Original Article

Serum Soluble OX40 as a Diagnostic and Prognostic Biomarker for Drug-Induced Hypersensitivity Syndrome/Drug Reaction with Eosinophilia and Systemic Symptoms

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What is already known about this topic? Drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DIHS/DRESS) is a severe drug reaction commonly associated with human herpesvirus 6 (HHV-6) reactivation. OX40 is involved in both T_H2-type inflammatory response and HHV-6 replication.

What does this article add to our knowledge? Serum soluble OX40 (sOX40) levels in patients with DIHS/DRESS in acute stage were elevated and significantly positively correlated with disease severity. In patients with DIHS/DRESS with HHV-6 reactivation, serum sOX40 levels correlated with peak HHV-6 viral loads.

How does this study impact current management guidelines? Serum sOX40 levels can be a useful diagnostic and prognostic marker for DIHS/DRESS. Elevated levels of serum sOX40 can also predict the extent of HHV-6 reactivation.

BACKGROUND: Drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DIHS/DRESS) is a severe adverse drug reaction commonly associated with the reactivation of human herpesvirus 6 (HHV-6). There are currently no adequate biomarkers for the early diagnosis and detection of DIHS/DRESS. Notably, OX40 (CD134) has an important role in allergic inflammation and functions as a cellular receptor for HHV-6 entry. We previously reported that the membrane-bound form of OX40 in $CD4^+$ T cells was upregulated in DIHS/DRESS.

OBJECTIVE: We sought to investigate the clinical significance of serum soluble OX40 (sOX40) in DIHS/DRESS.

METHODS: Serum sOX40 levels in patients with DIHS/DRESS (n = 39), maculopapular exanthema/erythema multiforme (n = 17), Stevens-Johnson syndrome/toxic epidermal necrolysis (n = 13), or autoimmune bullous diseases (n = 5), and levels in healthy volunteers (n = 5) were examined by enzyme-linked immunosorbent assay. Copy numbers of HHV-6, HHV-7, and cytomegalovirus in peripheral blood mononuclear cells were quantified using real-time PCR.

RESULTS: Serum sOX40 levels in patients with DIHS/DRESS in the acute stage were elevated in parallel with high OX40 expression on $CD4^+$ T cells. Serum sOX40 levels were significantly positively correlated with disease severity and serum levels of thymus and activation-regulated chemokine, IL-5, and IL-10. Human herpesvirus 6–positive patients had higher sOX40 levels than did HHV-6–negative patients, and serum sOX40 levels were correlated with HHV-6 DNA loads.

CONCLUSIONS: Serum sOX40 levels can be a useful diagnostic marker for DIHS/DRESS that reflect disease severity. Elevated serum sOX40 levels also predict HHV-6 reactivation in patients with DIHS/DRESS. © 2021 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2022;10:558-65)

Key words: *CD134; DIHS; DRESS; HHV-6; OX40; Thymus and activation-regulated chemokine*

INTRODUCTION

Drug-induced hypersensitivity syndrome (DIHS)/drug reaction with eosinophilia and systemic symptoms (DRESS) is a severe adverse drug reaction characterized by fever, generalized maculopapular erythema, liver dysfunction, and hematologic

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Abbreviations used
CMV- Cytomegalovirus
DIHS- Drug-induced hypersensitivity syndrome
DRESS- Drug reaction with eosinophilia and systemic symptoms
EM- Erythema multiforme
HHV- Human herpesvirus
MPE- Maculopapular exanthema
PBMC- Peripheral blood mononuclear cell
SJS- Stevens-Johnson syndrome
sOX40- Soluble OX40
TARC- Thymus and activation-regulated chemokine
TEN- Toxic epidermal necrolysis
T_{reg}- Regulatory T cell

abnormalities,[1] with a mortality rate of 5% to 10%.[2,3] These symptoms occur 2 to 6 weeks after the administration of a specific drug, such as carbamazepine, allopurinol, or salazosulfapyridine. In the case of antiepileptic drugs, it occurs in approximately one in 3,000 drug exposures.[4] It is usually associated with the reactivation of human herpesviruses (HHVs), including HHV-6 and HHV-7, and cytomegalovirus (CMV).[5,6] In particular, HHV-6 reactivation is detected in the vast majority of cases and is associated with the severity of DIHS/DRESS.[7,8] Systemic corticosteroids are the main treatment for DIHS/DRESS, although the optimal dose has not been established.[9,10] Because clinical manifestation in the early stage of DIHS/DRESS can resemble other, more common drug-induced reactions, the diagnosis is often delayed, leading to multiple organ failure and death.[2,3] It is therefore necessary to identify early diagnostic markers for this condition.

Human herpesvirus-6 latently infects monocytes and macrophages, whereas during the proliferation phase, it preferentially infects activated $CD4^+$ T cells to produce many copies of the viral genome.[11] OX40, also known as CD134, is a member of the TNF receptor superfamily. OX40 is expressed predominantly on activated lymphocytes, particularly $CD4^+$ T cells, and functions as a cellular receptor for HHV-6 entry.[12] The OX40 ligand (OX40L) is expressed on antigen-presenting cells and activated T cells.[13] Interaction of OX40-OX40L is required to generate long-term memory response, optimal T-cell activation, and T_H2 differentiation.[14-16] We have shown that OX40 and OX40L are upregulated in $CD4^+$ T cells and peripheral blood mononuclear cells (PBMCs), respectively, in patients with DIHS/DRESS. This suggests that OX40—OX40L interaction may be involved in activating $CD4^+$ T cells and initiating DIHS/DRESS.[17,18]

In a manner similar to that of other members of the TNF receptor superfamily, soluble OX40 (sOX40) may be produced by OX40 shedding, alternative splicing, or both.[19] Soluble OX40 has anti-inflammatory effects antagonistic to those of the membrane-bound form of OX40L, and it inhibits the inflammatory response, mimicking regulatory T-cell function.[20] Serum sOX40 levels are abnormal in various autoimmune and inflammatory diseases such as systemic sclerosis,[21] rheumatoid arthritis,[22] and atopic dermatitis,[23] although the role of sOX40 in these pathologies is unknown. In contrast, sOX40 levels have not been investigated in patients with DIHS/DRESS, although sOX40 may be involved in the pathophysiology of DIHS/DRESS, such as in HHV-6 reactivation and T_H2-type response. In this study, we focused on serum sOX40 levels and performed a retrospective longitudinal study in patients with DIHS/DRESS. Our data suggest that serum sOX40 levels may serve as a biomarker for early diagnosis and severity in DIHS/DRESS.

METHODS

Blood samples

We obtained blood samples from patients with DIHS/DRESS (n = 39), maculopapular exanthema (MPE)/erythema multiforme (EM) (n = 17), Stevens-Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN) (n = 13), and autoimmune bullous diseases (n = 5), as well as from healthy volunteers (n = 5), between October 2009 and July 2020 (see Table E1 in this article's Online Repository at www.jaci-inpractice.org). Drug-induced hypersensitivity syndrome (including atypical DIHS), DRESS (including possible, probable, and definite DRESS), SJS, overlapping SJS-TEN, and TEN were diagnosed based on previously described criteria.[24-26] Blood samples in the acute stage were taken an average of 7.7 days (range, 1-18 days) after the onset of skin eruption in DIHS/DRESS, 4.1 days (range, 1-13 days) in MPE/EM, and 7.2 days (range, 3-14 days) after the onset of SJS/TEN. Patients who visited our department more than 20 days after the onset of disease were excluded.

For a retrospective longitudinal study, we collected 79 samples from 22 patients with DIHS/DRESS. These patients had been observed for up to 32.5 ± 2.2 days (range, 19-58 days) after onset; each patient was tested two to four times during follow-up.

This study was approved by the Ethics Committee of Nara Medical University (Reference No. 1753). All studies using human materials were performed according to the principles of the Declaration of Helsinki. We obtained informed consent through the opt-out method because of the retrospective observational nature of this study.

Enzyme-linked immunosorbent assay and chemiluminescent enzyme immunoassay

We measured serum sOX40 levels with a human CD134/OX40 enzyme-linked immunosorbent assay (ELISA) kit (IBL, Gunma, Japan). Serum IL-5 and IL-10 levels were evaluated using commercial ELISA kits from R&D Systems (Minneapolis, Minn) and MyBioSource (San Diego, Calif), respectively. Serum levels of thymus and activation-regulated chemokine (TARC) (CCL17) (Sysmex Co, Kobe, Japan), IL-4 (BD Pharmingen, San Diego, Calif), and IFN-gamma (Thermo Fisher Scientific, Indianapolis, Ind) were analyzed by chemiluminescent enzyme immunoassay according to the manufacturers' protocols.

Flow cytometry

We analyzed the expression of OX40 on $CD4^+$ T cells in DIHS/DRESS using a FACSCalibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ) and CellQuest software (Becton Dickinson) as previously reported.[17,18] Peripheral blood mononuclear cells were isolated from whole blood by Ficoll density gradient centrifugation (GE Healthcare, Little Chalfont, UK) and stored at −80°C until use. To stain the cell surface of PBMCs, we used fluorescein isothiocyanate-conjugated anti-OX40 (clone ACT35, 1:10 dilution), phycoerythrin-conjugated anti-CD3 (UCHT1, 1:10), and

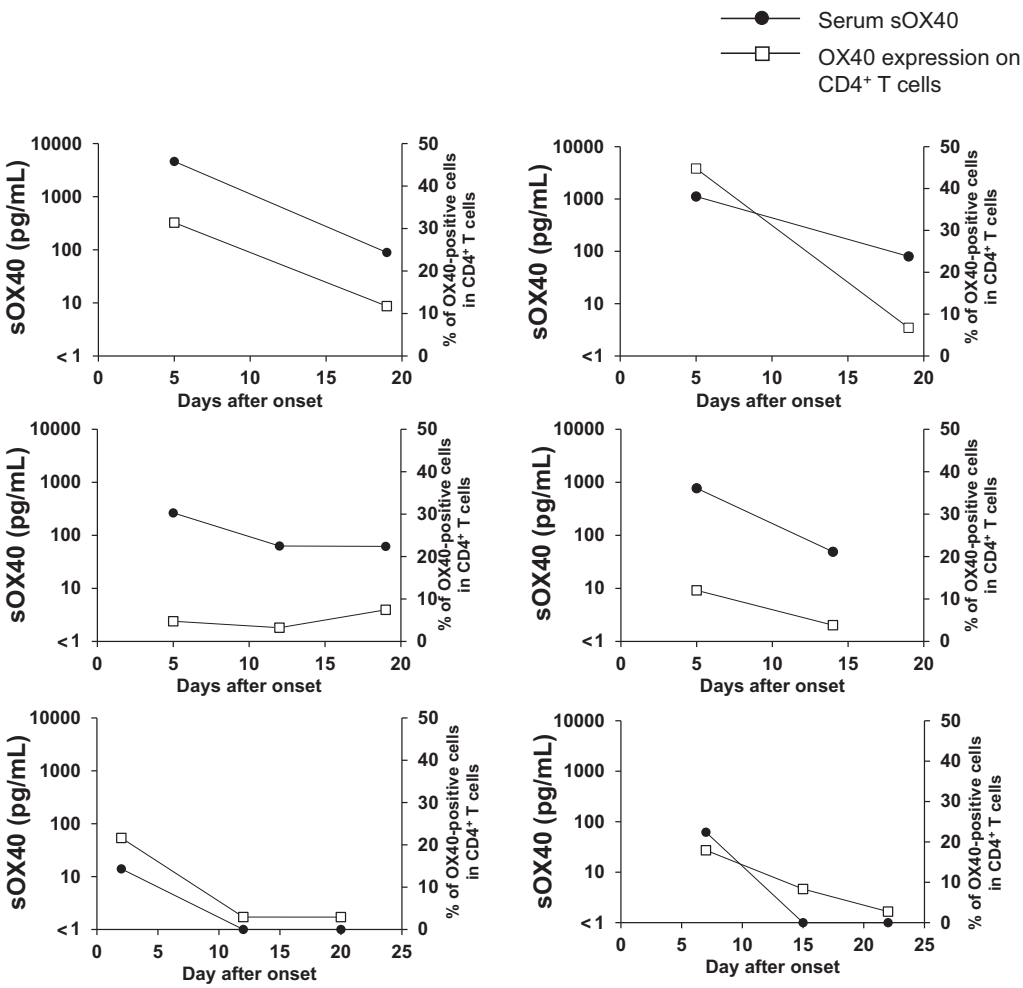

FIGURE 1. Time course of changes in serum soluble OX40 (sOX40) levels and cell surface expression of OX40 on $CD4^+$ T cells in six patients with drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms.

allophycocyanin-conjugated anti-CD4 (RPA-T4, 1:10) antibodies (all from BD Pharmingen).

Real-time polymerase chain reaction for quantitative analysis of HHV-6, HHV-7, and CMV DNA

We examined HHV-6, HHV-7, and CMV DNA copy numbers in PBMCs using real-time polymerase chain reaction (PCR), according to the methods described by Watzinger et al,[27] with some modifications. Peripheral blood was periodically obtained from the acute to the remission stage. We extracted DNA using a QIAamp DNA Blood mini-kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. All reactions were set up as singleplex PCRs at a total volume of 25 μL, containing 12.5 μL TaqMan Fast Advanced Master Mix (Thermo Fisher Scientific), primers at concentrations of 300 to 900 nM, and a 200-nM TaqMan probe (see Table E2 in this article's Online Repository at www.jaci-inpractice.org). The mixtures were amplified on a ABI StepOne real-time PCR system using the cycling parameters: 2 minutes at 50°C, 20 seconds at 95°C, and 53 cycles of 1 second at 95°C and 20 seconds at 60°C.

Scoring severity of DIHS/DRESS

We used the previously described DIHS/DRESS scoring system.[28] Clinical history, physical findings, and blood test results essential for scoring were retrospectively retrieved from the medical records.

Statistical analysis

We performed statistical analyses using EZR software (version 1.54, Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface for R (R Foundation for Statistical Computing, Vienna, Austria).[29] Mann-Whitney U test was applied to compare serum sOX40 levels between two independent groups. Correlations between serum sOX40 levels and various parameters, including cytokine levels and the severity score of DIHS/DRESS, were evaluated with Spearman rank correlation coefficient. Wilcoxon signed rank test was used to compare sOX40 levels in patients with DIHS/DRESS during acute and late phases. In all tests, effects were considered statistically significant when P was less than .05. Data are presented as the means ± standard errors of the mean. Receiver operating characteristic curves were plotted using SPSS software (version 28.0, IBM, Armonk, NY). The cutoff point was obtained

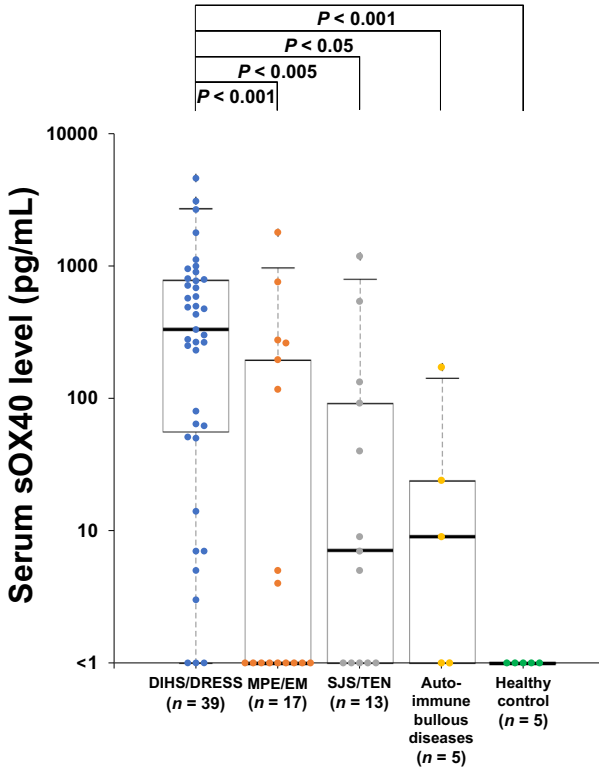

FIGURE 2. Serum soluble OX40 (sOX40) levels in 39 patients with drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DIHS/DRESS), 17 with maculopapular exanthema/erythema multiforme (MPE/EM), 13 with Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN), and five with autoimmune bullous diseases, and in five healthy controls.

according to the maximum Youden's index (sensitivity + specificity − 1).

RESULTS

Serum sOX40 level is proportional to fraction of OX40-expressing CD4$^+$ T cells in patients with DIHS/DRESS

We investigated the correlation between the percentage of OX40-expressing CD4$^+$ T cells and serum sOX40 levels using blood samples from six patients with DIHS/DRESS collected in the acute and late phases of the disease. In this analysis, we used flow cytometry data described elsewhere.[17] In all six patients, the percentage of OX40-expressing CD4$^+$ T cells in the acute stage was higher than that in the late stage. In each patient, serum sOX40 levels decreased in parallel with the downregulation of OX40 expression on CD4$^+$ T cells over the course of the disease (Figure 1). We confirmed that serum sOX40 reflects the percentage of OX40-expressing CD4$^+$ T cells in patients with DIHS/DRESS.

Elevated serum sOX40 levels in DIHS/DRESS

We examined whether serum sOX40 levels were elevated in DIHS/DRESS and other diseases. Serum sOX40 levels were significantly higher in patients with DIHS/DRESS in the acute stage (646 ± 151 pg/mL) compared with those in healthy controls ($P < .001$) or in patients with MPE/EM (201 ± 110 pg/mL; $P < .001$), SJS/TEN (155 ± 95 pg/mL; $P < .005$), or autoimmune bullous disease (41 ± 33 pg/mL; $P < .05$) (Figure 2). For the receiver operating characteristic curve for DIHS/DRESS versus MPE/EM, an area under the curve value of 0.781 (95% confidence interval, 0.638-0.923) and a cutoff of 7 pg/mL were obtained; the sensitivity and specificity were 0.872 and 0.647, respectively (see Figure E1, *A* in this article's Online Repository at www.jaci-inpractice.org). For the receiver operating characteristic curve for DIHS/DRESS versus SJS/TEN, an area under the curve value of 0.767 (95% confidence interval, 0.608-0.927) and cut-off of 231 pg/mL were found; the sensitivity and specificity were 0.667 and 0.846, respectively (Figure E1, *B*). There was no significant difference in sOX40 levels between patients with prior systemic steroid treatment (n = 20) and those without it (n = 19) (660 ± 164 pg/mL vs 632 ± 262 pg/mL; $P = .415$). These results indicate that the sOX40 level increases specifically in patients with DIHS/DRESS independent of the preceding treatment, and that sOX40 levels can be useful for distinguishing DIHS/DRESS from other disease groups.

Clinical correlations of serum sOX40 levels in DIHS/DRESS

To determine whether sOX40 is useful as a severity biomarker for DIHS/DRESS, we evaluated correlations between serum sOX40 levels and clinical severity and laboratory test findings. Serum sOX40 levels correlated positively with the severity score ($r_s = 0.527$; $P < .001$).

Previous studies showed that the serum TARC level is elevated in patients with DIHS/DRESS[30] and is associated with severity.[31] We found a positive correlation between sOX40 and TARC ($r_s = 0.597$; $P < .0001$). We next examined the correlations between sOX40 and cytokines that were reported to be elevated in DIHS/DRESS, such as IL-4, IL-5, and IL-10.[32] Serum sOX40 levels correlated positively with IL-5 ($r_s = 0.411$; $P < .05$) and IL-10 levels ($r_s = 0.608$; $P < .01$) but not with that of IL-4 ($r_s = 0.370$; $P = .0575$). There was no correlation between sOX40 and IFN-gamma ($r_s = 0.106$; $P = .606$).

Finally, serum sOX40 levels did not correlate with serum levels of creatinine and alkaline phosphatase, but they correlated with C-reactive protein ($r_s = 0.430$; $P < .01$) (Figure 3).

Longitudinal study of serum sOX40 levels in DIHS/DRESS

Serum levels of sOX40 differ in the acute and chronic phases of some diseases.[21,22] To investigate changes in serum sOX40 levels over time, we performed a longitudinal study of sOX40 levels in 22 patients with DIHS/DRESS from whom we could obtain serum samples over a long period. Serum samples in the acute stage were taken 2 to 18 days after the onset of DIHS/DRESS (average days, 8.7), whereas serum samples in the late stage were obtained at 19 to 58 days after onset (average days, 31.6). The interval between the acute and late stages in 22 patients was 23.5 ± 2.2 days (range, 13-56 days). There was a statistically significant difference between serum sOX40 levels at the acute and late stages ($P < .0005$, Wilcoxon signed rank test)

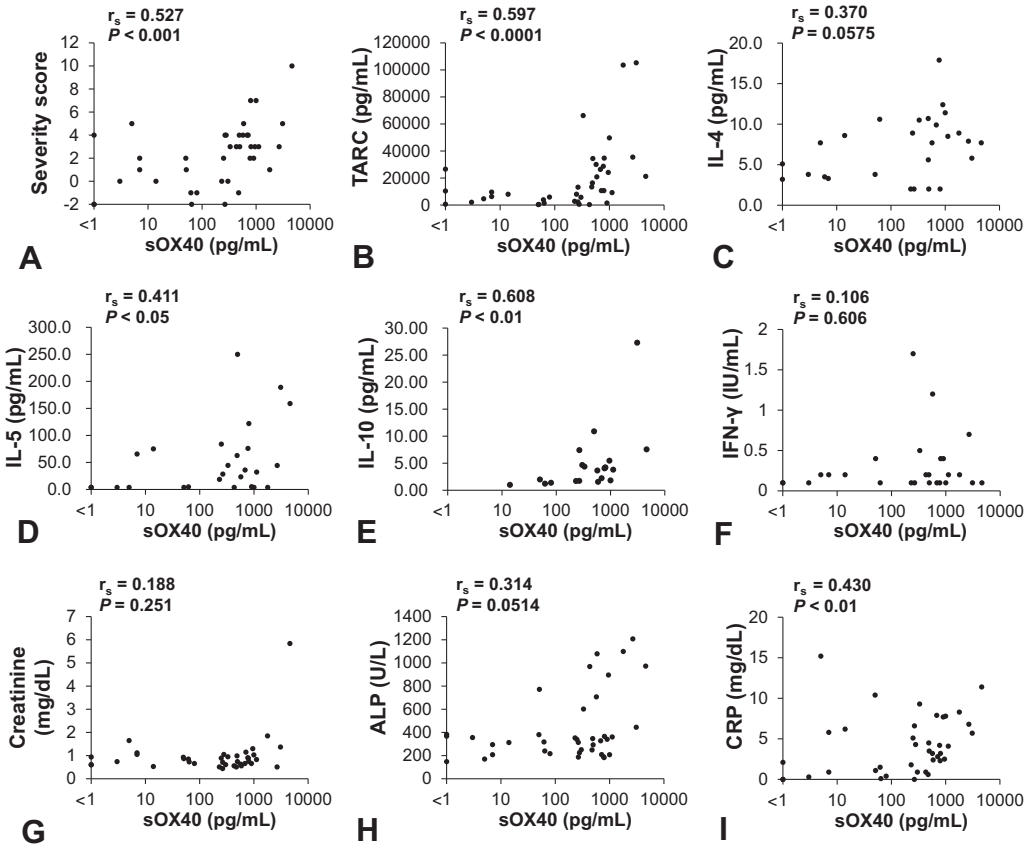

FIGURE 3. Correlations between serum soluble OX40 (sOX40) levels and (**A**) drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms severity score, and (**B**) serum levels of thymus and activation-regulated chemokine (TARC), (**C**) IL-4, (**D**) IL-5, (**E**) IL-10, (**F**) IFN-gamma, (**G**) creatinine, (**H**) alkaline phosphatase (ALP), (**I**) and C-reactive protein (CRP). Statistical analysis was performed by Spearman rank correlation test.

(Figure 4). Of 22 patients, 15 had elevated serum sOX40 levels in the acute phase, which decreased in the late phase. In five patients, serum sOX40 levels were less than 10 pg/mL throughout the acute and late phases. Serum levels in two patients increased in the late phase.

Relationship between reactivation of HHV-6 and serum sOX40 levels

Because OX40 functions as a cellular receptor for HHV-6 entry, we analyzed the correlation between the amount of HHV-6 and the serum sOX40 level. First, we measured sOX40 levels in patients with and without HHV-6 reactivation. Reactivation of HHVs (HHV-6, HHV-7, or CMV) was detected in all 39 patients with DIHS/DRESS. Of these, HHV-6 reactivation was identified in 33 patients. In 32 of these patients, HHV-6 DNA was detected in PBMCs by real-time PCR and one had elevated serum anti-HHV-6 IgG antibodies. Among the six patients without HHV-6 reactivation, two and four were positive for HHV-7 DNA and CMV DNA, respectively. Although the difference was not statistically significant, HHV-6—positive patients tended to have higher sOX40 levels than did HHV-6—negative patients ($P = .267$) (Figure 5, *A*). Furthermore, there was a positive correlation between sOX40 levels and HHV-6 DNA loads in the 32 HHV-6 DNA—positive patients ($r_s = 0.497$; $P < .005$) (Figure 5, *B*).

Kinetics of HHV-6 DNA copy numbers and serum sOX40 levels

Figure 6 shows the kinetics of HHV-6 DNA copy numbers in PBMCs and serum sOX40 levels for the two representative DIHS/DRESS patients with HHV-6 reactivation. In patient 1, serum sOX40 levels peaked on day 5 and HHV-6 reactivation was first observed on day 12. The HHV-6 viral loads increased and peaked on day 19. In patient 2, sOX40 peaked on day 9 and HHV-6 was first reactivated on day 15. The peak of HHV-6 DNA loads occurred on day 22. In both patients, at the peak of serum sOX40 levels, HHV-6 had not yet reactivated. C-Reactive protein appeared to decrease in parallel with sOX40.

In addition to these two patients, 12 others had four measurements of sOX40 during the acute and late phases, as shown in Figure E2 (in this article's Online Repository at www.jaci-inpractice.org). In six of the 12 patients shown in Figure E2, when elevated levels of sOX40 levels were observed, HHV-6 had not yet reactivated (patients 4, 7, 8, 9, 12, and 14)

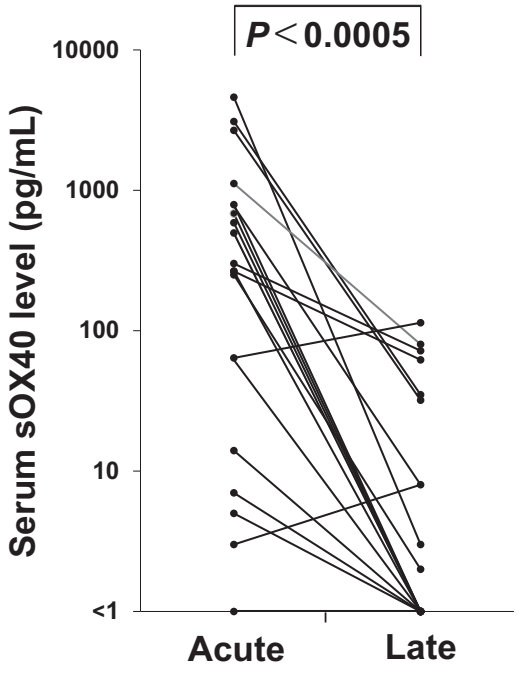

FIGURE 4. Elevated serum levels of soluble OX40 (sOX40) in acute phase of drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms. Blood samples in the acute phase were taken at 2 to 18 days after the onset of drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms, whereas blood samples in the late phase were obtained at 19 to 58 days after onset. Statistical analysis was performed using Wilcoxon signed rank test.

DISCUSSION

It is often difficult to distinguish DIHS/DRESS from other drug eruptions. Because DIHS/DRESS may cause multiple organ failure and death,[2,3] a reliable biomarker of this condition is critically needed. In this study, we observed that serum sOX40 levels were significantly elevated in patients with early-phase DIHS/DRESS compared with those in patients with MPE/EM, SJS/TEN, or autoimmune bullous disease, or in healthy volunteers. Furthermore, serum sOX40 levels were positively correlated with the severity score and C-reactive protein. Of 39 patients with DIHS/DRESS, three patients extremely elevated serum sOX40 died. We previously reported that OX40 expressed on $CD4^+$ T cells can be a useful biomarker for the diagnosis of DIHS/DRESS.[17] However, it is difficult to use the flow cytometry technique in daily clinical practice. In contrast, the serum sOX40 level can be easily measured by ELISA and applied to early diagnosis and prediction of the severity of DIHS/DRESS.

Eosinophils and TARC, one of the T_H2 chemokines, are elevated in the acute phase of DIHS/DRESS, indicating that DIHS/DRESS is associated with T_H2 polarizing conditions.[2,30,33] We previously showed that OX40-expressing $CD4^+$ T cells are more abundant in DIHS/DRESS. The OX40−OX40L interaction enhances T_H2 differentiation.[14] In this study, we revealed that serum sOX40 levels were positively correlated with serum levels of TARC and IL-5, a specific activator of eosinophils,[34] which suggests that upregulation of OX40 and elevated sOX40 levels may induce T_H2 polarization in DIHS/DRESS. In addition to T_H2 cells, regulatory T cells (Treg) are preferentially increased in the peripheral blood of patients with DIHS/DRESS at the acute stage.[35] We previously demonstrated that about 10% of OX40-expressing $CD4^+$ T cells express FoxP3, a Treg cell marker, in the early phase of DIHS/DRESS.[17] In the current study, we revealed a correlation between serum levels of sOX40 and IL-10, an immunosuppressive cytokine. Although it was not possible to establish the relative amounts of sOX40 and IL-10 that originated from T_H2 cells, Treg cells, or other cells, the current data suggest that sOX40 might be involved in Treg responses as well as T_H2.

Activated $CD4^+$ T cells are the preferential target of the fully permissive infection of HHV-6.[11] Although T cells are normally nonpermissive for HHV-6 infection, they became highly susceptible to such infection when OX40 was overexpressed.[12] Actually, serum sOX40 levels in DIHS/DRESS patients with HHV-6 reactivation tended to be higher than in patients without HHV-6 reactivation, which suggests that OX40 may have an important role in HHV-6 reactivation and proliferation. Besides DIHS, HHV-6 occasionally causes encephalitis upon reactivation after allogeneic hematopoietic stem cell transplantation (allo-HSCT). In patients who underwent allo-HSCT, upregulation of OX40 expression on T cells coincided with the time of the peak viral load.[36] In this study, by analyzing the kinetics of HHV-6 DNA copies in PBMCs and serum sOX40 levels in two DIHS/DRESS patients with HHV-6 reactivation, we found that serum sOX40 levels were elevated before the reactivation of HHV-6. The reason for the difference in HHV-6 viral load and serum sOX40 kinetics between patients who underwent allo-HSCT and those with DIHS/DRESS remains unclear. However, unlike in patients who underwent allo-HSCT, it is possible that in patients with DIHS, the administration of causative drugs activates T cells that express OX40, resulting in the stimulation of HHV-6 replication.

This study had some limitations. First, this was a single-center study with a small sample size. Second, in the DIHS/DRESS group, the period set as the acute stage was longer (1-18 days after onset) than that in the MPE/EM group (1-13 days) and SJS/TEN group (3-14 days). Third, the characteristics of patients and healthy volunteers, such as age and sex, differed among groups. A multicenter study with a larger sample size will be necessary to confirm our findings.

In conclusion, this study analyzed serum sOX40 levels in patients with DIHS/DRESS. The serum sOX40 level can be a useful marker for the early diagnosis of DIHS/DRESS as well as for prediction of the severity and presence of HHV-6 reactivation. Soluble OX40 may be involved in both T_H2-type inflammatory process and HHV-6 replication. Our study also contributes to a better understanding of the pathogenesis of DIHS/DRESS.

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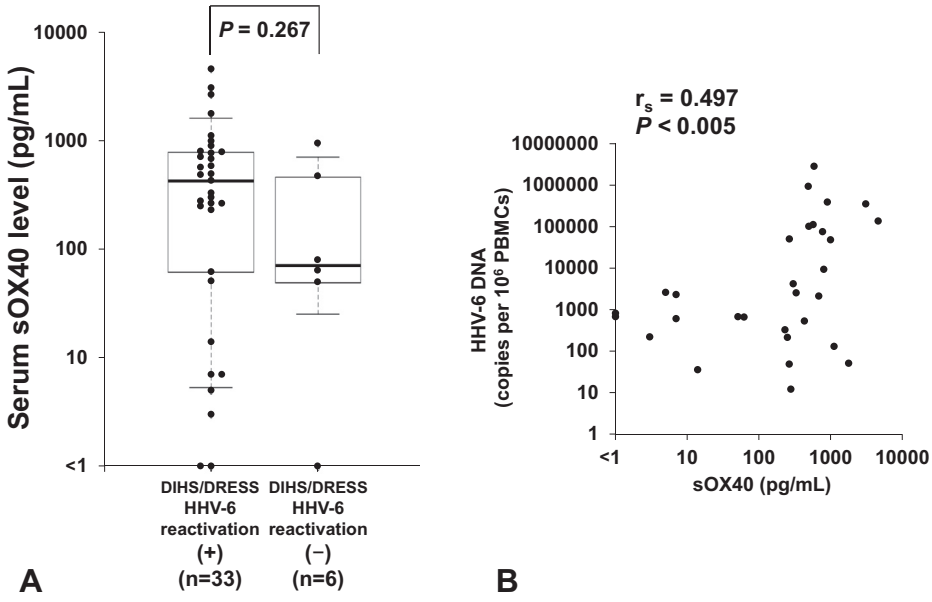

FIGURE 5. Relationship between serum soluble OX40 (sOX40) levels and reactivation of human herpesviruses (HHVs) in patients with drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DIHS/DRESS). (**A**) Serum sOX40 level in 33 patients with HHV-6 reactivation tended to be higher than in the six patients without HHV-6 reactivation ($P = .267$). (**B**) Correlation between serum sOX40 level and peak HHV-6 DNA copy number in HHV-6−positive patients with DIHS/DRESS.

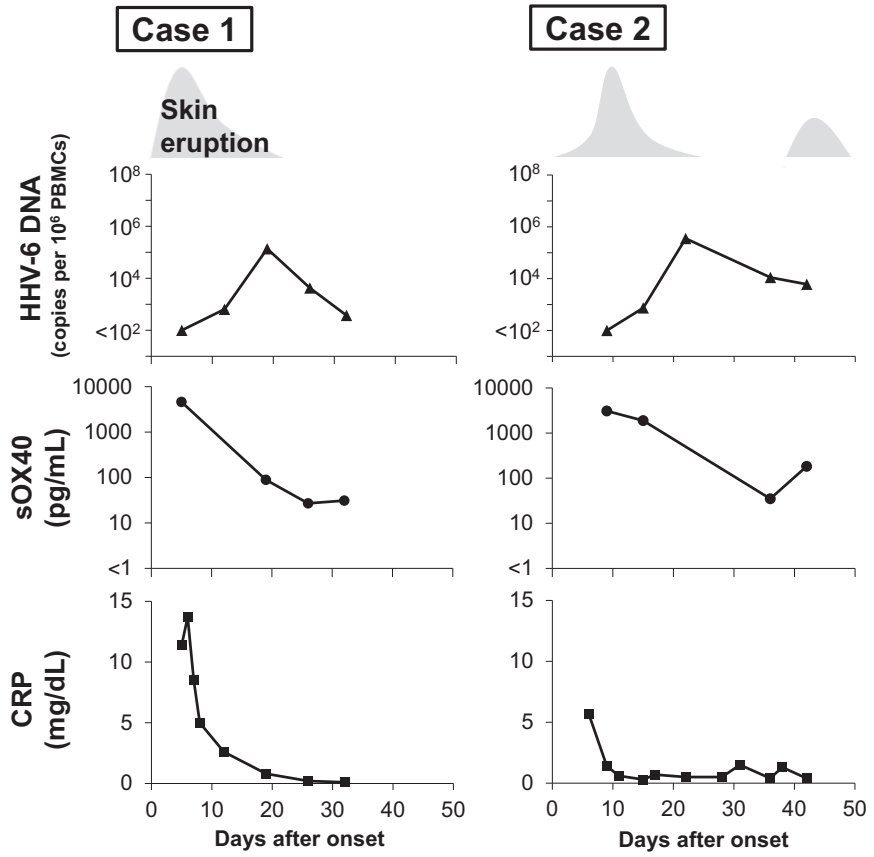

FIGURE 6. Time-dependent changes in skin eruption, human herpesvirus 6 (HHV-6) DNA loads, serum soluble OX40 (sOX40) levels, and serum C-reactive protein (CRP) levels of two representative cases of drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms. In both cases, at the peak of serum sOX40 levels, HHV-6 had not yet reactivated.

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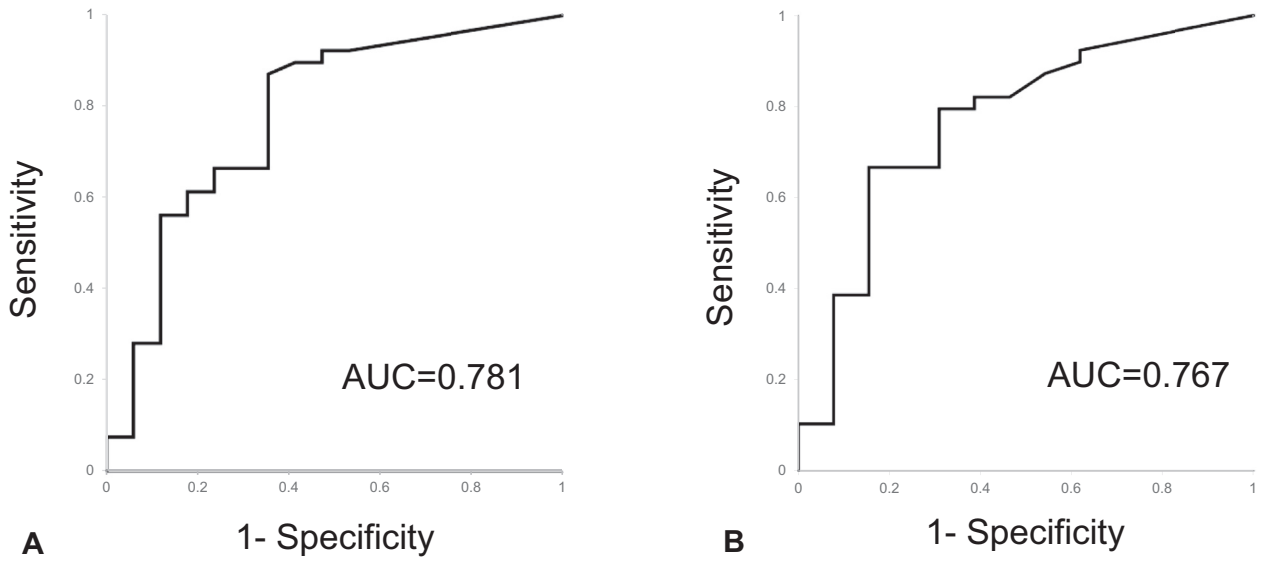

FIGURE E1. Receiver operating characteristic (ROC) curves for drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DIHS/DRESS) versus maculopapular exanthema/erythema multiforme and DIHS/DRESS versus Stevens-Johnson syndrome/toxic epidermal necrolysis. (**A**) From the receiver operating characteristic curve for DIHS/DRESS versus maculopapular exanthema/erythema multiforme, an area under the curve (AUC) value of 0.781 (95% confidence interval, 0.638-0.923) was obtained. (**B**) From the receiver operating characteristic curve for DIHS/DRESS versus Stevens-Johnson syndrome/toxic epidermal necrolysis, an AUC value of 0.767 (95% confidence interval, 0.608-0.927) was obtained.

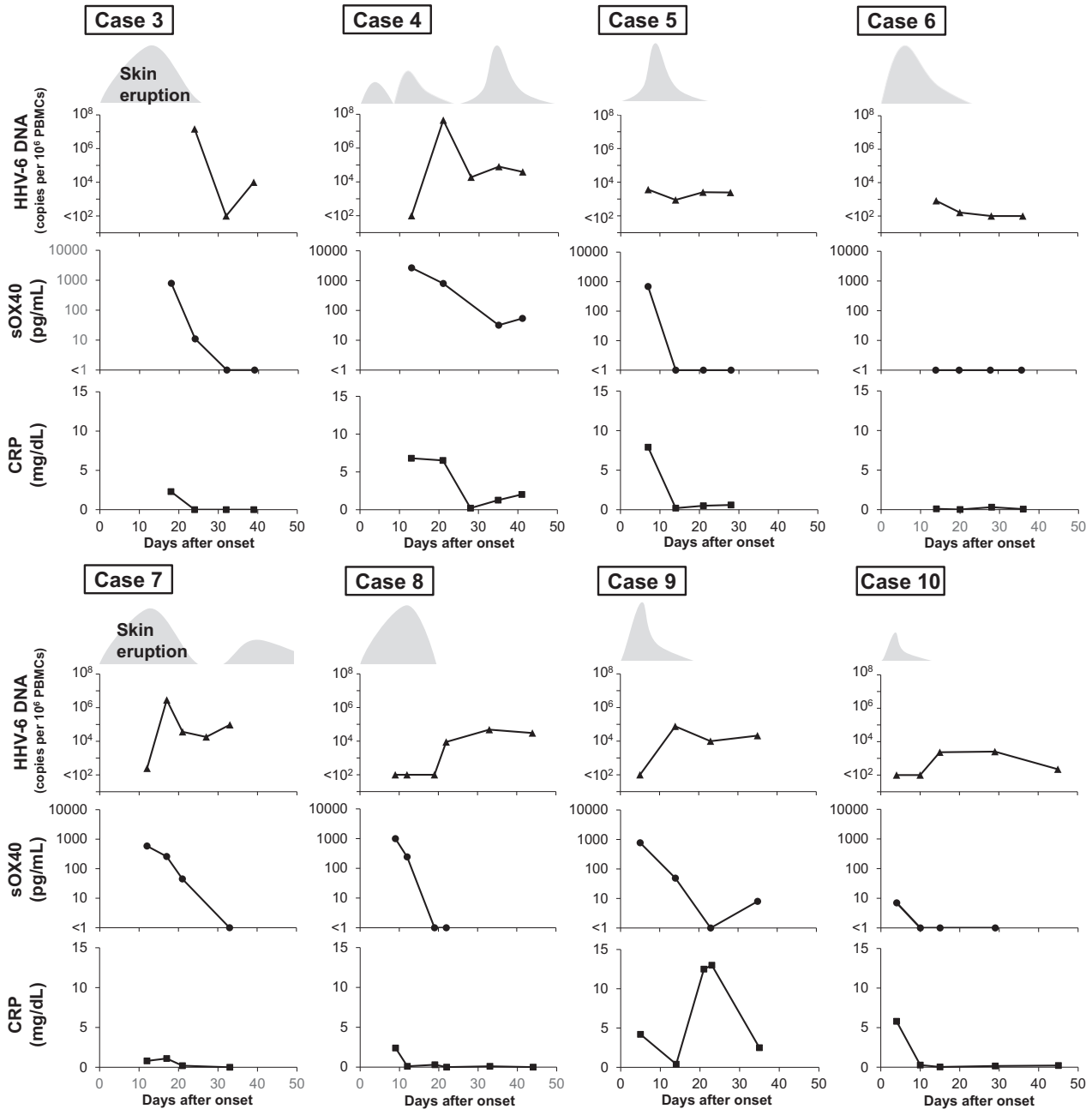

FIGURE E2. Relationships among skin eruption, human herpesvirus-6 (HHV-6) DNA loads, serum soluble OX40 (sOX40) (sOX40) levels, and serum C-reactive protein (CRP) levels in remaining 12 patients with drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms, except for two patients shown in Figure 6.

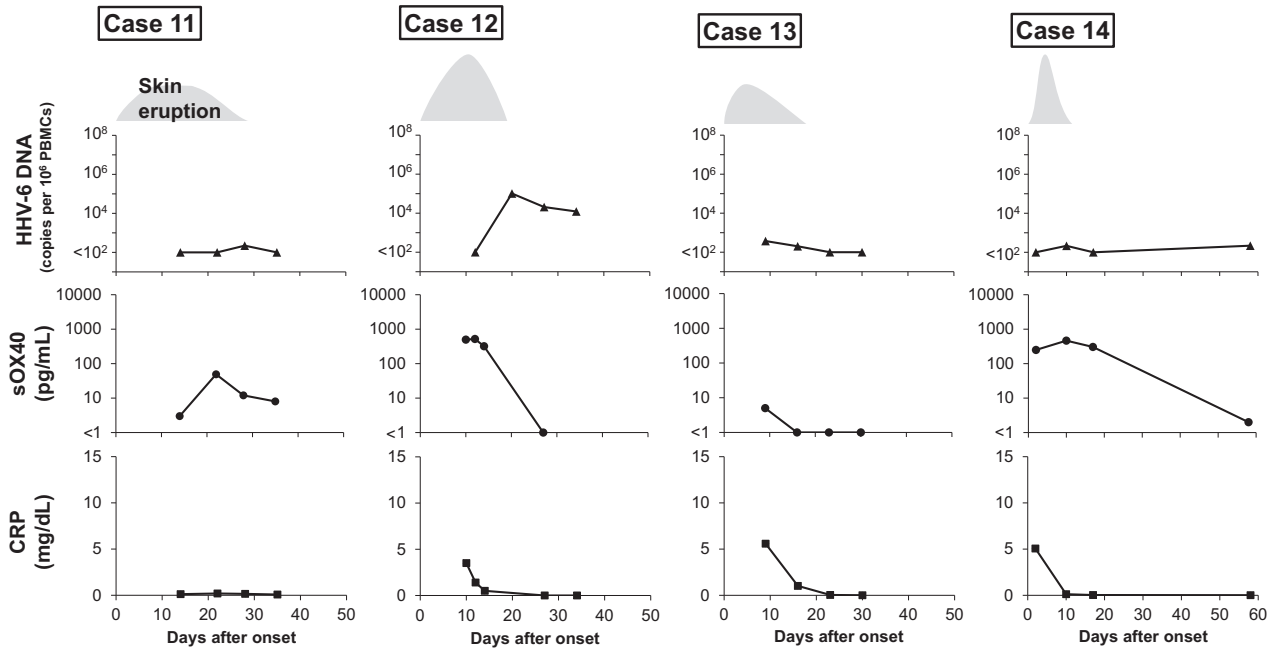

FIGURE E2. (*CONTINUED*).

TABLE E1. Features of patients with drug reaction with eosinophilia and systemic symptoms, maculopapular exanthema/erythema multiforme, Stevens-Johnson syndrome/toxic epidermal necrolysis, or autoimmune bullous disease, and healthy volunteers

		Sex	
Patient group	**Age, y (mean ± SD)**	**Male**	**Female**
Drug reaction with eosinophilia and systemic symptoms (n = 39)	57 ± 19.2	21	18
Maculopapular exanthema/erythema multiforme (n = 17)	68 ± 22.1	6	11
Stevens-Johnson syndrome/toxic epidermal necrolysis (n = 13)	66 ± 23.6	3	10
Autoimmune bullous diseases (n = 5), including bullous pemphigoid (n = 2), pemphigus vulgaris (n = 2), and pemphigus foliaceus (n = 1)	88 ± 32.4	4	1
Healthy volunteers (n = 5)	35 ± 3.8	3	2

TABLE E2. Sequence details of polymerase chain reaction primers and probes used

Virus type	Target	Primer type	Oligonucleotide sequence (5′-3′)	Nucleotide position
Cytomegalovirus	MIE protein	Forward primer	AAC TCA GCC TTC CCT AAG ACC A	2414-2435
		Reverse primer	GGG AGC ACT GAG GCA AGT TC	2470-2489
		Probe	CAA TGG CTG CAG TCA GGC CAT GG	2437-2459
Human herpesvirus-6	DNA polymerase gene	Forward primer	GAA GCA GCA ATC GCA ACA CA	57517-57536
		Reverse primer	ACA ACA TGT AAC TCG GTG TAC GGT	57568-57590
		Probe	AAC CCG TGC GCC GCT CCC	57544-57561
Human herpesvirus-7	Major capsid protein	Forward primer	CCC AAC TAT TTA CAG TAG GGT TGG TG	84230-84255
		Reverse primer	TTT AGT TCC AGC ACT GCA ATC G	84332-84353
		Probe	CTA TTT TCG GTC TTT CCA ATG CAC GCA (AS)	84258-84284