

## POTENTIAL EFFECT OF INTERFERON AND TREATMENT RECOMMENDATIONS AGAINST COVID-19

The antiviral effect of alpha interferon has been demonstrated whereas it has long been considered the first line of antiviral defense. Alpha interferon activates both the innate immune response against the virus and the inhibitory mechanism of viral replication, accounting for interferon-inducing genes and depressing the expression of the pathway JAK-STAT (*WM Schneider, et al. Annual Review of Immunology. 2014; 32: 513-545*).

Little is known about the effect of interferon specifically against coronavirus, although it is common for its known mechanism of action to be used against viral infections for which no therapies are available.

It has been reported that SARS-CoV reduces interferon expression by preventing INTERFERON-inducing genes such as STAT1 and MyD88 from being activated and that antiviral defense mechanisms detect the presence of the virus.

SARS coronavirus is known to efficiently suppress the induction of interferon through inhibition of the transcription factor IRF-3. Pretreatment of cells with interferon partially overturns the block of interferon expression imposed by the virus, thus restoring antiviral effector proteins, viral antigen presentation to the immunological system and other cytokines and chemokines (*Thomas Kuri. et al., Journal of General Virology (2009), 90, 2686–2694*).

The expression of NF- $\kappa$ B in turn mediates the expression of proinflammatory cytokines IL-6 and IL-8 and it has been reported that after SARS-CoV infection the expression of NF- $\kappa$ B increases as fast as at 12 hours (*T. Yoshikawa, et al. PLoS ONE, 5 (2010), p. E8729*). On the other hand, in a macaque model it has been shown that the induction of NF- $\kappa$ B is increased in adult monkeys associated with lung damage, than in young monkeys where, however, the high early expression of interferon is superior (*SL Smits, et al. PLoS Pathogens, 6 (2010), p. e100075*).

It has been shown that induction of interferon protects against MERS-CoV infection. Blocking INTERFERON-I prolongs virus clearance and increases the inflammatory response and decreases the T-cell-mediated cellular response. Administration of INTERFERON-I during the first day after inoculating mice with the virus (before the virus is reached peak of viral multiplication), prevented the death of the mice producing a subclinical infection. A late administration of the INTERFERON had no effect nor was it able to decrease infiltration by neutrophils, monocytes and macrophages into lung tissue. It failed to decrease the increase in proinflammatory cytokines or decrease the viral load, developing as a consequence pneumonia that led to the death of the mice (*R. Channappanavar, et al. J Clin Invest. 2019; 129 (9): 3625-3639* . <https://doi.org/10.1172/JCI126363>).

Recent articles have also shown that early interferon expression favors rapid induction of the neutralizing antibody response (*Marco de Giovanni, et al. Nature Immunology 2020*).

Another recent paper identify key differences in susceptibility to the Interferon response between SARS-CoV and SARS-CoV-2. The novel CoV is much more sensitive to Interferon pretreatment. Examining transcriptional factor activation and interferon stimulated gene (ISG) induction, SARS-CoV-2 in the context of type I interferon induces phosphorylation of STAT1 and increased ISG proteins. In contrast, the original SARS-CoV has no evidence for STAT1 phosphorylation or ISG protein increases even in the presence of type I interferon pretreatment. ([Doi: https://doi.org/10.1101/2020.03.07.982264](https://doi.org/10.1101/2020.03.07.982264))

Volunteers treated with an intranasal recombinant interferon or placebo and exposed to coronavirus and prophylactic intranasal interferon administration, effectively showed to shorten the duration and severity of coronavirus cold symptoms infection (*RB Turner., et al. The Journal of Infectious Diseases 1986: 154(3):443-447*). (*PG Higgins et al., Antimicrobial Agents and Chemotherapy. Nov 1983, p. 713-715*).

It is also known that as recommended by *Acta Obstet Gynecol Scand 2020; 00;1-4* in its article *Novel coronavirus disease (COVID-19) in pregnancy: What clinical recommendations to follow?*; it is stated nebulized interferon is recommended for pregnant women. In line with the above, *World Journal of Pediatrics Feb 2020* in its articles *“Diagnosis, treatment and prevention of 2019 coronavirus infection in children: experts’ consensus statement* and *“Pharmaceutical care recommendations for antiviral treatment in children with coronavirus disease 2019”* - recommended the use of nebulized interferon alpha 2 b.

The reference of use that we have received from our representation in China is that the interferon alpha 2b produced by the Cuban-Chinese Joint Venture “Changheber” (Changheber interferon) has been used nebulized and combined with other antivirals. In the case of the Changheber interferon, it has been used to reinforce the innate response mainly from medical personnel who are coping with the epidemic in a preventive manner.

It is also noteworthy, that several ongoing clinical studies are reported where this interferon is being administered to aerosolized nebulized patients and a study such as a nasal spray. However, these studies have not yet reported. Irrespective to the lack of rigorous efficacy trials demonstrating the effect of interferon to treated advanced ill-patients, it is claimed by Chinese and Cuban doctors the improvement of patients’ clinical outcome. The recommendation of the interferon for the treatment of coronavirus infected patients is included in several guidelines (*Recommendation guidelines of China, WHO, Singapore, South Korea, Spain, John Hopkins Medical Center, The Join Report of the WHO, and Chinese Commission for the epidemic follow up held from February 16-24*). *Interferon was also recommended by the Experts Committee for the treatment of COVID-19. The committee was integrated by the Group of Respiriology, Chinese Pediatric Society,*

*Chinese Medical Association, Chinese Medical Doctor Association Committee on Respiriology Pediatrics, China Medicine Education Association Committee on Pediatrics, Chinese Research Hospital Association, Committee on Pediatrics · Chinese Non-government Medical Institutions Association Committee on Pediatrics, China Association of Traditional Chinese Medicine, Committee on Children's Health and Medicine Research, China News of Drug Information Association, Committee on Children's Safety Medication, Global Pediatric Pulmonology Alliance, in a recent article of World Journal of Pediatrics dated on January 29. <https://doi.org/10.1007/s12519-020-00343-7>).*

The report of an open clinical study conducted in Wuhan, where Interferon was administered in nasal drops (2-3 drops / nostril / time, 4 times / day) for 28 days, to low-risk medical personnel (2415) and to those in the high-risk group (529) received rhIFN- $\alpha$  nasal drops combined with thymosin- $\alpha$ 1 (1.6 mg, hypodermic injection, once a week). The results were compared with the number of new cases in medical staff in the same areas of Hubei Province. The 28-day incidence of COVID-19 was zero in both the high- and low-risk groups. As controls, a total of 2035 medical personnel with confirmed COVID-19 pneumonia from the same area (Hubei Province) were observed. (Doi: <https://doi.org/10.1101/2020.04.11.20061473>)

Study conducted at Hôpital Cochin in Paris concluded that type-I Interferon deficiency in the blood is a hallmark of severe Covid-19 and could identify and define a high-risk population. (DOI: <https://doi.org/10.1101/2020.04.19.20068015>)

By the previous references the following treatments are recommended:

1. Interferon- $\alpha$  nebulization: interferon- $\alpha$  200,000–400,000 IU/kg or 2–4  $\mu$ g/kg in 2 mL sterile water, nebulization two times per day for 5–7 days.

2. Interferon- $\alpha$ 2b spray: applied for high-risk populations with a close contact with suspected 2019-nCoV infected patients or those in the early phase with only upper respiratory tract symptoms. Patients should use 1–2 sprays on each side of the nasal cavity, 8–10 sprays on the oropharynx, and the dose of interferon- $\alpha$ 2b per injection is 8000 IU, once every 1–2 hours, 8–10 sprays/day for a course of 5–7 days.

According to the Spanish guides issued on February 18<sup>th</sup>, it is recommended the use of nebulized interferon- $\alpha$ 2b 100.000–200.000 UI/kg for mild cases and 200.000–400.000 UI/kg for severe ill patients twice a day for 5–7 days.

Singapore National University in its indications of March 12, 2020 –it is also accrued the Chinese experience with interferon in its combined use with Kaletra (mixture of protease inhibitors: Lopinavir/Ritonavir).

In the report on the course of treatment to Covid19 by the Chinese authorities "Novel Coronavirus Pneumonia Diagnosis and Treatment Plan (Provisional 6th Edition)" ([https://www.chinalawtranslate.com/en/diagnostic-and-treatment-plan -6 /](https://www.chinalawtranslate.com/en/diagnostic-and-treatment-plan-6/)), dated 2020/02/19, expresses the following:

1. Treatment for mild cases includes bed rest, supportive treatments, and maintenance of caloric intake. Pay attention to fluid and electrolyte balance and maintain homeostasis. Closely monitor the patient's vitals and oxygen saturation.
2. As indicated by clinical presentations, monitor the hematology panel, routine urinalysis, CRP, biochemistry (liver enzymes, cardiac enzymes, kidney function), coagulation, arterial blood gas analysis, chest radiography, and so on. Cytokines can be tested if possible.
3. Administer effective oxygenation measures promptly, including nasal catheter, oxygen mask, and high flow nasal cannula.
4. Antiviral therapies: **Interferon-alpha (adult: 5 million units or equivalent can be added to 2ml sterile water for injection and delivered with a nebulizer twice daily)**, lopinavir/ritonavir (adult: 200mg/50mg/tablet, 2 tablets twice daily; the length of treatment should not exceed 10 days), ribavirin (recommended in combination with interferon or lopinavir/ritonavir, adult: 500mg twice or three times daily via IV, the length of treatment should not exceed 10 days), chloroquine phosphate (adult: 500mg twice daily; the length of treatment should not exceed 10 days), umifenovir (adult: 200mg three times daily; the length of treatment should not exceed 10 days). Pay attention to the adverse effects associated with lopinavir/ritonavir, such as diarrhea, nausea, vomiting and liver dysfunction, as well as interactions with other medications. The efficacy of the current medications in use will be evaluated in clinical application. Using 3 or more antiviral drugs is not recommended. Corresponding medication should be discontinued should intolerable side effects are present.

Finally, the current Cuban recommendation for patients is to use recombinant interferon alfa 2b (Heberon alfa 2b) 3 million of units intramuscular routes thrice a week. At the same time Cuban Ministry of Health approved the use of Heberon alfa 2b as nasal drops to potentiate the immunological response as a preventive administration in the medical staff at risk of infection with COVID-19.

Despite all these evidences, caution should be exercised in the use of interferon in the treatment of severe diseased patients, since the results in clinical studies with the use of interferon in viral pathogens have been inconsistent, including coronavirus infections. A pathogenic effect has also been reported in acute and chronic infections. In particular, interferon treatment of MERS patients failed to increase survival (*S. Shalhoud et al. J Antimicrob Chemother 2015; 70: 2129 –2132 and Al-Tawfig et al. IJID 2014; 20: 42-46*). In another study, the interferon extended life when evaluated at 14 days, but not at 28 days. (*A.S. Onrani, et al. Lancet Infect Dis 2014; 14: 1090–9517*).

**In Conclusions** all these evidences and previous studies suggest the possible usefulness of interferon alpha 2b preventively and at very early stages of infection. Everything seems

to indicate that very early in the infection it works and even better as a preventive, but with the more advanced infection cautious is advised. Reports of combinations with antivirals have not yielded positive results, although in the absence of options they continue to recommend it.

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