

Achievements in virus research

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Summary

This paper brings a brief survey of the achievements in virology as published from the second half of 20th century till now. Initially, virus research was performed at framework of microbiology and/or closely by related biological disciplines, such as parasitology, pathology, mycology and/or cytology. The reason why virologists have been involved in many related scientific disciplines, has been, in addition, because viruses infect not only great many mammalian species, but also lower vertebrates (such as birds and reptiles) and/or certain plants. Even though there could be difficult to assess such great variety of scientific information, a great proportion a great proportion of authors at least has attempted doing with success. Needless to mention that though precise origin of viruses has not been cleared till now, they have probably sequestered from cellular organelles and then sequently underwent to own (rather independent) evolution.

Keywords: Virus Evolution, Cellular Organelles, Host Infection, Biologic Sciences

Virology is a relatively novel experimental discipline, which has enriched the microbiology and related biological disciplines with a great amount of actual information. The last century (from year 1901 to 1999), has brought many discoveries, which fit to the formation of a new branch of microbial research, later on termed, “virology” Briefly, this was related to a substantial novel achievement, namely that chick embryo cells (CEC) have been separated by trypsinization from egg tissues and subsequently cultured at several virology departments. It has been recognized that even though the virus particle (later on called virion) is not a living entity, it is able to modify the susceptible cell, which it entered into. Namely, the virion blocks the cellular metabolism and forces its proteosynthesis machinery (especially the ribosomes) to produce novel viral components. Simple original meaning of the expression “virus” is poison. In our understanding, however, it is a particle called virion; more precisely it is a crystal-like structure (capsid) consisting of proteins which cover (wrap) the nucleic acid inside. The capsid consists of protein subunits, sticking together keeping the basic principles of symmetry. It protects a string-like nucleic acid molecule (either RNA or DNA) representing the viral genetic information capable to direct the synthesis of virion proteins. Two kind of virus particles have been recognized by electron microscopy: the so called, naked and/or enveloped ones (while the latter reveal two coat). To recognise them, special purification procedures, such as gradient centrifugation in CsCl, were introduced for virus purification. Soon thereafter, electron microscopy has been introduced allowing to observe the virus particles not only in the infected cells but also concentrated into

their extracts. The techniques for visualization of virus particles have been considerably improved introducing methods which demonstrated the viral components as antigens (as formed at various intervals post-infection) by using labeled antibodies, for example by immunofluorescence. In contrast to bacterial and/or animal cells, the virus particle has a single genetic information carrier (i.e. either an RNA and/or a DNA molecule, never both). By exception, the members of the Retrovirus family contain two RNA molecules. In DNA viruses, the size of the carrier filament varies considerably, since the members of genus Parvovirus possess a short nucleic acid equipped with 2 (two) genes only, while the long DNA of Poxvirus family members may have nearly 200 genes.

Within a relatively short period of time (namely from March 2020 to September 2021), a novel Coronavirus, later on designated Coronavirus 2019 (2019-CoV) has emerged (again in China, namely in the city Wuhan); the novel Coronavirus has caused a coronavirus disease (COVID) also termed COVID-19. The new isolates were called coronavirus 2 (CoV-2) and have been found causing a disease similar to that already described previously under name the severe acute respiratory syndrome (SARS) [22]. The outbreak of this virus has appeared at a different (Hubei) province having an exposure link to the market in Wuhan, namely to the South China Seafood City (ECSC) [12]. At this recent CoV-2 outbreak, many details have remained unclear, except that it again began in China and the fact that the novel virus was also transmitted by direct exposure [4]. Noteworthy, the Wuhan Fish and Wild animal Market continued selling living animals such as

poultry, bats, marmots and/or snakes, even though any of them might have been the source of infection [18]. Even though the majority of COVID-19 cases was reported from continental China, also the island of Taiwan has appeared involved [25]. Unlike to the previously (in year 2002 and/or 2003) described “classical” coronavirus (cCoV), which had been isolated in a different (Guandong) province, the novel virus (nCoV) quickly spread all over the world. Over 300 million people became infected, a proportion of whom (about 3-4 %) developed signs of a relatively severe lung disease. In association with its aggressive spread, WHO has declared the recent nCoV outbreak for a situation of public health emergency of international concern [15]. There became clear that already accepted and standard health measures may not be sufficient enough to prevent a quick and widespread (possibly worldwide) transmission of nCoV. Aiming to stop such dangerous outcome, additional measures were implemented, aiming to 1) slow down the spread of illness; 2) provide time (in any state) for better preparation of the health care system, business, educational organization, local health departments as well as the general public for the event if nCoV transmission of disastrous extent of would really occur; 3) characterize the COVID-19 in local public health guides [21]. Development and deployment of medical countermeasures, including precise diagnostics, therapeutic recommendations (not excluding the continued efforts for vaccine development) were put into the focus of interest [37]. In this respect, especially the asymptomatic cases should be diagnosed based on positive viral nucleic acid test results. Infections may occur without any COVID-19 symptoms, neither respiratory nor gastrointestinal symptoms, and without significant abnormalities on the chest radiograph [1]. Transmission of COVID-19 through asymptomatic carriers (i.e. via person-to-person contact) has been observed in several reports [33]. Guan et al. (2020) [16], by estimating a large sample, suggested that a median incubation period might be as short as 3 days on one hand but, by other hand, it could be as long as 24 days. Noteworthy, the mean incubation period has calculated for 5.2 days based on a range of 2.1 to 11.1 days.

Some patients with SARS, which were defined as laboratory-confirmed COVID-19 cases and had respiratory symptoms, the chest computed tomography (CT) did not reveal signs of pneumonia [32]. Another patients with pneumonia on chest radiograph (and defined as COVID-19 positive cases), had both respiratory symptoms as well as pneumonia [43]. The latter category of patients showed severe pneumonia (a respiratory rate over 30/minute, in addition the SpO₂ at 93% and the PaO₂/FiO₂ over 300 mmHg) along with a critical clinical condition, which was characterized by shock and respiratory failure requiring mechanical ventilation along with organ failure needing ICU management [5]. Patients with pneumonia were older, with a higher prevalence of smoking history, more underlying diseases, and were more likely to have fever, myalgia/fatigue, dyspnea, headache, and nausea/vomiting compared to patients with ARD (all differences are at p<0.05). In addition, pneumonia cases presented a higher white

blood cell count and neutrophil count, but had a reduced leukocyte count compared to ARD cases [24]. These patients received more antibiotics and antiviral therapy and were more likely to require oxygenation therapy, mechanical ventilator, renal replacement, and extracorporeal membrane oxygenation [27].

Person-to-person transmission of 2019-nCoV has been confirmed [30,42] and asymptomatic individuals have been identified as potential sources of infection [4,12]. The identification of COVID-19 cases and their contact persons along with immediately recommended measures, such as assessment and monitoring of travelers arriving from the areas with substantial probability of COVID-19 transmission, were similar to those introduced by a previous influenza virus pandemic [41]. The differences in infectivity of coronaviruses can be attributed to the differences in the rigidity of their shells which can be evaluated using computational tools for predicting intrinsic disorder predisposition of the corresponding viral proteins [13].

The estimated reproductive number of 0.3 was obtained from a small number of infected persons with imperfect information in the very early stages of the outbreak [34]; therefore the reproductive number of 2019-nCoV is likely to be similar to that of the 2002/2003 severe acute respiratory syndrome (SARS) coronavirus during the pre-intervention period (range, 2 to 3) and that of the 2009 pandemic A/H1N1 influenza virus in the United States (by a range from 1.3 to 1.7) [23,38]. Owing to these observations, the current control measures for 2019-nCoV, including the quarantine and an observation period of 14 days for suspected cases, can be considered for appropriate.

The structural proteins (and/or glycoproteins) by any Beta-coronavirus (B-CoV) strain are encoded by four regularly present structural genes, namely the spike glycoprotein (S, former E2), the envelope glycoprotein (E, former sM), the membrane glycoprotein (M, former E1) and the nucleocapsid protein (N) [36]. The 27.2 kb long single stranded negative sense viral RNA (vRNA) sequence has an untranslated region at its 5'-end (5'-UTR) along with a short leader sequence (LS) which continues into the two relatively long open reading frames (ORF 1a/b) encoding corresponding polyproteins (p1p1a and p1p1b). These become cleaved by an endogenous peptidase to form at least 10 non-structural viral polypeptides (nsp) involved in vRNA replication. Another four genes encode the structural proteins; their corresponding sequence is interrupted by regions specifying so called accessory proteins (in the case of CoV-2 they are the following: ORF 3, 6, 7a, 7b, 8 and 9b). One of them is located in between S and E sequences (ORF 3), the rest between M and N (with exception of ORF 9b which is positioned directly within the N sequence). The CoV RNA ends by a short untranslated region of the 3'-UTR sequence [27,39]. When comparing CoV-2 with the earlier isolated CoV strains, a key variation was found in the ORF 3 sequence region.

Taken together, the CoV genome might reveal the following genes: 5'-LS--p65/Plp1a/ORF1a/NSP1--Plp1b/ORF1b/NSP2 (poliovirus type protease)-NSP3--NSP4--NSP5--NSP6--NSP7--NSP8--NSP9 (RNA polymerase)--NSP10 (helicase/NTPase)--NSP11--NSP12--NSP13--S-E-M-N-3'

The life cycle of SARS-CoV-2 in the susceptible host cells begins by binding of the S protein to a corresponding cellular receptor, namely that for acetylcholinesterase (ACE2). After receptor binding, a conformation change within S protein facilitates the fusion of the virion membrane with the cell membrane, which activates a transportation pathway along the cellular endosomal reticulum (ER). The virus coded polymerase produces a series of sub-genomic mRNAs transcribed from the released vRNA by a process called discontinuous transcription. In the region of cellular ER and Golgi apparatus the set of newly formed transcripts is finally translated into relevant viral proteins. These along with the transcribed novel vRNA are subsequently assembled into new virions, which are via the cytoplasmic vesicles transported back to cell membrane in order to get released out of the cell.

There is no clinically approved antiviral drug or vaccine available to be used against COVID-19. As of now, there is no specific antiviral medication available for COVID-19 treatment, and also no vaccine is currently available. Health care providers generally treat the symptoms by using oxygen therapy for patients with severe infection. However, few broad-spectrum antiviral drugs have been evaluated. A potential antiviral treatment of human CoV has been recommended using drugs such as Lopinavir/Ritonavir (400 mg/100 mg), nucleoside analogues, neuraminidase inhibitors, Remdesivir, the peptide EK1, arbidol, RNA synthesis inhibitors (such as TDF, 3TC) and/or certain anti-inflammatory drugs including IFN- α (5 million Units/dose). IFN- α is a broad spectrum drug, which can be used, for example, to treat hepatitis B [21]. Lopinavir is a protease inhibitor showing anti-CoV activity in vitro. It has been used to treat infection by human immune deficiency virus, together with ritonavir as a booster. For SARS treatment, there was found that in contrast to ribavirin alone, patients treated with lopinavir/ritonavir as well as ribavirin had a lower risk of the so called acute respiratory distress syndrome (ARDS) and/or death [36]. Nevertheless, as shown in mouse experiments using the Middle East Respiratory Syndrome (MERS)-CoV, Remdesivir may have the best CoV treatment potential. Namely, it can effectively reduce the virus titer in infected mice, it even improves the lung tissue damage. Its effect may be better than that of the treatment using Lopinavir/Ritonavir combined with interferon [26].

China has relied on the use of the anti-viral drug Favilavir to treat the symptoms of COVID-19. This medication was initially developed by Toyama Chemical to treat nose and throat infections. Although the results of the study have not yet been published, it has been assumed that the drug has proven effective (at least in part) in treating symptoms of COVID-19 in a clinical trial of more than 70 patients with minimal side effects. Favilavir is another new antiviral drug that was approved in Japan in 2014

to treat influenza, but currently also has for treating COVID-19 [11], but not by the U.S. Food and Drug Administration (FDA). Remdesivir (GS-5734) is a broad-based antiviral drug originally designed to target Ebola and was developed by Gilead Sciences. It inhibits viral replication through premature termination of RNA transcription, which disrupts the virus's ability to reproduce. China announced that clinical trials of Remdesivir, have officially started in Wuhan to test its efficacy against COVID-19. Moreover, one clinical trial has also been approved by the FDA in the United States [22]. However, the efficacy and safety of Remdesivir in patients still need to be further clinical studies.

Chloroquine and Hydroxychloroquine are drugs used to treat malaria, as well as chemoprophylaxis; and certain inflammatory conditions to include rheumatoid arthritis, lupus and the blood disorder porphyria cutanea tarda, respectively. They have been approved by the FDA to be tested against COVID-19 [10]. Researchers have found that both drugs have in vitro activity against SARS-CoV and SARS-CoV-2, with hydroxychloroquine having relatively higher potency against SARS-CoV-2. Based on these results, currently in several countries including USA, chloroquine as well as hydroxychloroquine are recommended for treatment of the hospitalized COVID-19 patients. In a Chinese study, when chloroquine was tested on more than 100 patients, the results were superior as compared to a control drug; the exacerbation of pneumonia was inhibited, the lung-imaging findings improved, and the virus negative conversion promoted along with a shortened course of disease [19]. However, both chloroquine and hydroxychloroquine may cause relatively frequent side effects, such as worsening vision, nausea, digestive disorders not excluding heart failure in more severe cases. A man in Arizona died and his wife was in a critical condition after taking chloroquine just prophylactically, i.e. to prevent SARS-CoV-2 infection only.

Lopinavir/Ritonavir are sold under the name Kaletra by AbbVie and are designed to treat HIV (AIDS). To evaluate the efficacy of lopinavir/ritonavir for SARS-CoV-2 infection, 99 patients with positive infections were treated with lopinavir/ritonavir. No benefit was observed with lopinavir/ritonavir treatment compare to standard care [3]. However, in South Korea, a 54-year-old man was given a combination of these two medications and had a significant and substantial decrease in the levels of the β -coronavirus [24]. According to the WHO, there may be benefits to using lopinavir/ritonavir with other drugs such as interferon- β , oseltamivir or ribavirin [12].

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The lung involvement was focal but quite prominent at the involved regions; the lesions seen at histological examination were clearly distinct from what could be seen by a standard bacterial bronchopneumonia. Namely, what has occurred, was an interstitial lung disease, by which the interalveolar septi looked widely thickened, yielding a picture later on referred to as usual interstitial pneumonia (UIP). As already mentioned, the cardinal finding at UIP is the thickening of interalveolar septi followed with mononuclear cell infiltration, consisting mainly of lymphocytes. The capillaries of such septi were widened, occasionally accompanied with bleeding into alveolar cavity. The latter phenomenon was interpreted to occur due to probably damaged capillary endothelium lining. In addition, proliferation of type II alveolar pneumocytes could be noted, along with slight hyperplasia of the interalveolar and peribronchial connective tissue. The severity of the lung lesions might vary from mild and/or relatively focal to more confluent or even extensive, when involving the whole lung lobe. In such cases the clinical course corresponded to the syndrome of acute respiratory distress (ARD). Another patients not only showed pneumonia at roentgenological examination (positive chest computer tomography, CT), but might also develop severe organ failure, a state needing complex therapy as used for shock treatment [4]. Furthermore, the patients suffering of pneumonia might had pathological changes in blood, namely elevated levels of white blood cells, either polynuclear or mononuclear. The patients who developed pneumonia were, as a rule older; many of them were smokers and some of them suffered from an background disease. In such cases, the use of antibiotics and/or antiviral drugs has been obligatory in addition to oxygenation and mechanical ventilation of respiratory tract.

More frequently, the patients developed a mild respiratory disease with slight clinical signs, which state was referred to as Coronavirus Disease (CoViD) [9]. These patients had fever by a

probability of 67% to 98% accompanied with a relatively severe cough (statistically ranging from 43% up to 81%) associated with myalgia. Another patients revealed just mild respiratory problems (by a probability of 31% to 55%), which have resembled rather to influenza [6]. Finally, the infection could proceed under non-specific clinical signs (when revealing mild respiratory and/or slight gastrointestinal tract involvement in absence of lesions detectable at roentgenological examinations as seen by 3% to 11% of patients only (according to other statistical evaluations there were much more such cases, but their number never exceeded 44% of total) [2,18]. There should be noted, that cases showing neither clear-cut clinical symptoms nor evident roentgenological lesions (as confirmed at chest rtg examination), could be important from the epidemiological point of view [4]. A person, who feels neither sick, nor is aware of his infectiosity, would not stay at home and would keep any form of prevention.

More data describing the properties of Coronaviruses were obtained by analysing the recently emerged Coronaviruses (in year 2019, again in China, but at another city, called Wuhan and located in the Hubei province). To distinguish the new CoV isolates (prototype strains OC43, HKU and SARS) from the classical ones (229E and NL63), their vRNA molecules have been sequenced [35,40]. Comparing vRNAs sequences from 14 isolates (i.e. the majority out of the known 16 ones) a total of 129 mutations was detected. Based on these data, the isolated strains were divided into 4 (four) genera called Alpha, Beta, Gamma and Deltacoronavirus [2]. While the former two strains (Alpha- and Betacoronavirus) were coming mainly from bats, the latter ones (Gamma-and Deltacoronavirus) were avian in origin. Interestingly enough, also their geographical distribution differed accordingly, when the former came from Hong-Kong city area, while the latter from Bei-Jin (Peking) and/or Singapur.

Several details associated with the properties of nCoV isolated in China at year 2019 and/or 2020 remained still obscure, just their origin from the food market (at Wuhan) has shown an important analogy. It became clear that the novel strains can also spread from human to human, an important property closely resembling to that of the previously described isolates by nearly 20 year ago. There was quite typical for Chinese conditions that the Wuhan market has still remained in service [EU/EEA-third update; 2020, 7]. This might be the reason, along with the more aggressive properties of nCoV, that the latter virus spread very efficiently and quite soon all around the world, affecting several million people not only at continental China, but also at Taiwan [3] and elsewhere. Later on, the nCoV has „moved“ crossing the rest of the world, not even remaining limited to Eastern Asia. In contrast, the nCoV has quickly reached the surrounding states in India and also occurred in Australia, Japan, Canada, USA, Russia, on the Arabic peninsula as well as in East and European countries. The average incubation time of nCoV was estimated short (for 3 days), but a few cases could occur even 24 days after estimated (putative) infection. Nevertheless, the average incubation time has been calculated for 5.2 days, by estimated range limits of 2 to 11 days [4].

The man to man transfer of nCoV has been clearly demonstrated; especially persons showing no clinical symptoms could become the most dangerous source of infection (Diaz et al., 2005, 16). For this reason, air-travellers coming from endemic regions were carefully tested, with emphasis doing so already at airports [11]. The differences among the invasivity properties of individual isolates were attributed to differences in the sequence of virion surface proteins, especially that of S and M glycoproteins. Computer programmes were also used for testing the affinity of cell surface receptors are [8]. The so called virus reproduction number has been calculated either in association with the above mentioned small epidemic in 2003, but especially at the influenza A/H1N1 pandemic (year 2009 in USA). By latter, the reproduction number ranged in the limits of 1.3 do 1.7. Reflecting these data, the time interval for SARS caranteen has been calculated for 10 upto 14 days, what can be regarded for correct and effective eno ugh.

Local authorities were asked to publish statistical data illustrating the epidemiological situation at their region, namely to tell the number of hospitalized persons, not excluding those sick ones remaining home [9]. Finally, there was delared important to support the coresponding medical personal (including physisians and medical practioners) and provide financial help for their difficult, exhaustive and dangerous work. Neither last but not least, the development of efficient vaccine(s), which would be protective either by preventing disease or at least at milderding of its clinical coarse has been started by several companies. Briefly, a suitable vaccine is not expected not cause any undesired complications, or at least just some low grade side effects only. To achieve these goals, quick diagnostic technics, such as PCR test were introduced to be ready for use.

The structure of Coronavirus particles was first analysed by Betacoronavirus virions, which infect cattle. As already known, the nCoV particles are enveloped capsids which vRNA encodes 4 (four) basic proteins, namely the vRNA associated nucleoprotein (N), the two membrane (glyco)proteins called S/E2 (spike glykoprotein) and E/sM (membrane glycoprotein M/E1) and the capsid protein [16]. The above mentioned vRNA is a single strand molecule of positive polarity (26x103 nucleotides) equipped with a preceeding promoter sequence and the 5'-UTR end (UT, untranslated region). The short leader sequence is located in the front of the two open reading frames (ORF1a/ORF1b), which encode the polypeptides P11a and P11b. The long polypeptides in question are cleaved by an endogenous protease (peptidase). Another important nonstructural polypeptide is the RNA polymerase (RNAPoly) which replicates the vRNA molecule. The genes encoding the four structural proteins are interrupted with sequences encoding several accessory proteins (ORF3a, ORF3b, ORF6, ORF7a, ORF7b, ORF8a, ORF8b a ORF9). Some of these are located between S and E sequences (ORF3a/ORF3b), but the rest of them is located between genes encoding M a N proteins with exception of ORF9 located in the N-gene sequence. Finally, the vRNA molecule ends with an untranslated 3'-UTR sequence. In comparison with the classical c(CoV) isolates, important variations were found within the ORF3 sequence.

Until now, no effecient antiviral substance has been identified which would inhibit the replication of nCoV. Patients with severe repiratory symptoms are recomended to receive oxygen by means of a breathing device. Several medicaments have tested including antiviral drugs such as Lopinavir and/or Ritonavir in a dose of 100 mg and even 400 mg daily. In addition, several neuraminadase inhibitors as well as nucleoside analogues have been tested. Out of letter, Remdesivir, Arbidol and the EK1 peptide have been used by most investigators. Furthermore, vRNA replication inhibitors, such as TDF and/or 3TC have been used for therapeutic purpose. The widely acting IFNalpha, which up to now has been used mostly against B-hepatitis, has been tried as well. There is known that Lopinavirus is an efficient protease inhibitor, but this action has been clearly demonstarted in vitro only, thus its action in the organism should further analysed. Up to now, such treatment has been applied with success in cases of AIDS patients who ahd been positive for HIV (human immuno deficiency virus) infections. In latter patients, Ritonavirus has been found quite helpful. Up to now, there was also shown that a compination of Lopinavir with Ritonavir might be effective for SARS treatment, if a booster of Ribavirin has been added. The latter drug has been shown successful in the experimental treatment of mice, in which the development of pneumonitis could be hindered. Ribavirin has been used with success especially to boost the combined Lopinavir and Ritonavir therapy. In latter case there was better to use Ribavirin than IFNalpha [30]. Clearly, Remdesivir has been highly effective in experimental treatment of mice, in which the development of pneumonia could be fully avoided, when the animals had been infected with a closely related Coronavirus causing the disease called MERS (Middle East Respiratory Syndrome). The syndrome named MERS has been recognized among inhavitants of the Arabic Peninsula, where it is endmic in camels. In a comprehensive study, in which 400 camels were infected with MERS, also the corresponding receptor for the latter virus has been identified, namely the enzyme DPP4/8CD23 (dipeptidyl peptidase 4). It should be mentioned finally, in China the substance Filavir (develped by Toyama Chemicals company) has been also used for COVID-19 treatment [13] even though this drug has been yet approved by the U.S. Food and Drug Administration (FDA) office. Nevertheless, the substance Remdesivir/GS-5734 was shown eliciting a wide antiviral effect. It has been developed by the company Gilead Sciences for Ebola virus treatment. Remdesivir inhibits and/or regulates transcription; furthermore, this substance causes lock-down of vRNA synthesis as such, i.e. it very efficiently inhibits virus replication. In the case of emerging nCoV virus in Wuhan, the latter treatment (using Remdesivir) had been found effective, at according to the judgement of local athority. Based on these data, FDA recently has already approved the use of Remdesivir for human therapy. On the other hand, the affect of Remdesivir would need further careful analysis, as at least stressed by certain critical researchers.

Several details associated with the emergence of nCoV in years 2019 and/or 2020 in China have remained still obscure, but there became clear again that novel strains spread from human to human; in addition, their origin from the food market in Wuhan

showed another relevance to the origin of previously described isolates appearing nearly 20 year ago. There was quite typical for the Chinese conditions that the Wuhan market has remained in service [7]. This might be the reason, along with the properties of nCoV that the letter virus spread very efficiently, affecting many million people not only at the Chinese continent, but also in Taiwan [3]. Later on, the novel nCoV isolate has not remained limited to Eastern Asia, but quickly reached India, Australia, Japan, Canada, USA, Russia, spread to Arabic peninsula and then to near East not omitting the European countries. The average incubation time was estimated for 3 days, but cases could occur even by 24 days after putative infection. Nevertheless, the average incubation time has been calculated for 5.2 days, in the limits ranging from 2 to 11 days [15].

Due to quick spread of nCoV in the human population outlined above, the WHO has claimed the state of public danger. An attention of individual governments was drawn to the fact that hygiene measures acceptable before emergence of the recent pandemic might not be sufficient under novel conditions, i.e. not preventing the nCoV spread. The recommendations to individual governments were the following: 1. Paying attention to wide control and a unified and general accepted therapy of COVID; 2. Introducing the necessary legislative measures to prevent the virus spread within a reasonably short time interval; 3. To accept and introduce preventive measures for keeping public places (such as supermarkets, cinemas, public transport vehicles etc) safe for example by wearing masks in order to avoid formation of infectious air droplets (at exhalation) as well as to eliminate their inhalation. 4. Introducing additional measures to increase the safety of schools, nurseries, hospital wards etc; to achieve this, the corresponding and responsible local authorities should be asked to cooperate; 5. The population of regions in which nCoV has been found to circulate should be convinced to keep all the special rules helping to avoid and/or interrupt virus spread. 6. Finally great attention should be paid for getting the inhabitants continuously informed of the nCoV statistics and its possible presence in order to accept the temporarily introduced hygienic measures aiming to limit virus spread. As the transfer of nCoV has been clearly demonstrated, persons showing no clinical symptoms are the most frequent source of infection [9]. For this reason, especially travellers coming from endemic regions should be carefully tested, for example at airports [14]. The differences among properties of the individual isolates were attributed to differences in the sequence of virion surface proteins, especially that of S and M glycoproteins. Computer programmes used for testing the affinity of cell surface receptors are suitable for this purpose [12]. The so called virus reproduction number has been calculated either in association with above mentioned small epidemic of SARS in 2003, but especially during the influenza A/H1N1 pandemic in year 2009 at USA. By the latter, the reproduction number ranged in the limits of 1.3 to 1.7 [8]. Reflecting these data the time for SARS quarantine has been calculated for 10 to 14 days, what can be regarded for correct.

As recommended, the local authorities should be asked to publish

their statistical data illustrating the epidemiological situation at given region, especially for telling the number of hospitalized persons, stressing the number of heavily sick individuals [10]. Finally, there would be important to support the medical personal (including physicians and medical practitioners) who were involved in providing the corresponding medical care, in order to provide help to their difficult, exhaustive and dangerous work. Neither last not least, more attention has been paid to preparation of an efficient vaccine(s), which would either be protective for preventing disease or at least could milder its clinical course. A suitable vaccine is expected not to cause any undesired complications or at least induce some low side effects only [16]. A goal like this cannot be achieved in the absence of quick diagnostic techniques, especially not without PCR test.

The RNA molecule of viruses such as influenza, measles and/or infectious parotitis etc is stabilized by the help of several small basic proteins, also called histons and/or nucleoproteins; these not only do protect the nucleic acid, but they are able to stabilize its helical structure and, what is important, to neutralize its negative (acidic) charge. Of course, the number nucleoprotein peptides sticking to the helix is relatively high, in contrast to a single nucleic acid carrier molecule (with exception of Retroviruses which possess two copies of RNA). Large RNA viruses, such as influenza, measles and/or infectious parotitis, are formed by a helical filament, which is "stabilized" by the help of relatively short basic polypeptides, called histones. These, furthermore, neutralize its acidic charge. Of course, there are several nucleoprotein molecules distributed along a long single twisted (helical) RNA thread. The large RNA viruses, as members of Myxovirus family, are from outside protected by lipid membrane. This outer membrane may be covered by many glycosylated proteins (also called glycoproteins), which are inserted into outer membrane, which, for example, yields their typical appearance to coronavirus particles. The improved virus detection methods were associated with tremendous discoveries of novel viruses, that time designated newly emerging viruses. There has been clearly shown that the virus not only spreads from man to man but also from lower mammals and poultry to man. This way severe diseases like yellow fever and/or Ebola fever were recognized to have viral in origin. The diapazon of virus diseases, which 2 (two) centuries ago was limited just to the classical small-pox and/or cowpox, has now widened in a dramatic way. It was recognized that apes and also domestic animals not excluding poultry, might become the source of virus disease for human population. It became clear that the so called lymphotropic viruses (not excluding Retroviruses) might become activated and then can spread not only within the given body but also from it to whole human population. For example, in 1950 the viral origin of hemorrhagic fever with renal syndrome has been documented, along with its reservoir in small rodents. Alternatively, the Hantaan virus (family Bunyaviridae) has been identified which was followed by isolation of a closely related virus causing the Kyasanur Forest fever. Later on in 1958, the arenavirus Junin was isolated in Argentina, then a related arenavirus Machupo was detected in Bolivia (1966). In 1967 the filovirus Marburg was identified in Uganda, while in 1970 a novel arenavirus causing the

Lassa fever has been described in Western Africa. As not enough, in 1976 the filovirus Ebola has appeared in Sudan and in 1976 a filovirus (related to Ebola) was isolated in Zaire. Thereafter, in 1977 the Seul virus has been described and then (in 1989) an additional filovirus called Ebola/Reston was found. Furthermore, in 1993 the hantavirus Sin Nombre has suddenly appeared, while in 1994 the Sabia virus has emerged along with another Ebola-related filovirus at the Ivory Coast area (in Africa). Neither the so called „developed“ countries have remained saved from novel virus infections which became unexpectedly widespread. At the end of 70-ties the hepatitis C flavivirus has emerged in Europe and in the US. Then in 1988 the hepatitis E virus, earlier classified as hepatitis non-A/non B, has been isolated. In 1986 it became clear that human herpesvirus 6 (HHV-6), which causes fever and skin exanthema in children (exanthema subitum) may also spread in pandemic manner. In 1995 the viral etiology of Kaposi's sarcoma has been confirmed along with the identification of a novel human herpesvirus, called Herpesvirus 8 (HHV-8). The latter was frequent seen in patients, who showed symptoms of AIDS (acquired immune deficiency syndrome). Then new influenza pandemic has appeared in Russia as well as in China caused by the avian influenza A/H5N1 virus. The latter spread to man probably from wild ducks (which may be regarded for an universal source of naturally occurring viral recombinants), since at Chinese markets poultry might be sold alive.

By the end of year 2002, in the Chinese province Guangdong as well as in city of Hong-Kong, several thousand cases of an acute respiratory disease (ARD) have suddenly appeared. Since this disease might occasionally become lethal (when reaching a death rate of up to 9-10 %), it has been later designated, “severe acute respiratory syndrome (SARS)” [21]. The lung involvement in these cases even when focal was quite prominent. At histological examination, the lesions seen were clearly distinct from the standard bacterial bronchopneumonia. Namely, what has occurred, was the interstitial lung disease, in which the interalveolar septa get widely thickened, yielding a picture later on referred to as “usual interstitial pneumonia (UIP)”. As already mentioned, the cardinal finding by UIP is thickening of the interalveolar septa followed with their mononuclear cell infiltration (consisting mainly of lymphocytes). The capillaries of the thickened septa might be widened, occasionally accompanied with bleeding into alveolar cavity. This may occur from the probably damaged capillary endothelium lining. The severity of lung lesions could vary from mild and/or relatively focal to more confluent and even extensive (involving the whole lung lobe). In such cases, the clinical course corresponded to a syndrome of acute respiratory distress (ARD). Such patients showed pneumonia not only at roentgenological examination (positive chest computer tomography), but developed a severe organ failure, a state needing complex therapy similar to that used for shock treatment. Furthermore, some patients suffering of pneumonia might had blood changes, namely elevated levels of white blood cells (either polynuclear or mononuclear). Patients developing pneumonia were, as a rule, older; many of them were smokers and some of them suffered from an additional basic disease which decreased their immune competence. In such cases, the use

of antibiotics and/or antiviral drugs has been inevitable, in addition to oxygenation by mechanical ventilation in order to overcome the state of respiratory tract insufficiency.

More frequently, the infected patients developed a mild respiratory disease showing slight clinical signs, which state was referred to as Coronavirus Disease (CoViD). Such patients had fever by a probability of 67% to 98%, which had been accompanied with a relatively severe cough (statistically ranging from 43% up to 81%) associated with myalgia. Another patients revealed just mild respiratory problems (by a probability of 31% to 55%), which have resembled rather to influenza. Finally, the infection could proceed under non-specific clinical signs (when revealing mild respiratory and/or slight gastrointestinal tract involvement in absence of lesions detectable at roentgenological examinations as seen by 3% to 11% of patients only (according to other statistical evaluations there were much more cases like this, but their number never exceeded 44%). There should be noted, that cases showing neither clear-cut clinical symptoms nor evident roentgenological lesions (as confirmed at chest rtg examination), could be important from the epidemiological point of view. If an infected person does not feel sick, he is not aware of his infectivity; thus, he would not stay at home and/or keep any form of prevention. This might be the reason, along with the properties of nCoV that the latter virus spread very efficiently soon affecting many million people not only at Chinese continent, but also at Taiwan and then crossing the rest of the world; nCov has not remained limited to Eastern Asia, but quickly reached India, Australia, Japan, Canada, USA, Russia, the Arabic peninsula along with near East and European countries. The average incubation time was estimated for 3 days, but cases could occur even following 24 days of putative infection. Nevertheless, the average incubation time has been calculated for 5.2 days, when estimated to range in the limits of 2 to 11 days.

Data describing the properties of Coronaviruses were obtained from the recently emerged Coronaviruses (by the year 2019 and again in China, but at another city, namely in Wuhan, Hubei province) [11]. To distinguish the new CoV isolates (prototype strains OC43, HKU and SARS) from classical ones (229E and NL63), their vRNA has been sequenced. Comparing the vRNA sequences from 14 isolates (i.e. a majority out of 16) a total of 129 mutations was detected. Based on these data, the isolated strains were divided into 4 (four) genera called Alpha, Beta, Gamma and Deltacoronavirus. While the former two (Alpha-and Betacoronavirus) have been coming predominantly from bats, the latter (Gamma-and Deltacoronavirus) were rather avian in origin. Interestingly enough, also the geographical distribution of the novel isolates differed as well, when the former came from Hong-Kong city area, while the latter from Bei-Jin (Peking) and/or Singapur. Several details concerning the properties of nCoV isolated in the year 2019 and/or 2020 have remained still obscure, even though their origin (from food market at Wuhan) has been similar. When it became clear that the novel strains also spread among human, an additional important property resembled to the isolates described 20 year ago. Worth mentioning, the Wuhan market has remained in service too. Thus, neglecting the relevant

epidemiological measures seems quite typical for the Chinese conditions (EU/EEA-a third update). Probably for this reason, along with a more aggressive behavior of nCoV itself, the novel virus has widely spread all around the world, affecting many million people not only among continental China inhabitants, but also at Taiwan and additional countries. The nCoV has moved to rest of the world, not remaining limited to Eastern Asia. In contrast, it has quickly reached the surrounding states, namely India and then Australia, Japan, Canada, USA, Russia, the Arabic peninsula as well as Eastern and Western European countries. The average incubation time of nCoV was estimated for relatively short (for 3 days), but a few cases could occur even 24 days after estimated (putative) infection. Nevertheless, the average incubation time has been calculated for 5.2 days, by estimated range limits of 2 to 11 days. The man to man transfer of nCoV has been clearly demonstrated; especially persons showing no clinical symptoms could become the most dangerous source of infection. For this reason, air-travellers coming from endemic regions were carefully tested, with emphasis doing so already at airports. The differences among the invasivity properties of individual isolates were attributed to differences in the sequence of virion surface proteins, especially that of S and M glycoproteins. Computer programmes were also used for testing the affinity of cell surface receptors. The so called virus reproduction number has been calculated either in association with the above mentioned small epidemic in 2003, but especially at the influenza A/H1N1 pandemic (year 2009 in USA). By latter, the reproduction number ranged in the limits of 1.3 to 1.7. Reflecting these data, the time interval for SARS quarantine has been calculated for 10 to 14 days, what can be regarded for correct and effective enough.

Local authorities were asked to publish statistical data illustrating the epidemiological situation at their region, namely to report the official number of hospitalized persons, not excluding the sick ones who remained at home. For such reason a simple test has been introduced to recognize the agent in question. Finally, there was declared for obligatory to support the corresponding medical personal (including physicians and medical practitioners) including to provide additional financial help for their difficult, exhaustive and dangerous work. Neither last but not least, the development of efficient vaccine(s), which would be protective either by preventing disease or at least at milder of its clinical course has been started by several companies. Briefly, a suitable vaccine is not expected not cause any undesired complications, or at least just some low grade side effects only. To achieve these goals, quick diagnostic techniques, such as PCR test were introduced to be ready for use.

Until now, no efficient antiviral substance has been identified which would inhibit the replication of nCoV. Patients with severe respiratory symptoms are recommended to receive oxygen by means of a breathing device. Several medicaments have been tested including antiviral drugs such as Lopinavir and/or Ritonavir in a dose of 100 mg and even 400 mg daily. In addition, several neuraminidase inhibitors as well as nucleoside analogues have been tested. Out

of letter, Remdesivir, Arbidol and the EK1 peptide have been used by most investigators. Furthermore, vRNA replication inhibitors, such as TDF and/or 3TC have been used for therapeutic purpose. The widely acting IFN α , which up to now has been used mostly against B- hepatitis, has been tried as well. There is known that Lopinavir is an efficient protease inhibitor, but this action has been clearly demonstrated in vitro only, thus its action in the organism should further analysed. Up to now, such treatment has been applied with success in cases of AIDS patients who had been positive for HIV (human immunodeficiency virus) infections. In latter patients, Ritonavir has been found quite helpful. Up to now, there was also shown that a combination of Lopinavir with Ritonavir might be effective for SARS treatment, if a booster of Ribavirin has been added. The latter drug has been shown successful in the experimental treatment of mice, in which the development of pneumonia could be hindered. Ribavirin has been used with success especially to boost the combined Lopinavir and Ritonavir therapy. In latter case, there was better to use Ribavirin than IFN α . Clearly, Remdesivir has been highly efficient by experimental treatment of mice, in which the development of pneumonia could have been fully eliminated. In such experiments, the animals had been infected with a closely related Coronavirus causing called MERS (Middle East Respiratory Syndrome). The MERS has been originally recognized among the inhabitants of the Arabic Peninsula, where it had been found endemic in camels. In a comprehensive study, in which 400 camels were infected with MERS, also the corresponding receptor for the virus in question has been identified, namely an enzyme called DPP4/8CD23 (dipeptidyl peptidase 4). It should be mentioned that in China the substance Filavir (developed by Toyama Chemicals company) has also been used for COVID-19 treatment, even though this drug has been yet approved by the U.S. Food and Drug Administration (FDA) office. Nevertheless, the substance Remdesivir/GS-5734 was shown eliciting a wide antiviral effect. It has been developed by the company Gilead Sciences for Ebola virus treatment. Remdesivir inhibits and/or regulates transcription; furthermore, this substance causes lock-down of vRNA synthesis as such, i.e. it very efficiently inhibits virus replication. In the case of emerging nCoV virus in Wuhan, the latter treatment (using Remdesivir) had been found effective, according to the judgement of local authority. Based on these data, FDA recently has already approved the use of Remdesivir for human therapy. On the other hand, the effect of Remdesivir would need further careful analysis, as at least stressed by certain critical researchers.

Due to the above described quick spread of nCoV in the human population, WHO recently claimed the state of public danger. The attention of individual governments was drawn to the fact that the hygiene measures acceptable before the emergence of the recent pandemic might not be sufficient enough, since they could not prevent the nCoV spread. The recommendations to individual governments were the following: 1. Paying attention to wide control and a unified and general accepted therapy of COVID; 2. Introducing the necessary legislative measures to prevent the virus spread within a reasonably short time interval; 3. To accept and introduce preventive measures for keeping public places

(such as supermarkets, cinemas, public transport vehicles etc) safe for example by wearing masks in order to avoid formation of infectious air droplets (at exhalation) as well as to eliminate their inhalation. 4. Introducing additional measures to increase the safety of schools, nurseries, hospital wards etc; to achieve this, the corresponding and responsible local authorities should be asked to cooperate; 5. The population of regions in which nCoV has been found to circulate should be convinced to keep all the special rules helping to avoid and/or interrupt virus spread. 6. Finally great attention should be paid for getting the inhabitants continuously informed of the nCoV statistics and its possible presence in order to accept the temporarily introduced hygienic measures aiming to limit virus spread. As the transfer of nCoV has been clearly demonstrated, persons showing no clinical symptoms are the most frequent source of infection (Díaz et al., 2005). For this reason, especially travellers coming from endemic regions should be carefully tested, for example at airports. The differences among properties of the individual isolates were attributed to differences in the sequence of virion surface proteins, especially that of S and M glycoproteins. Computer programmes used for testing the affinity of cell surface receptors are suitable for this purpose. The so called virus reproduction number has been calculated either in association with above mentioned small epidemic of SARS in 2003, but especially during the influenza A/H1N1 pandemic in year 2009 at USA. By the latter, the reproduction number ranged in the limits of 1.3 to 1.7. Reflecting these data the time for SARS quarantine has been calculated for 10 to 14 days, what can be regarded for correct.

As recommended, the local authorities should be asked to publish their statistical data illustrating the epidemiological situation at given region, especially telling the number of hospitalized persons, stressing the number of heavily sick individuals. Finally, there has been claimed for important to introduce an additional support the medical personal (including physicians and medical practitioners) involved in the relevant medical care, namely providing all possible help to their difficult, exhaustive and dangerous work. Neither last not least, more attention should be paid to preparation of an efficient vaccine(s), which would prevent disease not only to mild the clinical disease. A suitable vaccine is expected not causing undesired complications, except of acceptable low side effects.

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