

Low Blood Concentration of Hydroxychloroquine Is a Marker for and Predictor of Disease Exacerbations in Patients With Systemic Lupus Erythematosus

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Objective. To study the possible relationship between whole-blood hydroxychloroquine (HCQ) concentrations and clinical efficacy of HCQ in patients with systemic lupus erythematosus (SLE).

Methods. Whole-blood HCQ concentrations were measured, under blinded conditions, in 143 unselected patients with SLE who had been receiving HCQ 400 mg daily for at least 6 months. The relationship of these concentrations to current disease activity and to subsequent exacerbations during 6 months of followup was investigated.

Results. At baseline, 23 patients had active disease (mean \pm SD SLE Disease Activity Index 12.4 ± 7.5). The mean whole-blood HCQ concentration in this group was significantly lower than that in the 120 patients with inactive disease (694 ± 448 ng/ml versus

$1,079 \pm 526$ ng/ml; $P = 0.001$). Among the 120 patients who had inactive disease at baseline, the mean HCQ concentration at baseline in the 14 (12%) who had disease exacerbations during followup was significantly lower than that in the patients whose disease remained inactive. Multivariate logistic regression showed that the HCQ concentration was the only predictor of exacerbation (odds ratio 0.4 [95% confidence interval 0.18–0.85], $P = 0.01$). Receiver operating characteristic curve analysis showed that a whole-blood HCQ concentration cutoff of 1,000 ng/ml had a negative predictive value of 96% for exacerbation during followup.

Conclusion. Low whole-blood HCQ concentrations are associated with SLE disease activity and are a strong predictor of disease exacerbation. Regular drug assaying and individual tailoring of treatment might help to improve the efficacy of HCQ treatment in patients with SLE.

Hydroxychloroquine (HCQ), an antimalarial drug, is a safe and effective therapy for systemic lupus erythematosus (SLE). The benefits of HCQ treatment have been demonstrated in a randomized, double-blind, placebo-controlled study of 47 SLE patients (1), in which the risk of clinical SLE flares rose 2.5-fold during a 6-month period after discontinuation of the drug. The relative risk of severe SLE exacerbations in patients taking placebo, compared with those who continued to take HCQ, was 6.1 (1).

Few data have been published on the pharmacologic management of HCQ therapy, and the optimal daily dosage in SLE is controversial. The average maintenance dosage recommended for SLE patients in the *Physicians Desk Reference* (2) is 200 mg once or twice per day (1). However, ophthalmologists have suggested that

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the daily dosage should not exceed 6.5 mg/kg, in order to avoid retinal toxicity.

HCQ levels can be quantified by high-performance liquid chromatography (HPLC), and a relationship between whole-blood concentrations of HCQ and clinical efficacy in patients with rheumatoid arthritis (RA) has been reported (3–5). We postulated that a similar relationship might exist in patients with SLE. To investigate this, we measured whole-blood HCQ concentrations in 143 unselected SLE patients who were all receiving HCQ, 400 mg/day. We first studied the relationship between HCQ concentrations and SLE activity on the day of HCQ assay (day 0), and then examined whether these baseline HCQ concentrations were predictive of flares during the subsequent 6 months.

PATIENTS AND METHODS

Patients. This study was a longitudinal evaluation of the HCQ pharmacokinetic–pharmacodynamic relationship in SLE patients. The study population consisted of 143 unselected outpatients and inpatients with SLE according to the American College of Rheumatology criteria (6), who were routinely followed up between June 2000 and March 2004 at the Internal Medicine Department of Pitié-Salpêtrière Hospital, Paris (the national referral center for SLE). All patients were age ≥ 16 years, and all had been receiving oral HCQ sulfate (Plaquenil; Sanofi-Winthrop, Paris, France) prescribed at a stable dosage of 400 mg/day for at least 6 months. For inclusion in the study, patients had to be easily able to contact their hospital physician if they developed symptoms of an SLE flare and had to be able to attend regular followup appointments at least every 6 months. Patients who were pregnant or had serious ophthalmic disorders were excluded. Written informed consent was obtained from all participants.

Outcome measures. The primary outcome measure was the occurrence of SLE flares, both on day 0 (the day of blood sampling for whole-blood HCQ assay) and during 6 months of followup (among patients who had inactive SLE on day 0).

Assessment of SLE flare. At each visit (at enrollment and during followup), all patients underwent a complete physical examination and laboratory testing (complete blood cell count, serum creatinine assay, urinalysis, C3 assay, antinuclear antibody test, and anti-double-stranded DNA [anti-dsDNA] antibody measurement by Farr assay). SLE flares were defined using the SLE Disease Activity Index (SLEDAI) (7); flare was denoted by a SLEDAI score of ≥ 6 . All patients were assessed clinically by physicians who were blinded with regard to the blood HCQ concentration.

Followup. Patients who were found to have active SLE on day 0 received standard care and were excluded from the second part of the study. Patients with inactive SLE on day 0 were followed up for 6 months. They were asked to contact their physician immediately if they developed symptoms of an SLE flare, and were promptly examined. Patients with clinically stable disease were assessed for SLE activity at the end of

the followup period. Additional visits occurred prior to the end of the followup period if considered necessary by the patient's physician. All HCQ-treated SLE patients at the Internal Medicine Department of Pitié-Salpêtrière Hospital undergo routine yearly ophthalmologic examinations, including electroretinography.

Pharmacokinetic analyses. Blood (10 ml) was collected in Vacutainers containing 125 units of heparin. The interval between the last ingestion of HCQ and blood sampling was recorded. Owing to the long terminal elimination half-life of HCQ (>40 days), within-day and inter-day variations in blood HCQ concentrations are small (refs. 3, 5, and 8, and Costedoat-Chalumeau N, et al: unpublished observations). As a result, all HCQ concentration data were retained for analysis, regardless of the time from last ingestion to sampling. Blood samples were stored at -20°C , which does not alter the results of HCQ measurements (Costedoat-Chalumeau N, et al: unpublished observations), and were analyzed once the patient had completed the study.

HPLC. For maximum sensitivity and reproducibility, HCQ concentrations were measured in whole blood (4). HCQ was assayed by HPLC with fluorometric detection as described by Tett et al (9), with minor modifications. The detection limit was 10 ng/ml, and the between-day and within-day coefficients of variation were $<8\%$.

Statistical analysis. To assess the predictive value of the blood HCQ concentration for SLE flare during the subsequent 6 months, we constructed a multivariate logistic regression model including age, sex, blood HCQ concentration, interval between the last ingestion of HCQ and blood sampling, corticosteroid therapy, C3 concentration, and anti-dsDNA status. Inclusion in the final model was determined by a backward stepwise process in which the likelihood ratio was used to evaluate the effect of omitting variables. Odds ratios for continuous predictive factors were standardized, thus expressing the risk associated with a 1-SD increase in the continuous predictive factor.

We also constructed a receiver operating characteristic (ROC) curve (plot of sensitivity versus 1 minus specificity) to determine whether baseline HCQ concentrations differed significantly between patients whose disease remained inactive and patients who had an SLE flare during the 6-month followup period. The area under the ROC curve and the 95% confidence interval (95% CI) were calculated by Hanley & McNeil curve comparison, using Analyse-it software (Leeds, UK).

Findings in patients who had SLE flares (on day 0 and/or during the 6 months of followup) and in patients whose disease remained inactive were compared by chi-square test or Fisher's exact test for categorical variables and by Student's *t* test for continuous variables (or Wilcoxon's rank sum test for non-normally distributed data). All *P* values are 2-sided. *P* values less than 0.05 were considered significant. Data were analyzed with SAS software, version 8.2 (SAS Institute, Cary, NC).

RESULTS

Patient characteristics. The demographic and disease characteristics of the 143 patients are shown in

Table 1. Demographic, clinical, and laboratory features of the SLE patients at baseline, by SLE activity status*

Baseline characteristic	Total SLE population (n = 143)	Patients with active SLE at baseline (n = 23)†	Patients with inactive SLE at baseline (n = 120)‡	P, patients with active disease vs. patients with inactive disease
Demographic characteristics				
Age, years	35 ± 11	31 ± 10	36 ± 11	0.05
Female, no. (%)	133 (93)	22 (96)	111 (92)	0.58
Current smokers, no. (%)	33 (23)	6 (26)	27 (22)	0.70
Body weight, kg	63 ± 12	62 ± 10	63 ± 13	0.70
Body mass index, kg/m ²	23 ± 4	22 ± 3	23 ± 4	0.34
Estimated creatinine clearance, ml/minute§	90 ± 24	94 ± 23	89 ± 24	0.40
Disease characteristics				
C3, gm/liter	0.91 ± 0.28	0.69 ± 0.29	0.95 ± 0.25	<0.0001
Anti-ds DNA positive, no. (%)	72 (50)	22 (96)	50 (42)	<0.0001
SLEDAI score	3.4 ± 5.2	12.4 ± 7.5	1.7 ± 1.6	<0.0001
Treatments				
HCQ¶				
Duration, years	5.2 ± 4.7	6.6 ± 4.1	4.8 ± 4.6	0.09
Once-daily regimen, no. (%)	51 (36)	7 (30)	44 (37)	0.56
Prednisone				
No. (%)	104 (73)	17 (74)	87 (72)	0.89
Dosage, mg/day	12.4 ± 10.4	17 ± 14	11 ± 9	0.13
Additional treatment, no. (%)				
Azathioprine	12 (8)	2 (9)	10 (8)	0.70
Cyclophosphamide	9 (6)	1 (4)	8 (7)	0.36
Methotrexate	2 (1)	1 (4)	1 (1)	0.27
HCQ concentration, ng/ml (range)	1,017 ± 532 (0–2,629)	694 ± 448 (0–1,560)	1,079 ± 526 (0–2,629)	0.001

* Baseline was the day of sampling for whole-blood hydroxychloroquine (HCQ) assay. Except where indicated otherwise, values are the mean ± SD. Anti-dsDNA = anti-double-stranded DNA; SLEDAI = Systemic Lupus Erythematosus Disease Activity Index.

† In the 23 patients with active SLE, the specific manifestations of SLE flares were skin rash (n = 10), active glomerulonephritis (n = 10), arthritis (n = 9), central nervous system involvement (n = 2), pleuritis (n = 1), and/or pericarditis (n = 1). Treatment intensification was required in 19 patients, including pulse methylprednisolone (n = 9), increased prednisone dosage (n = 15), and/or addition or increased dosage of an immunosuppressive agent (n = 8).

‡ Among patients with inactive disease, only 2 had clinical symptoms: 1 had worsening cutaneous lesions, and the other had alopecia. Treatment intensification at baseline was not required in any of these patients except the patient with cutaneous lesions, who was prescribed topical treatment.

§ Estimated with the Cockcroft-Gault formula.

¶ All patients received 400 mg of HCQ daily, in either a once-daily regimen or at 200 mg twice a day. The mean ± SD blood HCQ concentration at baseline was very similar between patients taking 2 200-mg pills once a day (1,005 ± 559 ng/ml) and those taking 1 pill twice a day (1,024 ± 519 ng/ml) (P = 0.74).

Table 1. The mean ± SD blood HCQ concentration on day 0 was 1,017 ± 532 ng/ml and was very similar between patients taking 2 pills once a day (1,005 ± 559 ng/ml) and those taking 1 pill twice a day (1,024 ± 519) (P = 0.74). We found large interindividual variations in blood HCQ concentrations, despite the fact that all patients were prescribed the same dosage (400 mg daily); similar variability has been observed in healthy volunteers (8) and in patients with RA (3–5). No retinal toxicity was observed during followup.

Association between low blood HCQ concentrations and SLE flares. On the day of blood HCQ assay (day 0), 23 of the 143 patients had active disease, with a mean ± SD SLEDAI score of 12.4 ± 7.5 (range 6–28). The specific manifestations of SLE flares were skin rash (n = 10), active glomerulonephritis (n = 10), arthritis

(n = 9), central nervous system involvement (n = 2), pleuritis (n = 1), and/or pericarditis (n = 1). In 19 patients the flares necessitated intensification of treatment, including pulse methylprednisolone (n = 9), increased prednisone dosage (n = 15), and/or addition or increased dosage of an immunosuppressive agent (n = 8). In contrast, treatment intensification at baseline was not needed for any of the patients with inactive SLE (except for 1 patient who was prescribed topical treatment for cutaneous lesions).

Characteristics of the patients grouped by disease activity at baseline are summarized in Table 1. Patients with active SLE had a lower mean blood HCQ concentration than patients with inactive SLE and, as expected, also had a lower mean C3 level and were more frequently positive for antibodies to double-stranded DNA.

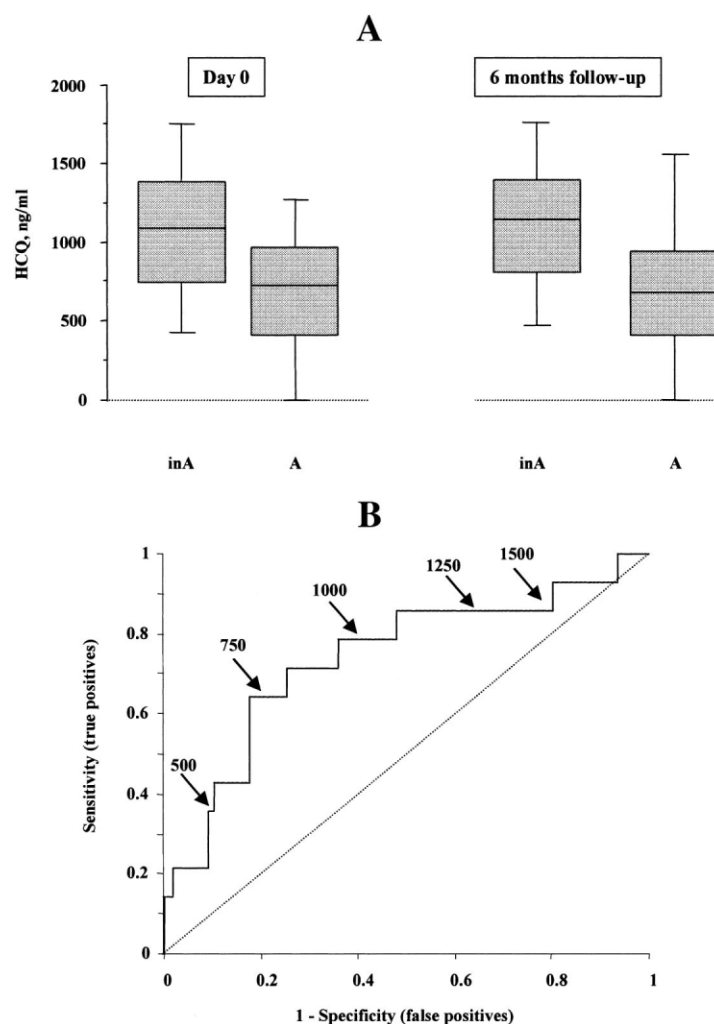


Figure 1. Whole-blood concentrations of hydroxychloroquine (HCQ) according to systemic lupus erythematosus (SLE) activity and receiver operating characteristic (ROC) curves of HCQ concentrations. **A**, Baseline (day 0) whole-blood HCQ concentrations in patients who had active SLE at baseline (A) and patients who had inactive SLE at baseline (inA), and baseline concentrations in patients who had SLE flares (A) and patients whose SLE remained inactive (inA) during the 6-month followup period. Only patients who had inactive SLE at baseline were included in the 6-month analysis. Data are shown as box plots. Each box represents the 25th to 75th percentiles. Lines outside the boxes represent the 10th and 90th percentiles. Lines inside the boxes represent the median. **B**, ROC curve estimates for the 120 patients who had inactive SLE on day 0. Sensitivity and 1 minus specificity (ROC curves) for the risk of SLE flare during the 6-month followup period are shown for various HCQ concentration cutoffs. Numbers with arrows are the HCQ cutoff values (in ng/ml).

On day 0 the mean \pm SD blood HCQ concentration was 694 ± 448 ng/ml in patients with active SLE and $1,079 \pm 526$ ng/ml in patients with inactive SLE ($P = 0.001$) (Table 1 and Figure 1A). HCQ was undetectable in 5 patients, of whom 3 had active SLE on the day of sampling. After exclusion of these 5 patients from the analysis, the mean blood HCQ concentration remained significantly lower in patients with active SLE (798 ± 381 ng/ml versus $1,097 \pm 511$ ng/ml; $P = 0.009$).

Low baseline blood HCQ concentrations are predictive of subsequent SLE flares. As indicated above, patients who were judged to have active SLE on day 0 received standard care and were excluded from the second part of the study. Among the 120 patients who had inactive SLE at baseline, 14 (12%) had a flare during the 6-month followup period, after a mean \pm SD of 3.5 ± 1.9 months (range 1.3–6.0 months). The specific clinical manifestations of SLE flares were arthritis ($n =$

Table 2. Univariate and multivariate analysis of factors potentially predictive of the occurrence of SLE flares during the 6-month followup period*

Baseline characteristic	Univariate analysis			Multivariate analysis		
	Patients with inactive SLE throughout followup (n = 106)	Patients with SLE flares during followup (n = 14)†	P	OR‡	95% CI	P
Demographic characteristics						
Age, years	36 ± 11	38 ± 9	0.36			
Female, no. (%)	98 (92)	13 (93)	0.96			
Current smokers, no. (%)	24 (23)	3 (21)	0.92			
Body weight, kg	63 ± 13	60 ± 6	0.41			
Body mass index, kg/m ²	23 ± 4	22 ± 3	0.60			
Estimated creatinine clearance, ml/minute§	89 ± 25	94 ± 20	0.47			
Disease characteristics						
C3, gm/liter	0.97 ± 0.25	0.81 ± 0.27	0.04	0.53§	0.25–1.13	0.14
Anti-dsDNA positive, no. (%)	40 (37)	10 (71)	0.02	4.12	0.99–17.25	0.053
Treatments						
HCQ¶						
Duration, years	4.8 ± 4.8	4.9 ± 4.1	0.96			
Once-daily regimen, no. (%)	41 (39)	3 (21)	0.22			
Prednisone						
No. (%)	78 (74)	9 (64)	0.47			
Dosage, mg/day	11.2 ± 8.5	14.2 ± 16.2	0.37			
Additional treatment, no. (%)						
Azathioprine	8 (8)	2 (14)	0.40			
Cyclophosphamide	7 (7)	1 (7)	0.94			
Methotrexate	1 (1)	0 (0)	0.99			
HCQ concentration, ng/ml (range)	1,128 ± 507 12–2,629	703 ± 534 0–1,857	0.006	0.4§	0.18–0.85	0.01

* Baseline was the day of sampling for whole-blood HCQ assay. Except where indicated otherwise, values are the mean ± SD. 95% CI = 95% confidence interval (see Table 1 for other definitions).

† In the 14 patients with active SLE, the specific manifestations of SLE flares were arthritis (n = 9), skin rash (n = 4), alopecia (n = 3), active glomerulonephritis (n = 1), pleuritis (n = 1), and/or pericarditis (n = 1).

‡ Odds ratio (OR) for an increase of 1 SD (~500 ng/ml for HCQ).

§ Estimated with the Cockcroft-Gault formula.

¶ All patients received 400 mg HCQ daily, in either a once-daily regimen or at 200 mg twice a day.

9), skin rash (n = 4), alopecia (n = 3), active glomerulonephritis (n = 1), pleuritis (n = 1), and/or pericarditis (n = 1). The mean ± SD SLEDAI score at the time of the flare was 7.2 ± 1.6 (range 6–10). In 13 of these 14 patients the flares necessitated intensification of treatment, including pulse methylprednisolone (n = 2), increased prednisone dosage (n = 9), and/or addition or increased dosage of an immunosuppressive agent (n = 4).

Compared with patients whose SLE did not flare during the 6-month followup, patients who had a flare during followup had a lower baseline mean blood HCQ concentration (mean ± SD 703 ± 534 ng/ml versus 1,128 ± 507 ng/ml; *P* = 0.006) (Table 2 and Figure 1A), a lower baseline mean C3 concentration (0.81 ± 0.27 gm/liter versus 0.97 ± 0.25 gm/liter; *P* = 0.04), and a higher frequency anti-dsDNA antibody positivity at baseline (71% versus 37%; *P* = 0.02) (Table 2). The SLEDAI score at baseline was also significantly higher in patients who had a flare during followup (2.8 ± 1.3

versus 1.5 ± 1.5; *P* < 0.01), but remained within the range of values defining inactive SLE (i.e., <6).

In multivariate logistic regression analysis, the baseline blood HCQ concentration was found to be the only independent predictor of subsequent flares (*P* = 0.01) (Table 2). For an increase in the blood HCQ concentration of 1 SD (~500 ng/ml), the risk of a subsequent flare fell by 60%. The odds ratio for a flare was 5.89 (95% CI 1.38–25.08) in patients with baseline blood HCQ concentrations <1,000 ng/ml. The predictive ability of baseline anti-dsDNA antibody status did not quite reach statistical significance (*P* = 0.053).

The ability of baseline blood HCQ concentrations to discriminate between patients who had flares during followup and patients whose SLE remained inactive was further assessed by ROC curve analysis (Figure 1B). The mean ± SD area under the ROC curve was 0.74 ± 0.08 (95% CI 0.58–0.89, *P* = 0.001). A cutoff of 1,000 ng/ml for the blood HCQ concentration had a

sensitivity of 79% and a specificity of 64% for predicting flares during the 6-month followup period. The negative predictive value of this cutoff was 96%.

Finally, given that the definition of SLE flare is controversial, the univariate and multivariate analysis were secondarily performed by defining SLE flare as an increase of ≥ 3 in the SLEDAI score between baseline and subsequent assessment. With this criterion for SLE flare, the HCQ concentration was the only predictor of flare in the subsequent 6 months, in both the univariate analysis ($P = 0.002$) and the multivariate analysis ($P = 0.004$) (data not shown). Furthermore, the results remained similar when analyses were performed using a SLEDAI score of ≥ 4 to define flare (data not shown).

DISCUSSION

We studied the relationship between the blood HCQ concentration and current and subsequent SLE disease activity in 143 unselected patients receiving HCQ at the same stable dosage. Low blood HCQ concentrations were strongly associated with ongoing disease activity. A similar relationship between blood concentrations of HCQ and its metabolites and clinical efficacy has been demonstrated in RA (3–5).

We also found that low baseline blood HCQ concentrations in patients with inactive SLE were strongly associated with the risk of developing SLE flares during the subsequent 6 months. This is consistent with the pharmacokinetic properties of HCQ, which has a long terminal elimination half-life (>40 days) and small within-day variations in blood concentrations (refs. 3 and 5, and Costedoat-Chalumeau N, et al: unpublished observations). Low C3 levels and anti-dsDNA positivity were also associated with the risk of development of SLE flare in univariate analysis, and have previously been identified in some studies, and not identified in other studies, as predictors of SLE exacerbation (10–15). In our multivariate analysis, the baseline blood HCQ concentration was the only predictor of subsequent SLE flares. SLE flares are currently difficult to predict, and the present results suggest that blood HCQ assay has major potential for this purpose. In addition, whereas there is no consensus on measures that should be taken when a patient's C3 level declines or anti-DNA antibodies are detected, low HCQ levels are readily amenable to direct intervention.

Whole-blood HCQ concentrations varied widely among the patients in our study, even though all had been prescribed the same daily dosage. We found that blood HCQ concentrations did not correlate with body

weight or body mass index. Similar variability in patients with RA has been reported (3–5). This may be explained in part by poor treatment compliance, since HCQ was undetectable in 5 of our patients, of whom 3 had active SLE on the day of sampling and 2 had flares during the subsequent 6 months. However, exclusion of these 5 patients did not affect our results: the mean blood HCQ concentration remained significantly lower in patients with active SLE. In addition, large interindividual variations in HCQ bioavailability have been found in healthy volunteers (8), suggesting that factors other than treatment compliance may contribute to the variability.

Accordingly, as recently emphasized by Wilkinson (16), differences in drug response among patients are common, often leading to challenges in optimizing the dosage regimen for an individual patient. This variability is multifactorial, entailing environmental, genetic, and disease determinants that affect the disposition of a given drug (16). This emphasizes the need for monitoring HCQ concentrations in whole blood, to detect both poor compliance with treatment and low HCQ levels due to individual pharmacokinetic parameters.

We used ROC curve analysis to determine the blood HCQ concentration associated with the lowest risk of SLE flare in the subsequent 6 months. A threshold value of 1,000 ng/ml provided the best trade-off between sensitivity and specificity, as well as a high negative predictive value for SLE flares (96%). We therefore propose 1,000 ng/ml as the target whole-blood HCQ concentration in patients with SLE.

This proposed cutoff raises questions regarding HCQ toxicity, and especially retinopathy. Indeed, in some patients, HCQ dosages >6.5 mg/kg/day will be required in order to reach a blood concentration of 1,000 ng/ml. However, no cases of retinopathy were observed in this study or in the study by Munster et al (4), even among patients who received HCQ at dosages of >6.5 mg/kg/day. Retinopathy is a rare adverse effect of HCQ, but no patients with known serious ophthalmic disorders at the time of enrollment were included in either study, meaning that a risk of adverse ophthalmic effects cannot be ruled out. We are currently conducting a study specifically designed to assess the relationship between blood HCQ concentrations and risk of retinal toxicity.

This study had some limitations. Some mild flares may not have been identified, because some patients were seen on only 2 occasions 6 months apart. Additionally, our definition of SLE flare may be disputable. The term “flare” has never been clearly defined in SLE and

there is no consensus on the best index or scoring system, or on a particular score. Our SLEDAI cutoff was determined before performing the analyses, and was chosen because of its utility for detecting clinically relevant flares that would generally necessitate treatment modification. Indeed, none of the 120 patients with inactive SLE according to our criteria required treatment intensification at baseline (except for 1 patient who was prescribed topical treatment for cutaneous lesions), whereas treatment intensification was required in 19 of 23 patients with active disease at baseline. Moreover, we repeated the analysis defining SLE flares either as a SLEDAI score of >3 or as an increase in SLEDAI score of ≥ 3 between baseline and subsequent assessment (14,15,17). In both cases, the blood HCQ concentration remained a strong, and the only, predictor of SLE flares in multivariate analysis.

In conclusion, these findings strongly suggest that routine assay of HCQ in whole blood might help to identify SLE patients who are at risk of disease exacerbation, and to optimize treatment efficacy. We are currently organizing a randomized prospective study designed to determine the potential benefits of individualized HCQ dosing schedules aimed at maintaining the whole-blood HCQ concentration above 1,000 ng/ml.

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