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1 **Low blood zinc concentrations in patients with poor clinical outcome**
2 **during SARS-CoV-2 infection: is there a need to supplement with Zinc COVID-19**
3 **patients?**

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27 **ABSTRACT**

28 Among 275 patients with COVID-19, we found that median blood zinc level was significantly
29 lower in patients with poor clinical outcome (N=75) as compared to patients with good
30 clinical outcome (N=200) (840 µg/L versus 970 µg/L; p< 0.0001), suggesting that zinc
31 supplementation could be useful for patients with severe COVID-19.

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33 **Keywords:** Zinc; SARS-COV-2; severe; clinical outcome; chloroquine; azithromycin

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52 **Introduction**

53 In the current situation of COVID-19 pandemic, drug repurposing may be the best option over
54 the development of novel drugs to fight this new acute infectious disease. Among existing
55 drugs that are currently under evaluation for treating COVID-19 infection,
56 hydroxychloroquine (HCQ) in combination with azithromycin (AZ) has been shown to be
57 effective and synergistic both *in vitro*¹ and *in vivo*². Using this combination, we have recently
58 treated 3,737 COVID-19 patients and observed a lower mortality and better outcome as
59 compared to other regimens in Marseille public hospitals³. In parallel with our studies, an
60 empiric regimen combining HCQ, AZ and zinc sulfate was recently proposed for treating
61 outpatients with a reduced hospitalization risk⁴. Zinc is an essential metal in human being and
62 is involved in a wide range of biological processes as a cofactor and signaling molecule⁵. In
63 *vitro*, zinc demonstrated its antiviral activity through inhibition of Severe Acute Respiratory
64 Syndrome 1 (SARS-CoV-1) RNA polymerase⁶. Moreover, zinc is well known as an essential
65 element for immune function and resistance to infections⁵. Zinc deficiency is very common,
66 even in developing countries and is associated to increased risk of infectious diseases
67 (pneumonia, diarrhea, malaria)⁵. Conversely, zinc supplementation is associated with a
68 significant decrease in mortality during severe pneumonia⁷. Finally, it has also been
69 demonstrated that chloroquine derivatives are ionophores for intracellular uptake of zinc into
70 the lysosomes⁵. Based on this knowledge and the fact that some of our patients from our
71 series of 3,737 patients had a poor clinical outcome, the purpose of this study was to assess
72 whether zinc concentration in COVID-19 patients was associated with the clinical outcome of
73 the disease.

74 **The study**

75 Patients were included as a part of a previous work³ and biochemical analysis were performed
76 as standard care. Patients were included between 2020, March, 1st and 2020, April, 16th, with

77 the following inclusion criteria; age >18 y.o., with at least one nasopharyngeal, specimen
78 positive for SARS-CoV-2 and a blood sample available for the **measurement of zinc**
79 **concentration** that was collected following the positive testing. Zinc administration before
80 during the admission was an exclusion criterion. Patients were previously categorized
81 according to their clinical outcome in two groups, as previously described³. Briefly, either
82 death or transfer to ICU or hospitalization for 10 days or more defined poor clinical outcome
83 (PClinO). Other patients were classified in the good clinical outcome (GO) group. The HCQ-
84 AZ group includes patients that received at least a 3-day course of HCQ-AZ. The other
85 regimens comprise shorter course of HCQ-AZ, AZ alone, HCQ alone, or none of these
86 molecules. The assessment of plasmatic zinc level was performed by inductively coupled
87 plasma-mass spectrometry (ICP-MS) using a Nexion ICP MS (Perkin Elmer, Waltham,
88 Massachusetts, USA). We performed Student t-test and Mann-Whitney U test, where
89 applicable, using GraphPad Prism 7.0 (GraphPad Software, La Jolla, USA) while we
90 performed the Chi-square test using the OpenEpi Platform (www.openepi.com). Logistic
91 regression was performed using XLStat 2016 (Addinsoft, Paris, France). Data from patients
92 were protected according to the European General Data Protection Regulation and follow the
93 MR004 processing registered under N° MR 5010010520.

94 A total of 275 patients were included (Table 1) with 200 (75%) that had a good clinical
95 outcome (GO group), and 75 (25%) with a poor clinical outcome (PClinO group) as
96 previously defined³. The median age was of 49.4 years old (standard deviation, sd, 16.2 y.o.)
97 and 64.1 years old (sd, 14.58 y.o.) in the PClinO and the GO group, respectively ($p < 0.0001$).
98 The mean plasmatic zinc level among the 275 patients was 961.9 $\mu\text{g/L}$ (sd 241.4 $\mu\text{g/L}$). No
99 difference was observed regarding the gender. The median plasmatic zinc level was
100 significantly lower in the PClinO group (840 $\mu\text{g/L}$) as compared to the GO group (970 $\mu\text{g/L}$)
101 ($p < 0.0001$) (Fig. 1). Two outliers (one belonging to each group) were excluded using the

102 ROUT method (Q=1%), resulting in a subsequent analysis of a total of 273 patients for which
103 the level of zinc remains significantly lower in the PClinO group ($p < 0.0001$). Due to the
104 significant difference in age between the PClinO and GO groups, we randomly paired by age
105 patients from the GO group (mean 61.1 y.o.; sd, 13.9) with those from the PClinO group
106 (mean, 61.1 y.o.; sd, 14.1) resulting a total of 114 patients (57 in each group) (Table S1). The
107 mean plasmatic zinc level was significantly lower in the PClinO group (843.6 $\mu\text{g/mL}$, sd
108 198.9) than in the GO group (930.3 $\mu\text{g/mL}$, sd 165.6) ($p = 0.013$) (Fig. 1C). We also aimed to
109 investigate if the therapeutics could affect zinc blood level. Of the 275 patients included, the
110 224 that complete the treatment combining hydroxychloroquine and azithromycin for at least
111 3 days exhibited higher but non-significant zinc blood levels (median 955.2 $\mu\text{g/L}$) when
112 compared to those that experienced another treatment (median 890 $\mu\text{g/L}$) ($p = 0.088$). In the
113 multivariate analysis we found that obesity ($p = 0.01$) (Figure 1B) and age ($p < 0.0001$) were
114 associated with the zinc status (Table S2). Only 25 subjects had zinc deficiency *stricto sensu*
115 (i.e., zinc plasmatic level $< 700 \mu\text{g/L}$). The proportions of patients with zinc deficiency was
116 higher in the PClinO group (21%) when compared to the GO group (4.5%) ($p < 0.0001$). Their
117 mean age was 69 y.o. (Sd=14.5 y.o). We show by using both multivariate and univariate
118 analysis that zinc deficiency is associated with older age ($p < 0.0001$) and obesity ($p = 0.011$)
119 (Table S3). Finally, we failed to correlate zinc status with the clinical presentation or the
120 symptoms recorded. Overall, our study demonstrates that patients with poor clinical
121 outcomes, although treated by the combination of HCQ + AZ, exhibit lower levels of
122 plasmatic zinc than those with good outcome during SARS-CoV-2 infection. This
123 phenomenon is not only due to the fact that patients in the PClinO group were older, as these
124 findings were comforted in a subgroup analysis with patients paired by age (Fig. 1C) (i.e.,
125 mean age of 61.1 y.o.). However, older patients are more likely to develop severe forms of
126 SARS-CoV-2 infection (Table 1)³. Zinc deficiency has been associated with severity during

127 SARS-CoV-2 infection⁹. The elderly are more frequently affected by zinc deficiency⁵ but the
128 diabetic patients and the obese people are also more likely to have a lower zinc status. In this
129 study, we identified only 25 patients suffering from zinc deficiency *stricto sensu*, mostly
130 belonging to the PClinO group (i.e., 64%). The zinc concentration was assessed from
131 K₂EDTA blood samples, which may overestimate zinc plasmatic level⁹. In our study zinc
132 deficiency was significantly associated with older age and obesity. These data raise the
133 question about the zinc supplementation in these populations. To the best of our knowledge,
134 impact of zinc supplementation in overweight individuals on the incidence or severity of
135 respirations has not been assessed. There are however several studies regarding the elderly.
136 Indeed, zinc supplementation was shown to significantly reduce the incidence of infections in
137 the older subjects, in particular regarding the “common cold” and the upper respiratory tract
138 infections⁵. Besides, zinc appears to reduce the duration of common cold when administered
139 24 hours following the onset of the symptoms⁵. Moreover, a high zinc level is associated with
140 a lower risk of pneumonia and a reduced duration of infection⁵. Importantly, oral
141 administration of zinc is safe as zinc intoxication is a very rare event. Taken together, these
142 elements suggest that zinc supplementation could be an empiric adjuvant therapy for COVID-
143 19 especially in high-risk patients. This proposal makes sense considering that chloroquine is
144 in fact a zinc ionophore⁵, inducing an increase in the uptake of zinc in cells and thus a higher
145 zinc concentration in lysosomes. These observations have led some physicians to propose a
146 regimen combining hydroxychloroquine, azithromycin and zinc for treating patients with
147 SARS-CoV-2 infection⁴. As a result, the authors report a significant reduction of
148 hospitalization when compared to the untreated group, with no cardiac side effects. The
149 conclusions are however difficult to interpret as the patient characteristics and clinical data
150 were however not available for the untreated group and could affect the outcome. In addition,
151 the impact of zinc supplementation on the clinical course remains unclear. However, a

152 retrospective study has recently shown that the addition of zinc to the chloroquine +
153 azithromycin regimen is associated with a higher rate of patients discharged home and a lower
154 mortality in patients that did not require ICU when compared to chloroquine + azithromycin
155 only¹⁰. In vitro works are ongoing to demonstrate if the addition of zinc may enhance the
156 efficacy of the combination of HCQ and AZ against SARS-CoV-2 viruses¹. Further clinical
157 studies would allow to evaluate how the zinc supplementation could prevent severe forms of
158 SARS-CoV-2 infections in high-risk patients, in particular the elderly and the obese
159 individuals.

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164 **Conflict of interest: none to declare**

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169 **FIGURE AND TABLES LEGENDS**

170 **Fig. 1. Plasmatic** zinc levels in COVID-19 patients according to the clinical outcome among
171 the 275 patients (1A) and among 114 patients paired by age (1C). The figure 1B highlights the
172 plasmatic zinc levels of the obese and non-obese patients among the 275 patients.

173 **Table 1.** Baseline characteristics according to clinical outcome of 275 COVID-19 patients at
174 IHU Méditerranée infection Marseille, France between 2020, March, 1st and 2020, April, 16th.

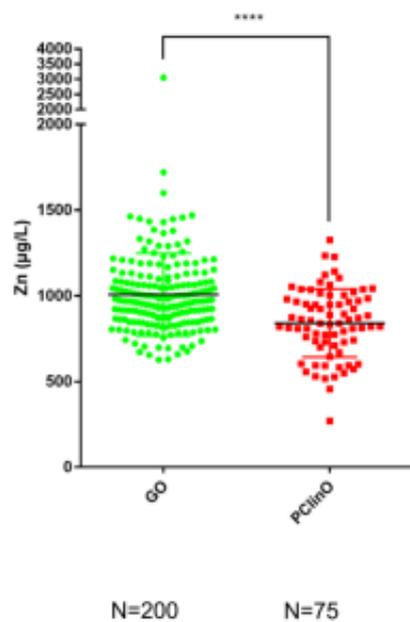
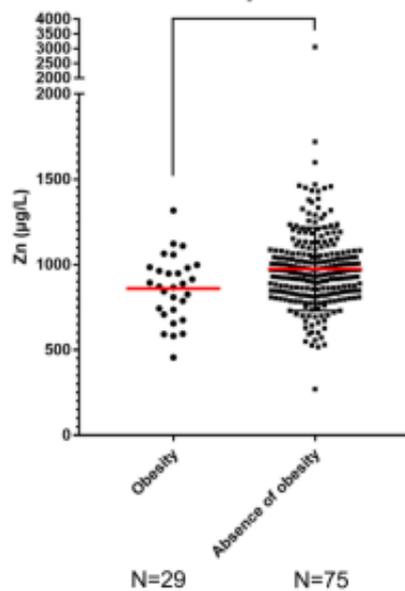
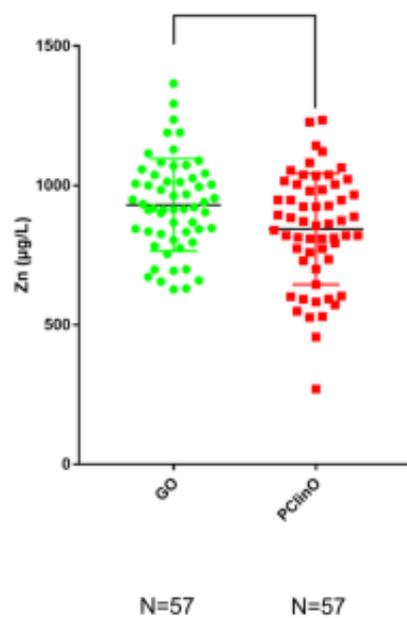
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1A**1B****1C**

	Good outcome	Poor clinical outcome	Total
	n (%)	n (%)	n (%)
Groupe size	200 (73%)	75 (27%)	275 (100%)
Age (years)			
Mean (SD)	49.4 (16.2)	64.1 (14.6)***	53.4 (17)
Median [Mean - Max]	49 [18-89]	63 [31-91]	54 [18 - 91]
Sex ratio (M/F)	0.74	1.34*	0.87
Time between diagnosis and Zn Level measurement			
Mean (SD)	2.61 (2.27)	2.17 (2.4)	2.5 (2.3)
Median [Mean - Max]	2 [0 - 15]	1 [0 - 9]*	2 [0 - 15]
Clinical manifestations			
Fever	39 (19.5)	28 (37.3)**	67
Cough	71 (35.5)	32 (42.7)	103
Rhinitis	27 (13.5)	8 (10.7)	35
Anosmia	32 (16)*	5 (6.67)	37
Ageusia	26 (13)	5 (6.67)	31
Dyspnea	29 (14.5)	14 (18.7)	43
Clinical classification (NEWS score)			
0-4 (low)	182 (91)***	31 (41.3)	213
5 -6 (medium)	8 (4)	14 (18.7)***	22
≥7 (high)	10 (5)	30 (40)***	40
Evolution			
Hospitalization	63 (31.5)	75 (100)****	138
Median days of hospitalization [Min - Max]	4 [1-9]	15 [1-40]****	
ICU	0 (0)	28 (37.3)****	28
Deaths	0 (0)	13 (17.3)****	13
Comorbidities			
Hypertension	45 (22.5)	36 (48)***	81
Diabetes mellitus	21 (10.5)	21 (28)***	42

Obesity	16 (8)	13 (17.3)*	29
Cancer	8 (4)	12 (16)**	20
Coronary disease	8 (4)	12 (16)**	20
Zinc blood level			
Mean (SD)	1007 (240.8)	841.1 (198.8)	961.9 (241.4)
Median [Mean - Max]	970.5 [627 - 3058]	840 [270 - 1326]***	947 [270 - 3058]
Therapeutics			
HCQ+AZ	178 (89)***	46 (61.3)	224 (81.5)
Other treatment	22 (11)	29 (38.7)***	51 (18.5)