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Title page

**Human herpes virus reactivations and dynamic cytokine profiles in patients with cutaneous adverse drug reactions—a prospective comparative study**

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## **Abstract**

**Background:** Sequential human herpes virus (HHV) reactivation is well-known in drug reaction with eosinophilia and systemic symptoms (DRESS), but such a phenomenon has seldom studied in other types of cutaneous adverse drug reactions (cADRs). Moreover, the association between viral reactivations and cytokine or chemokine changes is largely unknown. We aimed to evaluate the viral reactivation rates of HHV-6, HHV-7, Epstein–Barr virus (EBV), and cytomegalovirus (CMV) in different cADRs and their impacts on clinical prognosis. Cytokine and chemokine changes with viral reactivations were also examined.

**Methods:** A prospective study was conducted to monitor the viral statuses of patients with different cADRs by polymerase chain reaction and serum specific antibody titers. Changes in plasma cytokine and chemokine levels were also evaluated by sequential blood samples.

**Results:** Among the various cADRs, HHV-6 reactivation was only observed in DRESS, but EBV and CMV could be detected in other cADRs. Many proinflammatory cytokines and chemokines, including interleukin (IL)-1 $\beta$ , IL-2, IL-6, interferon- $\gamma$ , tumor necrosis factor- $\alpha$ , were significantly lower in DRESS patients with HHV-6 reactivation when compared to those without HHV-6 reactivation. In addition, these mediators were significantly lower before and during HHV-6 reactivation, compared to cytokine levels after HHV-6 reactivation in the same patient.

**Conclusion:** HHV-6 reactivation was only observed in DRESS patients, not in any other cADR. In DRESS patients, some proinflammatory cytokines were significantly lower before or during HHV-6 reactivation.

**Key words:** adverse drug reactions, Epstein–Barr virus, human herpes virus-6, proinflammatory cytokines, viral reactivation.

## INTRODUCTION

Drug reaction with eosinophilia and systemic symptoms (DRESS) or drug-induced hypersensitivity syndrome (DIHS) is associated with a specific type of severe adverse drug reaction. Typical  
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presentations include fever, rash, lymphadenopathy, leukocytosis with eosinophilia, atypical lymphocytosis, and visceral organ dysfunctions, all of which are easily reminiscent of viral infection.<sup>1</sup>

<sup>2</sup> Association between reactivation of human herpesvirus (HHV)-6 and DRESS was first reported in 1997.<sup>3</sup> Reactivations of several other HHVs in DRESS have also been reported afterward.<sup>4,5</sup> However, their roles in DRESS are still being debated. Some authors consider viral reactivation to be a secondary event occurring after culprit drugs induce an initial immunological reaction;<sup>6,7</sup> others consider DRESS to be the consequence of viral reactivation from its very beginning.<sup>8</sup> In addition, viral reactivation may be a complication of immunosuppressive therapy as several reports also found other HHVs reactivation in Stevens-Johnson syndromes (SJS) or toxic epidermal necrolysis (TEN).<sup>9,10</sup> Nevertheless, a comparative study to investigate the reactivation rates and clinical impacts of several HHVs in different cutaneous adverse drug reactions (cADRs) is still lacking.

Cytokine level changes associated with HHV-6 reactivation have been largely reported in DRESS and many other diseases.<sup>11,12</sup> In addition, differences of inflammatory cytokines (such as interleukin [IL]-1 $\beta$ , IL-6, IL-10, interferon [IFN]- $\gamma$ , and tumor necrosis factor [TNF]- $\alpha$ )<sup>13</sup> and cell-specific chemokines (such as IL-4, IL-5, IL-8, eotaxin, and chemokine [C-C motif] ligand 5 [CCL5])<sup>14</sup> were disclosed among various cADRs. However, the relationships between these mediators and viral reactivations are largely unknown. In contrast to prior studies in which investigations were mostly cross-sectional, we think dynamic changes of cytokine might provide more insight into the mechanism of viral reactivation.

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In this study, we aimed to prospectively examine viral reactivation rates of HHV-6, HHV-7, Epstein–Barr virus (EBV), and cytomegalovirus (CMV) in several cADRs, also their impacts on clinical prognosis, and to sequentially evaluate cytokine and chemokine changes associated with viral reactivation.

## **METHODS**

### **Inclusion of patients and sample collection**

We conducted a prospective study from September 2010 to June 2013 at a single medical center in Taiwan. Hospitalized patients who were preliminarily diagnosed as having DRESS, SJS/TEN, generalized bullous fixed drug eruption (GBFDE), or erythema multiforme majus (EMM) by dermatologists were included; patients with maculopapular eruption (MPE) and no systemic symptoms and signs during the same period were included as the control group. This study was approved by the Research Ethics Committee (REC) of National Taiwan University Hospital.

First blood samples were drawn within three days after admission or upon receiving a diagnosis of cADRs, and every week for at least 2 weeks thereafter. Patients were treated based on disease severity and clinical judgment, without interference from this study. The diagnostic criteria for DRESS, SJS/TEN, EMM, and GBFDE were previously described.<sup>15–17</sup> The index day was defined as the date of rash onset for DRESS, first bulla or mucosal erosion for SJS/TEN, first patch or bulla for

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GBFDE, and first target lesion for EMM. We defined the index day as “day one” for every included case.

#### **Detection of human herpesvirus deoxyribonucleic acid (DNA)**

Peripheral blood mononuclear cells (PBMCs) were purified and serum was separated immediately after blood samplings. DNA was extracted from PBMCs and serum samples using a QIAamp DNA Blood mini-kit (Qiagen Inc., Valencia, CA, USA). Extracted DNAs were stored at  $-80^{\circ}\text{C}$  and then subjected to a real-time polymerase chain reaction (PCR) for the detection of HHV-6, EBV, and CMV using the LightCycler® 480 System (Roche Applied Science, Indianapolis, IN, USA) or detection of HHV-7 by PrimerDesign® genesig (Southampton, UK).

#### **Detection of antibodies to HHV-6 and EBV**

Serum samples from patients were stored at  $-80^{\circ}\text{C}$  until use. Levels of anti-HHV-6 immunoglobulin G (IgG) were measured by enzyme-linked immunosorbent assays (ELISA) (Advanced Biotechnologies, Columbia, MD, USA). EBV serology profiles were also studied to evaluate titers of anti-EBV viral capsid antigen (VCA) IgM, IgG, and anti-EBV early antigen (EA) IgG by indirect immunofluorescence assays (Meridian Bioscience Inc., Cincinnati, OH, USA).

### **Criteria for viral reactivation**

Viral reactivation criteria were defined carefully according to the literature. HHV-6 reactivation was defined as detection of viral DNA in serum samples or a four-fold elevation in the serum level of anti-HHV 6 specific antibody.<sup>18</sup> EBV reactivation was established by one or more of the following: (1) four-fold elevation of anti-EBV VCA IgG or anti-EBV EA IgG without presence of anti-EBV VCA IgM, (2) positive serum EBV PCR, and (3) PBMCs with EBV viral loads higher than 1,000 copies/ $\mu$ g DNA.<sup>19, 20</sup> CMV and HHV-7 reactivation were defined as a positive serum or PBMC PCR.<sup>8,21</sup>

### **Cytokine measurements**

Patients' plasma were collected and then stored at  $-80^{\circ}\text{C}$  until assayed. Twenty-seven cytokines were measured using the Bio-Plex Human Cytokine 27-Plex panel in combination with the Bio-Plex Suspension Array System (Bio-Rad Laboratories Inc., Hercules, CA, USA).

### **Statistical analysis**

Differences in doses of systemic corticosteroids and cytokine levels between the groups were analyzed by unpaired Student *t*-tests, while personal differences in cytokine levels before and after

viral reactivations were compared by paired *t*-tests. All data were present as mean  $\pm$  standard error (SE). A *P* value of less than 0.05 was considered statistically significant for all tests.

## RESULTS

### **HHV-6 reactivation was observed only in DRESS patients while EBV and CMV reactivations were detected in various cADRs**

A total of 62 patients with DRESS (n=23), SJS/TEN (n=17), MPE (n=13), GBFDE (n=5), or EMM (n=4) were enrolled in this study. All patients survived the acute stage except one SJS-TEN overlap patient who died of shock and multiple organ failure with a severity of illness score (SCORTEN) of 5 at day 1 of hospitalization. Characteristics of the patients are summarized in Table 1.

HHV-6 reactivation was observed in ten DRESS patients (43.5%) but not in other cADR patients. Otherwise, EBV reactivation was detected in 35 patients distributed in all cADR groups. EBV reactivation rates were 73.9% for DRESS (17 in 23 patients), 29.4% for SJS/TEN (5 in 17 patients), 53.8% for MPE (7 in 13 patients), 80% for GBFDE (4 in 5 patients), and 50% for EMM (2 in 4 patients). CMV reactivation rates were 43.5% for DRESS (10 in 23 patients) and 11.8% for SJS/TEN (2 in 17 patients) and 0% for the other cADRs. Only two patients (1 SJS on day 5; 1 DRESS on day 94) had HHV-7 reactivation, which was not associated with clinical aggravation of symptoms.

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In the ten DRESS patients with reactivated HHV-6, nine showed a more than four-fold increase in anti-HHV-6 IgG titer while the tenth patient showed both positivity for HHV-6 DNA in serum (540 copies/ml) and a 2.9-fold increase in anti-HHV-6 IgG (Supplementary Table 1). Ten of the 23 DRESS patients experienced symptom flare-up or fluctuation of the disease course, which could be associated not only to HHV-6 reactivation but also to EBV and CMV. Clinical symptoms included fever, recurrent rash, liver function deterioration, or encephalitis with syndrome of inappropriate antidiuretic hormone secretion. In two DRESS patients, HHV-6 reactivation was subclinical. We compared clinical characteristics of DRESS patients with or without HHV-6 reactivation. DRESS patients with HHV-6 reactivation had higher RegiSCAR DRESS scores, more frequent flaring courses, and prolonged illness compared to those without HHV-6 reactivation (Table 2).

Most patients with reactivated EBV exhibited both a four-fold antibody elevation and positive quantitative-PCR in serum and PBMCs. EBV reactivation during acute stages was seldom present with clinical flare-up except in DRESS patients. The symptoms of HHV-6 and EBV reactivations in these patients were indistinguishable, but one of our patients displayed reappearance of atypical lymphocyte when only EBV reactivation was detected. No devastating CMV-related complication, such as colitis, skin ulcers or encephalitis, was documented in our patients.

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In these 23 DRESS patients, three (13.0%) developed long-term sequelae with follow-up duration between 6 months to 3 years. Two of them developed Hashimoto's thyroiditis and dermatomyositis individually, and the other one required life-time hemodialysis after allopurinol-induced DRESS. Intriguingly, both patients with autoimmune sequelae had viral reactivations of all three HHVs, and the other one with HHV-6 and EBV reactivations. All of them suffered from relapsing courses during their acute stages.

#### **Viral reactivation was not an initial event in cADRs**

No evidence of any viral reactivation was apparent in DRESS patients at very early stages. Seroconversion or four-fold increase in anti-HHV-6 antibodies occurred, on average  $22.3 \pm 2.7$  days, after the onset of DRESS. No patient had positive PCR results on PBMC or serum within 15 days after DRESS onsets (Supplementary Table 1). Four-fold increase of EBV antibodies or viral load peaks were observed  $27.9 \pm 2.4$  days,  $11.8 \pm 2.3$  days and  $9.1 \pm 1.0$  days after the onset of DRESS, SJS/TEN, and MPE, respectively (Figure 1). As for CMV, DNA in serum or PMBCs was positive by PCR  $36.9 \pm 3.9$  days after the index day in DRESS patients, while at day 17 and day 38 in two SJS/TEN patients.

#### **Viral reactivation was not related to the dosage of systemic corticosteroids**

The mean dose of systemic corticosteroids during acute stages was calculated by dividing cumulative corticosteroids that patients received within seven days after hospitalization, presenting as

equivalent prednisolone doses. Differences of mean doses of systemic corticosteroids between DRESS patients with or without viral reactivation were all non-significant: HHV-6, 1.1 vs. 1.3 mg prednisolone/kg/day ( $P = 0.6$ ); EBV, 1.1 vs. 1.5 mg prednisolone/kg/day ( $P = 0.07$ ); CMV, 1.0 vs. 1.4 mg prednisolone/kg/day ( $P = 0.1$ ). This phenomenon was also observed in the SJS/TEN patients: EBV, 2.0 vs. 1.7 mg prednisolone/kg/day ( $P = 0.6$ ); CMV, 3.4 vs. 1.6 mg prednisolone/kg/day ( $P = 0.4$ ). Besides, in two DRESS patients without corticosteroid treatments, one had all three kinds of viral reactivation, and the other had EBV and CMV reactivations.

#### **Proinflammatory cytokines and chemokines were significantly lower at the acute stage in DRESS patients with HHV-6 reactivation**

Acute stage (less than 10 days after index date) blood samples were used to compare levels of cytokines and chemokines in DRESS patients with or without HHV-6 reactivation. IFN- $\gamma$  ( $P = 0.048$ ), IL-1 receptor antagonist (IL-1Ra) ( $P = 0.017$ ), IL-1 $\beta$  ( $P = 0.009$ ), IL-2 ( $P = 0.004$ ), IL-6 ( $P = 0.025$ ), macrophage inflammatory protein-1 $\alpha$  (MIP-1 $\alpha$ ) ( $P = 0.013$ ) and TNF- $\alpha$  ( $P = 0.012$ ) were significantly lower in patients with HHV-6 reactivation than in those without HHV-6 reactivation (Figure 2). Only one chemokine, interferon-induced protein 10 (IP-10), was higher in the HHV-6 reactivation group than the non-HHV-6 reactivation group ( $P = 0.018$ ). When compared with SJS/TEN patients, IL-1Ra, IL-1 $\beta$ , IL-6 and TNF- $\alpha$  levels were significantly lower in DRESS patients with HHV-6 reactivation, and

IP-10 level was significantly higher. Most mean levels of cytokines/chemokines in DRESS patients with HHV-6 reactivation were lower than those of MPE patients, but without statistical significance (Supplementary Table 2). Furthermore, many proinflammatory cytokines were significantly lower before and during HHV-6 reactivation in DRESS patients, compared to those after viral reactivations (Figure 3). However, in DRESS patients without HHV-6 reactivations, none of the cytokines/chemokines had trough levels at referral time points and all of them declined afterwards (Supplementary Figure 1). IP-10 declined through the DRESS courses after acute stages (Figure 3). Lower proinflammatory cytokine/chemokine levels before and during viral reactivation were unique in DRESS patients with HHV-6 reactivation. All cytokines/chemokines were similar in any cADR group with or without EBV reactivation (data not shown).

## DISCUSSION

DRESS patients may experience multiple HHV reactivations, but the role of viral reactivation in DRESS is still debated.<sup>1</sup> In this study, HHV-6 reactivation was found only in DRESS, while EBV reactivation could be detected in various cADRs. Our findings support that the fluctuating course in DRESS patients might be attributed to HHV-6 and other HHV reactivations.<sup>1, 4, 5-7</sup> On the other hand, EBV reactivation is not associated with the development of clinical symptoms in SJS/TEN or MPE patients. DRESS patients with HHV-6 reactivation are prone to present more typical and severe

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symptoms, fluctuating courses and thus, prolonged illness. Besides, our data also indicate patients with multiple HHV reactivations warrant long term follow-ups and careful sequela surveys.<sup>22</sup>

HHV-6 reactivation rates in DRESS were variable in different studies, ranging from 45% to 62%, and even up to 85.7% in severe cases.<sup>8, 23, 24</sup> Such variation could be partially explained not only by using different diagnostic criteria for DRESS,<sup>1</sup> but also by adapting diverse methodologies and criteria defining viral reactivation in each study. The advantage of our present study is to evaluate three HHV reactivations using standardized methods and criteria for various cADRs in a single medical unit. Using quantitative PCR DNA tests on serum may not be as sensitive as using whole blood, so we also used serological criteria and quantitative PCR on PBMC to avoid false negative results in this study.

Our results demonstrated that herpesvirus reactivations are not the initial events in either DRESS or SJS/TEN. Most DRESS patients, if having HHV-6 reactivation, showed low titers of specific IgG in early stages, which then elevated after 2~4 weeks. EBV reactivation was never detected in patients' first samplings, and viral loads in serum or PBMC usually reached peak levels afterwards (Figure 1). For CMV, DNA was usually detectable more than 30 days after drug reactions. Thus, it is reasonable to suggest that viral reactivation occurs after the development of cADRs.

We partially clarified that whether or not viruses reactivate, it is not associated with the dose of systemic corticosteroids. However, we could not compare viral reactivation rates or viral loads in treated and non-treated groups because most of our patients received systemic corticosteroids at

early stages.<sup>10</sup> The above results indicate that HHV reactivation is neither the cause of cADRs nor a complication of systemic corticosteroids treatment, but might occur secondary to the development of a cADR.

In DRESS patients, we found that many proinflammatory mediators during acute stages were significantly lower in those with HHV-6 reactivation (Figure 2). Furthermore, the troughs of cytokine levels were usually detected before and during its reactivation in these patients (Figure 3). Although some studies stated higher or elevated inflammatory cytokines were observed in DRESS/DIHS patients or associated with HHV-6 reactivation,<sup>12, 25</sup> there may be some explanations for this discrepancy. First, prior studies compared their patients to healthy controls, but not comparing patients with or without HHV-6 reactivation.<sup>25</sup> Besides, prior series with limited case numbers could only show sporadic results which usually exhibited huge variations and discordance between individuals. Undeniably, we could not totally exclude the influence of systemic corticosteroids on cytokine levels in our patients. However, corticosteroids are not likely to be the cause because there was no difference in the doses of corticosteroids between our patients with or without HHV-6 reactivation.

Sugita et al. previously found that circulating plasmacytoid dendritic cells (pDCs) decreased in DIHS patients, especially around the time of viral reactivation.<sup>26,27</sup> Upon viral infection, stimulated pDCs are prompted to differentiate into DCs by autocrine IFN- $\alpha$  and TNF- $\alpha$ , and prime naïve CD4<sup>+</sup>T cells to produce IFN- $\gamma$  and IL-10.<sup>28</sup> Besides, pDCs preferentially secrete the proinflammatory

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chemokine MIP-1 $\alpha$ , which recruits mostly Th1 type effector cells and causes the production of other proinflammatory cytokines.<sup>29</sup> Therefore, decreased levels of proinflammatory cytokines/chemokines may result from decreased levels of pDCs and depress antiviral capacity in DRESS patients. After reactivation, HHV-6 may further modulate the release of these cytokines from PBMCs, including IFN- $\gamma$ , TNF- $\alpha$ , and IL-1 $\beta$ , which is reflected by their increased levels in blood.<sup>30,31</sup> In the literature, HHV reactivation was a frequent complication of anti-TNF- $\alpha$  treatment,<sup>32</sup> which indirectly indicates that decreased TNF- $\alpha$  level elevates the risk of viral reactivation.

In this study, IP-10 was the only cytokine/chemokine found to be at significantly higher levels in the HHV-6 reactivation group. IP-10 plays an important role in the recruitment of anti-viral specific cytotoxic T lymphocytes into the target tissue.<sup>33</sup> Serum and/or tissue expressions of IP-10 are increased in organ specific autoimmune diseases and also in interface dermatitis.<sup>34, 35</sup> Our novel finding may provide a link between HHV-6 reactivation, dermatopathological changes, and autoimmune sequelae in DRESS.

In conclusion, HHV-6 reactivation was only observed in DRESS patients; in contrast, EBV and CMV can reactivate in various cADRs. When comparing DRESS patients with or without HHV-6 reactivation, the viral reactivation group had lower proinflammatory cytokine levels during acute stages, and even displayed troughs before and during viral reactivation.

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- Accepted Article
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#### Figure legends

**Figure 1. EBV reactivations in DRESS, SJS/TEN, and MPE patients.**

EBV antibodies increased or viral load peaked at different periods after drug reactions in DRESS, SJS/TEN and MPE patients. However, viral reactivations at very early stages were unable to be observed in all patients.

**Figure 2. Proinflammatory cytokines and chemokines were significantly lower in DRESS patients with HHV-6 reactivation.** IFN- $\gamma$  ( $P = 0.048$ ), IL-1Ra ( $P = 0.017$ ), IL-1 $\beta$  ( $P = 0.009$ ), IL-2 ( $P = 0.004$ ), IL-6 ( $P = 0.025$ ), MIP-1 $\alpha$  ( $P = 0.013$ ) and TNF- $\alpha$  ( $P = 0.012$ ) were significantly lower in patients with HHV-6 reactivation than in those without HHV-6 reactivation. Otherwise, IP-10 showed higher level in the HHV-6 reactivation group, when compared to the non-HHV-6 reactivation group ( $P = 0.018$ ).

**Figure 3. Some proinflammatory cytokines and chemokines were significantly lower before and during HHV-6 reactivations in DRESS patients, compared to those after viral reactivations.** Mean levels of cytokines before, during and after HHV-6 reactivation were compared in DRESS patients with HHV-6 reactivation. Cytokine levels were significantly different after viral reactivation by paired  $t$ -test analyses.

**Table 1. Clinical characteristics of patients**

|                         | <b>DRESS</b>  | <b>SJS/TEN</b> | <b>MPE (n=13)</b> | <b>GBFDE</b> | <b>EMM</b>   |
|-------------------------|---------------|----------------|-------------------|--------------|--------------|
|                         | <b>(n=23)</b> | <b>(n=17)</b>  |                   | <b>(n=5)</b> | <b>(n=4)</b> |
| <b>M/F</b>              | 10/13         | 7/10           | 5/8               | 4/1          | 2/2          |
| <b>Age (years)</b>      |               |                |                   |              |              |
| Mean                    | 44.1          | 52.7           | 46.7              | 68.6         | 37.5         |
| Range                   | 16–75         | 30–79          | 17–78             | 39–88        | 33–46        |
| <b>Causative drugs</b>  |               |                |                   |              |              |
| Anticonvulsants         | 7             | 7              | 2                 | 0            | –            |
| Allopurinol             | 6             | 1              | 0                 | 0            | –            |
| Beta-lactam antibiotics | 1             | 1              | 2                 | 2            | 1            |
| Dapsone                 | 0             | 0              | 2                 | 0            | –            |
| Mexiletine              | 1             | 0              | 0                 | 0            | –            |
| NSAID                   | 1             | 3              | 3                 | 2            | –            |
| Sulfamethoxazole        | 1             | 0              | 3                 | 0            | –            |

|                                                  |               |               |               |               |               |
|--------------------------------------------------|---------------|---------------|---------------|---------------|---------------|
| Sulfasalazine                                    | 2             | 1             | 0             | 0             | –             |
| Vancomycin                                       | 1             | 1             | 0             | 0             | –             |
| *Others                                          | 3             | 3             | 1             | 1             | –             |
| <b>Incubation period (days)</b>                  |               |               |               |               |               |
| Mean                                             | 24.3          | 17.5          | 10.9          | 1.2           | 7             |
| Range                                            | 3–56          | 3–45          | 3–21          | 1–2           | –             |
| <b>Administration of systemic corticosteroid</b> |               |               |               |               |               |
| Yes/No                                           | 21/2          | 15/2          | 11/2          | 5/0           | 4/0           |
| † Mean dose $\pm$ SE                             | 1.3 $\pm$ 0.1 | 2.1 $\pm$ 0.3 | 1.0 $\pm$ 0.1 | 0.9 $\pm$ 0.2 | 0.9 $\pm$ 0.2 |

\*Others include: DRESS: linezolid (1 patient) and common cold drugs (2); SJS/TEN: esomeprazole (1), moxifloxacin (1), strontium ranelate (1); MPE: unknown (1); GBFDE: dicyclomine (1).

† Systemic corticosteroids that patients received within seven days after hospitalization, equivalent to prednisolone mg/kg/day

DRESS, drug reaction with eosinophilia and systemic symptoms; EMM, erythema multiforme majus; GBFDE, generalized bullous fixed drug eruption; NSAID, nonsteroidal anti-inflammatory drugs; MPE,

maculopapular eruption; SE, standard error; SJS/TEN, Stevens-Johnson syndrome/toxic epidermal necrolysis.

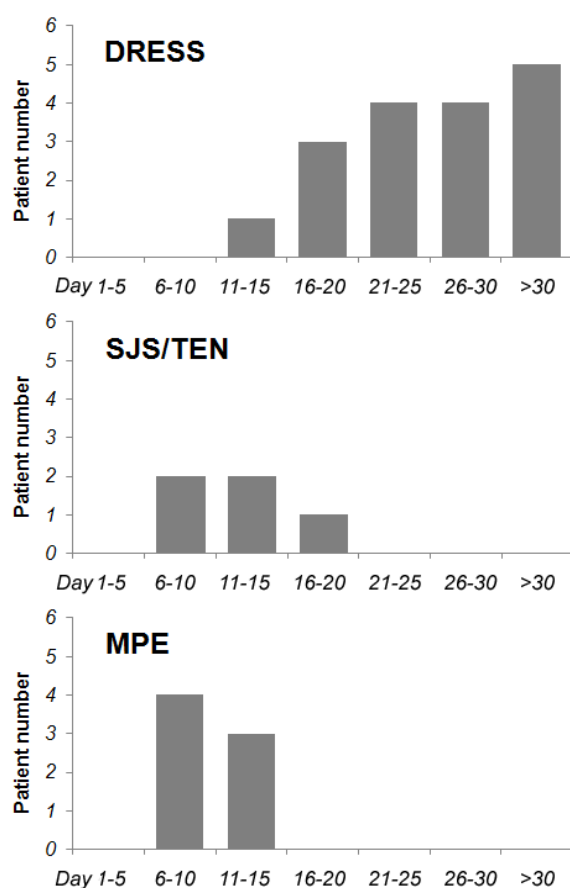
**Table 2. Clinical course of DRESS patients with or without HHV-6 reactivation**

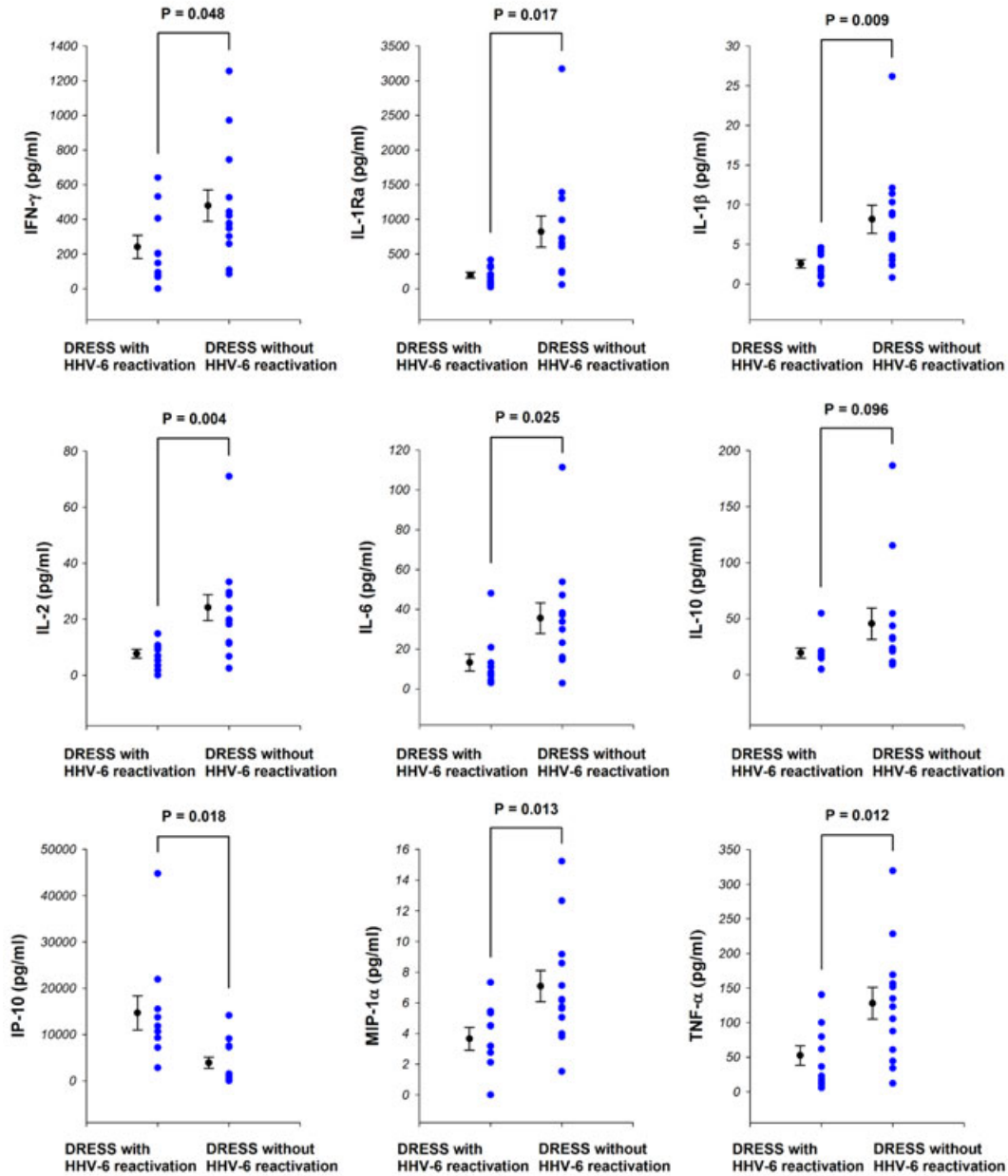
| Symptoms and signs<br>(patient number)    | With HHV-6<br>reactivation (n=10) | Without HHV-6<br>reactivation (n=13) | <i>P</i> value |
|-------------------------------------------|-----------------------------------|--------------------------------------|----------------|
| <b>Fever &gt;38.5°C</b>                   | 10                                | 6                                    | 0.003*         |
| <b>Lymphadenopathy</b>                    | 5                                 | 7                                    | 0.86           |
| <b>Eosinophilia<sup>a</sup></b>           | 9                                 | 10                                   | 0.42           |
| <b>Extreme eosinophilia<sup>b</sup></b>   | 7                                 | 7                                    | 0.45           |
| <b>Appearance of atypical lymphocytes</b> | 9                                 | 8                                    | 0.11           |
| <b>Hepatic involvement<sup>c</sup></b>    | 7                                 | 11                                   | 0.44           |
| <b>Renal involvement<sup>d</sup></b>      | 5                                 | 3                                    | 0.21           |
| <b>Average DRESS score<sup>e</sup></b>    | 6.5                               | 4.8                                  | 0.007*         |
| <b>Flaring courses<sup>f</sup></b>        | 8                                 | 2                                    | 0.001*         |

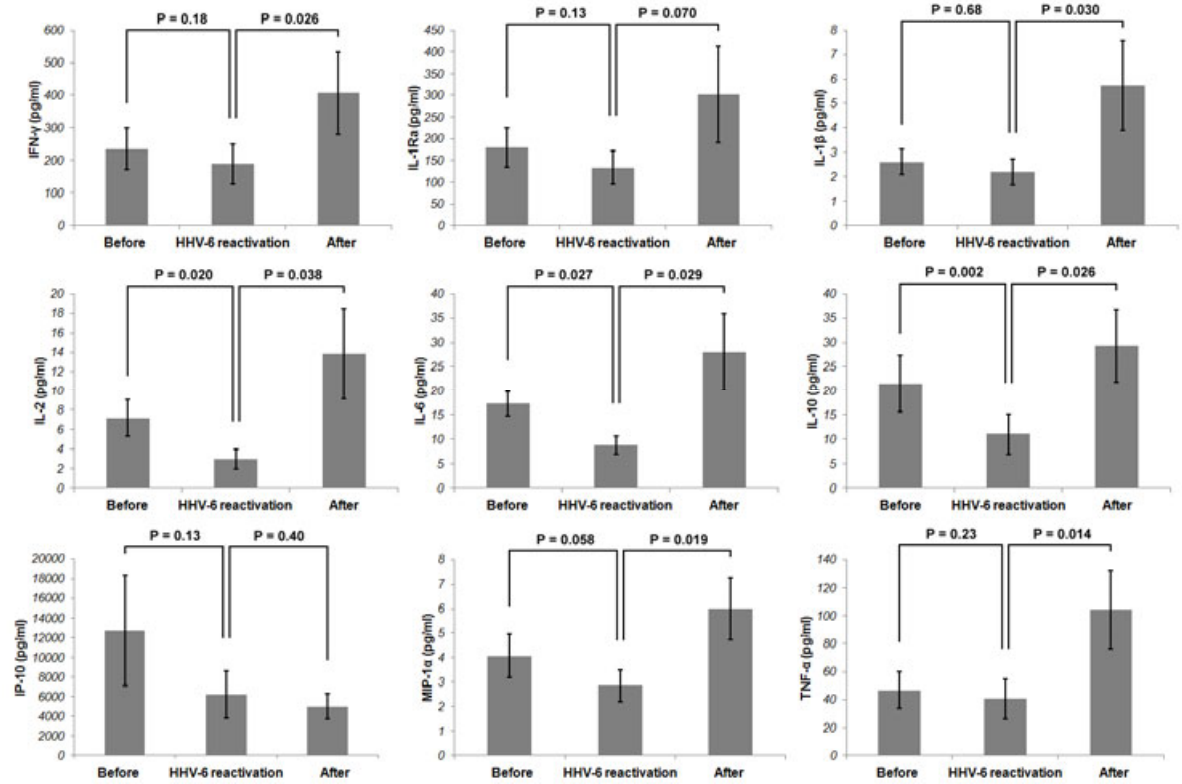
|                                            |          |          |         |
|--------------------------------------------|----------|----------|---------|
| <b>Duration of illness (Days, mean±SE)</b> | 37.5±3.5 | 20.4±1.9 | <0.001* |
| <b>Acquisition of long-term sequelae</b>   | 3        | 0        | 0.08    |

- Eosinophilia was scored if eosinophils  $\geq 700/\mu\text{L}$  or  $\geq 10\%$  if leukocyte  $< 4000/\mu\text{L}$ .
- Extreme eosinophilia was scored if eosinophils  $\geq 1500/\mu\text{L}$  or  $\geq 20\%$  if leukocyte  $< 4000/\mu\text{L}$ .
- Hepatic involvement was scored if alanine aminotransferase  $> 2\times$  upper normal limit, twice on successive dates.
- Renal involvement was scored if creatinine  $> 1.5\times$  patient's baseline on at least 2 successive dates.
- Adapting the scoring system of RegiSCAR for DRESS.
- Symptoms included recurrent fever, rash, abnormal hepatic enzymes, abnormal leukocyte differential counts, or other abnormalities without any definite cause.

\* *P* value of less than 0.05 was considered statistically significant.







## Supplementary Information

**Supplementary Table 1. Details of patients with viral reactivations in DRESS**

| Case no./<br>gender/age | HHV-6 (Day)                      | EBV (Day)                                                                                               | CMV (Day)                    | Clinical characteristics (follow-up duration)                                                                                     |
|-------------------------|----------------------------------|---------------------------------------------------------------------------------------------------------|------------------------------|-----------------------------------------------------------------------------------------------------------------------------------|
| 1/M/56                  | Serology (+) D34<br><br>sPCR (-) | Serology (+) CA IgG D24 1:40; D44 1: 320<br><br>pPCR (-)<br><br>sPCR (+) D52 750cp/ml                   | pPCR (-)<br><br>sPCR (+) D52 | DRESS score 5, recurrence of rash/ renal function deterioration on D31. No documented sequela (3Ys).                              |
| 2/M/55                  | Serology (+) D25<br><br>sPCR (-) | Serology (+) EA IgG D32 1:20; D39 1: 80<br><br>pPCR (+) D39 814 cp/μg DNA; D52 6408 cp/μg DNA; sPCR (-) | pPCR (+) D32<br><br>sPCR (-) | DRESS score 6, fever/ re-elevation of liver enzymes/ recurrence of rash on D27; fever/ rash on D51. No documented sequela (2.5Ys) |
| 3/M/49                  | Serology (+) D13<br><br>sPCR (-) | Serology (+) CA IgG D6 1:10(-); D14 1: 40<br><br>pPCR (+) D28 2576 cp/μg DNA<br><br>sPCR (-)            | pPCR (+) D58<br><br>sPCR (-) | DRESS score 5, re-elevation of liver enzymes on D13; recurrence of rash on D24. No documented sequela (1Y10Mos)                   |
| 4/F/37                  | Serology (+) D22                 | Serology (+) CA IgG D12 1:80; D45 1: 320                                                                | pPCR (+) D22                 | DRESS score 7, spiking fever on D17; slight                                                                                       |

|        |                                                        |                                                                                                      |                                   |                                                                                                                                                         |
|--------|--------------------------------------------------------|------------------------------------------------------------------------------------------------------|-----------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------|
|        | sPCR (-)                                               | pPCR (-)<br><br>sPCR (+) D50 595 cp/ml                                                               | sPCR (-)                          | re-elevation of liver enzymes on D49.<br><br>Hashimoto's thyroiditis 11Mos after DRESS.                                                                 |
| 5/F/49 | Serology (+) D18<br><br>sPCR (-)                       | Serology (-)<br><br>pPCR (-)<br><br>sPCR (+) D18 575 cp/ml                                           | pPCR (+) D18<br><br>sPCR (-)      | DRESS score 5. Developing dermatomyositis 6<br><br>Mos after DRESS.                                                                                     |
| 6/F/65 | Serology (+) D13<br><br>sPCR (-)                       | Serology (+) CA IgG D13 1:80; D25 1: 640<br><br>pPCR (-)<br><br>sPCR (+) D25 73 cp/ml; D46 710 cp/ml | pPCR (+) D61,<br><br>sPCR (+) D61 | DRESS score 8, fever/ re-elevation of liver<br><br>enzymes/ progression of rash/ re-appearance of<br><br>atypical lymphocytes on D10. No sequela (2Ys). |
| 7/M/29 | Serology (-) D29*<br><br>sPCR (+) D22 540<br><br>cp/ml | Serology (-)<br><br>pPCR (-)<br><br>sPCR (+) D22 75 cp/ml; D29 197 cp/ml                             | pPCR (-)<br><br>sPCR (-)          | DRESS score 8, re-elevation of liver enzymes on<br><br>D26. H/D 1Y2Mos after DRESS.                                                                     |
| 8/F/26 | Serology (+) D17<br><br>sPCR (-)                       | Serology (+) EA IgG D10 1:10(-); D26 1: 40<br><br>pPCR (-)<br><br>sPCR (+) D26 465 cp/ml             | pPCR (-)<br><br>sPCR (-)          | DRESS score 8, spiking fever/ recurrence of<br><br>rash/ seizure / consciousness disturbance/<br><br>SIADH on D10. No documented sequela (3Ys).         |

|         |                                  |                                                                            |                                  |                                                                                                                          |
|---------|----------------------------------|----------------------------------------------------------------------------|----------------------------------|--------------------------------------------------------------------------------------------------------------------------|
| 9/F/27  | Serology (+) D15<br><br>sPCR (–) | Serology (+) CA IgG D6 1:40; D29 1: 160<br><br>pPCR (–)<br><br>sPCR (–)    | pPCR (–)<br><br>sPCR (–)         | DRESS score 5, re-appearance of eosinophilia and slight elevation of liver enzymes on D23. No documented sequela (7Mos). |
| 10/F/75 | Serology (+) D37<br><br>sPCR (–) | Serology (–)<br><br>pPCR (–)<br><br>sPCR (–)                               | pPCR (+) D37<br><br>sPCR (–)     | DRESS score 8. No documented sequela (8Mos).                                                                             |
| 11/F/50 | Serology (–)<br><br>sPCR (–)     | Serology (+) CA IgG D34 1:10; D46 1: 80<br><br>pPCR (–)<br><br>sPCR (–)    | pPCR (+) D34<br><br>sPCR (–)     | DRESS score 5, re-appearance of eosinophilia on D39. Underlying AML, HBV carrier. No documented sequela (9Mos).          |
| 12/M/58 | Serology (–)<br><br>sPCR (–)     | Serology (–)<br><br>pPCR (–)<br><br>sPCR (+) D18 600 cp/ml; D26 2105 cp/ml | pPCR (+) D26<br><br>sPCR (+) D26 | DRESS score 5, spiking fever on D14, lipase elevation on D26. No documented sequela (8Mos).                              |
| 13/M/46 | Serology (–)<br><br>sPCR (–)     | Serology (–)<br><br>pPCR (–)                                               | pPCR (+) D30<br><br>sPCR (+) D30 | DRESS score 5. No documented sequela (10Mos).                                                                            |

|         |                              |                                                                                     |                          |                                                                                |
|---------|------------------------------|-------------------------------------------------------------------------------------|--------------------------|--------------------------------------------------------------------------------|
|         |                              | sPCR (–)                                                                            |                          |                                                                                |
| 14/F/53 | Serology (–)<br><br>sPCR (–) | Serology (–)<br><br>pPCR (+) D24 326 cp/μg DNA; D31 1382<br><br>cp/μg DNA; sPCR (–) | pPCR (–)<br><br>sPCR (–) | DRESS score 4. Underlying Evans syndrome.<br><br>No documented sequela (8Mos). |
| 15/F/34 | Serology (–)<br><br>sPCR (–) | Serology (+) CA IgG D13 1:160; D23 1: 640<br><br>pPCR (–)<br><br>sPCR (–)           | pPCR (–)<br><br>sPCR (–) | DRESS score 4. No documented sequela (3Ys).                                    |
| 16/F/33 | Serology (–)<br><br>sPCR (–) | Serology (–)<br><br>pPCR (–)<br><br>sPCR (+) D21 350 cp/ml                          | pPCR (–)<br><br>sPCR (–) | DRESS score 4. No documented sequela (3Ys).                                    |
| 17/F/28 | Serology (–)<br><br>sPCR (–) | Serology (+) CA IgG D14 1:160; D24 1: 640<br><br>pPCR (–)<br><br>sPCR (–)           | pPCR (–)<br><br>sPCR (–) | DRESS score 4. No documented sequela<br>(2Y9Mos).                              |
| 18/M/16 | Serology (–)                 | Serology (–)                                                                        | pPCR (–)                 | DRESS score 4. No documented sequela (2Ys).                                    |

|         |                              |                                                            |                          |                                               |
|---------|------------------------------|------------------------------------------------------------|--------------------------|-----------------------------------------------|
|         | sPCR (–)                     | pPCR (–)<br><br>sPCR (+) D16 130 cp/ml                     | sPCR (–)                 |                                               |
| 19/M/58 | Serology (–)<br><br>sPCR (–) | Serology (–)<br><br>pPCR (–)<br><br>sPCR (+) D19 585 cp/ml | pPCR (–)<br><br>sPCR (–) | DRESS score 4. No documented sequela (1Y1Mo). |

DRESS scores were calculated according to DRESS scoring criteria proposed by RegiSCAR.

\*Anti-HHV-6 antibody titer 2.9-fold elevation

AML, acute myeloid leukemia; BUN, blood urea nitrogen; BSA, body surface area; CA, capsid antigen; CMV, cytomegalovirus; D, day; DRESS, drug reaction with eosinophilia and systemic symptoms; EA, early antigen; EBV, Epstein–Barr virus; F, female; HBV, hepatitis B virus; H/D, hemodialysis; HHV, human herpesvirus; LCV, leukocytoclastic vasculitis; M, male; Mo, month; N/A, not available; NSAID, non-steroid anti-inflammatory drug; pPCR, polymerase chain reaction of peripheral blood mononucleated cells; SIADH, syndrome of inappropriate antidiuretic hormone secretion; sPCR, polymerase chain reaction of serum; Y, year.

**Supplementary Table 2. Comparisons of cytokines/chemokines between DRESS with HHV-6 reactivation to SJS/TEN and MPE**

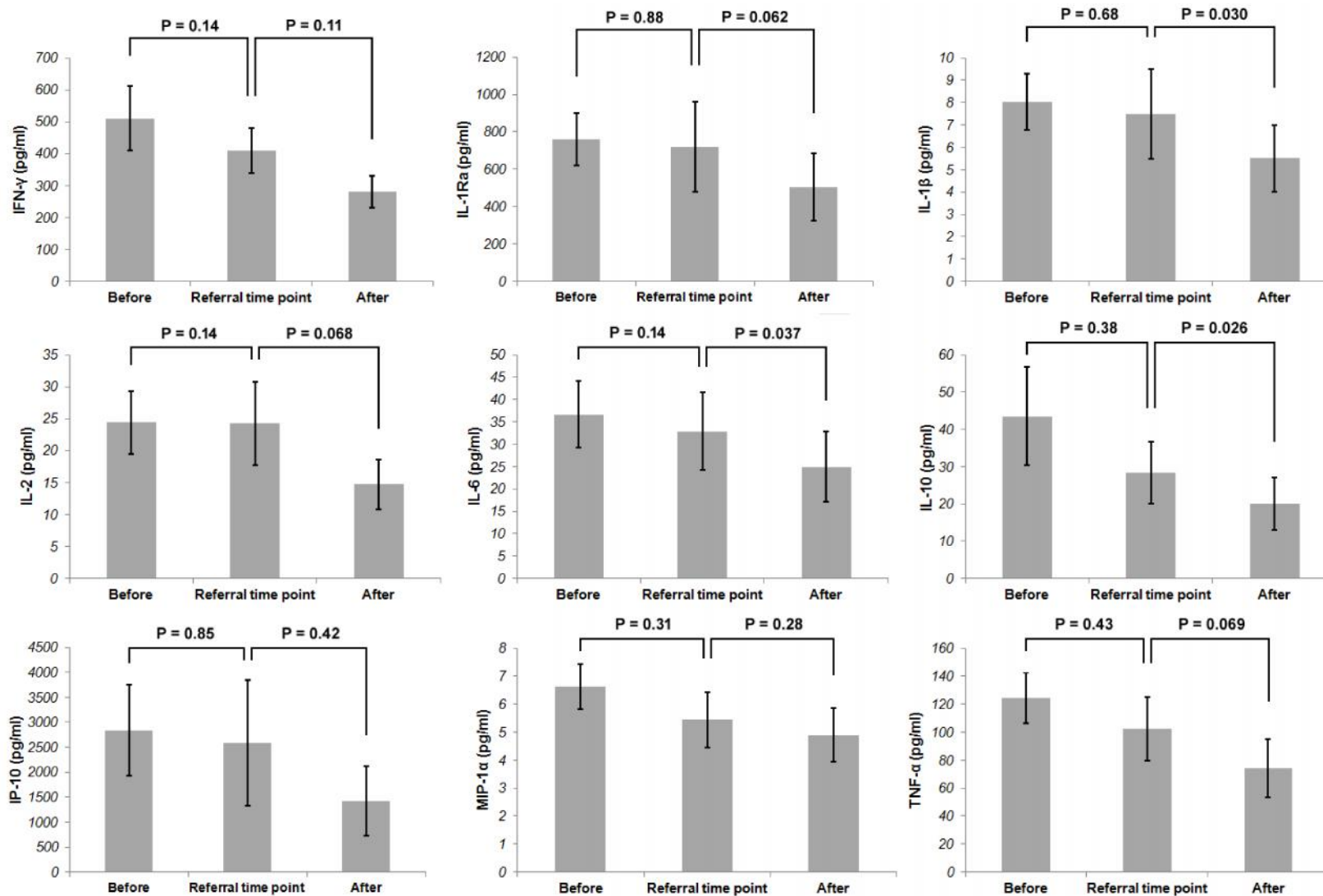
| Cytokine | *DRESS with HHV-6 reactivation | *SJS/TEN        | <sup>†</sup> <i>P</i> value | *MPE           | <sup>‡</sup> <i>P</i> value |
|----------|--------------------------------|-----------------|-----------------------------|----------------|-----------------------------|
| IFN-γ    | 236.75±68.31                   | 321.54±66.88    | 0.07                        | 207.72±57.41   | 0.61                        |
| IL-1Ra   | 179.32±41.41                   | 371.07±71.88    | <u>0.029</u>                | 513.03±257.17  | 0.22                        |
| IL-1β    | 2.40±0.51                      | 4.71±0.91       | <u>0.037</u>                | 7.22±2.68      | 0.10                        |
| IL-2     | 6.23±1.58                      | 11.65±2.55      | 0.08                        | 17.29±11.88    | 0.15                        |
| IL-6     | 12.70±4.28                     | 28.76±5.07      | <u>0.023</u>                | 20.33±8.11     | 0.42                        |
| IL-10    | 17.44±4.59                     | 19.91±3.79      | 0.68                        | 23.21±8.62     | 0.56                        |
| IP-10    | 14485.76±3750.88               | 5353.47±1110.35 | <u>0.04</u>                 | 2201.78±540.37 | <u>0.009</u>                |
| MIP-1α   | 3.52±0.75                      | 4.73±0.74       | 0.26                        | 3.92±0.90      | 0.74                        |
| TNF-α    | 48.76±14.34                    | 75.77±15.16     | <u>0.033</u>                | 64.76±23.72    | 0.26                        |

DRESS, drug reaction with eosinophilia and systemic symptoms; HHV-6, human herpesvirus 6; IFN, interferon; IP-10, interferon-induced protein 10; IL, interleukin; MIP, macrophage inflammatory protein; MPE, maculopapular eruption; SJS/TEN, Stevens-Johnson syndrome/toxic epidermal necrolysis; TNF, tumor necrosis factor

\*Represented as mean ± standard error

<sup>†</sup>*P* value comparing DRESS with HHV-6 reactivation and SJS/TEN

<sup>‡</sup>*P* value comparing DRESS with HHV-6 reactivation and MPE



**Supplementary Figure 1.** In DRESS patients without HHV-6 reactivation, we chose sampling days which were within day 14-28 and closest to day 21 to simulate timings of viral reactivation for comparing cytokine/chemokine levels before and after these referral time points. Interestingly, none of the cytokines/chemokines showed trough levels at referral time points and all of them declined afterwards.