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Review article

The SARS-CoV-2, a new pandemic zoonosis that threatens the world☆

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ABSTRACT

On December 31, 2019, an outbreak of pneumonia of unknown aetiology was detected in the city of Wuhan (China). A week later, a new coronavirus was isolated in these patients, initially designated as 2019-nCoV and subsequently SARS-CoV-2. This is a new virus that is much closer genetically to the coronavirus of bats than to human SARS. The new virus infects and replicates in the lung parenchyma pneumocytes and macrophages in which the ACE-2 cell receptor resides. He has now infected many more people than his predecessors (>85,000). From the clinical point of view, those infected have an average age of 55 years; the main symptoms are fever, dry cough, lymphopenia, dyspnoea, and pneumonia in its severe form. The overall lethality rate is 2–3% in China and 0.1% in cases detected outside of this country. The incubation period has been set at about 3 days (0–24 days). There are no specific antivirals or vaccines.

El SARS-CoV-2, una nueva zoonosis pandémica que amenaza al mundo

Palabras clave:
SARS-CoV-2
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RESUMEN

El 31 de diciembre de 2019 se detectó en la ciudad de Wuhan (China) un brote de neumonía de etiología desconocida. Una semana después se aisló en estos pacientes un nuevo coronavirus, designado inicialmente como 2019-nCoV y posteriormente, SARS-CoV-2. Este es un nuevo virus que está mucho más próximo genéticamente a los coronavirus de los murciélagos que del SARS humano. El nuevo virus infecta y se replica en los neumocitos y macrófagos del parénquima pulmonar en los que reside el receptor celular ACE-2. Ha infectado en estos momentos muchas más personas que sus predecesores (>85.000). Desde el punto de vista clínico, los infectados presentan una edad media de 55 años; los síntomas principales son fiebre, tos seca, linfopenia, disnea, y neumonía en su forma grave. La tasa

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de letalidad global se sitúa en el 2-3% en China y en el 0,1% en los casos detectados fuera de este país. El período de incubación se ha establecido en unos 3 días (0-24 días). No se dispone de antivirales específicos ni de vacuna.

In February 2018, the World Health Organisation (WHO) announced a list of priority diseases that would require in-depth study. These included SARS (severe acute respiratory syndrome) and MERS (middle east respiratory syndrome), as well what as what was classified as "disease X". This disease was defined as a new human pathology with epidemic or pandemic potential caused by an unknown pathogen. At this time, we should consider the new coronavirus (SARS-CoV-2) to be the first disease X (Wuhan pneumonia outbreak).[1]

Until 2019 there were two known coronaviruses that had also epidemically infected the human population. SARS-CoV appeared in 2002 in China's Guangdong province and spread throughout Southeast Asia. The last confirmed case was in September 2003. This virus infected about 8000 people and caused 774 deaths (9.5% fatality rate). Its infectivity (R0) rate was about 4, which facilitated its rapid spread.[2,3]

In 2012, a new coronavirus appeared in the Middle East that caused severe respiratory processes and was named MERS-CoV. In all cases involving this virus, an epidemiological link can be found with the Arabian Peninsula, although one major outbreak was exported to South Korea.[4,5] Unlike SARS-CoV, MERS-CoV is still circulating today and has a case fatality rate of about 35% and an R0 of below 1. It has, therefore not shown an excessive spread capacity, remaining confined to the geographical area of origin.[2–5]

On 31 December 2019, an outbreak of pneumonia of unknown aetiology was detected in the city of Wuhan, China, which was quickly reported to the WHO. One week later, on 7 January 2020, a new coronavirus, initially called 2019-nCoV,[6,7] was isolated from these patients. On February 11, 2020, the WHO named the disease COVID-19 (coronavirus disease-2019) and the causative coronavirus was provisionally called SARS-CoV-2.[8] However, Jiang et al.[9] suggested the name PARS (pneumonia-associated respiratory syndrome) and for the new coronavirus PARS-CoV, to maintain the terminology used for the two previous coronaviruses. After a few disagreements, the name TRAS (transmissible acute respiratory syndrome) was suggested and TRAS-CoV for the virus; however, this name was not confirmed. We must therefore consider that we are facing the third zoonotic epidemic caused by a coronavirus in the 21st century. That is why on 30 January 2020 the WHO declared this disease an international health emergency in order for all countries to prepare for it.[6–10]

Coronaviruses are capable of infecting humans, but their natural preferred hosts are a multitude of animal species (mammals, birds). Human coronavirus infection should be considered a zoonosis. They were first described in 1966 from the nasal secretions of a patient with rhinitis.[8,11] Those included in the alpha-coronavirus group, such as 229E (1a) and NL63 (1b), cause mild or moderate respiratory infections, and some among the beta-coronavirus group, such as OC43 (2a) and HKU1 (2a), also cause this type of infection, whereas the rest of this subgroup, such as SARS-CoV (2b) and SARS-CoV-2 (2b) and MERS-CoV (2c), cause epidemics and severe respiratory infections.[5,11,12]

SARS-CoV-2 is a new virus that belongs to the subfamily *Orthocoronavirinae*, the genus *Coronavirus* and the subgenus *Sarbecovirus* (beta-coronavirus, beta-2b) and within them to clade or lineage 2, which is much closer genetically to bat coronaviruses than to human SARS. The SARS-CoV-2 genome consists of single-stranded RNA of about 30,000 nucleotides and 6 ORF (open reading frames), identical to the rest of the coronaviruses, and several additional genes. Most of these genes are only 80% homologous with the former SARS-CoV virus; however, the genes involved in replication (ORF1ab) are 94% homologous with this virus.[2,13–15] The complete sequencing of the coronavirus genomes detected in patients, and especially the RNA-directed polymerase (RdRp) gene and the S (external spicule) gene, show that the human strains constitute a different lineage from SARS-CoV, but very close to the lineage detected in some bats (BatCoV RaTG13). The S protein of the new coronavirus shows <75% similarity with the other known coronaviruses but 93% identity with the bat coronavirus. These genetic similarities seem to confirm the origin of SARS-CoV-2, which would be some wild bat in the area. According to Zhou et al.,[7,14] this coronavirus would be a genetic recombinant between a bat strain (80%-85%) and that of another animal species (perhaps that of the intermediate host).

The S protein on the surface of coronaviruses is responsible for binding to the cell receptor and the fusion process, thus determining host tropism and transmission capacity in a new host, in addition to being the immunodominant antigen and the antigen most intensely recognised by the host's immune system.[16,17] For the S protein to be able to perform its function it must be hydrolysed by the pulmonary proteases resulting in the S1 fragment, responsible for binding to the receptor, and the S2 fragment, responsible for the fusion process. Although SARS-CoV and SARS-CoV-2 are found in different genetic lineages, they have around 50 amino acids preserved at position S1, while most of those from bats show important antigenic variations in this sensitive area.[12,13]

The capacity of the S1 protein to bind to the cell is located in the C-terminal domain of the cell. Phylogenetic studies of this area have shown that the SARS-CoV-2 protein is almost identical to that of SARS-CoV and is further removed from the homologous bat strain, which suggests an evolutionary process of adaptation to human cell receptors following the same process as that of SARS-CoV in 2002.[13] As with many other RNA genome viruses, the mutation rate of coronaviruses is 10 nucleotide substitutions/position/year, occurring mainly in the first replication cycles. It is therefore surprising that the

genetic sequences of SARS-CoV-2 from different patients are absolutely identical (99.9%). This data suggests that this new coronavirus originated from a single source in a very short period of time and was detected very early in the first days of human-human transmission.[13,16–19]

SARS-CoV-2 infects and efficiently replicates in pneumocytes, macrophages and dendritic cells in the deepest parts of the lung parenchyma where the ACE-2 (angiotensin converting enzyme II) cell receptor resides, which is used by this virus to bind to these cells and initiate the infectious process.[6,12,13] This cellular receptor is the same that SARS-CoV used to infect humans, therefore the pathophysiology of the new coronavirus at pulmonary level is probably very similar, with clear predominance of severe pneumonias and low involvement of the upper respiratory tract.[20–22]

The recent study by Wan et al.[16] indicates the possibility that a single mutation in the N501T position of the S1 protein may significantly increase the ability to bind to the ACE2 receptor; the evolution of this mutation should be monitored. It has also been shown, based on the affinity of the S1 protein for ACE2, that the new coronavirus is not capable of infecting the civet (SARS-CoV intermediary) or mice, and therefore they cannot be used as experimental models unless genetically modified. The animals that have shown capacity to be infected by SARS-CoV-2 are pigs, ferrets, cats and non-human primates, therefore they could be intermediate hosts and/or experimental models.[16–19]

According to the knowledge gained on the coronaviruses that cause SARS and MERS, one of the many species of bats that inhabit Southeast Asia, or perhaps the depths of Africa, should also be a natural reservoir of SARS-CoV-2. Genetic and phylogenetic analyses have shown a strong relationship with several coronaviruses of these mammals and very close with those related to the cause of SARS.[1–3,6]

Despite the importance of bats in the evolutionary biology of coronaviruses, in the case of the new coronavirus it does not appear that there has been direct transmission from this animal to humans.[7] The main reasons for this are: (a) the outbreak began in late December 2019, a period in which most bat species in the Wuhan region are hibernating; (b) according to Chinese epidemiologists, no bats were found or sold in the Huanan market (Wuhan) as it was a fish and seafood market, although other conventional mammals were found; (c) the genetic sequence identity of SARS-CoV-2 and its homologue bat-SL-CoVZC45 is less than 90%, indicating that it forms a phylogenetic branch distinct from humans, and therefore this bat virus and the bat SARS-like coronavirus (bat-SL-CoVZC21) cannot be considered the direct ancestors of the human virus; and (d) in previous coronaviruses causing human epidemics, it was always possible to find an intermediate host, and therefore in this case it must also exist.[13,18]

Two animal species appear to be intermediate host candidates, some snakes or a scaly mammal called a pangolin. The study by Ji et al.[17] has postulated that the new coronavirus is a recombinant strain between one from the bat and another from some snake species from the epidemic zone and that the affected zone corresponds to the 21,500–24,000 nucleotides of the gene encoding the S1 protein (a determinant of human tropism). The sequence of this reptile would have allowed the new virus to acquire the ability to infect humans. However, this is the first time that snakes have been described as hosts of coronaviruses and the study by Robertson and Jiang[23] does not confirm the previous findings.[17] On the other hand, Lam et al.,[24] through metagenomic analysis, have described for the first time the presence of genetic sequences in the Malaysian pangolin (*Manis javanica*) phylogenetically related to SARS-CoV-2, especially in the sequence that encodes the cellular ACE2 receptor-binding S protein. These authors believe that this mammal should be considered the intermediate host and removed from markets to prevent further zoonotic transmission.

The specific diagnosis of SARS-CoV-2 infection should be made by means of a real-time PCR assay that detects the specific targets of this virus (NP and E genes), together with the common RNA-polymerase (RdRp) gene.[24] Serology could be useful for identifying asymptomatic cases and for seroprevalence studies within a given population. This serological diagnosis (ELISA for IgG and IgM) has been designed using the nucleoprotein (NP) of bat coronavirus which has a genetic identity of 92% and does not show cross-reaction with the rest of the coronaviruses.[13,15,25] The isolation of this virus in cell cultures using the Vero and Huh7 cell lines can also be used as a diagnostic methodology. The cytopathic effect is detected after 3 days of incubation and can be detected by immunofluorescence directed against NP.[20]

A study by Zhang et al.[26] in 15 patients confirms the presence of SARS-CoV-2 in the pharynx, faeces and blood of patients, and therefore it could be transmitted via these three routes, although its presence has been detected only by molecular biology and its infectivity in a new host cannot be ascertained. They also observed how in patients with a negative pharyngeal smear the virus could be detected in faeces, especially 4–5 days after the onset of symptoms, producing oro-faecal transmission. According to these authors, infection could not be ruled out by a negative pharyngeal smear alone, although the airway remains the main route of transmission of the virus. In addition, the presence of an immune response in the form of IgM and specific IgG could be detected in all patients.[26]

SARS-CoV-2 has now infected many more people than its predecessors (>85.000). Several factors seem to have led to this rapid spread; on the one hand, the epicentre of the outbreak, the city of Wuhan, has about 11 million inhabitants and is the main communications hub in Hubei Province, which facilitates person-to-person contact and the export of cases to other cities. Its R0 is estimated at 2–3.5, therefore each patient may infect 2 or 3 new people. This value is similar to SARS (R0 2–5) and much higher than that of MERS (R0 < 1), but the number of people infected by the new coronavirus through human-to-human transmission is about 3–10 times higher than previous viruses.[3,10,18]

From a clinical point of view, the mean age of people with COVID-19 is 55 years. The main symptoms are fever (87.9%), dry cough (67.7%), lymphopenia (82.1%), dyspnoea and pneumonia in its severe form, diarrhoea is infrequent. The overall case fatality rate is 2%-3% in China and .1% in cases detected outside that country.[22,27] The incubation period has been set at about 3 days (interval 0–24 days).[2,18,22,27] Subsequent clinical and epidemiological analysis of 1099 laboratory-confirmed

cases has shown the mean age of patients to be somewhat lower (47 years) and 58.1% are male. Of these, only 1.18% had had direct contact with wild animals, 31.3% were residents of Wuhan and 71.8% were contacts with people from this city.[28] In the latest report from China's CDC,[29] out of 72,314 patients, only 61.8% were laboratory confirmed, 22.4% were suspected cases, 14.6% were clinically diagnosed cases and 1.2% were asymptomatic. Of the confirmed cases, the age range was 30–79 years (86.6%); 80.9% were considered moderate or mild cases, 13.8% severe and 4.7 very severe, of which 2.3% died. A total of 1716 health workers were infected, with .3% fatality.

The first cases described in early January 2020 were epidemiologically linked to a fish and live animal market in the city of Wuhan; but from the end of that month, transmission became predominantly person-to-person. This confirms the high capacity of this coronavirus to adapt to humans and to transmit efficiently between them. Genetic phenomena of adaptability, mutation and recombination with previous animal strains have probably participated in this adaptation, from which the new strain with special tropism for humans emerged. As the virus was new to humans, not to the natural world of viruses, the population lacked previous immunity and therefore the process of epidemic spread started.[15,21,22,28,29]

The presence of the virus in the oropharynx with a high viral load even before the onset of symptoms, although without proven ability to replicate in this area, is what determines the transmissibility of the virus. Obviously associated with the mechanisms that facilitate the spread of the virus are coughing, sneezing and expectoration, as well as the important role of direct contact through the hands or other fomites from an infected individual.[18,19] Zou et al.[30] studied the viral load of SARS-CoV-2 in the pharynx and nostrils of both symptomatic and non-symptomatic people and observed that the highest loads are detected from the time of onset of symptoms and that it is somewhat higher in the nostrils. It is especially relevant that the viral load of asymptomatic people is very similar to that of symptomatic people and can persist in some cases for up to 5 days, which supports asymptomatic people as possible efficient spreaders.

Ancestral genetic reconstruction studies carried out by Li et al.[19] on 12 human genome sequences of the new virus seem to indicate that this virus was already circulating in Wuhan on 9 November 2019, with a 95% credible interval, however, which places it between 25 September and 19 December 2019. Thus, the market and the possible presence of super-spreaders resulted in the rapid diffusion and spread of the virus among those attending it.

In SARS, while transmissibility only occurred from the onset of symptoms, fever and respiratory, it seems that SARS-CoV-2 can, as mentioned, transmit days before symptoms, and the possibility has been raised of asymptomatic spreaders or transmitters with a long excretion period.[3–5]

It seems that SARS-CoV-2 presents with three clinical patterns, beyond the asymptomatic cases: (a) a moderate upper respiratory tract infection with mild symptoms; (b) clinically and radiologically evident pneumonia; and (c) severe pneumonia associated with respiratory distress that could progress to respiratory failure and death of the patient (that 2% fatality). Severe pneumonia occurs more in men over 65 years of age and with comorbidities such as diabetes, pulmonary and cardiac pathologies.[22,28,29] One piece of data to be highlighted is that the child population under 15 years of age is rarely affected. There is as yet no clear physiological or virological explanation for this.[31] It also appears that although coronavirus infects pregnant women causing pneumonia, transmission of the virus to the foetus has not been described, and the few cases of detection of the virus in newborn infants have been due to direct transmission from the mother.[27]

Classically, Koch's postulates have been used to identify a new virus as the cause of a given disease, and were modified for viral disease by Rivers.[32] Currently the only criteria that have been met are that SARS-CoV-2 has been isolated, detected by analytical techniques (molecular biology) and cultured in vitro from respiratory samples from patients with suspected respiratory disease. However, no animal model is yet available to meet the remaining criteria, such as (a) demonstrating the ability of this new coronavirus to infect animals and cause them the same disease; (b) re-isolating the virus from experimentally infected animals; and (c) demonstrating an immune response to it. Despite this, virological, clinical and epidemiological evidence seems to indicate with some certainty that SARS-CoV-2 is the aetiological agent of the acute respiratory infection epidemic in Southeast Asia.[5]

Currently, in the absence of a vaccine, the only alternative that can be used in patients is treatment with antiviral drugs. Initially, drugs such as ribavirin, interferon, and the lopinavir-ritonavir combination have been studied both in vitro and in animal models. However, there is much debate as to the effectiveness of any of the drugs.[33]

Nucleoside analogues are a good alternative in some viral infections. These include remdesivir (GS-5734) which has shown therapeutic efficacy in infections caused by the Ebola virus, Nipah and the SARS and MERS coronaviruses.[34] Safety and bioavailability data obtained in Ebola patients could be the basis for its use in humans infected with SARS-CoV-2. Several clinical trials are currently underway in China to establish its efficacy in both non-hospitalised and severely ill hospitalised patients.[35]

We are therefore facing the challenge of a new pandemic of acute respiratory infection caused by a new coronavirus, SARS-CoV-2. We still do not know many of the virological, epidemiological and clinical aspects of this infection, and therefore as new studies appear, we will be able to update our knowledge. Once again, we are facing a new viral pandemic with no specific antivirals or a vaccine, and again only the classical epidemiological recommendations (isolation, surveillance and monitoring) will enable us to tackle this as we have other similar situations.

Conflict of interests

The author of this manuscript declares that he is a member of the editorial board of the journal *Vacunas*.

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