

University of Groningen

## Immunity to varicella-zoster virus in immunocompromised patients

Rondaan, Christien

**IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.**

*Document Version*

Publisher's PDF, also known as Version of record

*Publication date:*

2018

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Rondaan, C. (2018). *Immunity to varicella-zoster virus in immunocompromised patients*. University of Groningen.

### Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

### Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

*Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.*

Longitudinal analysis of varicella-zoster  
virus specific antibodies in Systemic Lupus  
Erythematosus: No association with  
subclinical viral reactivations or lupus  
disease activity

Christien Rondaan, Coretta C. van Leer, Sander van Assen,  
Hendrika Bootsma, Karina de Leeuw, Suzanne Arends,  
Nicolaas A. Bos, Johanna Westra

*Submitted*



## **ABSTRACT**

### **Introduction**

Systemic Lupus Erythematosus (SLE) patients are at high risk of herpes zoster. Previously, we found increased IgG-levels against varicella-zoster virus (VZV) in SLE patients compared to controls, while antibody levels against diphtheria and cellular immunity to VZV were decreased. We aimed to test our hypothesis that increased VZV-IgG levels in SLE result from subclinical VZV reactivations, caused by stress because of lupus disease activity or immunosuppressive drug use.

### **Methods**

Antibody levels to VZV (IgG, IgA, IgM), total IgG and VZV-DNA were longitudinally determined in serum of 34 SLE patients, using ELISA and PCR. Clinical data were retrieved from medical records. Reactivation of VZV was defined as at least five-fold rise in VZV-IgG or presence of VZV-IgM or VZV-DNA. Generalized estimating equations (GEE) was used to longitudinally analyse associations between antibody levels, lupus disease activity and medication use. SLE disease activity index (SLEDAI), anti-double stranded DNA and complement levels were used as indicators of lupus disease activity.

### **Results**

A VZV reactivation was determined in 11 patients (33%). In at least 5 of them, herpes zoster was clinically overt. No association between SLE disease activity or medication use and VZV-specific antibody levels was found. There was a weak association between total IgG and VZV-IgG.

### **Conclusion**

Our results indicate that increased VZV-IgG levels in SLE do not result from frequent subclinical VZV reactivations, and are not associated with lupus disease activity. Increased VZV-IgG can only partially be explained by hypergammaglobulinaemia.

## INTRODUCTION

Infectious diseases are an important cause of morbidity and mortality in patients with Systemic Lupus Erythematosus (SLE), an autoimmune disease that is characterized by the presence of autoantibodies and recurrent disease flares [1]. Herpes zoster (HZ), characterized by severe pain and rash in a dermatomal distribution, is one of the infectious diseases frequently affecting SLE patients. The incidence of HZ is reported to be 5 to 16 times higher in SLE patients than in the general population, and is associated with the use of immunosuppressive medication [2-5].

HZ is caused by reactivation of the varicella-zoster virus (VZV). After primary infection, known as varicella or chickenpox, VZV establishes latency in the sensory nerve ganglia [6-8]. Since almost the entire population of temperate countries has experienced a primary VZV infection in childhood, almost the entire adult population is at risk of HZ, occurring when cellular immunity to VZV wanes [9].

Previously, we found increased VZV-specific antibody levels of the immunoglobulin (Ig) G class in SLE patients compared to age-matched healthy controls, while cellular immunity to VZV was found to be decreased. This phenomenon could not be explained by polyclonal hypergammaglobulinaemia alone, which is often observed in SLE, because for diphtheria lower antibody levels were found in SLE patients than in age-matched healthy controls [10].

Also for other human herpes viruses that can establish latency such as Epstein-Barr virus (EBV) and cytomegalovirus (CMV), increased antibody levels have been described in SLE [11]. Antibody levels to several other infectious agents were shown not to differ or even be lower in SLE patients, compared to healthy controls [12]. Human herpes viruses, in particular EBV, have been implicated to play a role in development and relapsing course of SLE [13].

Over the years, different research groups have suggested that reactivation of VZV without overt clinical manifestations may play a role in the increased VZV-IgG level in SLE patients [10,14,15]. Indeed, it is known that a clinically evident reactivation of VZV, herpes zoster, leads to long-lasting rises in VZV-specific IgG levels [16,17]. Subclinical reactivations of VZV have been described in immunocompromised patients [18], and are known to occur under stressful circumstances, even in otherwise healthy persons [19,20].

In the present study antibody levels to VZV and presence of VZV-DNA were determined longitudinally in a retrospective cohort of SLE patients. We expected to find frequent subclinical VZV reactivations in SLE patients, able to account for their increased VZV antibody levels. We hypothesized that VZV reactivations in SLE patients would be potentiated by stress because of SLE disease activity or immunosuppressive drug use, and aimed to investigate the association between VZV reactivation, VZV antibody levels, lupus disease activity and immunosuppressive drug use.

## **MATERIALS AND METHODS**

### **Study population**

Serum samples, present in our biobank, from randomly selected SLE patients were used. Patients could be included in the study when they fulfilled American College of Rheumatology (ACR) criteria for SLE [21] and had a minimum age of 18 years at time of first serum sample. At least 5 samples per patient had to be present, with a minimum of 1 year between first and last sample. The study has been carried out in accordance with the principles of the Declaration of Helsinki. Approval by the medical ethics committee of the University Medical Centre Groningen was obtained and all patients gave informed consent.

### **Definition of VZV reactivation**

In line with the definition of Ljungman et al.[18], a subclinical reactivation was defined as an at least fivefold increase in VZV specific IgG antibody level or the occurrence of specific IgM (unless explained by presence of rheumatoid factor) without clinical signs of varicella or zoster. Moreover, we added the occurrence of VZV-DNA in serum to the existing definition. The definition of a clinical reactivation was the same as that of a subclinical one, but in the presence of clinical symptoms of herpes zoster. As VZV-specific IgA has been suggested to have some value in the diagnosis of herpes zoster,[16] we also analysed the course of antibodies of this isotype, but did not include it in the definition of a VZV reactivation.

### **VZV-specific antibody levels**

For determining VZV-specific IgG, IgA and IgM levels, an in-house glycoprotein ELISA was performed, which was validated previously [10]. VZV purified glycoproteins (EastCoastBio, North Berwick, USA) were used as antigen, and pooled human serum with known levels of anti-glycoprotein VZV was used as standard. Threshold for positivity was set at 100 mIU/ml for VZV-specific IgG levels, at 50 U/ml for VZV-specific IgA levels and at 15 U/ml for VZV-specific IgM levels, according to recommendations of a commercial kit used for validating the in-house ELISA's (Institut Virion\Serion).

### **Detection of VZV-DNA**

DNA was extracted from whole blood samples by the MagnaPure 96 system, using the MagnaPure 96 DNA and viral DNA small volume kit (Roche Applied Bioscience, Mannheim, Germany) according to the manufacturer's instructions. Detection of VZV-DNA was performed using a previously described protocol [22].

### **Total IgG antibody levels**

Total IgG antibody levels were determined using a commercial kit (Thermo Fisher Scientific, Waltham, USA), following the manufacturer's instructions.

## Symptoms of herpes zoster

Medical records from included patients were checked for a history of herpes zoster during follow-up period and at least 2 years before first sample, when possible.

## Statistics

Generalized estimating equations (GEE) was used to explore parameters associated with antibody levels over time. The exchangeable correlation matrix was used to take into account the within-patient correlations. In case residuals were non-normally distributed, dependent variable was log-transformed before entered into the analysis. Tested parameters included disease activity and immunosuppressive medication use over time. SLE disease activity index (SLEDAI, continuous and  $\geq 5$  for active disease), anti-double stranded (ds)DNA and complement levels (C3, C4) were used as indicators of lupus disease activity. Medication use was tested overall (use of any immunosuppressive drugs) and for different drugs separately when used by at least 5 patients. Lastly, longitudinal associations between VZV-IgG and total IgG was investigated.

To be able to compare results from this study with results from studies using a cross-sectional sample, also statistical analyses were done when including the first available patient samples only. Associations between antibody levels (VZV-specific IgG and IgA, and total IgG), patient characteristics, medication use and disease activity parameters were explored by determining Spearman's correlations coefficients in case of continuous variables and using Mann-Whitney U test to compare differences between groups.

Statistical analysis was performed using IBM SPSS Statistics 23 (SPSS, Chicago, IL, USA). Figures were made using GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA, USA). P-values  $\leq 0.05$  were considered significant.

## RESULTS

### Patient characteristics

Thirty-four SLE patients were eligible based on the inclusion criteria. One of these patients was found to be VZV seronegative and was therefore excluded from further analysis. Characteristics of the remaining 33 SLE patients are summarized in Table 1, including an overview of immunosuppressive medication use.

### Longitudinal measurement of total IgG antibodies and VZV-specific antibodies

There was considerable variation in levels of VZV-specific IgG and total IgG between patients. Twenty-eight of 33 patients exceeded the normal upper limit of 16.0 g/l of total IgG at one or multiple times during follow-up. A total of 17 (52%) SLE patients had positive VZV-specific IgA levels ( $\geq 50$  U/ml) at one or multiple time points during follow-up (data not shown). In two (6%) patients, levels of VZV-specific IgM above the threshold for

**Table 1.** Patient characteristics

	Total n=33
Female gender, no. (%)	29 (88)
Age at baseline (years), median (range)	39.2 (18.2-75.6)
Disease duration at baseline (months), median (range)	71.3 (0-272.2)
Follow-up duration (months), median (range)	41.4 (14.5-58.2)
Serum samples per patient, median (range)	11 (5-17)
Patients with active disease (SLEDAI $\geq$ 5 at any point during follow-up), no. (%)	23 (70)
Number of periods with SLEDAI score $\geq$ 5 in patients with active disease, median (range)	1 (1-4)
Immunosuppressive medication use at any time during follow-up, no. (%)   newly started or dose increase during follow-up, no. (%)	
Overall	33 (100)
Prednisone	26 (79)   20 (61)
Hydroxychloroquine	22 (67)   11 (33)
Azathioprine	17 (52)   9 (27)
Mycophenolate mofetil	10 (30)   7 (21)
Cyclophosphamide	5 (15)   5 (15)
Methotrexate	3 (9)   1 (3)
Cyclosporin	1 (3)   0 (0)
Ocrelizumab <sup>a</sup>	1 (3)   1 (3)

<sup>a</sup> Used in a study setting. SLEDAI = systemic lupus erythematosus disease activity index

positivity (15 U/ml) were found that could not be explained by presence of rheumatoid factor (data not shown).

### Reactivations of VZV

A total of 11 patients (33%) had a VZV reactivation during follow-up, according to the predefined definition (see methods) (Table 2). There were no patients with multiple VZV reactivations during the follow-up period. Patient characteristics, including disease activity and medication use, from patients that experienced a VZV reactivation did not differ from those who did not (data not shown). When reviewing medical records from all patients, clinically overt herpes zoster episodes were found in 5 patients, all of which met the predefined definition of reactivation (Table 2).

In three patients (SLE3, SLE17 and SLE26) VZV reactivation was closely related to a change in medication (Table 2).

Supplementary figures of antibody levels, presence of VZV-DNA and anti-dsDNA (as a measure of lupus disease activity) for all patients with a VZV reactivation are available online.

Table 2. Summary of findings in patients with a VZV-reactivation

Patient ID	≥5-fold rise VZV-IgG level	VZV-IgM positive	VZV-DNA present	VZV-IgA positive	HZ complaints noted in medical records	Alterations in medication use prior to reactivation
SLE2	✓		✓	✓	✓	10 months prior: Start of azathioprine
SLE3	✓		✓	✓	✓	3 months prior: Start of ocrelizumab and mycophenolate mofetil, increase in prednisone dose from 2.5 to 15 mg daily
SLE5	✓			✓		Stable for 18 months
SLE6		✓				No medication use at time of VZV-IgM positivity
SLE15	✓			✓	✓	No medication use prior to HZ
SLE17			✓			Two months prior: restart prednisolone
SLE23		✓				Stable for over 1 year
SLE26	✓				Unsure. At time of SLE flare.	1 month prior: Start of mycophenolate mofetil, increase in prednisone dose
SLE27	✓			✓		Stable for at least 2 years
SLE33			✓	✓		Stable
SLE34	Drop in VZV-IgG		✓	✓		Stable

VZV = varicella-zoster virus; SLEDAI = systemic lupus erythematosus disease activity index; HZ = herpes zoster



### *Rises in VZV-specific IgG antibody level*

Six patients had a  $\geq 5$ -fold rise in VZV-specific IgG level (Table 2). Total IgG levels were stable at the time of VZV-specific IgG rises, indicating that large rises in VZV-specific IgG levels were not a consequence of rises in total IgG levels (data not shown).

In four of six patients with VZV-IgG rise, clinical complaints matching herpes zoster were evident when reviewing medical records. For a fifth patient (SLE26), the VZV-IgG rise occurred during a severe SLE flare including skin manifestations and herpes zoster-like complaints could not be distinguished with certainty from the medical records retrospectively.

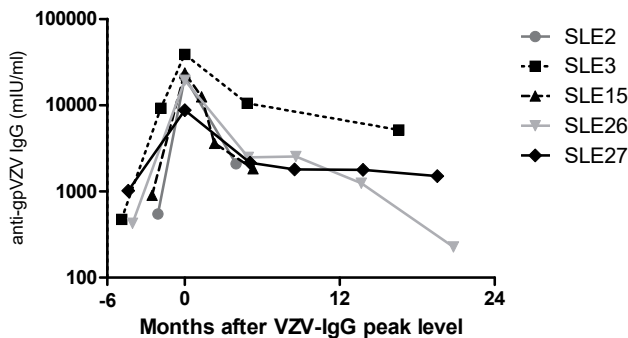
Figure 1 shows the last sample before  $\geq 5$ -fold VZV-specific IgG rise and course thereafter. Interestingly, VZV-specific IgG levels remained at increased levels for a long time and had not returned to original level after one year.

### *Presence of VZV-DNA in serum*

VZV-DNA was detected in serum of 5 patients. In two of those patients (SLE2 and SLE3) also a  $\geq 5$ -fold VZV-specific IgG level rise was seen and had clinically evident herpes zoster. In another patient (SLE34) a drop of VZV-specific IgG was seen at time of VZV-DNA presence.

### *Presence of VZV-specific IgM antibodies*

In two patients positive VZV-specific IgM levels were found. Patient SLE23 had a very high VZV-specific IgM value of 479 U/ml at one time point, rapidly decreasing at succeeding time points. Patient SLE6 had positive but decreasing VZV-specific IgM levels at the start of follow-up that lasted for over one year. At the same time VZV-specific IgG levels gradually decreased. No clinical signs of a VZV reactivation were present in these patients.



**Figure 1.** Last sample before  $\geq 5$ -fold rise and the VZV-specific IgG course thereafter in 5 SLE patients, presented on a log-scale. Because the VZV-IgG rise in patient SLE5 occurred in the last sample, this patient is not included in the graph.

## Presence of VZV-specific IgA antibodies

Levels of VZV-IgA above the threshold for positivity were found in 17 patients. The five patients with clinically evident herpes zoster all were VZV-IgA positive at that time. A rise in VZV-IgG usually co-occurred with a rise in VZV-IgA (Table 2).

## Longitudinal analysis of VZV-specific antibody levels

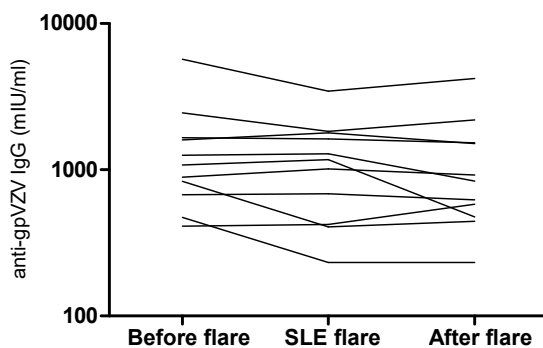
VZV-IgG levels were stable following SLE disease flare (including 11 patients with a minimum difference in SLEDAI of 4 points between intervals) (Figure 2).

Moreover, GEE analysis showed no significant associations between VZV-specific IgG levels and parameters of disease activity, including SLEDAI (continuous and  $\geq 5$  for active disease), levels of anti-dsDNA, and C3 and C4 levels (Table 3). After excluding patients with a  $\geq 5$ -fold rise in VZV-IgG level ( $n=6$ ), eliminating the possibly distorting effects of such a large rise to be able to assess the association between smaller variations in VZV-IgG and total IgG levels, a weak but statistically significant association was seen (B 0.008, 95% confidence interval 0.001 – 0.016,  $p=0.025$ ).

Significant but very weak associations were revealed between VZV-specific IgA levels and anti-dsDNA levels or VZV-specific IgG levels using GEE (data not shown).

## Parameters associated with total IgG levels over time

GEE analysis of variables of influence on total IgG levels was performed as a large variation was observed in total IgG levels between patients and also within patients over time, and a weak association between VZV-IgG and total IgG levels seemed to be present. This analysis revealed that overall medication use was inversely associated with total IgG levels. A negative association was shown for the use of prednisone, mycophenolate mofetil and cyclophosphamide.



**Figure 2.** Stable course of VZV-IgG surrounding SLE disease flare in 11 patients with a minimum difference in Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) of 4 points between intervals.

C3 and C4 were also negatively associated with total IgG levels. As C3 and C4 are known to indicate active disease when decreased, this inverse association indicates that patients with active disease have higher total IgG levels. This is in line with the positive association between total IgG levels and other measures of disease activity: SLEDAI (continuous and score  $\geq 5$  for active disease) and anti-dsDNA levels (Table 4).

**Table 3.** GEE analysis exploring the relationship of disease activity and medication use with VZV-IgG levels (log-transformed) over time in all patients and in patients without  $\geq 5$ -fold rise in VZV-IgG

	<b>B (95% CI)</b>	<b>p</b>
SLEDAI	-0.010 (-0.031 – 0.010)	0.315
Active disease (SLEDAI $\geq 5$ )	-0.031 (-0.116-0.053)	0.468
Anti-dsDNA	$9.45 \times 10^{-5}$ ( $-1.92 \times 10^{-4}$ – $3.81 \times 10^{-4}$ )	0.518
C3	0.123 (-0.241 – 0.486)	0.509
C4	0.033 (-0.972-1.038)	0.949
Medication use		
Overall (use vs. no use)	-0.001 (-0.108 - 0.107)	0.992
Prednisone (dose in mg/day)	-0.003 (-0.007 – 0.002)	0.240
Hydroxychloroquine (use vs. no use)	-0.036 (-0.120-0.049)	0.411
Azathioprine (dose in mg/day)	-0.001 (-0.002 – 0.001)	0.442
Mycophenolate mofetil (use vs. no use)	0.227 (-0.086 – 0.539)	0.155
Cyclophosphamide (use vs. no use)	-0.244 (-0.545 – 0.057)	0.113
Total IgG levels	0.006 (-0.003 – 0.015)	0.216

GEE = generalized estimating equations; CI = confidence interval; SLEDAI = systemic lupus erythematosus disease activity index; dsDNA = double stranded DNA.

**Table 4.** GEE analysis exploring the relationship of disease activity and medication use with total IgG levels in all patients

	<b>B (95% CI)</b>	<b>p</b>
SLEDAI	<b>0.389 (0.070 – 0.707)</b>	<b>0.017</b>
Active disease (SLEDAI $\geq 5$ )	<b>2.182 (0.323 – 4.04)</b>	<b>0.021</b>
Anti-dsDNA	<b>0.005 (0.001 – 0.009)</b>	<b>0.011</b>
C3	<b>-6.872 (-9.923 – -3.821)</b>	<b>&lt;0.0005</b>
C4	<b>-19.830 (-32.661 – -6.998)</b>	<b>0.002</b>
Medication use		
Overall (use vs. no use)	<b>-3.779 (-5.763 – -1.795)</b>	<b>&lt;0.0005</b>
Prednisone (dose in mg/day)	<b>-0.236 (-0.302 – -0.170)</b>	<b>&lt;0.0005</b>
Hydroxychloroquine (use vs. no use)	-0.210 (-2.178 – 1.759)	0.835
Azathioprine (dose in mg/day)	-0.502 (-2.940 – 1.937)	0.687
Mycophenolate mofetil (use vs. no use)	<b>-4.922 (-8.046 – -1.799)</b>	<b>0.002</b>
Cyclophosphamide (use vs. no use)	<b>-4.529 (-5.343 – -3.714)</b>	<b>&lt;0.0005</b>

GEE = generalized estimating equations; CI = confidence interval; SLEDAI = systemic lupus erythematosus disease activity index; dsDNA = double stranded DNA. Significant associations are presented in bold.

## Cross-sectional analysis using first available samples

To be able to compare our results with previous studies that used a cross-sectional approach, we analyzed the first included samples per patient in a cross-sectional manner. In this analysis, a significant correlation between VZV-specific IgG and IgA levels was found ( $\rho=0.404$ ), but not between VZV-IgG and total IgG. Another significant, but inverse correlation was determined between VZV-specific IgG levels and C4 levels ( $\rho=-0.360$ ). No significant correlations between other parameters and VZV-IgG levels were found. For VZV-IgA levels, no significant correlations were noted with parameters other than VZV-IgG. Significant negative correlations were demonstrated between total IgG levels and prednisone dose ( $\rho=-0.612$ ) and C3 levels ( $\rho=-0.459$ )

## DISCUSSION

The present study was initiated to clarify the cause of the elevated VZV-specific IgG levels in SLE patients. During longitudinal observation, in which 33 VZV-seropositive SLE patients were followed for a median duration of 41.4 months, 1 substantial ( $\geq 5$  fold) VZV-IgG rise was observed without clinical symptoms of herpes zoster. It therefore seems unlikely that subclinical VZV reactivations play an important causative role in the origin of increased VZV-IgG levels in SLE. Furthermore, longitudinal analysis using GEE did not reveal an association between lupus disease activity or medication use and VZV-specific antibody levels.-

In three patients we found a subclinical reactivation (1 positive VZV-IgM, 2 presence of VZV-DNA) while VZV-IgG remained stable. Substantial rises in VZV-specific IgG level were shown in 6 patients, almost all coinciding with clinical symptoms matching herpes zoster, as assessed retrospectively by reviewing medical records. The increased VZV-IgG levels were shown to be long-lasting, with high levels even after a year of follow-up. Also in healthy persons, VZV-specific IgG is known to remain increased for months and even 1 year after herpes zoster [16,17].

Medication use was found not to be associated with VZV-specific IgG levels over time, but was clearly shown to be negatively associated with total IgG levels. Although in three patients a close temporal relationship was seen between the start of a new drug and a VZV-reactivation, numbers are too low to draw firm conclusions.

When patients with a large rise in VZV-specific IgG values were excluded from the analysis, a weak association between VZV-specific IgG and total IgG levels was revealed, that was not found in the cross-sectional analysis. This suggests that there can be some influence of general increased B cell activity on the height of VZV-IgG values, but that this association is insufficient to completely explain the increased height of VZV-specific IgG values in SLE patients.

Our findings add valuable information to the debate on the role of human herpes viruses in the development and relapsing course of SLE. Especially EBV has been implicated to be involved [13]. Increased antibody levels and impaired cellular immunity to EBV in SLE patients [23,24] were found, similar to our previous findings for VZV [10]. The elevated

level of antibodies directed against lytic cycle antigens and an elevated level of EBV-DNA are suggested to indicate frequent EBV reactivations, and to contribute to the occurrence of SLE disease flares [23]. However, there is no direct evidence of this proposed link. The lack of an association between SLE disease activity and VZV-reactivations in our study suggest that there is no etiologic role of VZV reactivations in the relapsing disease course of SLE. Further research is needed to investigate whether this also holds true for other human herpes viruses.

Although herpes zoster is usually diagnosed based on clinical symptoms, the diagnosis can be challenging in cases when rash is absent (e.g. zoster sine herpette, VZV encephalitis). Elevated levels of VZV-specific IgA have been proposed to be of some diagnostic value in these cases [16,25]. VZV-specific IgA positivity was found in 17 out of 33 SLE patients at one or multiple time points during follow-up in our study, while only 5 patients were found to have clinically evident herpes zoster. Value of VZV-IgA in diagnosing herpes zoster therefore is questionable in SLE patients. VZV-specific IgM was shown to lack sensitivity when assessing its value in the diagnosis of herpes zoster in SLE patients.

This study has some limitations. Most importantly, caused by the retrospective study design, clinical data regarding herpes zoster complaints could be missing from medical records as herpes zoster infections do not necessarily come under attention of the treating specialist. Also, the study population consisted of patients with established disease, which made it impossible to study possible alterations in VZV-specific humoral immunity earlier in the disease course. Furthermore, the definition of a subclinical reactivation is arbitrary and intervals between measurements were not standardized. We believe to have used a broad definition, based upon the study of Ljungman et al.[18], including  $\geq 5$ -fold VZV-IgG rises, positivity of IgM and the addition VZV-DNA. A strength of the study is the use of GEE analysis in looking for associations between VZV antibody levels, disease activity and medication use. The GEE analysis was independent from the arbitrary definition of subclinical reactivation and this type of analysis is suitable for analyses of data with non-standardized intervals.

Concluding, our study results do not support the hypothesis that the increased VZV-specific antibody levels in SLE patients are caused by subclinical VZV reactivations or are related to SLE disease activity or medication use during established disease. Although generally increased B cell activity seems to influence the height of VZV-IgG values, this association is insufficient to completely explain the increased height of VZV-specific IgG values. Our findings might suggest an origin earlier in the disease process. Despite the limitation of a retrospective design, this study adds to the understanding of the humoral response to VZV in this patient group at increased risk of herpes zoster.

## ACKNOWLEDGEMENTS

The authors thank Alicia Borneman and Elisabeth Eelsing for their assistance in performing enzyme-linked immunosorbent assays, and dr. Fiona Maas for her help with GEE analyses.

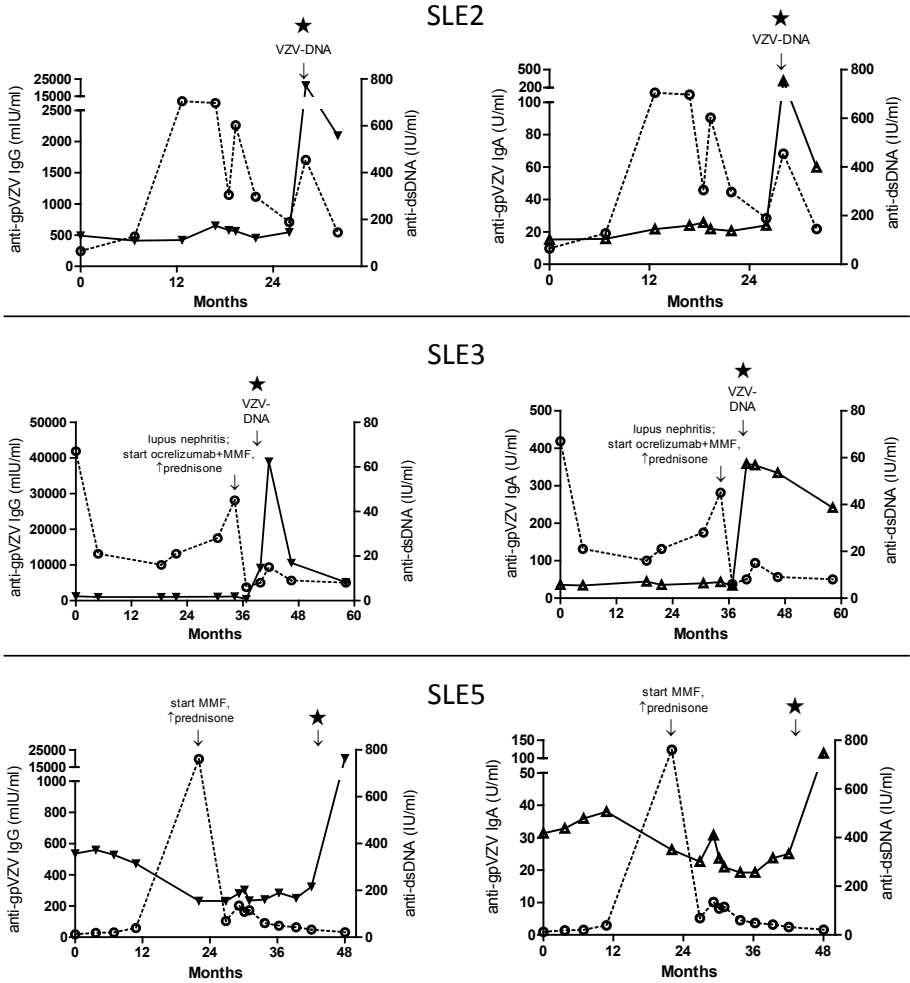
## REFERENCES

1. Borba EF, Ribeiro AC, Martin P, Costa LP, Guedes LK, Bonfa E. Incidence, risk factors, and outcome of Herpes zoster in systemic lupus erythematosus. *J Clin Rheumatol* 2010;16:119-22.
2. Kahl LE. Herpes zoster infections in systemic lupus erythematosus: risk factors and outcome. *J Rheumatol* 1994;21:84-6.
3. Manzi S, Kuller LH, Kutzer J, Pazin GJ, Sinacore J, Medsger TA, Jr, Ramsey-Goldman R. Herpes zoster in systemic lupus erythematosus. *J Rheumatol* 1995;22:1254-8.
4. Chakravarty EF, Michaud K, Katz R, Wolfe F. Increased incidence of herpes zoster among patients with systemic lupus erythematosus. *Lupus* 2013;22:238-44.
5. Ginzler E, Diamond H, Kaplan D, Weiner M, Schlesinger M, Seleznick M. Computer analysis of factors influencing frequency of infection in systemic lupus erythematosus. *Arthritis Rheum* 1978;21:37-44.
6. Bond D, Mooney J. A literature review regarding the management of varicella-zoster virus. *Musculoskeletal Care* 2010;8:118-22.
7. Heininger U, Seward JF. Varicella. *Lancet* 2006;368:1365-76.
8. Gershon AA, Gershon MD, Breuer J, Levin MJ, Oaklander AL, Griffiths PD. Advances in the understanding of the pathogenesis and epidemiology of herpes zoster. *J Clin Virol* 2010;48 Suppl 1:S2-7.
9. Oxman MN. Herpes zoster pathogenesis and cell-mediated immunity and immunosenescence. *J Am Osteopath Assoc* 2009;109:S13-7.
10. Rondaan C, de Haan A, Horst G, et al. Altered cellular and humoral immunity to varicella-zoster virus in patients with autoimmune diseases. *Arthritis Rheumatol* 2014;66:3122-8.
11. Rasmussen NS, Draborg AH, Nielsen CT, Jacobsen S, Houen G. Antibodies to early EBV, CMV, and HHV6 antigens in systemic lupus erythematosus patients. *Scand J Rheumatol* 2015;44:143-9.
12. Berkun Y, Zandman-Goddard G, Barzilai O, et al. Infectious antibodies in systemic lupus erythematosus patients. *Lupus* 2009;18:1129-35.
13. Draborg A, Izarzugaza JM, Houen G. How compelling are the data for Epstein-Barr virus being a trigger for systemic lupus and other autoimmune diseases? *Curr Opin Rheumatol* 2016;28:398-404.
14. Nagasawa K, Yamauchi Y, Tada Y, Kusaba T, Niho Y, Yoshikawa H. High incidence of herpes zoster in patients with systemic lupus erythematosus: an immunological analysis. *Ann Rheum Dis* 1990;49:630-3.
15. Park HB, Kim KC, Park JH, et al. Association of reduced CD4 T cell responses specific to varicella zoster virus with high incidence of herpes zoster in patients with systemic lupus erythematosus. *J Rheumatol* 2004;31:2151-5.
16. Schub D, Janssen E, Leyking S, et al. Altered phenotype and functionality of varicella zoster virus-specific cellular immunity in individuals with active infection. *J Infect Dis* 2015;211:600-12.
17. Cradock-Watson JE, Ridehalgh MK, Bourne MS. Specific immunoglobulin responses after varicella and herpes zoster. *J Hyg (Lond)* 1979;82:319-36.
18. Ljungman P, Lonnqvist B, Gahrton G, Ringden O, Sundqvist VA, Wahren B. Clinical and subclinical reactivations of varicella-zoster virus in immunocompromised patients. *J Infect Dis* 1986;153:840-7.

19. Cohrs RJ, Mehta SK, Schmid DS, Gilden DH, Pierson DL. Asymptomatic reactivation and shed of infectious varicella zoster virus in astronauts. *J Med Virol* 2008;80:1116-22.
20. Mehta SK, Cohrs RJ, Forghani B, Zerbe G, Gilden DH, Pierson DL. Stress-induced subclinical reactivation of varicella zoster virus in astronauts. *J Med Virol* 2004;72:174-9.
21. Tan EM, Cohen AS, Fries JF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982;25:1271-7.
22. van Doornum GJ, Guldemeester J, Osterhaus AD, Niesters HG. Diagnosing herpesvirus infections by real-time amplification and rapid culture. *J Clin Microbiol* 2003;41:576-80.
23. Draborg AH, Jacobsen S, Westergaard M, Mortensen S, Larsen JL, Houen G, Duus K. Reduced response to Epstein-Barr virus antigens by T-cells in systemic lupus erythematosus patients. *Lupus Sci Med* 2014;1:e000015,2014-000015. eCollection 2014.
24. Hanlon P, Avenell A, Aucott L, Vickers MA. Systematic review and meta-analysis of the sero-epidemiological association between Epstein-Barr virus and systemic lupus erythematosus. *Arthritis Res Ther* 2014;16:R3.
25. Doerr HW, Rentschler M, Scheifler G. Serologic detection of active infections with human herpes viruses (CMV, EBV, HSV, VZV): diagnostic potential of IgA class and IgG subclass-specific antibodies. *Infection* 1987;15:93-8.

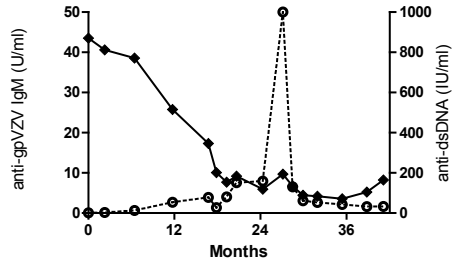
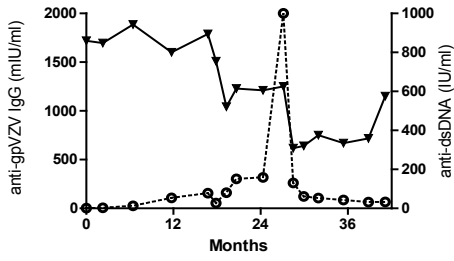
# SUPPLEMENTARY DATA

Supplementary Figures. Antibody levels, presence of VZV-DNA and anti-dsDNA (as a measure of lupus disease activity) for all patients with a VZV reactivation.

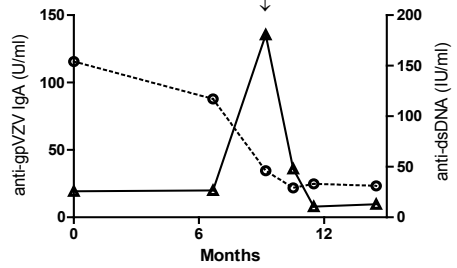
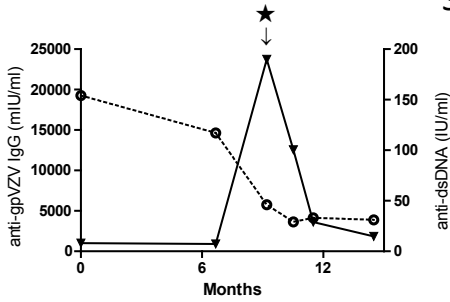




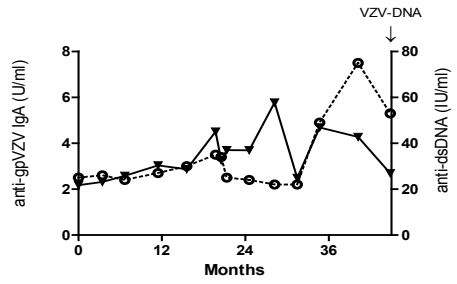
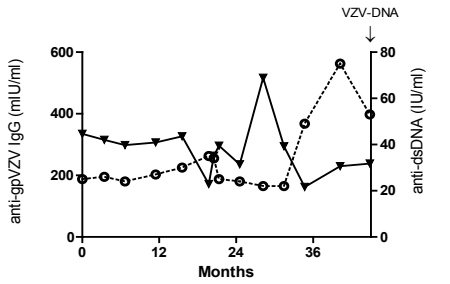
### SLE6



### SLE15



### SLE17



### SLE23

