Hydroxychloroquine daily dose, hydroxychloroquine blood levels and the risk of flares in patients with systemic lupus erythematosus

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ABSTRACT
Background Recent guidelines for SLE recommend using a hydroxychloroquine (HCQ) dose less than 5.0 mg/kg/day to reduce the risk of retinopathy. To determine if this dose reduction would have an impact on the clinical course of SLE, we compared flare incidence in a cohort of patients with SLE treated with two different oral HCQ dosages (≤5 mg/kg/day or >5 mg/kg/day). As a secondary analysis, we compared HCQ blood levels between the two different oral dosages, and evaluated the frequency of non-adherence in patients with SLE treated with HCQ.

Methods We identified a cohort of patients with SLE taking HCQ for at least 6 months and followed for 24 months. At study entry and 6 months later, a blood venous sample was taken to measure HCQ blood levels by liquid chromatography. Incidence of new SLE flares after recruitment was put in relation to daily HCQ dose and mean HCQ blood levels. Cox regression analysis served to identify factors associated with SLE flares.

Results 83 patients were enrolled. We observed 11 (16%) flares that developed in mean 14.8 months of follow-up. The difference in terms of flare rate and mean HCQ blood levels between the two oral dosages was not statistically significant. There was a trend (p=0.08) for high HCQ dose being associated with a lower flare rate. At Cox analysis, higher HCQ blood levels and older age at baseline were protective against flare occurrence, while concomitant immunosuppressant therapy showed significant positive association. HCQ blood levels did not correlate with prescribed HCQ dose.

Conclusion Patients with low oral HCQ dosage tend to have more flares, although the difference was not statistically significant. Higher HCQ blood levels were protective against flare occurrence. The risks and benefits must be balanced in choosing HCQ dose.

INTRODUCTION
SLE is an autoimmune systemic disease of multifactorial aetiology and with an unpredictable course. It can be characterised by the occurrence of flares, eventually leading to organ damage and an impaired quality of life.

WHAT IS ALREADY KNOWN ON THIS TOPIC
⇒ The American Academy of Ophthalmology recommends a maximum hydroxychloroquine (HCQ) dose of ≤5.0 mg/kg/day to reduce the risk of HCQ-induced retinopathy. However, many past studies evaluating the efficacy of HCQ in patients with SLE considered a dosage of at least 6.5 mg/kg ideal body weight per day.

WHAT THIS STUDY ADDS
⇒ This study suggests that reduction of daily HCQ dose to ≤5.0 mg/kg/day of actual body weight may have an impact on flare incidence in a group of patients with SLE. On the other hand, stable therapeutic HCQ levels were associated with lower rates of disease flares.

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HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY
⇒ The risks and benefits must be balanced in choosing HCQ dose. Measurement of HCQ in whole blood can help clinicians eventually adjust the daily dosage based on individual pharmacokinetic variability.

Hydroxychloroquine (HCQ), inexpensive and well-tolerated antimalarial drug, is now one of the most important medications for SLE, showing multiple benefits over several outcomes associated with the disease itself, as controlling disease activity, preventing flares and damage accrual, but also to its related comorbidities.1–9 HCQ binds strongly to melanin and can deposit in melanin-containing tissues such as the skin and the eyes, which might explain some side effects like retinopathy or skin hyperpigmentation.10

Currently, HCQ is prescribed according to body weight. The recommended daily dosage of HCQ has varied during the years. The 2016 American Academy of Ophthalmology recommendations stated that the safe daily dose of HCQ is no more than 5 mg/kg of actual body weight per day in SLE to decrease retinopathy...
occurrence. The current 2020 Joint Statement on HCQ and the 2019 European Alliance of Associations for Rheumatology (EULAR) guidelines confirmed the ophthalmological recommendations. An important concern with the current guidelines is that the efficacy of the recently considered a dosage of at least 6.5 mg/kg ideal body weight per day and it is unclear if this change in HCQ dose would be effective in controlling the disease. Moreover, this dosage is based on data from patients who effectively collected their medication. Unfortunately, less than half of the patients are taking HCQ as prescribed. Adherence to HCQ treatment is an unmet need in SLE, particularly for younger patients who may concern about adverse effects such as of retinopathy. Although non-adherence is an important issue, over two-thirds of rheumatologists are unaware of HCQ non-adherence. Low adherence rates to HCQ, if combined with a lower prescribed dose, may potentially lead to HCQ undertreatment.

Recently, measurement of HCQ in whole blood was proposed to help clinicians distinguish non-adherence versus lack of efficacy of HCQ and eventually adjust the daily dosage based on individual pharmacokinetic variability. Several studies underline a significant role for routine monitoring of HCQ levels as a measure of non-adherence.

Despite this finding, there is insufficient information on the overall clinical impact of incorporating routine testing of HCQ blood levels and potential benefits of individualising HCQ doses.

Therefore, the main aim of this study was to assess if the HCQ dose influenced the risk of SLE flare (less than or equal to 5 mg/kg per day or greater than 5 mg/kg per day). As a secondary analysis, we aimed to: (1) compare HCQ blood levels between the two different oral dosages, and (2) evaluate the frequency of non-adherence in patients with SLE treated with HCQ.

MATERIALS AND METHODS

Study patients
Consecutive adult patients admitted to the Rheumatology Unit of the University of Campania ‘L. Vanvitelli’ in Naples in a 24-month period starting in November 2014 were recruited. We selected all patients on HCQ therapy at baseline or during the follow-up.

Each of them signed an informed consent form and fulfilled the following inclusion criteria:
2. State of remission according to the 2021 Definitions of Remission in SLE (DORIS) criteria for at least 1 year.
3. Have a stable daily dose of HCQ for at least 6 months (200 or 400 mg every day).
4. Have a stable dose of glucocorticoids during the preceding 4 weeks.
5. Have had no modification of an immunosuppressant during the previous 2 months.

Exclusion criteria were patients who were pregnant, who planned a pregnancy and who were unable to sign the informed consent, with known or suspected non-adherence to the treatment, retinopathy, a severe cataract that made ophthalmological monitoring impossible, estimated glomerular filtration rate (calculated from serum creatinine according to the Cockcroft-Gault equation) lower than 60 mL/min, chronic alcoholism and liver failure. All participants provided written informed consent. Each patient, on admission, underwent a complete history taking, physical examination and laboratory investigations. We then categorised patients based on HCQ dose as either less than or equal to 5 mg/kg per day or greater than 5 mg/kg per day. The reasons underlying HCQ dose were not recorded, but low HCQ dose may have been due to reflect the 2016 American Academy of Ophthalmology guidelines, or low SLE activity.

Follow-up

Patients with SLE were followed for 24 months. Follow-up visits took place every 3 months. More frequent follow-up would be arranged for those with active disease or adverse events.

At study entry and 6 months later, a blood venous sample was taken to measure whole blood concentration of HCQ by liquid chromatography–tandem mass spectrometry. A mean HCQ value for each patient was then calculated. SLE activity and side effects were assessed for all patients. A disease activity score was obtained at each visit and an organ damage score was obtained annually. Patients were asked to contact their physician if they developed symptoms of an SLE flare and were then promptly examined. The primary outcome of interest of this study was the occurrence of flares. Flares were defined by the Safety of Estrogens in Lupus Erythematosus National Assessment-SLE Disease Activity Index (SLEDAI), and the SLICC/ American College of Rheumatology Damage Index of SLE was applied to estimate disease damage. Incidence of new SLE flares after recruitment was put in relation to daily HCQ dose according to body weight and mean HCQ blood levels. Remission was defined according to the DORIS criteria. The SLEDAI 2K was used to assess disease activity, and active patients (SLEDAI >6) or having a lupus flare at baseline visit were excluded. Body weight was assessed on every visit with the same scale. Ideal body weight was calculated according to the Devine formula. Ophthalmological testing (optical coherence tomography plus a visual field examination) was undertaken every year and signs and symptoms of drug toxicities eventually reported.
Patients were prescribed HCQ not exceeding 400 mg/day based on physician judgement. When prescribing more than 200 mg/day of HCQ, split dose was recommended. At study entry (T0) and 6 months later (T6), a blood venous sample was taken to measure whole blood concentration of HCQ. Patients who had to discontinue HCQ use due to intolerance were excluded. HCQ levels were serially quantified from EDTA whole blood by liquid chromatography–tandem mass spectrometry as previously described.22 A mean HCQ value for each patient was then calculated. The therapeutic range was 500–2000 ng/mL. This was chosen as our therapeutic range based on a review of the available literature.23 The patients were divided according to their blood level. Levels less than 100 ng/mL were considered to be consistent with complete non-adherence. Levels of 100–500 ng/mL were considered partially adherent, between 500 and 2000 ng/mL were therapeutic and greater than 2000 ng/mL were considered supratherapeutic. Incidence of new SLE flares after recruitment was put in relation to daily HCQ dose according to body weight and mean HCQ blood levels.

Statistical analysis
Categorical variables were analysed using the X² test. Continuous variables were analysed with Student’s unpaired t-test or with the Mann-Whitney U test as appropriate. The cumulative incidence of SLE flare on follow-up in the two groups of patients was calculated. Flare incidence rate was expressed as the number of events in the cohort divided by the total number of years at risk. Comparisons among the groups were performed by log-rank test. Correlation analysis between two variables was performed using Spearman’s rank correlation. The grading of correlation coefficients (r) can vary, but for the purposes of this study, 0.2–0.3=weak correlation, 0.3–0.7=moderate correlation and 0.7–1.0=strong correlation. Cox regression analysis served to identify factors associated with SLE flare occurrence in the overall cohort of patients according to HCQ dose. Factors found to be significant in univariable analysis were entered in the multivariable stepwise model. Statistical significance was set at p<0.05. Statistical analysis was performed with the MedCalc software, V.16.1.

RESULTS
Non-adherence by HCQ blood levels
Poor therapeutic adherence, reflected by at least one HCQ concentration ≤100 ng/mL value, was found in 17 (20%) patients. The proportion of non-adherent patients by drug level was 23% (10 of 42) in patients with a prescribed HCQ dose of ≤5 mg/kg/day and 17% (7 of 41) in patients with a prescribed HCQ dose of >5 mg/kg/day. Non-adherent patients differed from the other patients only for age at SLE diagnosis (20±8.5 vs 27±11.3 p=0.01) and concomitant immunosuppressant therapy (15 of 17 vs 10 of 66; p<0.0001). Patients labelled as non-adherent at study entry showed at T6 a significant higher HCQ blood concentration than baseline (median 0 ng/mL (range 0–99.4 ng/mL) vs median 515 ng/mL.
patients who had levels greater than 2000 ng/mL, 31 (37%) were considered partially adherent and 34 (42%) patients had mean HCQ value in therapeutic range. Of the 66 patients enrolled, 34 (42%) patients had mean HCQ value in therapeutic range, 31 (47%) were on immunosuppressants. No one has taken oral glucocorticoids. The median HCQ dosage was taken by 33 (50%) patients, while 17 (26%) of them had levels less than 500 ng/mL (range 0–1682 ng/mL); p<0.0001). Since our results could be influenced by the lowest non-therapeutic HCQ levels found in non-adherent patients, we excluded such patients from the analysis. Of the 66 patients enrolled, 34 (42%) patients had mean HCQ value in therapeutic range, 31 (37%) were considered partially adherent and patients who had levels greater than 2000 ng/mL considered in supratherapeutic range.

Demographic, clinical and laboratory features of patients

All the remaining 66 patients were Caucasian, mostly female (99%), with a mean age of 42 (±11.2) years and female (99%), with a mean age of 42 (±11.2) years and a mean SLE duration of 15.7 (±9.02) years. The entire population had a median SLEDAI of 2 (range 0–4) and a median SLICC of 0 (range 0–2). The median time of remission was 2 (1–11) years. Previous renal involvement has been observed in 31 (47%). Glucocorticoid therapy was taken by 33 (50%) patients, while 17 (26%) of them were on immunosuppressants. No one has taken oral or intravenous cyclophosphamide. The median HCQ treatment duration was 5 (0–32) years with median HCQ levels of 512.6 ng/mL (range 104.4–3105.6). Demographic, clinical and therapeutic features of the patients enrolled are illustrated in table 1.

Correlation of HCQ blood levels with HCQ oral prescribed dose and clinical characteristics

Blood HCQ levels did not correlate with the dose prescribed (p=0.05 (figure 1). Patients with greater body mass index tended to be treated with a higher daily dose of HCQ (r=0.40, p=0.0007). We stratified patients according to HCQ oral dose in two groups: ≤5 mg/kg vs >5 mg/kg per day. The HCQ dose of ≤5 mg/kg per day was assumed by 27 (41%) patients, with median HCQ blood levels of 580.8 ng/mL (121.6–1214.2). The HCQ dose of >5 mg/kg per day was assumed by 39 (59%) patients, with median HCQ blood levels of 617.8 ng/mL (104.4–3105.6). There was no difference in terms of proportion of patients with HCQ blood levels in therapeutic range between the two oral dosages (14 of 27 vs 20 of 39, p=0.963). No other significant differences were observed between the two populations (disease activity, damage index, age, disease duration, nephritis).

HCQ and SLE flares

Among the 83 patients enrolled, we excluded 17 patients who were severely non-adherent (HCQ concentration ≤100 ng/mL value). Out of the remaining 66 patients, we identified 27 (40.9%) patients who had a prescribed HCQ dose of ≤5 mg/kg/day and 39 (59%) patients with a prescribed HCQ dose of >5 mg/kg/day. There were no differences in patient characteristics at baseline between the two groups (table 1). Each of the 66 patients attended the scheduled follow-up visits, at least every 3 months for 24 months. We observed 11 (16%) flares that developed in mean 14.8 months of follow-up. A total of 7 of 27 (26%) patients taking an HCQ dose equal or less than 5 mg/kg/day had a flare (six mild/moderate flares and one severe flare). We registered only 4 of 39 (10%) flares in the other group, all mild/moderate flares, although the differences were not statistically significant (p=0.09). There was a trend (p=0.08) for high HCQ dose being associated with a lower flare rate (12.3 events per 100 person-years, 95% CI 4.98 to 25.5) vs HCQ ≤5 mg/kg/day (43.5 events per 100 person-years, 95% CI 11.8 to 111.5). However, comparing patients with and without flare, the former presented a lower mean HCQ blood levels (378.3±185.5 vs 782.7±646.7 ng/mL, p=0.005). Stable therapeutic HCQ levels above 500 ng/mL (in T0 and T6) were associated with no occurrences of disease flares (mild/moderate or severe). At Cox regression analysis, HCQ blood level was a significant independent risk for flare (HR 0.996, 95% CI 0.993 to 0.999, p=0.027). Moreover, older age at baseline was protective against flare occurrence (HR 0.93; p=0.001; 95% CI 0.89 to 0.97), while concomitant immunosuppressant therapy showed significant positive association (HR 3.66; p=0.002; 95% CI 1.59 to 8.43) (table 2). We also observed that longer time

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<tr>
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<td>Disease duration, years</td>
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HCQ, hydroxychloroquine; SDI, Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index of SLE; SLEDAI, SLE Disease Activity Index.
DISCUSSION
We carried out this observational study to compare the outcomes in terms of disease flares of a cohort of patients with SLE according to their prescribed dosage of HCQ (≤5 mg/kg/day or >5 mg/kg/day). In our cohort, we did not show significant differences in flare occurrence according to the different oral HCQ doses, even if we observed a tendency to have lower incidence of disease flares in patients taking more than 5 mg/kg/day. On the other hand, we found that stable therapeutic HCQ levels were associated with lower rates of disease flares. The latest guidelines from the American Academy of Ophthalmology and EULAR11,13 recommend the use of a lower dose of HCQ (maximum of 5 mg/kg of actual body weight per day) than previously used. This maximum dosage is controversial and is largely based on an ophthalmological study24 of nearly 2500 patients, mostly with rheumatoid arthritis and older than 50 years, who have used HCQ continuously for more than 5 years. As that study used pharmacy refill data, they evaluated the actual HCQ intake rather than prescribed dosages.14 However, disparity between the prescribed and actual intake dose is well-known, especially in young patients with SLE. A recent case-crossover study demonstrated that a higher risk of lupus flares was associated with HCQ dosing of 5 mg/kg per day or less, in accordance with current ophthalmological and rheumatology guidelines.25 Similar results were reported by a recent analysis of the SLICC cohort data showing that patients who reduced any HCQ dose had an increased risk of flaring, irrespective of the specific dose.26 On the other hand, other studies reported that HCQ dose reduction or withdrawal did not significantly increase the risk of flares.27,28 A limitation of those studies was the lack of information on medication adherence. Adherence to treatment is a problem in patients with SLE and is difficult to identify. Monitoring HCQ blood levels can be important to improve medication adherence in patients with lupus. We found that more than half of our patients have suboptimal adherence pattern. Levels of HCQ below 100 ng/mL in blood reflecting long-term non-adherence to the medication were found in 17 (20%) of our patients. Additionally, 32 (48%) patients showed HCQ concentrations <500 ng/mL in a visit, although it is possible that in this group, there may be individuals who, due to genetic differences in HCQ metabolism, are adherent but achieve lower blood levels. Moreover, HCQ blood levels improved at the second visit, demonstrating that when physicians discuss adherence with patients, it can improve their behaviours.

In our study, there was no correlation between the prescribed HCQ dose in mg/kg and the HCQ whole blood level. This may due to individual differences in HCQ metabolism or again to patients’ compliance. The lack of a correlation between HCQ dose and levels was already reported.29 Our data clearly indicate the need for personalised HCQ dosing approaches beyond empirical dosing recommendation through routinely HCQ blood level measurements.

In Cox regression analysis, we found that immunosuppressant therapy was associated with SLE exacerbation. Other cohort studies have reported that patients under immunosuppressive agents have higher flare risk.30,31 This could be explained as more aggressive treatment is generally required in case of more severe disease course. Moreover, we observed a protective role exerted by older age, consistent with previous evidence of longer periods of remission in elderly patients.32 This result may suggest that HCQ tapering may be relatively safe in seniors.

Our study is not without limitations. First of all, our population included a small sample size with mostly white patients and only one man. African descent and male patients, however, are known to have higher morbidity and mortality.33,34 Moreover, we studied patients in remission at baseline and hence a lower dose of HCQ could be used for maintenance. A bias by indication can be hypothesised, but it is difficult to quantify (are patients who maintained higher dosage more severe than patients in which physician decided to reduce the dosage?). In any case, we did not find any differences between these two groups and blood HCQ levels did not correlate with the dose prescribed in our study. Finally, there is still a lack of consensus on the HCQ level that would balance efficacy against toxicities. We defined 500 ng/mL as the minimal therapeutic level of HCQ which was considered as the target in other studies.23 However, other cut-offs have been used based on interviews of the patients for non-adherence35 and of pharmacokinetic/pharmacodynamics study for efficacy.36

In conclusion, our study suggests that patients who take the HCQ dosage of 5 mg/kg/day of actual body weight tend to have more exacerbation, although the differences were not statistically significant. The risks and benefits must be balanced in choosing the HCQ dose.

However, we found that low HCQ blood levels predicted lupus flares. Our study supports previous published studies reporting that periodical monitoring of HCQ levels might allow identification of early non-adherence, improve subsequent adherence, adjust the daily dosage based on individual pharmacokinetic variability and overall improve outcome in SLE. Further research focused on defining the optimal conditions for HCQ reduction is needed.

Correction notice The article has been corrected since it was published online. The co-author’s name has been corrected to Michele Iudici.

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