Letter to the Editor

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The friendly use of chloroquine in the COVID-19 disease: a warning for the *G6PD*-deficient males and for the unaware carriers of pathogenic alterations of the *G6PD* gene

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To the Editor,

In vitro data suggest that chloroquine (the most used antimalarial drug) inhibits SARS CoV-2 replication as many previous papers have shown as this molecule displays an *in vitro* activity against various viruses (e.g. chikungunya virus), although without any reliable benefit in animal models [1]. Nevertheless, there is a great debate regarding the usefulness and efficacy of this medication in anti-viral treatment: chloroquine is not included in the panel recommended for HIV treatment, while its modest effect in the therapy of human virus infection is reported for chronic hepatitis C. These findings did not allow for its inclusion in the standardised therapeutic protocols for hepatitis C patients [1, 2].

Furthermore, as reported by Touret et al. [1], the announcements of translational studies conducted in China during the current SARS-CoV-2 pandemic (without any data reported in a publication so far) do not clearly refer to a specific form of chloroquine, i.e. sulphate of chloroquine or hydroxychloroquine, the latter showing some *in vitro* activity against SARS-CoV-2 and immunomodulating properties [1, 2].

In addition to the questionable clinical antiviral effect, it is very important to focus on potential factors that can render the use of this drug less safe, particularly if it is not administered under medical control as it can happen in self-medication (particularly when the drug is offered online).

The benefits of chloroquine therapy strongly depend on: (i) the age of the patient; (ii) the clinical presentation and (iii) the stage of the COVID-19 disease. Noteworthy, the use of this drug is contraindicated in some conditions, particularly the glucose-6-phosphate dehydrogenase (*G6PD*) deficiency. The latter is a condition that has not been deeply taken into account particularly when, on the web, there has been a "viral" spread of news emphasizing the safe use and the free availability of chloroquine. Therefore, before establishing that the use of chloroquine in the treatment of SARS-CoV-2 could represent a good option in light of such announcements [1], we should not miss the following important information that limit the friendly access to the drug:

a) *G6PD* is a housekeeping enzyme that, in the red blood cell (RBC), guarantees the production of NADPH, which is required to preserve glutathione in the reduced state (GSH). The failure of this process due to the altered activity of *G6PD* impairs the ability of the RBC to counteract oxidative stress, which may determine haemolytic episodes (in some cases fatal) triggered by well-known foods and drugs [3]. This risk affects mainly the class II- and III-deficient subjects [4].
b) *G6PD* deficiency shows marked genetic heterogeneity. In this regard, we have reported more than 186 mutations [4] which were updated to about 220 by Gomez-Manzo et al. [5]. The clinical impact of such mutations is variable and includes either symptomatic or asymptomatic patients who display haemolytic crisis triggered by the assumption of oxidant substances, and asymptomatic subjects resistant to the effect of such stressors.
c) *G6PD* deficiency is a necessary but not sufficient condition for the occurrence of clinical manifestations: the incidence of clinically evident favism in a group of randomly selected enzymopenic individuals resulted to be less than 30% [6].

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d) Nevertheless, the estimated number of *G6PD*-deficient individuals is close to 400 million people worldwide, while the number of pathogenic gene variants is generally concentrated in a small group of variations that can be easily detected in reference laboratories. Thus, diagnostic molecular test can cover Mediterranean countries (the Mediterranean type), parts of Africa (the African type; *G6PD* A-) and parts of India and South East Asia.
e) We have previously reported two cases of completely asymptomatic males carrying the *G6PD* Ube/Konan and *G6PD* Murcia pathogenic variants, respectively, who normally ate fava beans and used drugs not recommended for *G6PD*-deficient subjects [6].
f) The gene defect is X-linked inherited, and therefore, males can be either hemizygous normal or hemizygous deficient, whereas females may be either homozygous normal, homozygous deficient or heterozygous. A heterozygous female will be a mosaic for cells expressing the wild-type enzyme and cells expressing a deficient variant, with a variable proportion of normal and deficient RBCs due to a random X-chromosome inactivation (lyonization). Therefore, the diagnosis in some female heterozygotes is difficult mostly when the not-appropriate biochemical screening assays are offered by laboratory services.
g) As the rate of deficient RBCs in heterozygote carriers is not predictable *a priori*, potential individual clinical complications can strongly be proportional to the fraction of the number of *G6PD*-imbalanced RBCs. Moreover, the majority of *G6PD*-deficient subjects do not show clinical manifestations in the steady state and the condition remains undetected until they are exposed to an exogenous haemolytic trigger such as bacterial or viral infections, ingestion of fava beans (favism) or drugs, mainly the hydroxychloroquine [3].

In female carriers of *G6PD* mutation, symptoms can become evident with age due to the imbalance of mechanisms of inactivation of chromosome X: these data should be carefully considered in patients with COVID-19 infection, in which the age represents the most important risk factor of the severe expression of the infection. Administering hydroxychloroquine or other chloroquine salts to elderly patients with *G6PD* deficiency represents a higher risk to generate adverse effects due to these medications.

Finally, we cannot predict the number of subjects potentially deficient of *G6PD* within a COVID-19-infected population. This information should be also shared with the scientific community particularly because a recent perspective study on more than 3000 healthy blood donors [7] identified a 1.1% of *G6PD*-deficient individuals, with haematological parameters of *G6PD* within the normal range. These percentages are superimposable to those verified in the past 10 years in our laboratory (unpublished data), as evaluated in normal individuals who underwent the *G6PD* screening to apply to military force selection call. We therefore agree with Maffi et al. [7] that also in Italy the presence of blood donors with *G6PD* deficiency is not a rare event and the class II severe variants are frequent. In this regard, we could expect that in the general population this prevalence is a bit higher than in the controlled cohorts like blood donors, considering that the global prevalence of *G6PD* deficiency is 4.9%, although epidemiological data are different in various areas. In fact, countries with the highest prevalence are Africa, Asia, the Mediterranean basin and the Middle East (mean frequencies 5–25%), while in Italy it is 0–3%, with a higher prevalence among the Sardinian population (the mean frequency is 7.5%, with peaks of 25–33% in some provinces) [7]. Lippi and Mattiuzzi also reported that "*the worldwide epidemiologic burden of inherited erythrocyte disorders remains particularly high*" [8].

We underline this as data coming from a screening performed between years 2014 and 2018 by the US Department of Defence (https://health.mil/News/Articles/2019/12/01/Prevalence-of-Glucose) on more than two million individuals reported a prevalence of 2.2% *G6PD*-deficient individuals (n = 49,897; male 2.3% and female 1.5%, respectively), with the highest rate of deficiency among the non-Hispanic black ethnic group. Obviously, the absence of a harmonised screening for *G6PD* deficiency worldwide can determine an underestimation of the true number of *G6PD*-deficient individuals and/or female carriers.

Thus, the population should have a filtered access to chloroquine, considering that: (i) the number of potential carriers of genetic defect is underestimated also among *G6PD*-deficient asymptomatic males, the latter gender being the major target of COVID-19; (ii) it would be paradoxical for an individual thinking that the use of such drugs can be protective, and without any COVID-19 infection, would lead to a severe side effect (like a severe haemolytic crisis) when using hydroxychloroquine or chloroquine salts.

To conclude, although the use of many old and recent drugs is "democratically" recommended on the web by lots of non-medical promoters, it would be important to remember that all subjects who will undergo hospitalised treatment with hydroxychloroquine should be at least tested for *G6PD* activity in the blood. Those following the information on the web and decide to use these drugs in

the self-medication path must refer to their family doctor before self-administration of the drug. Both medical and scientific communities should strongly emphasise these issues and spread these concerns on the web as a warning, against the non-medically guided information.

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References

1. Touret F, de Lamballerie X. Of chloroquine and COVID-19. Antiviral Res 2020;177:104762.
2. Cortegiani A, Ingoglia G, Ippolito M, Giarratano A, Einav S. A systematic review on the efficacy and safety of chloroquine for the treatment of COVID-19. J Crit Care 2020;S0883–9441:30390–7.
3. Minucci A, Giardina B, Zuppi C, Capoluongo E. Glucose-6-phosphate dehydrogenase laboratory assay: how, when, and why? IUBMB Life 2009;61:27–34.
4. Minucci A, Moradkhani K, Hwang MJ, Zuppi C, Giardina B, Capoluongo E. Glucose-6-phosphate dehydrogenase (G6PD) mutations database: review of the "old" and update of the new mutations. Blood Cells Mol Dis 2012;48:154–65.
5. Gómez-Manzo S, Marcial-Quino J, Vanoye-Carlo A, Serrano-Posada H, Ortega-Cuellar D, González-Valdez A, et al. Glucose-6-phosphate dehydrogenase: update and analysis of new mutations around the world. Int J Mol Sci 2016;E2069:1–15. Review.
6. Minucci A, Antenucci M, Giardina B, Zuppi C, Capoluongo E. G6PD Murcia, G6PD Ube and G6PD Orissa: report of three G6PD mutations unusual for Italian population. Clin Biochem 2010;43:1180–1.
7. Maffi D, Pasquino MT, Mandarino L, Tortora P, Girelli G, Meo D, et al. Glucose-6-phosphate dehydrogenase deficiency in Italian blood donors: prevalence and molecular defect characterization. Vox Sang 2014;106:227–33.
8. Lippi G, Mattiuzzi C. Updated worldwide epidemiology of inherited erythrocyte disorders. Acta Haematol 2019;24:1–7.