Galectin-7 as a potential biomarker of Stevens-Johnson syndrome/toxic epidermal necrolysis: identification by targeted proteomics using causative drug-exposed peripheral blood cells

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Clinical Implications

- Selected/multiple reaction monitoring targeted proteomics is a useful technique for identifying galectin-7, which could be a diagnostic marker of Stevens-Johnson syndrome and toxic epidermal necrolysis.

TO THE EDITOR:

Stevens-Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN) are severe cutaneous adverse drug reactions (cADRs) that can cause a life-threatening condition and late sequelae. SJS/TEN is defined as a spectrum of disease that is distinguished by the severity of epidermal necrosis and skin detachment.[1] In SJS, there is <10% epidermal detachment; in TEN, there is >30%. Once SJS/TEN develops, devastating conditions progress rapidly, and the mortality rate is high. With advances in medical technology, medical drugs have tended to increase in variety and use. As a result, the incidence of SJS/TEN and other drug eruptions has also increased.[2] However, the early diagnosis of SJS/TEN is often challenging, because at the initial stage, the clinical manifestations of severe drug eruptions resemble those of nonsevere ones. Several biomarkers have been reported for diagnosing SJS/TEN. For example, granulysin is a candidate for an early-diagnostic marker[3]; however, drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms, which are other types of severe cADRs, also show high serum levels of granulysin.[4] Biomarkers with greater specificity and sensitivity are urgently required for the early diagnosis of SJS/TEN.

Although proteomics analysis is a powerful tool for identifying specific disease markers, few reports involving such analysis have addressed specific SJS/TEN biomarkers. Because systemic inflammation is often induced in SJS/TEN, high levels of various inflammation-associated molecules might be detectable in sera. In addition, albumin hinders detecting small amounts of the serum proteins in proteomics. On initial drug stimulation, drug-specific lymphocytes secrete soluble factors. We hypothesized first that certain soluble factors could be secreted only by drug-specific lymphocytes of patients with SJS/TEN and not by those of nonsevere cADRs, and second that these could be biomarkers for SJS/TEN. Lymphocytes that specifically react with the causative drug then may remain in the peripheral blood of recovered patients with SJS/TEN, and on re-exposure to the causative drug, these lymphocytes could again secrete the key soluble factors. This study aims to use proteomics analysis to identify useful biomarkers for SJS/TEN diagnosis. To prevent the influence of albumin and to increase accuracy, we analyzed the supernatant of causative drug-exposed peripheral blood cells from patients with SJS/TEN, instead of their sera.

Peripheral blood mononuclear cells from patients with SJS/TEN (n = 3) or patients with nonsevere cADR (n = 3) were cultured with the causative drugs for 5 days, and then re-exposed with the causative drug. The causative drug and its exposure concentration were determined from lymphocyte transformation test data. After 1 day, supernatant was collected (Figure 1, *A*). The proteins in the supernatant were identified by mass spectrometry analysis. In each sample, approximately 200 to 500 proteins were identified (Figure 1, *B*; Table E1, available in this article's Online Repository at www.jaci-inpractice.org). Then the proteins that were found only in SJS/TEN and not in nonsevere cADR were selected (n = 47) as biomarker candidates (Table E2, available in this article's Online Repository at www.jaci-inpractice.org). We quantitated these proteins using selected/multiple reaction monitoring (SRM/MRM) with stable synthetic isotope-labeled peptides as an internal control. SRM/MRM is a quantitative technique that enables the targeted quantitation of potentially hundreds of proteins in a single analysis.[5] The SRM/MRM results revealed that 7 proteins—annexin A3, cathelicidin antimicrobial peptide (LL37), neutrophil gelatinase-associated lipocalin, calmodulin-like protein 5 (CALML5), galectin-7, interleukin-36γ, and S100A7—showed higher concentrations in the SJS/TEN samples than in the nonsevere cADR samples (ratios of 3:1 or more), and these 7 proteins have never been reported to be involved in SJS/TEN (Table I). Next, we performed an enzyme-linked immunosorbent assay (ELISA) to analyze the levels of these proteins in SJS/TEN sera (n = 7) and in normal control sera (n = 5). The ELISA found that CALML5 and galectin-7 were remarkably higher in the SJS/TEN sera than in the normal control sera (Figure 1, *C-F*). Finally, we compared the levels of these proteins in sera from patients with SJS/TEN (n = 24), patients with nonsevere cADR (n = 19), and normal controls (n = 8). Much higher levels of galectin-7 were observed (36.70 ± 43.75 ng/mL) in the SJS/TEN sera than in the nonsevere cADR sera (P = .005) or in the normal control sera (P = .0005) (Figure 1, *G*). In contrast, the levels of CALML5 were not higher in the SJS/TEN sera than in the nonsevere cADR sera (Figure 1, *H*). From the receiver operating characteristic (ROC) curve for normal control versus SJS/TEN, an area under the curve (AUC) of 0.945 and a cut-off point of 1.90 ng/mL were found (Figure E1, *A*, available in this article's Online Repository at www.jaci-inpractice.org). From the ROC curve for nonsevere cADR versus SJS/TEN, an AUC of 0.745

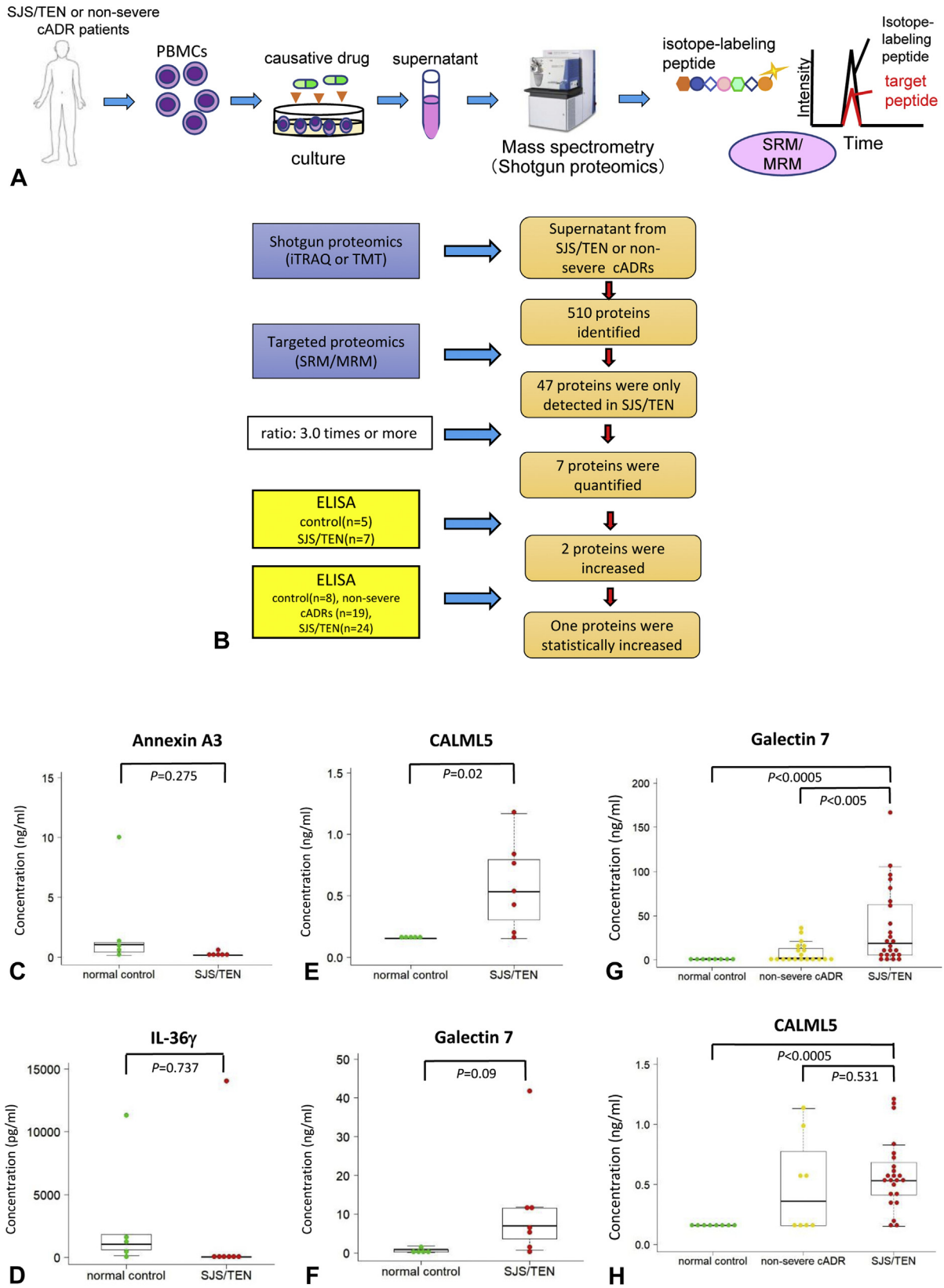
SJS/TEN or non-severe cADR patients
PBMCs
causative drug
supernatant
culture
Mass spectrometry (Shotgun proteomics)
isotope-labeling peptide
Isotope-labeling peptide
target peptide
Intensity
Time
SRM/ MRM
A
Shotgun proteomics (iTRAQ or TMT)
Targeted proteomics (SRM/MRM)
ratio: 3.0 times or more
ELISA control(n=5) SJS/TEN(n=7)
ELISA control(n=8), non-severe cADRs (n=19), SJS/TEN(n=24)
Supernatant from SJS/TEN or non-severe cADRs
510 proteins identified
47 proteins were only detected in SJS/TEN
7 proteins were quantified
2 proteins were increased
One proteins were statistically increased
B
Annexin A3
P=0.275
Concentration (ng/ml)
normal control
SJS/TEN
C
CALML5
P=0.02
Concentration (ng/ml)
normal control
SJS/TEN
E
Galectin 7
P<0.0005
P<0.005
Concentration (ng/ml)
normal control
non-severe cADR
SJS/TEN
G
IL-36γ
P=0.737
Concentration (pg/ml)
normal control
SJS/TEN
D
Galectin 7
P=0.09
Concentration (ng/ml)
normal control
SJS/TEN
F
CALML5
P<0.0005
P=0.531
Concentration (ng/ml)
normal control
non-severe cADR
SJS/TEN
H

TABLE I. High serum levels of proteins in causative drug-exposed peripheral blood mononuclear cells using SRM/MRM

Protein	Peptide	SJS/TEN	Nonsevere cADR	SJS/TEN: nonsevere cADR ratio
Neutrophil gelatinase-associated lipocalin	VPLQQNFQDNQFQGK	1.52	0.32	4.82
Cathelicidin antimicrobial peptide (LL37)	SSDANLYR	0.92	0.19	4.74
Protein S100-A7	GTNYLADVFEK	27.90	6.11	4.57
Interleukin-36 gamma	SDSVTPVTVAVITCK	1.15	0.29	3.96
Calmodulin-like protein 5	NLSEAQLR	0.59	0.16	3.81
Annexin A3	DYPDFSPSVDAEAIQK	0.36	0.10	3.47
Galectin-7	LDTSEVVFNSK	1.43	0.42	3.40

cADR, Cutaneous adverse drug reaction; *MRM*, multiple reaction monitoring; *SJS*, Stevens-Johnson syndrome; *SRM*, selected reaction monitoring; *TEN*, toxic epidermal necrolysis.

and a cut-off point of 5.08 ng/mL were found (Figure E1, *B*, available in this article's Online Repository at www.jaci-inpractice.org). The serum galectin-7 levels were significantly higher for the TEN cases than for the SJS cases ($P < .005$, Figure E2, available in this article's Online Repository at www.jaci-inpractice.org). We investigated the course of the serum galectin-7 level for each patient with SJS/TEN. In every case, the serum galectin-7 level was lower for the later phase (7 days or more after onset) than for the early phase (disease onset) regardless of whether the levels were high or low in the early phase (Figure E3, available in this article's Online Repository at www.jaci-inpractice.org). Furthermore, in every SJS/TEN case for which we collected serum before and after immunosuppressive therapy, the galectin-7 levels decreased after treatment (Figure E3). Moreover, we compared the concentration of galectin-7 in serum with that in blister fluids with the same patients' sample set (SJS: n = 3, TEN: n = 2). The concentrations of blister fluid were significantly higher than those of serum (serum: 29.94 ± 36.78 ng/mL, blister fluid: 2910 ± 1766 ng/mL). All the sera of autoimmune bullous disease and viral exanthema were below the detection limit. Taken together, these results suggest that galectin-7 could be a diagnostic marker of SJS/TEN.

Several serological factors such as soluble FasL[6] and IL-15[7] have been shown to reflect the activity or severity of SJS/TEN. In addition, granulysin, which elicits widespread keratinocyte death in SJS/TEN, has been shown to be a useful marker for early diagnosis.[3] Most of these factors were initially focused on for their own function, which might relate to SJS/TEN pathogenesis. In our study, we used targeted proteomics to analyze causative drug expose-induced factors. Our method allows the survey of SJS/TEN-specific proteins and the identification of "markers" that are not known to be associated with the pathogenesis. The investigation of the relationship to another marker, granulysin, showed no positive correlation between galectin-7 and granulysin. However, we did find a case with high granulysin and low galectin-7 (Figure E4, available in this article's Online Repository at www.jaci-inpractice.org). It is possible that a galectin-7/granulysin combined approach could be diagnostically useful. We need further investigation to conclude this.

Galectin-7, which is mainly expressed in stratified epithelia, has been described in epithelial tissues as being involved in apoptotic responses and in proliferation and differentiation. Galectin-7 has been shown to be either a proapoptotic factor or an antiapoptotic factor, indicating that galectin-7 activity in apoptosis varies according to the cellular context and/or the apoptotic stimulus. Galectin-7 overexpression occurs in apoptotic keratinocytes,[8] suggesting that galectin-7 is released from such keratinocytes in SJS/TEN, whereas another paper reported that galectin-7 itself is sufficient to induce apoptosis.[9] Our data indicate a possible contribution by galectin-7 to SJS/TEN etiology.

In conclusion, the novel method of SRM/MRM-targeted proteomics is useful for identifying a potential SJS/TEN biomarker and key candidates involved in SJS/TEN pathogenesis.

FIGURE 1. A, Schema of the study. Peripheral blood mononuclear cells were collected from patients who had recovered from SJS/TEN (n = 3) or nonsevere cADR (n = 3). They were cultured with causative drugs for 5 days, and then re-exposed with causative drug as previously reported (see Ref.E2, in this article's Online Repository at www.jaci-inpractice.org). One day later, supernatant was collected. Proteins in the supernatant were identified by mass spectrometry analysis using shotgun proteomics. Then we performed SRM/MRM analysis as a quantitative technique to narrow down the candidates. **B**, Schema of how the biomarker candidates were narrowed down. Supernatant was collected from causative drug-exposed peripheral blood mononuclear cells of SJS/TEN or nonsevere cADR. Mass spectrometry–based proteomics analysis identified 510 proteins. Targeted proteomics (SRM/MRM) was performed for the proteins that were detected only in the SJS/TEN samples. Seven proteins were found to be upregulated (ratio of SJS/TEN to nonsevere cADR = 3.0 or more). These were measured in the sera of patients with SJS/TEN (n = 7) or healthy control sera (n = 5), and only 2 of the 7 proteins were found to be significantly increased. Finally, measurement of the sera of patients with SJS/TEN (n = 24), sera of patients with nonsevere cADR (n = 19), and normal control sera (n = 8) found statistically elevated levels of only 1 protein in SJS/TEN. Serum levels of biomarker candidates: **C**, annexin A3; **D**, IL-36γ; **E**, CALML 5; and **F**, galectin-7, from patients with SJS/TEN and healthy donors are shown. Serum levels of **G**, CALML 5 and **H**, galectin-7 from patients with SJS/TEN (n = 24), patients with nonsevere cADR (n = 19), and healthy donors (n = 8) are shown. The scales are different for each galectin-7 and CALML 5 figure. *cADR*, Cutaneous adverse drug reaction; *ELISA*, enzyme-linked immunosorbent assay; *iTRAQ*, isobaric tag for relative and absolute quantitation; *MRM*, multiple reaction monitoring; *PBMC*, peripheral blood mononuclear cell; *SJS*, Stevens-Johnson syndrome; *SRM*, selected reaction monitoring; *TEN*, toxic epidermal necrolysis; *TMT*, tandem mass tags.

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METHODS

Patient's samples

All patients with Stevens-Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN) had severe drug reactions meeting the criteria for SJS/TEN as previously described.[E1] The patients with nonsevere cutaneous adverse drug reaction (cADR) included those with maculopapular exanthema and erythema multiforme, but excluded those with other adverse drug reactions, such as drug-induced hypersensitivity syndrome/drug rash with eosinophilia and systemic symptoms and acute generalized exanthematous pustulosis. SJS/TEN cases were treated by immunosuppressive therapy including high dose of corticosteroid and intravenous immunoglobulin. The patients with nonsevere cADR had recovered with steroid ointments and antihistamines, without systemic glucocorticoids. Sera were collected at onset or a few time points. Peripheral blood mononuclear cells were taken from patients at least 6 months to 5 years after the complete remission of symptoms. Sera were collected from 8 patients with autoimmune bullous disease (4 bullous pemphigoid, 2 pemphigus foliaceus, 1 pemphigus vulgaris, and 1 acquired epidermolysis bullosa) and from 4 patients with viral exanthema. In addition, blister fluid samples were collected from patients with SJS/TEN. The protocols were approved by the local ethics committee of Niigata University and Hokkaido University. Informed consent was obtained from all patients.

Culture supernatant

Peripheral blood mononuclear cells (PBMCs) were prepared from patient's blood using Ficoll-Paque PLUS (GE Healthcare Life Sciences, Buckinghamshire, UK). Isolated PBMCs were exposed to causative drugs and then cultured in Roswell Park Memorial Institute complete medium for 5 days to allow the proliferation of drug-specific T cells. The cells were re-exposed to the same causative drugs for 1 day so as to increase the number of drug-specific T cells,[E2] and then culture supernatant was collected. The drug concentration was determined from the data of a lymphocyte transformation test.[E3]

Sample preparation

Culture supernatant (3 mL) was centrifuged at 2000 × *g* for 10 minutes at 4°C, and debris was removed. Proteins were precipitated with trichloroacetic acid (TCA), and solubilized with phase-transfer surfactant (PTS) solution[E4,E5] in the presence of sodium deoxycholic acid and N-lauroyl sarcosinate at 95°C for 5 minutes followed by sonication for 1 minute. The protein concentration in the samples was determined using the DC Protein Assay Kit (Bio-Rad, Hercules, Calif). Proteins were reduced with 1/20 volume of 100 mM dithiothreitol (DTT) in 50 mM $NaHCO_3$ at room temperature (RT) for 30 minutes, and alkylated with 1/20 volume of 550 mM iodoacetic acid in 50 mM $NaHCO_3$ at RT for 30 minutes. The alkylated samples were digested with 1% trypsin (Roche) overnight at 37°C, and then treated using acetic ether to remove the PTS reagent. After digestion, peptides were desalted, concentrated, and prefractionated using C18-SCX stage Tips.[E6]

LC-MS/MS

LC-MS/MS analysis was performed using an LTQ-Orbitrap XL (Thermo Fisher Scientific, Bremen, Germany) with a nano-LC interface (AMR, Tokyo, Japan), a Paradigm MS2 (Michrom Bioresources, Auburn, CA), and an HTC PAL autosampler (CTC Analytics, Zwingen, Switzerland). Samples were injected into a trap column (0.3 × 5 mm, L-column ODS; Chemicals Evaluation and Research Institute [CERI]) and separated on an analytical column (0.1 × 200 mm in-house Tip Column packed with L-column2 C18 particles; CERI). Solvent A (2% acetonitrile, 0.1% formic acid) and solvent B (90% acetonitrile, 0.1% formic acid) were used in the mobile phase, and the injected peptides were eluted using a gradient from 5% to 95% solvent B at a flow rate of 500 nL/minute in 60 minutes. The 8 most abundant precursor ions in the MS scan were selected for subsequent MS/MS analysis in the linear ion trap after precursor fragmentation by collision-induced dissociation.

Protein identification

Raw data from the LC-MS/MS analysis were examined using Proteome Discoverer ver.1.3 (Thermo Fisher Scientific) with Mascot v2.4 (Matrix Science, London, UK) against UniProt/SwissProt (release 2014_03), which contains 20,129 sequences of *Homo sapiens*. The search parameters were as follows: precursor mass tolerance was 7 ppm, fragment ion mass tolerance was 0.6 Da, the enzyme was trypsin, and 1 missed cleavage site was allowed. The carboxymethylation of cysteine was chosen for the fixed modification. The oxidation of methionine was chosen for variable modifications. The percolator program was used to calculate the false discovery rate (FDR) of the identified peptides. Only peptides with FDR <1.0% were considered for high-confidence peptide identification. A minimum of 2 unique peptides meeting the criteria were required for protein identification.

Selected/multiple reaction monitoring (SRM/MRM) analysis

We used SRM/MRM to confirm the differential expression of selected proteins that were identified only in SJS/TEN samples from LC-MS/MS. SRM/MRM was performed as previously described.[E7,E8] The peptide sequence was mainly selected from the unique peptide sequences identified in our LC-/MS/MS experiments. One or 2 peptides per protein target were used in the SRM/MRM analysis. Stable synthetic isotope-labeled peptides (SI peptides) with a C-terminal [$^{13}C_6$, $^{15}N_4$]-arginine or [$^{13}C_6$, $^{15}N_2$]-lysine residue (isotopic purity>99%) were purchased from Greiner Bio One (Frickenhausen, Germany).

First, the SI peptide mixture was analyzed by the above-mentioned LC-MS/MS method using LTQ Orbitrap-XL to acquire MS data. A preliminary SRM/MRM-transition list for SI peptides was created from the MS data acquired using Pinpoint ver.1.0 (Thermo Fisher Scientific). The SI peptide mixture was then analyzed using a TSQ-Vantage triple quadrupole mass spectrometer (Thermo Fisher Scientific) with a nano-LC interface (AMR), a Paradigm MS2 (Michrom Bioresources), and an HTC PAL autosampler (CTC Analytics). The data were analyzed using Pinpoint software to optimize parameters such as collision energy and acquire the retention time of each SI peptide. The timed-SRM/MRM method (retention time window of ±2 minutes) was created using these parameters and was optimized. Finally, 4 optimal transitions per peptide were selected for quantitation using SRM/MRM.

For quantitation using SRM/MRM, a culture supernatant sample after TCA precipitation was solubilized with PTS solution, reduced by DTT, alkylated with iodoacetamide, and then digested with trypsin as described above. The digested peptide (2 μg) was dissolved in acetonitrile and trifluoroacetic acid. The SI peptide mixture was added to the trypsin-digested sample and analyzed using the above-described optimal timed-SRM/MRM method with TSQ-Vantage. The area ratio of the endogenous peptide to the SI peptide was calculated using the transition peak area measured with Pinpoint software. The average of these ratios for more than 2 transitions was first calculated, and then the average ratio of 2 technical replicates of an individual sample was determined as the relative quantitative value of the target peptide. Statistical analysis of the area ratios was performed using the *t* test.

Enzyme-linked immunosorbent assay (ELISA)

Serum concentrations of galectin-7 were assayed using the galectin-7 Human ELISA Kit (Abcam, Cambridge, UK) according to the manufacturer's instructions. The minimum detectable dose of galectin-7 was 20 pg/mL. Other ELISA kits were human annexin A3 (LSBio, Seattle, Wash), NGAL (Cloud-Clone, Katy, Tex), CALML5 (Cloud-Clone), IL-36G (Sigma-Aldrich, St. Louis, Mo), and LL-37 (MyBioSource, San Diego, Calif).

Statistical analysis

Receiver operating characteristic curve analysis was performed to determine a cutoff value for galectin-7 using SPSS software (ver.25; IBM Corp., Armonk, NY). The cutoff point was determined by Youden's index.

TABLE E1. Number of proteins in supernatant identified by mass spectrometry analysis

SJS/TEN			Nonsevere cADR		
No. 1	No. 2	No. 3	No. 1	No. 2	No. 3
390	389	278	475	227	349
Total 510 (including overlap)			Total 529 (including overlap)		

cADR, Cutaneous adverse drug reaction; *SJS*, Stevens-Johnson syndrome; *TEN*, toxic epidermal necrolysis.

TABLE E2. Proteins in causative drug-exposed peripheral blood mononuclear cells using SRM/MRM

Protein	Peptide	SJS/TEN1	SJS/TEN2	SJS/TEN3	SJS/TEN4	Nonsevere cADR1	Nonsevere cADR2	Nonsevere cADR3	Nonsevere cADR4	SJS/TEN average	Nonsevere cADR average	SJS/TEN/ nonsevere cADR	*t*-test
Neutrophil gelatinase-associated lipocalin	VPLQQNFQDNQFQGK	3.33	0.12	0.23	2.41	0.22	0.26	0.31	0.48	1.52	0.32	4.82	0.183
Cathelicidin antimicrobial peptide	SSDANLYR	1.48	0.10	0.19	1.92	0.07	0.19	0.18	0.33	0.92	0.19	4.74	0.166
Protein S100-A7	GTNYLADVFEK	95.43	4.28	4.69	7.21	9.58	2.44	6.74	5.66	27.90	6.11	4.57	0.372
Interleukin-36 gamma	SDSVTPVTVAVITCK	3.44	0.23	0.37	0.56	0.44	0.16	0.35	0.21	1.15	0.29	3.96	0.305
Calmodulin-like protein 5	NLSEAQLR	1.69	0.11	0.20	0.36	0.13	0.07	0.28	0.14	0.59	0.16	3.81	0.286
Annexin A3	DYPDFSPSVDAEAIQK	0.18	0.08	0.75	0.43	0.06	0.06	0.13	0.17	0.36	0.10	3.47	0.138
Galectin-7	LDTSEVVFNSK	4.09	0.29	0.73	0.62	0.39	0.13	0.70	0.46	1.43	0.42	3.40	0.304
Annexin A3	SEIDLLDIR	0.28	0.11	1.00	0.61	0.08	0.08	0.19	0.26	0.50	0.15	3.29	0.133
Interleukin-36 gamma	DQPIILTSELGK	10.18	0.83	1.06	1.66	1.65	0.58	1.52	0.83	3.43	1.15	2.99	0.353
Galectin-7	AVVGDAQYHHFR	0.33	0.04	0.06	0.07	0.04	0.03	0.05	0.05	0.12	0.04	2.83	0.295
Heat shock protein beta-1	QLSSGVSEIR	2.12	0.96	0.77	0.86	0.78	0.23	0.65	0.54	1.18	0.55	2.14	0.112
Plakophilin-1	LLQSGNSDVVR	0.80	0.86	0.56	0.63	0.25	0.29	0.46	0.41	0.71	0.35	2.04	0.005
Heat shock protein beta-1	VSLDVNHFAPDELTVK	1.01	0.28	0.35	0.30	0.25	0.11	0.36	0.27	0.48	0.25	1.94	0.251
Serpin B12	IGFIEEVK	0.80	0.26	0.25	0.23	0.26	0.11	0.25	0.19	0.39	0.20	1.92	0.244
Interferon-induced guanylate-binding protein 1	SYQEHLK	1.17	6.20	5.31	2.87	2.10	2.57	1.02	2.46	3.89	2.04	1.91	0.173
Fascin	VTGTLDANR	0.08	0.15	0.96	0.19	0.21	0.15	0.31	0.06	0.34	0.18	1.91	0.472
Plakophilin-1	SPNQNVQQAAAGALR	0.65	0.61	0.47	0.41	0.24	0.22	0.29	0.37	0.53	0.28	1.90	0.009
Fascin	YSVQTADHR	0.45	1.07	7.04	1.22	1.55	1.27	2.15	0.42	2.44	1.35	1.81	0.515
Serpin B12	ADLTGISPSPNLYLSK	0.65	0.17	0.21	0.22	0.21	0.10	0.22	0.17	0.31	0.18	1.79	0.282
Interferon-induced guanylate-binding protein 1	LQEQEQLLK	0.49	2.81	2.00	1.41	0.86	1.35	0.43	1.15	1.68	0.95	1.77	0.215
Protein POF1B	NLEQENQNLR	0.63	0.22	0.20	0.24	0.13	0.13	0.21	0.26	0.32	0.18	1.76	0.246
Carbonic anhydrase 1	GGPFSDSYR	59.47	0.42	0.17	1.35	1.33	0.65	1.13	32.87	15.35	8.99	1.71	0.717
Testican-2	IQIQEAAK	0.45	0.77	0.16		0.25	0.16	0.36	0.31	0.46	0.27	1.70	0.278
Gasdermin-A	VEGDVDVPK	0.62	0.16	0.17	0.21	0.22	0.13	0.22	0.15	0.29	0.18	1.60	0.371
Carbonic anhydrase 1	ESISVSSEQLAQFR	99.07	0.62	0.21	2.48	2.11	0.78	1.63	59.91	25.59	16.11	1.59	0.751
Ribonuclease T2	ELDLNSVLLK	0.16	0.08	0.18	0.19	0.12	0.09	0.11	0.06	0.15	0.09	1.59	0.105
Zinc finger CCCH domain-containing protein 18	ASDLEDEESAAR	0.09	0.14	0.13	0.20	0.06	0.10	0.11	0.11	0.14	0.09	1.48	0.132

Zinc finger CCCH domain-containing protein 18	SQDQDSEVNELSR	0.30	0.42	0.41	0.51	0.14	0.31	0.31	0.37	0.41	0.28	1.46	0.101
Ribonuclease T2	HGTCAAQVDALNSQK	0.02	0.01	0.02	0.03	0.02	0.01	0.01		0.02	0.01	1.43	0.164
Testican-2	LEQQACLSSK	0.38	0.70	0.15	0.07	0.23	0.14	0.29	0.27	0.32	0.23	1.39	0.555
Gasdermin-A	ALETVQER	1.36	0.41	0.40	0.47	0.53	0.31	0.68	0.42	0.66	0.48	1.36	0.507
Cytochrome c oxidase subunit 6B1	GGDISVCEWYQR	3.23	3.35	3.17	8.18	1.61	2.28	4.68	5.66	4.48	3.56	1.26	0.577
Cytochrome c oxidase subunit 6B1	NCWQNYLDFHR	0.22	0.25	0.25	0.65	0.15	0.17	0.38	0.41	0.34	0.28	1.24	0.613
Thyroid hormone receptor-associated protein 3	GSFSDTGLGDGK	1.45	2.13	2.18	2.80	0.83	2.15	1.30	2.86	2.14	1.78	1.20	0.521
Thimet oligopeptidase	DAASGEVVGK	0.93	0.86	1.08	1.27	0.71	1.19	0.76	0.82	1.04	0.87	1.19	0.288
Serine/arginine-rich splicing factor 2	VGDVYIPR	2.80	4.34	2.47	4.21	2.00	3.57	1.55	5.10	3.46	3.05	1.13	0.683
14 kDa phosphohistidine phosphatase	WAEYHADIYDK		0.03	0.02	0.03	0.02	0.03	0.02	0.02	0.02	0.02	1.13	0.521
Fructose-1,6-bisphosphatase isozyme 2	IYSLNEGYAK	1.02	7.64	13.85	13.09	17.36	7.19	6.25	1.32	8.90	8.03	1.11	0.853
Thyroid hormone receptor-associated protein 3	ASAVSELSPR	0.21	0.52	0.62	0.61	0.14	0.48	0.27	0.94	0.49	0.46	1.06	0.891
Spectrin beta chain, non-erythrocytic 1	LLQLTEK	0.68	0.55	1.26	0.86	0.24	0.44	1.41	1.08	0.84	0.79	1.06	0.891
RNA-binding protein 8A	GFGSEEGSR	0.49	0.52	0.65	0.69	0.31	0.89	0.44	0.61	0.59	0.56	1.04	0.858
Splicing factor 3A subunit 1	QSDDEVYAPGLDIESSLK	0.27	0.32	0.21	0.47	0.21	0.43	0.24	0.36	0.32	0.31	1.03	0.921
Zinc finger CCCH-type antiviral protein 1	ATDLGGTSQAGTSQR	0.06	0.31	0.13	0.29	0.09	0.20	0.10	0.40	0.20	0.20	0.99	0.988
Heterogeneous nuclear ribonucleoprotein F	VHIEIGPDGR	3.53	10.10	2.91	5.45	5.07	7.44	4.41	5.47	5.50	5.60	0.98	0.958
Zinc finger CCCH-type antiviral protein 1	ASLEDAPVDDLTR	1.05	5.18	2.42	4.62	1.40	3.15	1.42	7.56	3.32	3.38	0.98	0.971
Serine/threonine-protein kinase 10	ALEEDLNQK	0.34	1.27	1.16	1.72	0.64	1.57	0.56	1.88	1.12	1.16	0.97	0.933
Putative protein FAM10A4	VAAIEALNDGELQK	0.10	0.12	0.05	0.10	0.07	0.16	0.07	0.09	0.09	0.10	0.96	0.890
Basigin	FFVSSSQGR	0.41	0.98	0.82	1.27	0.94	1.03	1.17	0.52	0.87	0.91	0.95	0.849

(continued)

TABLE E2. *(Continued)*

Protein	Peptide	SJS/TEN1	SJS/TEN2	SJS/TEN3	SJS/TEN4	Nonsevere cADR1	Nonsevere cADR2	Nonsevere cADR3	Nonsevere cADR4	SJS/TEN average	Nonsevere cADR average	SJS/TEN/ nonsevere cADR	*t*-test
Secernin-1	AIIESDQEQGR	0.72	1.22	0.91	1.67	1.47	0.91	1.24	1.18	1.13	1.20	0.94	0.781
Chromobox protein homolog 1	NSDEADLVPAK	0.95	0.78	0.60	0.84	0.66	1.23	0.74	0.82	0.79	0.86	0.92	0.643
Ras-related protein Rab-10	AFLTLAEDILR	0.26	0.72	0.25	0.44	0.40	0.71	0.30	0.43	0.42	0.46	0.91	0.780
Putative protein FAM10A4	AIDLFTDAIK	1.24	2.07	1.13	1.59	1.17	3.05	1.14	1.26	1.51	1.66	0.91	0.780
Small nuclear ribonucleoprotein Sm D3	VAQLEQVYIR	2.26	3.22	4.02	3.87	1.88	6.35	1.47	5.25	3.34	3.74	0.89	0.768
Heterogeneous nuclear ribonucleoprotein F	ATENDIYNFFSPLNPVR	0.96	2.76	0.99	1.60	1.52	2.78	1.10	1.71	1.58	1.78	0.89	0.727
Echinoderm microtubule-associated protein-like 4	VQQQEDEITVLK	0.22	0.74	0.28	0.42	0.48	0.72	0.24	0.44	0.41	0.47	0.88	0.722
Lysosomal alpha-glucosidase	VTSEGAGLQLQK	0.83	2.59	1.74	2.08	4.46	1.50	1.62	0.67	1.81	2.06	0.88	0.790
Lysosomal alpha-glucosidase	AGYIIPLQGPGLTTTESR	0.32	1.05	0.65	0.77	1.63	0.78	0.60	0.22	0.70	0.81	0.87	0.760
Serine/threonine-protein kinase 10	IQVAQEK	0.35	0.60	0.60	0.87	0.49	0.95	0.61	0.77	0.60	0.71	0.86	0.514
FYN-binding protein	SGPTPPTSENEQK	0.32	0.43	0.52	0.51	0.28	0.89	0.29	0.63	0.45	0.52	0.86	0.641
Phosphoglycerate mutase 2	VLIAAHGNSLR	1.87	4.22	5.25	8.61	6.13	8.90	3.93	4.56	4.99	5.88	0.85	0.636
Ras-related protein Rab-10	NIDEHANEDVER	0.20	0.40	0.20	0.24	0.33	0.35	0.21	0.34	0.26	0.31	0.85	0.447
Basigin	GSDQAIITLR	0.34	0.56	0.52	0.69	0.71	0.74	0.84	0.47	0.53	0.69	0.76	0.177
Eukaryotic translation initiation factor 5A-1	VHLVGIDIFTGK	2.51	3.61	3.47	2.72	3.04	7.77	2.79	3.28	3.08	4.22	0.73	0.386
Leukosialin	TGALVLSR	0.57	0.57	0.85	1.02	0.82	0.96	1.33	1.03	0.75	1.04	0.73	0.114
FYN-binding protein	TTAVEIDYDSLK	1.07	1.15	0.76	0.79	0.82	2.49	1.07	1.85	0.94	1.56	0.61	0.168
Echinoderm microtubule-associated protein-like 4	WFVLDAETR	0.15	0.26	0.20	0.16	0.44	0.61	0.13	0.29	0.19	0.37	0.52	0.146
Histone H2B type 1-O	ESYSIYVYK	0.16	0.37	2.50	0.19	0.18	3.43	0.10	6.30	0.80	2.50	0.32	0.326

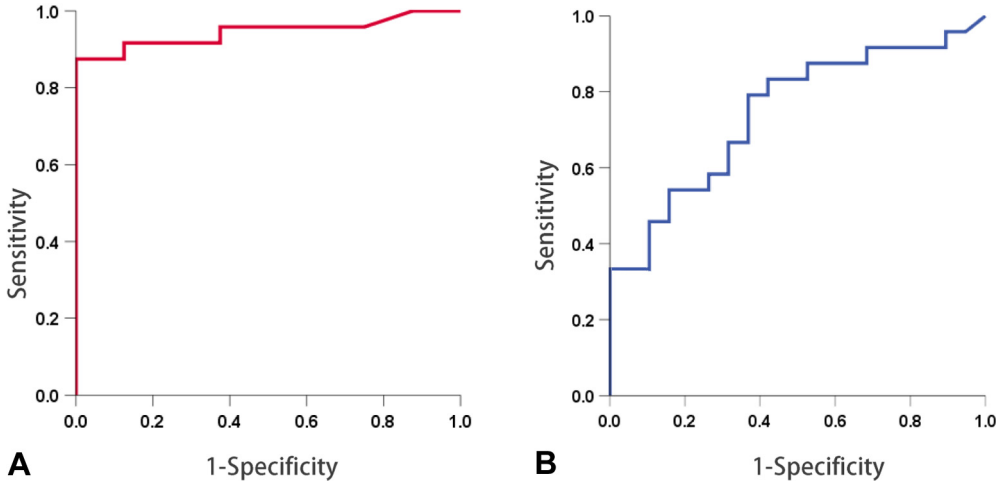

FIGURE E1. ROC curves for normal control versus SJS/TEN and nonsevere cADR versus SJS/TEN. **A**, From the ROC curve for normal control vs SJS/TEN, an AUC of 0.945 and a cutoff point of 1.90 ng/mL are found. **B**, From the ROC curve for nonsevere cADR vs SJS/TEN, an AUC of 0.745 and a cutoff point of 5.08 ng/mL are found. *AUC*, Area under the curve; *cADR*, cutaneous adverse drug reaction; *ROC*, receiver operating characteristic; *SJS*, Stevens-Johnson syndrome; *TEN*, toxic epidermal necrolysis.

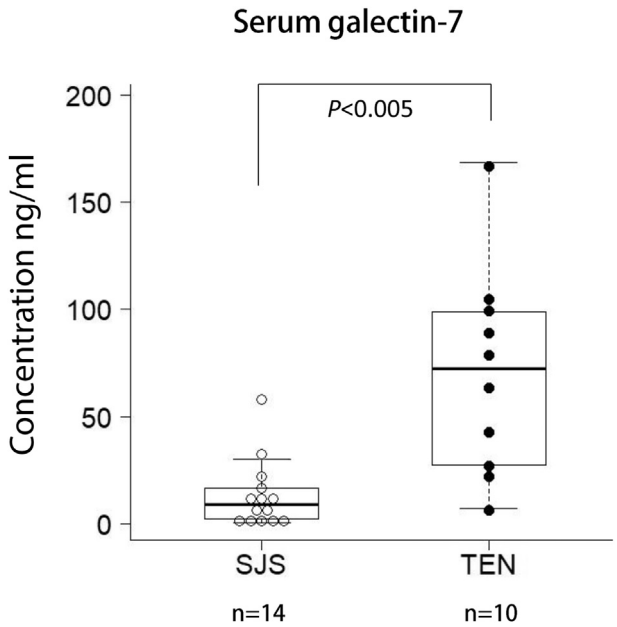

FIGURE E2. The serum galectin-7 levels for TEN and SJS. The serum galectin-7 levels are significantly higher for the TEN cases than for the SJS cases ($P < .005$). *SJS*, Stevens-Johnson syndrome; *TEN*, toxic epidermal necrolysis.

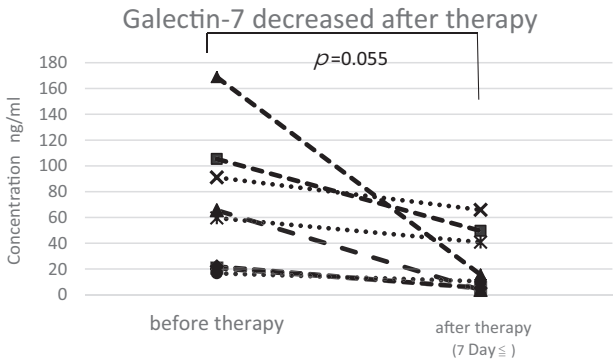

FIGURE E3. The course of serum galectin-7 level for each patient with SJS/TEN. In every case, the serum galectin-7 level is lower in the later phase (7 days or more after onset) than in the early phase (disease onset), regardless of whether they are high or low in the early phase. Furthermore, all cases were treated by immunosuppressive therapy including high-dose corticosteroid. *SJS*, Stevens-Johnson syndrome; *TEN*, toxic epidermal necrolysis.

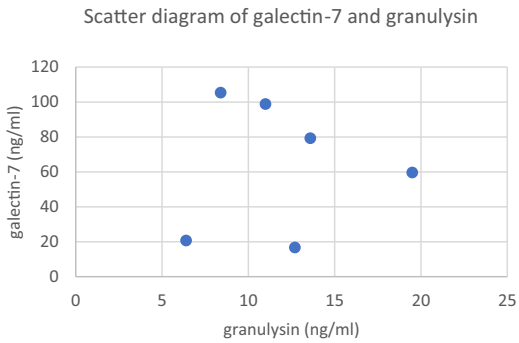

FIGURE E4. Scatter diagram of galectin-7 and granulysin. The relationship between galectin-7 and another SJS/TEN marker, granulysin, was investigated. A scatter diagram of patients with SJS/TEN (n = 6) shows no positive correlation between galectin-7 and granulysin ($r = 0.019$, $P = .51$). In addition, only 1 of 6 SJS/TEN cases shows a high level of granulysin (over 10 ng/mL) and a low level of galectin-7. *SJS*, Stevens-Johnson syndrome; *TEN*, toxic epidermal necrolysis.

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