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NOTE

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Diagnosis of severe fever with thrombocytopenia syndrome (SFTS) in a cat with clinical findings resembling lymphoma

Mitsuhiro IRIE[1)]*, Takuma MIYOSHI[1)], Akira HIRAMOTO[1)], Masahiko HIRATA[2)], Masamine TAKANOSU[3)], Eun-Sil PARK[4)] and Ken MAEDA[4)]

[1)]Shikoku Veterinary Medical Center, Kagawa, Japan
[2)]IDEXX Laboratories Japan Incorporation, Tokyo, Japan
[3)]Nasunogahara Animal Clinic, Tochigi, Japan
[4)]National Institute of Infectious Diseases, Tokyo, Japan

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ABSTRACT. A two-year-old male domestic cat showed lethargy, tonic-clonic convulsion, and mucosal jaundice. Upon admission, blood examination indicated severe neutropenia and thrombocytopenia, and ultrasonography revealed diffuse splenomegaly with a honeycomb appearance and abdominal lymph nodes enlargement in addition to a decrease in cardiac blood flow indicating a shock condition. Cytology of the spleen showed a cell population composed of immature large lymphoid cells with distinct nucleoli, suggesting lymphoma. The cat received symptomatic treatments but died four hours later. Reverse transcriptase polymerase chain reaction assay of the spleen sample indicated the presence of severe fever with thrombocytopenia syndrome (SFTS) virus S gene segment. Clinical features of this case that was diagnose as SFTS were similar to lymphoma. Therefore, pet owners and veterinary workers should be protected against infection of SFTS.

KEYWORDS: feline, lymphoma, public health, severe fever with thrombocytopenia syndrome

Severe fever with thrombocytopenia syndrome (SFTS) is a recently discovered infections disease caused by *Dabie bandavirue* (formerly SFTS virus; SFTSV), which has become epidemic in East Asian countries, including China [24, 25], Korea [6], Vietnam [21] and Japan [19]. SFTSV is a negative-sense single-stranded RNA virus classified in the genus *Bandavirus* of the family *Phenuiviridae* [22].

In Japan, many human SFTS patients have been reported mainly in the southwestern region [17]. The incubation period following SFTSV infection ranges from six days to two weeks, after which human patients develop fever, digestive tract symptoms, headache, myalgia, lymphadenopathy, neurological symptoms, and bleeding tendency. The estimated fatality rate of SFTSV infection in humans has been reported as 5% up to 40% [17].

Antibodies to SFTSV have been detected in wild and domestic animals such as goats, deer, cattle, dogs, and cats in SFTS-endemic areas [3, 7, 8, 13, 18, 20]. SFTSV is thought to circulate in an enzootic environment and to have a tick–vertebrate-tick cycle [8]. In 2017, fatal SFTS was confirmed in two captive cheetahs in a zoo located in western Japan [9]. Moreover, in the same year, the Ministry of Health, Labor and Welfare of Japan published information on a dog and a cat with fever and asthenia, which were infected with SFTSV. Then, Matsuu *et al*. [10] reported a study on 24 client-owned cats infected with SFTSV who showed clinical signs, including anorexia and lethargy. The mortality rate in the cats naturally infected with SFTSV were reported as high as 62.5% [10]. Acute onset of anorexia, lethargy, fever, and vomiting was commonly found in cats that developed SFTS [10].

Because veterinary medical staff has a risk of SFTSV transmission from infected animals, especially in the areas where SFTS is endemic, it is necessary to understand the clinical presentation of SFTS in animals to prepare prevention measures for SFTSV. Herein, we report a cat that showed clinical findings resembling lymphoma and was finally diagnosed with SFTS after death.

The case was a two-year and three-month-old male mongrel cat. When the cat returned home after spending five days outdoors, the cat was lethargic and anorectic. Just after admission to our veterinary clinic, the cat showed tonic-clonic convulsion with other clinical sings including wobbling, decreased consciousness, and jaundice of the visible mucosa. Physical examination indicated hypothermia (35.1°C), heart rate of 130 /min, and respiratory rate of 30 /min. Furthermore, severe decrease of the femoral artery pressure was noted. Emergency ultrasonography showed a decrease in cardiac blood flow, and the patient was judged to be in a serious shock condition overall. Abdominal ultrasonography revealed generalized splenomegaly with spotted hypoechoic areas

*Correspondence to: Irie, M.: vetirie@me.com, Shikoku Veterinary Medical Center, 3308-5 Ikenobe, Miki-cho, Kita-gun, Kagawa 761-0701, Japan

distributed throughout the organ, a so-called honeycomb appearance (Fig. 1), and slightly swollen intraperitoneal lymph nodes. Ultrasound-guided fine-needle aspiration (FNA) of the spleen revealed a uniform population of large lymphoid cells containing a round nucleus with a fine chromatin pattern and distinct nucleolus (or nucleoli), corresponding a characteristic cytology appearance of lymphoma (Fig. 2). Mitotic figures were sometimes found, and phagocytosis by macrophages was commonly observed (Fig. 2). A tentative diagnosis of lymphoma was made in an emergency condition, and fluid therapy was immediately started for the initial treatment of shock.

Blood examination revealed profound thrombocytopenia (21×10^3 /µl) and leukopenia (240 /µl). Blood chemistry revealed hypoglycemia (30 mg/dl) and increased levels of bilirubin (8.4 mg/dl), aspartate transaminase (203 U/l), urea nitrogen (69 mg/dl), creatinine (2.0 mg/dl), and inorganic phosphorus (>16.1 mg/dl) (Table 1). Moreover, severe metabolic acidosis in venous blood gas analysis (Table 1) and increased blood lactic acid level were found. Even after initiating the fluid therapy, the cat gradually underwent unconsciousness, recumbency, and further hypothermia, and died four hours later. Due to the emergency status, other tests including feline immunodeficiency virus (FIV) antibody test and feline leukemia virus (FeLV) antigen test could not be performed.

Tissue specimens by autopsy could not be obtained from the cat. Meanwhile, lymphocyte clonality was assessed using a reverse transcriptase polymerase chain reaction (PCR) assay using the FNA sample of the spleen. Genomic DNA was extracted from cells aspirated from the spleen and subjected to PCR for the rearrangement of T-cell receptor γ chain (TCRγ) [12] and immunoglobulin heavy chain (IgH) [2, 11, 23] genes. Rearrangement of the immunoglobulin kappa chain locus was also separately analyzed for this case (Takanosu *et al.*, manuscript in preparation). Clonal rearrangement was not identified in any of the antigen receptor genes examined.

Further effort was needed to identify the cause of leukopenia and thrombocytopenia observed in the cat.

In a recent study reported by Matsuu *et al*. [10], 24 cats with acute onset of anorexia and lethargy were found to be infected with SFTSV in the western region of Japan [10]. Thrombocytopenia (91.7%), leukopenia (79.2%), and elevated serum total bilirubin levels (94.7%) were commonly observed [10]. The case described in this study showed anorexia, lethargy, and convulsion that have been described as common clinical signs reported in cats naturally infected with SFTSV [10]. Blood examination results, including thrombocytopenia, leukopenia, and elevated total bilirubin, were similar to those reported in cats that developed SFTS. Furthermore, the location of the cat's owner and our clinic was the western region of Japan where SFTS was known to be endemic [17]. At the time of admission, we did not find any tick infestation on the body of the cat; however, since the cat was free to go out from the owner's house, the cat conceivably had many opportunities to be infested by ticks that can transmit SFTSV.

Although fresh blood sample from this case was not stored, spleen cells obtained by FNA on a glass slide were available for detection of SFTSV by PCR. The spleen cell RNA was extracted with ISOGEN (Nippon Gene, Toyama, Japan) according to the manufacturer's instructions. Total RNA was dissolved in 20 µl diethylpyrocaronate (DEPC) -treated water (Nippon Gene). RT-PCR was performed using the Superscript III one-step RT-PCR system with platinum Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA) according to our previous study [14]. Two types of S segment-specific primer sets, [5′- GACACAAAGTTCATCATTGTCTTTGCCCT- 3′ and 5′-TGCTGCAGCACATGTCCAAGTGG-3′]and[5′-GCCATCTGTCTTCTTTTTGCG-3′and5′-AGTCACTTGCAAGGCTAAGAGG-3′], reported in our previous study [14] were used for PCR. The results showed that the case was positive for the SFTSV (Fig. 3).

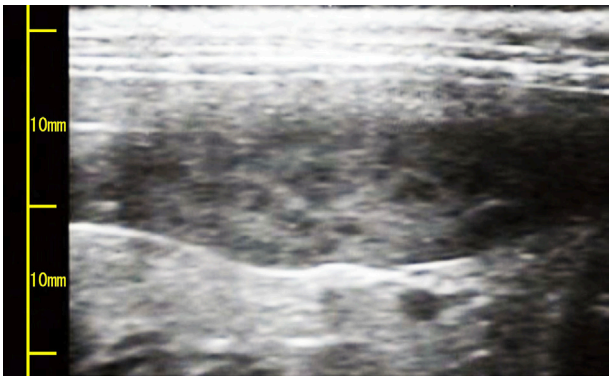

Fig. 1. Abdominal ultrasonography. Spotted hypoechoic areas distributed throughout spleen showing honeycomb appearance.

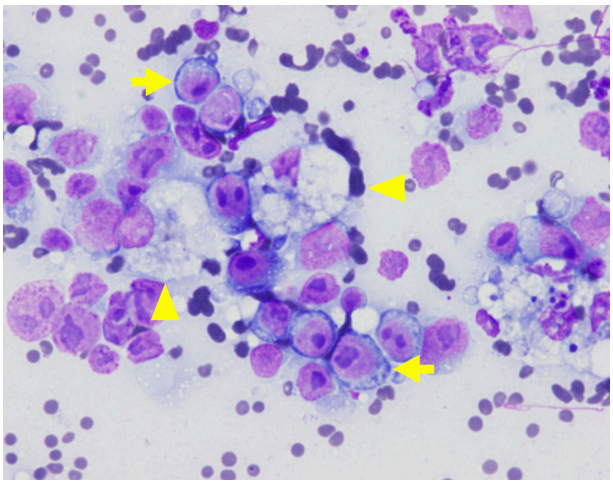

Fig. 2. Cytology of the fine needle aspiration samples of spleen (Wright-Giemsa stain, ×400). Cell population of round cells having a large nucleus containing a centrally located nucleolus (arrows) resembling "Immunoblasts". Cluster of lymphoid cells with a round nucleus containing one or two nucleolus and several macrophages showing hemophagocytosis (arrowheads).

Table 1. Results of complete blood cell count, serum chemistry analysis, and venous blood gas analysis in the cat case examined in this study

RBC ($\times 10^6$ /μl)	11.92		RBC: red blood cell
PCV (%)	47.4		PCV: packed cell volume
Hemoglobin (g/dl)	14.6		
MCV (fl)	39.8		MCV: mean corpuscular volume
MCHC (g/dl)	30.8		MCHC: mean corpuscular hemoglobin concentration
WBC (/μl)	240		WBC: white blood cell
Segmented neutrophils (/μl)	100		
Lymphocytes (/μl)	70		
Monocytes (/μl)	10		
Eosinophils (/μl)	30		
Basophils (/μl)	30		
Platelets ($\times 10^3$/μl)	21		
BUN (mg/dl)	69		BUN: blood urea nitrogen
CRE (mg/dl)	2		CRE: creatinine
ALP (U/l)	<10		ALP: alkaline phosphatase
ALT (U/l)	64		ALT: alanine aminotransferase
AST (U/l)	203		AST: aspartate aminotransferase
GGT (U/l)	0		GGT: γ-glutamyltransferase
TP (g/dl)	7.7		TP: total protein
ALB (g/dl)	2.8		ALB: albumin
T-Bil (mg/dl)	8.4		T-Bil: total bilirubin
T-Cho (mg/dl)	78		T-Cho: total cholesterol
Glu (mg/dl)	30		Glu: glucose
Ca (mg/dl)	7.8		Ca: calcium
P (mg/dl)	>16.1		P: phosphate
Na (mEq/l)	158		Na: sodium
K (mEq/l)	4.8		K: potassium
Cl (mEq/l)	124		Cl: chloride
		(Reference value)	
pH	7.27	(7.36 ± 0.02)	
$PvCO_2$ (Torr)	30.6	(43 ± 4)	$PvCO_2$: mixed venous carbon dioxide
PvO_2 (Torr)	39.8	(53 ± 10)	PvO_2: mixed venous oxygen pressure
HCO_3^- (mmol/l)	13.7	(23 ± 2)	HCO_3^-: Bicarbonate ion
Lactate (mmol/l)	4.4	(0–1 to 1.7)	

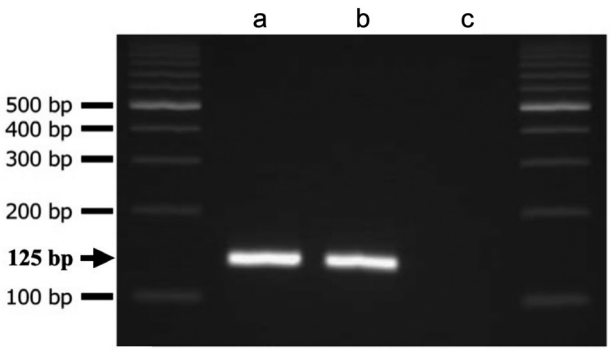

Fig. 3. Gel electrophoresis of the DNA samples amplified by RT-PCR using severe fever with thrombocytopenia syndrome virus S-segment-specific primers. A band of 125 bp was generated from the fine-needle aspiration sample of the spleen (**a**) as in the positive control (**b**) but not in negative control (**c**).

Although fever was commonly found in cats with SFTS (68.2%) [10], the cat in the present study was hypothermic. At the time of presentation, the cat was in shock and already in moribund state. Therefore, it was speculated that the cat had experienced pyrexia before getting into shock.

Initially, we suspected a diagnosis of lymphoma from the cytology findings of the FNA sample of the spleen. These cells looked like immunoblasts, which are observed in a type of high–grade lymphoma in Kiel classification for lymphomas [1]. Although the uniform population of the large lymphoid cells was indicative of lymphoma, the presence of a relatively large number of macrophages showing phagocytosis admixed to the lymphoid cells was not a typical finding of lymphoma. Autopsy findings of the human SFTS patients in Japan included severe necrotizing lymphadenitis with massive necrosis, the depletion of lymphocytes, and severe infiltration of the lymph nodes by histiocytes and immunoblasts [19]. Moreover, hemophagocytosis was prominent in lymph nodes, bone marrow, and spleen in human patients who suffered from SFTS [19]. We reported that four of the six cats experimentally

infected with SFTSV became moribund about one week after virus infection [15]. Histologically, lesions were found mainly in the lymph nodes and spleen, showing necrotizing inflammation. We identified that B-cell lineage at a stage between germinal center B-cells and immunoblasts were infected with SFTSV [15]. A number of immunoblast-like cells found in the present case naturally infected with SFTSV could be B-lymphoid cells activated by SFTSV.

Ultrasonographic findings such as splenomegaly with scattered hypoechoic foci and enlargement of the pleural number of intraperitoneal lymph nodes are common in cats with lymphoma. However, the present study elicits a warning that such findings can also be found in cats suffered from SFTS.

Matsuu *et al*. [10] reported that SFTSV RNA was detected in the urine, conjunctiva, and oral and rectal swabs from the cats affected with SFTS, and they were considered as a possible source of infection. SFTS should be considered as a zoonotic disease that can transmit from cats to humans [5, 26], while person-to-person transmission of SFTSV has been demonstrated [4, 16]. When some cats that go outdoors show rapidly debilitating clinical signs with fever and jaundice, it is necessary to consider a possible infection of SFTSV, especially in the endemic area of SFTS like southwestern Japan. Accordingly, when veterinary clinic receives feline cases that might be infected with SFTSV especially in the SFTSV-endemic areas like southwestern Japan, appropriate personal protective equipment for infection (hoods, masks, face guards, protective clothing, and gloves) should be equipped in the facility to protect the staff at risk of SFTS from infected animals.

The feline case described in this study was tentatively diagnosed with lymphoma in an emergency, but diagnosis of SFTS was later confirmed from the result of RT-PCR assay for SFTSV. Thus, the case report here could be a warning of SFTSV transmission from cats to humans. Understanding the clinical features of cats suffered from SFTS will help to avoid the hazard of SFTSV transmission from animals.

POTENTIAL CONFLICTS OF INTEREST. The authors have nothing to disclose.

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