CASE REPORT

REVISED Case Report: Use of hydroxychloroquine and N-acetylcysteine for treatment of a COVID-19 patient [version 2; peer review: 2 not approved]

Previously titled: Use of hydroxychloroquine and N-acetylcysteine for treatment of a COVID-19 positive patient

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Abstract

There is worldwide concern for lack of specific therapy against the novel *Betacoronavirus* severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This case report presents the results of a pharmacological intervention aimed at modulating the inflammatory effects of coronavirus disease 2019 (COVID-19), in an effort to avoid the use of mechanical ventilation. A COVID-19 positive patient was admitted with multisystem organ dysfunction, including acute respiratory insufficiency, and was treated with a combination of low oral doses of hydroxychloroquine and intravenous N-acetylcysteine (NAC). The combination therapy resulted in noticeable clinical improvement and a quantifiable decrease of several of the inflammatory markers measured, in particular ferritin levels, C-reactive protein (CRP) and lactic acid. He also developed pulmonary embolism (PE) and deep vein thrombosis (DVT), both known side effects of COVID-19 infection. Following thrombolysis and heparinization his clinical evolution continued a positive trend until discharge.

The therapeutic approach utilized in this case suggests that early intervention not only decrease acute organ dysfunction but also may decrease the need for mechanical ventilation in COVID-19 positive patients.

Keywords

COVID-19, Hydroxychloroquine, N-Acetylcysteine

Open Peer Review

Approval Status **XX**

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Any reports and responses or comments on the article can be found at the end of the article.

This article is included in the **Emerging Diseases** and **Outbreaks** gateway.

This article is included in the **Coronavirus** collection.

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REVISED Amendments from Version 1

Our revised article contains added information to provide in depth information in support of the patient's evolution, and the impact of the therapeutic interventions. Discussion was added regarding the impact of research and published articles in the matter of HCQ/NAC viral treatment.

Any further responses from the reviewers can be found at the end of the article

Background

A novel coronavirus, SARS-CoV-2, isolated in January 2020¹ was implicated as the cause of COVID-19 that resulted in an outbreak of pneumonia in Wuhan, China. Human to human transmission has reached pandemic levels with cases infecting millions of individuals resulting in significant morbidity and mortality. Although multiple therapies have been proposed against SARS-CoV-2 virus, no clear consensus exists on the best approach for treatment^{2,3}.

The human body requires an efficient innate immune system in the airway mucosa to respond to viral or bacterial antigens and preserve tissue homeostasis. SARS-CoV-2 enters the human airway in a process reminiscent of other viruses^{4,5}. The virus invades healthy cells, replicates, and leads to cellular necrosis⁶. Neutrophils are essential for a proper innate response to antigens derived from cellular necrosis⁷. We previously demonstrated that restoring the capacity of the innate immune system by modulating neutrophil activity with hydroxychloroquine (HCQ) and N-acetylcysteine (NAC) was sufficient to ameliorate local tissue effects of cellular necrosis and inflammation⁷. HCQ is a well-known therapy for certain inflammatory autoimmune diseases such as rheumatoid arthritis and lupus erythematosus and has significant impact on Toll-like receptor 9 (TLR-9) activity⁸. NAC has been used as an antioxidant, as

a modulator of inflammatory responses due to its actions on NF- κ B⁹, as a mucolytic agent, and for the treatment of acetaminophen-induced liver failure¹⁰. In a patient with SARS-CoV-2 infection, we used oral low-dose HCQ in combination with intravenous NAC in an effort to modulate the inflammatory response secondary to COVID-19.

Case report

We describe a 54-year-old Caucasian male patient, with past medical history significant for hypertension, hyperlipidemia, and obesity, who tested positive for SARS-CoV-2 by reverse-transcriptase-polymerase-chain-reaction (RT-PCR) 11 days prior to his admission on mid April, 2020 (Table 1) at Holy Family Hospital in Methuen, Massachusetts. Following transfer from another hospital, he was admitted to the Intensive Care Unit with shortness of breath, body aches, fever, diaphoresis, tachypnea, low oxygen saturation of 92% requiring oxygen supplementation via non-rebreather mask, elevated lactic acid of 7.6 (0.5–2.2 mmol/L), and hyperglycemia with blood glucose of 402 (<100 mg/dL fasting). His vital signs included initial blood pressure of 92/62 mmHg (72 MAP), respiratory rate of 48 bpm, heart rate 120–130 bpm, and temperature of 97°F.

Initial laboratory work-up showed elevations of inflammatory markers common to patients diagnosed with COVID-19^{7–9} (Table 2); particularly lymphocytes 900 (850–3900 cells per mm³), high-sensitivity C-reactive protein 149.2 (1.0–3.0mg/L), D-dimer 16.47 (<0.5 μ g/ml FEU), lactate dehydrogenase 1579 (102–266 U/L), and serum ferritin 23713 (30–400 ng/L). A noticeable decrease in lactic acid 7.6 to 2.4 (0.5–2.2 mmol/L) was observed in the first 24 hours of treatment. He also showed signs of multi-system end-organ damage, as evidenced by elevations in alanine aminotransferase 1017 (14–63 U/L), aspartate amino transferase 852 (15–41 U/L), and serum creatinine 1.4 (0.6–1.4 mg/dL). At presentation, lung auscultation was remarkable for scattered rales, chest radiograph was unremarkable, and abdominal exam was normal.

Table 1. Demographic and Clinical Characteristics.

Demographic Characteristics	
Age – year	54
Sex	Male
BMI (kg/m ²)	32
Initial findings	
Medical history	Hyperlipidemia, hypertension, benign prostatic hyperplasia, degenerative disc disease, no history of thrombosis
Symptoms at presentation	Fever, body aches, malaise, dyspnea
Days from disease onset to presentation	21
Disease severity	Critical
Serial (Q6H) lactic acid measurements (mmol/L)	7.6 → 5.6 → 3.3 → 2.4

Table 2. Laboratory Findings and Imaging Results.

Characteristic	Day 0*	Day 1	Day 3	Day 5	Day 6	Day 10
White cell count (per mm ³)	17600	17900	18100	14600	12300	6400
Differential count (per mm ³)						
Total neutrophils	15000	14700	15800	12300	9900	4000
Total lymphocytes	900	2000	1100	1300	1400	1700
Total monocytes	900	1000	1000	700	700	600
Platelet count (per mm ³)	284	212	177	207	213	302
Hemoglobin (gm/L)	145	127	108	99	103	108
Albumin (gm/L)	31	30	28	23	ND†	27
Alanine aminotransferase (U/L)	1017	1520	693	277	ND	81
Aspartate aminotransferase (U/L)	852	998	116	61	ND	55
Lactate dehydrogenase (U/L)	1579	ND	380	281	288	533
Serum creatinine (mg/dL)	1.4	1.1	1	0.9	0.8	0.8
Estimated creatinine clearance (mL/min)	74	96	107	117	131	131
Fibrinogen (mg/dL)	472	ND	309	464	625	534
D-dimer (µg/mL FEU)	16.47	>20	>20	10.41	10.03	3.82
Serum ferritin (µg/L)	23713	ND	4590	2430	2359	2416
Procalcitonin (ng/mL)	0.29	ND	0.19	0.32	0.3	ND
High sensitivity C-reactive protein (mg/L)	149.2	ND	ND	ND	ND	64
Imaging features	No acute pulmonary process	Bilateral pulmonary emboli; Patchy ground-glass bilateral infiltrates	Improved aeration	Minimally increased opacities	Patchy densities in the upper lobes	ND

* Day 0 denotes assessments on the morning prior to N-acetylcysteine administration

† Not determined

In the ICU he received empiric antibiotic therapy that included Azithromycin 500 mg IV (1 dose) and Vancomycin 2750 mg IV (divided doses), while the results of bacterial cultures were obtained. Antibiotic therapy was reassessed and discontinued due to very low probability of bacterial infection (patient afebrile, negative cultures, low procalcitonin levels). Culture data was reported as follows: On April 14, MRSA PCR swab (negative); blood cultures (negative); April 17, sputum with light growth normal respiratory flora; April 20, tracheal aspirate showed heavy growth of *Serratia Marcescens*, for which he received levofloxacin. No vasopressors were utilized in the medical care of this patient. Other prescribed medications included gastrointestinal prophylaxis (protonix 40 mg/po), propofol and fentanyl during the intubation period, anti-anxiolytics, laxatives and cough medications as needed.

Despite escalating oxygen requirements, intubation was delayed as the patient was assessed to be stable. The patient was

prescribed HCQ 400 mg, given as a single oral dose, and NAC intravenously at 75 mg/kg over 4 hours, then 35 mg/kg over 16 hours, followed by 17 mg/kg over 24 hours on Day 2. Prophylactic anticoagulation was started with subcutaneous heparin 5000 Units q8H. An additional 200 mg dose of HCQ was given on Day 2. No cardiac arrhythmia was noticed with either dose of HCQ, with the highest measured corrected QT interval documented at 0.49 (0.36–0.44 seconds).

This patient initially experienced progressive clinical improvement; however, bilateral pulmonary embolism (PE) and right lower extremity popliteal deep venous thrombosis were diagnosed in the setting of persistently elevated D-dimer. A heparin infusion was started, and PE embolization was complicated by severe hypoxemia requiring mechanical ventilation. After three days of mechanical ventilation and catheter-directed thrombolysis, he was successfully extubated and transferred to a general medicine floor on Day 7. The patient was discharged

home on Day 12 with stable vital signs, normalizing laboratory values, and on therapeutic anticoagulation with rivaroxaban. SARS-CoV-2 RT-PCR prior to discharge was negative.

Discussion

SARS-CoV-2 infection is characterized by multisystem organ involvement as illustrated in the present case yet no universally accepted standard therapy is available. It is theorized that SARS-CoV-2 causes the human immune system to overcompensate in response to infection and inflict collateral damage on itself, as evidenced by the abnormalities in inflammatory markers^{11–13}. COVID-19 also appears to increase the risk of thrombotic events^{14,15}. In this case the initial presentation with fever, diaphoresis, tachypnea, low oxygen saturation, neutrophilia and borderline lymphopenia would suggest an infectious process. Presumption of bacterial infection was addressed with 3 doses of antibiotics administered empirically (Azithromycin 500 mg IV 1 dose, Vancomycin 2750 mg IV divided doses), until culture results were obtained. Antibiotics were discontinued after culture reports found no bacterial presence and with low PCT levels, the possibility of bacterial infection was minimal. The only positive culture revealed was *Serratia Marcescens* 6 days after ICU admission and was treated with levofloxacin. The combination of elevated inflammatory markers (CRP, Ferritin, LFT's) and SARS-CoV-2 positive results suggest a viral source for the clinical manifestations of this patient. After the patient received HCQ/NAC, clinical improvement was seen, and laboratory reports progressively normalized. When considering the fast decline of the severe symptoms that followed HCQ/NAC administration, versus natural progression of this disease; or the impact of a single dose of Azithromycin in the evolution of this patient; we concluded that HCQ/NAC offers the best scientific explanation for the observed improvement. Previous work has shown that HCQ and NAC can modulate the innate immune system^{7,8}, as well as reduce hypercoagulability and inhibit thrombosis^{16,17}. We recognize that HCQ has been associated with a higher risk of cardiac abnormalities and fatal heart rhythms¹⁸; however, our low-dose strategy (600 mg total) allowed us to take advantage of its potential benefits and long half-life. HCQ may impact viral activity at multiple levels by blocking viral entry into the cells, interfering with endosomal maturation, and lysosomal transport^{19–21}.

NAC, modulates the activity of the innate immune system by controlling the overproduction of proinflammatory cytokines at the NF- κ B level, by mitigating cellular damage working as an antioxidant, and replenishing glutathione stores necessary for cellular protection during acute injury. NAC also has potential direct and indirect antiviral activity^{22,23}, and has shown synergy if combined with HCQ in the treatment of inflammation related to cellular toxicity⁷. NAC has been shown to be safe at doses up to 980 mg/kg over 48 hours when used for acetaminophen overdose¹⁰.

Because of this, we theorized that the administration of HCQ and NAC would be well tolerated and have favorable effects on patient outcomes.

Conclusions

Although there is controversy on the benefits of HCQ in the treatment of viral infections, results of *in vitro* studies have shown its activity against viral infections including SARS-CoV-2 infection. The clinical studies available have contradictory results with some in support and others against the benefit of HCQ in SARS-CoV-2 infection, however, they all have in common the use of excessive amounts of the medication, naturally inducing toxicity and thus likely negating the therapeutic benefits. Multimodal antiviral therapies such as the one presented in this case report need to be considered to better treat viral infections. Therapeutic interventions with modulators of the innate immune system such as HCQ (low doses) and NAC, are necessary to mitigate the effects of multisystem organ dysfunction observed in viral infections including SARS-CoV-2. Avoidance of mechanical ventilation may represent a secondary benefit of this therapy. A large randomized clinical trial is warranted to further evaluate the benefits of HCQ/NAC combination during SARS-CoV-2 infection.

Consent

Written informed consent for publication of their clinical details was obtained from the patient.

Data availability

Underlying data

All data underlying the results are available as part of the article and no additional source data are required.

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Vui Heng Chong

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The authors report a case of COVID-19 with respiratory compromised that was tipped over by pulmonary embolism from lower limb venous thrombus. The patient also had hepatic compromised in this case probably hemodynamic related given the admission parameters with hypotension and metabolic acidosis. Hepatitis secondary to COVID-19 is a possibility. The focus of the authors in this case report is that initiation of HCQ and NAC, the authors theorized had contributed to the recovery of the patient.

There is a lack of information; not certain if there were additional therapies provided? Antimicrobials (for sepsis given the high CRP), lopinavir/ritonavir, azithromycin, and inotropes. Based on the information provided, the patient also had septic shock and co-infection must be considered.

There is strong evidence and is now widely accepted that HCQ does not work in COVID-19. NAC use in COVID-19 is novel and there are several publications that hypothesis its role. NAC replenishes glutathione stores and helps mitigate the oxidative injuries during inflammatory process not just for COVID-19 but also other diseases.

There is nothing concrete in the information presented that supports the authors' claim as the recovery may well have been related to the hemodynamic supportive provided.

Is the background of the case's history and progression described in sufficient detail?

No

Are enough details provided of any physical examination and diagnostic tests, treatment given and outcomes?

No

Is sufficient discussion included of the importance of the findings and their relevance to

future understanding of disease processes, diagnosis or treatment?

No

Is the case presented with sufficient detail to be useful for other practitioners?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Internal medicines with interest in gastroenterology, hepatology and infectious diseases

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 19 Aug 2020

Carlos Puyo

August 18, 2020

Drs. Bin Cao, M.D., and Vui Heng Chong, M.D.
Reviewers F1000Research

Dear Drs, Cao and Chong:

We are very grateful for your insightful comments on our Case Report "**Use of hydroxychloroquine and N-Acetylcysteine for treatment of a COVID-19 patient**". We have given careful consideration to the points raised by you during the review process. The case report has been adjusted to reflect our comments as indicated in the manuscript using a tracking system.

We look forward to your response and hope to complete the publication of our Case Report in *F1000Research*.

Sincerely,

Carlos A. Puyo, M.D., FCCP

Specific comments are listed below:

1. According to the background and discussion part, the authors' inference for the potential efficacy of the two drugs are mainly established on evidence from pre-clinical experiments. However, clinical evidences from COVID-19 or other respiratory infectious diseases should also be mentioned and discussed in the manuscript.

- For hydroxychloroquine, the results of several studies have been released. The RECOVERY trial was reported to find no clinical benefit from use of

hydroxychloroquine in hospitalized COVID-19 patients (28-day mortality, length of hospitalization, etc.)¹. A published RCT in BMJ from Tang et al found that the use of hydroxychloroquine did not increase the proportion of virus negative conversion². Also, the interim analysis of WHO solidarity trial showed little or no reduction in the mortality of hospitalized COVID-19 patients in the hydroxychloroquine arm and WHO thus discontinued the hydroxychloroquine arm for hospitalized patients³. Although the dosage of hydroxychloroquine varies in different trials and might be higher than the dosage used in this case report (400mg/600mg per day), it is hard to attribute the efficacy to hydroxychloroquine given the accumulating clinical evidences. The authors should provide clinical evidences in the manuscript in discussion to defend their hypothesis.

- 1. Response to reviewers:** The release of several studies promoting or discrediting the use of chloroquine or hydroxychloroquine for the treatment of COVID-19 has created confusion in the medical community. Here, the reviewer duly noted studies showing lack of or questionable therapeutic benefit^{1,2}, however, there is no mention of studies that have shown benefit^{3,4,5,6,7}. The RCT by Tang, et al, contains several shortcomings among them the use of an open label instead of a double blind approach, potentially introducing bias by the researchers. Similarly, the underpowered sample size and enrollment of patients in more advance stages of the disease likely influence negatively their assessment of the true potential value of HCQ in the disease progression. In the RECOVERY trial mentioned by the reviewer, the doses of HCQ 2400 mg in 24 hours followed by 1600 mg daily for at least 9 days, are indeed higher than for malaria treatment, thus exposing patients to unnecessary risks. It is unclear what scientific bases were utilized to determine the exceedingly high doses of HCQ administered. The Indian Council of Medical Research alerted the WHO about the excessive doses of HCQ (4 times higher) used in the RECOVERY trial, thus, possibly introducing bias in the comparative analysis. The RECOVERY trial also has some interesting findings regarding their unexplained very high mortality data in the control group (23.6%), compared to the 12.7% and 13.5% reported by others in treating high acuity hospitalized patients^{4,8}.

Interpretation of HCQ studies is difficult, in part due to the variety of end points utilized by the researchers. For instance some studies used mortality or pneumonia¹, while others used negative viral conversion² as the end points of the studies, thus making a comparative analysis on the effects of HCQ a complex one. In some cases reputable journals (Lancet and others) had to RETRACT anti-HCQ studies⁹⁻¹⁰ for a variety of reasons, further confounding the data analysis<https://c19study.com/>.

Discussions regarding the clinical use of HCQ¹¹ need to account for the intracellular activity of this compound to avoid toxicity and to attain therapeutic benefit. Infections due to SARS-CoV-2 alter cellular homeostasis leading to cytotoxicity and cell death. HCQ may impact viral activity at multiple levels by blocking viral entry into the cells, interfering with endosomal maturation, and lysosomal transport⁵. Furthermore, HCQ may also serve as an immune-modulator through its action on cytokine activity¹². The human oral absorption of HCQ/CQ reach significant concentrations in various tissues including the lungs, and has been reported to be 200-700 times higher than plasma levels¹³. HCQ given at a dose of 6-6.5mg/kg per day would generate serum levels of 1.4-1.5 mM in humans¹⁴, sufficient to

inhibit SARS-CoV-2 infection^{3,5}. Also the prolong half-life of HCQ (1440 hours) and the distribution on intercellular compartments (1300 hours)¹⁵, needs to be accounted as a factor in prescribing the treatment. Ultimately, the therapeutic goal in our treatments is to contain the viral invasion and prevent amplification of the infectious and inflammatory damage. Generation of reactive oxygen species (ROS) capable of inducing cellular apoptosis, necrosis and tissue destruction has been documented in viral infections¹⁶, and SARS-CoV-2 is not an exception. We have shown in an *in vitro* sterile injury model of cellular necrosis (neutrophils) that HCQ was capable of modulating ROS, and Toll-like receptor 9 (TLR9) activity mediated by the released of Damage Associated Molecular Patterns (DAMPs) in particular mitochondrial DNA (mtDNA)¹⁷. Other studies have shown that cellular injury mediated by viral invasion may release other DAMPs such as High Mobility Group B1 (HMGB1) during cellular damage¹⁸. Activity of HCQ on other inflammatory pathways such as cyclic-GMP-AMP (cGAMP) synthase (cGAS), or by interfering with interferon activity for example, needs to be analyzed as factors involved in generation of acute or chronic symptoms induced by viral infections¹⁹.

We are in urgent need to use HCQ at effective low doses as indicated by various basic science research groups thus preventing the occurrence of clinical toxicity observed worldwide. There is no sufficient evidence for or against completely eliminating HCQ as a therapeutic intervention during SARS-CoV-2. HCQ in combination with NAC needs to be considered a possible option to treat viral diseases. Studies that have used HCQ need to be analyzed for their potential benefit in post-viral chronic disease.

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- For N-acetylcysteine, the authors also mainly provide evidence from pre-clinical experiments. If there is no direct evidence from COVID-19, the authors should carefully review the literature and present possible indirect clinical evidence from severe pneumonia or viral pneumonia (if there is any).

Response to reviewers: N-Acetylcysteine (NAC) is an antioxidant, precursor of reduced glutathione, and modulator of transcription nuclear factor- κ B (NF- κ B) activity with a potential therapeutic profile for use in prevention or as adjuvant treatment for SARS-CoV-2. Viral infections induce cytotoxicity, cell death, overproduction of ROS and cytokines, thus promoting cellular and organ damage. NAC interferes with viral replication of human coronaviruses HCoV-229E¹, influenza H5N1², human immunodeficiency virus (HIV)³, and respiratory syncytial virus (RSV)⁴, among others. Recently, it was shown that NAC inhibited SARS-CoV-2 replication by binding to Cys-145, which is an active site for the main protease

(Mpro) involved in viral activity^{5,6}. Several *in vitro* studies have shown other potential mechanisms of NAC anti-viral activity that include: NF- κ B modulation (cytokine modulation)⁷, interference with angiotensin II type 1 receptors⁸, direct inhibition of furin (protease involved in the viral fusion process)⁹, and interaction with disulfite bonds¹⁰.

Pneumonia is a severe manifestation of SARS-CoV-2, SARS-CoV-1, and Middle East Respiratory Syndrome (MERS-CoV), with all sharing similar CT-scan findings of bilateral patchy infiltrates or ground glass opacities. NAC may modulate the effects of viral pneumonia induced by the cytotoxic effects of viral proliferation, cellular apoptosis, necrosis and excessive production of ROS that promote release of proinflammatory cytokines^{7,10}.

Other proposed mechanisms of action related to NAC include preservation of T cell activity and inhibition of the NOD-LRR- and pyrin domain-containing protein 3 (NLRP3) inflammatory pathway in monocytes and macrophages during viral respiratory infections^{11,12}. Promising results have been reported with the use of NAC for influenza, ARDS and VAP as measured by length of hospital stay and disease severity^{13,14}.

The therapeutic potential for NAC during viral infections and in particular in SARS-CoV-2 is supported by *in vitro* and indirectly by clinical studies (coronavirus, influenza). We are proposing the use of NAC during viral infections as adjuvant therapy with broad impact on viral activity, with low toxicity and cost. Certainly, further studies are necessary to document the cellular activity as well as the clinical benefits that NAC may provide.

The combination of low dose HCQ (total dose 600mg) and short-term NAC administered IV had a positive impact on the treatment of this patient and merits evaluation in large scale randomized clinical studies. The administration of both compounds orally during preintubation periods may attenuate viral symptomatology and possibly decrease the need for intubation.

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2. Response to reviewers: The clinical manifestations of viral or bacterial infections as initially observed in this patient with a qSOFA score of 2, multiorgan dysfunction and systemic inflammatory response syndrome (SIRS) as indicated by various inflammatory markers, are consistent with high risk of death.

Fluctuation of neutrophils and lymphocyte counts during bacterial or viral infections are used in general to support empiric antibiotic therapy. However, in the case presented and due to the absence of identifiable bacteria (see case report addendum) and with a positive SARS-CoV-2 RT-PCR a decision to treat the viral infection was made.

In this case initial neutrophilia and borderline lymphopenia would suggest a bacterial process, however low PCT indicates otherwise, thus antibiotic therapy was not warranted. Inflammatory markers as seen in the current case (CRP, Ferritin), highlights the acuity of the process, and the decreased observed over a few days correlated with the clinical improvement of this patient. CRP in particular is an acute phase reactant, indicative of inflammation, produced in the liver and may support the diagnosis of sepsis if other indicators of infection are also present. In this particular case there was a disconnect between elevated CRP and low PCT, raising the possibility of an inflammatory process rather than an infectious condition. Secondly, the initial cultures reported negative findings, observation supporting a non-infectious inflammatory process. Although co-infections may occur during viral infections, the possibility of a therapeutic benefit of the 3 doses of antibiotics administered empirically (Azithromycin 500 mg IV 1 dose, Vancomycin 2750 mg IV divided doses), is difficult to ascertain since cultures were reported negative. The only positive culture revealed was *Serratia Marcescens* 6 days after ICU admission and was treated with levofloxacin. Several markers of inflammation and the clinical improvement observed was dramatic after the introduction of HCQ/NAC in the treatment of this patient. Aside from the presence of deep venous thrombosis and pulmonary embolism every other inflammatory parameter measured had a positive progression.

There is no clear distinction between viral or bacterial respiratory infections based only on clinical manifestations such as fever, cough, shortness of breath, and fatigue, therefore the symptomatology exhibited by this patient was considered SARS-CoV-2 infection, in the absence of positive bacterial cultures.

We remain confident that the use of HCQ/NAC altered positively the clinical evolution of this patient and would suggest that this therapy merits further evaluation in randomized trials, with focus on early treatment of the SARS-CoV-2 infection.

Competing Interests: None

Reviewer Report 09 July 2020

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Bin Cao

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² Institute of Respiratory Medicine, Chinese Academy of Medical Sciences, Beijing, China
³ Department of Respiratory Medicine, Capital Medical University, Beijing, China
⁴ Tsinghua University-Peking University Joint Center for Life Sciences, Beijing, China

Puyo et al. presented the clinical course of a severe COVID-19 patient treated by hydroxychloroquine, N-acetylcysteine and supportive care. The insufficient details and case report nature of the study hamper a reliable causal inference for the efficacy and safety of hydroxychloroquine and N-acetylcysteine. Given the information provided in the manuscript, the influence of other factors (natural disease progression, effects of other treatments, etc.) cannot be excluded. Although we are in urgent need of treatment for COVID-19 in such a pandemic, inappropriate interpretation of the result may be misleading and cause unnecessary resources wasting at this time point. It should be very cautious to try to associate the recovery of the patient with these two drugs.

Specific comments are listed below:

1. According to the background and discussion part, the authors' inference for the potential efficacy of the two drugs are mainly established on evidence from pre-clinical experiments. However, clinical evidences from COVID-19 or other respiratory infectious diseases should also be mentioned and discussed in the manuscript.
 - For hydroxychloroquine, the results of several studies have been released. The RECOVERY trial was reported to find no clinical benefit from use of hydroxychloroquine in hospitalized COVID-19 patients (28-day mortality, length of hospitalization, etc.)¹. A published RCT in BMJ

from Tang et al found that the use of hydroxychloroquine did not increase the proportion of virus negative conversion². Also, the interim analysis of WHO solidarity trial showed little or no reduction in the mortality of hospitalized COVID-19 patients in the hydroxychloroquine arm and WHO thus discontinued the hydroxychloroquine arm for hospitalized patients³. Although the dosage of hydroxychloroquine varies in different trials and might be higher than the dosage used in this case report (400mg/600mg per day), it is hard to attribute the efficacy to hydroxychloroquine given the accumulating clinical evidences. The authors should provide clinical evidences in the manuscript in discussion to defend their hypothesis.

- For N-acetylcysteine, the authors also mainly provide evidence from pre-clinical experiments. If there is no direct evidence from COVID-19, the authors should carefully review the literature and present possible indirect clinical evidence from severe pneumonia or viral pneumonia (if there is any).

2. Details of the case: The authors only described in detail about hydroxychloroquine and N-acetylcysteine and details of other treatments were not provided. The authors should provide the details of all the important treatments to give readers a whole picture of the patient's disease course. For example:

- COVID-19 patients are often presented with normal or decreased white blood cell (WBC) counts and lymphocyte counts^{4,5}. This patient had elevated WBC count, elevated neutrophil count and normal lymphocyte count (according to the provided normal range). Though the procalcitonin was lower than 0.5 ng/mL, the possibility of co-bacterial infection could not be excluded. The authors should provide results of lower respiratory tract etiological testing (sputum bacteriology, etc.) and blood culture. Besides, whether the patient received antibiotics and (if so), the details of antibiotics should also be described.
- According to the clinical information provided in the manuscript, the patient's qSOFA score at admission was 2 (Blood Pressure 92/62mmHg; Respiratory Rate 48 times per minute). The patients showed signs of sepsis or even septic shock. However, the provided information was not enough, and I am wondering whether the diagnosis of sepsis was made across the disease course and whether guideline-based treatment was given. More details should be described. Besides, if sepsis-related treatment is given, the role of hydroxychloroquine and N-acetylcysteine should be further questioned.
- "Prophylactic anticoagulation was started with subcutaneous heparin". The dosage of heparin should be specified.

3. Please distinguish between the disease name "COVID-19" and the virus name "SARS-CoV-2", and use the right word in the manuscript. For example, "COVID-19 positive patient" should be revised to "COVID-19 patient"; "COVID-19 enters the human airway in a process reminiscent of other viruses" should be revised to "SARS-CoV-2 enters..."; "COVID-19 infection" should be revised to "SARS-CoV-2 infection"; "COVID-19 RT-PCR" should be revised to "SARS-CoV-2 RT-PCR".

The objective of the case report is to present hypothesis for the potential efficacy of hydroxychloroquine and N-acetylcysteine. However, given the rationale mentioned above, at this stage, the information provided in the manuscript did not support such hypothesis.

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Full Text

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Is the background of the case's history and progression described in sufficient detail?

No

Are enough details provided of any physical examination and diagnostic tests, treatment given and outcomes?

No

Is sufficient discussion included of the importance of the findings and their relevance to future understanding of disease processes, diagnosis or treatment?

No

Is the case presented with sufficient detail to be useful for other practitioners?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Pulmonary and critical care medicine; Infectious diseases

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 19 Aug 2020

Carlos Puyo

August 18, 2020

Drs. Bin Cao, M.D., and Vui Heng Chong, M.D.
Reviewers F1000Research

Dear Drs, Cao and Chong:

We are very grateful for your insightful comments on our Case Report "**Use of hydroxychloroquine and N-Acetylcysteine for treatment of a COVID-19 patient**". We have given careful consideration to the points raised by you during the review process. The case report has been adjusted to reflect our comments as indicated in the manuscript using a tracking system.

We look forward to your response and hope to complete the publication of our Case Report in *F1000Research*.

Sincerely,

Carlos A. Puyo, M.D., FCCP

Specific comments are listed below:

1. According to the background and discussion part, the authors' inference for the potential efficacy of the two drugs are mainly established on evidence from pre-clinical experiments. However, clinical evidences from COVID-19 or other respiratory infectious diseases should also be mentioned and discussed in the manuscript.

- For hydroxychloroquine, the results of several studies have been released. The RECOVERY trial was reported to find no clinical benefit from use of hydroxychloroquine in hospitalized COVID-19 patients (28-day mortality, length of hospitalization, etc.)¹. A published RCT in BMJ from Tang et al found that the use of hydroxychloroquine did not increase the proportion of virus negative conversion². Also, the interim analysis of WHO solidarity trial showed little or no reduction in the mortality of hospitalized COVID-19 patients in the hydroxychloroquine arm and WHO thus discontinued the hydroxychloroquine arm for hospitalized patients³. Although the dosage of hydroxychloroquine varies in different trials and might be higher than the dosage used in this case report (400mg/600mg per day), it is hard to attribute the efficacy to hydroxychloroquine given the accumulating clinical evidences. The authors should provide clinical evidences in the manuscript in discussion to defend their hypothesis.

1. **Response to reviewers:** The release of several studies promoting or discrediting the use of chloroquine or hydroxychloroquine for the treatment of COVID-19 has created confusion in the medical community. Here, the reviewer duly noted studies showing lack of or questionable therapeutic benefit^{1,2}, however, there is no mention of studies that have shown benefit^{3,4,5,6,7}. The RCT by Tang, et al, contains several shortcomings among them the use of an open label instead of a double blind approach, potentially introducing bias by the researchers. Similarly, the underpowered sample size and enrollment of patients in more advance stages of the disease likely influence negatively their assessment of the true potential value of HCQ in the disease progression. In the RECOVERY trial mentioned by the reviewer, the doses of HCQ 2400 mg in 24 hours followed by 1600 mg daily for at least 9 days, are indeed higher than for malaria treatment, thus exposing patients to unnecessary risks. It is unclear what scientific bases were utilized to determine the exceedingly high doses of HCQ administered. The Indian Council of Medical Research alerted the WHO about the excessive doses of HCQ (4 times higher) used in the RECOVERY trial, thus, possibly introducing bias in the comparative analysis. The RECOVERY trial also has some interesting findings regarding their unexplained very high mortality data in the control group (23.6%), compared to the 12.7% and 13.5% reported by others in treating high acuity hospitalized patients^{4,8}.

Interpretation of HCQ studies is difficult, in part due to the variety of end points utilized by the researchers. For instance some studies used mortality or pneumonia¹, while others used negative viral conversion² as the end points of the studies, thus making a comparative analysis on the effects of HCQ a complex one. In some cases reputable journals (Lancet and others) had to RETRACT anti-HCQ studies⁹⁻¹⁰ for a variety of reasons, further confounding the data analysis <https://c19study.com/>.

Discussions regarding the clinical use of HCQ¹¹ need to account for the intracellular activity of this compound to avoid toxicity and to attain therapeutic benefit. Infections due to SARS-CoV-2 alter cellular homeostasis leading to cytotoxicity and cell death. HCQ may impact viral activity at multiple levels by blocking viral entry into the cells, interfering with endosomal maturation, and lysosomal transport⁵. Furthermore, HCQ may also serve as an immune-modulator through its action on cytokine activity¹². The human oral absorption of HCQ/CQ reach significant concentrations in various tissues including the lungs, and has been reported to be 200-700 times higher than plasma levels¹³. HCQ given at a dose of 6-6.5mg/kg per day would generate serum levels of 1.4-1.5 mM in humans¹⁴, sufficient to inhibit SARS-CoV-2 infection^{3,5}. Also the prolong half-life of HCQ (1440 hours) and the distribution on intercellular compartments (1300 hours)¹⁵, needs to be accounted as a factor in prescribing the treatment. Ultimately, the therapeutic goal in our treatments is to contain the viral invasion and prevent amplification of the infectious and inflammatory damage. Generation of reactive oxygen species (ROS) capable of inducing cellular apoptosis, necrosis and tissue destruction has been documented in viral infections¹⁶, and SARS-CoV-2 is not an exception. We have shown in an *in vitro* sterile injury model of cellular necrosis (neutrophils) that HCQ was capable of modulating ROS, and Toll-like receptor 9 (TLR9) activity mediated by the released of Damage Associated Molecular Patterns (DAMPs) in particular mitochondrial DNA (mtDNA)¹⁷. Other studies have shown that cellular injury mediated by viral invasion may release other DAMPs such as High Mobility Group B1 (HMGB1) during cellular damage¹⁸. Activity of HCQ on other inflammatory pathways such as cyclic-GMP-AMP (cGAMP) synthase (cGAS), or by interfering with interferon activity for example, needs to be analyzed as factors involved in generation of acute or chronic symptoms induced by viral infections¹⁹.

We are in urgent need to use HCQ at effective low doses as indicated by various basic science research groups thus preventing the occurrence of clinical toxicity observed worldwide. There is no sufficient evidence for or against completely eliminating HCQ as a therapeutic intervention during SARS-CoV-2. HCQ in combination with NAC needs to be considered a possible option to treat viral diseases. Studies that have used HCQ need to be analyzed for their potential benefit in post-viral chronic disease.

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doi:10.1016/j.cytogfr.2014.06.006

- For N-acetylcysteine, the authors also mainly provide evidence from pre-clinical experiments. If there is no direct evidence from COVID-19, the authors should carefully review the literature and present possible indirect clinical evidence from severe pneumonia or viral pneumonia (if there is any).

Response to reviewers: N-Acetylcysteine (NAC) is an antioxidant, precursor of reduced glutathione, and modulator of transcription nuclear factor- κ B (NF- κ B) activity with a potential therapeutic profile for use in prevention or as adjuvant treatment for SARS-CoV-2. Viral infections induce cytotoxicity, cell death, overproduction of ROS and cytokines, thus promoting cellular and organ damage. NAC interferes with viral replication of human coronaviruses HCoV-229E¹, influenza H5N1², human immunodeficiency virus (HIV)³, and respiratory syncytial virus (RSV)⁴, among others. Recently, it was shown that NAC inhibited SARS-CoV-2 replication by binding to Cys-145, which is an active site for the main protease (Mpro) involved in viral activity^{5,6}. Several *in vitro* studies have shown other potential mechanisms of NAC anti-viral activity that include: NF- κ B modulation (cytokine modulation)⁷, interference with angiotensin II type 1 receptors⁸, direct inhibition of furin (protease involved in the viral fusion process)⁹, and interaction with disulfide bonds¹⁰. Pneumonia is a severe manifestation of SARS-CoV-2, SARS-CoV-1, and Middle East Respiratory Syndrome (MERS-CoV), with all sharing similar CT-scan findings of bilateral patchy infiltrates or ground glass opacities. NAC may modulate the effects of viral pneumonia induced by the cytotoxic effects of viral proliferation, cellular apoptosis, necrosis and excessive production of ROS that promote release of proinflammatory cytokines^{7,10}. Other proposed mechanisms of action related to NAC include preservation of T cell activity and inhibition of the NOD-LRR- and pyrin domain-containing protein 3 (NLRP3) inflammatory pathway in monocytes and macrophages during viral respiratory infections^{11,12}. Promising results have been reported with the use of NAC for influenza, ARDS and VAP as measured by length of hospital stay and disease severity^{13,14}. The therapeutic potential for NAC during viral infections and in particular in SARS-CoV-2 is supported by *in vitro* and indirectly by clinical studies (coronavirus, influenza). We are proposing the use of NAC during viral infections as adjuvant therapy with broad impact on viral activity, with low toxicity and cost. Certainly, further studies are necessary to document the cellular activity as well as the clinical benefits that NAC may provide. The combination of low dose HCQ (total dose 600mg) and short-term NAC administered IV had a positive impact on the treatment of this patient and merits evaluation in large scale randomized clinical studies. The administration of both compounds orally during preintubation periods may attenuate viral symptomatology and possibly decrease the need for intubation.

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2. Response to reviewers: The clinical manifestations of viral or bacterial infections as initially observed in this patient with a qSOFA score of 2, multiorgan dysfunction and systemic inflammatory response syndrome (SIRS) as indicated by various inflammatory markers, are consistent with high risk of death.

Fluctuation of neutrophils and lymphocyte counts during bacterial or viral infections are used in general to support empiric antibiotic therapy. However, in the case presented and due to the absence of identifiable bacteria (see case report addendum) and with a positive SARS-CoV-2 RT-PCR a decision to treat the viral infection was made.

In this case initial neutrophilia and borderline lymphopenia would suggest a bacterial process, however low PCT indicates otherwise, thus antibiotic therapy was not warranted.

Inflammatory markers as seen in the current case (CRP, Ferritin), highlights the acuity of the process, and the decreased observed over a few days correlated with the clinical improvement of this patient. CRP in particular is an acute phase reactant, indicative of inflammation, produced in the liver and may support the diagnosis of sepsis if other indicators of infection are also present. In this particular case there was a disconnect between elevated CRP and low PCT, raising the possibility of an inflammatory process rather than an infectious condition. Secondly, the initial cultures reported negative findings, observation supporting a non-infectious inflammatory process. Although co-infections may occur during viral infections, the possibility of a therapeutic benefit of the 3 doses of antibiotics administered empirically (Azithromycin 500 mg IV 1 dose, Vancomycin 2750 mg IV divided doses), is difficult to ascertain since cultures were reported negative. The only positive culture revealed was *Serratia Marcescens* 6 days after ICU admission and was treated with levofloxacin. Several markers of inflammation and the clinical improvement observed was dramatic after the introduction of HCQ/NAC in the treatment of this patient. Aside from the presence of deep venous thrombosis and pulmonary embolism every other inflammatory parameter measured had a positive progression. There is no clear distinction between viral or bacterial respiratory infections based only on clinical manifestations such as fever, cough, shortness of breath, and fatigue, therefore the symptomatology exhibited by this patient was considered SARS-CoV-2 infection, in the absence of positive bacterial cultures.

We remain confident that the use of HCQ/NAC altered positively the clinical evolution of this patient and would suggest that this therapy merits further evaluation in randomized trials, with focus on early treatment of the SARS-CoV-2 infection.

Competing Interests: None

Comments on this article

Version 2

Reader Comment 25 May 2021

Ed J. van Hezik, WCC at SEA, Waterfront at Sea, The Netherlands

The authors have updated their findings in a new manuscript "N-Acetylcysteine to Combat COVID-19: An Evidence Review" Therapeutics and Clinical Risk Management 2020-11 | journal-article, please see <https://doi.org/10.2147/TCRM.S273700>

Now it offers a fresh view on NAC (the previous discussion about HCQ effect can be deleted).

In addition NAC can be considered as a H₂S donor ,in this way offering a strong antiviral effect on enveloped MRA virus like CoV2.

For info please see

N-acetylcysteine (NAC) and Hydrogen Sulfide (H₂S) in Coronavirus Disease 2019 (COVID-19).

Antioxid Redox Signal. 2021 <https://doi.org/10.1089/ars.2020.8247>

Adapted Incremental Treatment Plan in COVID-19 - version nov 2020

DOI: <https://doi.org/10.13140/RG.2.2.19955.04643>

N-acetylcysteine: a convenient rationale for COVID-19. Consideration of antiviral H₂S for inclusion in one of the ANTICOV or WHO master protocol

<https://doi.org/10.13140/RG.2.2.26361.19044>

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Competing Interests: the author has no conflicting /competing interests, no funding.

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