RESEARCH BY AN INTERNATIONAL SCIENTIFIC GROUP. COVID-19: LET'S TRY TO UNDERSTAND WHERE WE ARE AND WHAT WE HAVE LEARNED ABOUT THE CORONAVIRUS

Abstract

The ongoing coronavirus pandemic is one of the worst health crises of our century: new estimates from the World Health Organization show that the full death toll associated directly or indirectly with the COVID-19 pandemic between 1 January 2020 and 31 December 2021 was approximately 14.9 million (range 13.3 million to 16.6 million) [3]. Doctors and researchers have discovered many things about the characteristics of the coronavirus, from the way it attacks cells to the disproportionate response of our immune system, which sometimes leads to severe symptoms and requires intensive care. Other aspects of the virus and COVID-19 are still elusive or not fully understood by scientists. Some questions about the coronavirus haven't yet found convincing answers.

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Where does the coronavirus come from?

After months of study and analysis, most researchers agree that SARS-CoV-2 originated in "horseshoe" bats (genus Rhinolophus), in a region of China, perhaps Yunnan, or another. Asian country like Myanmar, Laos or Vietnam.

Based on the genetic characteristics of the current coronavirus and other similar viruses, the researchers suspect that SARS-CoV-2 may have passed on to us after an intermediate transition to another species. In recent months there has been much talk of pangolins, animals whose scales are used for some preparations in China and which are often found in wild species markets, despite being a protected species. Some studies have detected the presence of coronaviruses that share an ancestor with SARS-CoV-2, but this does not imply that there has been a passage of the virus from pangolins to humans.

The hypotheses about the Wuhan laboratory

Another hypothesis about what could have caused the pandemic is a laboratory accident or rather the accidental escape of coronavirus-infected bats by the National Biosafety Laboratory in Wuhan, certified according to BSl-4 standards and criteria (level biosecurity 4 for the study of the world's most dangerous pathogens and emerging diseases). Coronavirus experiments on bats captured in Yunnan caves are known to have been conducted in the virology institute of this center, funded by a US government $ 3.7 million grant. Also, according to the Washington Post, in 2018 some US diplomats in Beijing had written a dossier on the Wuhan research center highlighting the danger of studies conducted on bat coronaviruses and the risk of a pandemic [2].

It would not be the first time that an epidemic has arisen from laboratory experiments (as in the case of Marburg syndrome), however it is a hypothesis that must be verified, otherwise it remains only a hypothesis.

Comparing the trend of Covid-19 and many of the great pandemics of the past, such as Spanish, Asian, avian and swine influences, we can observe that all of them originated in Asia, and then spread to the rest of the world, first passing through the Europe, then in the Americas and finally in Africa, Australia and Oceania.

The probability of transmission and infectivity of the Sars-CoV-2 virus cannot be changed in the absence of adequate therapy or a vaccine, while a timely diagnosis can serve to contain the number of infections.

It is clear that this diagnosis can only be made by means of a Pcr laboratory test (of the "polymerase chain reaction" for the study of viral RNA following nasopharyngeal swab or endotracheal aspiration or bronchoalveolar lavage), but since it is impossible to subject the whole population to these tests, on the recommendation of the World Health Organization (WHO) at first, only those who had had contact in some way with people from China or other countries, where the pandemic initially occurred. Subsequently, due to the lack of the necessary reagents, in Italy (as already in China) it was decided to test only those who had, in addition to the symptoms, high fever for several days without benefiting from the common antipyretics. Finally, there have been cases of deaths with symptoms compatible with Covid-19, but without tests, of elderly people in "nursing homes for the elderly" or of people who died at home (without having received adequate care and subjected to a tampon) or people who had worked in contact with Covid patients. This being the case, it is clear that the death toll of Covid-19 in our country is largely underestimated.

Gender Differences in COVID-19: Possible Mechanisms

Evidence gathered so far explicitly shows that there are important differences in the onset, clinical manifestations, responses to treatments and outcomes of diseases common to men and women. And this also seems to emerge in the context of the COVID-19 pandemic. Indeed, the statistics found around the world speak for themselves: SARS-Cov-2 infection produces different effects in men and women. To explain this phenomenon, some general hypotheses have been advanced, including: a greater tendency of men to smoke (risk factor for contracting the infection and to develop a more serious clinical picture of the disease) a stronger habit of women to devote themselves significant space of their daily life to personal hygiene an immune response, innate and adaptive, more prompt and effective in women than in men. However, we must also highlight the differences between women and men as we begin to get into the mechanisms behind the infection. Differences that can be both hormonal and genetic. The virus responsible for COVID-19 penetrates into our cells by binding to a receptor called ACE2 (Angiotensin Converting Enzyme 2), an enzyme that regulates arterial vasoconstriction and is found on the cells of the lung epithelium where it protects the lung from damage caused by infections. , inflammation and stress. When the virus binds to ACE2 and enters the cell, it decreases its expression and thus removes it from its protective function. In women of childbearing age, estrogens are able to increase the presence of the ACE2 receptor so that this enzyme, even after infection, is able to carry out its protective function, in particular towards the lungs. Conversely, androgens appear to play an opposite role in influencing the expression of cellular enzymes involved in the subsequent phases of the virus attack on the receptor, favoring the later phases of lung cell infection. Finally, it is known that in female cells there are two X chromosomes while in male cells there are one X chromosome and one Y chromosome. In female cells, therefore, to prevent the redundant expression of the products of the genes present in double copies on the X chromosomes, there is a physiological random inactivation of one of the two chromosomes. However, chromosomal portions remain that escape inactivation and the genes present in these areas may be overexpressed in women. ACE2 is encoded precisely in these regions of the X chromosome that escape the inactivation of one of the two X chromosomes, thus supporting the hypothesis of a greater expression of this protein in the lungs of women. Over time, it will therefore be important to carry out specific studies, even retrospective ones, to evaluate the role of sex hormones in the differences.

Why do people react in such different ways?

In some infected people, the coronavirus (SARS-CoV-2) does not seems to cause any kind of problems and does not lead to the manifestation of symptoms, while in other individuals it can lead to serious complications, with serious breathing difficulties that in some cases can be fatal.

A research conducted on about 4 thousand people in Italy and Spain has made it possible to identify some genetic characteristics that, according to the authors, could provide an answer to the variety with which COVID-19 presents itself. Patients who developed severe respiratory symptoms were more likely to have two genetic variants, absent in people who did not develop symptoms. A variant concerns the mechanisms that determine one's blood group, while another concerns a protein in cell membranes, which is exploited by the coronavirus to evade the cell's defenses, inject its own genetic material into it and then start the processes by which multiplies.

However, these variants do not appear to have the potential to determine the severity of COVID-19 on their own. For this reason, other researchers are working to study the genetic characteristics of individuals who have become ill, but who are less than 50 years of age and should therefore be less at risk than older people.

Can one think of the fact that the activity of a virus depends, yes, on its properties, but also and perhaps above all on the characteristics of the host, that is, of the man who is infected?

In Italy, the hypothesis was made of a possible link between the spread of the coronavirus and atmospheric pollution by relating the high concentration of the virus in the Po Valley and the pollution that characterizes it, this area being recognized as one of the most polluted. in Europe. Here the air pollution could probably act both as a vector, facilitating the transport of the virus, and as an amplifier of its effects on the lung. A study was recently published by Harvard researchers (Xiao Wu, 2020) in the United States, which highlighted the relationship between long-term exposure to Pm2.5 and the risk of death from Covid-19. There would be an excess of mortality of 15% of the total population, for an increase of 1g / m3 in the atmospheric concentration of Pm2.5. This could explain the greater spread of the virus in the northern regions, compared to the rest of Italy.

The theory has long been highlighted according to which in the inhabitants of more polluted areas, in whose atmosphere a very fine particulate matter (pm 2.5) abounds, ACE2 is overexpressed, a defense protein which, however, has also proved to be the main gateway to SARS Cov-2 in host cells. A protective action against pollution could, therefore, have created greater accessibility to the pathogen in some more particularly exposed individuals, compared to others residing in less polluted areas.

To provide a further contribution of sustainability and credibility to this reasoned and "personalized" approach to the diversified incursions of the new Coronavirus, there is a further very recent evidence gained in the Netherlands where some researchers, studying the trend of viral infection in pairs of siblings severely affected by the coronavirus despite their young age, they discovered a very clear link between the expression of a gene (TLR7) that plays a strategic role in the functioning of the immune system and the severity of the patient's response to SARS Cov-2. Basically, given that the TLR7 (Toll-like receptor 7) gene triggers in humans the mechanisms that lead to the production of so-called interferons, that is, those proteins that play a leading role in the defense against viral infections, we have given that subjects with malfunctioning TLR7 genes, as it was in the case of the brothers examined, produce few interferons with consequent inadequate response of the immune system to the attack of the new coronavirus. It is clear that such finding attributes a strategic role in the affirmation of the disease to the genetic peculiarities of individuals rather than to the viral agent.

Harmful and protective role of ACE inhibitors

It should be noted that, in both cases, these are still pure hypotheses under study and that, therefore, it is absolutely not appropriate for patients suffering from various cardiovascular problems or arterial hypertension to suspend, or worse still, interrupt the treatment. Currently, a clinical study has been approved in which the possibility that the administration of recombinant ACE2 protein plays a role in restoring the renin-angiotensin-aldosterone axis will be evaluated, thus contributing to the containment of lung damage in patients with COVID-19. In the

meantime, however, a suppression of the therapy for hypertension, for unfounded reasons, could result in damage that must absolutely be avoided.

By reviewing the available evidence, some researchers from the University of Salerno confirmed the hypothesis that ACE2 represents the vector of the virus within cells. But, in their article published in the scientific journal Frontiers in Pediatrics, they also brought to light a hitherto little-considered possibility. In addition to being a receptor anchored to the cells, the ACE2 protein is present in the blood, in a soluble form. This second "face" of the enzyme would be formed in the synthesis process of ACE2: at the end of the translation process, which converts the RNA into the finished and finished protein. Unlike that present on the cell surface, ACE2 circulating in the blood could be an "ally" in case of infection. With a mechanism reminiscent of that of neutralizing antibodies, the soluble form of the ACE2 receptor could intercept the circulating virus and prevent or at least attenuate its binding to the cell, which instead exposes the membrane form of the ACE2 protein.

The potential for transmissibility: The R0 parameter

An important parameter that allows us to evaluate the progress of the infection is R0, or the basic reproduction number, which indicates the data of secondary infections that an infected individual can transmit in a population completely susceptible to a new pathogen (in this case SARS-CoV-2). It measures the potential transmissibility of the disease in the absence of containment measures. The higher the R0, the more easily the infectious disease can spread: if R0 is equal to or greater than 2, it means that each positive infects two people on average; an R0 of less than 1 indicates that the epidemic can be contained. According to the WHO, the R0 value of Covid-19 is between 1.3 and 3.8 based on data collected by research institutions around the world. That of Sars was between 2 and 4 with an average of about 3 and that of Mers less than 1.

This parameter depends on the probability of transmission by single contact between an infected person and a susceptible person, the number of contacts of the infected person and the duration of the infectivity (which, according to Chinese studies, can reach up to 50 days, while here in Italy a Bolognese girl tested positive until day 75). After the introduction of containment measures, it is preferred to use RT, which indicates the number of secondary infections that can occur, after these measures have been introduced in a specific territory.

Epidemics and Pandemics: The triggers

What are the factors that can trigger an epidemic / pandemic? One of these is certainly the colonization of a new environment, especially following deforestation. Indeed, this brings humans into contact with potential wild animals, reservoirs of viruses that they would not normally encounter. Another factor is urbanization which often leads to a high population density and is characterized by pockets of poverty on the outskirts of large cities. Poor personal or environmental hygiene often due to the lack of adequate sewage systems and / or drinking water. The loss of immunity in a population ("herd immunity") due to the reduction in the number of vaccinations or the mutation of pathogens. The abuse of antibiotics with the consequent emergence of resistance to them. Lifestyle change which can include drug abuse, new sexual and / or dietary habits. The globalization of trade and travel in general which makes every part of the world reachable in a few hours, contributing to the spread of vectors of etiological agents everywhere. The use of pesticides, which has eradicated some diseases in some areas of the world, but

has favored their spread in others, especially following the appearance of vector insects resistant to the same pesticides. Finally, possible laboratory accidents.

In some Italian hospitals such as the Sacco of Milan and the Giovanni XXIII of Bergamo, autopsies were performed and it was thus understood that the greatest damage from Covid is not to the lungs, but to the circulatory system with the formation of thrombus, which slow down circulation blood, which once it reaches the lungs no longer allows proper ventilation. Covid would therefore be an inflammatory blood disease. Thus, drugs that prevent blood clots, such as heparin, can provide invaluable help. The result obtained from the autopsies therefore highlighted that it was a mistake first of all to hospitalize patients only when they had reached the "air hunger" situation and, therefore, to intubate them for mechanical ventilation. According to a Wall Street Journal survey based on British NHS data, 58.8% of intubated Covid patients died. In New York, 88% of the 320 mechanically ventilated patients died. According to a study by the Policlinico di Milano published by Giacomo Grasselli in the Journal of the American Medical Association, almost one Covid patient was intubated on two stumps, this is because mechanical ventilation can worsen pre-existing lung damage.

Looking for antibodies

As for health checks, the most used test is that of the nasopharyngeal swab, which allows a first analysis of the presence of the virus using the PCR (Polymerase Chain Reaction) technique for the identification of viral RNA. A more accurate test is that of the collection of biological samples from the lower respiratory tract (sputum, endotracheal aspiration, broncho-alveolar lavage). Additional biological samples such as blood, urine and faeces are collected to monitor the presence of the virus in various areas of the body. The serum analysis also allows to evaluate the quantity of antibodies to Sars-CoV-2 and thanks to it it is possible to observe the increase in the values of immunoglobulin M (IgM, indicating an infection in the initial state) and immunoglobulin G (IgG, advanced infection). In addition to the rapid sampling of a drop of blood using the lancing device, a method that gives an instant but not always reliable result, the serum sampling is currently carried out with blood sampling in a vein and the analysis is performed with the Elisa method, which results are more reliable, especially since some diagnostic kits test antibodies directed against the spike protein (S1 domain), the most specific and least conserved region of the Covid-19 coronavirus. Other kits identify antibodies against the virus's nucleocapsid protein N, a longer-lasting protein common to other coronaviruses, which commonly infect humans (such as the common cold), so cross-reactivity giving false positives is possible. To obtain a result as reliable as possible, some laboratories have started to test not only IgM and IgG, but also IgA, the antibodies present in the secretions, which play a fundamental role in creating an initial immune response to the virus at the mucosal level including that of the respiratory system.

Do you become immune to the coronavirus?

To date we do not know if our immune system is able to maintain a memory of SARS-CoV-2, after recovery, nor for how long this possible immunity may last. So far, the presence of neutralizing antibodies has been detected — which play a central role in preventing a new infection by blocking some virus proteins — but it has been observed that their levels tend to decrease significantly after a few weeks from becoming infected.

However, there is the possibility that these antibodies will remain present in more significant quantities in people in whom the coronavirus had managed to

replicate abundantly, causing a more serious infection. Something similar was observed with SARS-CoV-1, the coronavirus that causes SARS and has several things in common with the current one: neutralizing antibodies in those recovered from SARS tended to disappear after a few years, but in patients who they had suffered a more consistent infection and lasted much longer, with the possibility of finding its presence even over 10 years from the first contact with the virus.

However, it is currently unclear what is the minimum level of neutralizing antibodies necessary to prevent SARS-CoV-2 from causing a new infection, or in any case to make COVID-19 present with less significant symptoms in a person who was already been ill. Also for this reason, several researchers are orienting their studies towards other mechanisms of the immune system that include other cells, compared to antibodies alone.

However, from current knowledge it seems that the systems to prevent a new infection last a few months, after the coronavirus has occurred. However, the immune system should develop protection to reduce the extent of the symptoms of the disease, in the case of a second infection.

The virus has mutated alarmingly

Viruses change continuously in their replication process, due to errors that can occur in the transcription processes of their genetic material. Although we tend to think of a mutation as something negative, in reality in most cases mutations do not lead to significant changes or changes that make a virus more dangerous: in fact, the opposite is often true.

For months, researchers have been monitoring the way the coronavirus varies and evolves, as it spreads around the world, between different populations and places with different environmental and hygienic conditions. Several studies have reported the presence of a European variant of the coronavirus, derived from that originating in China, which has progressively spread to other areas of the world, becoming the most common.

The variant was studied in the laboratory, where it showed a greater ability to cause infection than other versions of the coronavirus. To date, however, it is not clear whether this feature, found in vitro on cell cultures, also applies to humans.

So what are the drugs that really work?

A key role is that of the excessive inflammation mechanism associated with a cytokine release syndrome. Based on this survey, and with a view to preventing severe forms in patients in home isolation, a therapy proposal to be followed at home, inexpensive and could easily be prescribed by family doctors, was developed. The combination is as follows: hydroxychloroquine (an old antimalarial drug), azithromycin (antibiotic with immunomodulating effect) and celecoxib, an anti-inflammatory with activity against type 2 cyclo-oxygenase (COX-2, an enzyme that determines the release of cytokines).

Two different dosages of hydroxychloroquine have been hypothesized for those under 65 without risk factors and for those over 65 and / or with concomitant diseases. All patients should take potassium and magnesium supplements to avoid the risk of cardiac arrhythmias, a possible side effect of the combined hydroxychloroquine-azithromycin.

Anti-HIV drugs, which are talked about a lot, are not mentioned.

The experience gained in Italian clinical centers regarding the use of lopinavir / ritonavir (and other medicines that act in a similar way) shows, in a relevant percentage of patients, a poor tolerability to therapy and there is also a widespread perception of lack of effectiveness. Lopinavir, as well as darunavir, are drugs designed to target a typical target of the HIV virus, which is not present in Sars-CoV-2.

Management of patients with severe forms of Covid-19

The efficacy of biological drugs (tocilizumab, sarilumab, anakinra), to be administered in the hospital, is being evaluated positively, evaluating each individual case, and under strict medical supervision.

There is a new drug that is added to the list of those potentially effective in combating COVID-19, the infection triggered by the SARS-CoV-2 coronavirus. This is Nafamostat mesylate, a medicine with anticoagulant, antiviral and anti-cancer properties used in Japan for the treatment of acute pancreatitis and other conditions. It is part of the family of so-called protease inhibitors, or viral enzymes that produce proteins, and it is for this reason that these drugs are also used against the HIV virus (responsible for acquired immunodeficiency syndrome or AIDS) and that of hepatitis C (HCV). In the specific case of COVID-19, Nafamostat mesylate would be able to block the fusion between the SARS-CoV-2 coronavirus and human cells, effectively preventing the invasion and replication of the virus, at the basis of the potentially lethal pathology.

Determining the possible efficacy of Nafamostat mesylate was a team from the Research Center for Infectious Diseases of the Institute of Medical Sciences at the University of Tokyo. The same results came from another international research team, led by German scientists from the Infection Biology Unit — German Primate Center of the Leibniz Institute for Primate Research, who collaborated with colleagues from the University of Göttingen and the Sechenov University of Moscow. and several other institutes.

The Japanese researchers, coordinated by professors Jun-ichiro Inoue and Mizuki Yamamoto, had already demonstrated in the laboratory the efficacy of Nafamostat mesylate against MERS-CoV, the coronavirus "cousin" of the new and responsible for MERS (Middle East respiratory syndrome — Middle Eastern Respiratory tract Syndrome), so they decided to test its potential also against SARS-CoV-2.

But how exactly does the drug work? To explain this, the scientists first showed how the coronavirus penetrates human cells. SARS-CoV-2, underline Inoue and colleagues, is wrapped in a double lipid layer studded with the so-called "spikes" (protein S), which it uses to hook onto the ACE2 receptor of human cells and "unhinge" them like a picklock , allowing invasion and replication. When this process is initiated, protein S is broken down into S1 and S2 components by a protease derived from human cells, which scientists believe to be Furin. "S1 then binds to its receptor, ACE2 — explain the scholars — the other fragment, S2, is split by TMPRSS2, a serine protease of the human cell surface, with consequent fusion of the membrane. According to Hoffmann et al., ACE2 and TMPRSS2 are essential in airway cells to determine SARS-CoV-2 infection.

In a 2016 study, as specified, the same Japanese research group had observed in vitro tests the efficacy of the drug Nafamostat mesylate against MERS-CoV; the drug, in simple terms, was able to prevent the fusion between the pathogen and human cells. In the new experiment, based on 293FT cells (derived from the human fetal kidney) expressing both ACE2 and TMPRSS2, the same result was obtained by testing SARS-CoV-2 proteins. The active ingredient is able to suppress the fusion between the human cell membrane and that of the virus, effectively preventing its aggression and replication. One of the most interesting details of the research is that Nafamostat mesylate was found to be effective at one-tenth the concentration of Camostat, a related drug that has the same antiviral ability. The first is administered intravenously, the second orally. Scientists think they can be used alone or in combination as needed. Of course, the results will need to be confirmed in clinical (human) trials that the University of Tokyo will launch this month, but the safety of the drugs has long been known so there is

a lot of optimism. The details of the Japanese research were presented in a press release from the Tokyo university, those of the German investigation, which came to similar conclusions, were published in the journal Cell.

In conclusion, other hypotheses, some of which also focus on the role of bacteria in the intestinal and respiratory microbiota, are gradually being added to the set of accredited evidence in favor of a non-generalist and even less fatalistic reading of CoViD-19, in the management of which even a patient-centered approach becomes more urgent than the virus and its misunderstood oddities.

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