

# Effect of Add-on Hydroxychloroquine Therapy on Serum Proinflammatory Factor Levels in Patients with Systemic Lupus Erythematosus With or Without Lupus Nephritis

Risa Wakiya (✉ [risa-wakiya@med.kagawa-u.ac.jp](mailto:risa-wakiya@med.kagawa-u.ac.jp))

Kagawa University <https://orcid.org/0000-0003-1998-548X>

Kiyo Ueda

Kagawa University

Shusaku Nakashima

Kagawa University

Hiroshi Shimada

Kagawa University

Tomohiro Kameda

Kagawa University

Mai Mahmoud Fahmy Mansour

Kagawa University

Mikiya Kato

Kagawa University

Koichi Sugihara

Kagawa University

Yusuke Ushio

Kagawa University

Norimitsu Kadowaki

Kagawa University

Hiroaki Dobashi

Kagawa University

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## Research article

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# Abstract

**Background:** We investigated the effects of add-on hydroxychloroquine (HCQ) therapy on the expression of proinflammatory cytokines and other factors in systemic lupus erythematosus (SLE) patients with low disease activity.

**Methods:** Patients who had low disease activity of at least 3 months duration were included. Patients with a history of lupus nephritis (LN+) must have been in remission for at least 3 months prior to enrollment. Serum levels of interferon  $\alpha$ , S100A8, S100A9, tumor necrosis factor (TNF)  $\alpha$ , interleukin (IL)-2, IL-6, IL-8, vascular endothelial growth factor (VEGF)-A, Monocyte Chemoattractant Protein-1, macrophage inflammatory protein-1 $\alpha$ , IL-1 $\beta$ , Interleukin 1 receptor antagonist (IL-1ra), and Granulocyte Colony Stimulating Factor were measured immediately before and 3 months after treatment with oral HCQ treatment.

**Results:** Of the 42 patients enrolled in the study (4 males, 38 females, mean age  $\pm$  standard deviation age  $41.4 \pm 13.3$  years), 19 patients had a history of lupus nephritis but were currently in remission (LN+), and the remaining 23 patients had no history of LN (LN-). Serum levels of IL-1ra, S100A8, and S100A9 at baseline were significantly higher in the LN+ group compared with the LN- group ( $p=0.0092$ ,  $p=0.012$ , and  $p=0.0043$ , respectively). In the full cohort, HCQ treatment led to significantly reduced serum levels of TNF- $\alpha$ , IL-6, VEGF-A, IL-1ra, IL-2, S100A8, and S100A9, and to decreased, albeit not significantly, levels of IL-8 and MIP-1 $\alpha$ . The HCQ-induced changes in serum IL-8, IL-1ra, S100A8, and S100A9 levels were greater for patients in the LN+ group than those in the LN-group ( $p=0.0039$ ,  $p=0.0011$ ,  $p=0.0201$ , and  $p=0.0092$ , respectively).

**Conclusion:** Add-on HCQ treatment decreased several proinflammatory cytokines levels in SLE patients with low disease activity, especially those with LN. The ability of HCQ to reduce IL-8 levels in patients with a history of LN suggests that HCQ treatment may improve the prognosis of LN.

## Introduction

Systemic lupus erythematosus (SLE) is a chronic systemic autoimmune disease in which multiple organ systems can be damaged by autoantibodies, immune complexes, and inflammation [1]. The pathogenesis of SLE results from the complex interplay of immunological, genetic, and environmental factors [2]. Several studies have identified associations between levels of various proinflammatory cytokines and SLE disease activity with specific clinical manifestations. In particular, dysregulation of interferons (IFNs) has been suggested to be involved in the pathogenesis of SLE. Accordingly, transcriptome analysis has confirmed that numerous IFN-stimulated genes are upregulated in the peripheral blood mononuclear cells of patients with SLE, and serum IFN- $\alpha$  levels are elevated in SLE patients in a manner that correlates with disease activity and severity [3–5].

Bauer et al. reported that levels of IFN and IFN-inducible chemokines, such as macrophage inflammatory protein-1 (MIP-1), monocyte chemoattractant protein-1 (MCP-1) and interferon-inducible protein-10, correlate

with disease activity, as measured by various disease activity indices such as erythrocyte sedimentation rate and anti-dsDNA antibody titers [6, 7]. Other proinflammatory cytokines that have been shown to correlate with SLE disease activity include tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin (IL)-6, IL-8, IL-10, and vascular endothelial growth factor (VEGF) [8–12]. Recent work has reported that myeloid-related proteins (MRPs), also known as damage-associated molecular patterns, may be involved in the pathogenesis of multiple autoimmune diseases [13, 14]. In SLE patients, serum levels of these proteins, which include MRP8, MRP14, and S100 proteins, are positively correlated with disease activity, especially lupus nephritis (LN) [15]. Histological analyses have also shown these proteins to be expressed in renal tissues in SLE patients, with levels proportional to the severity of LN [16, 17].

Despite these studies, the associations between serum levels of proinflammatory cytokines and disease characteristics remain to be fully elucidated. Moreover, there is a need to identify biomarkers to aid in the diagnosis and monitoring of disease activity in patients with SLE, especially those with involvement of the kidneys, the major organs involved in SLE. However, the effects of many SLE treatments on proinflammatory cytokine levels remain unclear.

Hydroxychloroquine (HCQ) is a common add-on treatment for SLE patients with low disease activity (LDA), although the European League Against Rheumatism recommended HCQ for all patients with SLE in 2019 [18]. HCQ was approved for the treatment of SLE in Japan in July 2015, and since then, it has been prescribed as an add-on treatment for many SLE patients on immunosuppressants, especially women of child-bearing age. Although the mechanisms may be unclear, there is evidence for multiple beneficial effects of HCQ in SLE [19]. Willis et al. showed that HCQ therapy resulted in significant clinical improvement in a manner that strongly correlated with reductions in IFN- $\alpha$  levels [20]. Our previous study suggested that HCQ modulated the expression of S100 proteins in SLE patients with LDA [21]. However, little is known about the effects of HCQ on biomarkers in SLE patients with LDA. In the present study, we investigated the effect of add-on HCQ on the expression of proinflammatory cytokines and other serum factors in SLE patients with LDA who were receiving immunosuppressants.

## Patients And Methods

### Patients

This was a single-center prospective study. We enrolled subjects who were diagnosed with SLE using the American College of Rheumatology criteria [22] or the Systemic Lupus Collaborating Clinics criteria [23] and began HCQ treatment for the first time between September 2015 and March 2019. All patients had a  $\geq 3$ -month history of LDA prior to enrollment, defined as (i) a SELENA-SLEDAI score of  $\leq 8$  with no activity in major organ systems, such as renal involvement, neuropsychiatric SLE, cardiopulmonary involvement, and vasculitis; (ii) current prednisolone or equivalent dose of  $\leq 10$  mg per day; and (iii) well-tolerated maintenance doses of other immunosuppressants. Pregnant women and patients who were not currently in complete renal remission [24], regardless of LN history, were excluded. Patients who began anti-thrombotic therapy or add-on immunosuppressants after starting the HCQ treatment were excluded

from the study. Informed consent was obtained from all participants. The study was approved by the ethical committee of Kagawa University (Heisei30-047).

## Treatment and outcomes

Patients were administered oral HCQ sulfate (Plaquenil; Sanofi-Winthrop, Paris, France) continuously for at least 3 months. HCQ was administered at a dose based on ideal body weight (IBW) calculated using the modified Broca's method: 200 mg daily for patients with IBW < 46 kg; 200 mg and 400 mg on alternate days for IBW  $\geq$  46 kg and < 62 kg; and 400 mg daily for IBW  $\geq$  62 kg. Clinical parameters (age, gender, HCQ dose, immunological biomarkers, disease activity indices, and skin scores) were recorded before and after HCQ treatment. Disease activity was evaluated using the SELENA-SLEDAI 2011 criteria [25]. Cutaneous disease activity was evaluated using the Cutaneous Lupus Erythematosus Disease Area and Severity Index (CLASI) [26]. In accord with the CLASI improvement criteria of Klein et al. [27], the principal investigator designated CLASI as improved, unchanged, or worse compared with the previous visit. Patients classified as improved were defined as "CLASI responders" and those classified as unchanged or worse were defined as "CLASI non-responders". Immunological activity was determined by measuring serum levels of complement factors (C3, C4, CH50), anti-double stranded DNA (dsDNA) antibodies, and total white blood cell counts, lymphocyte counts, and platelet counts. Specific ELISA kits were used to measure serum S100A8, S100A9 (CircuLex ELISA Kits, MBL, Woburn, MA, USA), and IFN- $\alpha$  (Human IFN alpha Platinum ELISA Kit, Thermo Fisher Scientific Inc., Carlsbad, CA, USA) according to the manufacturers' instructions. Serum TNF- $\alpha$ , IL-2, IL-6, IL-8, VEGF-A, MCP-1, MIP-1 $\alpha$ , IL-1 $\beta$ , Interleukin 1 receptor antagonist(IL-1ra), and Granulocyte Colony Stimulating Factor (G-CSF) were measured using a multiplex immunoassay (Luminex Assay, R&D Systems).

## Statistical analysis

Data are presented as the mean  $\pm$  standard deviation (SD) unless otherwise noted. Immunological biomarkers and proinflammatory cytokine levels were compared using Student's t test for continuous variables or the Wilcoxon signed-rank test for non-normally distributed data. Comparisons between groups were performed using the Wilcoxon rank sum test. All *p* values were two-sided, and a *p* value < 0.05 was considered significant. Data were analyzed using JMP® 13 software (SAS Institute, Cary, NC, USA).

## Results

### Baseline characteristics

Forty-two SLE patients (38 women, 4 men) with sustained LDA of at least 3 months duration were enrolled in this study. Their baseline characteristics are shown in Table 1. The mean ( $\pm$  SD) age was 41.4  $\pm$  13.3 years, the mean disease duration was 14.8  $\pm$  11.8 years, the mean SELENA-SLEDAI score was 3.7

$\pm 2.0$ , and the mean CLASI activity score was  $3.1 \pm 3.1$ . Of the 42 patients, 19 had a history of LN and had been in complete remission for at least 1 year.

Table 1  
Baseline characteristics of SLE patients enrolled in the study (n = 42)

Characteristics	n = 42
Female, no(%)	38(90)
Age, years, mean $\pm$ SD	41.4 $\pm$ 13.3
Disease duration, years, mean $\pm$ SD	14.8 $\pm$ 11.8
Past involvement	
Skin involvement	37 (88)
Renal involvement	19 (45)
Duration of CR free, years	6.1 $\pm$ 5.2
NPSLE	2 (5)
Complication of APS	7 (17)
Positive rate of autoantibody	
Anti-Sm	8 (19)
Anti-RNP	20 (48)
Anti-SS-A	20 (48)
Anti-SS-B	9 (21)
Lupus anticoagulant	9 (21)
Anti-cardiolipin	16 (38)
Anti- $\beta$ 2GPI	3 (7)
Disease activity	
SELENA-SLEDAI score	3.7 $\pm$ 2.0
CLASI activity score	3.1 $\pm$ 3.1 (n = 27)

\*<sup>1</sup> Two patients received multiple immunosuppressants.

\*<sup>2</sup> Four patients received antiplatelet agent and anticoagulant agent.

\*<sup>3</sup> Anti-dsDNA positive means anti ds-DNA titer increases over 12 IU/ml

\*<sup>4</sup> Low complement means any of C3, C4 and CH50 decreases to less 68 mg/dl, less 12 mg/dl, 30 U/ml.

APS: Anti-phospholipid antibody syndrome, NPSLE: neuropsychiatric SLE,

Characteristics	n = 42
CLASI damage score	0.5 ± 1.3 (n = 27)
anti-dsDNA positive, no(%) *3	15 (36)
anti-dsDNA (IU/mL)	15.7 ± 18.2
C3 (mg/dL)	80.9 ± 23.7
C4 (mg/dL)	16.0 ± 7.9
CH50 (U/mL)	35.2 ± 8.7
low complement, no(%) *4	22 (52)
White Blood Cell (/μL)	4960.5 ± 1630.6
Lymphocytes (/μL)	1208.0 ± 673.9
Platelet (× 10 <sup>4</sup> /μL)	21.5 ± 7.3
Concomitant immunosuppressive treatments	
Prednisone	
No. (%)	36 (86)
Median Dosage, mg/day (range)	4.7 (1–10)
Other immunosuppressant*1	
Tacrolimus	13 (31)
Mycophenolate mofetil	7 (17)
Cyclosporine A	2 (5)
Azathioprine	2 (5)
Mizoribine	1 (2)
Methotrexate	1 (2)
*1 Two patients received multiple immunosuppressants.	
*2 Four patients received antiplatelet agent and anticoagulant agent.	
*3 Anti-dsDNA positive means anti ds-DNA titer increases over 12 IU/ml	
*4 Low complement means any of C3, C4 and CH50 decreases to less 68 mg/dl, less 12 mg/dl, 30 U/ml.	
APS: Anti-phospholipid antibody syndrome, NPSLE: neuropsychiatric SLE,	

## Baseline serum levels of proinflammatory factors

Serum levels of IL-1ra, S100A8, and S100A9 at baseline were significantly higher in SLE patients with a history of LN (LN+) compared with those without a history of LN (LN-) ( $p = 0.0092$ ,  $p = 0.012$ , and  $p = 0.0043$ , respectively; Fig. 1). There were no significant differences between the LN+ and LN- groups in the baseline levels of other proinflammatory cytokines (Fig. 1). Some proinflammatory cytokines were not detected in all SLE patients; for example, serum IFN- $\alpha$  and IL-1 $\beta$  were detected in only 6 and 15 of the 42 patients, respectively (Fig. 1).

## Serum levels of proinflammatory factor after HCQ treatment

We analyzed serum levels of proinflammatory cytokines at 3 months after initiation of HCQ treatment. As shown in Fig. 2, TNF- $\alpha$ , IL-6, VEGF-A, IL-1ra, IL-2, S100A8, and S100A9 decreased significantly after HCQ treatment. Although IL-8, MIP-1 $\alpha$ , and IL-1 $\beta$  levels were also decreased, the difference from baseline was not significant. In the patients with detectable levels of MCP-1 ( $n = 42$ ), G-CSF ( $n = 37$ ), and IFN- $\alpha$  ( $n = 6$ ) at baseline, no change after add-on HCQ treatment was detected (Fig. 2). The HCQ-induced changes in IL-8, IL-1ra, S100A8, and S100A9 levels were significantly greater in the LN+ group compared with the LN- group (Fig. 3). As for association with proinflammatory factors, changes in IL-8, IL-1 $\beta$ , IL-1ra and IL-2 levels correlated with those of serum S100 protein (Table 2). No associations were detected between changes in serum proinflammatory cytokine levels and immunological biomarkers or disease activity scores (Supplemental Material Table).

Table 2  
Associations between changes in serum factor levels and S100 proteins after hydroxychloroquine treatment

	$\Delta$ S100A8		$\Delta$ S100A9	
	r	P	r	P
$\Delta$ TNF- $\alpha$	0.0412	0.8116	0.178	0.2988
$\Delta$ IL-6	0.205	0.1933	0.204	0.1939
$\Delta$ IL-8	<b>0.318</b>	<b>0.0489</b>	0.279	0.0859
$\Delta$ MCP-1	-0.261	0.0948	0.00227	0.9886
$\Delta$ VEGF-A	0.180	0.2724	0.147	0.3722
$\Delta$ IL-1 $\beta$	0.280	0.5915	<b>0.840</b>	<b>0.0364</b>
$\Delta$ IL-1ra	<b>0.513</b>	<b>0.0005</b>	<b>0.574</b>	<b>&lt; 0.0001</b>
$\Delta$ MIP-1a	0.0704	0.7116	0.181	0.3373
$\Delta$ IL-2	<b>0.366</b>	<b>0.0397</b>	<b>0.393</b>	<b>0.0261</b>
$\Delta$ G-CSF	-0.0683	0.6879	0.0344	0.8400
$\Delta$ IFN- $\alpha$	0.663	0.3373	0.693	0.3070
<i>P</i> and r values were determined by univariate analysis.				
P values < 0.05 are indicated in bold.				

## Discussion

Previous studies have demonstrated significant changes in many proinflammatory cytokines, especially IL-1, IL-6, TNF- $\alpha$ , and IFNs, in SLE patients [28, 29]. However, serum levels of most of the proinflammatory cytokines measured here were not associated with disease activity indices such as, SLEDAI and CLASI, or with serum immunological biomarkers, most likely because our study only included SLE patients with LDA.

We detected decreases in serum levels of TNF- $\alpha$ , IL-6, VEGF-A, IL-1ra, IL-2, MIP-1 $\alpha$ , IL-8, IL-1 $\beta$ , S100A8, and S100A9 after add-on HCQ treatment. Our results differ from those of Monzavi et al., who reported no change in serum IL-8 levels after treatment of newly diagnosed SLE patients with HCQ [30]. This difference, together with the observed lack of response of other factors, such as MCP-1, to HCQ treatment, is likely to be due to the inclusion of only patients with LDA. Plasma and urinary IL-8 levels are reportedly associated with LN activity [8, 31–33], and serum IL-6, IL-8 and IL-18 levels have been proposed to be useful predictors of relapse in SLE [34]. We found that HCQ had a greater effect on serum IL-8 levels in patients with a history of renal involvement compared with patients with no history, suggesting that HCQ

may help improve the prognosis and prevent relapse of LN. Of note, it is possible that even renal lesions considered to be in remission may express elevated levels of proinflammatory cytokines.

Despite its common use, the mechanism of action of HCQ in autoimmune diseases is unclear. HCQ is a weak base and is known to raise the pH of acidic intracellular vesicles and interfere with their physiological functions, including autophagy and antigen processing [35]. In addition, HCQ interferes with intracellular signaling, which may suppress the response to engagement of the innate Toll-like receptors (TLRs), thereby inhibiting the production and release of cytokines and promoting apoptosis in lymphocytes and endothelial cells [36, 37]. Thus, both acidification of endosomal vesicles and increased lymphocyte apoptosis following HCQ treatment may contribute to the decreased production of proinflammatory cytokines. In addition, HCQ-mediated inhibition of TLR activation suppresses the activity of plasmacytoid dendritic cells and autoreactive B cells in SLE patients [37], leading to a reduction in inflammation. Indeed, Sacre et al. demonstrated that HCQ treatment of SLE patients reduced the ability of plasmacytoid dendritic cells to produce IFN- $\alpha$  and TNF- $\alpha$  in response to TLR-9 and TLR-7 stimulation *in vivo* [38]. We believe that the mechanism of cytokine reduction by HCQ in the present study may be mediated by these effects.

We previously reported that HCQ modulates serum levels of S100A8 and S100A9 in SLE patients with LDA [21]. S100 proteins are components of neutrophil extracellular traps (NETs), which play an important role in the pathogenesis of SLE [39] [40]. In addition, chloroquine and HCQ have been reported to inhibit NETs *in vivo* and *in vitro* [41, 42], which may suggest a mechanism for the regulation of S100 proteins. S100A8 and S100A9 proteins upregulate the expression of proinflammatory cytokines such as IL-6 and IL-8 [43, 44]. Therefore, HCQ-mediated modulation of S100 proteins may also be involved in the suppression of proinflammatory cytokine expression in SLE patients.

There are several limitations to this study. First, we did not monitor HCQ adherence by measuring blood HCQ levels. Second, whether the change in proinflammatory cytokine levels were a direct result of add-on HCQ treatment is difficult to determine unequivocally because some patients were receiving other immunosuppressants. Third, the sample size was small due to the strict inclusion and exclusion criteria. Finally, serum IFN- $\alpha$  levels were undetectable in most patients. Nevertheless, our study has merit because it is the first to demonstrate the effect of add-on HCQ treatment on proinflammatory cytokines in SLE patients with LDA.

## Conclusions

We found that add-on HCQ treatment decreased the levels of several proinflammatory cytokines in SLE patients with LDA, especially those with LN. Our results suggest that HCQ treatment may reduce IL-8 expression in patients with LN in remission, which could improve the renal prognosis.

## List Of Abbreviations

Hydroxychloroquine; HCQ, systemic lupus erythematosus; SLE, lupus nephritis; LN+, tumor necrosis factor; TNF, interleukin; IL, vascular endothelial growth factor; VEGF, Interleukin 1 receptor antagonist; IL-1ra, no history of LN; LN-, interferons; IFNs, macrophage inflammatory protein-1; MIP-1, monocyte chemotactic protein-1; MCP-1, myeloid-related proteins; MRPs, lupus nephritis; LN, low disease activity; LDA, ideal body weight; IBW, Cutaneous Lupus Erythematosus Disease Area and Severity Index; CLASI, anti-double stranded DNA; dsDNA, Granulocyte Colony Stimulating Factor; G-CSF, standard deviation; SD, Toll-like receptors; TLRs, neutrophil extracellular traps; NETs

## **Declarations**

### **Ethical Approval and Consent to participate**

The study was approved by the ethical committee of Kagawa University (Heisei30-047) and prospectively registered. All participants gave their written informed consent prior to entering the study. The study was conducted in accordance with the Declaration of Helsinki.

### **Consent for publication**

Not applicable.

### **Availability of supporting data**

The dataset supporting the conclusions of this article is available upon reasonable request.

### **Competing interests**

The authors declared no conflicts of interest.

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### **Authors' contributions**

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be submitted for publication.

RW and HD planned the study and wrote the manuscript. RW conducted the study and interpreted the results together with KU, SN, HS, TK, MK. YU, KS and HD. MFMM and NK reviewed the manuscript for intellectual content. The authors read and approved the final manuscript.

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## References

1. Liu Z, Davidson A. Taming lupus-a new understanding of pathogenesis is leading to clinical advances. *Nat Med*. 2012;18:871–82.
2. Chasset F, Arnaud L. Targeting interferons and their pathways in systemic lupus erythematosus. *Autoimmun Rev*. 2018;17:44–52.
3. Bennett L, Palucka AK, Arce E, Cantrell V, Borvak J, Banchereau J, et al. Interferon and granulopoiesis signatures in systemic lupus erythematosus blood. *J Exp Med*. 2003;197:711–23.
4. Chiche L, Jourde-Chiche N, Whalen E, Presnell S, Gersuk V, Dang K, et al. Modular transcriptional repertoire analyses of adults with systemic lupus erythematosus reveal distinct type I and type II interferon signatures. *Arthritis Rheumatol*. 2014;66:1583–95.
5. Ytterberg SR, Schnitzer TJ. Serum interferon levels in patients with systemic lupus erythematosus. *Arthritis Rheum*. 1982;25:401–6.
6. Bauer JW, Baechler EC, Petri M, Batliwalla FM, Crawford D, Ortmann WA, et al. Elevated serum levels of interferon-regulated chemokines are biomarkers for active human systemic lupus erythematosus. *PLoS Med*. 2006;12:e491.
7. Bauer JW, Petri M, Batliwalla FM, Koeuth T, Wilson J, Slattery C, et al. Interferon-regulated chemokines as biomarkers of systemic lupus erythematosus disease activity: a validation study. *Arthritis Rheum*. 2009;60:3098–107.
8. Idborg H, Eketjäll S, Pettersson S, Gustafsson JT, Zickert A, Kvarnström M, et al. TNF- $\alpha$  and plasma albumin as biomarkers of disease activity in systemic lupus erythematosus. *Lupus Sci Med*. 2018; doi:10.1136/lupus-2018-000260.
9. Davas EM, Tsirogianni A, Kappou I, Karamitsos D, Economidou I, Dantis PC. Serum IL-6, TNF $\alpha$ , p55 srTNF $\alpha$ , p75srTNF $\alpha$ , srlL-2 $\alpha$  levels and disease activity in systemic lupus erythematosus. *Clin Rheumatol*. 1999;18:17–22.
10. Mao YM, Zhao CN, Liu LN, Wu Q, Dan YL, Wang DG, et al. Increased circulating interleukin-8 levels in systemic lupus erythematosus patients: a meta-analysis. *Biomark Med*. 2018;12:1291–1302.
11. Grondal G, Gunnarsson I, Ronnelid J, Rogberg S, Klareskog L, Lundberg I. Cytokine production, serum levels and disease activity in systemic lupus erythematosus. *Clin Exp Rheumatol*. 2000;18:565–70.
12. Adhya Z, El Anbari M, Anwar S, Mortimer A, Marr N, Karim MY. Soluble TNF-R1, VEGF and other cytokines as markers of disease activity in systemic lupus erythematosus and lupus nephritis. *Lupus*. 2019;28:713–21.
13. Hammer HB, Odegard S, Fagerhol MK, Landewé R, van der Heijde D, Uhlig T, et al. Calprotectin (a major leucocyte protein) is strongly and independently correlated with joint inflammation and damage in rheumatoid arthritis. *Ann Rheum Dis*. 2007;66:1093–7.
14. Kane D, Roth J, Frosch M, Vogl T, Bresnihan B, FitzGerald O. Increased perivascular synovial membrane expression of myeloid-related proteins in psoriatic arthritis. *Arthritis Rheum*. 2003;48:1676–85.

15. Tydén H, Lood C, Gullstrand B, Jönsen A, Ivars F, Leanderson T, et al. Pro-inflammatory S100 proteins are associated with glomerulonephritis and anti-dsDNA antibodies in systemic lupus erythematosus. *Lupus* 2017;26:139–49.
16. Frosch M, Vogl T, Waldherr R, Sorg C, Sunderkotter C, Roth J. Expression of MRP8 and MRP14 by macrophages is a marker for severe forms of glomerulonephritis. *J Leukocyte Biol.* 2004;75:198–206.
17. Tantivitayakul P, Benjachat T, Somparn P, Leelahavanichkul A, Kittikovit V, Hirankarn N, et al. Elevated expressions of myeloid-related proteins-8 and -14 are danger biomarkers for lupus nephritis. *Lupus* 2016;25:38–45.
18. Fanouriakis A, Kostopoulou M, Alunno A, Aringer M, Bajema I, Boletis JN, et al. 2019 update of the EULAR recommendations for the management of systemic lupus erythematosus. *Ann Rheum Dis.* 2019;78:736–45.
19. Ruiz-Irastorza G, Ramos-Casals M, Brito-Zeron P, Khamashta MA. Clinical efficacy and side effects of antimalarials in systemic lupus erythematosus: a systematic review. *Ann Rheum Dis.* 2010;69:20–8.
20. Willis R, Seif AM, McGwin G Jr, Martinez-Martinez LA, González EB, Dang N, et al. Effect of hydroxychloroquine treatment on pro-inflammatory cytokines and disease activity in SLE patients: data from LUMINA (LXXV), a multiethnic US cohort. *Lupus* 2012;21:830–5.
21. Wakiya R, Kameda T, Ueeda K, Nakashima S, Shimada H, Mansour MF, et al. Hydroxychloroquine modulates elevated expression of S100 proteins in systemic lupus erythematosus. *Lupus* 2019;28:826–33.
22. Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum.* 1982;25:1271–7.
23. Petri M, Orbai AM, Alarcón GS, Gordon C, Merrill JT, Fortin PR, et al. Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. *Arthritis Rheum.* 2012;64:2677–86.
24. Bertsias GK, Tektonidou M, Amoura Z, Aringer M, Bajema I, Berden JH, et al. Joint European League Against Rheumatism and European Renal Association-European Dialysis and Transplant Association (EULAR/ERA-EDTA) recommendations for the management of adult and paediatric lupus nephritis. *Ann Rheum Dis.* 2012;71:1771–82.
25. Petri M, Kim M.Y, Kalunian K.C, Grossman J, Hahn B.H, Sammaritano L.R, et al. Combined oral contraceptives in women with systemic lupus erythematosus *N Engl J Med.* 2005;353:2550-2558
26. Albrecht J, Taylor L, Berlin JA, Dulay S, Ang G, Fakharzadeh S, et al. The CLASI (Cutaneous Lupus Erythematosus Disease Area and Severity Index): an outcome instrument for cutaneous lupus erythematosus. *J Invest Dermatol.* 2005 ;125:889-94.
27. Klein R, Moghadam-Kia S, LoMonico J, Okawa J, Coley C, Taylor L, et al. Development of the CLASI as a tool to measure disease severity and responsiveness to therapy in cutaneous lupus erythematosus. *Arch Dermatol.* 2011;147:203–8.

28. Willis R, Smikle M, DeCeulaer K, Romay-Penabad Z, Papalardo E, Jajoria P, et al. Clinical associations of proinflammatory cytokines, oxidative biomarkers and vitamin D levels in systemic lupus erythematosus. *Lupus* 2017;26:1517–27.
29. Umare V, Pradhan V, Nadkar M, Rajadhyaksha A, Patwardhan M, Ghosh KK, et al. Effect of proinflammatory cytokines (IL-6, TNF- $\alpha$ , and IL-1 $\beta$ ) on clinical manifestations in Indian SLE patients. *Mediators Inflamm.* 2014;385297.
30. Monzavi SM, Alirezai A, Shariati-Sarabi Z, Tavakol Afshari J, Mahmoudi M, Dormanesh B, et al. Efficacy analysis of hydroxychloroquine therapy in systemic lupus erythematosus: a study on disease activity and immunological biomarkers. *Inflammopharmacology* 2018;26:1175–82.
31. Lit LCW, Wong CK, Tam LS, Li EKM, Lam CWK. Raised plasma concentration and ex vivo production of inflammatory chemokines in patients with systemic lupus erythematosus. *Ann Rheum Dis.* 2006;65:209–15.
32. Reyes-Thomas J, Blanco I, Putterman C. Urinary biomarkers in lupus nephritis. *Review Clin Rev Allergy Immunol.* 2011;40:138–50.
33. El-Shehaby A, Darweesh H, El-Khatib M, Momtaz M, Marzouk S, El-Shaarawy N, et al. Correlations of urinary biomarkers, TNF-like weak inducer of apoptosis (TWEAK), osteoprotegerin (OPG), monocyte chemoattractant protein-1 (MCP-1), and IL-8 with lupus nephritis. *J Clin Immunol.* 2011;31:848–56.
34. Ruchakorn N, Ngamjanyaporn P, Suangtamai T, Kafaksom T, Polpanumas C, Petpisit V, et al. Performance of cytokine models in predicting SLE activity. *Arthritis Res Ther.* 2019; doi:10.1186/s13075-019-2029-1.
35. Schrezenmeier E, Dörner T. Mechanisms of action of hydroxychloroquine and chloroquine: implications for rheumatology. *Nat Rev Rheumatol.* 2020;16:155–66.
36. Rainsford KD, Parke AL, Clifford-Rashotte M, Kean WF. Therapy and pharmacological properties of hydroxychloroquine and chloroquine in treatment of systemic lupus erythematosus, rheumatoid arthritis and related diseases. *Inflammopharmacology* 2015;23:231–69.
37. Lenert PS. Targeting Toll-like receptor signaling in plasmacytoid dendritic cells and autoreactive B cells as a therapy for lupus. *Arthritis Res Ther.* 2006;8:203.
38. Sacre K, Criswell LA, McCune JM. Hydroxychloroquine is associated with impaired interferon-alpha and tumor necrosis factor-alpha production by plasmacytoid dendritic cells in systemic lupus erythematosus. *Arthritis Res Ther.* 2012;14:R155.
39. Salemme R, Peralta LN, Harika Meka S, Pushpanathan N, Alexander JJ. The role of NETosis in systemic lupus erythematosus. *J Cell Immunol.* 2019;1:33–42.
40. Urban CF, Ermert D, Schmid M, Abu-Abed U, Goosmann C, Nacken W, et al. Neutrophil extracellular traps contain calprotectin, a cytosolic protein complex involved in host defense against *Candida albicans*. *PLoS Pathog.* 2009; doi:10.1371/journal.ppat.1000639.
41. Zhang S, Zhang Q, Wang F, Guo X, Liu T, Zhao Y, et al. Hydroxychloroquine inhibiting neutrophil extracellular trap formation alleviates hepatic ischemia/reperfusion injury by blocking TLR9 in mice. *Clin Immunol.* 2020; doi:10.1016/j.clim.2020.108461.

42. Smith CK, Vivekanandan-Giri A, Tang C, Knight JS, Mathew A, Padilla RL, et al. Neutrophil extracellular trap-derived enzymes oxidize high-density lipoprotein: an additional proatherogenic mechanism in systemic lupus erythematosus. *Arthritis Rheumatol.* 2014;66:2532–44.
43. Crowe LAN, McLean M, Kitson SM, Garcia Melchor E, Patommel K, Cao HM, et al. S100A8 & S100A9: Alarmin mediated inflammation in tendinopathy. *Sci Rep.* 2019; doi:10.1038/s41598-018-37684-3.
44. Gao H, Zhang X, Zheng Y, Peng L, Hou J, Meng H. S100A9-induced release of interleukin (IL)-6 and IL-8 through toll-like receptor 4 (TLR4) in human periodontal ligament cells. *Mol Immunol.* 2015;67(2 Pt B):223–32.

## Figures

Figure 1.

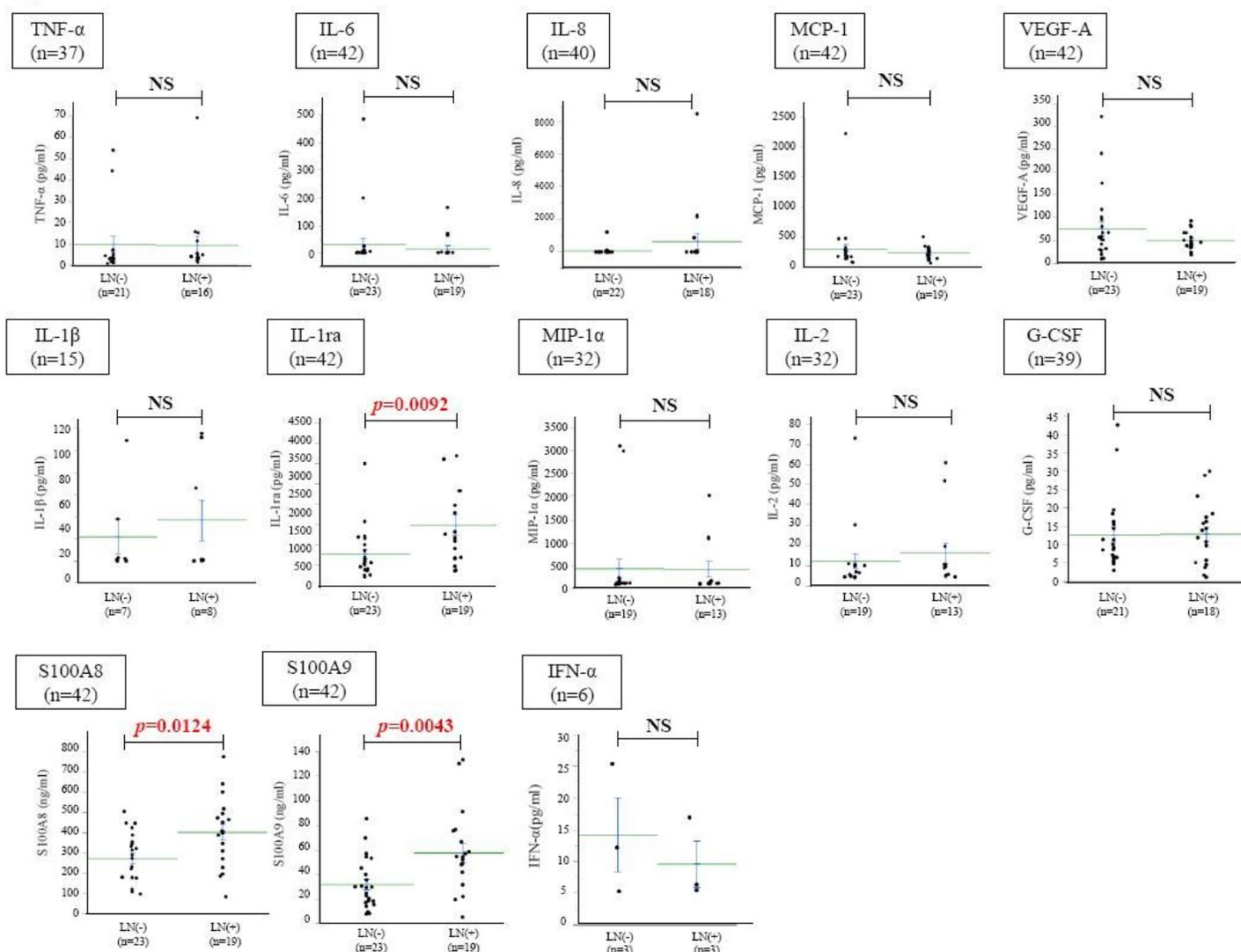


Figure 1

Baseline serum cytokine levels in SLE patients. Serum cytokine levels in SLE patients with (+) or without (-) a history of lupus nephritis (LN) were measured by specific ELISA prior to hydroxychloroquine treatment. Circles represent individual patients, and the green bar indicates mean value. NS: Not significant. P values were determined by the Wilcoxon rank sum test.

Figure 2.

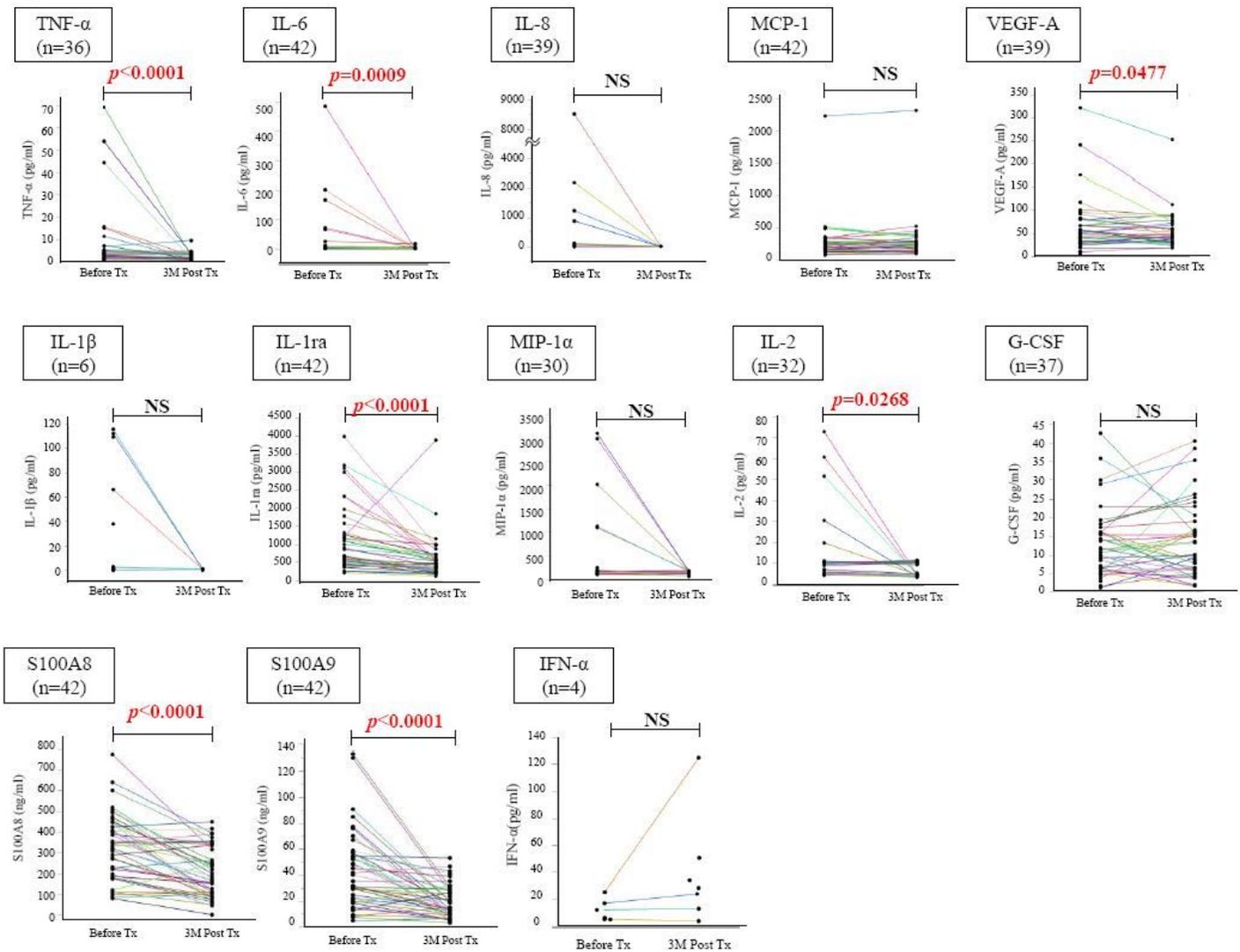


Figure 2

Serum cytokine levels before and after hydroxychloroquine treatment. Serum levels of the indicated cytokines and factors were measured before or after 3 months (3M Post) treatment (Tx) with hydroxychloroquine. Colored lines represent individual patients. NS: not significant. P values were determined by the Wilcoxon signed-rank test

Figure 3.

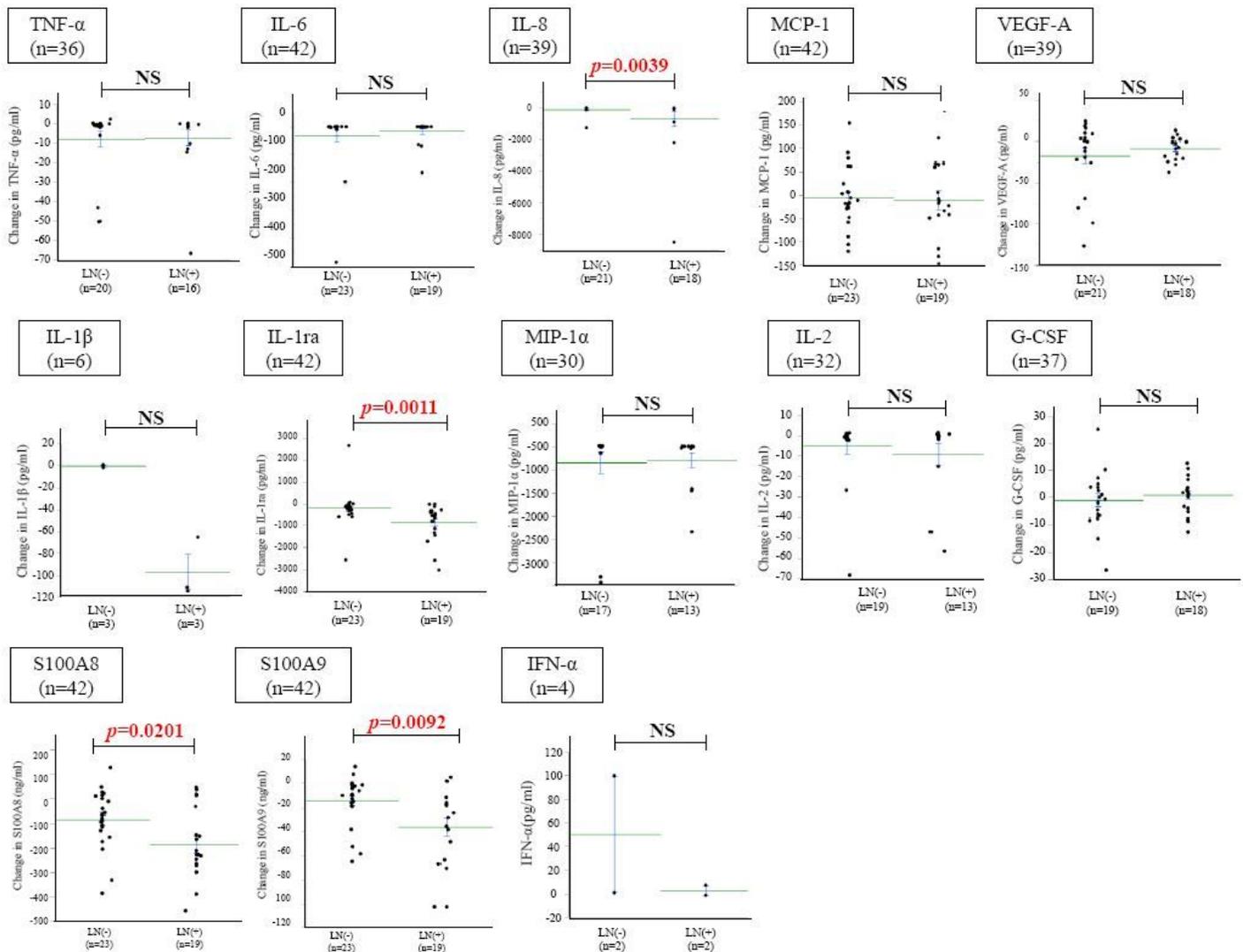


Figure 3

Magnitude of hydroxychloroquine treatment effects in patients according to lupus nephritis history. Serum levels of the indicated factors were measured before and 3 months after treatment with hydroxychloroquine and the difference between the two values is plotted for patients with (+) or without (-) a history of lupus nephritis. Circles represent individual patients, and the green bar indicates mean value. NS: not significant. P values were determined by the Wilcoxon rank sum test.

## Supplementary Files

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