WELBLU CLINICAL SUMMARY

Potential for Treatment of New Hybrid Covid -19 Variant Infection based on Mechanism of Action.

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Abstract

Forty-nine patients who were positive for SARS-CoV-2 were treated using. Three viral genes (gene RdRP, gene E, and gene N) were detected to follow the viral presence's positivity. The average of the patients tested negative after three days of treatment.

The control group, ninety-five patients without WELBLU treatment, who receive the traditional treatment for COVID, have an average of 21 days to tested negative for the SARS-CoV-2 virus. In the WELBLU group, only two patients were positive for six days (the maximum lapse of positivity); on the other hand, one person was positive for 49 days in the control group. The goal of the study was to determine the efficacy of WELBLU (Liposomal Methylene Blue with microparticles) and Photodynamic Therapy as a possible treatment in patients with COVID-19 for the eradication of the virus.
This Studywas aimed to determine if WELBLU has an effect on the positivity or negativity to SARS-CoV-2 in outpatients with viral Ct lower to 32. And to propose WELBLU as a novel COVID-19 treatment.

Material and Methods.

Patients groups:

Group A (experimental group): 49 Patients with the following characteristics were included: at least 1 of the following symptoms: headache, nausea, dyspnea, myalgia, vomiting. Likewise, that they met the inclusion criteria and none were for exclusion or elimination.

Group B (control group): 95 Patients were selected with the same characteristics as the intervention group.

The study was carried out in the Adult Emergency Area (Covid Area) assigned for this purpose by previously assigned and trained personnel, at the Lic. Adolfo López Mateos Regional Hospital and at the Ignacio Zaragoza Regional Hospital, both belonging to the Institute of Social Security and Services for State Workers (ISSSTE) in Mexico City, and in the BSL-3 at the Biomedical Innovation Department of CICESE.

In both groups, the clinical history was taken, after a detailed explanation of the protocol, if the patient met the inclusion criteria, an invitation was made, if the patient accepted, signed the informed consent. All patients could receive, at the discretion of their treating physician, a conventional treatment that could include analgesics, anti-inflammatories, antibiotics, steroids, antiplatelet agents and anticoagulants.

RT-PCR Test

A Nasopharyngeal and buccopharyngeal sample were taken from each patient using a swab. Bout swabs were placed in viral transportation medium for the RNA extraction. The obtained RNA was used as a template in the reverse transcription and amplification of the SARS-CoV-2 E gene (Corman et al., 2020). For this amplification, qPCRBIO probe 1 step Go No-ROX (PCR biosystems) kit was used in a final volume of 20 µl. Amplification conditions were: Retro-transcription at 50 °C for 15 min, denaturation at 95°C for 2 min, followed by 45 cycles for 15 s at 95°C, 30 s at 60 °C acquiring the fluorescence at this step. The RT-qPCR reactions were conducted using a 96-well and optical adhesive film (Bio-Rad, CA, USA) on a CFX96 Real-Time PCR Detection System (Bio-Rad).

The patient was referred every 24 hours and the procedure was repeated at the same time until the RT-PCR was negative (maximum seven days).

Inclusion criteria

People over 18 years of age, of both sexes. With a diagnosis of Covid-19 corroborated by PCR and CT less than 32 in gene E. With at least 1 of the following symptoms: fever, headache, nausea, dyspnea, myalgia, vomiting, diarrhea, anosmia, ageusia, or dysgeusia. With Oxygen Saturation> 90 by oximetry. No history of allergic reaction to methylene blue.

Exclusion criteria

Diagnosis of cancer at any stage and of any type. Treatment with immunosuppressive drugs (cyclophosphamide, azathioprine, mycophenolate, cyclosporine, chlorambucil, tacrolimus and rapamycin). Decompensated comorbidities (diabetes and ischemic heart disease). Patients with severe renal and hepatic impairment (estimated glomerular filtration rate <= 30 ml / min) or patients receiving continuous renal replacement therapy, hemodialysis, peritoneal

dialysis. Immunosuppressed patient: all patients with a history of HIV positive, a history of any autoimmune inflammatory rheumatic disease, and a history of kidney transplantation were considered immunosuppressed. Pregnant or lactating women.

Ethical considerations.

The project was an investigation of greater risk to the minimum and adhered to the provisions of the General Health Law and its regulations on Research Matters and Guidelines of the International Conference on Harmonization on Good Clinical Practices. Participation was voluntary and confidential. The project was also submitted to the Ethics and Research Commissions of the hospitals that were selected for the study.

WELBLU treatment.

Once the diagnosis of Covid-19 was confirmed by the positive RT-PCR test with a CT lower than 32, the blood was drawn to perform the laboratory tests described above.

The protocol was started on day 1 with the sublingual **WELBLU** treatment, which consisted of: Taking a nasopharingeal sample for RT-PCR and CT measurement, then the patient was placed with a photodynamic device on wrist and 1 mL of the previously **WELBLU** activated. **WELBLU** activation was done using Low Level Light Therapy by 10 minutes.

Activated **WELBLU** solution applied sublingually, keeping it under the tongue for 10 minutes before swallowing. Simultaneously, light phototherapy was started on wrist for 50 minutes.

Once this stage had started, the patient was summoned every 24 hours at the same time for seven days and the procedure was repeated until the RT-PCR test was negative.In the event that the patient was a woman of reproductive age, a rapid pregnancy test was requested before starting treatment. If this was positive, the patient was no longer be part of the protocol.

Analysis of data.

Pairwise comparisons were made between the intervention group and the control group using parametric or non-parametric hypothesis tests based on the distribution of the data and the fulfillment of the assumptions of the models.

RESULTS.

Forty-nine positive patients for SARS-CoV-2 were treated using **WELBLU**. An RT-PCR test was performed every day to determine if the patient was still positive. Most of the patients turn negative after two days of treatment with **WELBLU** (figure 1 A, B, and C).

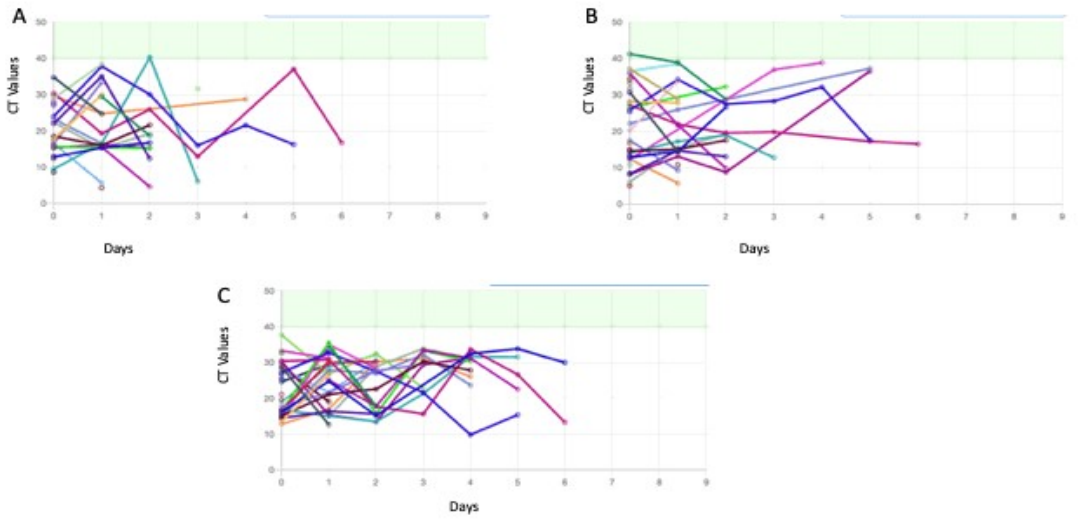


Fig 1.Days of positivity of the **WELBLU** treated patients. A) days of positivity through RdRP viral gene amplification. B) days of positivity through E viral gene amplification; C) days of positivity through N viral gene amplification.

In the control group, ninety-five patients without treatment were analyzed by RT-PCR. They were tested every seven days until they turn negative for all three viral tested genes. The average to have a negative test was twenty-five days. One patient was tested positive for seventy-seven days, and another one was positive for sixty-four days (data not shown).

There was a statistical difference between the treated group and the untreated group (control). As we can see in figure 2, the treated group showed a small difference between individuals. The negative control group showed an open range between three and fifty-five days, with an average of two days for the treated group and twenty-five days for the untreated group.

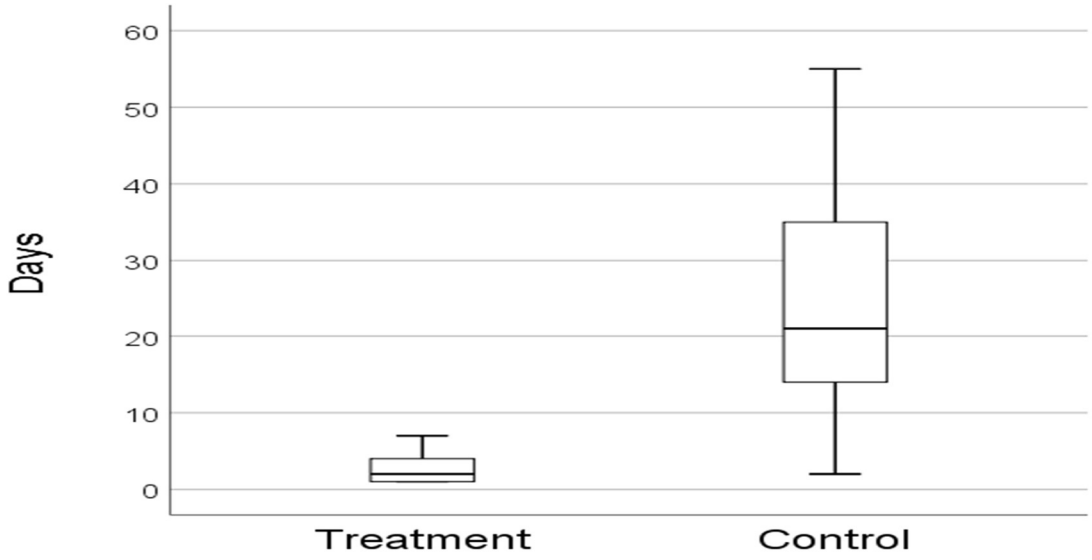


Figure 2. Distribution of positive days for the treated group and the untreated group (control).

Discussion:

The treatment of 49 COVID-19 positive outpatients with **WELBLU**, shows a significant difference with the non-treated group. We have analyzed three different viral genes; gene E, gene RdRP, and gene N. All of these genes show a fast elimination of the virus in the treated group. For gene E, the average of negativity was 1.5 days; for gene RdRp the average was also 1.5 days, and for gene N the average was three days. 48% of the patients were male, and 51% were female. There was a nonstatistical difference in the treatment between male and female

We can determine that the time to get negative to SARS-CoV-2 was three days when WELBLU was used as a treatment. On the other hand, the non-treated group has a negative average of 25 days, this is consistent with previous works (Rattan &Ahmad, 2019). As we mentioned before, **WELBLU** group shown a small difference of days that they were positive, and the untreated group, exhibit a wide range of distribution, just like it was reported previously (Cowling&Leung, 2020).

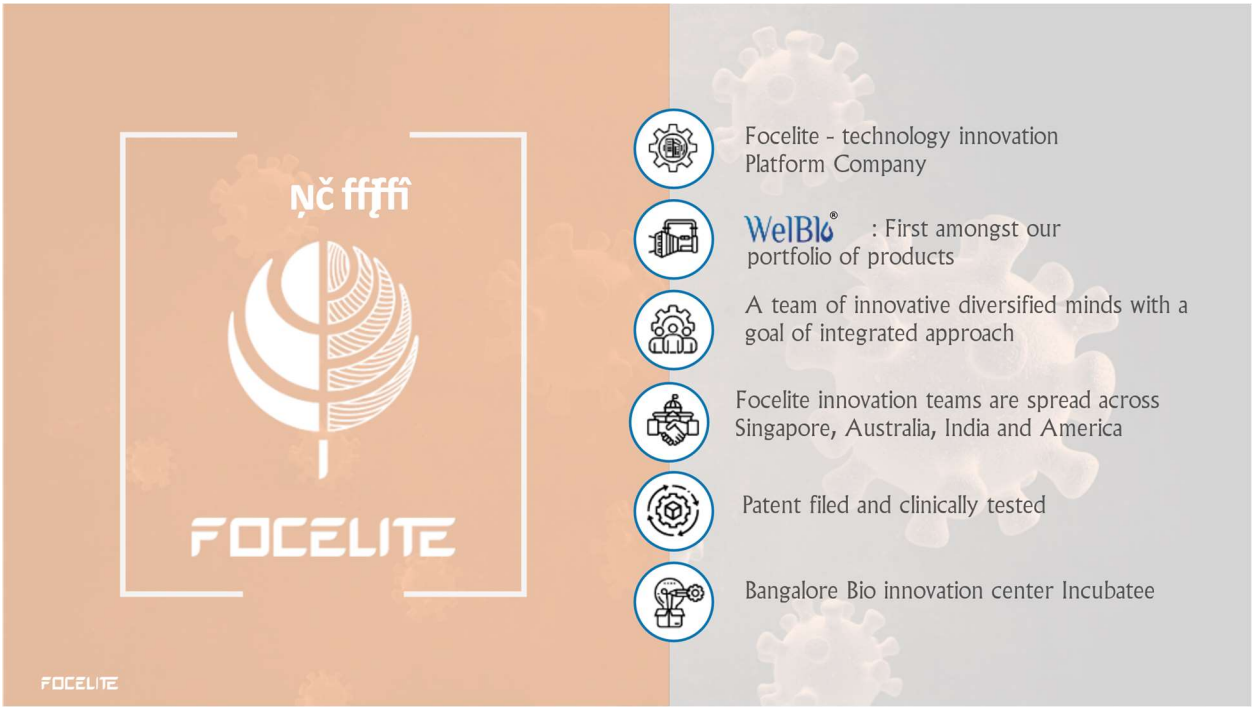
Just like normal infected persons, all the untreated patients have presented at least one of the following symptoms for at least two weeks: fever, headache, nausea, dyspnea, myalgia, vomiting, diarrhea, anosmia, ageusia, or dysgeusia (Cowling & Leung, 2020; Wu & McGoogan, 2020). On the other hand, most of the patients treated with **WELBLU** didn´t present any COVID-19 symptoms one day after the

treatment's first application. None of the treated group died for COVID-19 causes, while the untreated patients around the world, show several dead's (Baud *et al.*, 2020;Wu & McGoogan, 2020).

After the **WELBLU** treatment, the most relevant result is that none of the 49 treated patients were hospitalized, and none of them need external oxygenation. With this, we can conclude that **WELBLU** can be used as an effective treatment for COVID-19 that turns negative all the outpatients, even with an initial Ct value of 12 for viral genes. **WELBLU** treatment can considerably

- Potential Therapy for future variant of COVID infection based on mechanism of action.
- Reduce the number of hospitalized patients and reduce patients side effect, with the advantage of cost reduction to the National Health System.

Further studies with hospitalized patients are required to determine the effectiveness of **WELBLU** in patients with severe symptoms.



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