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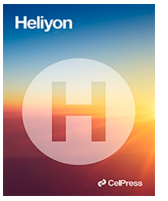

Prompt diagnosis and appropriate treatment of Japanese spotted fever: A report of three cases

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ABSTRACT

Background: Japanese Spotted Fever (JSF) is a Spotted Fever Group (SFG) rickettsiosis caused by *Rickettsia japonica*. More than 300 cases are diagnosed annually in Japan, and the number of reported cases has been increasing. Correct diagnoses depend on the triad of symptoms and signs, including fever, rash, and eschar, which can be seen at the site of vector bites. JSF is not life-threatening if treated appropriately without diagnostic delay but there are some fatal cases every year. This negligence leads to disseminated intravascular coagulation (DIC) and multiple organ failure (MOF), and poor prognoses, consequently. Prompt diagnosis of JSF is difficult when the aforementioned triad of signs and symptoms is not initially present.

Case report: This report describes three JSF cases: an 87-year-old woman with fever, shock, pancytopenia, DIC, and MOF; a 79-year-old man with fever and difficulty in movement; and a 78-year-old man with fever, general fatigue, and appetite loss. All patients had a rash and eschar, which led to prompt diagnosis and appropriate treatment immediately. All patients were treated without any complications.

Why should an emergency physician be aware of this?: As mentioned above, JFS can be fatal with delayed diagnoses and treatment initiations. The key for a prompt diagnosis is to recognize the triad of symptoms and signs, which are not often present initially, and it makes JSF diagnosis challenging. Repeated comprehensive physical examinations are essential for prompt diagnosis and improve prognosis of JSF.

1. Introduction

Spotted fever group (SFG) rickettsioses (spotted fevers) are a group of diseases that are caused by rickettsial infections, spread through the bite of infected mites and ticks [1], and have a worldwide distribution [2]. Rickettsial diseases are very common globally; they have been reported to be the second most common cause of non-malarial febrile illness in the Southeast Asia region after dengue infection [3,4]. Recent environmental changes, including climate change and globalization, have contributed to the expansion and invasion of ticks into new geographical areas, where spotted fevers can be diagnosed [5–8]. The advancement in molecular genetics in the past decades has improved more identification and characterization for several novel SFG rickettsiae and their association with tick

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reservoirs and numerous tick-borne rickettsioses throughout the world [5]; however nonspecific clinical symptoms of rickettsial infections and limited access to molecular diagnostic tools in reference laboratories prohibit the prompt diagnoses and treatments [5]. The actual incidence of tick-borne rickettsioses is predicted to be much higher, and the case fatality rate of SFG rickettsioses remains high in many parts of the world [5,9–11].

Japanese Spotted Fever (JSF) is an SFG rickettsiosis caused by *Rickettsia japonica*, first diagnosed in Japan in 1984 [12]. The number of annual total cases of JSF was between 30 and 60 before 2006, but it has been increasing and has been constantly over 300 since 2017 [12]. JSF infections have a pattern in their geographical distributions; the area used to be in the western to southern part of Japan, but has been spreading out, and it is now also covering the northern area of Japan [13]. Patients are mainly infected during their activities outside [14], and the incubation period is 2–8 days [13–15]. The triad of the signs and symptoms of JSF are fever, rash, and eschar at the site of vector (arthropod) bites [16], but this triad is not always recognized clearly [15]. Although the triad is not always observed, the triad is a very important clue for prompt and right diagnoses of JSF, and delayed diagnoses are caused by failure of reaching thoughts of JFS as the diagnoses due to incomplete triad. The major diagnostic methods are serological tests and Polymerase Chain Reaction (PCR) tests, and the former of which is the most commonly used method for diagnosis [13–16]. The mortality rate of JFS is not high [17] when diagnosed promptly and initiated treatment immediately, same as other SFG, except for Rockey Mountain spotted fever (RMSF) [8,18]. However, JSF can be life-threatening due to disseminated intravascular coagulation (DIC) and multiple organ failure (MOF) subsequently, due to the delay in the initiation of adequate treatment [13,19,20], same as other rickettsioses [8,21,22]. In severe SFG rickettsial cases, organ failures, such as pulmonary involvement, pre-renal azotemia, neurological involvement, can be the cause of fatality [8]. Tetracycline is very effective for JSF.

This is a case series in which JSF was successfully diagnosed and treated without complications.

Informed consents were obtained from patients for the publication of all images, clinical data and other data in this paper.

2. Case Reports

2.1. Case 1

An 87-year-old woman with a medical history of aortic valve stenosis, for which she had undergone a transcatheter aortic valve implantation 9 months prior, acute coronary syndrome, complete atrioventricular block, hypertension, and dyslipidemia visited a clinic with the chief complaint of a 1-day history of fever. She was so active that she went to her garden to grow some vegetables. She lived in Oda, Shimane prefecture, Japan. Covid-19 antigen test was negative and the patient was transferred from primary clinic to the nearest general hospital due to dehydration. As her condition was complicated with an underdiagnoses of shock and DIC, she was transferred to our hospital for further investigations and treatments on the same day (day 0). In the Emergency Room (ER), she was distressed with a mild loss of consciousness (Glasgow Coma Scale 13: E3V4M6). Her blood pressure was 108/50 mmHg, with continuous noradrenaline administration, heart rate was 80/min, respiratory rate was 17/min, SpO2 was 97 % (room air), and body temperature was 37.8 °C (100 °F). There were petechial rashes on the trunk, arms, legs, palms, and soles, and was an eschar on her left arm (Fig. 1), which was not recognized in the previous hospital. Laboratory studies revealed pancytopenia, DIC, elevated liver enzymes, elevated creatine kinase, renal impairment, hyponatremia, and elevated inflammatory markers (Table 1). The DIC score was 9 points, according to DIC diagnostic criteria established by the Japanese Association for Acute Medicine (JAAM DIC diagnostic criteria). There were no remarkable abnormalities in either the imaging tests or the electrocardiogram (ECG). Based on her symptoms and laboratory data, JSF was suspected, and minocycline (200mg/day, intravenously) administration was initiated. She was admitted to the critical care ward, noradrenaline was continued, and thrombomodulin and human anti-thrombin III were initiated. After admission, the shock status and hyperthermia persisted. On day 3, tachycardia with atrial fibrillation worsened her hypotension and catecholamine demand increased so that landiolol was initiated. On day 4, hydrocortisone and vasopressin were initiated owing to

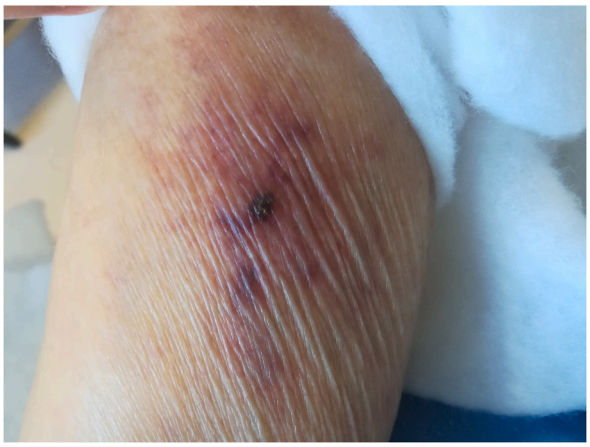

Fig. 1. There was an eschar on her left arm.

Table 1
Clinical characteristics and laboratory data on admission of patients with JSF.

		case 1	case 2	case 3
Symptoms				
Fever (°C)		37.8	35.5	37.3
Rash		+	+	+
Eschar		+	+	+
Headache		+	–	+
Laboratory data	normal range			
Leukocytes (/μL)	3.30–8.60	2.43	12.49	3.83
Hemoglobin (g/dL)	11.6–14.8	8.8	17.4	12.1
Platelet (x10^3/μL)	158–348	36	253	90
PT-INR	0.9–1.10	1.22		1.33
APTT (sec)	25.0–39.0	57.4		43.6
Fibrinogen (mg/dL)	200–400	93		
FDP (μg/mL)	≦ 5.0	207		9.2
D dimer (μg/dL)	≦ 1.0	57.2		
AST (IU/L)	13–30	120	30	73
ALT (IU/L)	7–23	58	25	54
LDH (IU/L)	124–222	711	295	420
ALP (IU/L)	38–113		121	80
γGTP (IU/L)	13–64		88	26
T.Bil (IU/L)	0.4–1.5	0.7	0.4	0.8
CK (mg/dL)	41–153	465	26	332
BUN (mg/dL)	8.0–20.0	44.8	16.9	20.9
Cre (mg/dL)	0.46–0.79	2.08	0.89	1.24
Na (Eq/L)	138–145	124	133	130
K (Eq/L)	3.6–4.8	3.8	4.4	3.7
Cl (Eq/L)	101–108	93	97	98
CRP (mg/dL)	<0.14	11.11	5.33	19.29
Urine protein	–	1+		
Urine blood	–	2+		

*"+" means that the signs are positive.
**We used the fully automatic urine analyzer with "Uro Paper αiii" as the test filter paper, from Eiken Chemical Co.,LTD.(https://www.eiken.co.jp/en/). Concerning Urine protein, "±" means ~15 mg/dL, "1+" means ~30 mg/dL, "2+" means ~100 mg/dL, "3+" means ~300 mg/dL, and "4+" means ~1000 mg/dL. Concerning Urine blood, "±" means 1–10 cells/high power field(HPF), "1+" mans 11–20 cells/HPF, "2+" means 21–50 cells/HPF, "3+" mans 51–250 cells/HPF.

delayed shock status, which continued until day 8. Due to her low cardiac function and infusion for shock treatment, she required high-flow nasal cannula oxygen therapy from day 4 to day 12. Receiving a PCR test result for *R. japonica*, which was positive for blood samples on day 4 (Table 2). Piperacillin/tazobactam, which was initiated immediately after admission for coverage of bacteriemia by other organisms, was stopped and minocycline was continued for 14 days. Serological test results were also consistent with JSF (Table 3). Her status improved beyond thrombocytopenia, which became not life-threatening, and she was transferred to another general hospital for continuous rehabilitation on day 21.

2.2. Case 2

A 79-year-old man with a medical history of diabetes mellitus and dyslipidemia, with no recent drug or supplement initiation, visited the ER with the chief complaint of a 1-day history of fever and difficulty of movement. He lived in Izumo, Shimane prefecture, Japan. He often visited bamboo grove near his house. The vital signs were normal, including body temperature (35.5 °C, 95.9 °F), and no abnormality except rashes in his trunk and extremities. A careful physical examination revealed an eschar on his right arm (Fig. 2). Laboratory data revealed elevated white blood cell counts, biliary enzymes, and inflammatory markers (Table 1). Imaging examination

Table 2
PCR tests results of patients with JSF.

		PCR[a]		
	material	*Orientia tsutsugamushi*	*Rickettsia japonica*	*Huaiyangshan banyangvirus*
case 1	blood	negative	positive	negative
case 2	blood	negative	judgement pending[b]	negative
	eschar	negative	positive	negative
case 3	blood	negative	positive	negative
	eschar	negative	positive	negative

[a] A duplex real-time Polymerase Chain Reaction (PCR) assay targeting the 16S ribosomal DNA, for the assessment of *R. japonica* and *Orientia tsutsugamushi* infection was conducted.
[b] PCR tests consist of two-times experiments. "Judgement pending" means that one experiment was positive but the other was negative.

Table 3
Indirect immunofluorescence assay (IFA) tests results of patients with JSF.

IFA (Indirect immunofluorescence assay)*				
	IgG		IgM	
	on admission	2 wks later	on admission	2 wks later
case 1	<1:20	1:320	<1:20	≧1:640
case 2	<1:20	≧1:640	<1:20	1:320
case 3	<1:20	≧1:640	<1:20	≧1:640

Indirect immunofluorescence assays (IFAs), employing *R. japonica* (strain YH)-infected cells as antigens, were conducted utilizing patient sera.

revealed no signs of cholecystitis. With a suspicion of JSF due to a history of visiting a bamboo grove 3 days prior to the fever, symptoms including eschar, and other examination data, he was admitted to the ward, and minocycline (200mg/day, intravenously) was initiated. After admission, there were no worsening symptoms, including fever. The PCR test result for *R. japonica* with a blood sample was "judgement pending" but one with an eschar sample was positive (Table 2). He was discharged on day 7 in good general condition. The treatment with minocycline was continued for 10 days, converting to oral administration from day 7. Serological test results were consistent with the diagnosis (Table 3).

2.3. Case 3

A 78-year-old man with a medical history of diabetes mellitus, hypertension, dyslipidemia, a pacemaker implantation, and coronary spasm angina, visited the ER with chief complaints of intermittent fever, general fatigue, and appetite loss. Two days prior to this visit, he visited the ER with 2-day-history of posterior neck pain and fever. As the vital signs and physical examination findings were normal, and laboratory data showed elevated *C*-Reactive Protein (CRP) (8 mg/dL) and hyponatremia (133 Eq/L), celecoxib was prescribed for a suspected diagnosis of Crowned dens syndrome. He visited the ER as the symptoms did not improve in 2-day interval. He occasionally went to his garden to grow up some vegetables. He lived in Izumo, Shimane prefecture, Japan. At the ER, he was distressed with general fatigue, but there was no disturbance of consciousness (Glasgow Coma Scale score 15). Blood pressure was 96/48 mmHg, heart rate was 80 beats/min, respiratory rate was 24/min, SpO2 was 92 % (room air), and body temperature was 37.3 °C (99.1 °F). There were rashes on his face, trunk, and extremities and an eschar on his left leg (Fig. 3). He remembered being bitten by an

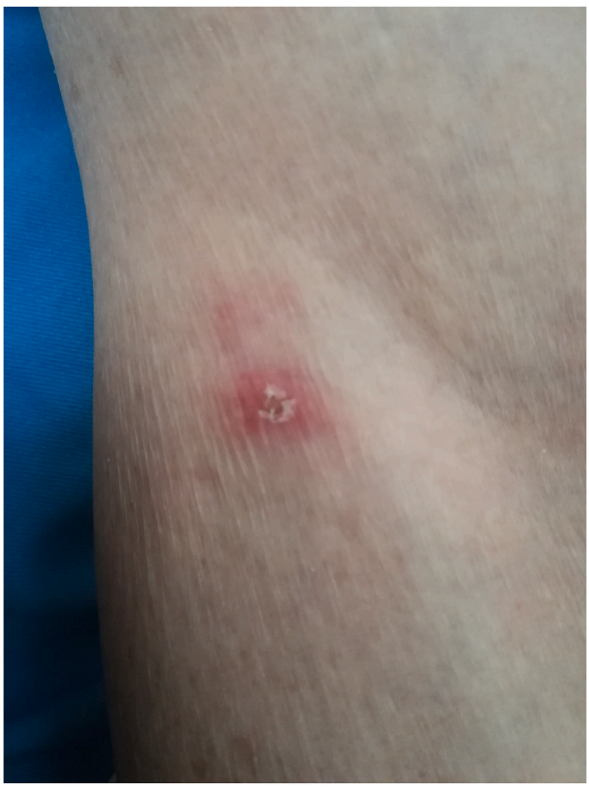

Fig. 2. There was an eschar on his right arm.

insect 8 days prior. Laboratory studies revealed thrombocytopenia, elevated liver enzymes, elevated creatine kinase, mild renal impairment, hyponatremia, and elevated inflammatory markers (Table 1). There were no remarkable abnormalities both in the imaging tests and ECG. Because JSF was suspected based on his symptoms and laboratory data, minocycline (200mg/day, intravenously) was initiated. After admission, noradrenaline was needed for 3 days, and there were two episodes of bloody diarrhea with elevated FDP; however, these symptoms were controlled with no pharmaceutical treatment. The platelet count reached the normal range on day 5, and the CRP levels peaked out on day 6. The PCR test result with both a blood sample and an eschar sample for *R. japonica* were positive (Table 2). The patient was discharged on day 13 in good general condition. Minocycline treatment was continued for 14 days, converting to oral administration from day 8. Serological tests also indicated the presence of JSF (Table 3).

2.4. Detailed methods for diagnosis

In the cases of this article, we used the eschar samples, which were peeled off with sterilized forceps, and blood samples collected intravenously. We made formal requests to Shimane Prefectural Institute of Public Health and Environmental Science for the following molecular biological and immunological tests. A duplex real-time Polymerase Chain Reaction (PCR) assay targeting the 16S ribosomal DNA, as developed by Kawamori et al. [23], for the assessment of *R. japonica* and *Orientia tsutsugamushi* infection was conducted. The presence or absence of infection was determined by the amplification curve and the quantification cycle (Cq).

Primer Sequence (5′ to 3′)
OR-F GGAGCATGCGGTTTAATTCG.
OR-R GCCATGCAACACCTGTGTGT.
Probe Sequence (5′ to 3′)
Rj (*R. japonica*)-VIC VIC-CGGATCGCAGAGATG-MGB.
Ot (*Orientia tsutsugamushi*)-FAM FAM-AATGGAGACATTTTTCTTC-MGB.

The acute serum samples obtained upon admission and the convalescent serum samples collected within 14-days post-admission were examined. Indirect immunofluorescence assays (IFAs), employing *R. japonica* (strain YH)-infected cells as antigens, were conducted utilizing patient sera, as described previously [23]. The serum samples were deemed positive when there was a fourfold or greater elevation in antibody titers between the paired sera, indicative of seroconversion.

3. Discussion

JSF is not a life-threatening disease if treated appropriately without diagnostic delay [20]; however, some fatal cases are reported

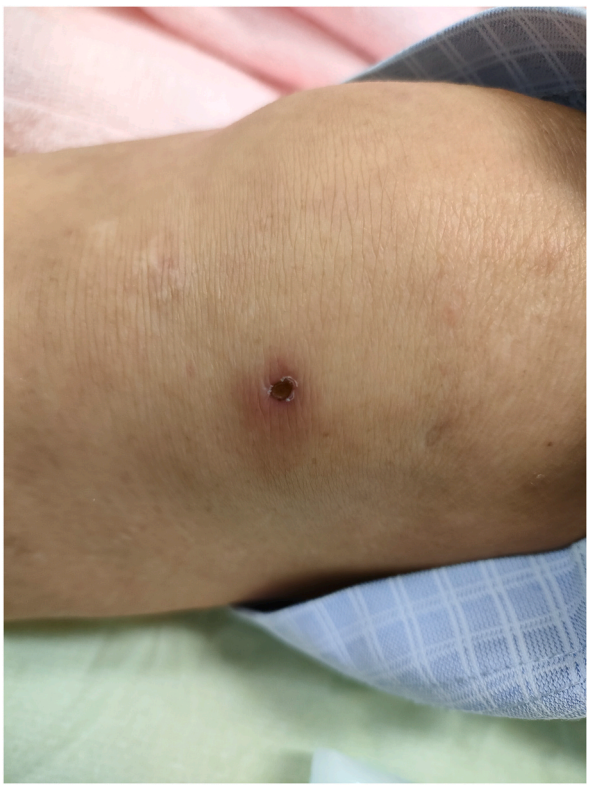

Fig. 3. There was an eschar on his left knee.

[20,24,25], and delayed treatment initiation is one of the factors in fatal cases [20,26,27]. Kodama et al. [27] reported that cases with DIC or organopathy such as respiratory failure and disturbance of consciousness tended that it took more than 6 days before therapy initiated [20,28]. It makes considerably difficult to make a prompt diagnosis without the triad of signs and symptoms, which are often not all present initially, due to the time difference between the emergence of fever and rash. As a single physical examination cannot always reveal them, multiple careful, encompassing physical examinations, from the top of the head to the tiptoes, which will reveal the eschar, can lead to prompt diagnoses of JSF. In Case 1, a prior examination did not reveal a rash or eschar, which led to a delayed treatment initiation. In Case 3, the patient had a fever in advance of the rash, which made it difficult to diagnose JSF at the first visit to the ER. As three of them were from Shimane prefecture and they had a history of regular activities outside, considering the geographical and seasonal distributions of JSF, patients' behavioral histories, such as activities in mountainous areas or grassy places, and travel histories [8], are also very important for prompt diagnoses and appropriate treatment initiations. Fever is very important as clinical features of JSF. According to a report of 31 JSF, 100 % of patients had fever [2]. In all three cases in this article, however, high fever was not observed. According to a 55-JSF-patients' report, 94.5 % had fever (≧39 °C; 38.2 %, ≧38 °C; 90.9 %, ≧37.5 °C; 94.5 %) [25]. JFS patients are not necessarily febrile, and it might be related to the severity of the disease. Other symptoms, such as headache, shaking chills, malaise, are useful for early suspicion of JSF [2,25]. An elevation of CRP, leukocytosis or leukopenia, thrombocytopenia, and hepatic dysfunction are commonly seen among JSF patients [15,19,20]; however, these are not specific findings. Hyponatremia, which was recognized in all 3 cases, could be an indicator of JSF [29,30], although hyponatremia is also a nonspecific finding. A slight positive reading for protein and occult blood in urinalysis may lead to a misdiagnosis of urinary tract infection [2]. Not only JSF, SFG are often underdiagnosed as SFG are obligate intercellular bacteria whose culture in vitro is complicated, long, expensive and often reserved to specialized laboratories equipped with BSL3 level containment facilities [3,31]. Serological tests and PCR tests are key examinations for the right diagnosis; the former is the primary method to detect SFG [32]. Serological tests provide important information regarding the distribution of SFG in community- or hospital-based settings [3]; however it takes at least 2 weeks before the exam the test results are reliable as we have to wait for the adequate antibodies production. The choice of diagnostic cut off has may greatly influence the serological results [3,33]. PCR tests are very useful especially for the early detection of infection before the development of detectable antibodies [3], which are the target for serological tests. The merit of PCR tests is their accurate STG identification, possibilities of discovery of novel species, its affordability and reproducibility, less time-consumption with high specificity and sensitivity especially in the early phase of infection [3,34]. PCR tests may also detect the transient nature of these pathogens present in the circulating samples [3,35]; however, PCR tests requires expertise and techniques, which have limitations as false-negative results [3]. The positivity rate for eschar PCR result is higher than that for blood PCR [25], and this is consistent to the result of PCR tests with *R. rickettsii*, which is the cause of RMSF, which belongs to the spotted fever group as JSF. Eschars are suitable sample type for PCR, compared to serum or plasma, because these samples are less than optimal due to fewer infected cells in the materials [3]. Repeated comprehensive physical examinations could lead to prompt diagnosis and treatment, but new diagnostic modalities, such as a diagnostic scoring system or a rapid diagnostic examination kit, are needed. Minocycline should be considered immediately when JSF is suspected [8,20], even if only slightly, or before the definite diagnoses, as it gets severe quickly without appropriate treatment [20]. Adding to it, Tetracycline antibiotics are a considerably safe drug with no serious side effects and only contraindicated for children [36].

There are several limitations in this article. First, this article has only three cases so that it is difficult to generalize diagnosis and treatment of JSF according to those results. Second, all the case were observed in limited rural area so that there might be geographical bias. Third, all the patients in the cases were relatively old so that it is possible that the prognoses might be different from those among young people.

4. 5.Why should an emergency physician be aware of this?

As JSF could be life-threatening if treated appropriately and without diagnostic delay, it is essential to improve the physicians' diagnostic techniques and wide recognition that JSF has a fatal risk without prompt and appropriate treatments [20]. Recognition of clinical symptoms and knowledge of the epidemiology, detailed history taking regarding exposure to potential vectors are the key to right diagnoses, as it is sometimes very difficult to recognize the bites from these arthropods [8]. Repeated comprehensive physical examinations, which are considered less serious these days, are, of course, one of the most important keys for prompt diagnoses and better prognoses of JFS. Considering the increasing number of cases and expanding area recently, there should be more chances for physicians to encounter JSF cases, even outside of Japan. There is a case series of JSF from China, which includes one fatal case due to a delay in diagnosis [37]. This article is promisingly available for prompt diagnoses and better prognoses of JFS all over the world.

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Data availability statement

The dataset is not registered in any publicly available repository as this is a case series and it is not thought to be favorable to disclose the detailed data of each patient. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

Rie Sato: Writing - review & editing, Writing - original draft, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Noriaki Yamada:** Supervision, Methodology, Data curation, Conceptualization. **Nobuhiro Kodani:** Data curation, Conceptualization. **Tetsuya Makiishi:** Data curation, Conceptualization. **Yoshiaki Iwashita:** Writing - review & editing, Supervision, Methodology, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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