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Re-tasking the use of pre-existing medications and potential therapeutic options for coronavirus disease (COVID-19): systematic review of clinical studies

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SUMMARY With the emergence of coronavirus disease 2019 (COVID-19) in late December 2019, many clinical studies on a group of the pre-existing medications have been conducted to treat this disease. The purpose of this review was to compile the clinical evidences on the use of the pre-existing medications and potential therapeutic options for the management of COVID-19. We reviewed the literature to highlight the clinical studies on the use of these medications to be available as a scientific overview for further perspectives. Inadequate clinical evidences are available to be affirmed for the repurposing of old medications, and large scale clinical studies are needed to be carried out to further confirm the use of these agents. The clinical use of these medications should be well explained and follow the framework of Monitored Emergency use of Unregistered Interventions (MEURI) of World Health Organization (WHO).

Keywords COVID-19, clinical studies, hydroxychloroquine, pneumonia, SARS-CoV-2

1. Introduction

In late December 2019, a novel strain of coronavirus was identified in a group of patients in Wuhan city of China (*1*). It was preliminarily named as 2019 novel coronavirus (2019-nCoV), then the virus has officially been named as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (*2*). Despite local measures by Wuhan the virus has spread beyond China. On the 11th of March 2020 World Health Organization (WHO) announced a Coronavirus disease 2019 (COVID-19) as a pandemic disease. There is a diversity in the severity of COVID-19, ranging from mild respiratory tract symptoms to severe or fatal pneumonia. As of 26th of April 2020 coronavirus has affected a total of 2,920,877 cases and lead to 203,272 deaths in 212 countries.

Outbreaks of coronavirus groups have been recorded in the last twenty years other than Wuhan's new coronavirus (2019-nCoV). Six other coronavirus strains are known to infect human with different origin and transmission dynamics. Among these, only severe acute respiratory syndrome coronavirus (SARS-CoV) in China in 2003 and middle east respiratory syndrome coronavirus (MERS-CoV) in Saudi Arabia in 2012 were with high mortality rate (*3*) and the other four coronaviruses (HCoV-229E, HCoV-NL63, HCoV-HKU1, and HCoV-OC43) can only cause mild respiratory infections.

Studies on SARS-CoV and MERS-CoV helped in identifying potentially effective therapies such as: remdesivir, lopinavir/ritonavir, interferon and convalescent plasma. Limited information is available on SARS-CoV-2, however several insights may be gained from its more well-known family member, SARS-CoV (*4*). The available evidence, and experience from SARS-CoV and MERS-CoV suggests several approaches to manage and limit the spread of SARS-CoV-2. One of these approaches is by targeting the binding protein of the virus with the host receptor which will disrupt the replication and will modulate the immunity of the individual. Coronavirus is an enveloped, positive-strand RNA virus, it is covered with club-shaped glycoproteins which look like 'crowns', or 'halos' so it is called a coronavirus. It forms coronal protrusions at the edges.

For all coronaviruses including SARS-CoV-2, at least three structural proteins are shared on the membrane. First protein is spike (S); the spike protein of these coronaviruses binds angiotensin converting enzyme-2 (ACE-2), which is highly abundant in the lungs and heart, leading to respiratory and potential

cardiovascular damage (*5*). Angiotensin converting enzyme-2 (ACE-2) serves as the cellular entry point for coronaviruses (*6*). Second structural protein is the membrane protein (M) and third one is small membrane protein (E). Another four functional proteins were found in almost all coronaviruses: 3-chymotrypsin-like protease, papain-like protease, RNA-dependent RNA polymerase (RdRp) and helicase. A high genomic similarity between SARS-CoV-2 and the previous coronaviruses particularly SARS-CoV has been noticed, it has been found that they share 82% RNA sequence identity, and their RNA-dependent RNA polymerase (RdRp) shares 96% sequence identity with SARS-CoV-2 (*4*). Therefore, drugs targeting viral RdRp proteins of SARS-CoV are likely to be effective for SARS-CoV-2. To date and according to the World Health Organization (WHO), the Centers for Disease Control and Prevention (CDC), and the Food Drug Administration (FDA) there are currently no medications or vaccines proven to be effective for the treatment or prevention of SARS-CoV-2 (*7-9*).

Large number of the *in vitro*, *in vivo*, preclinical, and clinical studies have been conducted and they have reported certain agents that displayed strong antiviral potential of which some have been permitted to be used in an attempt to combat the disease in clinical trials, including the pre-existing medications that were used for the treatment of other diseases with the aim of repurposing them to treat COVID-19. Some of these studies have demonstrated a particular therapeutic intervention in a clinical basis that could not provide a strict clinical evidence. For this reason, the current review sets out to compile the clinical evidences on the use of pre-existing medications and potential therapeutic options for the management of COVID-19 since the emergence of the outbreak in late December 2019 till April 2020 to be available as a scientific overview for further perspectives. Table 1 summarized some repurposing efforts for the existing approaches in treatment of COVID-19 through demonstrating their clinical efficacy and safety.

2. Repurposing of chloroquine/hydroxychloroquine for use as treatment of COVID-19

During any outbreaks of epidemic and pandemic levels, repurposing of the pre-existing drugs is a common practice due to difficulty in development of new drug at that time being. Although repurposing of the old medications for treatment of COVID-19 with safe profile was not apprehended by the virologists, many clinical studies on those medications were carried out in several Chinese hospitals and their clinical efficacy were also being evaluated in many studies (*10*). Some protocols have included the recommendation for using them (*9,11*). The factual evidence for the effectiveness of chloroquine/hydroxychloroquine in COVID-19 is currently very limited. The first clinical outcome of treating over 100 patients with chloroquine phosphate at 500 mg twice daily for 10 days was documented on February 17, 2020 by the State Council of China in a news briefing, the results were superior to the control without serious adverse reaction and was prevented the exacerbation of pneumonia, improved lung imaging findings, and reduced clinical duration of disease markedly, thereafter chloroquine phosphate has been designated as a re-tasking strategy for COVID-19 with a marked efficacy and tolerability in treating severe pneumonia related to COVID-19 in China (*12*).

Moreover, Gautret and his colleagues in France (*13*) have conducted the first clinical open label non randomized controlled trial on 36 patients diagnosed with COVID-19 (20 hydroxychloroquine-treated patients and 16 control patients), the treated patients received 600 mg of hydroxychloroquine daily (200 mg, three times per day during ten days), and azithromycin has been given to six patients to protect against a possible superimposed bacterial infection. The patients in other centers did not receive hydroxychloroquine and served as controls, they received only supportive management. The results of this clinical study demonstrated that on day 6, patients in the treatment group were significantly more likely to test negative for the virus than patients in the control group (70% *vs*. 12.5% virologically cured, $p < 0.001$) and all of the six patients who were treated with a combination of hydroxychloroquine and azithromycin tested negative on day 6. The authors concluded that despite its small sample size their clinical study demonstrated that in the hydroxychloroquine-treated group there was a significant reduction and disappearance of viral load and it was also found that azithromycin synergized its effect.

Subsequent to the promising results of these first clinical trials, guidelines published recommending the treatment of COVID-19 using chloroquine/hydroxychloroquine. The National Health Commission of the People's Republic of China published their recommendation in mid-February, suggesting to treat patients with 500 mg chloroquine phosphate twice per day, for a maximum of 10 days (*14*). In order to guide and standardize the use of chloroquine in the treatment of the new coronavirus pneumonia, the Guangdong Provincial Department of Science and Technology and the Guangdong Provincial Health and Health Commission's chloroquine treatment of new coronavirus pneumonia multi-center collaboration group, developed the expert consensus after fully discussing the diagnosis of the new coronavirus for patients with pneumonia of mild, common and severe, after chloroquine contraindications were ruled out, chloroquine phosphate tablets could be used 500 mg each time, twice daily for 10 days. Due to the findings from the above studies, China then recommended the

Table 1. Characteristics of the clinical studies re-tasking pre-existing medications for management of the coronavirus disease 2019 (COVID-19)

Drug /Approach	Type of clinical study	Dosage	Intervention group	Comparison group	Outcome measures	*Ref.*
Chloroquine Phosphate	Multicenter clinical study	500 mg twice daily for 10 days	Chloroquine Phosphate	Control group	Detail not included however the results were superior to the control	*12*
Hydroxychloroquine (HCQ) and Azithromycin (Azi)	Open label non-randomized clinical study	HCQ; 200 mg, 3 times/day for 10 days, Azi; 500 mg on day1followed by 250 mg/day, the next four days	20 patients received HCQ; Azi were added to 6 of them	16 control patients received supportive management	Virological clearance, clinical follow-up, side effects.	*13*
Hydroxychloroquine (HCQ)	Randomized clinical study (A pilot study)	400 mg per day for 5 days	HCQ plus conventional treatments	Control group: conventional treatment	Virological clearance (rRT-PCR-SARS-CoV-2) in respiratory pharyngeal swab on days 7, adverse drug event	*16*
Hydroxychloroquine (HCQ)	Randomized clinical study	400 mg/day; (200 mg twice daily) for 5 days	HCQ plus standard treatment	Standard treatment, with or without corticosteroids	TTCR, clinical characteristics, and radiological results	*17*
Hydroxychloroquine (HCQ)	Multicenter,randomized, open-label, parallel clinical study	Laoding dose = 1,200 mg daily for 3 days, maintenance dose = 800 mg for 2 - 3 weeks	HCQ plus standard care	Standard care	Negative conversion rate of COVID-19 virus, improvement rate of clinical symptoms.	*18*
Remdesivir	Case report	I.V. Remdesivir	I.V. Remdesivir for 7 days	NA	Cycle threshold values (Ct), Patient's rRT-PCR-SARS-COV-2 in nasopharyngeal and oropharyngeal swabs	*26*
Lopinavir-ritonavir	Open-label randomized clinical study	lopinavir-ritonavir (400 - 100 mg) twice daily for 14 days	Lopinavir-ritonavir treated group (n = 99)	Conventional therapy group n = 100)	Time to clinical improvement, clinical status seven-category ordinal scale on days 7 and 14, mortality at day 28, the duration of mechanical ventilation, the duration of hospitalization	*33*
Convalescent plasma (CP)	Pilot clinical study	One dose of 200 mL CP obtained from recently COVID-19 recovered patients	Severe patients confirmed by rRT-PCR-SARS-COV-2 received One dose of 200 mL CP	Pre- and post CP transfusion comparison.	The outcome measures: safety of CP transfusion, clinical improvement and amelioration of laboratory values within 3 days after CP transfusion	*41*
Convalescent plasma (CP)	Uncontrolled case series	2 consecutive transfusions of 200 to 250 mL of ABO blood type-compatible convalescent plasma	400 mL of convalescent plasma in total on the same day it was obtained from the donor. The patients received antiviral agents continuously until the SARS-CoV-2 viral loads became negative.	Pre- and post CP transfusion comparison.	Clinical symptoms, changes in body temperature, organ failure assessment (SOFA) score, virology parameters, hematological and biochemistry parameters, the ratio of the partial pressure to fraction of inspired oxygen (PAO_2/FIO_2)	*42*

rRT-PCR-SARS-CoV-2: real-time reverse-transcriptase-polymerase-chain-reaction; ABO: blood type; TTCR: Time to clinical recovery.

use of chloroquine in prevention and management of COVID-19 (*12*). In Italy, the L. Spallanzani National Institute for the Infectious Disease published their recommendations for treatment of COVID-19 on the 17th of March, which included the provision of 400 mg of hydroxychloroquine per day or 500 mg chloroquine per day, in combination with another antiviral agent (*15*). Additionally, in a systematic review on the safety and efficacy of chloroquine the authors identified and mentioned 23 ongoing clinical trials on chloroquine in COVID-19. The ongoing trials varied in study design, severity of the disease in the target population and in dosing and duration of the treatment (*10*).

The current review further elaborated one pilot study of using hydroxychloroquine in treatment of patients with COVID-19 (*16*). The study prospectively enrolled 30 patients with confirmed COVID-19 after informed consent at Shanghai Public Health Clinical Center had been obtained. The patients were randomized 1:1 to hydroxychloroquine group and the control group. Patients in hydroxychloroquine group were given 400 mg hydroxychloroquine per day for 5 days plus conventional treatments, while those in the control group were given conventional treatment only. The primary outcome measure was a negative conversion rate of the real-time reverse transcription PCR testing for SARS-CoV-2 (rRT-PCR-SARS-CoV-2) in respiratory pharyngeal swab on days 7 after randomization. The results showed no significant difference in outcomes between those who received the drug and those who received conventional treatment. The main findings are one patient developed severe disease and the authors stated that developing severe disease did not appear related to the medication. One week after hospitalization, 13 (86.7%) of patients in the experimental group and 14 (93.3%) of patients in the usual care group tested negative. This difference was not statistically significant, (p value > 0.05) median time for Patients' temperatures to return to normal was comparable in both groups. Disease progression in chest CT images was statistically comparable between both groups (5 (33.3%) of the hydroxychloroquine treated-group and 7 (46.7%) of the conventional group). At two weeks, all patients in both groups tested negative and showed improvement in their symptoms, regarding side effects short-term diarrhea and abnormal liver function occurred in 4 (26.7%) of the hydroxychloroquine group and 3 (20%) of the control group. The rate of adverse events was similar in both groups. The authors recommended larger size trials and they estimated that a trial would require 784 patients with no drop-outs to determine whether hydroxychloroquine definitively results in better or worse outcomes.

Recently in a larger scale, efficacy of hydroxychloroquine in patients with COVID-19 in a randomized clinical trial has been evaluated in 62 patients in another hospital of China (*17*). All patients were randomized in a 1:1 ratio, each group was of 31 patients. Standard treatment was given for both group (oxygen therapy, antiviral agents, antibacterial agents, and immunoglobulin, with or without corticosteroids), hydroxychloroquine-treated group received 5-day hydroxychloroquine (400 mg/day; 200 mg twice daily), time to clinical recovery (TTCR), clinical characteristics, and radiological results were assessed at baseline and 5 days after treatment to evaluate the effect of hydroxychloroquine. The results showed that TTCR, the body temperature recovery time and the cough remission time were significantly shortened in the hydroxychloroquine treated group. A larger proportion of patients from hydroxychloroquine treated group 25 (80.6%) versus 17 (54.8%) in control group showed improvement confirmed by chest CT. Four patients in the control group found to have to severe illness. However, only 2 hydroxychloroquine treated patients found to have mild adverse reactions including skin rash and headache. The results suggested that the use of hydroxychloroquine could significantly shorten TTCR and promote the absorption of lung lesion. They concluded that despite the efficacy, tolerability and affordability of hydroxychloroquine, the detrimental effects of this drug cannot be ruled out.

Most recently another clinical study designed in a randomized, open-label, multicenter, parallel trial has been conducted in China. The study enrolled 150 COVID-19 patients from 16 designated COVID-19 treatment centers of three provinces in China to assess the effectiveness and safety of hydroxychloroquine (*18*). Half of the patients (n = 75) were allocated to receive standard care of the hospitals alone while the other half (n = 75) to receive standard care plus hydroxychloroquine. Hydroxychloroquine was given as 1,200 mg loading dose daily for three days then followed by 800 mg as maintenance dose daily for duration of two weeks for mild/moderate to three weeks for severe patients.

The primary endpoint was a negative conversion rate of COVID-19 virus while the secondary endpoints included a 28-days improvement rate of clinical symptoms. The result of the study showed that almost collectively a 28-day negative conversion rate of the virus was similar between the two groups. The Kaplan-Meier estimate for negative conversion rate was 85.4% versus 81.3%, p = 0.341. Additionally, no difference in mitigation of symptoms in a 28-day duration was seen between the two studied groups. The authors stated that using hydroxychloroquine with the standard care did not provide any additional virologic response and its effect on the mitigation of symptoms was more evident when the confounding effects of other anti-viral agents were removed. Further they emphasized on that the adverse effects of hydroxychloroquine should be closely monitored. Despite the larger scale of the latest clinical study (n = 150) on re-tasking

hydroxychloroquine against COVID-19, the efficacy and safety of hydroxychloroquine need to be further monitored.

Moreover, additional investigation on the safety and efficacy of chloroquine and hydroxychloroquine has been included in this systematic review (*19*) to demonstrate the preference of hydroxychloroquine over the chloroquine although the antimalarial activity of hydroxychloroquine is equivalent to that of chloroquine. Hydroxychloroquine is preferred over chloroquine for its lower ocular toxicity (*20*). The authors focused on retinopathy and QT-prolongation as a safety and risk concern for chloroquine and hydroxychloroquine comparison, they identified retinopathy as a dose-limiting adverse effect of hydroxychloroquine (21) they also highlighted the advantages of hydroxychloroquine over chloroquine concerning its easily obtainability, less drug interaction with other protease inhibitors especially lopinavir/ritonavir as a clarification for the preference of hydroxychloroquine.

Furthermore, the prophylaxis role of chloroquine against COVID-19 has been supported based on the preliminary clinical evidences of chloroquine in COVID-19 and an optimal dosing regimen to reach a preventive effect of chloroquine against SARS-CoV-2 inhibition in respiratory tissues with acceptable safety profile was formulated as stated by the two authors Raymond Chang and Wei-Zen Sun in their review articles (*22*).

3. Repurposing of remdesivir for use as treatment of COVID-19

With the emergence of the COVID-19 it had been suggested that remdesivir might be an option for the therapy of patients with COVID-19 (*4*). Remdesivir is a broad-spectrum antiviral agent, is a nucleotide analog inhibitor of RNA-dependent RNA polymerases (RdRps). It is a monophosphoramidate prodrug and is an adenosine analog. Although currently there are no approved antiviral medications for the treatment of COVID-19. Preclinical data with the nucleotide analogue remdesivir and its safety profile encourages repurposing the use of this drug as a treatment for COVID-19 (*23*). This drug shows a wide range of antiviral activities against several RNA viruses (*24*) including SARS-CoV and MERS-CoV. Remdisivir was originally developed for the treatment of Ebola virus disease (EVD) (*25*). It was synthesized and developed by Gilead Sciences in 2017 as a treatment for Ebola virus infection. The first case of COVID-19 in Washington, USA (although transmission dynamics and the full spectrum of clinical illness was not fully understood) was compassionately treated with I.V. remdesivir for the progression of pneumonia on day 7 of hospitalisation and the case has been reported as case study by Holshue ML *et al*. (*26*). The authors in this study demonstrated cycle threshold values (Ct) (lower cycle threshold value indicates higher viral load) as an indicator for a decline in a viral load of the patient. Patient's rRT-PCR-SARS-COV-2 in nasopharyngeal and oropharyngeal swabs remained positive at 4 days after the administration of remdesivir, but the authors recorded a trend in the decline of viral load in nasopharyngeal swabs. Despite the patient's initial mild symptoms, the Ct values (18 to 20 in nasopharyngeal specimens and 21 to 22 in oropharyngeal specimens) on illness day 4 suggest high levels of viral load in these specimens. Nasopharyngeal and oropharyngeal specimens obtained on illness days 11 and 12 showed a trend toward decreasing levels of viral load (day 11, Ct was 33-34; and day 12 Ct was 37-40 for nasopharyngeal swab). The oropharyngeal specimen tested negative for SARS-CoV-2 on day 12. The patient's condition improved and no obvious side effects were observed. The rRT-PCR-SARS-CoV-2 results for serum obtained on these dates are still pending. The authors in this case report highlighted the necessity to identify the pathophysiology, duration of viral stay and other features of COVID-19 to provide more information on clinical management. Although it is not plausible to conclude the direct antiviral effect of remdesivir on enhanced clearing of viral loads in the respiratory tract only by a case report, it indeed suggests a promising therapeutic effect of remdesivir.

Currently there are two phase 3 randomized, double-blind, placebo controlled multicenter clinical trials ongoing in China. These trials have been submitted to ClinicalTrials.gov and are designed to assess the safety and efficacy of parenteral remdesivir in hospitalized adults with mild-to-moderate and severe COVID-19 (*27*). The number of the planned-recruited cases is 308 and 452, respectively. A treatment protocol will be 200 mg loading dose on first day, then 100 mg maintenance doses per a day for nine successive days in both studies. This protocol of remdesivir therapy was used in the randomized clinical trial of Ebola virus disease (*28*). Clinical efficacy of remdesivir on COVID-19 still unknown, and researchers are waiting the final outcomes of these ongoing clinical trials.

4. Repurposing lopinavir-ritonavir protocol as a treatment in COVID-19

Previous experiences and preclinical studies on other coronaviruses (SARS-COV, MERS-CoV) identified lopinavir as a potential therapy against COVID-19. Lopinavir, a human immunodeficiency virus (HIV) type 1 aspartate protease inhibitor, has *in vitro* inhibitory activity against SARS-COV (*29*). Ritonavir is used in lower doses to enhance the activity of other protease inhibitors by improving their bioavailability, plasma half-life and inhibiting their metabolism. Lopinavir is now available only in combination with ritonavir to

improve bioavailability and plasma half-life of lopinavir through the inhibition of cytochrome P450. Lopinavir has activity, both *in vitro* (*30*) and in an animal model (*31*) against MERS-CoV, and case reports have suggested that the combination of lopinavir-ritonavir with ribavirin and interferon alfa resulted in virologic clearance and survival (*32*). After the emergence of COVID-19 and the urgent need of an antiviral therapy, also because lopinavir was clinically available for HIV-1 infection and a study of lopinavir plus the protease inhibitor ritonavir demonstrated clinical efficacy for SARS-CoV, researchers conducted an open-label randomized trial at one hospital in China (*33*). They recruited 199 adult COVID-19 patients (assigned randomly to the lopinavir-ritonavir treated group (n = 99) and conventional therapy group (n = 100)). The conventional group received a standard care alone while the treated group received oral lopinavir-ritonavir (400-100 mg) twice daily for 14 days. The results showed that the treated group and those receiving standard care did not differ significantly in time to clinical improvement, duration of intensive care unit stay, days of mechanical ventilation, or days of oxygen support. Patients who received lopinavir-ritonavir had lower 28-day mortality (19% *vs*. 25%), but the differences between the groups was not significant. The results of rRT-PCR-SARS-CoV-2 testing of throat swabs did not differ between the two groups. The authors concluded no clinical advantage of using lopinavir-ritonavir treatment over the standard care, they stated that further clinical trials are needed to confirm the effectiveness of these medications in COVID-19 treatment.

5. Convalescent plasma uses as a potential therapy for COVID-19

Convalescent plasma (CP) therapy, is a classic adaptive immunotherapy, has been used in a therapy for many infectious diseases since last century. In the last twenty years, CP therapy was well used in the management of SARS, MERS, with reasonable efficacy and safety (*34*). In 2014, the use of CP collected from patients who had recovered from Ebola virus disease was recommended by WHO as an empirical treatment during outbreaks (*35*). A protocol for the use of CP in the treatment of MERS was established in 2015 (*36*). No adverse events were observed. The possible mechanism of action of CP is antibody associated suppression of viraemia. Because the virological and clinical characteristics are sharing similarities among SARS, MERS, and COVID-19 (*37*), scientists believed that CP therapy might be a promising treatment option as COVID-19 rescue (*38*). A viewpoint by Casadevall A and Pirofski LA has argued the use of convalescent serum as an option for COVID-19 treatment (*39*). Furthermore, there are reports that CP was used for therapy of patients with COVID-19 in China during the current outbreak (*40*). Feasibility of CP transfusion to treat severe patients was investigated in one pilot study conducted prospectively in China (*41*). The study enrolled ten severe patients confirmed by rRT-PCR-SARS-CoV-2. One dose of 200 mL CP obtained from recently COVID-19 recovered patients with the neutralizing antibody titers above 1:640 was transfused to the patients as an addition to maximal supportive care and antiviral agents. The primary outcome measure was the safety of CP transfusion. The second outcome measures were the clinical improvement and amelioration of laboratory values within 3 days after CP transfusion. The results showed that CP was well tolerated and could increase or maintain the neutralizing antibodies at a high level in a significant manner, leading to the removal of viremia in 7 days. There was improvement in clinical symptoms of the patients as well as other laboratory parameters promptly within 3 days. Radiological examination showed different degrees of absorption of lung lesions within 7 days. Although the results were promising the authors of this study affirmed the necessity of conducting randomized clinical trial.

Furthermore, most recently an uncontrolled case series which included 5 critically ill COVID-19 patients with acute respiratory distress syndrome (ARDS) who met the inclusion criteria were reported in China (*42*). CP-transfusion was given between 10 and 22-day post admission. Clinical symptoms were compared pre and post CP transfusion. The main outcome measures were changes in body temperature, organ failure assessment (SOFA) score, virology parameters, hematological and biochemistry parameters, the ratio of the partial pressure to fraction of inspired oxygen (PAO_2/FIO_2) pre and post CP transfusion. The results showed that following the CP-transfusion, temperature reduced in 4 patients, SOFA score decreased, and PAO_2/FIO_2 increased after 12 days of CP-transfusion. Viral load detected by rRT-PCR-SARS-CoV-2 test became negative and ARDS resolved in 4 patients and specific ELISA and neutralizing antibody titers for SARS-CoV-2 increased on 7-day post transfusion. After 14 days of treatment, 3 out of the 5 patients were withdrawn from mechanical ventilation, hospitals stay for two patients was 37 days then the patients discharge with a stable condition, while three patients have been discharged after (53, 51, and 55 days).

The authors stated that despite the promising effect of CP in those critically ill patients the sample size and the uncontrolled study design were the main limitation for the study to be declared as an affirming therapy therefore this approach necessitate to be evaluated in further randomized control trial to confirm its potential effectiveness for COVID-19.

6. Conclusion

Few clinical evidences are available to be affirmed for

the repurposing of chloroquine, hydroxychloroquine, remdesivir, lopinavir/ritonavir and convalescent plasma as a treatment and/or prophylaxis for COVID-19. Further large scale randomized control clinical studies are needed to assure the use of these agents as antiviral therapy for COVID-19 although *in vitro*, *in vivo*, preclinical trials and safety profile for these old drugs are promising. Medication repurposing may be supported by expert judgments, however clinical use of these drugs in patients with COVID-19 should be clearly justified and should follow the framework of Monitored Emergency Use of Unregistered Interventions (MEURI) or approval of a clinical trials as stated by the World Health Organization.

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Original Article

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Increase of lymphocytes and eosinophils, and decrease of neutrophils at an early stage of anti-PD-1 antibody treatment is a favorable sign for advanced malignant melanoma

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SUMMARY The advent of immune checkpoint inhibitors such as anti-PD-1 antibodies had a striking impact on the treatment for advanced malignant melanoma. However, less than half of the patients benefited from those antibodies, and biomarkers that could sensitively differentiate responders from non-responders are urgently needed. Herein, we explored such biomarkers by retrospectively analyzing clinical data from patients with advanced malignant melanoma treated with nivolumab and pembrolizumab. We found that anti-PD-1 antibody was especially effective for those with metastasis only to soft tissues. Although no significant difference was found in the baseline value of relative neutrophil count (RNC), relative lymphocyte count (RLC), neutrophil to lymphocyte ratio (NLR), and relative eosinophil count (REC) between responders and non-responders, responders after anti-PD-1 therapy revealed the increase of lymphocytes and eosinophils and the decrease of neutrophils within the first 6 weeks of the treatment. We also calculated the change of RNC and RLC 3 weeks and 6 weeks after the initiation of the therapy and designated as NΔ3-LΔ3 and NΔ6-LΔ6 respectively. NΔ3-LΔ3 was significantly decreased in responders, which suggest that the neutrophil decrease and lymphocyte increase after as early as 3 weeks of anti-PD-1 therapy might be a useful clinical indicator. In addition, the difference of NΔ6-LΔ6 between responders and non-responders was even more robust. These data suggest that change of RNC, RLC, and REC together with the combination of NΔ3-LΔ3 and NΔ6-LΔ6 might be a useful tool for early and sensitive biomarkers for anti-PD-1 therapy.

Keywords malignant melanoma, nivolumab, pembrolizumab, relative neutrophil count, relative lymphocyte count, biomarker

1. Introduction

The introduction of anti-PD-1 antibody has dramatically changed the treatment for advanced malignant melanoma. Since the advent of nivolumab in July 2014, nivolumab and pembrolizumab are widely used worldwide for unresectable or metastatic melanomas. Although the clinical effects of anti-PD-1 antibodies are unprecedented, anti-PD-1 antibodies are effective only in about 30% of Japanese patients with advanced malignant melanoma (*1*). Under the present circumstances, there is only limited information regarding which type of patients this treatment protocol is more beneficial. Biomarkers for predicting the therapeutic effects of anti-PD-1 antibodies are still obscure, and being actively studied throughout the world.

Sex might affect the therapeutic effect, and men have longer overall survival (OS) and progression free survival (PFS) than women (*2*). In contrast, age is not known to affect the prognosis. Immune checkpoint inhibitors (ICI) have been reported to prolong OS in both young and elderly patients (cut-off 65-70 years), and no significant difference in the average age was found between responders and non-responderrs (*3*). Elevated lactate dehydrogenase (LDH), elevated C-reactive protein (CRP), and Eastern Cooperative Oncology Group performance status (ECOG PS) ≥ 1 have been reported to shorten OS (*4*), and patients with no metastases other than soft tissue and lung have been reported to fare well (*5*).

One of the promising biomarkers is blood cell count. For example relative eosinophil count (REC) ≥ 1.5 was reported to be associated with good OS (*5*). In addition, reduction of neutrophil to lymphocyte ratio (NLR) 6 weeks after the first dose of anti-PD-1 antibody suggests better responses (*6*), and an increase of 30% or more in NLR within the first two cycles of anti-PD-1 antibody was associated with worsening of OS and reduction of

time to treatment failure (*7*).

To elucidate the factors and biomarkers that could sensitively discriminate responders and non-responders, we conducted a retrospective single-center study of patients with unresectable malignant melanoma.

2. Materials and Methods

2.1. Patient

Sixteen patients with unresectable malignant melanoma (stage III-IV) who received nivolumab or pembrolizumab treatment from February 2015 to March 2020 were enrolled in this study. Twelve patients received nivolumab and 4 patients received pembrolizumab. A patient who discontinued treatment after the first dose and was transferred to another hospital was excluded. Relevant prognostic factors that included age, gender, stage, ECOG PS, presence or absence of BRAF mutation, metastatic site, LDH, CRP, RLC, RNC, NLR, and REC were analyzed to measure the treatment response among those patients. Within 21 days prior to anti-PD-1 antibody administration, patients underwent a blood test as a baseline. LDH, CRP, RNC, RLC, NLR, and REC were measured at 3 weeks and 6 weeks after initiation of treatment, and their time-courses were monitored. Five patients had a blood draw at week 4 instead of week 3. Three patients had blood draws at 7 or 8 weeks instead of week 6. This study has been approved by the Research Ethics Committee of St. Marianna University School of Medicine.

2.2. Treatment and response

Nivolumab was started at 2 mg/kg every 3 weeks in 9 patients. Three of them was later changed to 240 mg/body every 2 weeks. In the other 3 patients, the dose started at 240 mg/body every 2 weeks. Pembrolizumab was started at 2 mg/kg every 3 weeks in 4 patients, and one of them was later changed to 200 mg/body every 3 weeks. Patients were classified as responders (complete response: CR + partial response: PR) or non-responders (stable disease: SD + progressive disease: PD) using Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 at 6 months after anti-PD-1 antibody administration.

2.3. Statistical analysis

Fisher's exact test was used to compare the ratio of nominal variables between two independent groups. Repeated measures analysis of variance was used to compare continuous variables before, 3 weeks after, and 6 weeks after administration. A *t*-test was used to compare continuous variables before and after administration. A *p*-value of 0.05 or less was considered statistically significant. All data was analyzed using EZR (*8*).

3. Results

3.1. Patient characteristics

The characteristics of the 16 patients are shown in Table 1. The average age was 74.6 years (range 51-88 years), with 7 (43.8%) patients aged 75 years or older and 9 (56.3%) younger than 75 years. The gender was 8 males (50%) and 8 females (50%). The stage was 8 in stage 3 (50%) and 8 in stage 4 (50%). The ECOG PS was 11 in PS 0 (68.8%), 4 in PS 1 (25%), and 1 in PS 2 (6.3%). Three (18.8%) had BRAF mutations and 13 (68.8%) did not. Patients with low-cumulative sun damage (CSD) were 6 cases (37.5%), high-CSD were 2 cases (12.5%), and 7 patients were low to no-CSD, of which 4 were acral (25%) and 3 were mucosal (18.8%). The primary site was unknown in 1 patient (6.3%). Ten (62.5%) patients had metastasis of lymph nodes/soft tissue only, while 6 (37.5%) had metastasis to other organs. Nine (56.3%) patients had received prior therapy, including 6 in adjuvant interferon-β (IFN-β) (37.5%), 2 in adjuvant DAV-Feron (dacarbazine, nimustine, vincristine, and interferon-β) (12.5%), and 3 in dabrafenib + trametinib (18.8%), and 1 patient (6.3%) had dacarbazine (including duplicates). Baseline LDH ranged from 121 to 886 (U/L). Five patients (31.3%) exceeded the reference value (115-230), and 11 patients (68.8%) were below the reference value. Baseline CRP ranged from less than 0.03 to 1.32 (mg/dL). Four patients (25%) exceeded the reference value (< 0.3), and 12 (75%) were below the reference value (Table 1).

Table 1. Patient demographics

Factor	Category	*n* (%)
Age	< 75	7 (43.8)
	> 75	9 (56.3)
Gender	Male	8 (50.0)
	Female	8 (50.0)
Stage	III	8 (50.0)
	IV	8 (50.0)
ECOG PS	0	11 (68.8)
	1	4 (25.0)
	2	1 (6.3)
BRAF mutation	presence	3 (18.8)
	absence	13 (68.8)
Primary site	Low-CSD	6 (37.5)
	High-CSD	2 (12.5)
	Low to no-CSD	
	acral	4 (25)
	mucosal	3 (18.8)
	Unknown	1 (6.3)
Site of metastasis	only lymph node and soft tissue	10 (62.5)
	other	6 (37.5)
Prior therapy	adjuvant IFN-β	6 (37.5)
	adjuvant DAV-Feron	2 (12.5)
	Dabrafenib + Trametinib	3 (18.8)
	Dacarbazine	1 (6.3)
Baseline LDH	< 230	5 (31.3)
	> 230	11 (68.8)
Baseline CRP	< 0.3	4 (25)
	> 0.3	12 (75)

3.2. Immune-related adverse events (irAE)

IrAEs of Grade 2 or higher include: hypoadrenocorticism (Grade 2) in 1 patient, hypophysitis (Grade 3) in 1, hypothyroidism (Grade 2) in 3, and type 1 diabetes in 1 (Grade 3), 1 with liver dysfunction (Grade 3), 1 with pancreatitis (Grade 2), 1 with renal dysfunction (Grade 3), and 1 with interstitial pneumonia (Grade 3). Six of the patients had vitiligo and 2 had erythema (Table 2).

3.3. Clinical responses and survival

The response to the anti-PD-1 antibody was 2 in CR, 4 in PR, 1 in SD, and 9 in PD. Six patients (37.5%) were responders (CR + PR), and 10 patients (62.5%) were non-responders (SD + PD) (Table 3).

3.4. Assessment of pretreatment prognostic factors

Patients with lymph node/soft tissue metastases responded better than patients with other organ metastases (p = 0.044). The average age of responders was 77.8 years, and the average age of non-responders was 72.7 years, with no significant difference (p = 0.342). No significant difference in treatment response was found by gender, stage, ECOG PS of 0 or more, and the presence or absence of BRAF gene mutation (p = 1, 0.119, 1, and 1, respectively). There was also no significant difference in treatment response by baseline LDH and baseline CRP (p = 0.588 and 1, respectively). No significant difference was also noted when the patients were divided by baseline RNC, RLC, NLR, and REC (p = 0.683, 0.121, 0.269, and 0.3, respectively) (Table 4).

Table 2. IrAE of Grade 2 or higher

IrAE	Grade	*n*
hypoadrenocorticism	2	1
hypophysitis	3	1
hypothyroidism	2	3
type 1 diabetes	3	1
pancreatitis	2	1
renal dysfunction	3	1
interstitial pneumonia	3	1

Table 3. Clinical responses

	n	Response	*n*
Responder	6 (37.5%)	CR	2 (12.5%)
		PR	4 (25.0%)
Non-responder	10 (62.5%)	SD	1 (6.3%)
		PD	9 (56.3%)

3.5. Changes of biomarkers through the early phase of the treatment

As responders did not show any significant differences in the baseline of various biomarkers, we next examined the sequential changes of these biomarkers before, 3 weeks after, and 6 weeks after anti-PD-1 therapy. Notably, RNC was significantly decreased and RLC was significantly increased in responders compared to non-respoders after 6 weeks (p = 0.024, 0.00038, respectively). Reflecting the increase of RLC and the decrease of RNC, NLR was significantly decreased in responders (p = 0.018) (Figure 1). However, no significant difference was found in LDH and CRP (p = 0.382, 0.265, respectively).

3.6. Difference of NΔ3-LΔ3 and NΔ6-LΔ6 between responders and non-responders

As for RLC and RNC, we noticed the difference between responders and non-responders steadily widened from baseline to week 3, and then to week 6. Therefore, we calculated the change of RNC and RLC values to find more sensitive early biomarkers that could differentiate responders from non-respomders. We designated the value of RNC at week 3 minus its baseline value as NΔ3, and the value of RLC at week 3 minus its baseline value as LΔ3. In a similar manner,

Table 4. Assessment of prognostic factors and early biomarkers before the therapy

Factor	Responder (n = 6)	Non-responder (n = 10)	*P*
Lymph node/soft tissue metastases only[*1]	6	4	0.044
Age	77.8 (64-88)	72.7 (51-83)	0.342
Gender (male)	3	5	1
Stage (IV)	1	7	0.119
ECOG PS (> 0)	2	3	1
BRAF mutation	1	2	1
Baseline LDH (> 230)	1	4	0.588
Baseline CRP (> 0.3)	2	3	1
Baseline RNC[*2]	63.0 (40.5-72.2)	61.1 (49.5-69.1)	0.683
Baseline RLC[*2]	23.7 (19.3-28.0)	27.2 (20.7-35.2)	0.121
Baseline NLR[*2]	2.7 (1.6-3.7)	2.3 (1.4-3.3)	0.269
Baseline REC[*2]	1.77 (0.3-3.4)	1.03 (0.5-2.08)	0.3
REC > 1.75 (6 weeks after administration)[*2]	3	0	0.044

[*1]One case with only local recurrence is not included. [*2]One data is missing.

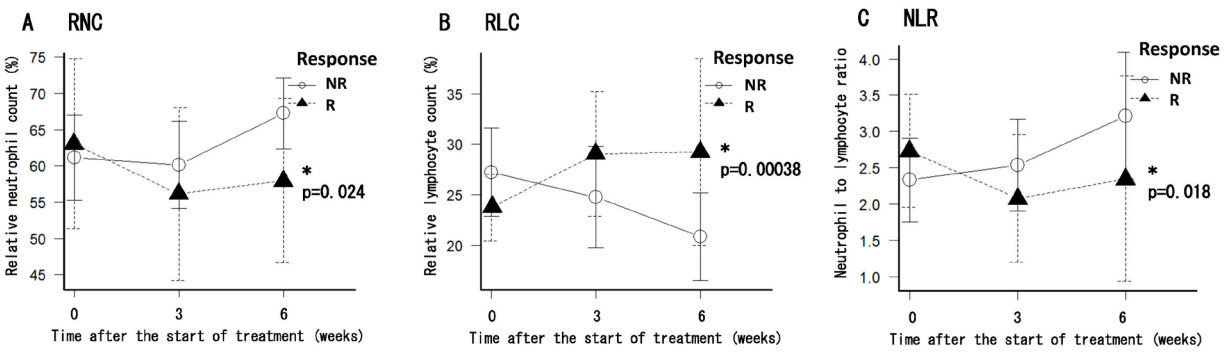

Figure 1. Time course of RNC, RLC, and NLR at 0, 3, and 6 weeks after anti-PD-1 antibody treatment. (A) RNC decreased in responders, and increased in non-responders. The difference between responders, and non-responders was significant at week 6 (p = 0.024). **(B)** RLC increased in responders, whereas it decreased for non-responders. The difference between responders, and non-responders was significant at week 6 (p = 0.00038). **(C)** NLR was significantly decreased in responders (p = 0.018).

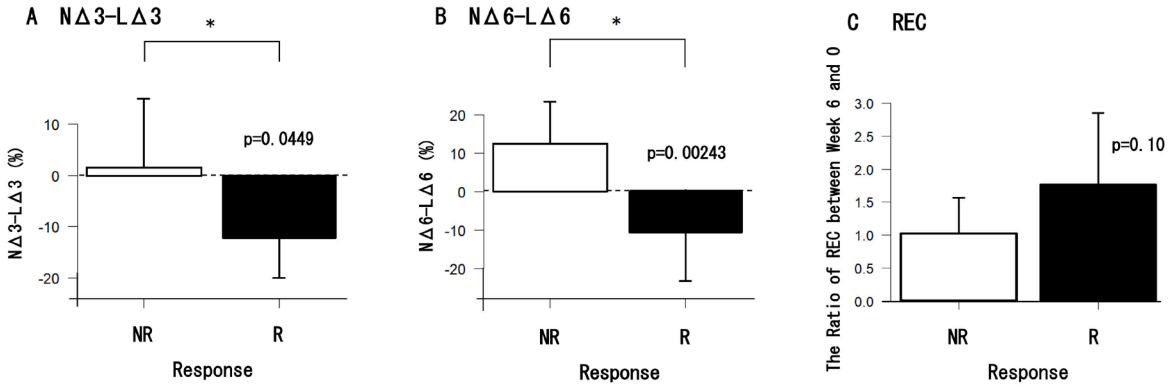

Figure 2. Difference of NΔ3-LΔ3 and NΔ6-LΔ6 between responders and non-responders. (A) The value of NΔ3-LΔ3 at week3 was decreased in responders. The difference between responders, and non-responders was significant (p = 0.044). **(B)** The value of NΔ3-LΔ3 at week6 was decreased in responders and increased in non-responders. The difference between responders, and non-responders was significant (p = 0.00234). **(C)** The ratio of REC between week 6 and its baseline tended to be increased in responders compared to non-responders (p = 0.10).

NΔ6 was designated as the value of RNC at week 6 minus its baseline, and LΔ6 as the RLC at week 6 minus its baseline. We found that NΔ3-LΔ3 was significantly decreased in responders (-12.3%; range from -1.7 to -20.5) compared to non-responders (1.4%; range from -8.9 to 35.1) (p = 0.0449). The change of NΔ6-LΔ6 was even more striking, with -10.6% in responders compared to +12.4% in non-responders (Responders range from 5.5 to -21.8. Non-responders range from -4.4 to 27.9. p = 0.00243) (Figure 2).

Of note, while the change between NΔ3-LΔ3 and NΔ6-LΔ6 was subtle in responders, NΔ6-LΔ6 (+12.4%) was substantially increased compared to NΔ3-LΔ3 (+1.4%) in non-responders. In addition, the ratio of REC between week 6 and its baseline tended to be increased in responders compared to non-responders (p = 0.10). In 3/6 of responders, the value of REC at 6 weeks was more than1.75 times of baseline REC, whereas there were none (0/10) in non-responders (p = 0.044) (Table 4).

These data demonstrate that within 6 weeks of anti-PD-1 treatment, neutrophils tend to decrease and lymphocytes tend to increase in responders, and non-responders show the opposite tendency.

4. Discussion

In the current study, we found that responders after anti-PD-1 therapy revealed the increase of lymphocytes and eosinophils and the decrease of neutrophils within the first 6 weeks of the treatment. We also generated more sensitive clinical indicators, NΔ3-LΔ3 and NΔ6-LΔ6, that discriminate between responders and non-responders. NΔ3-LΔ3 was significantly decreased in responders, which suggest that the neutrophil decrease and lymphocyte increase after as early as 3weeks of anti-PD-1 therapy might be a useful clinical indicator. In addition, the difference of NΔ6-LΔ6 between responders and non-responders was even more robust. These data suggest that the combination of NΔ3-LΔ3 and NΔ6-LΔ6 might be a useful tool for early (NΔ3-LΔ3) and sensitive (NΔ6-LΔ6) dection of anti-PD-1 efficacy.

Several predictive factors for better ICI responses have recently been reported, such as sex, age,

performance status, and site of metastasis. In our study, no significant difference was found in terms of age, sex, and performance status between responders and non-responders. As for the site of metastasis, metastasis to soft tissue alone was associated with good response as previously reported (*5*). Therefore, it is preferable that ICI could be administered before metastasizing to distant organs.

LDH and CRP has been reported as useful biomarkers (*4*). However, we could not find significant difference between responders and non-responders. Instead, we could confirm the usefulness of REC, and the increase of REC at 6 weeks suggested the better response.

The hallmark of current studty is to show the usefulness of NΔ3-LΔ3 and NΔ6-LΔ6. Although the baseline NLR exceeding 2.2-5 indicated the sign of poor prognosis in previous studies (*6,7,9*), we could not detect significant difference regarding the baseline NLR. However, the increase of RLC, and the decrease of RNC and NLR during the course of anti-PD-1 therapy were associated with better treatment response. In previous studies, the reduction of NLR at week 6 suggested the better response (*6*), and the increse of NLR within the first two cycles of anti-PD-1 antibody indicated worsening of OS (*7*). We utilize this notion and calculated NΔ3-LΔ3 and NΔ6-LΔ6. NΔ3-LΔ3 reflects the change of neutrophils and lymphocytes at week 3 and could be used as an early biomarker, and NΔ6-LΔ6 reflects the change at week 6 and could be used as a sensitive biomarker to judge the effect. Although this formula was somewhat complicated and this retrospective study consisted of only limited number of patients, NΔ3-LΔ3 and NΔ6-LΔ6 could be useful to estimate the therapeutic effect of anti-PD-1 antibodies at an earlier stage.

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Original Article

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High-dose vitamin B supplementation for persistent visual deficit in multiple sclerosis: a pilot study

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SUMMARY The aim of this study is to investigate the potential neuroprotective effect of high-doses vitamins B1, B6 and B12 in patients with relapsing-remitting multiple sclerosis (RRMS) and persistent visual loss after acute optic neuritis (AON). Sixteen patients (20 eyes) diagnosed with RRMS and visual permanent disability following AON were enrolled for the present open, pilot study. Each patient was treated with oral high-doses 300 mg of vitamin B1, 450 mg of vitamin B6 and 1,500 mcg of vitamin B12, as add-on treatment to concomitant disease-modifying therapies (DMTs) for consecutive 90 days. Outcome measures were to determine changes from baseline to month three in visual acuity (VA) and visual field (VF) testing, with correlations with clinical parameters. Logistical regression was performed to evaluate predictors of final VA. A statistically significant improvement was registered in visual acuity ($p = 0.002$) and foveal sensitivity threshold (FT) ($p = 0.006$) at follow-up compared to baseline. A similar trend was demonstrated for mean deviation (MD) ($p < 0.0001$), and pattern standard deviation (PSD) ($p < 0.0001$). Age at the time of inclusion was positively correlated with latency time (rho = 0.47, $p = 0.03$), while showing a negative correlation with visual acuity (rho = – 0.45, $p = 0.04$) and foveal sensitivity threshold (rho = – 0.6, $p = 0.005$) at follow up. A statistically significant correlation was demonstrated between foveal sensitivity threshold and visual acuity at baseline (rho = 0.79, $p < 0.0001$). In a linear regression model, the main predictor of visual acuity at follow up was the foveal sensitivity threshold (B = 1.39; $p < 0.0001$). Supplemental high-dose vitamins B1, B6 and B12 resulted as effective therapy to improve visual function parameters in MS-related visual persistent disability.

Keywords B Vitamins group, visual function, neuroprotection

1. Introduction

Multiple sclerosis (MS) is an autoimmune neurodegenerative disorder associated with chronic inflammation of the central nervous systems (CNS), resulting in characteristic demyelination and axonal damage. With an increased incidence between 20 and 40 years, it represents the most common acquired disabling neurological disease in young adults, and females appear to be more frequently diagnosed than males by 2-3:1 ratio. MS affects approximately 2.5 million people worldwide, with a tendency of prevalence data to considerably differ from one country to another, and registered highest percentages in North America (140 per 100.000) and Europe (108 per 100.000) (*1-3*). Although the etiopathogenesis is still unclear, genetic profiles predominantly involving immune response genes (*HLA-DRB1, HLA-DPB1, HLA-A TNF* genes), as well as inflammatory and environmental factors including Epstein-Barr virus infection, tobacco exposure, vitamin deficiencies and high saturated fat/carbohydrate diet are currently recognized among the most relevant causative agents (*4-9*).

The main efforts in MS therapy are primarily directed at developing immunomodulatory and immunosuppressive agents for reducing the frequency of new episodes, however, the persistence of symptoms in most patients, drugs side effects and limited long-term efficacy emphasized the relevance for complementary treatments.

The B vitamins are known to effectively modulate the repair and maintenance of lipids, including myelin, and to protect against axonal hypoxia through enhanced ATP production. Of note, their deficiency appear to have detrimental effects in terms of occurrence and progression of multiple sclerosis (*10-14*). In recent years, B vitamins supplementation has attracted growing interest as far as incidence, progression and potential

reversal in MS, demonstrating improved neurological conditions even in absence of documented deficiency (*15-23*). To date, limited clinical studies included sensitive measures of visual function to evaluate vitamin B efficacy at restoring neurological damage in patients with MS (*21-23*). High-dose vitamin B12 effectively improved visual evoked potentials (VEP) as evaluated in 6 patients with chronic progressive MS on concomitant disease modifying therapies (DMTs) (*21*). Unlike favorable preliminary results, no significant improvement in visual acuity was indeed observed in a recent randomized controlled trial on high-dose biotin in patients suffering from progressive MS-related visual disability on DMTs (*22,23*). Thus, the re-myelinating effects of B vitamins are somewhat promising for irreversible optic nerve damage in MS, but currently lack of sufficient insight and decisive results.

Moreover, in spite of the reported neurologic synergistic effects of B1 (thiamine), B6 (pyridoxine), and B12 (cyanocobalamin) (*24-27*), there is no available data on their concomitant use in MS. Furthermore, there is no literature evaluating visual function in relapsing-remitting MS type (RRMS) on B vitamins supplementation.

The present study was designed to investigate the efficacy of three months-treatment with high-dose vitamins B1, B6, and B12 in combination, as adjunctive to immunomodulatory therapy in eligible patients diagnosed with RRMS, to improve visual function parameters in persistent visual loss following acute optic neuritis (AON).

2. Materials and Methods

A pilot study was carried out on 16 consecutive patients (20 eyes) (13 F/3 M), mean age 36.31 years ± 7.34 standard deviation (SD), with a diagnosis of relapsing-remitting (RR) MS according to the 2017 revised McDonald criteria, and presenting with a history of one or more previous episodes of AON and persistent impairment in visual symptoms and visual field testing (*28*). Patients were enrolled from May 2019 to October 2019 at the University of Rome 'Sapienza', Umberto I Hospital, Italy.

Patients were all stable on disease modifying therapies (interferon-β-1b, glatiramer acetate, fumarate, and teriflunomide) at the time of inclusion, and in course medications were allowed throughout the study.

Exclusion criteria were: (1) previous diagnosis of glaucoma and any other optic neuropathy; (2) poor reliability indices of visual field tests (scores exceeding 20% of fixation losses or false negative/false positive errors); (3) any other previous or coexisting ocular condition that could interfere with functional outcomes including *i.e.* media opacities, amblyopia, uveitis, congenital ocular malformations, retinal and macular diseases, refractive defects greater than ±6 D (spherical equivalent); (4) patients who had undergone intraocular surgery, other than cataract surgery, more than the preceding 6 months; (5) bilateral VA ≤ 1/20; (6) relapse (among which acute optic neuritis) occurred less than 6 months before inclusion; (7) unstable VA in the 6 months prior to inclusion; (8) treatment for MS introduced < 3 months prior to inclusion.

Each patient underwent a comprehensive ophthalmological examination at baseline and at scheduled 3-months follow-up time, including clinical history, measurement of the best-corrected visual acuity by using the standardized, 70-letter Early Treatment Diabetic Retinopathy Study chart (Chart 'R', Precision Vision, La Salle, IL, USA) at 4 m distance, evaluation of intraocular pressure by using the Goldmann applanation tonometry, slit-lamp biomicroscopy, mydriatic indirect fundus biomicroscopy, and automated perimetry with the Humphrey Field Analyzer (Carl Zeiss Meditec, Dublin, CA, USA) and Swedish Interactive Threshold Algorithms standard strategy (program 30-2). The parameters evaluated on visual field testing were mean deviation (MD, dB), pattern standard deviation (PSD, dB) and foveal sensitivity threshold (FS, dB). Two consecutive visual field tests were carried out for each patient, and only the second was considered for the aim of the present study. The examination of patients, along with the interpretation of instrumental results, was performed by two independent experienced ophthalmologists. Acquisition of instrumental data was conducted by certified optometrists. The time elapsing between the last episode of acute optic neuritis to the time of inclusion was calculated for each patient and expressed as 'latency time'.

All patients were treated with oral high-doses of 300 mg vitamin B1 (thiamine), 450 mg vitamin B6 (pyridoxine) and 1500 mcg vitamin B12 (cyanocobalamin) administered as three capsules daily for 90 consecutive days. Employed dosages appear as safe for short-term periods as per current knowledge (*29-31*). No additional drugs other than ongoing immunomodulatory agents and vitamin B complex were introduced during the period of treatment.

The study was approved by the Ethics Committee of the Sapienza University of Rome. The study followed the tenets of the Declaration of Helsinki and all patients signed informed consent.

3. Results

Data were collected on a Microsoft Office Excel worksheet. The normality of data was assessed by Shapiro-Wilk test. Correlations were assessed by using calculation Spearman's rho. Differences between unpaired variables were assessed through Mann-Whitney test, while differences between paired variables were performed by Wilcoxon test. A linear regression model to evaluate predictors of visual acuity at follow-

up was produced. A value of $p \leq 0.05$ was considered statistically significant. Intraobserver and interobserver agreement was evaluated with the K Cohen Coefficient. The statistical analysis was performed using Graph Pad vers. 8.0.2 and SPSS vers. 24 on the Windows 10 Home edition platform. None of the enrolled patients experienced any serious adverse event during the whole follow-up period. Results from all visual field examinations performed on the affected eye at baseline were abnormal, and most consisted of central visual field defects, according to current literature. In detail, central or cecocentral-type scotomas were detected in the majority of patients (12 patients, 14 eyes), whereas non-central scotomas such as paracentral and arcuate defects, were identified in 4 patients and 6 eyes.

There was a statistically significant improvement of visual acuity (44.8 ± 11.7 *vs.* 50.3 ± 12.4; $p = 0.002$) and foveal sensitivity threshold (FT) (28.2 ± 7.4 *vs.* 30.6 ± 6.2; $p = 0.006$) at follow-up (T1) if compared to baseline (Figures 1 and 2). Similarly, there was a statistical significant reduction of mean deviation (MD) (– 7.12 ± 4.9 *vs.* – 5.45 ± 4.3; $p < 0.0001$), and PSD (5.78 ± 3.5 *vs.* 3.88 ± 2.5; $p < 0.0001$) at follow-up in comparison to baseline (Figures 3 and 4). Age at the time of inclusion showed a statistically significant correlation with the latency time (rho = 0.47, $p = 0.03$), with visual acuity (rho = – 0.45, $p = 0.04$) and with foveal sensitivity threshold (rho = – 0.6, $p = 0.005$) at follow up. A positive correlation was demonstrated between foveal sensitivity threshold and VA at baseline (rho = 0.79, $p < 0.0001$).

The results of the bivariate correlations are shown in Table 1. In a linear regression model, the main predictor of visual acuity at follow up was the foveal sensitivity threshold, which retained a statistically significant positive association (B = 1.39; CI (95%) = 0.93-1.85; $p < 0.0001$). The intraobserver and interobserver agreement in clinical and instrumental assessment was excellent (Kappa = 0.92).

4. Discussion

Our results, based on clinical and automated perimetry data, demonstrated that daily administration of high doses vitamin B1 (thiamine), vitamin B6 (pyridoxine) and vitamin B12 (cyanocobalamin) for consecutive 90 days significantly improved visual acuity, FT, MD and PSD in patients with RRMS and visual permanent disability following documented episodes of AON. The disease generally manifests itself thorough time-varying attacks of neurological dysfunction with sensory, motor, autonomic, cognitive, or neuropsychiatric symptoms, followed by partial or complete recovery (*32,33*). AON is an inflammatory and demyelinating disorder of the optic nerve that occurs in up to 50% of patients with MS, and distinctively presents itself as an acute monocular loss of vision, though it can possibly show bilateral simultaneous or sequential involvement (*34-36*).

The pivotal field defect in AON is a widespread depression of sensitivity, and visual field testing typically reveals a central scotoma, although bitemporal hemianopia, paracentral scotoma, arcuate and altitudinal deficits have also been reported. Vision

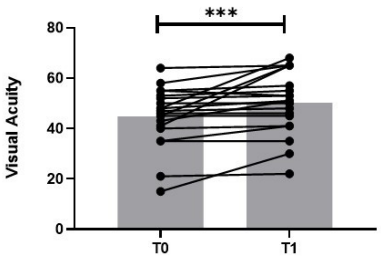

Figure 1. Comparison between visual acuity of enrolled subjects at baseline and follow-up. $^{***}p = 0.002$.

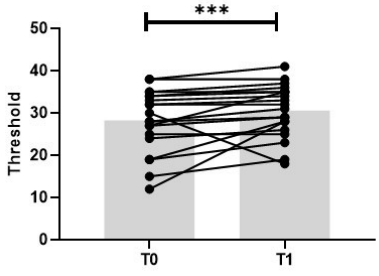

Figure 2. Comparison between foveal threshold of enrolled subjects at baseline and follow-up. $^{***}p = 0.006$.

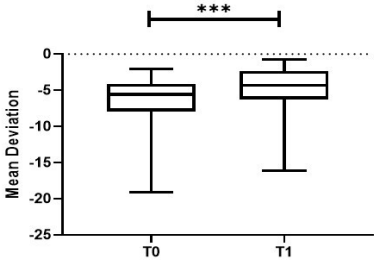

Figure 3. Comparison between mean deviation at baseline and follow-up. $^{***}p < 0.001$.

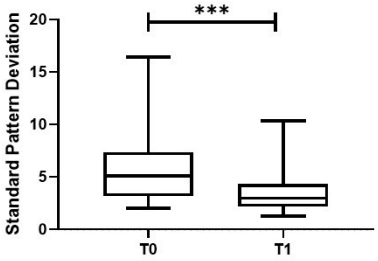

Figure 4. Comparison between pattern standard deviation at baseline and follow-up. $^{***}p < 0{,}001$.

Table 1. Spearman's rho correlation

Items	Age	Latency Time	VA0	VA1	FT0	FT1	MD0	MD1	PSD0	PSD1
Spearman's rho										
Age										
Correlation Coefficent	1.000	0.473*	-0.250	-0.452*	-0.522*	-0.600**	0.121	-0.126	0.112	0.320
Sig.2 (2-tailed)		0.035	0.289	0.045	0.018	0.005	0.612	0.596	0.639	0.169
N	20	20	20	20	20	20	20	20	20	20
Latency Time										
Correlation Coefficent	0.473*	1.000	-0.234	-0.350	-0.343	-0.543*	0.005	-0.212	0.277	0.460*
Sig.2 (2-tailed)	0.035		0.320	0.130	0.130	0.013	0.982	0.371	0.237	0.041
N	20	20	20	20	20	20	20	20	20	20
VA0										
Correlation Coefficent	-0.250	-0.234	1.000	0.706**	0.791**	0.495*	0.248	0.210	-0.340	-0.370
Sig.2 (2-tailed)	0.289	0.320		0.001	0.000	0.026	0.291	0.374	0.164	0.109
N	20	20	20	20	20	20	20	20	20	20
VA1										
Correlation Coefficent	-0.452*	-0.350	0.706**	1.000	0.791**	0.756**	0.413	0.540*	-0.288	-0.573**
Sig.2 (2-tailed)	0.045	0.130	0.001		0.000	0.000	0.070	0.014	0.218	0.008
N	20	20	20	20	20	20	20	20	20	20
FT0										
Correlation Coefficent	-0.522*	-0.343	0.791**	0.791**	1.000	0.838**	0.271	0.374	-0.405	-0.648**
Sig.2 (2-tailed)	0.018	0.130	0.000	0.000		0.000	0.247	0.104	0.077	0.002
N	20	20	20	20	20	20	20	20	20	20
FT1										
Correlation Coefficent	-0.600**	-0.543*	0.495*	0.756**	0.838**	1.000	0.122	0.344	-0.325	-0.707**
Sig.2 (2-tailed)	0.005	0.013	0.026	0.000	0.000		0.608	0.138	0.162	0.000
N	20	20	20	20	20	20	20	20	20	20
MD0										
Correlation Coefficent	0.121	0.005	0.248	0.413	0.271	0.122	1.000	0.851**	-0.558*	-0.432
Sig.2 (2-tailed)	0.612	0.982	0.291	0.070	0.247	0.608		0.000	0.011	0.057
N	20	20	20	20	20	20	20	20	20	20
MD1										
Correlation Coefficent	-0.126	-0.212	0.210	0.540*	0.374	0.344	0.851**	1.000	-0.463*	-0.551*
Sig.2 (2-tailed)	0.596	0.371	0.374	0.014	0.104	0.138	0.000		0.040	0.012
N	20	20	20	20	20	20	20	20	20	20
PSD0										
Correlation Coefficent	0.112	0.277	-0.340	-0.288	-0.405	-0.325	-0.558*	-0.463*	1.000	0.752**
Sig.2 (2-tailed)	0.639	0.237	0.164	0.218	0.077	0.162	0.011	0.040		0.000
N	20	20	20	20	20	20	20	20	20	20
PSD1										
Correlation Coefficent	0.320	0.460*	-0.370	-0.573**	-0.648**	-0.707**	-0.432	-0.551*	0.752**	1.000
Sig.2 (2-tailed)	0.169	0.041	0.109	0.008	0.002	0.000	0.057	0.012	0.000	
N	20	20	20	20	20	20	20	20	20	20

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed). VA: Visual Acuity, FT: Foveal Sensitivity Threshold, MD: Mean Deviation, PSD: Pattern Standard Deviation.

loss typically extends over hours or days, and peaks within 1 or 2 weeks. The maximum recovery after the peak is usually reached within the first 3 months and rarely continues beyond 6 months following a relapse (*34,35,37*). Currently, MS treatment only relies on immunomodulatory therapeutics aimed at reducing the frequency of new episodes, and the introduction of complementary neuroprotective approaches directly acting on neuronal damage still remains challenging. In recent years, vitamins and dietary supplements have attracted growing interest as far as incidence, progression and potential reversal in MS (*15-23,38-40*).

The B vitamins refer to a biochemically heterogeneous group of water soluble molecules: B1 (thiamine), B2 (riboflavin), B3 (nicotinamide), B5 (pantothenic acid), B6 (pyridoxine), B7 (biotin), B9 (folic acid), B12 (cyanocobalamin), required as cofactors for the metabolism of fatty acids, amino acids, neurotransmitters, myelin, and nucleic acids, and for providing energy to support neurons in such mechanisms. In particular, vitamin B1, B6, and B12 have demonstrated to considerably contribute to neuro-immune homeostasis and appeared to be firmly involved in MS pathogenesis (*41*). Intriguingly, vitamin B12 deficiency and MS share similarities in terms of clinical presentation, including megaloblastic anemia or macrocytosis, and MRI findings (*38, 42*).

A synergistic effect of vitamins B1, B6, and B12 for improving neurologic functions has been demonstrated based on overlap in several biochemical pathways (*24-27,41*). Vitamin B12 operates as a cofactor for methylmalonyl coenzyme A (CoA) mutase (MCM), an enzyme that catalyzes the reversible isomerization of l-methylmalonyl-CoA to succinyl-CoA. If MCM turns

out to be defective, it occurs an upstream accumulation of methylmalonyl CoA and its precursor, propionyl CoA, with subsequent propionyl CoA replacing succinyl CoA in the Krebs cycle. As a result, effective long-chain lipid synthesis is hindered, and aberrant short lipid chains end up constituting the myelin sheath (*41*). In addition, vitamin B12 exerts a decisive role in the homocysteine remethylation process to methionine within folate metabolism, and its deficiency is thereby associated with reduced synthesis of phospholipids and increased levels of serum homocysteine. In accordance, increased plasma homocysteine levels in MS patients have been reported (*43,44*). Moreover, homocysteine can be removed by conversion to cysteine (amino-acid having role in myelin formation) with vitamin B6 as a cofactor, this underlying the strict interplay between B6 and B12 vitamins (*45*). Furthermore, vitamin B6 serves as a cofactor in sphingolipid and neurotransmitter synthesis and in free radicals scavenging activity (*46,47*). Similarly, studies suggest that vitamin B1 is involved in the synthesis of myelin, nucleic acids, and several types of neurotransmitters, and shows antioxidant activity (*41*). B12 in addition to interferon-beta improved demyelination and reduced astrocytosis, resulting in near normal motor function in experimental autoimmune encephalomyelitis (EAE) mice (*15*). Similar results were achieved with B12 supplementation in combination with paclitaxel in EAE animals (*16*). Intramuscular B12 administration for 24 weeks in 138 patients with MS resulted in improvement by 2 Guy's neurological disability scale (GNDS) points in a randomized placebo controlled, double blind study (*19*). In a pilot study on 15 patients with MS in remitting phase experiencing fatigue, high doses thiamine resulted in fatigue regression despite normal blood thiamine levels (*17*). Despite mentioned encouraging results, a few studies investigated visual function parameters as manageable model for evaluating the potential neuroprotective effects of B vitamins in MS. It has been estimated that one-third of MS patients show persistent visual symptoms after AON, with significant reduction in quality of life (*48*). The effects of massive vitamin B12 supplementation (oral 60 mg/day for 6 months and intravenous 5 mg vitamin B 12 every day for 14 days before oral administration in 3 patients) in 6 patients with progressive MS on either low dose prednisolone or azathioprine, resulted in improved visual and brainstem auditory evoked potentials at 3 and 6 months follow-up, with bilateral re-appearance of P100 wave after treatment in one case (*21*). None of the patients had low serum vitamin B12 levels when entering the study and no one experienced any adverse effect. An open-label pilot study based on high-dose biotin administration (oral 300 mg/day for a mean duration of 9.2 months) in progressive MS, showed encouraging preliminary results, inclusive of visual function assessment, in terms of reversing disease progression and reducing chronic disability (*22*). However, in a recent randomized 6-months controlled trial, high-dose biotin did not significantly improve visual acuity in MS-related chronic visual loss compared to placebo, with 70.7% of patients on concomitant DMTs (*23*). We reported a statistically significant improvement of visual acuity ($p = 0.002$) and foveal sensitivity threshold ($p = 0.006$) at 3-months follow-up time (T1) compared to baseline (T0). Similarly, a significant reduction was observed for MD ($p < 0.0001$), and PSD ($p < 0.0001$) at follow-up, in comparison to baseline. Interestingly, age at the time of inclusion showed a statistically significant correlation with the latency time (rho = 0.47, $p = 0.03$) at follow up. This could be explained by the fact that patients younger at onset have demonstrated to experience larger number of relapses before progression, in accordance with literature (*49*). A positive correlation was demonstrated between foveal sensitivity threshold and VA at baseline (rho = 0.79, $p < 0.0001$), and it appears as interesting information since there are described cases with FT decline and relatively good visual acuity in the context of subclinical optic neuritis or cases with good FT measurements but very reduced vision as per involvement of factors other than optic neuropathy (*50*). Linear regression analysis showed that baseline foveal sensitivity threshold was the only variable significantly correlated with final visual acuity, with a statistically significant positive association (B = 1.39; CI (95%) = 0.93-1.85; $p < 0.0001$). We speculate that the relevance of foveal threshold for prediction of visual outcome could be related to the frequent involvement of central visual field in MS patients, and accordingly in our sample.

In conclusion, the current work indicates the possible effectiveness of high-dose B1, B6 and B12 vitamins in patients with MS-related visual permanent disability. The favorable findings from our open, pilot study might open new perspectives for reaching placebo-controlled randomized trials based on B vitamins, and particularly B1, B6 and B12 in combination, as complementary therapeutic against RRMS visual impairment. The results so far presented on vitamin B12 and biotin in progressive MS then, could allow for extending the investigation to progressive forms of disease. An insight on biochemical pathways through which B vitamins promote re-myelination and inhibit inflammation in neurodegenerative diseases including MS, clearly indicates their potential to influence the course of disease by improving and even reversing neurological fixed and disabling damage. Therefore, novel neuroprotective approaches are expected to support existing relapse-preventative strategies in MS, with regard to optic nerve axonal and neuronal degeneration and related impairment in visual quality of life.

Ethics Approval and Consent to Participate:
All procedures performed in studies involving human participants were in accordance with the ethical

standards of the institutional research committee of Sapienza University of Rome and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Consent for publication:
Informed consent to publish personal or clinical details along with any identifying images was obtained from study patients.

Data Availability Statement:
The data used to support the findings of this study are available from the corresponding author upon request.

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Original Article

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Comparative efficacy and safety of Verbascox® – a proprietary herbal extract capable of inhibiting human cyclooxygenase-2 – and celecoxib for knee osteoarthritis

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SUMMARY The aim of this randomized, single-blind, active-controlled pilot study was to investigate the clinical efficacy of oral supplementation with Verbascox®, a proprietary herbal extract capable of inhibiting human cyclooxygenase-2 (COX-2), in patients with mild-to-moderate osteoarthritis (OA) of the knee. Patients in the control group (n = 50) did not undergo any treatment (watchful waiting). Patients in the Verbascox® group (n = 50) received oral supplementation (800 mg/day) with the herbal extract for 2 weeks. The final study group consisted of patients (n = 50) who received celecoxib, a known pharmacological inhibitor of COX-2, 200 mg/day for 2 weeks. Examining physicians and laboratory personnel were blinded to group assignment, whereas patients were unblinded. All participants were evaluated using standard measures of pain relief and improvement in functional capacity at baseline, after 1 week, and at the end of the 2-week treatment course. Moreover, serum levels of substance P (SP), a member of the tachykinin family of neuropeptides involved in pain perception, were measured at the three time points. Both Verbascox® and celecoxib reduced pain, improved functional capacity, and lowered serum SP levels at 2 weeks compared with baseline, without significant inter-arm differences. Both Verbascox® and celecoxib showed a limited number of treatment-emergent adverse events. In summary, oral supplementation with Verbascox® (800 mg/day) in patients with mild-to-moderate OA of the knee is as effective and safe as a standard therapeutic dose of celecoxib in terms of pain relief and improvement in functional capacity after a 2-week treatment course.

Keywords phytomedicine, pain, functional capacity

1. Introduction

The knee is the most common joint localization of symptomatic osteoarthritis (OA) (*1*). Knee OA, affecting more than 250 million people worldwide, has significant effects on patient function and considerable societal costs in terms of morbidity (*e.g.*, work loss and joint replacement) (*2*). The results of the OA process are cartilage degradation and synovial inflammation; these features are associated with the development of symptoms of pain, stiffness, and functional disability (*1,2*). In the current paradigm, the structural changes represent the disease, whereas the symptoms of aching, discomfort, pain, and stiffness are the reasons whereby patients seek medical care (*3*).

Current treatment of OA is based on symptom management, primarily pain control (*4*). Clinical guidelines recommend the use of oral non-steroidal anti-inflammatory drugs (NSAIDs) in patients with persistent symptoms (*5*). Although conventional NSAIDs are the most frequently prescribed medicines for OA, they are characterized by numerous potential adverse effects including gastrointestinal bleeding, cardiovascular side effects, and risk of nephrotoxicity (*6*). To overcome these issues, the use of selective cyclooxygenase-2 (COX-2) inhibitors (coxibs) – which offer the advantage of an anti-inflammatory and analgesic activity similar to that of conventional nonselective NSAIDs but with a more favorable profile in terms of adverse event – has gained momentum (*7,8*). Celecoxib is a selective NSAID indicated for the treatment of the signs and symptoms associated with OA (*9*). Its efficacy in relieving pain

and inflammation and improving physical function in patients with OA has been established (*10*), and it has a better gastrointestinal tolerability profile compared with nonselective NSAIDs (*11*).

Recent years have witnessed a growing interest in natural compounds as promising alternatives to synthetic COX-2 inhibitors (*12,13*). We have previously shown that Verbascox®, a proprietary herbal extract from *Lippia citriodora* and *Plantago lanceolata* titred in verbascoside (a natural polyphenol known for the high antioxidant power; ≥ 5%) and aucubin (a naturally occurring iridoid glycoside; ≥ 2%), inhibits LPS-stimulated expressions of COX-2 in human neutrophils in a dose-dependent fashion (*14*). Based on its *in vitro* activity, we hypothesized that Verbascox® may exert significant anti-inflammatory and analgesic effects by acting as a specific non-pharmacological COX-2 inhibitor.

Here, we sought to investigate the clinical usefulness of oral supplementation with Verbascox® in patients with mild-to-moderate OA of the knee. The primary aim of this pilot study was to compare the magnitude of pain relief and improvement in functional capacity of patients who received oral supplementation with Verbascox® compared with those who were treated with celecoxib, a known pharmacological inhibitor of COX-2 (*9*). We also measured serum levels of substance P (SP), a member of the tachykinin family of neuropeptides involved in pain perception (*15*), as a biochemical marker of treatment response in the study participants.

2. Materials and Methods

2.1. Patients

This research was a randomized, single-blind, active-controlled pilot study of 150 consecutive patients (117 women and 33 men). Patients who were 40-75 years of age with a clinical diagnosis of OA of the knee according to the American College of Rheumatology criteria (*16*) were eligible. Inclusion criteria were as follows: 1) presence of symptoms for at least one month; 2) number of leukocytes in synovial fluid < 2,000/mL; 3) pain on a visual analog scale (VAS) ≥ 2 at rest; and 4) duration of stiffness in the morning ≤ 30 min. The following exclusion criteria were applied: 1) pregnancy or breastfeeding; 2) positive history for major renal, hepatic, cardiac, gastrointestinal, or hematologic disorders; 3) presence of malignancies; 4) presence of neurologic or psychiatric disorders; 5) atopy or allergic disorders; 6) diabetes mellitus or other endocrine disorders; 7) coagulation disturbances; 8) positive history for peptic ulcer; 9) use of corticosteroids in the four weeks preceding the study; and 10) use of NSAIDs in the two weeks preceding the study. According to the Kellgren-Lawrence Grading System (*17*), 61.3% and 38.7% of the study patients had a score of 1 and 2, respectively. The patients were therefore classified as having mild-to-moderate OA of the knee. The study protocol complied with the tenets of the Declaration of Helsinki and was approved by the local ethics committee (approval number E04/18). Before the study, each patient was informed about the purpose of the study and signed informed consents were obtained.

2.2. Materials

Verbascox® was supplied by LaBiotre srl (Tavarnelle Val di Pesa, Italy). The oral supplement tested in this study was in tablet form containing 800 mg of the proprietary extract. Celecoxib 200 mg oral capsules were from Pfizer (New York, NY, USA).

2.3. Procedures

The study period was two weeks. At baseline, body mass index (BMI) and duration of pain were collected from all participants. Patients (n = 150) were randomly divided into three study groups (1:1:1 ratio). The random allocation sequence was generated by a computer program. Patients in the control group (n = 50) did not undergo any treatment (watchful waiting). Patients in the Verbascox® group (n = 50) received oral supplementation (800 mg/day; one tablet) with the herbal extract for 2 weeks. The final study group consisted of patients (n = 50) who received celecoxib (200 mg/day; one capsule) for 2 weeks. Examining physicians and laboratory personnel were blinded to group assignment, whereas the study patients were unblinded. All participants were asked to suspend other treatments during the study course. A total of three assessments were performed (baseline, at one week, and at the end of the 2-week treatment course).

2.4. Clinical endpoints

The clinical endpoints included: 1) patient's assessment of arthritis pain score (VAS) at rest (range: 0-10; where 0 is no pain and 10 is worst pain), 2) patient's assessment of arthritis pain score (VAS) upon movement (range: 0-10; where 0 is no pain and 10 is worst pain), 3) range of motion (degrees), and 4) the Western Ontario and McMaster Universities (WOMAC) index score (*18*). The WOMAC index scores consists of subscales that measure pain (range: 0-20; where 0 is no pain and 20 is worst pain), stiffness (range: 0-8, where 0 is no stiffness and 8 is worst stiffness), and physical functioning (range: 0-68, where 0 is best functioning and 68 is worst functioning). The resulting total composite score ranges from 0 to 96 (*19*).

2.5. Measurements of serum substance P levels

Venous blood samples were collected in serum separator tubes at each assessment. Blood was allowed to clot

Table 1. Baseline characteristics of the three study groups

Items	No treatment (n = 50)	Supplementation with Verbascox® 800 mg/day (n = 50)	Treatment with celecoxib 200 mg/day (n = 50)	p
Age, years	57.1 ± 5.3	56.8 ± 5.6	57.4 ± 5.5	0.31
Women/men	39/11	40/10	38/12	0.89
Body mass index, kg/m^2	25.7 ± 2.4	25.8 ± 2.2	26.0 ± 2.5	0.48
Pain duration, months	3.1 ± 0.4	2.9 ± 0.6	3.2 ± 0.5	0.51
Pain at rest, VAS (0-10)	2.6 ± 0.3	2.7 ± 0.4	2.6 ± 0.4	0.59
Pain on movement, VAS (0-10)	3.7 ± 0.6	3.9 ± 0.7	3.8 ± 0.6	0.79
Range of motion (degrees)	133 ± 10	132 ± 9	134 ± 10	0.81
WOMAC scores				
Pain	5.6 ± 0.6	5.8 ± 0.8	5.7 ± 0.8	0.73
Stiffness	1.4 ± 0.3	1.5 ± 0.4	1.4 ± 0.3	0.68
Physical function	20.1 ± 2.4	20.9 ± 3.6	20.5 ± 3.2	0.75
Total	27.1 ± 3.3	28.2 ± 4.0	27.6 ± 3.8	0.59

Data are expressed as means and standard deviations or as counts. Abbreviations: VAS, visual analogue scale; WOMAC, Western Ontario and McMaster Universities.

at room temperature for 30 min and then centrifuged at 1,000 × g for 15 min. Serum was removed and aliquots were kept frozen at −80°C until measurements. Serum SP levels were assayed using an enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's protocol (R&D Systems, Minneapolis, MN, USA). Absorbance at 450 nm was measured on an ELISA plate reader (PerkinElmer, Waltham, MA, USA). The detection limit of this assay was 25 pg/mL, and intra- and interassay coefficients of variation were 8% and 13%, respectively. All samples were processed simultaneously at the end of the study by laboratory personnel blinded to clinical data.

2.6. Safety

Safety was assessed by recording treatment-emergent adverse events and changes from baseline in clinical laboratory tests, vital signs, and physical examinations, all of which were administered at visits on weeks 1 and 2. Clinically relevant changes in laboratory values were defined as: aspartate aminotransferase (AST) and/or alanine transaminase (ALT) ≥ 3 × upper limit of normal (ULN), creatinine ≥ 1.3 × ULN, blood urea nitrogen (BUN) ≥ 2 × ULN, hematocrit decrease ≥ 5 percentage points from baseline, and hemoglobin decrease ≥ 2 g/dL from baseline (*20*).

2.7. Statistical analysis

Categorical variables are expressed as counts and percent frequency and were compared using the chi-square test. Continuous variables are given as means ± standard deviations and were compared across the three assessment points using one-way analysis of variance (ANOVA) followed by *post-hoc* Newman-Keuls tests. All analyses were performed using GraphPad Prism, version 7.0 (GraphPad Inc., San Diego, CA, USA) and SPSS 17.0 (SPSS Inc., Chicago, IL, USA). Two-tailed p values < 0.05 were considered statistically significant.

3. Results

3.1. Patient characteristics

The baseline characteristics of the three study groups are shown in Table 1. There were no significant intergroup differences in terms of age, sex, BMI, pain duration, pain at rest, pain upon movement, range of motion (degrees), and WOMAC index scores. Laboratory safety parameters (AST, ALT, creatinine, BUN, hematocrit, and hemoglobin) at baseline were all within the normal range (data not shown). The study sample may therefore be considered representative of a clinical population of patients with mild-to-moderate OA of the knee in need for pharmacological treatment.

3.2. Clinical endpoints

No patient withdrew from the study. The clinical endpoints in the three study groups are shown in Table 2. No significant differences over time were observed in the control arm (watchful waiting). Compared with baseline values, celecoxib significantly outperformed Verbascox® with regard to all clinical endpoints at one week. However, no significant differences were evident between the Verbascox® group and the celecoxib group at the end of the 2-week study period. Figure 1 compares the changes in the WOMAC index total score in the Verbascox® and celecoxib groups along the 2-week study course.

3.3. Serum substance P levels

Table 3 summarizes the temporal variations in serum SP levels in the three study groups. No significant differences over time were observed in the control arm (watchful waiting). At one week, celecoxib, but not Verbascox®, produced a statistically significant reduction in serum SP levels compared with baseline values. However, no significant differences were evident

Table 2. Temporal course of clinical endpoints in the three study groups

Items	No treatment (n = 50)			Supplementation with Verbascox® 800 mg/day (n = 50)			Treatment with celecoxib 200 mg/day (n = 50)		
	Baseline	One week	End of the study	Baseline	One week	End of the study	Baseline	One week	End of the study
Pain at rest, VAS (0-10)	2.8 ± 0.3	2.8 ± 0.2	2.5 ± 0.3	2.7 ± 0.4	2.5 ± 0.7	1.7 ± 0.5*	2.9 ± 0.4	2.0 ± 0.3*,†	1.5 ± 0.4*
Pain on movement, VAS (0-10)	3.7 ± 0.6	3.9 ± 0.8	3.8 ± 0.7	3.9 ± 0.7	3.6 ± 0.9	2.4 ± 0.6*	3.8 ± 0.6	2.9 ± 0.5*,†	2.2 ± 0.4*
Range of motion (degrees)	133 ± 10	135 ± 13	131 ± 12	132 ± 9	141 ± 18	168 ± 21*	134 ± 10	160 ± 15*,†	173 ± 19*
WOMAC scores									
Pain	5.6 ± 0.6	5.8 ± 0.7	5.5 ± 0.9	5.8 ± 0.8	5.2 ± 1.4	3.3 ± 0.9*	5.7 ± 0.8	4.1 ± 0.9*,†	3.0 ± 0.9*
Stiffness	1.4 ± 0.3	1.6 ± 0.3	1.5 ± 0.2	1.5 ± 0.4	1.3 ± 0.4	0.8 ± 0.3*	1.4 ± 0.3	1.0 ± 0.2*,†	0.7 ± 0.1*
Physical function	20.1 ± 2.4	22.5 ± 2.7	21.8 ± 2.6	20.9 ± 3.6	18.7 ± 2.5	11.9 ± 2.0*	20.5 ± 3.2	14.6 ± 2.1*,†	11.4 ± 1.7*
Total	27.1 ± 3.3	29.9 ± 3.8	28.8 ± 3.6	28.2 ± 4.0	25.2 ± 3.1	16.0 ± 2.7*	27.6 ± 3.8	19.7 ± 2.9*,†	15.1 ± 2.2

Abbreviations: VAS, visual analogue scale; WOMAC, Western Ontario and McMaster Universities. Data are expressed as means and standard deviations. *$p < 0.001$ *versus* baseline. †$p < 0.001$ *versus* the Verbascox® arm at one week.

Table 3. Temporal course of serum substance *P* levels in the three study groups

Items	No treatment (n = 50)			Supplementation with Verbascox® 800 mg/day (n = 50)			Treatment with celecoxib 200 mg/day (n = 50)		
	Baseline	One week	End of the study	Baseline	One week	End of the study	Baseline	One week	End of the study
Serum substance P, pg/mL	423 ± 85	451 ± 96	444 ± 72	445 ± 91	423 ± 80	312 ± 77*	436 ± 95	358 ± 78*,†	299 ± 64*

Data are expressed as means and standard deviations. *$P < 0.001$ *versus* baseline. †$P < 0.001$ *versus* the Verbascox® arm at one week.

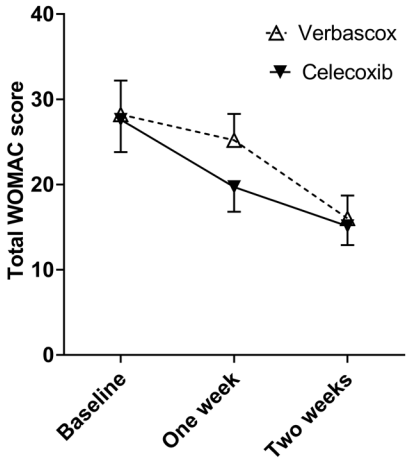

Figure 1. Comparison of changes in the WOMAC index total score in the Verbascox® and celecoxib groups along the 2-week study course. Error bars represent standard deviations.

Table 4. Treatment-emergent adverse events in the Verbascox® and celecoxib arms

Items	Supplementation with Verbascox® 800 mg/day (n = 50)	Treatment with celecoxib 200 mg/day (n = 50)
Gastrointestinal adverse events		
Dyspepsia	5 (10%)	4 (8%)
Nausea	1 (2%)	2 (4%)
Constipation	0 (0%)	2 (4%)
Diarrhea	2 (4%)	0 (0%)
Flatulence	2 (4%)	1 (2%)
Other adverse events		
Headache	2 (4%)	3 (6%)
Dizziness	1 (2%)	0 (0%)
Pruritus	0 (0%)	1 (2%)

Data are expressed as number of patients (percentages in parenthesis).

between the Verbascox® group and the celecoxib group at the end of the 2-week study period – with both treatment arms showing similar reductions compared with baseline values.

3.4. Safety

Treatment-emergent adverse events in the Verbascox® and celecoxib arms occorred sporadically and did not differ significantly in the two groups (Table 4). Clinically relevant changes in laboratory values were not observed in any of the study patients regardless of the treatment arm.

4. Discussion

In this randomized, single-blind, active-controlled pilot study, we found that supplementation with Verbascox® (800 mg/day) in patients with mild-to-moderate OA of the knee is as effective and safe as a standard therapeutic dose of celecoxib in terms of pain relief and improvement in functional capacity after a 2-week

treatment course, although celecoxib was more rapidly effective. Interestingly, the temporal course of serum SP reduction followed a similar pattern, suggesting that the clinical effects of both Verbascox® and celecoxib could at least in part mediated by a decrease in serum levels of this biochemical mediator of pain and inflammation. Supplementation with Verbascox® for two weeks was safe, with treatment-emergent adverse events being sporadic and similar to those observed in the celecoxib arm and none of them leading to withdrawal. Notably, there were no changes from baseline in clinical laboratory safety tests in both the Verbascox® and celecoxib groups.

Although several herbal inhibitors of COX-2 have been developed (*12,13*), to date there has been little direct evidence comparing such extracts with celecoxib, one of the most commonly used coxibs. The efficient analgesic effect of celecoxib in knee OA begins within two days of treatment initiation, and taking 200 mg/day is known to ensure an efficient control of pain (*21*). We therefore utilized this dosage for the active-controlled treatment arm.

Using *in vitro* experiments, we have previously shown that Verbascox® is capable of inhibiting COX-2 in a dose-dependent fashion (*14*). In the current study, the comparable clinical efficacy of Verbascox® and celecoxib was shown by similar scores in VAS at rest, VAS upon movement, range of motion, and WOMAC index total score at 2 weeks, although celecoxib acted more rapidly – with several improvements being already evident at one week. We believe that two potential explanations can be offered for the more rapid efficacy of celecoxib. First, our *in vitro* experiments indicated that celecoxib is a more potent inhibitor of COX-2 than Verbascox® (*14*). Second, it is possible that Verbascox® and celecoxib may differ in terms of pharmacokinetic properties – with steady state plasma concentrations possibly being reached more rapidly by the former. Additional studies are required to shed more light on the pharmacokinetics of Verbascox®.

Verbascox® may be clinically effective against mild-to-moderate OA not only in light of its capacity to inhibit COX-2, but also because of other active properties of its components. Verbascoside is indeed characterized by a high antioxidant power (*22*), whereas aucubin can attenuate tumor necrosis factor-α-induced inflammatory responses (*23*). The possibility that Verbascox® may exert additional COX-2-independent antioxidant and anti-inflammatory effects would explain why its clinical effectiveness was found to be similar to that of celecoxib at 2 weeks, despite being a less potent inhibitor of COX-2 (*14*). Importantly, the amount of point reductions in the WOMAC index total score at the end of the study (from 28.2 ± 4.0 to 16.0 ± 2.7 in the Verbascox® group and from 27.6 ± 3.8 to 15.1 ± 2.2 in the celecoxib group) should be considered as a clinically relevant improvement. Accordingly, the minimum clinically important differences in the WOMAC index total score have been reported to be 9.5-10.1 for OA of the knee and hip (*21*). In our study, improvements in clinical symptoms in both study arms were paralleled by a significant reduction in serum levels of SP, a neuropeptide released from sensory nerves that exerts different pro-inflammatory effects (*15*). It is notable that SP signaling is not only involved in pain perception but is also capable of upregulating COX-2 expression (*24,25*). In recent years, growing evidence has shown a role of SP in human joint disease including OA (*26,27*). It is therefore feasible that the reduction in SP levels can contribute to the anti-inflammatory and pain-mitigating effects of both Verbascox® and celecoxib.

There are several limitations to this study which need to be mentioned. First, the relatively small sample size may give rise to overestimation of treatment effects (*28*), Moreover, continuous endpoints and self-reported outcomes can also lead to potential bias. To circumvent these issues, we also used objectively measured clinical (range of motion) and biochemical (SP) endpoints. Finally, our study should be considered as an exploratory analysis; the follow-up time was short and independent replication is needed to extend and confirm our results. Patients with OA of the knee patients should be treated on an individual basis according to each patient's disease characteristics, based on clinical trial data. Larger studies that include a placebo group or a cross-over design will be required to further elucidate the clinical usefulness of Verbascox® in patients with inflammatory joint disorders.

These caveats notwithstanding, our results suggest that supplementation with Verbascox® and treatment with celecoxib for 2 weeks have similar effects in reducing symptoms of mild-to-moderate OA of the knee, although celecoxib was more rapidly effective. Reduction in serum SP levels followed a similar temporal pattern and could be at least in part responsible for the observed clinical effects.

Conflict of interest:

This study was partly funded by LaBiotre srl (Italy). The sponsor had no influence in the performance, analysis, and interpretation of the study.

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Brief Report

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Malnutrition as an important risk factor for drug-induced liver injury in patients on anti-tubercular therapy: an experience from a tertiary care center in South India

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SUMMARY Drug-induced liver injury (DILI) due to anti-tubercular treatment (ATT) leads to increased morbidity and mortality in patients with tuberculosis (TB). The aim of this study was to find the impact of malnutrition on the development of DILI. This was a prospective cohort study (September 2017 to August 2019) in which all newly diagnosed in-patients with tuberculosis above the age of 18 years were included. Those patients with a body mass index (BMI) of < 18.5 kg/m^2 were considered malnourished. The patients were monitored for the development of DILI. Liver function tests were done at the baseline (before initiation of ATT), on the third day and at discharge in all the patients. Chi-square tests and conditional multiple logistic analysis was performed to identify risk factors associated with DILI. Out of the 319 subjects who were enrolled, a total of 138 patents chose to follow up at our hospital. A total of 14 patients (10%) developed DILI. The median time to onset of DILI was found to be ten days. Extra-pulmonary TB, low BMI and high baseline liver enzyme levels had a significant association with the development of DILI ($p < 0.05$). Low serum albumin had increased odds ratio but wasn't statistically significant. Malnutrition is an important risk factor for TB-DILI.

Keywords aspartate transaminase, alanine transaminase, body mass index, tuberculosis, adverse drug reaction, nutrition

1. Introduction

Tuberculosis (TB) is an epidemic of massive proportions and is a significant cause of concern in a developing country like India. With an incidence of approximately 2.8 million cases in 2018, India accounts for almost a quarter of the world's TB affected population (*1*). Malnutrition in TB is both a cause and an effect. Malnutrition leading to dysfunction of the immune system is one of the most frequent causes of secondary immunodeficiency worldwide (*2*). It must be recognized as one of the driving reasons for the slower decline of the TB epidemic in India despite all the steps taken nationally towards TB eradication (*3*). Given the multifaceted nature of this two conditions, tuberculosis and malnutrition, it is of great importance to understand the link between the two and ensure that high-quality treatment can be provided to these patients with minimal interruptions. Drug-induced liver injury (DILI) is a significant cause of treatment interruption in people on anti-tubercular treatment (ATT) (*4*). This leads to increased morbidity and mortality among affected patients. It is hence imperative to consider the implications that coincident malnutrition may have on the impact of DILI in patients with tuberculosis, especially considering that the incidence of DILI is far more in developing countries where malnutrition is rampant (*5*). The study aimed therefore, to find the impact of malnutrition on the development of DILI.

2. Materials and Methods

This was a cohort study conducted between September 2017 and August 2019 at a tertiary care hospital in South India after taking approval from the Institute's Ethics committee (approval number: 660/2017). All newly diagnosed in-patients with tuberculosis (pulmonary and extra-pulmonary) above the age of 18 years were included in the study. Those patients with a past history of tuberculosis were excluded. A brief clinical history,

examination and baseline laboratory parameters of all the recruited patients were obtained and recorded according to a pre-defined questionnaire. Those patients with a body mass index (BMI) of < 18.5 were considered malnourished. This was done according to Asian classification. After ATT was started, the patients were monitored for the development of symptoms of DILI. Liver function tests were done at the baseline, on the third day and at discharge in all the patients. The frequency of LFT was increased depending on the risk factors and development of symptoms. This was done at the treating physician's discretion. DILI was defined as an increase in serum aminotransferase level > 5 times the upper limit of normal (ULN) in asymptomatic individuals and > 3 times the ULN in symptomatic patients. In patients diagnosed with DILI, ATT medications were withheld and were reintroduced sequentially once liver function tests were normalized. The offending drug was identified and replaced with an alternative drug as per the judgement of treating physician. Those patients who chose to continue treatment at our hospital were followed up until the end of the study period.

Data analysis: Chi-square tests and conditional multiple logistic analysis was performed to identify risk factors associated with DILI.

3. Results and Discussion

In this study, a total of 319 patients were enrolled. A total of 188 of these were diagnosed with pulmonary TB (PTB) (58.9%), and the remaining 131 were diagnosed with extrapulmonary TB (EPTB) (41.06%). Majority of the subjects of the total cohort were male (male, 209; female, 110) and belonged to the age group of 40-49 years. The mean age was 46.3 ± 16.62 years. PTB was prevalent in the age group 40-49, whereas EPTB was prevalent in the 20-29 age group. It was found that 35.7% of these subjects were under-nourished. The mean BMI was 20.3 ± 4.01. The proportion of individuals with low BMI was significantly higher in PTB than in EPTB (45% *vs*. 22%, $p < 0.001$).

Out of the 319 subjects, 181 (56.7%) were transferred out to their respective directly observed treatment short-course chemotherapy (DOTS) centre while the remaining 138 (43.2%) chose to follow up at our hospital.

Of the 138 patients who were managed at our hospital, a total of 45 (32.6%) of these patients were cured, 65 (47.1%) completed treatment, 19 (13.8%) were still on ATT, and a total of nine (6.5%) patients died during treatment. A total of 14 patients (10%) developed DILI. All patients who developed DILI were initially started on four-drug ATT. The median time to onset of DILI was found to be ten days. The primary drug attributed to causing this liver injury was found to be pyrazinamide, seen in about 8 of the 14 cases. This was followed by rifampicin, seen in 2 cases. Others were undetermined.

A total of 20% of the patients who were malnourished developed DILI while only 5.4% of the patients with a BMI of > 18.5 kg/m^2 developed DILI. BMI was found to have a significant association with DILI. EPTB and high baseline transaminase levels were also found to have a significant association with DILI (Table 1). However, other attributed risk factors such as pre-existing liver disease, low serum albumin levels, female sex and human immunodeficiency virus (HIV) coinfection were not associated with increased risk for DILI. Low serum albumin had increased odds ratio but wasn't statistically significant.

DILI is a possible adverse outcome of the consumption of ATT. DILI is one of the leading causes of treatment

Table 1. Chi-square test and conditional multivariate logistic regression analysis to determine factors associated with DILI

Items	DILI, No. (%)	non-DILI, No. (%)	*p*-value	OR (95% CI)
Sex			0.827	1.137 (0.702-2.088)
Male	9 (10.6)	76 (89.4)		
Female	5 (9.4)	48 (90.6)		
Type			0.034	0.15 (0.35-0.64)
PTB	4 (6)	63 (94)		
EPTB	10 (14.1)	61 (85.9)		
BMI			0.025	-
< 18.5	9 (20)	36 (80)		
18.5 - 22.9	2 (3.8)	50 (96.2)		
≥ 23	3 (7.3)	38 (92.7)		
Albumin			0.207	2.024 (0.38-6.22)
< 3.5	7 (14.6)	41 (85.4)		
≥ 3.5	7 (7.8)	83 (92.2)		
HIV	2 (20)	8 (80)	0.284	0.414
HBV	0 (0)	2 (100)	0.632	1.016
HCV	0 (0)	2 (100)	0.632	1.016
CLD	0 (0)	5 (25)	0.491	1.051
Baseline ALT	56 ± 95.9	27.7 ± 28.3	0.056	0.99 (0.98-1.0)

*DILI, drug-induced liver injury; OR, odds ratio; CI, confidence interval; BMI, body mass index; HIV, human immunodeficiency virus; HBV, hepatitis B virus; HCV, hepatitis C virus; CLD, chronic liver disease; ALT, alanine transferase.

Table 2. Comparison of DILI and attributed risk factors among various studies

Studies	Location of study	No. of patients enrolled (N)	Duration of study (years)	Incidence of DILI (%)	Risk factors attributed	Median time of onset (days)
Current study	Manipal, India	134	2	14 (10)	Low BMI, EPTB, high baseline ALT	10
Saha *et al*. (2008-2012) (*8*)	Vellore, India	252	5	26 (9.5)	Nil	
Latief *et al*. (2012-2015) (*7*)	Jammu & Kashmir, India	200	3	16 (8.4)	Female gender, EPTB	< 14
Naidoo *et al*. (2009-2012) (*11*)	South Africa	94	3		Age >35 years, Low Albumin	31
Abbara *et al*. (2010-2014) (*9*)	England	1,529	4	105 (6.9)	Low BMI, HIV-TB coinfection, Alcohol, High baseline ALP	12.5
Tweed *et al*. (2018) (*10*)	London	1,927		58 (3)	Older age, Asian ethnicity	

*DILI, drug-induced liver injury; BMI, body mass index; HIV, human immunodeficiency virus; ALT, alanine transferase; ALP, alkaline phosphatase; TB, tuberculosis; EPTB, extrapulmonary tuberculosis.

interruption in patients on ATT. The risk factors of DILI in previously published studies have been compiled in Table 2. According to Saukkonen *et al*., the incidence of DILI attributable to ATT is 5-33% (*6*). A total of 10% of the patients developed DILI in our study. Similar results were observed in studies conducted in India by Latief *et al*. (2012-2015), and Saha *et al*. (2008-2012) wherein the incidence rates for DILI were found to be 8.4% and 9.5% respectively (*7,8*). The median time of onset of DILI was found to be within ten days of starting the treatment with ATT. This correlates well with similar studies done by Abbara *et al*. wherein it was found to begin within 12.5 days of treatment initiation (*9*). Other studies from India have also reported DILI onset to be common in the first two weeks of starting ATT (*7,8*). Therefore, patients with risk factor, who are started on ATT, should be followed for the first two weeks of treatment with LFTs. This will help in early identification of DILI.

Three of the five first-line ATT drugs have been known to cause hepatotoxicity, *i.e*. isoniazid, pyrazinamide, and rifampicin. Pyrazinamide has been documented as being the most hepatotoxic, according to Saukkonen *et al*. (*6*). The drug that most commonly attributed to the development of DILI in our study was also pyrazinamide (in 8 of the 14 patients).

Malnutrition plays a two-fold role in increasing the morbidity and mortality rates of people suffering from TB. Nutritional depletion has a major impact on immune function, and loss of CMI is not an advantageous factor in an individual fighting an invasive mycobacterial infection. Primary malnutrition is known to not only increase the frequency of occurrence but also to exacerbate clinical manifestations of TB. In addition to ATT, these patients require an adequate supply of nutrition during the treatment/ recovery phase. In our study, patients with low BMI were significantly at a higher risk of developing DILI. Malnutrition increases the risk of developing DILI due to altered drug metabolism pathways (*9*).

Increasing age has been reported as a risk factor of DILI in several studies. This is because of the age-related decline in liver function. A study was done by Naidoo *et al*., found a higher incidence of DILI in patients above the age of 35 years (*10*). Similar results were observed by Tweed *et al*., who found that DILI was more common in the elderly. In our study, the age difference between the two groups was statistically insignificant (*p*-value 0.261). No association with gender and DILI was found in our study, though certain studies done in North India have found an association with female gender and DILI (*7*). Interestingly, the same study found an association with EPTB and DILI, as was the case in our study. This may be due to subclinical involvement of the liver as a part of EPTB. However, the exact reason for the same remains unclear. Though HIV-TB coinfection and association with DILI have been reported previously, this association was not found in our current study. Similar results have also been observed in a study done by Tweed *et al*. in England (*11*). In that study, an increased number of HIV-TB coinfection patients showed elevated liver enzymes, but that result was not statistically significant (*7*). A study was done in South India, which also failed to find such an association (*7*). Sharma *et al*. reported low serum albumin as an independent risk factor for the development of DILI (*12*). Low serum albumin level as a risk factor for DILI was reported in studies done in South Africa as well (*10*). However, in our study and other studies done in India, low albumin level was not found to be a risk factor for the development of DILI (*7,8*).

In conclusion, malnutrition, presence of EPTB and pre-existing increased liver enzymes are an important

risk factor for TB-DILI. Further studies are needed to evaluate whether nutritional interventions, along with ATT, will help in mitigating this risk.

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Commentary

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Can mesenchymal stem cell therapy be the interim management of COVID-19?

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SUMMARY COVID-19 pandemic has accounted for ~ 4.3 million confirmed cases and ~ 292,000 deaths (till 12th May, 2020) across the globe since its outbreak. Several anti-viral drugs such as RNA dependent RNA polymerase inhibitors (remdesivir, favipiravir, ribavirin), protease inhibitors (lopinavir, ritonavir) and drugs targeting endocytic pathway (hydroxychloroquine) are being evaluated for COVID-19 but standard therapeutics yet not available. Severe health deterioration in critically ill patients is characterized by pulmonary edema, severe respiratory distress, cytokine storm and septic shock. To combat cytokine storm, immune-therapy targeting IL-1, IL-2, IL-6 and TNFα are being evaluated and one of the promising immune-modulator is the mesenchymal stem cells (MSCs) that can surmount the severity of COVID-19 infections. Recent studies have shown that MSC-therapy significantly dampens the cytokine storm in critically ill COVID-19 patients. This communication endows with the insight of stem cell therapy and summarizes the recent studies on COVID-19 patients.

Keywords novel coronavirus disease, SARS-CoV-2, cytokine storm, MSC-therapy

The entire world is in state of emergency due to outbreak of novel corona virus disease 2019 (COVID-19) that first reported in Wuhan, China in December 2019. It became a global health emergency due to non-availability of vaccine or specific therapeutic drugs. Till May 12, 2020 there were 4,337,602 cases worldwide among which 1,408,636 cases were from USA, making it an epicenter for this pandemic (as per worldometers.info). This ripple of COVID-19 has severely impacted the global economy and healthcare system. Currently the mainstay of COVID-19 management is symptomatic treatment and mechanical ventilation for critically ill patients. Medications include anti-viral drugs like remdesivir, ribavirin, favipiravir, lopinavir/ritonavir, hydroxychloroquine, and arbidol which blocks the entry of virus to host cell, and other drugs such as IFN-α2a, IFN-β1b, tocilizumab which inhibits the viral exocytosis leading to regulation of immune response (*1*). However, till date, there's no COVID-19 approved drug available for the treatment. This necessitates a prodigious effort to understand the pathophysiology of the virus, develop efficient therapeutic modality as well as sensitive and specific rapid diagnostics in order to combat COVID-19 pandemic.

Coronaviruses (CoV) belongs to the Coronaviridae family of viruses, which is subdivided into 4 classes *i.e*. alpha, beta, gamma and delta. The beta-coronavirus group includes Middle East Respiratory Syndrome (MERS-CoV), Severe Acute Respiratory Syndrome (SARS-CoV) and the novel SARS-CoV-2 the major causative agent for COVID-19 (*2*). The whole genome sequencing of SARS-CoV-2 shows a similarity of 96.2% to a bat betacoronavirus (SARSSr-CoV; RaTG13) while comparatively less similar to SARS-CoV (~ 79%) and MERS (~50%) (*3*). Similar to SARS-CoV and MERS, SARS-CoV-2 attacks the respiratory tract causing severe respiratory distress, pulmonary edema and viral pneumonia. However, in severe cases, SARS-CoV-2 affects heart, kidney, liver, GI-system, leading to multiple organ failure and eventually death in some cases (*4*). SARS-CoV-2 is an enveloped, positive sense RNA virus with genome encoding various glycoproteins, including the glycosylated spike (S) protein. This S-protein binds to angiotensin I converting enzyme 2 receptor (ACE-2) in host cell. Also, ACE-2 receptor is highly expressed on the surface of lung alveolar type II cells (AT-2) and capillary endothelium. In addition, AT-2 cells also express type II transmembrane serine protease (TMPRSS211) which facilitates priming of S-protein and in turn the invasion of virus inside the host cell (*5*). Unfortunately, ACE-

2 receptors are also expressed on other tissues like kidney, liver, heart and digestive system organs; thus explaining the rapid progression towards systemic inflammatory conditions as observed in critically ill patients. The silver lining is that thymus, bone marrow, spleen, lymph node and macrophages do not express ACE-2 receptors (*6*); this implies that exploiting immuno-therapeutic approaches to target SARS-CoV-2 virus infection pathway can be feasible and may provide better treatment outcomes.

The clinical course of SARS-CoV-2 begins with robust viral replication accompanied with mild symptoms such as fever, cough, headache and the second phase exhibit high grade fever, difficulty in breathing and pneumonia-like symptoms. The progression to third stage is mediated by inflammatory cytokines (IL-2, IL-6, IL-8, TNF-α, G-CSF, GM-CSF), chemokines (MCP-1, MIP1α, IP10) and massive infiltration of inflammatory cells. This 'cytokine storm' is the hallmark of third stage SARS-CoV-2 pathogenesis in critically ill patients inducing pulmonary edema, dysfunction of air-exchange, acute respiratory distress syndrome (ARDS), cardiac injury and secondary infections which ultimately leads to death (*7*). A number of studies with drugs targeting GM-CSF, IL-6, IL-1, IL-2 and TNF-α is already in pipeline which aims to dampen the inflammatory response in COVID-19 patients. Mesenchymal stem cells (MSCs) are known for their immuno-modulatory properties such as secretion of anti-inflammatory cytokines/chemokines, anti-apoptotic effect and their ability to repair damaged epithelial cells. Their inherent nature to migrate towards injured lungs and secretion of paracrine factors which protects and repair alveolar cells; make MSCs a potential therapeutic option for COVID-19 treatment.

In recent years, MSCs have been widely employed from basic research to clinical trials (*8,9,10*), especially for immune-mediated inflammatory diseases such as systemic lupus erythematous (SLE) (*11*) and graft versus-host disease (GVHD) (*12*). The success of MSCs is attributed to their ability to modulate immune response directly via interaction with host immune cells or indirectly through paracrine secretion of various cytokines (*8,13*). MSCs modulate immune response by regulating the function and proliferation of various immune cells, inhibits monocyte differentiation into dendritic cells (DCs) which results in upregulation of regulatory cytokines and downregulation of inflammatory cytokines (*14*). Previous studies suggested that systemic administration of MSC resulted in reduction of H5N1 influenza virus-induced mortality in older patients with severe pulmonary illness (*15*). Also, in patients with H7N9 induced ARDS, a significant improvement in survival rate was observed (*16*). So far, MSC transplantation in human subjects with diverse disease conditions has not showed any severe adverse events (*17*). Therefore, it is plausible that MSC-therapy can be used to treat COVID-19 patients.

Recently, Leng Z *et al*. conducted a pilot study on 7 confirmed COVID-19 pneumonia patients presented with mild, common, severe and critically ill disease condition (*18*). Clinical grade MSCs were injected intravenously (1×10^6 cells/kg body weight) and follow-up for 14 days. A significant reduction in clinical symptoms and pneumonia infiltration was observed in chest CT of critically ill COVID-19 patient within 2-4 days of MSC-therapy. An increase in peripheral lymphocyte levels, decrease in C-reactive protein (CRP), drastic disappearance of activated cytokine-secreting immune cells (CXCR3+CD4+T-cells, CXCR3+CD8+T-cells and CXCR3+NK-cells) and restoration of

Table 1. Ongoing MSC-based clinical trials for COVID-19 treatment

Cell source	Clinical trial number	Reference
Human allogeneic MSCs (Source Unknown)	ChiCTR2000030224 ChiCTR2000030835	*http://www.chictr.org.cn*
	NCT04288102 NCT04299152	*https://clinicaltrials.gov*
Human Umbilical Cord derived MSC	ChiCTR2000030866 ChiCTR2000030138	*http://www.chictr.org.cn*
	NCT04273646 NCT04269525 NCT04252118	*https://clinicaltrials.gov*
Wharton's Jelly derived MSC	ChiCTR2000030088 NCT04313322	*http://www.chictr.org.cn* *https://clinicaltrials.gov*
Dental pulp MSC	ChiCTR2000031319 NCT04302519	*http://www.chictr.org.cn* *https://clinicaltrials.gov*
Ruxolitinib in combination with MSCs	ChiCTR2000029580	*http://www.chictr.org.cn*

regulatory DC cell population (CD14+CD11c+CD11b^{mod} regulatory DC cell) to normal levels was observed after day 6 of MSC transplantation. Additionally, an increased level of anti-inflammatory cytokine IL-10 and significantly decreased levels of serum pro-inflammatory cytokine TNF-α indicated efficientregulation of cytokine storm in COVID-19 patients on MSC transplantation. Furthermore, the absence of ACE-2 receptor and TMPRSS2 on the transfused MSCs affirmed that they cannot get infected with SARS-Cov-2, suggesting the usefulness of MSC-therapy in COVID-19 infection.

A case study reported that a 65y/o type-II diabetic, hypertensive COVID-19 positive female with severe pneumonia, acute gastrointestinal bleed and ARDS was treated with MSCs (*19*). Initially, the patient did not respond to any anti-viral drug and thereafter progressed towards multiple organ injury. At this stage, the patient was intravenously injected with 3 doses of human umbilical cord MSC (hUC-MSC, 50×10^6 cells/dose). After second cell infusion, ventilator was removed as the vital stats had improved with gradual decrease in serum albumin and CRP levels. CT images showed no pneumonia patches by the end of MSC-therapy. These results suggest that hUC-MSC can be beneficial to patient who are resistant to anti-viral drugs. Owing to the therapeutic potential of MSCs in viral infections and its immense capability to regulate immune cells to ameliorate the cytokine storm, a few studies have been initiated to evaluate their efficiency for COVID-19 treatment (Table 1). Seeing the promising prospective of MSC-therapy and to meet the large-scale demand of cGMP produced stem cells, US-based biotech companies (Mesoblast, Athersys) have taken up the challenge for mass production of clinical grade MSCs (*20*).

In summary, it appears that MSC-therapy through its immuno-modulatory properties suppresses the over activated immune system and promotes the tissue repair of alveolar cells in lung microenvironment of SARS-CoV-2 infected patients. Although the data derived from recent studies are encouraging, but the major limitations are the small-sized patient enrollment and thus necessitating larger randomized control trials to establish the effectiveness and safety of MSC-therapy in SARS-Cov-2 infection. It has also been observed that most of the clinical trials for COVID-19 treatment have used allogeneic stem cell source. With the vast available knowledge with reference to the mechanism of action of MSCs and their effective potencies at a specific disease stage makes MSCs as an ideal therapeutic candidate.

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Targeting lymphocyte Kv1.3-channels to suppress cytokine storm in severe COVID-19: Can it be a novel therapeutic strategy?

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SUMMARY In the midst of a pandemic, finding effective treatments for coronavirus disease 2019 (COVID-19) is the urgent issue. In "chronic inflammatory diseases", the overexpression of delayed rectifier K^+-channels (Kv1.3) in leukocytes is responsible for the overactivation of cellular immunity and the subsequent cytokine storm. In our previous basic studies, drugs including chloroquine and azithromycin strongly suppressed the channel activity and pro-inflammatory cytokine production from lymphocytes. These findings suggest a novel pharmacological mechanism by which chloroquine, with or without azithromycin, is effective for severe cases of COVID-19, in which the overactivation of cellular immunity and the subsequent cytokine storm are responsible for the pathogenesis.

Keywords COVID-19, cytokine storm, lymphocyte, Kv1.3-channels, chloroquine

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Most patients are either asymptomatic or develop only mild to moderate symptoms, such as fever, dry cough and shortness of breath, which often improve spontaneously with supportive treatment alone. However, some patients develop fatal pneumonia with acute respiratory distress syndrome (ARDS) or are occasionally complicated by multiple organ failure due to generalized thrombotic microangiopathy as the result of systemic vasculitis (*1*). Concerning the mechanisms, the over-activation of leukocytes and the uncontrolled release of pro-inflammatory cytokines, such as interleukin (IL)-6, IL-1, IL-2, IL-10, tumor necrosis factor (TNF)-α and interferon (IFN)-γ, are thought to be responsible (*2,3*). This phenomenon is termed "cytokine storm" characterized by an excessive response of the immune system.

"Chronic inflammatory diseases" is a disease category in which over-stimulated cellular immunity is responsible for the pathogenesis (*4*). Besides infectious diseases and autoimmune disorders, studies indicate that common diseases, such as metabolic disorders and cancer, are also included in this disease category. Recently, we have demonstrated in animal studies that that the cytokine storm can occur in advanced-stage kidney diseases, such as end-stage renal disease and interstitial nephritis (*5,6*). In these animal models, leukocytes including T-lymphocytes and macrophages were markedly increased within the kidneys and the cytokine levels, such as IL-2 and TNF-α, were actually elevated. In these leukocytes, since delayed rectifier K^+-channels (Kv1.3) were over-expressed, and since pharmacological blockade of the channels actually ameliorated the disease progression, they were thought to be the primary trigger of the overactivation of cellular immunity and the subsequent cytokine storm.

In the midst of a pandemic, finding effective treatments for COVID-19 is an urgent issue. Despite the limited evidence, some clinical studies have suggested the efficacy of chloroquine with or without azithromycin for COVID-19 patients with severe respiratory failure (*7-9*). In addition to the direct effect of chloroquine on reducing the viral replication (*10*), in vitro studies demonstrated its inhibitory effect on the production of cytokines from leukocytes (*7,11*). Regarding the mechanisms of such anti-inflammatory properties of chloroquine, recent reports suggest the involvement of glycogen synthase kinase-3β (GSK3β) or Toll-like receptors (TLRs) (*12,13*). In our previous patch-clamp studies using murine thymocytes, both chloroquine and azithromycin strongly suppressed the activity of lymphocyte Kv1.3-channels and thus reduced the production of inflammatory cytokines (*14,15*). These findings provide an additional pharmacological mechanism by which chloroquine with or without azithromycin was effective for severe cases of COVID-19, where the overactivation of cellular immunity and the subsequent cytokine storm were responsible for their pathogenesis (Figure 1).

Additionally, in our series of patch-clamp studies

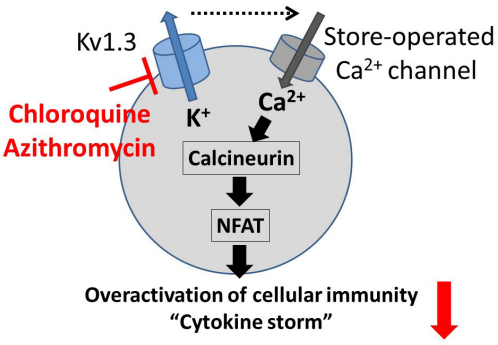

Figure 1. Roles of Kv1.3-channels in the activation pathway of T lymphocytes and as the targets of chloroquine and azithromycin. Kv1.3-channels promote calcium influx and trigger the proliferation and activation of lymphocytes (*4*). The increased cytosolic calcium concentration stimulates the phosphatase calcineurin, which dephosphorylates the nuclear factor of activated T cells (NFAT), causing its accumulation in the nucleus and binding to the promoter region of cytokine-encoding genes. Both chloroquine and azithromycin inhibit Kv1.3-channels (*14,15*).

so far, we also revealed the inhibitory properties of nonsteroidal anti-inflammatory drugs, anti-hypertensive drugs, anti-cholesterol drugs and anti-allergic drugs on lymphocytes Kv1.3-channels (*16-18*). Considering such pharmacological properties, these commonly used drugs may also be beneficial in the treatment of COVID-19, since the channel blockade reduces the cytokine production and thus suppresses the cytokine storm.

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Prospects for development of group purchasing organizations (GPOs) in China within the context of national centralized drug procurement

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SUMMARY Healthcare group purchasing organizations (GPOs) are considered to play an integral role in the healthcare supply chain by keeping prices low and helping all members of the healthcare system achieve their objectives. China has been exploring GPOs in the field of drug procurement since 2015, and there are currently three GPO models in Shanghai, Shenzhen, and Guangzhou. Although the three models operate differently and they have each been examined, they have all achieved certain results and demonstrated the ability to control drug expenditures. In 2018, the National Healthcare Security Administration implemented a national centralized drug procurement policy, also known as the 4 + 7 procurement policy ("4+7 Policy"). This policy context has also led to changes in the strategy for development of GPOs in China. GPOs need to explore strategies that do not overlap with the scope of 4 + 7 procurement, and they need to develop dynamic and personalized procurement plans that are more in line with first-line clinical practices to have a synergistic effect in combination with the "4+7 Policy." In the future, GPOs will grow rapidly in China. The number of GPOs need to be increased to prevent monopolies, and GPOs need to expand their diversified value-added services to perform more tasks in terms of supply chain management and data analysis.

Keywords group purchasing organizations (GPOs), centralized drug purchasing, China

1. Definition of a group purchasing organization (GPO) and its institution worldwide

The first group purchasing organization (GPO) was established in the US in 1910. Since then, GPOs have been expanding from healthcare to assorted industries, such as groceries, electronics, industrial manufacturing, and agriculture. In the field of healthcare, GPOs help healthcare providers – such as hospitals, nursing homes, surgical centers and clinics, and home healthcare agencies – realize savings and efficiencies by aggregating purchasing volume and using that leverage to negotiate discounts with manufacturers, distributors, and other vendors. Hospitals and other healthcare providers use group purchasing to obtain the best products at the best value (*1*). Besides the US, GPOs negotiate and manage supply agreements for partner hospitals in European countries like Germany, Austria, and the Netherlands (*2*).

GPOs claim that hospitals can reduce supply expenses by an average of 10-15%. The savings are projected to be upwards of $864 billion over a ten-year period ending in 2022 (*3*).

2. The institution of GPOs in China

2.1. Current status and effect

The healthcare system is facing tremendous changes in the regulatory environment and competitive pressures such as reimbursement and payment policies (*4,5*). After many years of development, China's current drug procurement model is "government-led and implemented at the provincial level," allowing a variety of drug procurement models to be explored at the provincial level. In addition to the volume procurement pilot program led by the National Healthcare Security Administration, there are many provincial (city) -based online drug procurement models, including the drug exchange model, the joint limited-price sunshine procurement model, the cross-region joint procurement model, and the GPO model. China started adopting the GPO model in 2015 to further reduce drug expenditures and reduce distribution costs.

There are currently three models of GPOs for drug procurement in China (Table 1):

i) *Shanghai model*. Launched in June 2016 by the

Table1. Current status of three group purchasing organization (GPO) models in China

Items	Model characteristics	Shenzhen	Shanghai	Guangzhou
Current Exploration	GPO Entity	Shenzhen Quanyaowang Technology Co., Ltd. (a for-profit GPO)	Shanghai Medical and Health Affairs Service Center under the guidance of the Shanghai Health Reform Office (a not-for-profit GPO)	The Guangzhou social health insurance department, Guangzhou healthcare security administration, and Guangzhou Public Resources Trading Center (a not-for-profit GPO)
	Launch Time	July, 2016	June, 2016	October, 2018
	Area Covered	Mainly in Shenzhen, also includes 10 cities in Guangdong Province and other Midwestern cities in China	Only in Shanghai	Mainly in Guangzhou, also includes several cities in Guangdong Province
	Relationship to provincial bidding and drug procurement platforms	Independent GPO platform; parallel to the provincial platform	Drugs selected from among bid winners at the provincial level and traded on the provincial platform	Led by the Guangzhou healthcare security administration; independent GPO platform; parallel to the provincial platform
	Drugs procured	Drugs (covering low-price drugs in short supply, first-aid medications, special medications for women and children, and 80% of the drugs most often purchased in Shenzhen)	Drugs (antibacterials and often used drugs covered by health insurance)	Drugs (covering low-price drugs in short supply, first-aid medications, and special medications for women and children)
Effect	Decrease in the percentage of drug expenditures	1st Batch: 15.8% 2nd Batch: 22.57%	1st Batch: 64% 2nd Batch: 53% 3rd Batch: 54%	Not published
Problem and Risk	Antitrust Risk	Single service provider	Transaction boycott	Profit model and motivation for commissioned companies are unclear

Shanghai Medical and Health Development Foundation, five tertiary hospitals and six district-affiliated hospitals and counties in Shanghai jointly established the Shanghai Public Medical Facility Alliance for Group Drug Procurement. Shanghai launched a total of 3 rounds of GPO procurement. The drugs included were mostly antibacterials and often used drugs covered by health insurance, with an average decrease of 60% in drug procurement prices (*6*).

ii) *Shenzhen model*. Drawing on the experience of foreign GPO models, Shenzhen first introduced a profitable third-party drug GPO, namely the Shenzhen Quanyaowang Technology Co., Ltd., which was responsible for platform construction, operation, and maintenance. The Shenzhen GPO was officially launched in July 2016. A total of 2 rounds of GPO procurement were implemented in Shenzhen and the drugs included were drugs in short supply and drugs purchased in large amounts. Drug expenditures decreased by an average of 21.99% (*7*).

iii) *Guangzhou model*. The Guangzhou GPO model, which was launched in October 2018, established a three-tiered management system of leadership, supervision, and implementation. That is, GPO procurement is organized by the local municipal social health insurance department and monitored by the local healthcare security administration. In addition, the Guangzhou Public Resources Trading Center is responsible for the construction, operation, and maintenance of the Guangzhou GPO platform. Three companies have been included in the bidding for commissioned price negotiating.

2.2. Challenges

First, the differences between the Chinese model and the international model have led to questions about the reasonable compliance of the Chinese model. At present, the three GPO models are preliminary, and local improvements have been made to the internationally accepted GPO model of operation. Therefore, there has been much debate about whether such models are GPO models in nature, especially considering the profit model (a contract management fee or membership fee as the major source of income for the international model, a difference in purchase price for the Shenzhen GPO, and no cash flow involved in drug procurement for the Guangzhou GPO) and procurement entities (customized procurement needs from medical facilities for the international model, and government-specified drug lists for the Shenzhen GPO). In addition, the Shanghai and Shenzhen models have also undergone antitrust investigations and rectifications due to heavy government intervention in the design framework and questions about the legality and compliance of existing GPO practices.

Second, the status of GPO development in China is unclear because of the implementation of the 4 + 7 procurement policy (the "4+7 Policy"). The announcement of the "4+7 Policy" in November 2018 marked the start of the national volume procurement model, leading to uncertainty regarding the status and future development of other procurement models. One key question is how the procurement drug lists previously determined by the three GPO platforms can be updated and adjusted under the current national volume procurement model. This question is especially important to the Shenzhen GPO since its current list covers the top 80% of drugs purchased by city hospitals in 2015. Whether the current drug list will need to be expanded in the future is unclear, and the basis for a policy to continuously implement the GPO model is unsure. That said, determining whether the simultaneous implementation of the regional GPO model and the national "4+7 Policy" has a synergistic effect is difficult. For example, distinguishing between the effects of respective policies is difficult when two policies are implemented simultaneously since the Guangzhou GPO has adopted negotiated procurement similar to the approach used in the national"4+7 Policy," which uses the power of the healthcare security administration to bargain with manufacturers.

3. The future of GPOs rests on the "4+7 Policy"

3.1. The strategic position of GPOs

The government needs to promptly address the issue of "expensive medical treatment" through the "4+7 Policy." Problems with drug circulation in China include the marked skewing of interests related to drug circulation, the complicated circulation of drugs, disordered ordering, imperfect mechanisms of drug purchasing, and a low level of market adoption (*8*). There is no "one-size-fits-all" solution due to differences in economic factors, disease spectra, geographical conditions, government capacity, and other aspects. Opportunities and room are available for any methods of procurement, including GPOs.

First, GPOs should compensate for dislocation and competition. Drugs to treat tumors, chronic conditions, and common diseases are included in the national procurement catalog under the "4+7 Policy." These drugs are expensive and used in large volumes. GPOs can adjust various types of procurement orders by focusing on drugs not included in the "4+7 Policy" purchase list by offering drugs in demand by medical facilities in a market-oriented model. The procurement cycle of GPOs is dynamic while that of the "4+7 Policy" is one year. GPOs will establish a market environment with fair competition and reflect actual drug prices in the market.

Second, co-ordination between GPOs and the "4+7 Policy" will have a synergistic effect. One potential risk of implementation of the "4+7 Policy" is a supply shortage due to the "single source" and "volume-price linkage" model (*9*). According to international experience, one of the chief advantages of using GPOs is to help prevent and mitigate drug shortages (*10*). The Shenzhen GPO is a successful example. The traditional model has been improved locally by neither buying nor selling an item but by acting solely as an intermediary (*11*). The Shenzhen GPO has built a platform for supply chain integration and management on which to purchase drugs in short supply, low-cost drugs, and first-aid medications. Moreover, GPOs increase hospitals' awareness of new products and technology and foster competition among suppliers within the context of controlling healthcare costs (*12*).

3.2. Prospects for development of GPOs

GPOs have attracted attention worldwide, and they have become an effective way to relieve pressure on healthcare insurance in China. GPOs play a strategic role in the purchasing process, and they act to keep prices low and help all members of the healthcare system achieve their objectives (*11,13*). GPOs will need to be rapidly instituted and operated in the Chinese market. Firstly, more GPOs should be fostered to prevent a monopoly. There is a risk that all hospitals only use one GPO in China. This differs from the international approach: most hospitals use multiple GPOs, so they use at least two GPOs to negotiate with suppliers (*14-16*). In addition, GPOs will need to expand their diversified and value-added services. Besides price negotiations and procurement, GPOs perform other tasks such as drafting individual contracts, assessing new healthcare technologies, supply chain management, and data analysis (*12*). Eventually, the GPO market will be structured with perfect competition to allow many types of GPOs to co-exist, including large national GPOs, small to medium-sized regional GPOs, GPOs with comprehensive services, and GPOs for special procurement.

Some flaws and deficiencies with the development of GPOs in China need to be addressed. The first step is to set appropriate rules for access. The defining elements of the international GPO model should be improved and adjusted in accordance with national conditions, which include the nature of the institution (for-profit or non-profit), the composition and source of profit, the extent of profit, the type of contract, and the role of the institution. The next step is to establish a diversified supervision and management mechanism. In terms of monitoring bodies, the healthcare, justice, and commerce sectors and industry associations need to create a mechanism for common management with multilateral participation. Monitoring should review contracts and profit and oversee the proportion and cost of purchases from a GPO by each medical facility (*17*).

4. Conclusion

Three GPOs models have been explored and implemented in three cities in China. Although the GPOs face challenges, they have all achieved certain results and demonstrated the ability to control drug expenditures. GPOs have contributed to the healthcare supply chain by keeping prices low. The status of GPO development in China is unclear because of the implementation of the "4+7 Policy." GPOs need to explore strategies that do not overlap with the scope of 4 + 7 procurement, and they need to develop dynamic and customized procurement plans that are more in line with first-line clinical practices in order to have a synergistic effect in combination with the "4+7 Policy."

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Letter

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Traditional Chinese medicine to treat COVID-19: the importance of evidence-based research

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SUMMARY Coronavirus disease 2019 (COVID-19) broke out in 2019 and spread rapidly around the world, causing a global pandemic. Traditional Chinese medicine has a history of more than 2,000 years in the prevention and treatment of epidemics and plagues. In guidelines on fighting COVID-19, the National Health Commission (NHC) has recommended some traditional Chinese medicines (TCM), including Jinhua Qinggan granules, Lianhua Qingwen capsules, XueBijing injections, a Qingfei Paidu decoction, a Huashi Baidu decoction, and a Xuanfei Baidu decoction. Based on current results, TCM has displayed some efficacy in combating COVID-19. However, TCM faces many challenges in terms of being recognized around the world. Therefore, evidence-based research is crucial to the development of TCM.

Keywords traditional Chinese medicine, COVID-19, evidence-based research

Coronavirus disease 2019 (COVID-19) broke out in 2019 and spread rapidly around the world, causing a global pandemic. As of June 30, 2020, more than 10 million confirmed cases of COVID-19 have been documented globally. Unfortunately, there is still no specific vaccine or drug approved for the treatment of COVID-19 (*1*). Traditional Chinese medicine (TCM) has a history of more than 2,000 years in the prevention and treatment of epidemics and plagues. In light of its experience treating SARS and H1N1 influenza, the National Health Commission (NHC) has recommended TCM as a strategy for COVID-19 treatment.

As of March 4, 2020, the NHC has issued 7 versions of its guidelines for diagnosis and treatment of COVID-19. TCM to prevent and control COVID-19 has been included since the 3rd version. Based on the current results, TCM has displayed some efficacy in combating COVID-19 (*2*). The public is well aware of the clinical efficacy and safety of TCM for treatment of COVID-19, and numerous studies in that respect have been conducted. As of June 20, 2020, more than 1,000 articles are available on PubMed when searching with the keywords "COVID-19" and "Chinese medicine", and about 150 clinical trials on TCM for treatment of COVID-19 (20.0% of the total trials) have been registered on the Chinese Clinical Trial Registry (*3*).

According to data presented by the National Administration of Traditional Chinese Medicine on March 23, 2020, 74,187 patients diagnosed with COVID-19 (accounting for 91.5% of the sample) have used TCM in China, and the total efficacy of TCM is as high as 90% based on observations (*4*). Moreover, "three patented Chinese medicines and three TCM prescriptions" have proven to be efficacious in treating COVID-19. The three patented Chinese medicines are Jinhua Qinggan granules, Lianhua Qingwen capsules, and XueBijing injections, and the the three TCM prescriptions are a Qingfei Paidu decoction, a Huashi Baidu decoction, and a Xuanfei Baidu decoction (*5*).

Jinhua Qinggan granules have been found to have equivalent efficacy to that of oseltamivir in combating H1N1 influenza (*6*). Patients with COVID-19 taking Jinhua Qinggan granules recovered faster than patients who did not take the granules, testing negative for coronavirus more than two days sooner (*7*). Therapeutic efficacy was significantly higher in patients with COVID-19 taking Lianhua Qingwen capsules (n = 147) and Arbidol (umifenovir) (n =148) than that in patients taking Arbidol alone (80.95% vs. 64.86%, p = 0.002), and the conversion rate to severe disease in patients taking the capsules was significantly lower than that in patients taking Arbidol alone (14.29% *vs*. 23.65%, p = 0.040) (*8*). A Qingfei Paidu decoction has also displayed noteworthy efficacy in treating COVID-19. Two hundred and fourteen patients with confirmed COVID-19 in four provinces took 3 courses of the decoction (*9*). More than 60% of those patients displayed obvious improvement in symptoms

and computed tomography (CT) findings, and the remaining 30% were in stable condition without disease progression.

Although TCM has played an important role in fighting COVID-19 in China, it faces many challenges in terms of being recognized around the world. For example, the journal "Nature" not long ago published an article on its website questioning traditional Chinese medicine; David Cyranoski thought it was dangerous to support therapies that have yet to be proven safe and efficacious (*10*). Therefore, quality clinical trials and basic research must be conducted in order to advance TCM.

In comparison to the process of treatments being developed "from the laboratory to the clinic" in Western medicine, TCM has first found to be effective in its long history of clinical use, following an opposite process "from the clinic to the laboratory". The gradual development of TCM is based on the constant accumulation and summarization of experience in clinical practice. With the development of evidence-based medicine, the rapid transition of and transformation from experience-based medicine to evidence-based medicine have become crucial to the development of TCM. Therefore, a series of prospective cohort studies or randomized controlled trials (RCTs) should be conducted to evaluate the efficacy of TCMs, yielding reliable evidence for use of TCM to prevent or treat COVID-19 or similar emerging respiratory infectious diseases in the future (*11*). Moreover, well-designed *in vitro* cell experiments and *in vivo* animal studies need to be conducted and multiple modern technologies, including molecular biology, proteomics, metabolomics, genomics, and network pharmacology, should be used to confirm the ingredients, targets, and mechanisms of TCM (*12*). In addition, researchers should look into the safety of TCM (*13*).

In conclusion, more solid scientific grounds are needed if TCM is to play a bigger role on the world stage, and TCM still has a long way to go. With hope, further research will study the standardization, evaluate the safety, and explore the mechanism of action of TCM in the treatment of COVID-19 and other diseases. TCM and Western medicine should be able to complement one another and improve health worldwide.

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Letter

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Devoting attention to the management of community-acquired pneumonia for the elderly

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SUMMARY Community-acquired pneumonia (CAP) is the third leading contributor to lost disability-adjusted life years worldwide, and this is especially true in the elderly population. In order to reduce the burden of disease, effective management of CAP is crucial to public health in terms of maintaining and promoting the health of the elderly and involves safe drug use, vaccinations, early treatment in the ICU, and health education. Since the long-term mortality of CAP is particularly high in the elderly, biomarkers and a predictive diagnostic model of CAP should be developed in future research.

Keywords community-acquired pneumonia (CAP), management

Community-acquired pneumonia (CAP) causes significant morbidity and mortality, and this is especially true in the elderly population. This represents a growing problem for an aging population and an era of longevity. One study in Japan found that the median treatment costs for CAP were ¥536,538 for inpatients and ¥38,490 for outpatients (*1*). In addition, the median total healthcare costs for CAP were $11,549 (IQR: $6,060-21,613) for patients who were ≥ 65 years of age, with an excess cost of approximately $9,500 per year due to CAP (*2*). Therefore, effective management of CAP the elderly, including the clinical manifestations of atypical pneumonia, methods of assessing the severity of the disease, appropriate care settings, and the management of complications, is of paramount importance.

Although patients with CAP are generally expected to briefly recover to their state before contracting pneumonia, many patients still suffer from a loss of functional independence and a severe deterioration in health status for a long time after diagnosis. In order to reduce the burden of disease, effective management of CAP is crucial to public health in terms of maintaining and promoting the health of the elderly.

Safe drug use: For the elderly, drug safety is very important. Adverse reactions caused by irrational drug use accounted for 1/7 of the causes of death in the elderly, with an incidence of 15-20%. This can lead to an increase in drug-related diseases and also cause a decline in the quality of life for the elderly. A retrospective and observational study reviewed data on the history of disease in 632 elderly patients with CAP over a period of 5 years in the Kyrgyz Republic (*3*). Results indicated that 33.5% of patients took drugs with adverse drug reactions (ADRs) that exceeded their benefit and that 24.5% of patients took drugs with a high level of danger. Therefore, the treatment of CAP must consider the aging process, the variability of the effects of drugs, and the principle of rational use of drugs to improve the efficiency, safety, and individualization of drug treatment.

Vaccination: Given the high treatment costs of CAP and its risks of hospitalization in the elderly population, priority must be given to prophylactic vaccination against Streptococcus pneumoniae. S. pneumoniae is the most common pathogen causing CAP both in elderly and younger adult patients. According to CDC guidelines, the pneumococcal polysaccharide vaccine is recommended for all adults older than 65 years of age and for persons who are 2 years and older and at a high risk for contracting pneumococcal disease (*4*). In addition, the influenza vaccine can both prevent primary influenza pneumonia as well as secondary bacterial pneumonia in the elderly. Large cohort studies have indicated that vaccination against influenza can significantly reduce the risk of influenza infection and mortality in elderly patients, although the effect varies depending on comorbidities and demographic factors. Although there have been concerns about the protection provided by and the safety of vaccination in patients with poor immune function or who have only recently recovered from pneumonia, the two vaccines are still recommended.

Early treatment of extremely elderly patients

in the ICU: Elderly patients often have endocrine or cardiovascular conditions, chronic obstructive pneumonia, or other chronic diseases, causing organ reserve function to decline. The organ reserve function of the elderly over 80 years of age is more than 50% lower than that of normal adults (*5*). The repeated use of antibacterials increases drug resistance, and a variety of bacteria often invade the body simultaneously, resulting in infection; at the same time, the use of some drugs is limited due to the reduced functioning of various systems. The probability that elderly patients will develop pneumonia increases further when the patient's condition deteriorates, the patient suffers major trauma (traffic accident, surgery, severe stress, *etc*.), or the patient receives chemotherapy drugs or immunosuppressants. The physical characteristics of elderly patients with CAP make them susceptible to developing severe pneumonia. Moreover, elderly patients with severe pneumonia deteriorate rapidly progress and they have a poor prognosis and high mortality. Therefore, treatment of elderly patients with pneumonia in the ICU should be considered.

Health education for the elderly: Health education is a teaching process that changes the cognition and behavior of patients and family members through a change in knowledge, attitudes, and beliefs and that facilitates the acquisition of certain skills. Systematic education of care recipients to foster physiological, psychological, and social adaptability is an organic union of knowledge, practices, and principles (*6*). Health education is a treatment factor that can improve a patient's understanding of a disease and compliance with care, that can improve the treatment of disease and reduce complications, and that can improve the quality of life and ability for self-care of patients. At the same time, health education strengthens the relationship between nurses and patients, reducing the incidence of medical disputes and increasing the trust in and satisfaction with nursing that patients and their families have.

Expectations for research on CAP: The long-term mortality of CAP is particularly high in the elderly, so CAP needs to be effectively prevented in the elderly. Biomarkers play an important role in the mortality of and prognosis for elderly patients with CAP (*7*). Although the role, utility, and effectiveness of each biomarker need to be determined, they will help to determine the prognosis for and care of elderly patients with CAP and to determine whether admission is necessary and the most appropriate treatment for these vulnerable individuals. In addition, the creation of a predictive diagnostic model of CAP has also had a positive effect on the management of CAP in the elderly (*8*).

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