Herpes Virus-8.

Human herpes virus (HHV)-8, which infects B-cells and expresses a viral homolog of IL-6, drives the disease in immunocompromised MCD patients [11,12]. There is also a significant cohort of MCD patients around the world that are HHV-8-negative and the etiology of disease is unknown [13–15]. These cases are referred to as idiopathic MCD (iMCD) [6]. Possible etiologies in these patients include a virus other than HHV-8, paracrine secretion of cytokines by a small population of neoplastic cells, or autoinflammatory mechanisms [6]. The unknown etiology of these iMCD cases presents a significant challenge with regards to designing appropriate treatment regimens.

Recently, Takai et al. [16] reported three patients who shared a constellation of clinical symptoms that have been called TAFRO syndrome: thrombocytopenia (T), anasarca (A), fever (F), reticulin fibrosis (R), and organomegaly (O). Of note, one of these three patients underwent a lymph node biopsy demonstrating hyaline-vascular-like changes consistent with MCD. These patients responded well to immunosuppressive therapy with cyclosporine and prednisolone. Takai and colleagues proposed that this novel clinical entity represents a group of systemic inflammatory disorders rooted in autoimmunity [16]. Since this initial description in 2010, an additional 11 cases of TAFRO syndrome have been reported [14,17–22], and most recently, a Caucasian case was reported in Europe [23]. All of these patients in which a lymph node biopsy was performed have demonstrated histopathology consistent with MCD. Physicians in the United States of America (USA) have reported having iMCD patients with TAFRO features (Personal Communications, van Rhee, F; Personal Communications, Uldrick, T). However, these descriptions of TAFRO in the USA have not been published, and a larger recognition and reporting of TAFRO is needed outside of Japan.

To investigate the clinical entity of TAFRO syndrome and further characterize its histopathological findings, we have analyzed the largest-to-date series of 25 patients diagnosed with HHV-8 negative or iMCD, demonstrating the TAFRO syndrome (TAFRO-iMCD). This is the largest series of patients reported to have TAFRO syndrome, and the first description of TAFRO in the USA.

Methods

Patients. Patients who had been diagnosed with HHV-8-negative iMCD were selected from pathology files in the Department of Pathology, Okayama University from 1999 to 2013 for careful medical record review by a team of physicians. Twenty-three Japanese patients were found to demonstrate at least three out of five TAFRO clinical symptoms and characteristic TAFRO-iMCD lymph node histopathology, such as atrophic germinal centers with enlarged nuclei of endothelial cells, proliferation of endothelial venules with enlarged nuclei in interfollicular zone, and small numbers of mature plasma cells [18]. Twelve of these cases were referred to our institution because their physicians suspected TAFRO syndrome. Two of the cases have been reported previously [17,18]. We also included two patients from the USA, who also were found to have iMCD and demonstrate all five TAFRO clinical features. They were both born in the USA and currently reside there. One is of European descent and has been reported previously [14], while the other patient is of Sri Lankan descent.

As a control, 19 cases of HHV-8 negative MCD with PC-subtype, were selected from the same Okayama University pathological files. These cases are herein referred to as iMCD-NOS (not otherwise specified).

The use of samples and the medical records (clinical history, treatmentm and survival data) in our study was approved by the Institute Review Board (IRB) at Okayama University. Written informed consent was waived by our institutional review board, since our study was limited to the use of excess human tissue samples and medical records.

Latency-associated nuclear antigen-1 (LANA-1) was performed by immunohistochemistry of paraffin-embedded lymph node to assess HHV-8 status. Polymerase chain reaction (PCR) for HHV-8 DNA in the blood was also tested in eight cases of TAFRO-iMCD. For all patients and controls, alternative diagnoses for iMCD were excluded as determined by clinical features, blood cultures, serum immunoelectrophoresis, and autoantibody tests. The differential diagnosis includes infectious diseases, POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, M-protein, and skin pigmentation), lymphoma, and rheumatologic diseases, such as systemic lupus erythematosus (SLE).

Definition of TAFRO symptoms. Thrombocytopenia was defined as a platelet count < 100×10^3/μL at the time of diagnosis. Fever was defined as temperature of >38.0° C (100.4F). Anasarca was defined as the presence of pleural fluids and ascites on computed tomography. Organomegaly included lymphadenopathy, hepatomegaly, or splenomegaly, which was also evaluated by radiologists using computed tomography (CT). Reticulin fibrosis was evaluated via bone marrow biopsy (Table I).

Histological and immunohistochemical examination. Paraffin-embedded lymph node and bone marrow biopsy specimens were cut into 4 μm sections and stained with hematoxylin/eosin and reticulin silver impregnation, respectively. The lymph node sections were immunohistochemically stained using an automated immunostainer (Ventana Medical Systems, Tucson, AZ, USA). Tissue sections were subjected to standardized heating pretreatment for antigen retrieval with antibodies specific for CD20 (L26; 1:200; Novocastra, Newcastle, UK); CD3 epsilon (PS1; 1:50; Novocastra); CD21 (1F8; 1:20; Dako, Carpinteria, CA,USA); CD10 (56C6; 1:50; Novocastra); bcl2 (3.1; 1:200; Novocastra); CD138 (MI15; 1:100; Dako); kappa light chains (kp-53; 1:100; Novocastra); lambda light chains (HP-6054; 1:200; Novocastra) and HHV-8 (13B10; 1:25; Novocastra). *In situ* hybridization (ISH) of kappa and lambda light-chains was performed by an automated Bond Max stainer (Leica Biosystems, Melbourne, Australia).

Statistical analysis. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria) [24]. Comparisons of the clinical parameters (platelet count, serum level of CRP, immunoglobulin G (IgG), immunoglobulin A (IgA), immunoglobulin M (IgM), lactate dehydrogenase (LD), alkaline phosphatase (ALP), and IL-6) in TAFRO syndrome, and iMCD-NOS were performed with Fisher's exact test for categorical variables and the Mann–Whitney U test for continuous variables. All *P*-values were two sided, and *P*-values of 0.05 or less were considered statistically significant.

Results

Clinical features

TAFRO syndrome group (TAFRO-iMCD). Clinical features are summarized in Table II. The TAFRO-iMCD group included 14 males

TABLE II. Clinical Characteristics and Laboratory Data, TAFRO-iMCD and iMCD-NOS

	TAFRO-iMCD (n=25) Median	(range)	iMCD-NOS (n=19) Median	(range)	*P* value
Age (y.o)	50	(23–72)	47	(28–68)	N.S
Gender (M:F)	14: 11		12: 7		N.S
PS≧2	76.5%	(13/17)	0%	(0/11)	*P*<0.01
Anasarca	96.0%	(24/25)	0%	(0/14)	*P*<0.01
Fever	84.0%	(21/25)	18.2%	(2/11)	*P*<0.01
Reticulin fibrosis	81.3%	(13/16)	N.E		N.E
Abdominal pain	32.0%	(8/25)	N.E		N.E
Plt ($\times 10^3\mu L$)	43	(14–171)	339	(206–500)	*P*<0.01
Hb (g/dL)	9.1	(6.2–16.3)	11.0	(5.8–13.2)	*P*=0.12
Alb (g/dL)	2.3	(1.1–3.5)	2.8	(1.8–4.3)	*P*=0.01
CRP (mg/dL)	14.9	(0.8–30.2)	5.9	(1.5–16.9)	*P*=0.01
IgG (mg/dL)	1,476	(880–2,824)	4,775	(2,176–8,380)	*P*<0.01
IgA (mg/dL)	225.7	(129–432)	621	(258–997)	*P*<0.01
IgM (mg/dL)	74	(37–257)	229	(129–726)	*P*<0.01
LD (IU/L)	210	(110–424)	114	(85–138)	*P*<0.01
ALP (IU/L)	469	(102–2,388)	266	(174–1,240)	*P*<0.01
IL-6 (pg/mL)	16.2	(6.0–67.3)	25.9	(8.6–113)	*P*=0.17
VEGF (pg/mL)	305	(<20–1,410)	N.E		N.E

PS, performance status; Anasarca, pleural fluid and ascites; Fever: >38.0°C (100.4F),.
Plt, Blood platelet count; Hb, Hemoglobin; Alb, Albumin; CRP, C-reactive protein; IgG, Immunoglobulin G; IgA, Immunoglobulin A; IgM, Immunoglobulin M; LD, lactate dehydrogenase; ALP, alkaline phosphatase; IL-6, Interleukin-6 (serum normal value; <5.0 pg/mL); VEGF, Vascular endothelial growth factor (plasma normal value, <115 pg/mL); N.S, not significant; N.E, not evaluated.

and 11 females. The median age of disease onset was 50 years old (range, 23–72). Twenty-three patients were of East Asian origin, whereas one patient was South Asian and one Caucasian. The median duration between disease onset and time to diagnosis by lymph node biopsy was 6 weeks (range, 1–70 weeks). General condition at diagnosis tended to be poor; Eastern Cooperative Oncology Group (ECOG) performance status (PS) was greater than one for 76.5% (13/17) of patients. The median duration between diagnosis and the last follow-up for data collection was 9 months (range: 0–91 mos.).

Since bone marrow biopsy was only performed in 16 cases, all five criteria could only be assessed in those 16 cases. Nine of those TAFRO-iMCD cases fulfilled all five TAFRO criteria. The remaining 16 cases met at least three of four criteria. The frequency of each complication at diagnosis was as follows: thrombocytopenia (21/25), anasarca (24/25), fever (21/25), reticulin fibrosis (13/16), and organomegaly (25/25). Thirty-two percent (8/25) of TAFRO-iMCD cases reported abdominal pain, and three cases experienced painful lymphadenopathy. The median duration between diagnosis and the last follow-up for data collection was 9 months (range: 0–91 mos.).

iMCD control group with plasma cell type (iMCD-NOS). Twelve males and seven females with iMCD-NOS served as controls from the same Okayama University pathological files. The median age of disease onset was 47 years old (range: 28–68). All cases were of Asian origin. The general condition at diagnosis tended to be better than in the TAFRO-iMCD group, as all patients were PS 0 or 1. None of the patients exhibited anasarca. 2/11 cases demonstrated fever and all 19 cases had organomegaly (Table II).

Laboratory features

Laboratory data are presented in Table II. Both groups commonly demonstrated microcytic anemia, hypoalbuminemia, and elevated serum CRP. CRP levels were significantly higher (P=0.01) in the TAFRO-iMCD group compared to the MCD-NOS group. Patients in the TAFRO-iMCD group demonstrated severe thrombocytopenia whereas none of the iMCD-NOS cases had thrombocytopenia. The median platelet count was 43 $\times$ $10^3/\mu L$ (range 14–171 $\times$ $10^3/\mu L$) for the TAFRO-iMCD group and 339 $\times$ $10^3/\mu L$ (206–500 $\times$ $10^3/\mu L$) for the iMCD-NOS group (P <0.01). Serum platelet associated immunoglobulin G were checked in only 10 cases of TAFRO-iMCD, and elevated in nine cases (Median 144, range 12.5–1,340 ng/10^7cells). Median serum LD was not elevated in either group at time of diagnosis. Serum ALP at diagnosis was elevated in 19/24 cases (79.2%) of TAFRO-iMCD cases (median: 469 IU/L, range: 102–2,388 IU/L) without corresponding elevations in transaminases, bilirubin, or LD. The median ALP level was significantly (P<0.01) lower yet still elevated in iMCD-NOS group (median: 266 IU/L, range: 174–1,240 IU/L). All five TAFRO-iMCD cases that were tested for ALP isozymes demonstrated hepatogenous-specific isozyme.

Several cases of TAFRO-iMCD showed progressive acute kidney failure and five cases needed temporary hemodialysis because of uremic symptoms during the course of disease. Median serum creatinine level of TAFRO-iMCD cases at diagnosis was 0.96 mg/dL (range 0.52–6.08 mg/dL). None of the TAFRO-iMCD cases demonstrated polyclonal hypergammaglobulinemia (median IgG level 1,476 mg/dL, range 880–2,842 mg/dL). By contrast, 17 of the 19 cases of iMCD-NOS showed marked hypergammaglobulinemia with IgG levels greater than 3,500mg/dL (median IgG level 4,775 mg/dL, range 2,176–8,380 mg/dL) (P<0.01).

The median serum IL-6 level at diagnosis was greater for iMCD-NOS (25.9 pg/mL, range: 8.6–113 pg/mL) than for the TAFRO-iMCD group (16.2 pg/mL, range: 6.0–67.3 mg/dL), but these differences were not statistically significant. The median level of plasma vascular endothelial growth factor (VEGF) level was 305 pg/mL in the 16 cases of TAFRO-iMCD that measured VEGF (range <20–1,410 pg/mL), which is approximately three times the upper limit of normal. VEGF was not measured for the iMCD-NOS cases.

Histologic and immunohistochemical findings

Lymph nodes. All 44 patients had one or more lymph nodes samples available for further analysis. All of the 42 Japanese TAFRO-iMCD and iMCD-NOS cases were collected and reviewed at the Department of Pathology, Okayama University. Both cases from the USA were reviewed at National Institutes of Health/National Cancer Institute. All cases of TAFRO-iMCD and iMCD-NOS were tested and found to be negative for HHV-8 by immunohistochemistry for LANA-1. HHV-8 DNA was also not detected by PCR in the blood in all eight cases of TAFRO-iMCD in which this test was performed.

TAFRO-iMCD patients exhibited several common histopathological findings. Nearly all lymph nodes obtained at diagnosis were only slightly enlarged in size with regards to length in greatest diameter

(median: 9 mm, range: 6–14mm). The characteristic histopathological findings of the TAFRO-iMCD group included atrophic germinal centers, expansion of the interfollicular zone, proliferation of highly dense endothelial venules, and relatively few mature plasma cells (Fig. 1a–e). HV features such as penetrating blood vessels were present but not as prominent as usually seen in HV or mixed-type iMCD. Architectural features typical of unicentric hyaline vascular CD were not observed. Also, enlarged nuclei were found in proliferating endothelial cells in the germinal centers and interfollicular zone of TAFRO-iMCD cases (Fig. 1a–d). Immunohistochemical studies showed that the follicular dendritic cell (FDC) networks tended to be expanded or disrupted in the interfollicular zone of TAFRO-iMCD cases (Fig. 1f).

The histological findings in TAFRO-iMCD were quite different from iMCD-NOS. All cases of iMCD-NOS showed classically reported PC-type features, including diffuse marked interfollicular plasmacytosis, prominence of germinal centers, and preservation of overall lymph node architecture [25]. No light chain restrictions were detected by *in situ* hybridization or immunohistochemical staining in either group (Table I).

Bone marrow. Bone marrow biopsy or aspiration samples were available in 22/25 cases with TAFRO-iMCD. Aspirates only were available in six cases. No samples demonstrated infiltration of neoplastic cells. In two cases, biopsy yielded a dry tap and no cellular material was obtained. Of the 22 TAFRO-iMCD bone marrow samples available, 13 were hypercellular (Fig. 1g–i), six were normocellular, and three were hypocellular. Megakaryocytic hyperplasia with slight atypia such as multiple and widely separated nuclei was observed in 12 cases (Fig. 1i). Micromegakaryocytes were not increased obviously. Significant plasmacytosis was not observed.

Myelofibrosis (MF) was scored using a scale from 0 to 3 according to the European consensus on bone marrow fibrosis grading [26]. 13/16 cases were positive for reticulin fibrosis and all 13 cases were classified MF-1 with a very loose network of reticulin fibers (Fig. 1j). Bone marrow samples from iMCD-NOS cases were not tested.

Clinical management and follow-up

Corticosteroids, such as prednisolone, methylprednisolone, and dexamethasone, were used as first-line therapy in 23/25 TAFRO-iMCD cases. 11/23 cases (47.8%) responded well to initial corticosteroids therapy alone. Because of acute deterioration, one case required multidrug therapy at the time of diagnosis with a regimen consisting of rituximab, tocilizumab, cyclophosphamide, and etoposide. One case showed spontaneous remission with only pleural effusion drainage and nonsteroidal anti-inflammatory treatment.

Twelve patients received additional therapy because of poor response to corticosteroids, and nine improved with additional therapies. Some patients received more than one additional treatment regimen. Additional treatments that were used included cyclosporine ($n = 7$); tocilizumab ($n = 6$); rituximab ($n = 4$); siltuximab (anti-IL-6 monoclonal antibody, $n = 1$); thalidomide ($n = 1$); vincristine ($n = 1$); sirolimus ($n = 1$); VDT-ACE-R (bortezomib, dexamethasone, thalidomide, adriamycin, cyclophosphamide, etoposide, and rituximab, $n = 1$); and intravenous injection of immunoglobulin (IVIG) ($n = 1$). One seriously ill patient that was refractory to corticosteroids, rituximab, and siltuximab therapy responded well to a cycle of VDT-ACE-R, but relapsed 15 months later while on siltuximab maintenance administered every 3 weeks. The patient then responded to another cycle of VDT-ACE-R, but relapsed 16 months later while on siltuximab every 3 weeks and weekly VDT maintenance. The patient received a third round of VDT-ACE-R, which induced another complete remission. He is now receiving maintenance IVIG and sirolimus and has been in a complete remission for 21 months. Plasma exchange therapy was used in two cases. Seven cases with severe acute renal failure required temporary dialysis during flares, which was discontinued following multidrug treatment.

The median follow-up period was 9 months (range, 0–91 mos.). Three patients died during the observation period because of progression of disease or sepsis. Three other patients experienced disease flares as they were weaned from immunosuppressive agents (In Supporting Information Table I Treatments and outcomes of TAFRO-iMCD). No patients developed lymphoma, Kaposi sarcoma, or any other malignancies during the follow-up period.

Discussion

We have analyzed the clinical features and histopathological characteristics of 23 Japanese cases and two US cases of HHV-8-negative MCD that demonstrated TAFRO clinical symptoms (TAFRO-iMCD). Relative to iMCD-NOS, TAFRO-iMCD is characterized by a more aggressive clinical course, corticosteroid-refractoriness, thrombocytopenia, higher frequency of anasarca, elevated level of ALP, and normal gammaglobulin levels. These unique clinical and laboratory features suggest that TAFRO-iMCD is a distinct entity within the larger entity of iMCD.

Two great challenges in the diagnosis and treatment of iMCD are the spectrum of nonspecific symptoms and the unclear etiology of the disease. By comparison, HHV-8-positive MCD is easier to diagnose, because positive LANA-1 staining in the presence of characteristic MCD lymph node histopathology is specific for HHV-8-positive MCD. Since B-cells host HHV-8, treatment is targeted at CD20, and B-cell depletion is highly effective [27]. However, iMCD does not have a positive diagnostic biomarker and can demonstrate a wide spectrum of symptoms, which makes the disease extremely difficult to diagnose. Further, once the disease is diagnosed, little is known about the mechanisms of pathogenesis to guide treatment decisions.

Our report advances the understanding of iMCD by addressing the challenges posed by nonspecific symptoms and unclear etiology. With regards to the symptoms of iMCD, we have identified a group of iMCD patients with both TAFRO pathological characteristics and core TAFRO symptoms, which are highly represented in affected patients. Identifying a homogeneous group within iMCD is critical, because it simplifies recognition of disease and diagnosis. Whereas HHV-8-associated MCD is readily recognized through the sensitivity of the LANA-1 staining for the HHV-8 virus and the specificity of "Castleman-like" histopathological features combined with positive LANA-1, so too may TAFRO-iMCD be readily recognized through its easily identifiable clinical symptoms and pathological features. In addition to aiding diagnosis, subclassification of iMCD based on clinical features will help to inform prognosis, appropriate treatments, and new targets for future therapies.

To this end, we propose that MCD can be further classified beyond HHV-8 status. HHV-8 negative iMCD may be further stratified into iMCD with TAFRO features or iMCD-NOS. Based on our series and other iMCD reports, we propose the following diagnostic criteria for iMCD with TAFRO features (Table III). TAFRO-iMCD patients must meet the histopathological criteria, three major criteria and one or more minor criteria. Excluding diseases from the differential diagnosis is necessary. Diseases that should be excluded include rheumatologic diseases such as SLE, infectious diseases such as acute Epstein-Barr Virus, and neoplastic diseases such as lymphoma, POEMS syndrome and cancer. Recently, a newly described hyper inflammatory cytokine syndrome associated with HHV-8 or Kaposi sarcoma-associated herpesvirus (KSHV) infection has been described as KSHV inflammatory cytokine syndrome (KICS) [28]. This clinical condition is characterized by elevated IL-6 because of replicating HHV-8 and presents with clinical inflammatory symptoms similar to MCD. However, these patients do not have characteristic MCD