

**Pathological characteristics of severe fever with
thrombocytopenia syndrome (SFTS) virus in a patient infected
with SFTS virus from a sick cat's bite**

Running title: Pathology in a cat-associated SFTS patient

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ABSTRACT: A woman in her 50s showed symptoms of fever, loss of appetite, vomiting, and general fatigue 2 days after she was bitten by a sick cat, which had later died, in Yamaguchi prefecture, western Japan, in June 2016. She subsequently died of multiorgan failure, and an autopsy was performed to determine the cause of death. However, the etiological pathogens were not quickly identified. The pathological features of the patient were retrospectively re-examined, and the pathology of the regional lymph node at the site of the cat bite was found to show necrotizing lymphadenitis with hemophagocytosis. The pathological features were noticed to be similar to those of patients reported to have severe fever with thrombocytopenia syndrome (SFTS). Therefore, the lymph node section was retrospectively tested immunohistochemically for SFTSV antigen, which revealed the presence of SFTSV antigen. The sick cat also showed similar symptoms and laboratory findings similar to those shown in human SFTS cases. It is highly possible that the patient was infected with SFTSV through the sick cat's bite. If a patient gets sick in an SFTS-endemic region after a cat bite, SFTS should be considered in the differential diagnosis.

Keywords: severe fever with thrombocytopenia syndrome, cat, companion animals, viral hemorrhagic fever, pathology

1. Introduction

Severe fever with thrombocytopenia syndrome (SFTS) (formerly SFTS virus, SFTSV) is caused by the Dabie bandavirus, which belongs to the *Bandavirus* genus (formerly *Phlebovirus* genus) of the *Phenuiviridae* family (formerly *Bunyaviridae* family). SFTS was first discovered in China [1,2] and was then reported to be endemic to Japan [3], South Korea [4], Taiwan [5], and Vietnam [6]. Patients with SFTS are usually infected with SFTSV through a tick bite such as that from *Haemaphysalis longicornis* or *Amblyomma testudinarium* [7].

The case fatality rate of patients with SFTS in Japan is reported to be approximately 30% [8,9]. Notably, there is always a risk for people living in SFTS-endemic regions to suffer from SFTS, a fatal disease with a high fatality rate.

Recently, it has been reported that cats including cheetahs were infected with and sickened by SFTSV [10,11]. Furthermore, patients with SFTS who were infected by sick cats have also been reported [12].

A woman who died from multiorgan failure of unknown cause was retrospectively diagnosed as having SFTS following a pathological examination. A sick cat bit her on her left hand, and symptoms appeared 2 days later. The cat also died of multiorgan failure. In this study, the clinical and pathological characteristics of the patient with SFTS who was infected by a cat positive for SFTSV infection were examined.

2. Materials and Methods

2.1 Patient

A woman in her 50s became ill after she was bitten by a sick cat. She was retrospectively diagnosed as having SFTS as described below. The clinical course comprising her symptoms, laboratory findings including total blood cell (TBC) counts, serum chemistry, computed tomography imaging, and postmortem examination were retrospectively retrieved from her medical records.

2.2 Cat

The clinical course and laboratory findings of the cat that bit the reported patient were retrospectively retrieved from its medical records.

2.3 Measurement of SFTSV genome load with real-time RT-PCR in blood

To measure copy numbers of the SFTSV S segment in sera, quantitative one-step reverse-transcription polymerase chain reaction (RT-PCR) was performed as described previously [13].

2.4 Antibody detection with indirect immunofluorescence assay

Immunofluorescence assay using SFTSV-infected cells was performed to evaluate the presence of IgM and IgG to SFTSV as

described previously [14].

2.5 Measurement of SFTSV genome load with real-time RT-PCR in tissues

The SFTSV copy number was determined by a quantitative real-time RT-PCR on RNA samples extracted from paraffin-embedded sections (10 μm ; $\times 3$) as described previously with some modifications [3,16]. Briefly, RNA was extracted using a Pure Link FFPE RNA isolation kit (Invitrogen, Carlsbad, CA), and RT-PCR was performed using a QuantiTect Multiplex RT-PCR Kit (Qiagen, Hilden, Germany) and Agilent Mx3000P system (Agilent, Santa Clara, CA) according to the manufacturer's protocol. Quantitative real-time RT-PCR amplified the N region within the S segment of the SFTSV genome. The amount of human β -actin mRNA in the RNA extracted from each section was also determined and used as an internal reference for normalization. The relative copy number of SFTSV RNA was calculated using the β -actin mRNA copy number, estimated at 1500 copies/cell, as previously described [3]. The following primers and labeled probe were used to amplify the SFTSV-N region: Primers, forward (SFTS-F2: 5'-CCCTGATGCCTTGACGATCT-3') and reverse (SFTS-R2b: 5'-TGATTGGGTGAGGGACACAAAGTT-3'); probe 5'-(FAM) TTGCCTCGAGTCAGGGCAAAGACAA (BHQ1)-3'.

2.6 Pathological and immunohistochemical analyses

Histopathological studies of formalin-fixed and paraffin-embedded specimens were performed using hematoxylin-eosin staining. Immunohistochemical detection of the SFTSV nucleoprotein antigen (SFTSV-NP) was performed on paraffin-embedded sections, as previously described [3,15]. After deparaffinizing with xylene, sections were rehydrated in ethanol and immersed in PBS. Antigens were retrieved by hydrolytic autoclaving for 10 min at 121°C in 10 mmol/L sodium citrate-sodium chloride buffer (pH 6.0). After cooling, the sections were immersed in PBS. Endogenous peroxidase was blocked by incubation in 0.3% hydrogen peroxide in methanol for 30 min. After washing in PBS, the sections were incubated with normal goat serum for 5 min and then with rabbit polyclonal antibody against SFTSV-NP overnight at 4°C. After three washes in PBS, the sections were incubated with peroxidase-labeled polymer-conjugated anti-rabbit immunoglobulins (EnVision/HRP, Dako) for 30 min at room temperature. Peroxidase activity was detected by development with diaminobenzidine containing hydrogen peroxide. Nuclei were counterstained by hematoxylin.

2.7 Ethical statement

Serum samples were used for virological analysis after obtaining written informed consent from the responsible family members. All of the protocols and procedures were approved by the Research and Ethical Committees of the National Institute

of Infectious Diseases (No. 825).

3. Results

3.1 Patient presentation

A previously healthy woman in her 50s living in Yamaguchi prefecture in western Japan was bitten by an ill cat, which she cared for, in early summer 2016. The cat bit her on her left hand, and swelling occurred at the site of the bite. She became feverish on Day 2, with the day she was bitten considered Day 0. She showed symptoms of loss of appetite, fatigue, and vomiting on Day 3. She visited the Kanmon Medical Center and underwent a physical examination on Day 5. Her consciousness was clear. Her body temperature was 39.3°C, blood pressure was 122/48 mmHg, and pulse rate was 77 beats per min. The site of the cat bite did not show any abnormal lesions except for the bite-associated scars. The physician decided to treat her on an outpatient basis. Her laboratory findings on Day 5 are shown in Table 1. Most of the parameters were within a normal range including serum chemistry except for the presence of leukocytopenia and thrombocytopenia. Because her symptoms had worsened, she visited the hospital again on Day 8 and was hospitalized. Most of the laboratory findings at this time had become abnormal, suggesting that her physical condition had worsened further (Table 1). Her leukocytopenia had progressed with a white blood cell count of $0.5 \times 10^3/\mu\text{L}$ with 64% neutrophils,

32% lymphocytes, 1% monocytes, and 3% atypical cells. Liver-associated enzyme levels had also elevated. Chest computed tomography imaging revealed the presence of an enlarged lymph node in the left axially fossa and hepatic enlargement. A bone marrow aspiration test revealed the presence of hemophagocytosis.

Table 1

Sequential laboratory data of the patient with SFTS.

| Categories | Normal range | Day 5 | Day 8 | Day 9 | Day 11 |
|--|--------------|-------|-------|-------|--------|
| Total blood cell counts | | | | | |
| WBC ($\times 10^3$ cells/ μ L) | 3.3-8.6 | 1.9 | 0.5 | 0.5 | 3.1 |
| Platelet ($\times 10^3$ cells/ μ L) | 158-348 | 133 | 67 | 52 | 49 |
| RBC ($\times 10^6$ cells/ μ L) | 3.86-4.92 | 4.50 | 4.67 | 4.61 | 5.14 |
| Serum chemistry | | | | | |
| TP (g/dL) | 6.6-8.1 | 6.8 | 6.1 | 5.4 | 5.7 |
| ALB (g/dL) | 4.1-5.1 | 4.1 | 3.5 | 3.1 | 2.9 |
| TB (mg/dL) | 0.40-1.50 | 0.64 | 0.71 | 0.58 | 1.85 |
| AST (U/L) | 13-30 | 23 | 494 | 1210 | 3784 |
| ALT (U/L) | 7-23 | 14 | 169 | 376 | 961 |

| | | | | | |
|-----------------------|-------------|------|-------|-------|-------|
| LDH (U/L) | 124-222 | 187 | 940 | 1584 | 5021 |
| ALP (U/L) | 106-322 | 189 | 201 | 220 | 425 |
| γ -GTP (U/L) | 9-32 | 15 | 23 | 26 | 85 |
| BUN (mg/dL) | 8.0-20.0 | 14.3 | 17.6 | NT | 31.4 |
| CRE (mg/dL) | 0.46-0.79 | 0.57 | 0.58 | 0.52 | 0.90 |
| Na (mmol/L) | 138-145 | 133 | 134 | 133 | 130 |
| K (mmol/L) | 3.6-4.8 | 4.2 | 3.6 | 3.6 | 4.5 |
| Cl (mmol/L) | 101-108 | 96 | 93 | 96 | 94 |
| CRP (mg/dL) | 0.00-0.14 | 0.04 | 0.03 | 0.06 | 0.04 |
| PT (second) | 10.5-15.5 | NT | 14.2 | 14.4 | 15.0 |
| APTT (second) | 30.0-40.0 | NT | 65.3 | 76.7 | 82.1 |
| Fibrinogen (mg/dL) | 150.0-450.0 | NT | 229.0 | NT | 203.0 |
| D-dimer (μ g/mL) | 0.00-0.40 | NT | 11.51 | 12.88 | 9.07 |

Abbreviations: SFTS, severe fever with thrombocytopenia syndrome; WBC, white blood cells; RBC, red blood cells; TP, total protein; ALB, albumin; TB, total

bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; ALP, alkaline phosphatase; γ -GTP, gamma glutamyl transpeptidase; BUN, blood urea nitrogen CRE, creatinine; Na, sodium; K, potassium; Cl, chloride; CRP, C-reactive protein; PT, prothrombin; time; APTT, activated partial thrombin time; NT, not tested.

Administration of methylprednisolone was initiated as treatment for the hemophagocytosis. Her consciousness deteriorated on Day 9, and her creatinine level increased, indicating kidney dysfunction. She died of multiorgan failure on Day 12 despite intensive care including respiratory support via artificial mechanical ventilation and a blood purification procedure.

3.2 Cat presentation

The patient had found a cat near her house on Day -2 and noticed that it was sick. The cat seemed to have lost its appetite, was frequently vomiting, and its breathing did not seem to be regular. She took care of the cat and brought it to a veterinary hospital on Day -1. While taking care of the cat, it bit her on Day 0. The laboratory findings, TBC count, and serum chemistry of the cat are shown in Table 2. Severe leukocytopenia and thrombocytopenia were present. The data also indicated liver injury because of the abnormally high values of ALT and hyperbilirubinemia. The cat died of unknown causes.

Table 2

Total blood cell counts and serum chemistry of the sick cat.

| Categories | Normal range | Values |
|--|--------------|--------|
| Total blood cell counts | | |
| WBC ($\times 10^3$ cells/ μ L) | 2.07-17.02 | 0.87 |
| Platelet ($\times 10^3$ cells/ μ L) | 151-600 | 12 |
| RBC ($\times 10^6$ cells/ μ L) | 6.54-12.20 | 9.76 |
| Serum chemistry | | |
| TP (g/dL) | 5.2-8.2 | 7.7 |
| ALB (g/dL) | 2.2-3.9 | 2.6 |
| ALT (U/L) | 12-130 | 234 |
| ALP (U/L) | 14-192 | < 10 |
| TB (mg/dL) | 0-0.9 | 5.3 |
| BUN (mg/dL) | 13-33 | 25 |
| CRE (mg/dL) | 0.6-1.6 | 1.1 |
| Na (mmol/L) | 150-165 | 149 |
| K (mmol/L) | 3.7-5.9 | 3.1 |
| Cl (mmol/L) | 115-156 | 110 |
| Urinalysis | | |
| Protein | Negative | ++ |
| Hematuria | Negative | +++ |

Abbreviations: WBC, white blood cells; RBC, red blood cell; TP, total protein; ALB, albumin; ALT, alanine

aminotransferase; ALP, alkaline phosphatase; TB, total bilirubin; BUN, blood urea nitrogen CRE, creatinine; Na, sodium; K, potassium; Cl, chloride.

3.3 Dynamics of SFTSV loads and immunological responses in sera

The SFTSV copy number on Day 5 was 2.0×10^4 copies/mL and reached a maximum of 2.7×10^9 copies/mL on Day 11. No IgM and IgG antibodies reactive with SFTSV were detected in any sera collected during the course of the patient's hospitalization.

3.4 Pathological findings

Gross pathology

Gross examination on autopsy revealed mild bilateral pleural effusion (L 200 mL; R 100 mL), retention of ascites (600 mL), liver congestion, splenic congestion, and many enlarged lymph nodes (short-axis diameter of ~7 mm) throughout the mesentery, omentum, and para-aortic regions. The stomach contained 300 mL of blood clots, and gastric mucosa showed severe congestion in the absence of ulceration.

Histopathology and immunohistochemistry

Histopathological analyses showed infiltration of numerous atypical large lymphocytes in red pulp and periarteriolar sheaths, white pulp depletion with massive nuclear debris, necrotic debris, hemophagocytosis, and congestion in the spleen

(Fig. 1A). Spleen, liver, multiple lymph nodes, bone marrow, kidney, thyroid, adrenal gland, and lung were positive for SFTSV antigen in the immunohistochemistry (IHC) analysis. Numerous atypical lymphocytes positive for SFTSV antigens were present in the spleen (Fig. 1B). In liver, viral antigens were detected in the sinusoids amid a background of hemophagocytosis, centrilobular necrosis, hemorrhage, and mild lymphocytic inflammation around the portal tracts (Fig. 1C, D). The lymph nodes in the left subclavian regions showed focal necrotizing lymphadenitis with viral antigens positive within necrotic regions (Fig. 1E, F). IHC assays for *Bartonella henselae* were negative in the lymph nodes. Atypical large lymphoid cells with viral antigens were present in follicles of the mesentery and subcarinal lymph nodes (Fig. 1G, H). Prominent hemophagocytosis was also evident in these lymph nodes and bone marrow (Fig. 1I, J).

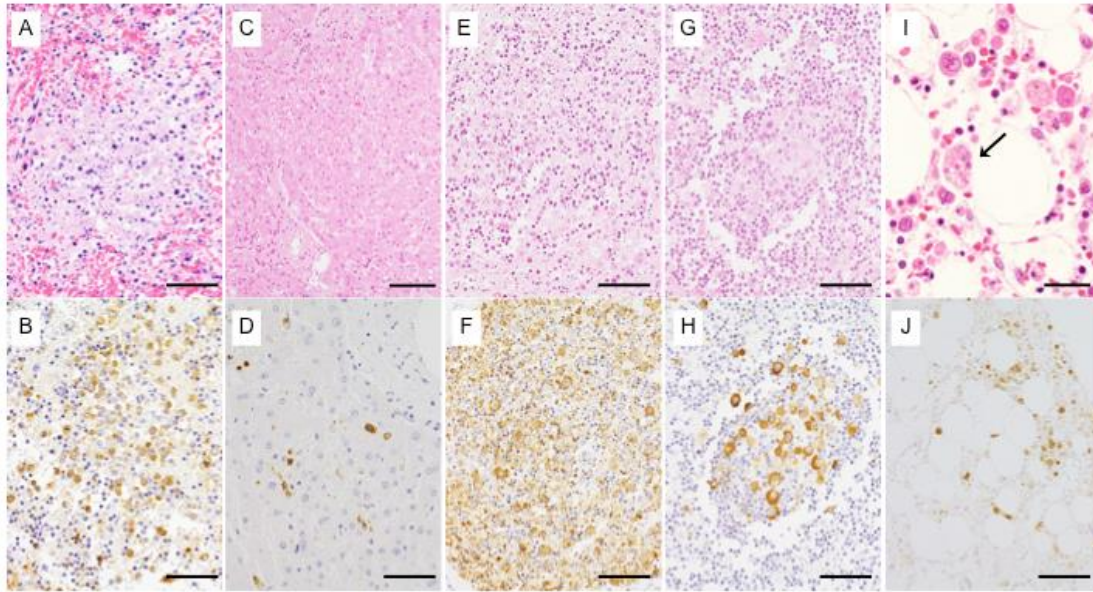


Fig. 1. Pathological findings of the patient with SFTS who was possibly infected with SFTSV from a cat also infected with SFTSV. Histopathological and immunohistochemical evaluation of spleen, liver, bone marrow, and lymph nodes. (A) Spleen: atypical lymphocyte infiltration and necrotic foci. (B) SFTSV antigen detected in the atypical large lymphocytes. (C) Liver: centrilobular necrosis and hemorrhage. (D) SFTSV antigen detected in Kupffer cells and atypical large lymphoid cells in sinusoids in liver. (E) Subclavian lymph nodes: focal necrotizing lymphadenitis. (F) SFTSV antigen detected in necrotic debris and large lymphocytes in necrotic foci. (G) Subcarinal lymph nodes: atypical large lymphocytes in follicles. (H) SFTSV antigen detected in the follicles of the subcarinal lymph nodes. (I) Bone marrow: prominent hemophagocytosis (arrow). (J) SFTSV antigen detected in atypical large lymphocytes. Routine hematoxylin and eosin stain: A, C, E, G, I. immunohistochemical staining with

anti-SFTSV NP-specific antibody: B, D, F, H, J. Scale bar
measurements: A, B, D-H, J, 60 μm ; C, 100 μm ; I, 20 μm .

SFTSV RNA measurement in organs

SFTSV-RNA was detected in all organs and tissues tested, and the numbers of SFTSV-RNA copies/cell calculated using the β -actin mRNA copy number are shown in Table 3. The level of SFTSV genome copies was consistent with the IHC results.

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Table 3

Distribution of SFTS viral NP antigen and SFTS viral RNA in formalin-fixed and paraffin-embedded tissue sections from the autopsied patient.

| Tissue section | Measurement of SFTSV genome with quantitative real-time reverse transcription PCR assay | | | IHC (SFTSV-NP) |
|----------------|---|-------------------------------------|--------------------|-------------------|
| | SFTSV RNA (copies/reaction) | β -actin (copies/reaction) | Copies/cell* | |
| Spleen | 4.73×10^4 | 7.96×10^3 | 8.91×10^3 | ++++ |
| Liver | 2.53×10^3 | 5.81×10^3 | 6.53×10^2 | ++ |
| Adrenal gland | 6.08×10^1 | 1.98×10^3 | 4.62×10^1 | ++ |
| Bone marrow | 1.23×10^0 | 4.36×10^1 | 4.24×10^1 | ++ |

| | | | | |
|----------------------------|--------------------|--------------------|--------------------|----|
| Left subclavian lymph node | 2.08×10^2 | 8.83×10^3 | 3.54×10^1 | ++ |
| Lung | 9.52×10^1 | 5.85×10^3 | 2.44×10^1 | + |
| Thyroid | 4.02×10^1 | 4.80×10^3 | 1.26×10^1 | + |
| Mesenteric lymph node | 3.88×10^1 | 4.99×10^3 | 1.17×10^1 | ++ |
| Kidney | 4.81×10^1 | 8.51×10^3 | 8.48×10^1 | + |
| Subcarinal lymph node | 1.90×10^1 | 6.19×10^3 | 4.61×10^1 | ++ |

Abbreviations: SFTS, severe fever with thrombocytopenia syndrome; NP, nucleoprotein antigen; SFTSV, SFTS virus; IHC: immunohistochemistry.

*(Copies/cell): $\text{copy/cell} = \text{SFTSV}/\beta\text{-actin} \times 1500$.

4. Discussion

In the present patient, findings associated with hemophagocytosis were present in bone marrow and lymph nodes. Necrotizing lymphadenitis in lymph nodes and hemophagocytosis in bone marrow are the relatively specific and common findings in the pathology of SFTS patients [16]. The presence of necrotizing lymphadenitis and hemophagocytosis in bone marrow are reported to be positive in fatal patients with SFTS [3,15-20]. Because the presence of these factors was observed in the patient, her pathological materials were further examined for SFTSV infection.

The data in Table 3 indicate that SFTSV replicated predominantly in the spleen in this patient. SFTSV antigen was detected with IHC analyses in regional lymph nodes such as the subclavian and subcarinal lymph nodes, indicating that SFTSV replicates dominantly in the regional lymph nodes (Fig. 1E, H). It was unclear whether SFTSV replicated in the non-regional lymph nodes of the patient because these lymph nodes were not tested for SFTSV antigens in the IHC analyses. Similar pathological findings have already been reported in another SFTS patient, in whom regional lymph nodes were enlarged and SFTSV was detected in these nodes but not in the non-regional lymph nodes [3]. SFTSV antigen was detected in all organs tested in the present patient. Other reports also noted that SFTSV

antigens were positive in most of the organs tested as shown in the present patient [17,18]. There were no pathological characteristics specific to our patient. All of her pathological features were similar to those reported so far [3,15-20].

However, the incubation time in the present patient was only 2 days. It is possible that the incubation time might be shorter in a patient with SFTS if infected through the bite of an SFTSV-infected cat. Whether the morbidity and mortality of patients with SFTS who are infected through the bite of an SFTSV-infected cat are different from those of patients infected through a tick bite should be addressed in further studies.

The clinical signs and laboratory findings of SFTSV-infected cats were characterized in 24 cats with confirmed SFTSV infection in western Japan [10]. All cats showed clinical signs of anorexia and lethargy, with fever and vomiting in 68% and 42%, respectively, and the case fatality rate was 63%. Laboratory characteristics included a TBC count indicating thrombocytopenia and leukocytopenia and elevation of serum total bilirubin, amyloid A, and CPK. Another study summarizing the cases of 3 sick cats infected with SFTSV, showed that fever and loss of appetite were the typical signs, and the TBC count revealed the presence of thrombocytopenia and leukocytopenia

[12].

The clinical signs and laboratory findings of the cat who bit the present patient were similar to those previously reported. Therefore, it is highly possible that this patient was infected with SFTSV from the sick cat, although the cat was not specifically diagnosed as having SFTSV infection virologically.

A male veterinarian in western Japan was also infected with SFTSV by a sick cat [12]. The patient had taken care of sick mammals, including three cats suspected of having SFTSV infection. A partial nucleotide sequence of the SFTSV genome amplified with RT-PCR from a blood sample of the patient was identical to that detected in the cats. According to the report, the partial SFTSV genome sequence detected in the three cats was identical to each other.

The present patient suffered from SFTS-like disease 2 days after being bitten by a sick cat. However, SFTS was not included in the differential diagnosis because it has not been well noticed that sick cats could be a vector for the transmission of SFTSV to humans. The pathological examination following the patient's autopsy made it possible to diagnose her retrospectively as having SFTS. The first case of a patient with SFTS diagnosed in Japan was autopsied to determine the cause of death [3]. The patient was diagnosed as having SFTS

retrospectively by isolation of SFTSV from blood samples. Pathological examinations including IHC for SFTSV infections were performed to clarify the characteristics of SFTSV infections in humans, indicating that the presence of necrotizing lymphadenitis in regional lymph nodes and hemophagocytosis in bone marrow were characteristic to SFTS [3]. The present study confirms the importance of performing autopsy on patients dying of unknown causes. Domestic cats live with humans, and humans have close contact with their cats. Some cat owners let their domestic cats outdoors, which can result in an increased risk of the cats being infected with SFTSV through a tick bite.

The present study revealed that there is a risk of being infected with SFTSV not only through tick bites but also through close contact with or being bitten by a sick cat infected with SFTSV. If a patient gets sick in an SFTS-endemic region after a cat bite, SFTS should be considered in the differential diagnosis. Further, cat owners and veterinary personnel in SFTS-endemic regions should be advised to treat sick cats with careful infection control measures and not to be bitten by or have direct and close contact with cats sick due to unknown causes.

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