Histopathological diagnosis of Japanese spotted fever

Japanese spotted fever (JSF) is caused by infection of *Rickettsia japonica* via larval tick bite, and lethal cases are reported yearly in southwest Japan. The methods of diagnosing JSF by immunohistochemistry (IHC) and real-time PCR (RT-PCR) using formalin-fixed, paraffin-embedded skin biopsy specimens have been established. Two mouse monoclonal antibodies (S3 and X1) are used for IHC, and the 17k genus common antigen gene serves as the target of RT-PCR. IHC of skin biopsy specimens shows coarse dots in the cytoplasm of endothelial cells and macrophages. For IHC, the eschar is the most suitable target, while scabs are often insufficient. The diagnosis of JSF can be made by IHC and/or RT-PCR earlier than serology.

Ref.-1: Mahara F. Japanese spotted fever: report of 31 cases and review of the literature. Emerg Infect Dis 1997; 3(2): 105-111. doi: 10.3201/eid0302.970203

Ref.-2: Tamakuma K, et al. Histopathological diagnosis of Japanese spotted fever using formalin-fixed, paraffin-embedded skin biopsy specimens. Usefulness of immunohistochemistry and real-time PCR analysis. Clin Microbiol Infect 2012; 18(3): 260-267. doi: 10.1111/j.1469-0691.2011.03569.x

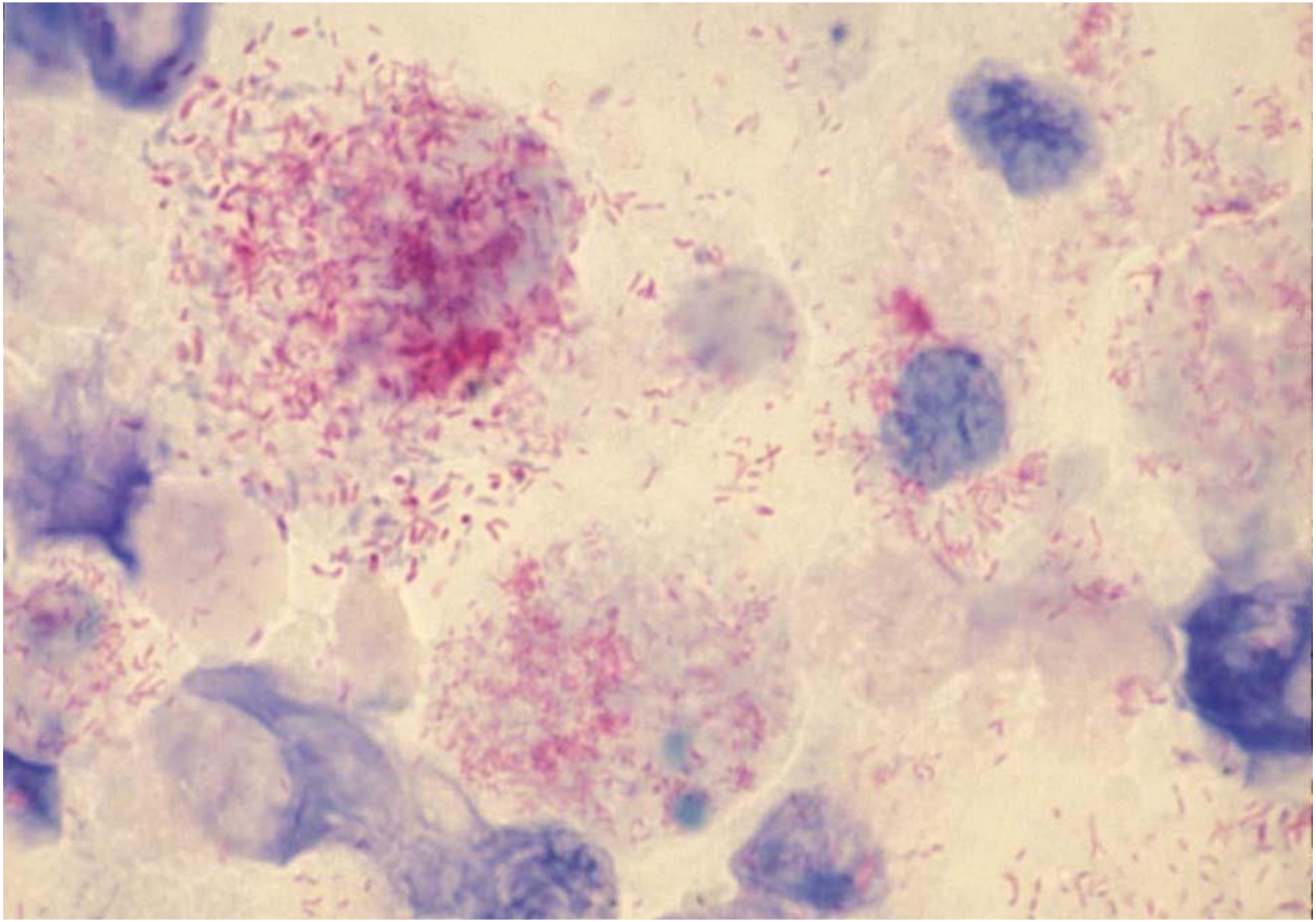
Weil-Felix reaction in rickettsiosis

Disease	OX19	OX2	OXK
Epidemic typhus	++++	+	-
Murine typhus	++++	+	-
Tsutsugamushi disease	-	-	+++
Spotted fever group	+	++++	—
	++++	+	—
Q fever	-	-	-

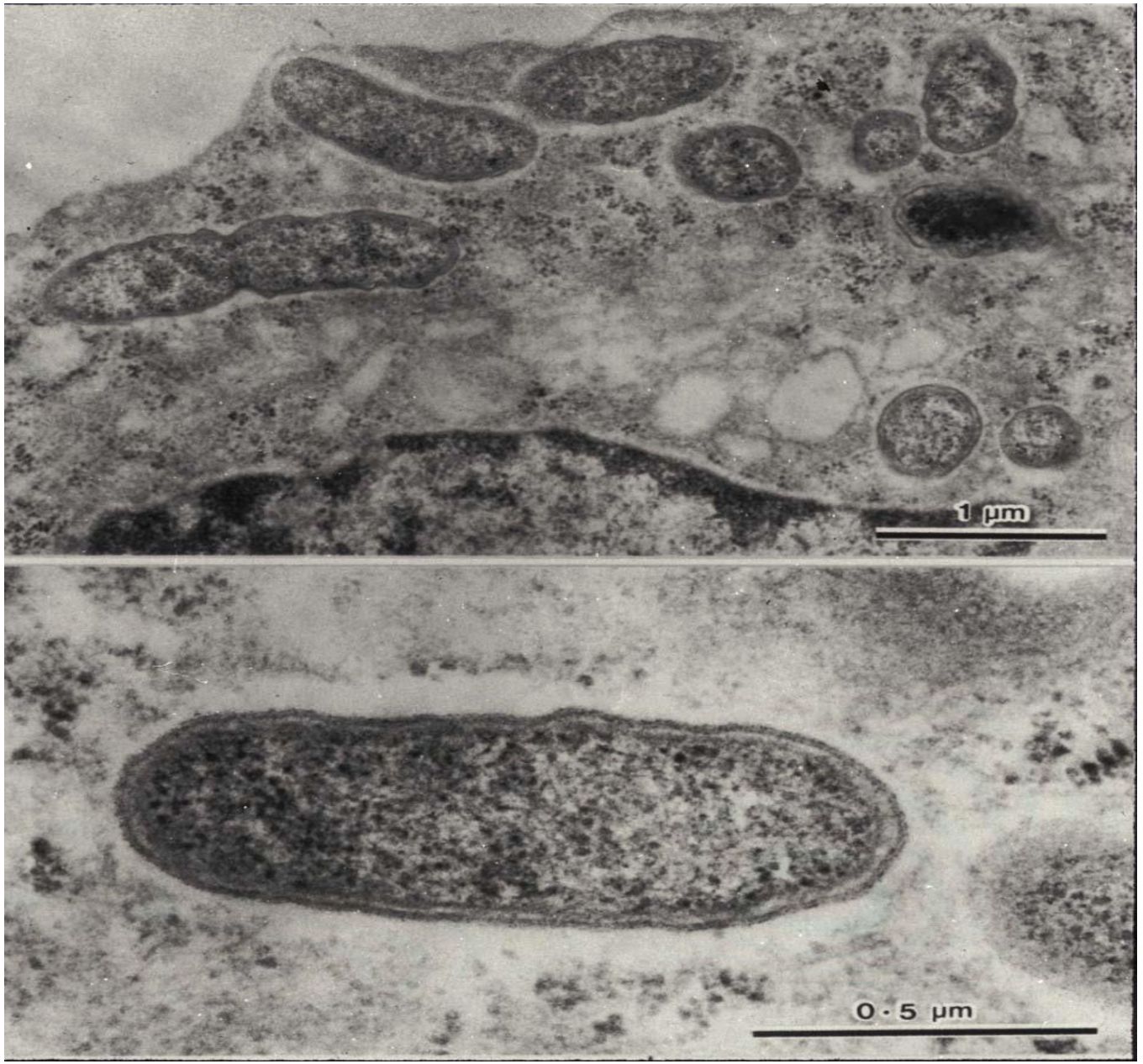
Case: a 63 year-old female patient

Weil-Felix reaction	Results	Unit	Normal range
OXK antigen	<10	dilution	<20
OX19 antigen	<10	dilution	<80
OX2 antigen	×640	dilution	~

Larval ticks: *Haemaphysalis flava*



Rickettsia japonica infecting macrophages (Gimenez staining)



Ultrastructure of *Rickettsia Japonica*

Clinical features of Japanese spotted fever

1) Japanese spotted fever (JSF) is seen along the southern coast of Japan in the spring through late autumn.

2) Clinical features of JSF resemble those of Tsutsugamushi disease (a traditional and endemic mite-mediated infection throughout Japan (Tsutsuga means a disease, mushi means a worm), including high fever, eschar (the site of tick bite) and skin rash. JSF may be life-threatening. Combination of tetracycline and new quinolone is effective.

3) SJF is transmitted by a variety of larval ticks, including *Haemaphysalis flava.*

Immunohistochemical detection of *R. japonica* antigens

Cases: Representative 4 cases of serologically confirmed JSF

Skin biopsy: Formalin-fixed, paraffin-embedded sections sampled from the eschar and rash

Immunoperoxidase staining: Amino acid polymer method

Mouse Mab: S3 and X1 (culture medium), diluted at 1:10

*Both Mabs cross-react with rickettsiae of the spotted fever group, but NOT react with *R. typhi and Orientia tsutsugamushi.*

Pretreatment for immunostaining: Pressure cooking in 10 mM citrate buffer, pH 7, for 10 minutes

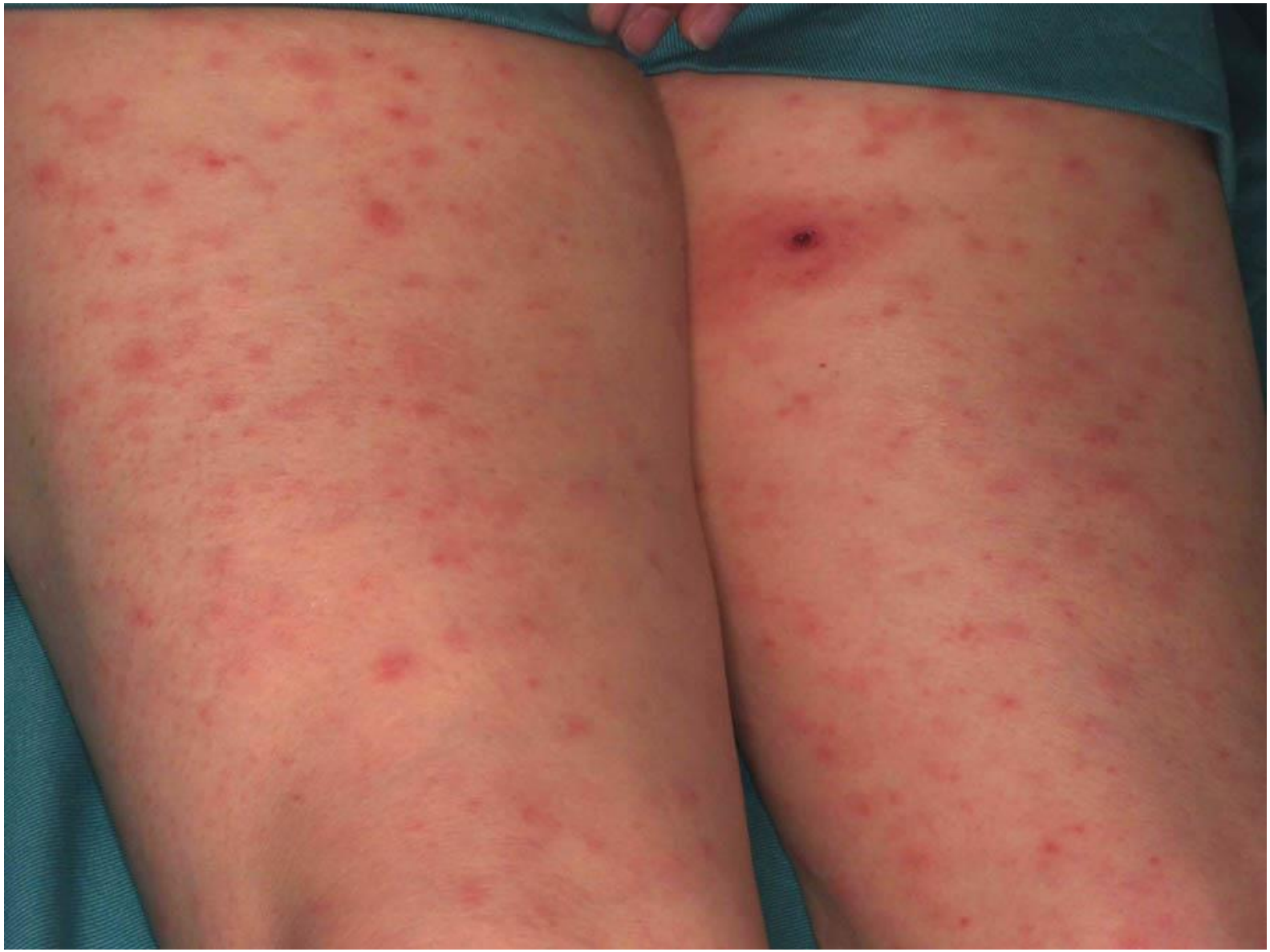
Case presentation

Case 1. 78 y-o F
Minomycin administered on the same day.
Biopsy taken from eschar and rash 1 day later.

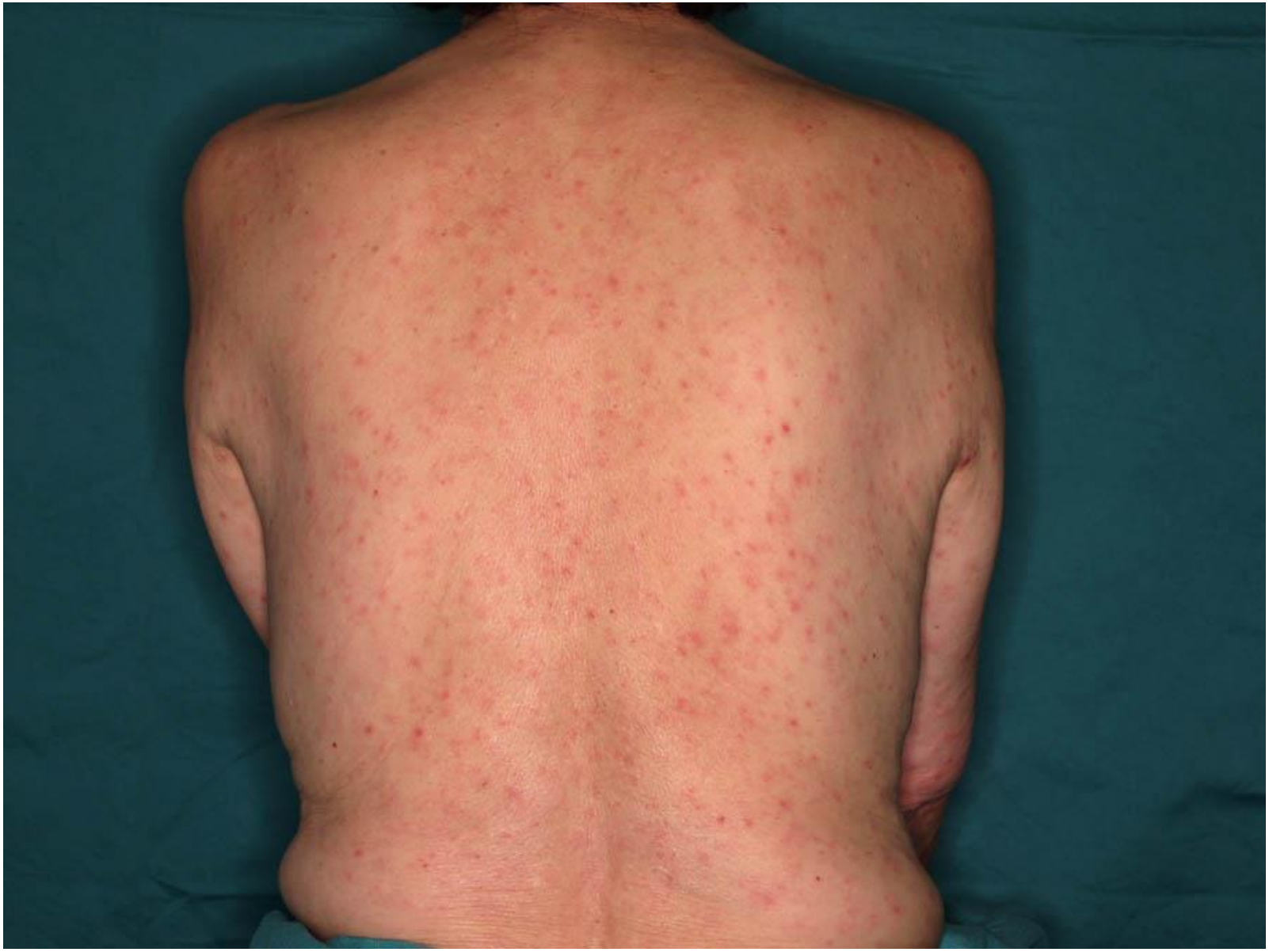
Case 2. 77 y-o M
Minomycin administered on the same day.
Biopsy taken from eschar and rash 4 days later
(fever alleviated).

Case 3. 51 y-o M
Minomycin administered on the same day.
Biopsy taken from rash on the next day.

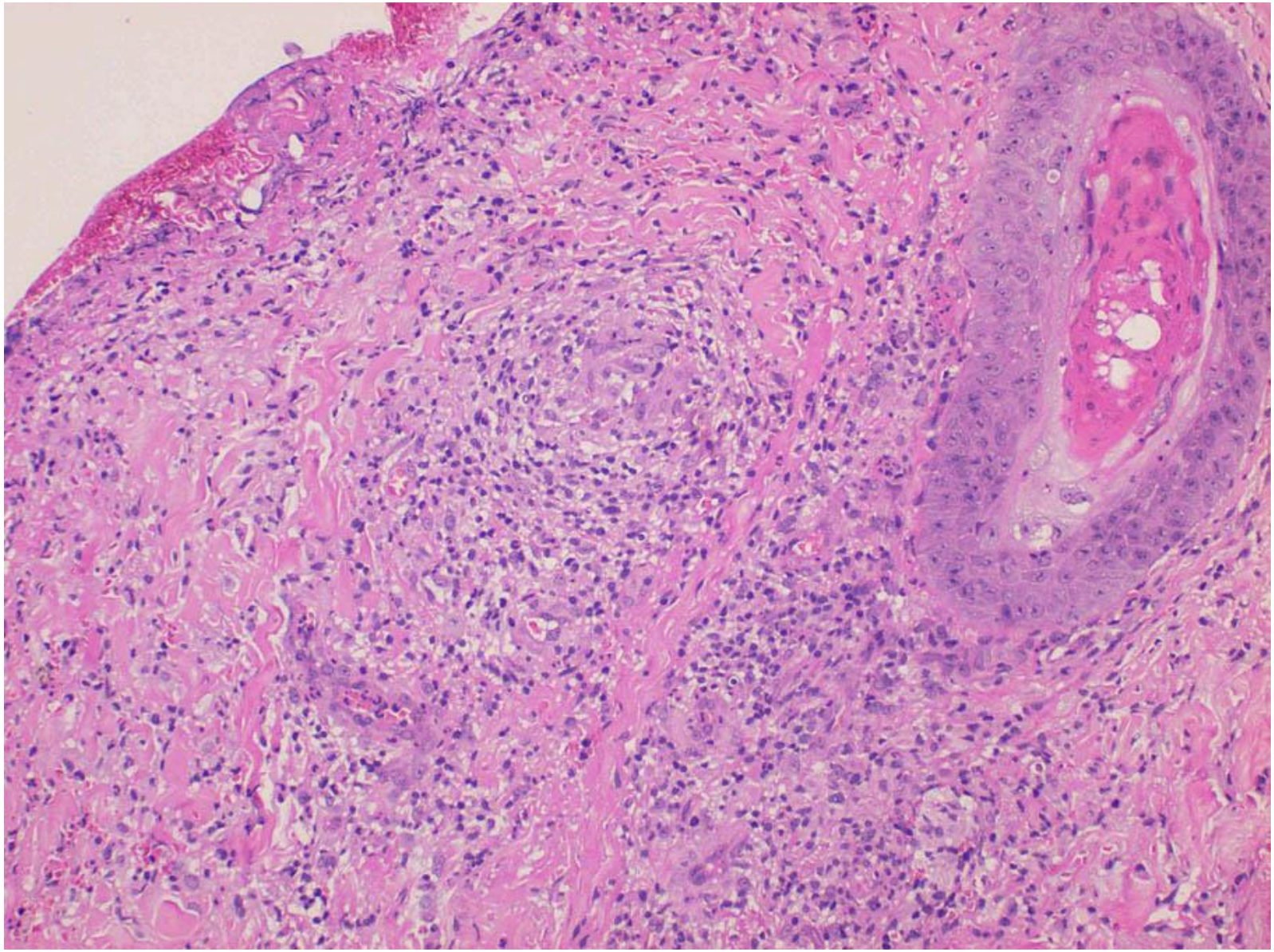
Case 4. 65 y-o F
Minomycin administered on the same day.
Biopsy taken from eschar and rash 4 days later
(fever alleviated).



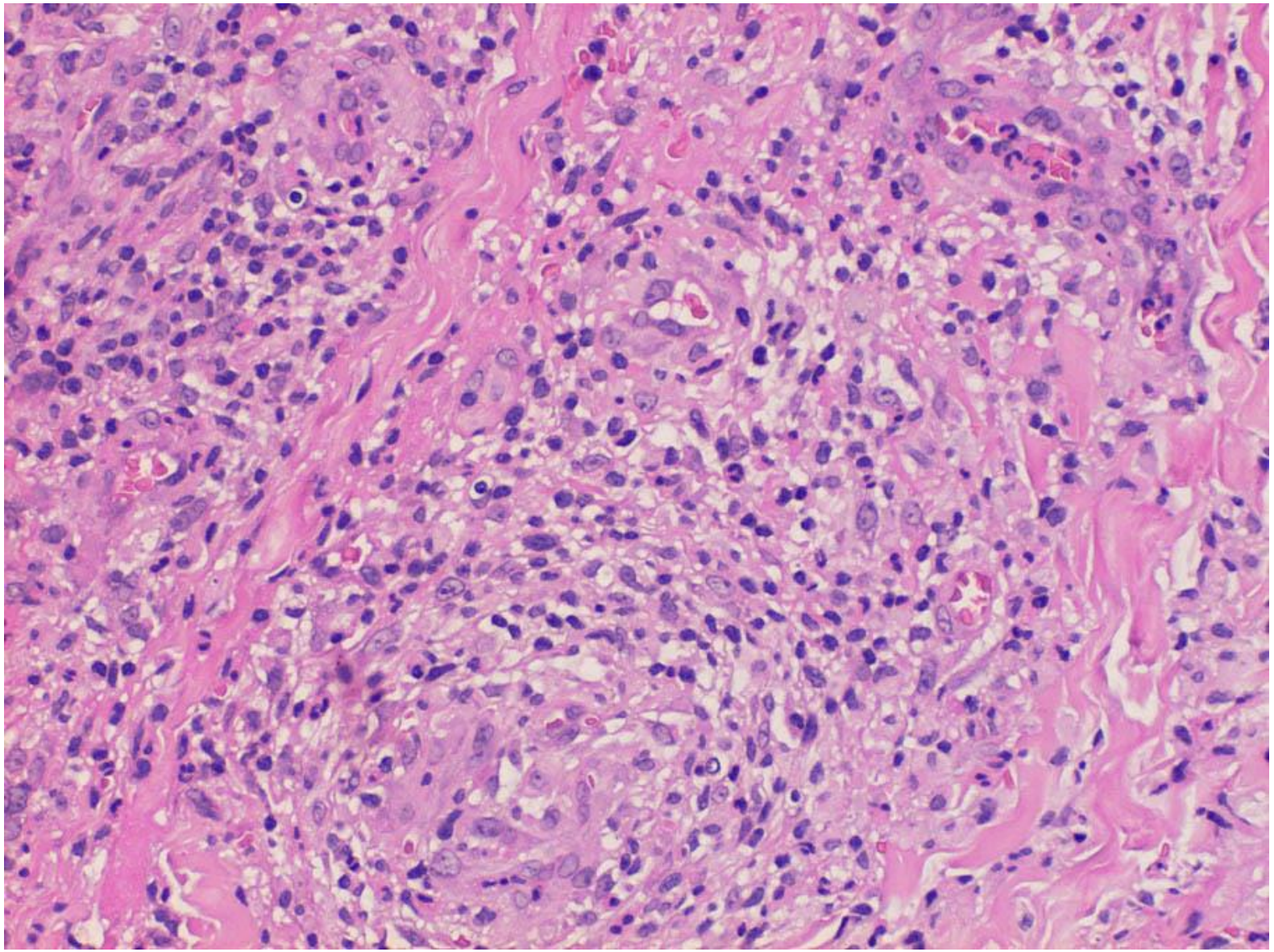
Case 1. Eschar and rash of JSF



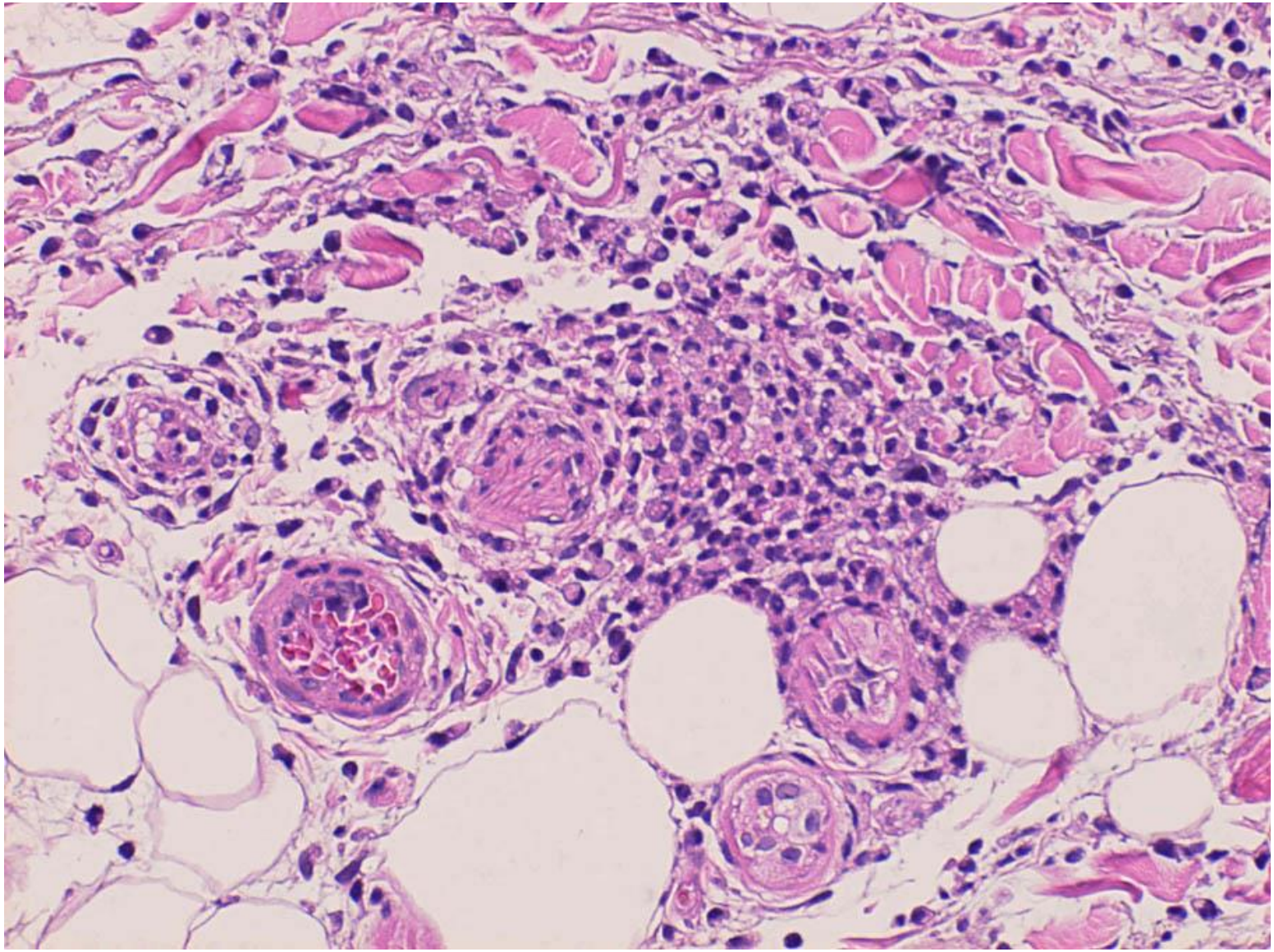
Case 1. Rash of JSF on the back



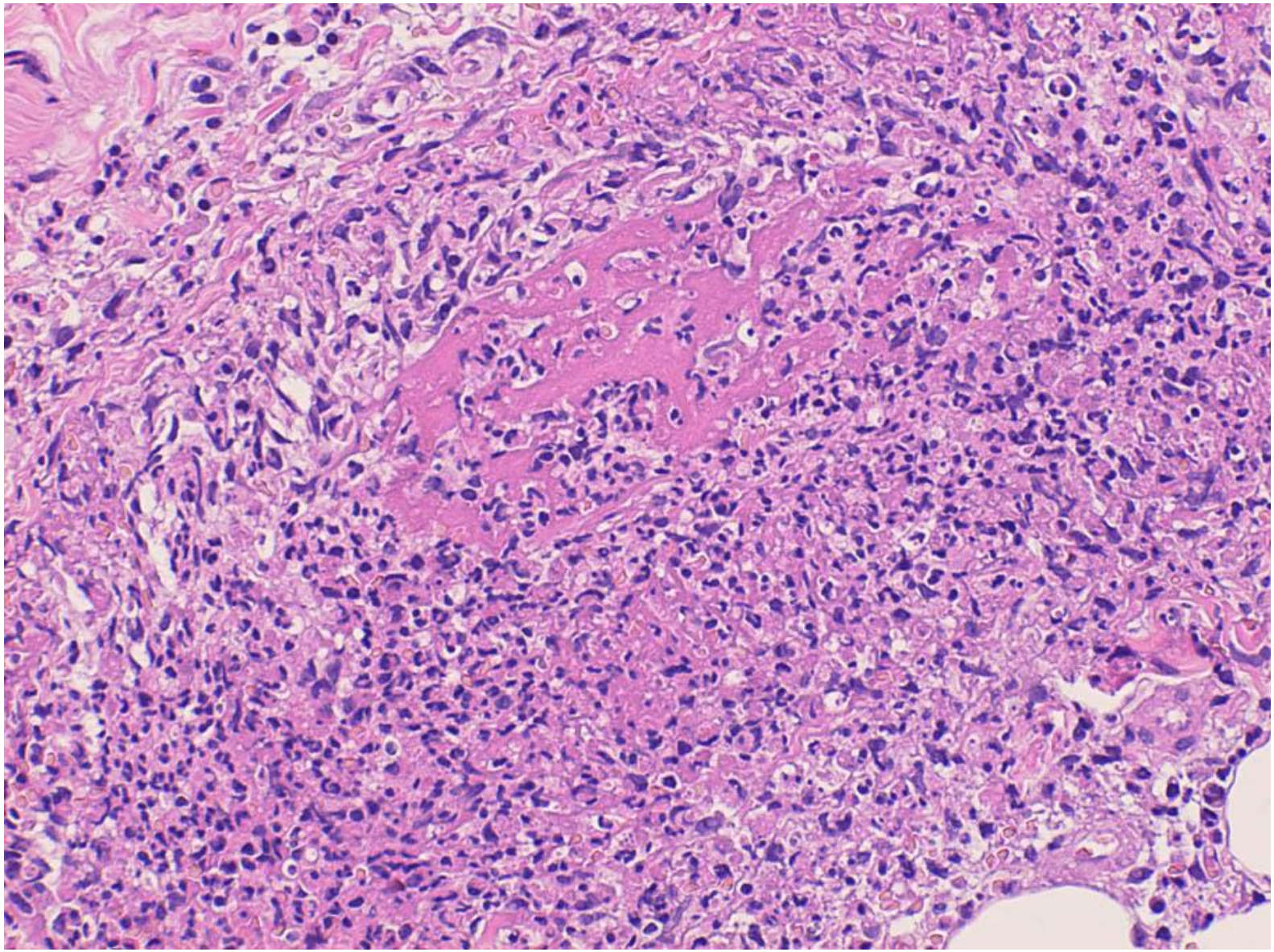
Case 4. Skin biopsy from the eschar of JSF (H&E)



Case 4. Skin biopsy from the eschar of JSF (H&E)



Case 2. Biopsy from the skin rash of JSF (H&E)



Case 1. Biopsy from the skin rash of JSF (H&E): necrotizing angiitis

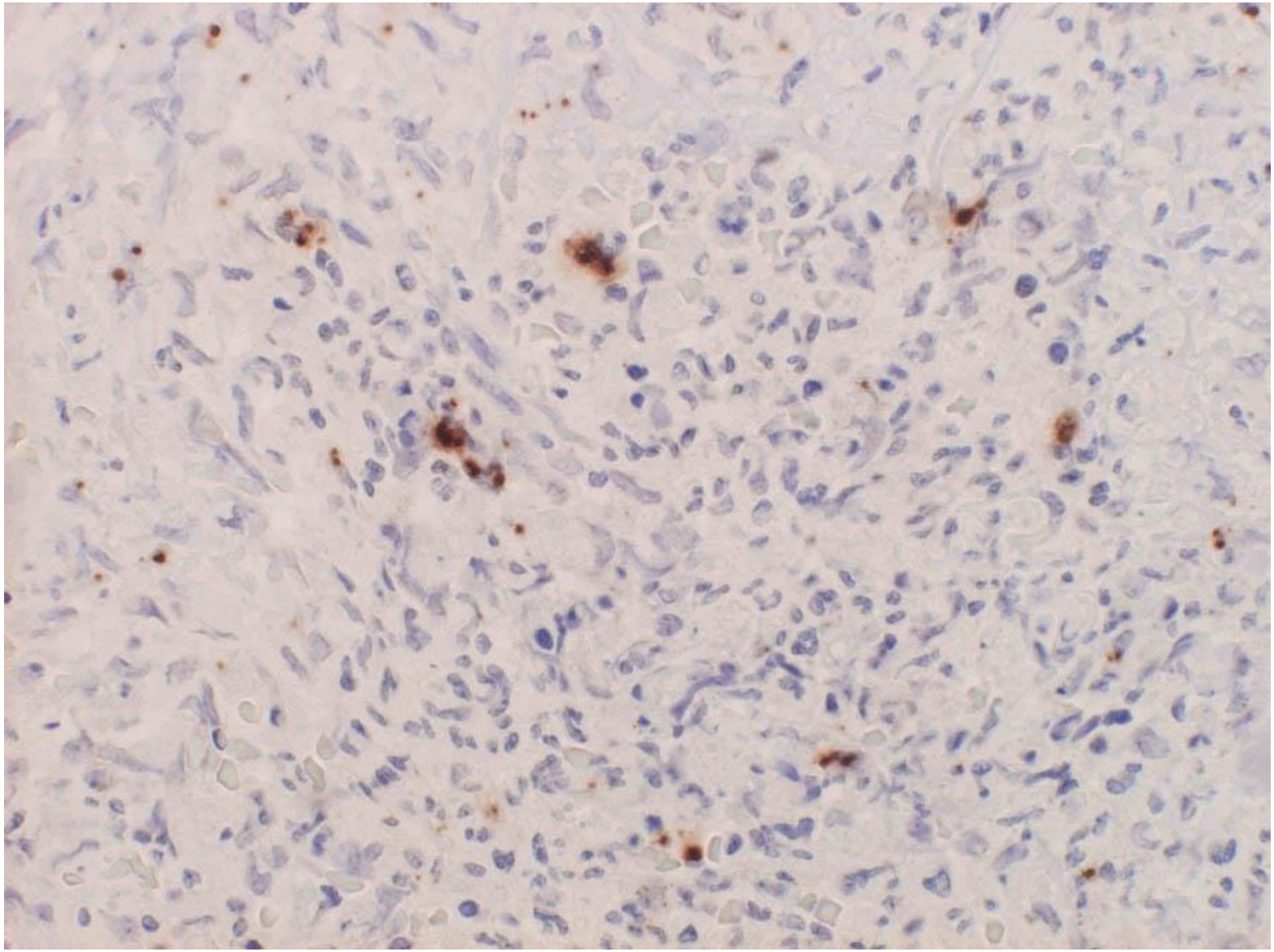
Histolopathological findings

1. **Eschar**: Necrotizing ulceration.

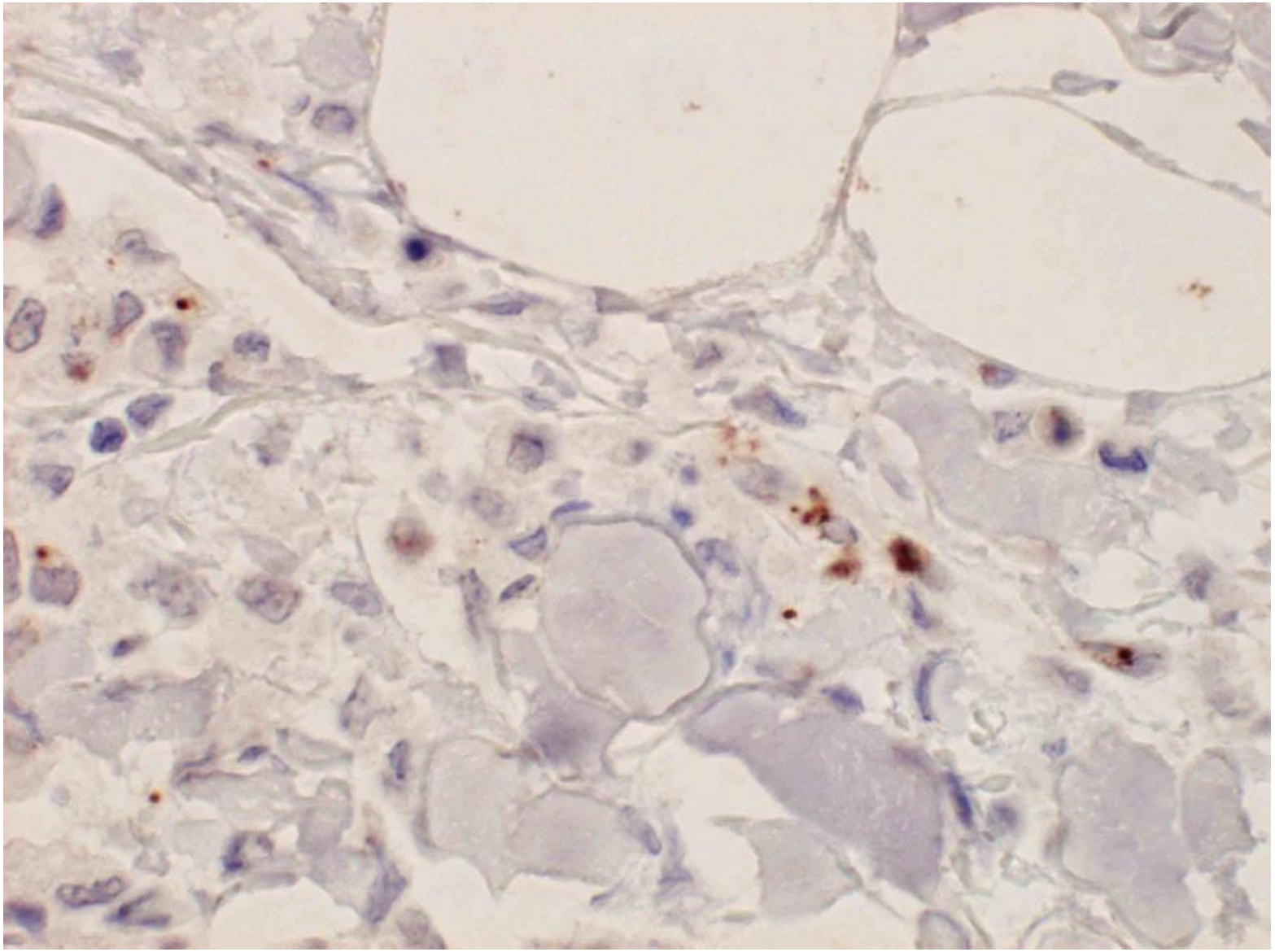
   Infiltration of macrophages and perivascular accumulation of lymphocytes

2. **Skin rash**: Perivascular accumulation of lymphocytes

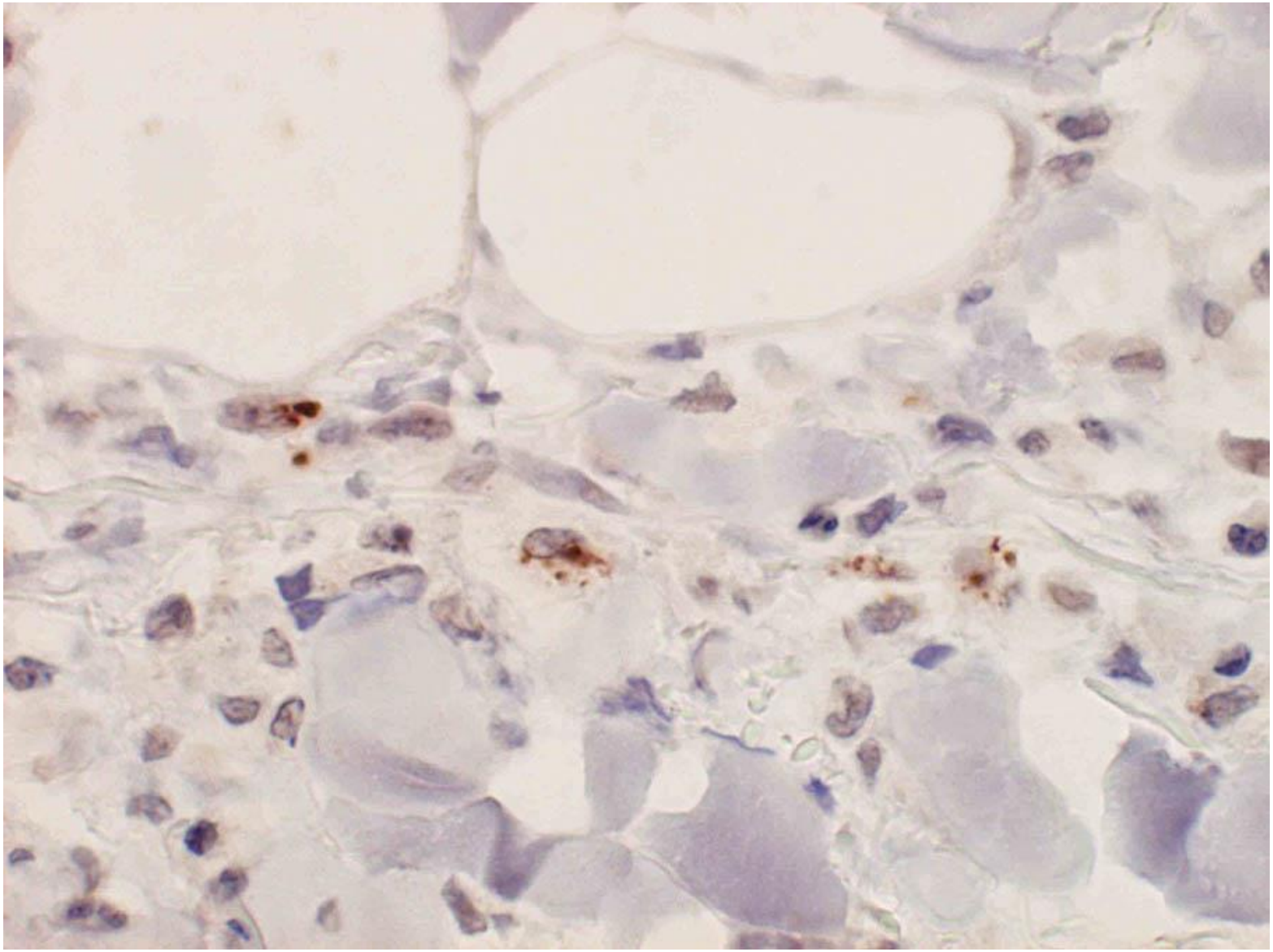
   In one case, necrotizing angiitis with fibrinoid necrosis noted



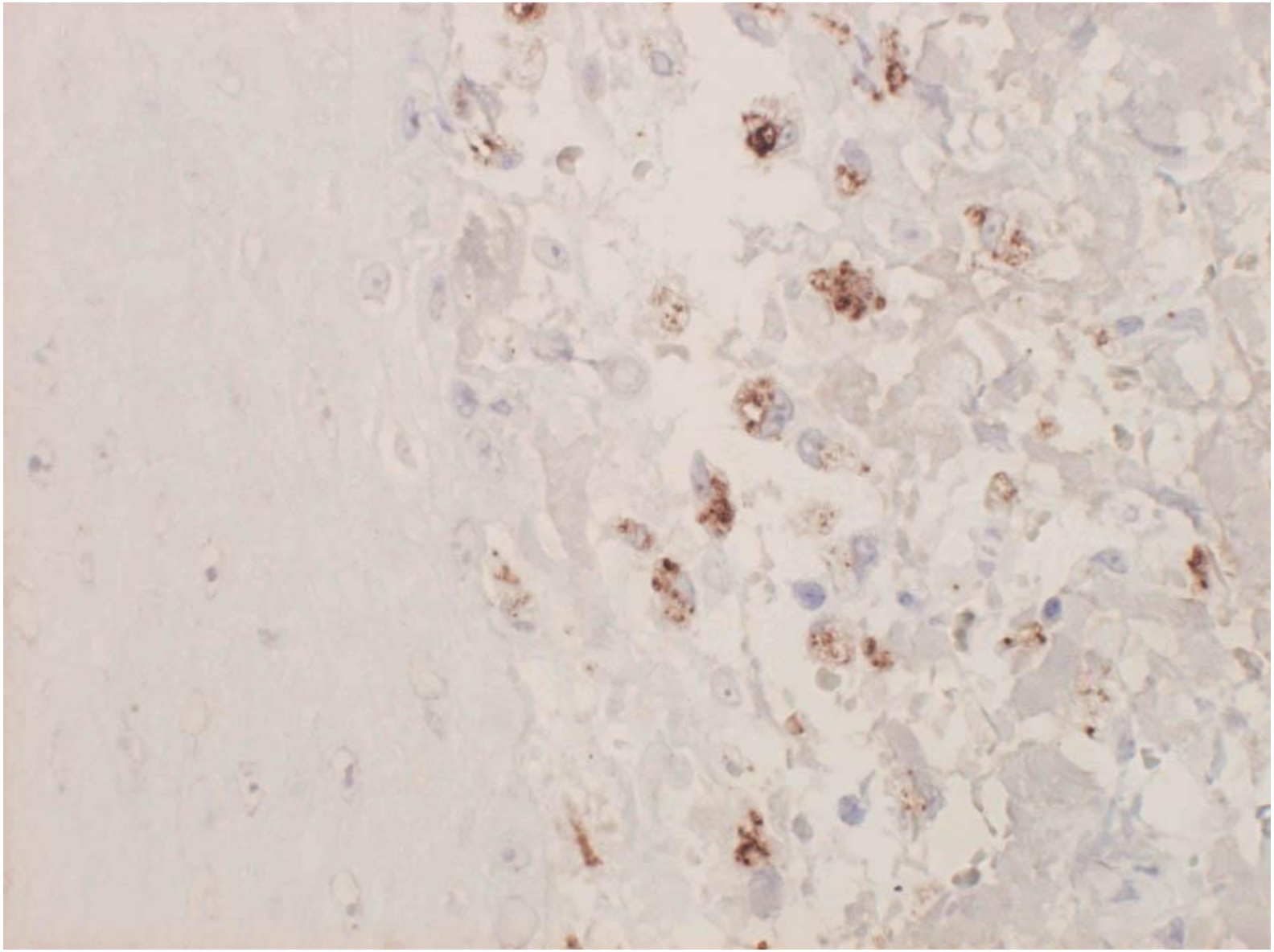
Case 1. Biopsy from the rash of JSF (immunostaining using Mab S3): necrotizing angiitis



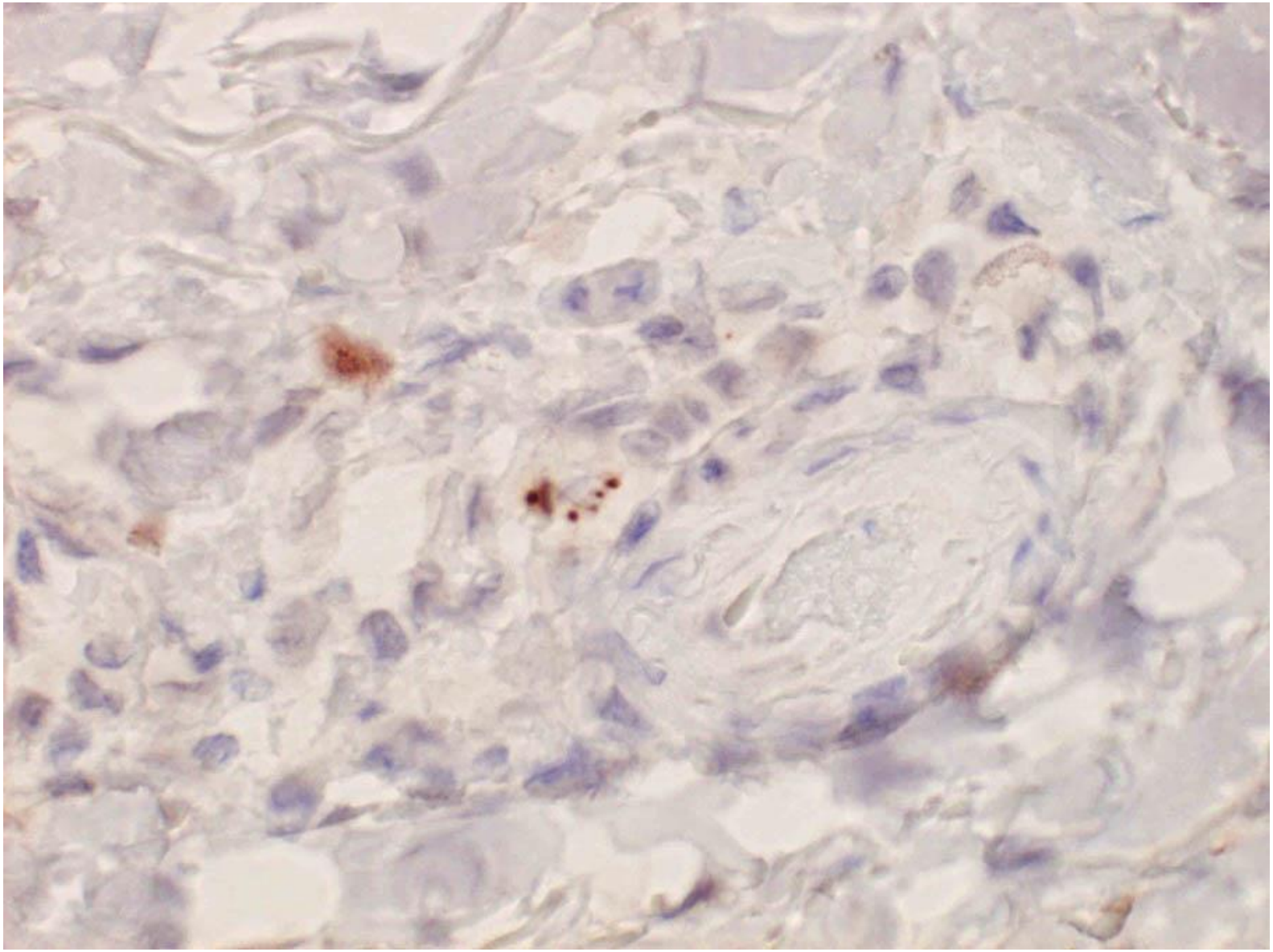
Case 1. Biopsy from the rash of JSF (immunostaining using Mab S3)



Case 1. Biopsy from the rash of JSF (immunostaining using Mab X1)



Case 2. Biopsy from the eschar of JSF (immunostaining using Mab S3)



Case 2. Biopsy from the rash of JSF (immunostaining using Mab S3), afebrile stage

Summary of Immunoperoxidase Staining

	Days after Minomycin Ad.	Eschar	Skin rash
Case 1	1 day	+	+ (necr. angiitis)
Case 2	4 days (afebrile)	+	+
Case 3	1 day	+	~
Case 4	4 days (afebrile)	+	−

(The same results obtained with Mabs S3 and X1)

Conclusions

1. Immunostaining for rickettsial antigens in routinely processed skin biopsy specimens was useful in early diagnosis of JSF in 3–4 days.

2. Rickettsial antigens were demonstrated after use of Minomycin and even after alleviation of fever.

3. Necrotizing angiitis was observed in the skin rash in case 1.

4. Heat–induced epitope retrieval was necessary for identification of rickettsial antigens. Both Mabs showed the same staining pattern.

5. The Mabs were cross–reactive with rickettsiae of spotted fever group and thus not specific for *R. japonica*, but Tsutsugamushi disease can be distinguished, because of the lack of cross–reactivity with *O. tsutsugamushi.*

When you detect a new disease, do not use the name of country or city

Disease	*Endemic areas*
1. Japanese spotted fever	Korea, Thailand
2. Japanese encephalitis	South eastern Asia
3. Japanese schistosomiasis	China and Philippines