

Increased soluble Fas ligand levels in patients with Stevens-Johnson syndrome and toxic epidermal necrolysis preceding skin detachment

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Background: It is difficult to distinguish the early phase of Stevens-Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN) from other ordinary types of drug-induced skin reactions (ODSRs). Levels of several serum soluble factors, including soluble Fas ligand (sFasL), have been reported to be increased in patients with SJS/TEN; however, the marker to predict the onset of SJS/TEN before the development of skin detachment or mucosal lesions has not been identified.

Objective: We sought to determine whether sFasL might be a useful marker in the early stages of SJS/TEN.

Methods: Sera of 19 patients with SJS and 16 patients with TEN at 1 or multiple time points were obtained from Japanese multiple hospitals. The disease onset (day 1) was defined when erosion/ulceration of the mucocutaneous or ocular lesion first developed. For the investigation of soluble factors, including sFasL, TNF- α , IFN- γ , IL-6, and sCD40 ligand, we used ELISAs and Cytometric Bead Arrays.

Results: Before disease onset (day -4 to approximately -2), 7 samples were available, and we detected the highest concentrations of sFasL in 5 (71.4%) of 7 patients. Increased sFasL levels decreased rapidly within 5 days of disease onset. In all 32 patients with ODSRs and 33 healthy control subjects, no increase of sFasL levels was detected. Other soluble factor concentrations did not show significant difference with those seen in patients with SJS/TEN before disease onset and ODSRs. **Conclusion:** The sFasL levels of sera in patients with SJS/TEN are significantly increased before development of skin detachment, mucosal lesions, or both. (J Allergy Clin Immunol 2008;122:1016-1021.)

Key words: Soluble Fas ligand, Stevens-Johnson syndrome, toxic epidermal necrolysis

Toxic epidermal necrolysis (TEN) and Stevens-Johnson syndrome (SJS) are 2 of the most severe cutaneous adverse reactions and life-threatening diseases.^{1,2} They are considered part of the same spectrum of diseases but with different severities. In SJS

Abbreviations used

EM:	Erythema multiforme
FasL:	Fas ligand
MPR:	Maculopapular rash
ND:	Not detectable
ODSRs:	Ordinary types of drug-induced skin reactions
sCD40L:	Soluble CD40 ligand
SJS:	Stevens-Johnson syndrome
TEN:	Toxic epidermal necrolysis

the skin detachment area is less than 10% of the body surface area, whereas patients with TEN have more extensive lesions.³ Although they are rare diseases (2 cases per million population per year in patients with TEN), their mortality rates are high; the mortality for TEN is up to 20% to 25%, for example.⁴ At the early stage, clinical presentations of SJS/TEN are very similar to those of ordinary types of drug-induced skin reactions (ODSRs). As a consequence of this, diagnosis of SJS/TEN is difficult, and the start of treatment is often delayed, resulting in the high mortality rates of SJS/TEN. In addition, the number of patients with SJS/TEN has increased recently, especially among patients with HIV.⁵ Therefore elucidation of the pathogenesis of SJS/TEN and their effective treatments is urgently required. However, the pathomechanisms of these diseases are still not understood fully.

In patients with SJS/TEN, blister formation and widespread skin detachment are characteristically observed. Histologically, marked keratinocyte apoptosis is seen in lesional skin. Some soluble factors inducing keratinocyte apoptosis, such as TNF- α , IL-6,⁶ and soluble CD40 ligand (sCD40L),⁷ were expected to be of concern in the pathogenesis of SJS/TEN. However, it is difficult to explain the characteristic clinical course and histologic distinction based on these factors alone. For example, TNF- α is an inflammatory cytokine and can be increased in various inflammatory diseases, such as systemic lupus erythematosus,⁸ and fulminant hepatic failure.⁹ Therefore it is unlikely that TNF- α is a specific factor that induces keratinocyte apoptosis in patients with SJS/TEN.

In 1998, Viard et al¹⁰ reported that activation of the apoptosis receptor Fas through Fas ligand (FasL) is an initial important step leading to keratinocyte apoptosis in patients with TEN. They assumed that both Fas and FasL were derived from keratinocytes and that FasL expressed by keratinocytes led to apoptosis of keratinocytes in patients with TEN.¹⁰ We have previously indicated that levels of soluble FasL (sFasL) in serum of patients with SJS/TEN were increased, sFasL was secreted by causal drug-stimulated PBMCs,¹¹ and sera of patients with TEN with

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TABLE I. Hospitals and institutes that cooperated with the study

Hospital or institute, department of dermatology	Physicians
JR Sapporo Railway Hospital	Maki Kasai, MD, Kazuo Kodama, MD
Kurusu Dermatological and Urological Clinic	Yukie Kurisu, MD
Kushiro Dermatological Clinic	Koichi Adachi, MD
Oji General Hospital	Mitsuhito Ota, MD
Hakodate Central General Hospital	Mamoru Yaosaka, MD, Koichi Yokota, MD
Tohoku University Graduate School of Medicine	Hiroshi Suzuki, MD, Setsuya Aiba, MD
Gunma University Graduate School of Medicine	Yayoi Nagai, MD, Osamu Ishikawa, MD
Kakegawa City General Hospital	Shingo Suzuki, MD
Keio University School of Medicine	Rena Okada, MD, Tomotaka Sato, MD, Masayuki Amagai, MD
Teikyo University School of Medicine	Tomonori Takekoshi, MD, Shinichi Watanabe, MD
Kawaguchi Kogyo General Hospital	Katsuyuki Nishigori, MD
Yokohama City University Medical Center	Yuko Ikezawa, MD, Katuyuki Kawano, MD, Shinobu Mohri, MD
International Goodwill Hospital	Hiromichi Yamada, MD
Faculty of Medicine, University of Yamanashi	Mirei Kanzaki, MD, Tatsuyoshi Kawamura, MD, Shinji Shimada, MD
Social Insurance Chukyo Hospital	Ayuko Ijima, MD, Toshikazu Usuda, MD
Chita-Kosei General Hospital	Atsuko Ono, MD
Ogaki Municipal Hospital	Mariko Seishima, MD
Fukui Red Cross Hospital	Saeko Fujikawa, MD, Youichi Nishimura, MD
Kyoto University Graduate School of Medicine	Miki Tanioka, MD, Yoshiki Miyachi, MD
Nara Medical University	Takaya Fukumoto, MD, Hideo Asada, MD
Otemae Hospital	Eriko Mabuchi, MD
Kobe Rosai Hospital	Hideki Shimizu, MD
Wakayama Medical University	Takashi Yoshimasu, MD, Fukumi Furukawa, MD
Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Science	Kazuyasu Fujii, MD, Keiji Iwatsuki, MD
Graduate School of Biomedical Sciences, Hiroshima University	Keiji Nagata, MD, Michihiro Hide, MD
Onomichi General Hospital	Kenichi Morimoto, MD
Shimane University School of Medicine	Sakae Kaneko, MD, Eishin Morita, MD
Mitoyo General Hospital	Akemi Seno, MD
The University of Tokushima Faculty of Medicine	Rinko Majima, MD, Kenji Hirose, MD, Shin-ichi Ansai, MD, Seiji Arase, MD
Tokushima Red Cross Hospital	Yoshio Urano, MD
Kyusyu University, Graduate School of Medical Sciences	Rieko Kinoshita, MD, Masataka Furue, MD
Iizuka Hospital	Toshikatsu Igata, MD

high levels of sFasL induced apoptosis in cultured keratinocytes.¹¹ From these facts, it seems highly probable that sFasL produced by causal drug-stimulated PBMCs directly mediates keratinocyte apoptosis in patients with SJS/TEN.

In addition, sFasL levels are not increased in patients with other types of drug eruptions, such as erythema multiforme (EM)-type drug eruption,^{8,9} which is in the same spectrum of diseases as SJS/TEN, although it is a mild variant without mucosal lesions and skin detachment.³ These reports suggested that an increase of sFasL levels in sera of patients with SJS/TEN could be considered a marker of SJS/TEN to distinguish this condition from ODSRs.

The aim of this study was to determine whether sFasL could be a useful marker of SJS/TEN at the early stage, when patients have no skin detachment or mucosal lesions. We assembled serum samples of patients with SJS/TEN and detailed clinical information from hospitals across Japan. We analyzed the correlation of sFasL and other cytokine concentrations with clinical manifestations and disease courses.

METHODS

Patients

Sera of 19 patients with SJS (10 men and 9 women; average age, 44.8 ± 25.0 years) and 16 patients with TEN (10 men and 6 women; average age, 47.9 ± 23.4 years) were obtained from multiple Japanese institutions (Table I). All patients had actively progressing reactions meeting the criteria for TEN or

SJS, as previously defined.³ Twenty patients underwent skin biopsy to confirm the diagnosis. All biopsy samples showed typical histologic features, including numerous cases of keratinocyte apoptosis. SJS refers to cases with mucosal erosions and epidermal detachment of less than 10% of the body surface area, and TEN refers to those with more than 10% involvement. We assembled the clinical information of patients: sex, age, and percentage of lesion involvement (Table II). All samples of patients with SJS/TEN obtained before day 1 were collected by chance as samples of ordinary types of drug reactions in that time. We examined the sera that were taken for a routine laboratory test and preserved for further examination.

Sera of 32 patients with ODSRs (10 men and 22 women; average age, 50.4 ± 21.8 years) and 33 healthy control subjects (19 men and 14 women; average age, 29.9 ± 3.1 years) were also obtained. ODSRs included maculopapular-type and EM-type reactions and excluded other adverse drug reactions, including drug-induced hypersensitivity syndrome/drug rash with eosinophilia and systemic symptoms and acute generalized exanthematous pustulosis.

The protocols were approved by Ethical Committee at Hokkaido University Graduate School of Medicine.

Analysis of transition of sFasL with clinical course

In this research we conditionally defined that day 1 stands for disease onset of SJS/TEN, when erosions/ulceration of mucocutaneous or ocular lesions appeared. Day -1 is 1 day before the onset of SJS/TEN, when only erythema or papules are presented without erosions/ulcerations of mucocutaneous or ocular lesions. We examined concentration of serum sFasL at 5 times: day -4 to -2, day -1 to 2, day 3 to 5, day 6 to 10, and after day 11.

TABLE II. Patient information: percentage of involvement and sFasL concentration of sera in patients with SJS/TEN

Patient no.	Diagnosis	Age (y)	Sex	Percentage of lesion involvement	sFasL (pg/mL)				
					Day -4 to -2	Day -1 to 2	Day 3 to 5	Day 6 to 10	≥Day 11
1	SJS	15	F	3				ND	
2	SJS	17	F	<10			ND		ND
3	SJS	85	F	<10		112		85	83
4	SJS	31	M	5		111	59	ND	ND
5	SJS	49	F	Only mucosal lesion			57	ND	ND
6	SJS	27	F	<10	232	53		ND	
7	SJS	80	M	5	51	ND			
8	SJS	19	M	<10		136		92	
9	SJS	25	M	Only mucosal lesion			ND		
10	SJS	13	M	<10			ND	ND	
11	SJS	61	M	<10		83			139
12	SJS	36	M	<10				ND	ND
13	SJS	25	F	Only mucosal lesion	124	109		40	
14	SJS	71	F	<10			ND		
15	SJS	26	M	<10		51			
16	SJS	65	F	Only mucosal lesion		68	50	ND	ND
17	SJS	68	F	<10		ND			
18	SJS	79	M	5		ND		ND	
19	SJS	60	M	<10			ND	ND	ND
20	TEN	41	F	40	126		84		ND
21	TEN	71	M	40		ND	ND	ND	ND
22	TEN	9	M	95				ND	
23	TEN	63	M	90		67	ND		ND
24	TEN	54	F	20		ND	ND		ND
25	TEN	21	F	50		74	48	42	55
26	TEN	75	M	60			54	ND	
27	TEN	66	F	70	127	ND	ND		
28	TEN	55	M	40			ND	ND	ND
29	TEN	55	F	70		65			
30	TEN	6	M	60			42		
31	TEN	81	M	65		ND			
32	TEN	17	M	20	336	129	49		ND
33	TEN	56	M	90	ND	51	ND	ND	42
34	TEN	43	M	35				ND	
35	TEN	54	F	70				ND	55

F, Female; M, male; ND, less than 38 pg/mL.

Detection of sFasL and other soluble factors

For sFasL detection, we used ELISAs (MBL, Nagoya, Japan). We also used ELISAs (R&D Systems, Minneapolis, Minn) for sCD40L, TNF- α , IFN- γ , and IL-6 detection, and the Cytometric Bead Array (BD PharMingen, San Diego, Calif) for TNF- α , IFN- γ , and IL-6 detection. Each sample was analyzed in duplicate by means of ELISA. In Cytometric Bead Arrays, measurement of cytokines was carried out by means of flow cytometry (FACScalibur; BD Immunocytometry Systems, San Jose, Calif).

The limits of detection in ELISAs were as follows: sFasL, 38 pg/mL; sCD40L, 312 pg/mL; TNF- α , 15.6 pg/mL; IFN- γ , 15.6 pg/mL; and IL-6, 3.1 pg/mL. This was also applied to the limit of concentration measured by means of the Cytometric Bead Array.

Statistical analysis

Results are shown as means $\pm$ SD. The Student *t* test was used for comparison of paired conditions.

RESULTS

sFasL levels in sera of patients with ODSRs and healthy control subjects

We first measured the sFasL concentrations in the sera of 33 healthy volunteers. sFasL was not detected in the sera of 19

(57.6%) volunteers (cutoff level, 38 pg/mL; Fig 1, A; Table III). The range of sFasL concentrations of healthy volunteers was from the cutoff level to 67 pg/mL, and the mean was 42.8 ± 8.2 pg/mL. In this study we set the normal range of sFasL as less than 100 pg/mL. This level is almost the same as previously reported.^{12,13} In 32 patients with ODSRs, sFasL levels of sera were within the normal range (<100 pg/mL; Fig 1, A). The range was from not detectable (ND) (<38 pg/mL) to 81 pg/mL. The mean was 45.0 ± 11.1 pg/mL. There was no significant difference between sFasL levels of sera in healthy control subjects and patients with ODSR (*P* = .19).

sFasL Levels in patients with SJS/TEN

We then measured sFasL concentrations in the sera of patients with SJS/TEN (*n* = 35; SJS, *n* = 19; TEN, *n* = 16) and analyzed the correlation of sFasL levels with disease course (Fig 1, A). We examined concentrations of serum sFasL at 5 times: day -4 to day -2, day -1 to day 2, day 3 to day 5, day 6 to day 10, and after day 11.

sFasL levels were significantly increased in 5 (71.4%) of the 7 sera taken from day -4 to day -2 (ND to 336 pg/mL; mean, 147.76 ± 104.4 pg/mL) compared with levels in patients with ODSR and healthy control subjects (*P* = .020 and .019,

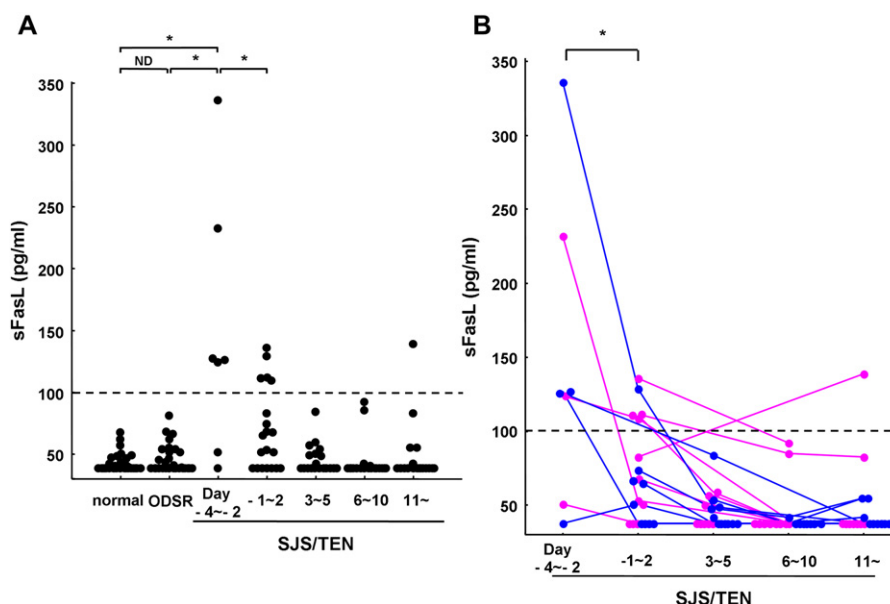


FIG 1. Increased serum sFasL levels of patients with SJS/TEN. **A**, sFasL levels of healthy control subjects, patients with ODSRs, and patients with SJS/TEN, with disease courses. In patients with SJS/TEN, we examined concentrations of serum sFasL at 5 times: day -4 to day -2, day -1 to day 2, day 3 to day 5, day 6 to day 10, and after day 11. sFasL levels were significantly increased before onset of disease, day -4 to day -2, compared with those in patients with ODSRs and healthy control subjects (* $P < .05$). **B**, Time course of serum sFasL levels in patients with SJS/TEN. Increased sFasL levels in patients with SJS/TEN rapidly decreased to the normal range within 5 days after onset.

TABLE III. Number of patients and percentage of patients with increased sFasL, TNF- α , IFN- γ , IL-6, and sCD40L levels

	Day	sFasL	TNF- α	IFN- γ	IL-6	sCD40L
Healthy control subjects		0/33 (0.0%)	0/25 (0.0%)	0/24 (0.0%)	0/20 (0.0%)	0/20 (0.0%)
Patients with ODSR		0/32 (0.0%)	4/20 (20.0%)	4/20 (20.0%)	13/20 (65.0%)	4/20 (20.0%)
Patients with SJS/TEN	-4~-2	5/7 (71.4%)	1/4 (25.0%)	3/4 (75.0%)	4/4 (100.0%)	0/7 (0.0%)
	-1~2	5/20 (25.0%)	4/11 (36.4%)	4/11 (36.4%)	11/11 (100.0%)	1/14 (7.1%)
	3~5	0/19 (0.0%)	3/12 (25.0%)	1/12 (8.3%)	12/12 (100.0%)	2/15 (13.3%)
	6~10	0/20 (0.0%)	2/11 (18.2%)	2/11 (18.2%)	7/11 (63.6%)	2/12 (16.7%)
	11~	1/17 (5.9%)	2/12 (18.7%)	1/12 (8.3%)	8/12 (66.7%)	1/13 (7.7%)

respectively). High sFasL levels were detected in only 5 (25.0%) of 20 samples taken from day -1 to day 2 (ND, approximately 136 pg/mL; mean, 68.8 ± 33.4 pg/mL). From day 3 to day 5, no increased sFasL levels were detected in any samples (ND, approximately 84 pg/mL; mean, 45.3 ± 11.9 pg/mL). These data indicated that the peak of sFasL levels would be a few days before disease onset of SJS/TEN, and sFasL levels already decreases before onset. In almost all cases increased sFasL levels are decreased to within the normal range about 5 days after onset (Fig 1, B). sFasL levels in patients with TEN and SJS show no significant differences in all phases, suggesting the degree of increased sFasL concentration did not correlate with disease severity.

Other soluble factor levels in the sera of patients with SJS/TEN, patients with ODSR, and healthy control subjects

Next we examined TNF- α , IFN- γ , IL-6, and sCD40L levels in sera of healthy control subjects, patients with ODSR, and patients with SJS/TEN patients. In all healthy control subjects we did not detect serum TNF- α (ND, <15.6 pg/mL; Fig 2, A; Table III), although we detected high TNF- α concentrations in the sera of

25.0% of patients with SJS/TEN before onset (ND, approximately 33.8 pg/mL; mean, 20.2 ± 9.1 pg/mL) and 20.0% of patients with ODSR (ND, approximately 411.9 pg/mL; mean, 40.6 ± 89.7 pg/mL). The peak of TNF- α levels in patients with SJS/TEN was detected from day -1 to day 2 (ND, approximately 72.7 pg/mL; mean, 29.9 ± 22.0 pg/mL). Its level was less than that seen in patients with ODSR, but it was not significantly different ($P = .31$). In addition, there was no significant difference between healthy control subjects and patients with ODSR.

IFN- γ levels of sera in almost all healthy control subjects (95.8%) were not increased, and the range was ND (<15.6 pg/mL) to 18.3 pg/mL (mean, 15.7 ± 0.55 pg/mL; Fig 2, B). IFN- γ levels were increased in 20.0% of ODSR samples; and the range was ND to 778.7 pg/mL, and the mean was 68.5 ± 176.8 pg/mL. During day -4 to day -2 in patients with SJS/TEN, increased IFN- γ levels (ND, approximately 57.6 pg/mL; mean, 31.4 ± 18.6 pg/mL) were detected in 75.0% of samples. However, importantly, there was no significant difference between patients with ODSR and patients with SJS/TEN before onset (day -4 to day -2, $P = .19$). No obvious peak was found in the disease course of patients with SJS/TEN. IFN- γ levels of sera in healthy control

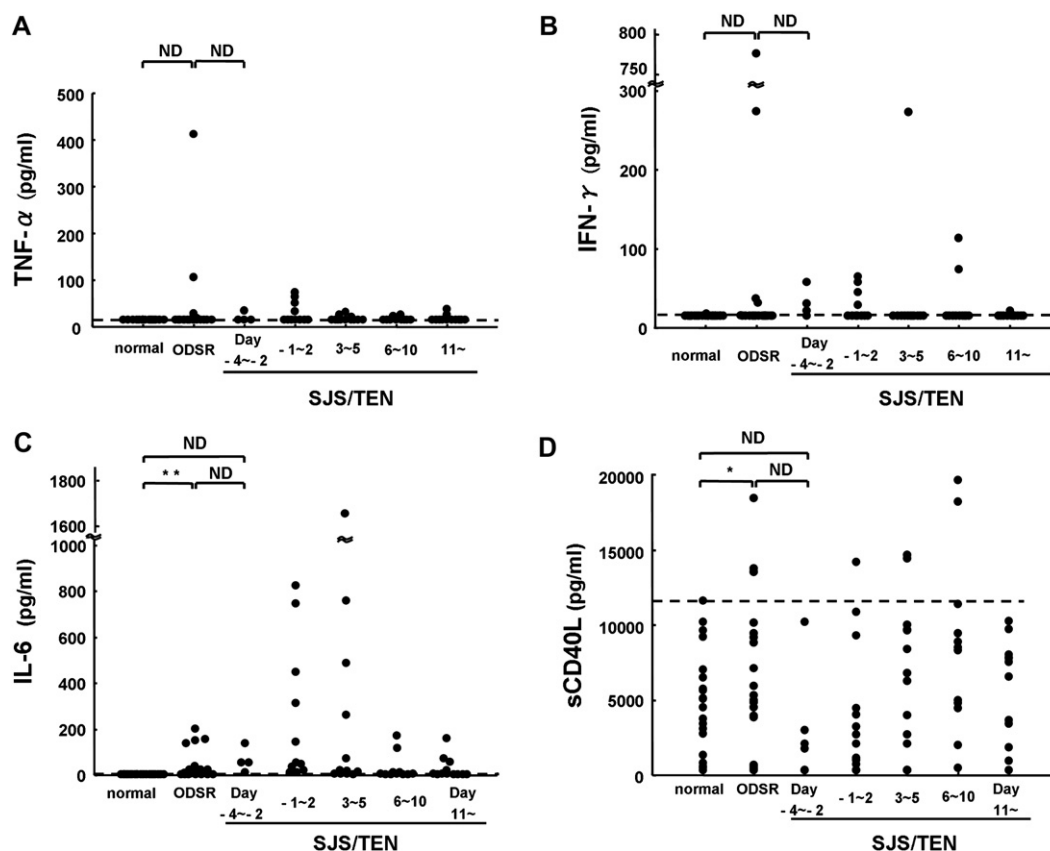


FIG 2. Serum levels of TNF- α , IFN- γ , IL-6, and sCD40L. **A**, TNF- α levels in sera of healthy control subjects, patients with ODSRs, and patients with SJS/TEN, with disease courses. High TNF- α concentrations were detected in sera of 25.0% of patients with SJS/TEN before onset (ND, approximately 33.8 pg/mL; mean, 20.2 ± 9.1 pg/mL) and 20.0% of patients with ODSRs (ND, approximately 411.9 pg/mL; mean 40.6 ± 89.7 pg/mL). There was no significant difference between patients with SJS/TEN and patients with ODSRs. **B**, In 20.0% of samples from patients with ODSRs, IFN- γ levels were increased (ND, approximately 778.7 pg/mL; mean, 68.5 ± 176.8 pg/mL). From day -4 to day -2, 75.0% of samples showed high IFN- γ levels (ND, approximately 57.6 pg/mL; mean, 31.4 ± 18.6 pg/mL) in patients with SJS/TEN. There was no significant difference between patients with ODSRs and patients with SJS/TEN between day -4 and day -2. **C**, In 65.0% of samples of patients with ODSRs, IL-6 levels were increased (ND, approximately 202.9 pg/mL; mean, 41.1 ± 63.5 pg/mL) and significantly increased compared with those seen in healthy control subjects (** $P < .01$). Before onset, day -4 to day -2, the IL-6 level increased in all samples (approximately 11.9–139.6 pg/mL; mean, 64.7 ± 53.7 pg/mL), whereas they did not significantly increase compared with those of patients with ODSRs. **D**, In 20.0% of samples from patients with ODSRs, sCD40L levels were increased (approximately 314–18424 pg/mL; mean, 6955 ± 5092 pg/mL). sCD40L concentrations in patients with SJS/TEN taken before onset, from day -4 to day -2, were not increased (ND, approximately 10,197 pg/mL; mean, 2559 ± 3525 pg/mL) and were lower than those of patients with ODSRs (** $P < .05$).

subjects, patients with ODSR, and patients with SJS/TEN were not significantly different (Fig 2, B).

Similarly to TNF- α and IFN- γ concentrations, serum IL-6 concentrations of all healthy control subjects were at quite low levels, and the range was ND (<3.1 pg/mL) to 3.6 pg/mL (mean, 3.1 ± 0.1 pg/mL; Fig 2, C). In patients with ODSR, the range of IL-6 was ND to 202.9 pg/mL, and the mean was 41.1 ± 63.5 pg/mL, which was a significant increase compared with that seen in healthy control subjects ($P = .007$). Patients with SJS/TEN had the highest IL-6 concentrations, 274.6 ± 493.0 during day 3 to day 5 (approximately 4.1–1639.3 pg/mL). The levels of IL-6 in patients with SJS/TEN seemed to increase along with disease severity. Before onset, day -4 to day -2, IL-6 levels increased in all samples (approximately 11.9–139.6 pg/mL, mean, 64.7 ± 53.7 pg/mL); however, they did not significantly increase compared with levels in patients with ODSR ($P = .24$).

sCD40L levels of healthy control subjects excessively varied from ND (<312 pg/mL) to 11,623 pg/mL (mean, 4832 ± 3422 pg/mL; Fig 2, D). In patients with ODSR, the range of sCD40L levels was 314 to 18,424 pg/mL, and the mean was 6955 ± 5092 pg/mL. The concentrations of sCD40L in patients with ODSR were not significantly increased compared with those of healthy control subjects ($P = .066$). sCD40L concentrations in patients with SJS/TEN taken before onset, from day -4 to day -2, were ND to 10,197 pg/mL. The mean was 2559 ± 3525 pg/mL, which was significantly less than those of patients with ODSR ($P = .012$). The mean level of sCD40L tended to be increased in later stages.

Serum sFasL levels and disease course in representative cases

There were 7 cases in which serum samples were available before disease onset. Herein we present the 3 representative cases

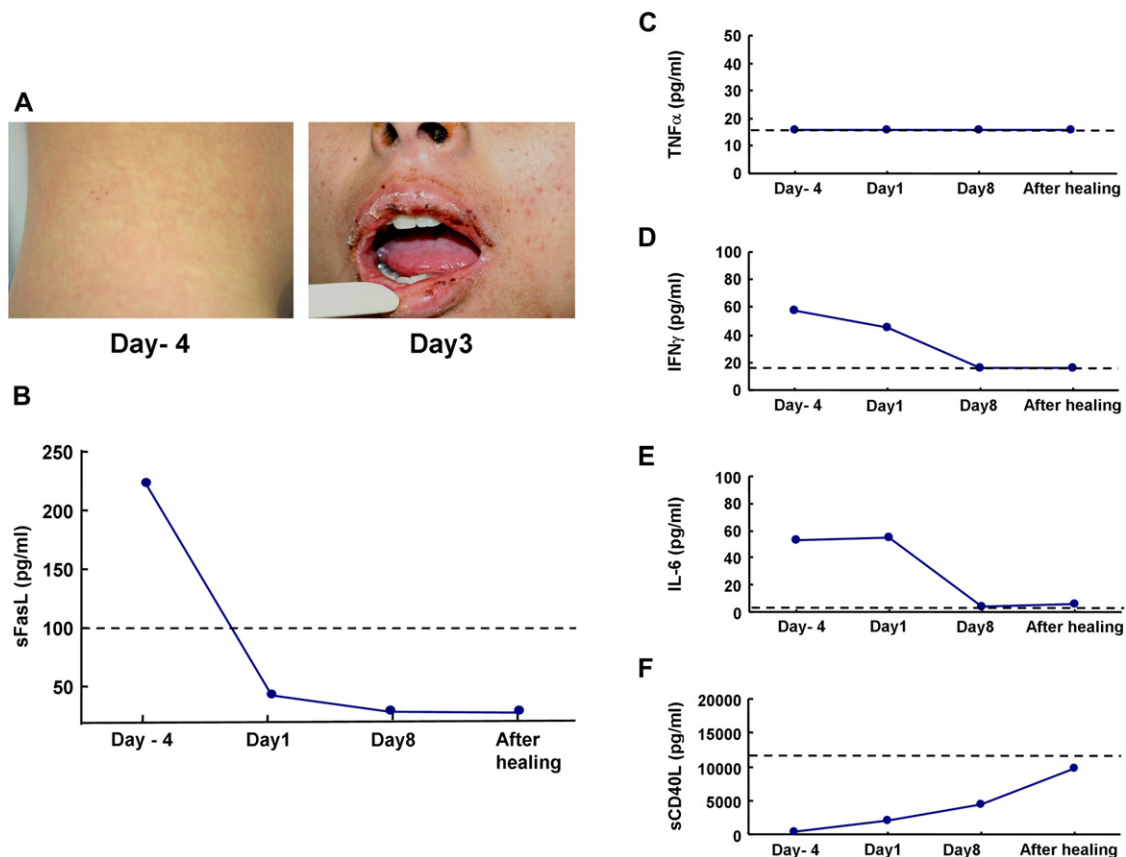


FIG 3. Case 1 (patient 6). **A**, clinical presentation of the patient at day -4 and day 1. **B**, sFasL levels of sera with disease course. sFasL levels were increased up to 232 pg/mL on day -4 when she presented with only erythema without oral lesions and skin detachment. When an oral lesion developed (day 1), sFasL levels had already decreased to normal levels and remained at these levels until her eruption was cured (day 11). **C-F**, TNF- α , IFN- γ , IL-6, and sCD40L levels of sera with disease course. In her serum IFN- γ and IL-6 levels increased on day -4 and day 1 and then decreased to within normal levels.

(SJS, $n = 1$; TEN, $n = 2$) in which serum sFasL levels were increased before disease onset.

Case 1 (patient 6). A 27-year-old woman presented with small, fresh, reddish erythema on her whole body accompanied by fever and liver dysfunction a few days after she had taken alprazolam, flunitrazepam, paroxetine hydrochloride, and ethyl loflazepate (Fig 3, A). Although prednisolone (20 mg/d) was started, oral mucosal erosion appeared, and skin detachment developed on her knees 10 days later. She was admitted to Fukui Red Cross Hospital, given a diagnosis SJS, and treated with a higher dose of corticosteroid (betamethasone, 10 mg/d). Her mucosal lesion, skin detachment, and liver function improved within a few days.

We measured the soluble factor levels of sera taken on day -4, day 1, and day 8 and after healing (day 11; Fig 3, B-F). sFasL levels were increased up to 232 pg/mL on day -4, when the patient presented with erythema but without oral lesions and skin detachment. When oral lesions developed (day 1), sFasL levels were already decreasing toward normal levels and remained at this lower level until her eruption was cured (day 10; Fig 3, B).

IFN- γ and IL-6 levels were increased on day -4 (IFN- γ , 57.6 pg/mL; IL-6, 52.9 pg/mL) and day 1 (IFN- γ , 44.9 pg/mL; IL-6, 54.7 pg/mL) and then gradually decreased (Fig 3, D and E).

Case 2 (patient 20). A 41-year-old woman had trifacial neuralgia and was started on treatment with carbamazepine and mecobalamin. One month after starting these medications, she

presented with a rash and fever. These medications were stopped, and she was treated with a systemic steroid (hydrocortisone sodium succinate, 100 mg/d); however, her eruptions and liver function were exacerbated. About 2 weeks after she first presented with a rash, vesicles and skin detachment developed on her legs and buttock (Fig 4, A). She was admitted to Shimane University School of Medicine, and the diagnosis of TEN was made. She was treated with a high dose of corticosteroid, including methylprednisolone pulse therapy (1000 mg). Her eruption gradually cleared, and liver function also recovered.

We tested sFasL levels of sera taken on day -3, day 5, and day 20 and after healing (4 months after; Fig 4, B). Although we detected increased sFasL levels on day -3 (126 pg/mL), sFasL levels gradually decreased. We also investigated other soluble factor levels in the sera (Fig 4, C-F). The peak of TNF- α was 25.4 pg/mL on day 20, that of IL-6 was 72.1 pg/mL on day 20, and that of sCD40L was 14,424 pg/mL on day 5. The IFN- γ level was slightly increased on day -3 (21.5 pg/mL) but soon decreased.

Case 3 (patient 27). A 66-year-old woman with chronic myelocytic leukemia was treated with imatinib mesilate. After 2 months of therapy, erythema and scales abruptly appeared on her whole body. Extensive skin detachment was derived from widespread erythema (Fig 5, A), and she consulted at Fukui Red Cross Hospital. She also had a high fever and liver dysfunction. A diagnosis of TEN was made, and combination therapy with a high-dose

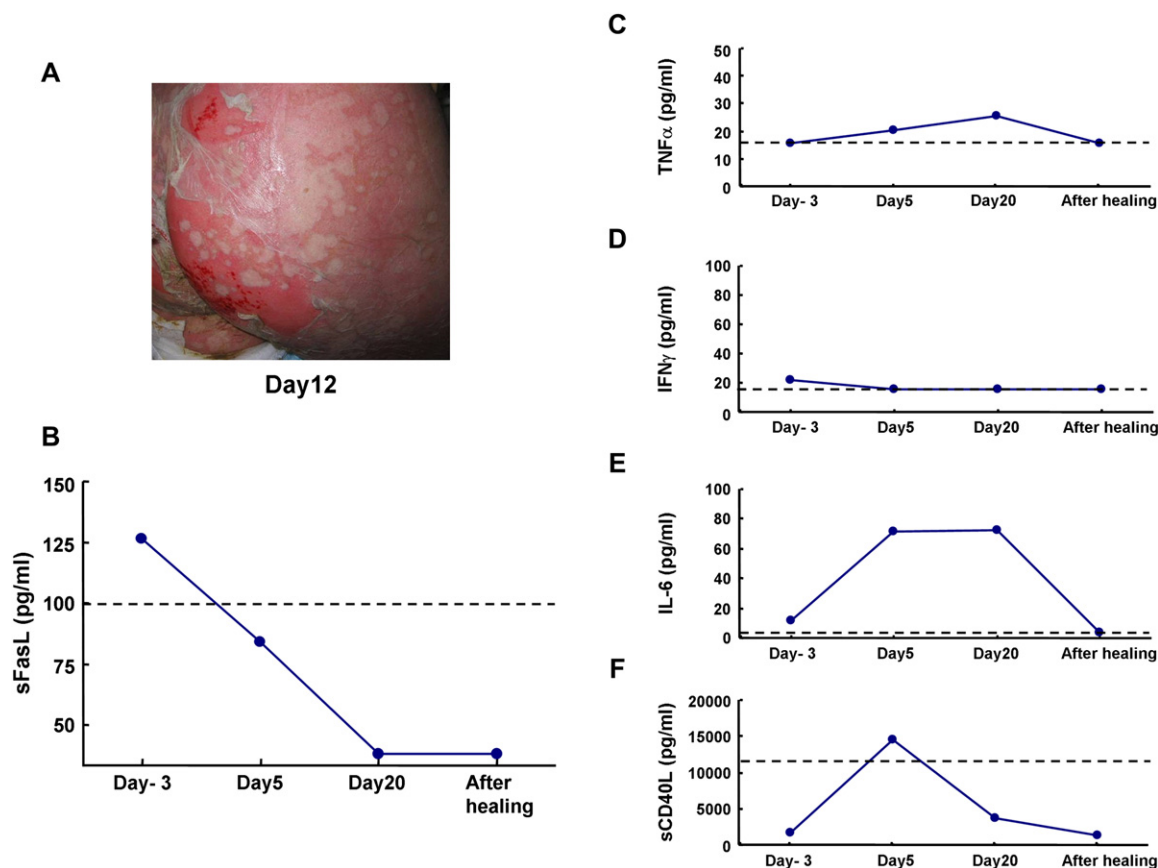


FIG 4. Case 2 (patient 20). **A**, clinical presentation of the patient at day 12. **B**, sFasL level of sera with disease course. Although increased sFasL levels were detected on day -3 (126 pg/mL), they decreased to normal ranges on day 20 and after healing. **C-F**, TNF- α , IFN- γ , IL-6, and sCD40L levels in sera with disease course. The peak of TNF- α was 25.4 pg/mL on day 20, that of IL-6 was 72.1 pg/mL on day 20, and that of sCD40L was 14,424 pg/mL on day 5. IFN- γ levels were slightly increased on day -3 (21.5 pg/mL) and soon decreased.

systemic corticosteroid (betamethasone, 5 mg/d) and intravenous γ -globulin (25 g/d) was started. Despite these therapies, she died on day 5 from disseminated intravascular coagulation caused by sepsis.

sFasL and other cytokine levels were measured on day -3, day 2, and day 5 (Fig 5, B-F). An increased sFasL level was detected on day -3 (127 pg/mL; Fig 5, B), although it had decreased on day 2 and day 5 to less than 100 pg/mL (Fig 5, B). In this case the TNF- α level increased on day 5 (24.8 pg/mL; Fig 5, C), and the IFN- γ level increased before onset (Fig 5, D) on day -3 (30.7 pg/mL). The sCD40L level was not increased (Fig 5, F) at any time points. IL-6 levels were considerably increased on day 2 to day 5 (825.2 and 488.4 pg/mL, respectively; Fig 5, E). It has been reported that serum IL-6 levels are increased in patients with sepsis,¹⁴ which appeared to be the case in this patient.

DISCUSSION

Serum samples of 35 patients with SJS/TEN were measured for sFasL, TNF- α , IFN- γ , IL-6, and sCD40L levels. We detected the highest concentrations of sFasL (>100 pg/mL) before disease onset (appearance of erosion, bulla, or mucous lesion) in 5 of 7 patients whose serum samples were available. Increased sFasL levels decreased rapidly within 5 days from disease onset. No

increase of sFasL level to greater than 100 pg/mL was detected in any of the samples from 32 patients with ODSRs or the 33 healthy control subjects. Compared with those of patients with ODSRs, TNF- α , IFN- γ , IL-6, and sCD40L concentrations of patients with SJS/TEN did not significantly increase before disease onset.

The sampling number of sera of patients with SJS/TEN in our study ($n = 35$) is larger than in the previously published articles and the first to track serum sFasL levels along with disease course, as far as we know. In this research we investigated sFasL concentrations from before SJS/TEN onset in particular. We detected increased sFasL levels in 71.4% of patients with SJS/TEN (5/7 cases) before onset (day -4 to day -2). After onset, the rate of patients with SJS/TEN with high sFasL levels was gradually decreased, although skin detachment, mucosal lesions, or both were extended (25.0% at day -1 to day 2 and 0% at day 3 to day 10). However, only 7 patients could be analyzed for sFasL concentration before onset (day 1) of SJS/TEN in our study. It is necessary to examine more samples of patients with SJS/TEN before onset to confirm the increase in sFasL concentration. Additionally, in most patients with SJS/TEN, therapy with systemic steroids was already started before onset. Thus it is unclear that treatment influenced the sFasL levels.

Patients with SJS/TEN have had several critical problems associated with the clinical aspects. Most patients in the early

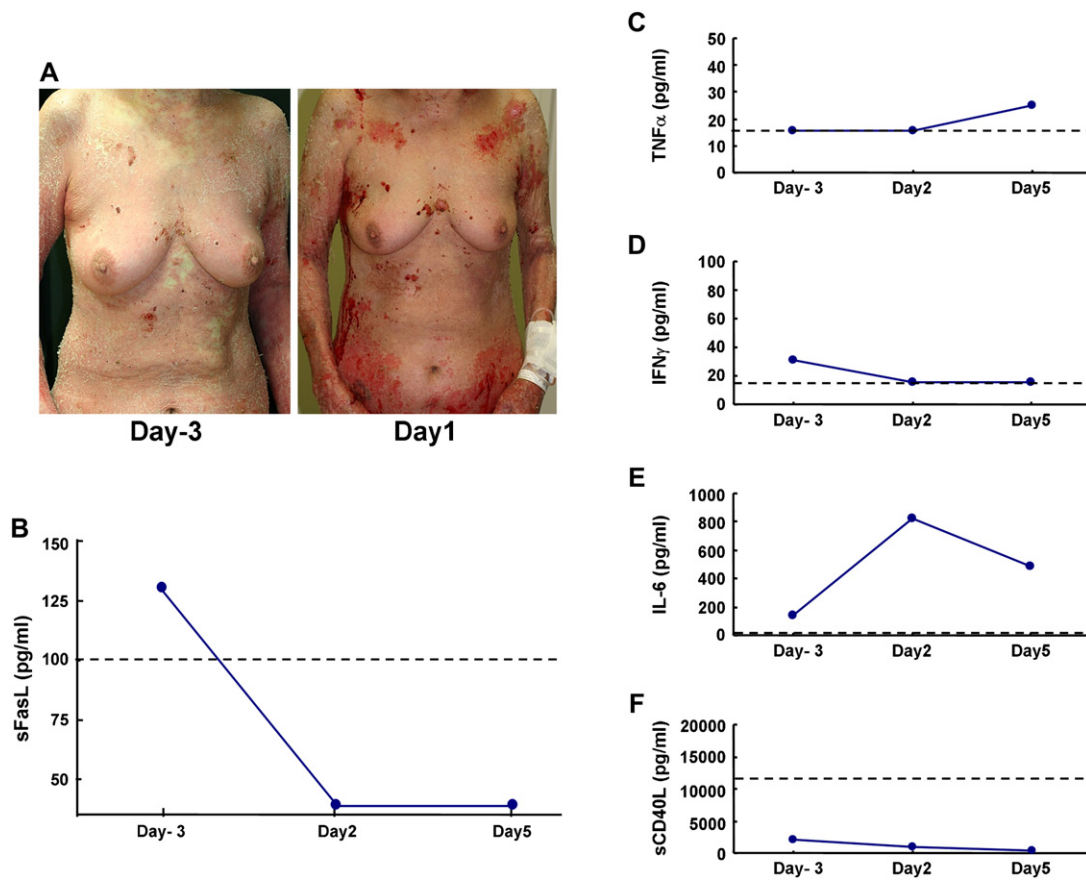


FIG 5. Case 3 (patient 27). **A**, Clinical presentation of the patient at day -3 and day 1. **B**, sFasL level of sera with disease course. Increased sFasL levels were detected on day -3 (127 pg/mL) and were decreased on day 2 and day 5 to less than 100 pg/mL. **C-F**, TNF- α , IFN- γ , IL-6, and sCD40L levels in sera with disease course. TNF- α increased on day 5 (24.8 pg/mL), and IFN- γ levels increased before onset at day -3 (30.7 pg/mL). sCD40L levels were not increased in any samples. IL-6 concentrations were considerably increased on day 2 (825.2 pg/mL).

stages of SJS/TEN have no mucosal lesions or skin detachment. Therefore clinical presentations of patients with SJS/TEN in the early stages are quite difficult to distinguish from those of patients with other mild types of drug eruptions. Although some patients with SJS/TEN take a relatively long time for skin detachment or mucous lesions to develop, a majority of patients with SJS/TEN rapidly advance within a few days from initial skin lesion appearance. Delay of diagnosis is likely to prolong the start of appropriate treatment, resulting in the high mortality seen with these diseases.

Thus far, a number of attempts have been made to distinguish early SJS/TEN from other mild types of drug eruption by using soluble markers. Wolkenstein et al⁶ detected high concentrations of TNF- α and IL-6 in sera and blister fluid of patients with SJS/TEN. The median value of TNF- α in sera was 36 pg/mL (range, 2-432 pg/mL), and that of IL-6 was 278 pg/mL (70-20,087 pg/mL). Caproni et al⁷ showed increased serum sCD40L levels (12.52 ± 5.82 ng/mL) in patients with SJS/TEN. Indeed, our experiments showed that values both soluble factors were increased in patients with SJS/TEN; however, values of these soluble factors did not increase significantly in patients with SJS/TEN before onset compared with those seen in patients with ODSRs. From our results, we speculate that increased sFasL levels in patients without skin detachment and mucosal erosion can be an important marker to predict the development of SJS/TEN.

Viard et al¹⁰ first reported increased sFasL concentrations in the sera of patients with TEN ($n = 5$) and Fas-FasL system-induced keratinocyte apoptosis in patients with TEN. They showed that increased sFasL concentrations were not detected in patients with drug-induced maculopapular rash (MPR; $n = 5$). We also previously showed that increased sFasL concentration were detected in the sera of patients with SJS/TEN ($n = 22$) and sFasL levels were not increased in EM-like drug eruptions ($n = 14$).¹¹ On the other hand, 2 recent articles reported that high concentrations of sFasL were indicated in other types of drug eruptions, such as MPR. Caproni et al⁷ showed that a statistically significant increase in sFasL levels was found for patients with both EM ($n = 24$) and SJS/TEN ($n = 7$), whereas no differences were found between patients with EM and those with SJS/TEN. Stur et al¹⁵ showed that high sFasL levels were detected in sera from patients with MPR. They explained the reasons for this discrepancy as induction of a larger number of patients with MPR ($n = 42$) and sampling during the early course of the disease. In our study we explored sera from 32 patients with ODSRs, and all serum samples were taken within 5 days from the initial skin eruption. Therefore we considered that a high enough number of samples was measured and that timings of sampling were appropriate. Stur et al¹⁵ also showed that sFasL levels were increased in sera from patients with SJS ($n = 2$), whereas sFasL

levels were low in patients with TEN ($n = 4$). Because sFasL levels of patients with SJS/TEN were quickly returned to undetectable levels in a few days, we speculate one of the reasons why significant increased sFasL levels could not be detected in patients with SJS/TEN compared with patients with EM or MPR in previous reports might be due to the delay of sampling. If the samples were collected after the onset of disease, then sFasL levels would have already decreased. Furthermore, they reported that sFasL concentrations were increased in patients with drug-induced MPR or SJS/TEN but not increased in patients with viral exanthema. In 35 patients with SJS/TEN in our study, there was only 1 virus-induced case. Sera samples of this virus-induced case were taken after disease onset, and the sFasL level was less than 100 pg/mL. Further experiments will be necessary to determine whether sFasL levels were different between patients with drug-induced SJS/TEN and those with virus-induced SJS/TEN.

We thought that increased sFasL levels would be one of the important factors in SJS/TEN; however, other factors might also influence the pathogenesis of SJS/TEN. Several articles have reported that genetic factors connected drug hypersensitivity, drug metabolism, and the immune response, including HLA genotypes.¹⁶⁻¹⁹ Chung et al¹⁶ indicated that alleles within the HLA region occurred at increased frequency in patients with carbamazepine-induced SJS; in particular, HLA-B*1502 was present in 100% of patients with carbamazepine-induced SJS but in only 3% of carbamazepine-tolerant patients and in 8.6% of the general population.

It has been reported that serum sFasL levels are increased in other diseases, such as chronic hepatitis B,²⁰ chronic graft-versus-host disease,²¹ adult Still's disease,²² silicosis,²³ and SLE.²⁴ Therefore increased serum sFasL levels do not always indicate the risk of cutaneous and mucosal fragility induced by keratinocyte apoptosis. In these diseases increased sFasL levels induced cell apoptosis in specific target organs. It is still unclear why the sensitivity to sFasL on each organ cell is different in these diseases. Further studies are necessary to clarify this. Additionally, in almost all these diseases, cell apoptosis lies in the background of their pathogenesis, so that it is certain that increased sFasL levels cause apoptosis on any organ tissue cells. Genetic factors can also influence drug metabolism and immune response, including cutaneous and mucosal keratinocyte susceptibility of sFasL. Particularly in patients with cutaneous adverse drug reactions, high sFasL levels in sera might be indicative of a more severe reaction, as seen in patients with SJS/TEN.

In conclusion, we have shown significantly increased sFasL levels in sera of patients with SJS/TEN before disease onset. From this, early diagnosis of SJS might be made by monitoring serum sFasL levels in patients with cutaneous adverse drug reactions who are suspected of progressing to SJS/TEN. Furthermore, more concise studies are expected to clarify the relation of increased sFasL levels and the risk of SJS/TEN in patients with adverse drug reactions.

Clinical implications: sFasL might be a useful marker of SJS/TEN at very early phases to distinguish from ordinary types of drug-induced skin reactions.

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