Susceptibility to COVID-19 in Patients Treated With Antimalarials: A Population-Based Study in Emilia-Romagna, Northern Italy

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Objective. To evaluate the susceptibility to coronavirus disease 2019 (COVID-19) in patients with autoimmune conditions treated with antimalarials in a population-based study.

Methods. All residents treated with chloroquine (CQ)/hydroxychloroquine (HCQ) from July through December 2019 and living in 3 provinces of Regione Emilia-Romagna were identified by drug prescription registries and matched with the registry containing all residents living in the same areas who have had swabs and tested positive for severe acute respiratory syndrome coronavirus 2 (SARS–CoV-2). Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated.

Results. A total of 4,408 patients were identified. The prevalence of patients receiving antimalarials was 0.85 per 1,000 men and 3.3 per 1,000 women. The cumulative incidence of testing during the study period was 2.7% in the general population and 3.8% among those receiving CQ or HCQ, while the cumulative incidence of testing positive was 0.55% in the general population and 0.70% among those receiving CQ/HCQ. Multivariate models showed that those receiving CQ/HCQ had a slightly higher probability of being tested compared to the general population (OR 1.09 [95% CI 0.94–1.28]), the same probability of being diagnosed as having COVID-19 (OR 0.94 [95% CI 0.66–1.34]), and a slightly lower probability of being positive once tested (OR 0.83 [95% CI 0.56–1.23]). None of the differences were significant.

Conclusion. Our findings do not support the use of antimalarials as a prophylactic treatment of COVID-19.

INTRODUCTION

Given the increasingly widespread use of the antimalarial drugs chloroquine (CQ) and hydroxychloroquine (HCQ), not only as therapy but also as prophylaxis for coronavirus disease 2019 (COVID-19) (1–4), there is an immediate unmet need to obtain insights into their efficacy, particularly because of their potential toxicity (5).

Antimalarial drugs are well-known, disease-modifying antirheumatic drugs (DMARDs) used in the treatment of several autoimmune conditions such as rheumatoid arthritis (RA), juvenile idiopathic arthritis (JIA), systemic lupus erythematosus (SLE), discoid lupus erythematosus (DLE), and other off-label uses including antiphospholipid syndrome and primary Sjögren’s syndrome. In addition to their immunomodulatory capacity, these drugs protect patients with inflammatory rheumatic diseases against infection. For example, in SLE, the duration of antimalarial treatment is a protective factor against infections (6). Antimalarials have also been reported to inhibit severe acute respiratory syndrome...
coronavirus 2 (SARS–CoV-2) in vitro (7,8). Therefore, because of their immunomodulatory and antiviral effects, these drugs have been proposed to be repurposed not only for the treatment of COVID-19, but also for the primary prophylaxis in healthy subjects living in highest risk areas.

Patients with autoimmune conditions who received long-term treatment with antimalarials before the onset of SARS-CoV-2 infection, potentially represent the best candidates to test the efficacy of these drugs in preventing symptomatic COVID-19 (9,10). In these patients, CQ and HCQ accumulate at the cell and tissue level, including in the lungs, where they may exert an antiviral effect, although it is unclear whether such antiviral action may be achieved using the standard therapeutic doses of antimalarials (7,8,11). We decided to evaluate, in a population-based study, the risk of COVID-19 in patients treated with antimalarials before the start of the infection in a large geographic area (3 provinces of Emilia-Romagna) with a high rate of spread of COVID-19.

PATIENTS AND METHODS

**Study population.** The 3 provinces included in the catchment areas (Bologna, Modena, and Reggio Emilia) have 2,251,903 residents. We identified all resident populations who had been prescribed CQ or HCQ during the period from July 1 through December 31, 2019, via the local drug prescription registries. The database is updated every 3 months. Those receiving CQ or HCQ were cross-referenced with the archive of residents who had oral nasopharyngeal swabs for SARS–CoV-2 reverse transcriptase–polymerase chain reaction (RT-PCR) testing and with the COVID-19 registry. All residents in the study areas who have had oral nasopharyngeal swabs since February 21, 2020, the date of diagnosis of the first COVID-19 case in Italy, are registered in a local registry. Those who tested positive were included in the COVID-19 registry, with data collected at the local level and gathered at the national level (12,13).

With a few exceptions, swabs were performed only in symptomatic subjects. Therefore, all patients included in the COVID-19 registry are considered to be COVID-19 patients. Initially, only patients who had contact with other SARS–CoV-2 patients were tested, but after the second week of the outbreak, all patients with symptoms compatible with COVID-19 were tested with RT-PCR on oral nasopharyngeal samples.

A fiscal code (a government-issued identification number used in Italy) was used to identify and match patients treated with antimalarial agents and those with COVID-19 infection. We used data updated on May 13, 2020. In Emilia-Romagna, the epidemic curve peaked in the last third of March and then decreased. At the end of the study period, the cumulative incidence of COVID-19 in the general population was 0.48%, 0.54%, and 0.9%, in Bologna, Modena, and Reggio Emilia, respectively.

The cumulative incidence of COVID-19 increased exponentially with age, with women showing a slightly higher incidence. Those receiving CQ or HCQ had almost the same probability of being diagnosed as having COVID-19 as the general population (OR 0.94 [95% CI 0.66–1.34]). The probability of being positive once tested was slightly, albeit nonsignificantly, lower among those receiving CQ or HCQ than in the general population (OR 0.83 [95% CI 0.56–1.23]).
DISCUSSION

In a recent observational study involving a large sample of consecutive patients who had been hospitalized in New York City with COVID-19, HCQ use was not associated with a significantly higher or lower risk of intubation or death (14). Although these results may be affected by prescription bias, with patients with severe disease receiving the drug, they do not support the use of HCQ at present, outside of randomized clinical trials testing its efficacy. Furthermore, a randomized trial did not demonstrate a significant benefit of HCQ as postexposure prophylaxis for COVID-19 (15). Accordingly, the Italian Medicines Agency (AIFA), in addition to other regulatory national agencies, has recently stopped the use of HCQ both for treatment of and prophylaxis for COVID-19, outside of clinical trials.

Our study is the first population-based study in a geographic area with a high level of spread of COVID-19 to evaluate if antimalarials might be effective in preventing symptomatic COVID-19 in a large number of patients (n = 4,408) treated with long-term CQ or HCQ for autoimmune conditions. These drugs have been reported to have antiviral activity in vitro against SARS–CoV-2; in particular, they seem able to block or decrease viral replication in a time- and concentration-dependent manner, as well as to inhibit the fusion of the virus to the cell membrane (7,8). Taken together, these effects have prompted suggestions for the use of antimalarials as prophylactic treatment of COVID-19. However, in our study, those individuals receiving antimalarials had the same probability of being diagnosed as having COVID-19 as the general population; therefore, our study does not support a role for CQ or HCQ in the prevention of COVID-19.

Table 1. Cumulative incidence of testing for severe acute respiratory syndrome coronavirus 2 and of testing positive, by age, sex, and use of hydroxychloroquine or chloroquine

| Age, years | Men | Women | Tested, no (%) | Tested positive, no. (%) |
|------------|-----|-------|----------------|-------------------------|
| <40        | 47  | 318   | 1 (2.1)        | 9 (2.8)                 |
| 40–49      | 84  | 483   | 2 (2.4)        | 19 (3.9)                |
| 50–59      | 152 | 671   | 2 (1.3)        | 29 (4.3)                |
| 60–69      | 162 | 707   | 6 (3.7)        | 9 (1.3)                 |
| 70–79      | 254 | 781   | 14 (5.5)       | 33 (4.2)                |
| 80–89      | 151 | 500   | 7 (4.6)        | 30 (6.0)                |
| ≥90        | 24  | 74    | 3 (12.5)       | 4 (5.4)                 |
| Overall    | 874 | 3,534 | 35 (4.0)       | 133 (3.8)               |

Table 2. Adjusted odds ratios of being tested for severe acute respiratory syndrome coronavirus 2, testing positive, and testing positive if tested in Emilia-Romagna, Italy between March 2020 and May 2020*

| Cumulative incidence of being tested | Cumulative incidence of testing positive | Probability of being positive, if tested |
|-------------------------------------|---------------------------------------|----------------------------------------|
| Individuals taking antimalarials    |                                       |                                        |
| Men                                 | 1.09 (0.94–1.28)                      | 0.94 (0.66–1.34)                      |
| Women                               | 1.24 (1.22–1.26)                      | 1.05 (1.01–1.09)                      |
| Age, years                          |                                       |                                        |
| <40                                 | 1 (referent)                          | 1 (referent)                          |
| 40–49                               | 1.82 (1.77–1.87)                      | 2.19 (2.05–2.34)                      |
| 50–59                               | 1.90 (1.85–1.95)                      | 2.70 (2.54–2.87)                      |
| 60–69                               | 1.58 (1.54–1.63)                      | 2.56 (2.4–2.74)                       |
| 70–79                               | 1.80 (1.75–1.86)                      | 2.88 (2.69–3.08)                      |
| 80–89                               | 3.45 (3.35–3.55)                      | 5.42 (5.08–5.78)                      |
| ≥90                                 | 7.72 (7.45–8.01)                      | 10.84 (10.02–11.74)                   |

* Values are the adjusted odds ratio (95% confidence interval).
HCQ in preventing symptomatic COVID-19 at the dosage used to treat autoimmune conditions. The maximum prescribed dosage of HCQ, the most commonly used antimalarial, is 400 mg daily. Safety is a major concern at higher doses.

The probability of those receiving CQ or HCQ being tested for SARS–CoV-2 was slightly increased, while the probability of those who were taking CQ or HCQ receiving a positive swab once tested was slightly lower. These differences are compatible with an increased propensity to test patients with autoimmune conditions who are considered at higher risk of infection, including patients with less typical symptoms or at lower risk of COVID-19. However, the differences were minimal and not significant and cannot have impeded the observation of an important prophylactic effect of antimalarials. In particular, the 95% CI suggests that a reduction larger than one-third is extremely unlikely.

Among patients who were followed up for at least 4 weeks, we observed a high rate of fatality (18%) in the Emilia Romagna COVID-19 population, which outlined the severity of the disease among our patients (16). We cannot rule out the possibility that a group of patients with asymptomatic or mildly symptomatic COVID-19 may have been tested; however, such a high case fatality rate suggests that patients with asymptomatic disease did not represent a substantial part of our COVID-19 registry.

Only 3.6% of the patients were treated with a single pack of antimalarials, possibly prescribed as antimalarial prophylaxis in travelers, suggesting that most patients were treated long-term for autoimmune conditions and therefore, with regard to the accumulation of the drugs in the cells and tissues related to long-term treatment, our patients represented an ideal population for evaluating the prophylactic effectiveness of antimalarials.

This study has many limitations, but also some strengths. First, the number of patients with COVID-19 was too small to provide definitive conclusions; however, our study is the first population-based study on this topic, the case ascertainment was accurate using 2 reliable sources, and we examined a large population of patients (>4,000 patients) who received long-term antimalarials. However, we compared the incidence of COVID-19 in patients with autoimmune conditions with that of the general population, and we could adjust only for sex and age. The 2 populations are not comparable with regard to health conditions and possibly also for their probability of being infected by SARS–CoV-2 and developing COVID-19. In fact, the underlying autoimmune condition and immunosuppressive treatment could have influenced the susceptibility or the course of the infection. It is worth noting that, at least for susceptibility, we did not observe any impact of prolonged use of biological DMARDs or targeted synthetic DMARDs (17). Finally, we cannot exclude the possibility that higher dosages of CQ or HCQ than those used in autoimmune diseases could be effective in treating COVID-19. Balevic et al. showed that patients receiving HCQ treatment for rheumatic diseases are unlikely to achieve total serum or plasma concentrations shown to inhibit SARS-CoV-2 in vitro; however, patients receiving HCQ long term may have tissue concentrations far exceeding serum/plasma levels (18).

In conclusion, our study did not show a prophylactic effect of antimalarial for symptomatic COVID-19 in a large population of patients with autoimmune conditions. If confirmed in larger observational studies, these results do not support the rationale for conducting large trials.

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AUTHOR 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 published. Dr. Salvarani had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Salvarani, Sandri, Bajocchi, Galli, Muratore, Boiardi, Pipitone, Cassone, Croci, Marata, Costantini.

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REFERENCES

1. Gautret P, Lagier JC, Parola P, Hoang VT, Meddeb L, Mailhe M, et al. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. Int J Antimicrob Agents 2020;56:105949.
2. Million M, Lagier JC, Gautret P, Colson P, Fournier PE, Amrane S, et al. Early treatment of COVID-19 patients with hydroxychloroquine and azithromycin: a retrospective analysis of 1061 cases in Marseille, France. Travel Med Infect Dis 2020;35:101738.
3. Hernandez AV, Roman YM, Pasupuleti V, Barboza JJ, White CM. Hydroxychloroquine or chloroquine for treatment or prophylaxis of COVID-19: a living systematic review. Ann Intern Med 2020;173:287–96.
4. Spinelli FR, Ceccarelli F, di Franco M, Conti F. To consider or not antimalarials as a prophylactic intervention in the SARS-CoV-2 (COVID-19) pandemic [letter]. Ann Rheum Dis 2020;79:666–7.
5. Schrezenmeier E, Dörner T. Mechanisms of action of hydroxychloroquine and chloroquine: implications for rheumatology [review]. Nat Rev Rheumatol 2020;16:155–66.
6. González-Echavarri C, Capdevila O, Espinosa G, Suárez S, Marín-Ballvé A, González-León R, et al, on behalf of RELES, Autoimmune Diseases Study Group GEAS. Infections in newly diagnosed
Spanish patients with systemic lupus erythematosus: data from the RELES cohort. Lupus 2018;27:2253–61.

7. Yao X, Ye F, Zhang M, Cui C, Huang B, Niu P, et al. In vitro antiviral activity and projection of optimized dosing design of hydroxychloroquine for the treatment of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Clin Infect Dis 2020;71:732–9.

8. Wang M, Cao R, Zhang L, Yang X, Liu J, Xu M, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro [letter]. Cell Res 2020;30:269–71.

9. Mathian A, Mahevas M, Rohmer J, Roumier M, Cohen-Aubart F, Amador-Borrero B, et al. Clinical course of coronavirus disease 2019 (COVID-19) in a series of 17 patients with systemic lupus erythematosus under long-term treatment with hydroxychloroquine [letter]. Ann Rheum Dis 2020;79:837–9.

10. Cassione EB, Zanframundo G, Biglia A, Codullo V, Montecucco C, Cavagna L. COVID-19 infection in a northern-Italian cohort of systemic lupus erythematosus assessed by telemedicine. Ann Rheum Dis 2020;79:1382–3.

11. Kang CK, Seong MW, Choi SJ, Kim TS, Choe PG, Song SH, et al. In vitro activity of lopinavir/ritonavir and hydroxychloroquine against severe acute respiratory syndrome coronavirus 2 at concentrations achievable by usual doses. Korean J Intern Med 2020;35:782–7.

12. Onder G, Rezza G, Brusaferro S. Case-fatality rate and characteristics of patients dying in relation to COVID-19 in Italy. JAMA 2020;323:1775–6.

13. Riccardo F, Ajelli M, Andrianou XD, Bella A, del Manso M, Fabiani M, et al. Epidemiological characteristics of COVID-19 cases in Italy and estimates of the reproductive numbers one month into the epidemic. medRxiv. April 2020. URL: https://doi.org/10.1101/2020.04.08.20056861.

14. Geleris J, Sun Y, Platt J, Zucker J, Baldwin M, Hripcsak G, et al. Observational study of hydroxychloroquine in hospitalized patients with COVID-19. N Engl J Med 2020;382:2411–8.

15. Boulware DR, Pullen MF, Bangdiwala AS, Pastick KA, Lofgren SM, Okafor EC, et al. A randomized trial of hydroxychloroquine as postexposure prophylaxis for COVID-19. N Engl J Med 2020;383:517–25.

16. Rossi PG, Broccoli S, Angelini P, for the Emilia-Romagna COVID-19 working group. Case fatality rate in patients with COVID-19 infection and its relationship with length of follow up [letter]. J Clin Virol 2020;128:104415.

17. Salvarani C, Bajocchi G, Mancuso P, Galli E, Muratore F, Boiardi L, et al. Susceptibility and severity of COVID-19 in patients treated with bDMARDS and tsDMARDs: a population-based study. Ann Rheum Dis 2020;79:986–8.

18. Balevic SJ, Hornik CP, Green TP, Cloowe ME, Gonzalez D, Maharaj AR, et al. Hydroxychloroquine in patients with rheumatic disease complicated by COVID-19: clarifying target exposures and the need for clinical trials. J Rheumatol 2020. E-pub ahead of print.

Errata

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In the article by Xu et al in the August 2020 issue of Arthritis & Rheumatology (Interleukin-17A Is Produced by CD4+ but Not CD8+ T Cells in Synovial Fluid Following T Cell Receptor Activation and Regulates Different Inflammatory Mediators Compared to Tumor Necrosis Factor in a Model of Psoriatic Arthritis Synovitis [pages 1303–1313]), a second institutional affiliation of one of the authors was inadvertently omitted from the title page footnotes. Dr. Dominique Baeten’s information should have read “Academic Medical Center and UCB Pharma, Amsterdam, The Netherlands.” Dr. Baeten was not, however, employed by UCB Pharma at the time of his work on the study reported in the August 2020 issue.

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In the letter by Bertin et al in the November 2020 issue of Arthritis & Rheumatology (Anticardiolipin IgG Autoantibody Level Is an Independent Risk Factor for COVID-19 Severity [pages 1953–1955]), two errors were inadvertently introduced in copyediting. The sentence “To this end, levels of IgG and IgM anticardiolipin antibodies (aCLs) and anti–β2-glycoprotein I (anti–β2GPI) autoantibodies were measured using real-time polymerase chain reaction in serum samples from 56 COVID-19 patients with severe acute respiratory syndrome coronavirus 2 (SARS–CoV-2)” (page 1953, right column) should have read “To this end, levels of IgG and IgM anti–β2-glycoprotein I (anti–β2GPI) and anticardiolipin (aCL) autoantibodies were measured by enzyme-linked immunosorbent assay in serum samples from 56 COVID-19 patients who were positive for severe acute respiratory syndrome coronavirus 2 (SARS–CoV-2) by reverse transcriptase–polymerase chain reaction.” The sentence “Except for 1 patient who presented with a history of stroke, no other IgG aCL–positive patient with a severe manifestation of COVID-19 presented with a history of thrombosis, which suggests that positivity for aCL could be attributed to infection with SARS–CoV-2” (page 1954, right column) should have read “Except for 1 patient who presented with a history of stroke, no other IgG aCL–positive patient with a severe manifestation of COVID-19 presented with a history of thrombosis, which suggests that positivity for aCL could be attributed to severe infection with SARS–CoV-2.”

We regret the errors.