SARS-CoV-2 PANDEMIC: A NARRATIVE REVIEW ON LESSONS AND VIEWPOINTS ARISING FROM BOTH THE HISTORY OF MEDICINE AND FROM THE BIOLOGICAL BEHAVIOUR OF OTHER WELL-KNOWN VIRUSES

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Abstract

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is the etiological agent of the current pandemic worldwide. The pathological condition induced by this pathogen is known as COVID-19 disease. SARS-CoV-2 associated pandemic has been defined as a "public health emergency of international concern" by the International Health Regulation Emergency Committee of the World Health Organization. To date, considerable efforts are in progress to develop more advanced strategies against SARS-CoV-2. Despite the numerous scientific studies published, our knowledge regarding this pathogen is still incomplete, as this virus has been identified only recently. Therefore, scientific investigation of the SARS-CoV-2 has been possible only for a short period of time and effective management of the serious forms of this disease is still lacking. Considerable efforts are in progress worldwide with the purpose to develop more advanced strategies against this pathogen. In this review, we have analyzed the structural and the biological SARS-CoV-2 characteristics and those of other well-known RNA viruses, with the aim to identify possible similarities and analogies between all these pathogens, may be a very useful approach. These infectious agents have been widely studied since several years ago and, a large series of scientific reports are available in the literature regarding this topic. Therefore, focusing on the Human Immunodeficiency Virus (HIV), Hepatitis C Virus (HCV) and Influenza viruses (IVs), we have collected their historical data, clinical manifestations, pathogenetic mechanisms and related infections. Taking advantage of the results of our research, we have assembled this narrative review, with the aim to get useful insights and lessons from HIV, HCV and IVs characteristics and, consequently, to transfer the obtained knowledge to the study of SARS-CoV-2 biology. There are well known differences between all these pathogens. In particular, they present a distinct mode of transmission, as SARS-CoV-2 and Influenza viruses are airborne pathogens, whereas HIV and HCV are bloodborne infectious agents. However, these viruses exhibit some potential common

clinical manifestations and pathogenetic mechanisms and their understanding may contribute to establishing preventive measures and new therapies against SARS-CoV-2. Accordingly, we have analysed and discussed the following points: 1) the biology, the pathogenesis and the clinical manifestations of SARS-CoV-2, HIV, HCV and IVs in mankind; 2) the onset and spreading of pandemics caused by respiratory viruses according to a perspective historical point of view; 3) the possible development of a persistent SARS-CoV-2 reservoir worldwide; 4) the possibility of SARS-CoV-2 reinfection/reactivation; 5) the possible involvement and impact of climatic factors in increasing the risk of SARS-CoV-2 spreading.

Key-words: Coronavirus, COVID-19, Hepatitis C Virus (HCV), Human Immunodeficiency Virus (HIV), Influenza viruses, ribonucleic acid (RNA), SARS-CoV-2

Introduction

At the end of 2019 an epidemic, caused by a novel single-stranded ribonucleic acid (RNA) human-infecting coronavirus, defined SARS-CoV-2, has broken out in Wuhan, Hubei Province in China and it subsequently spread worldwide. This virus induces a pathological condition, known as the Coronavirus Disease 19 (COVID-19). It is characterized both by different degrees of severity [1] and by a wide spectrum of signs and symptoms, including cough, sore throat, fever, shortness of breath, sudden onset of anosmia, ageusia or dysgeusia, nausea or vomiting and diarrhea [1]. This epidemic has been declared a “public health emergency of international concern” by the International Health Regulations Emergency Committee of the World Health Organization [2]. A dramatic situation is evolving, with the spreading of SARS-CoV-2 quickly increasing in all Nations across the globe and with a consequent growing up of morbidity and mortality in human population. This pandemic has already caused about two million deaths around the world. Despite the strong efforts of researchers, currently, only some drugs potentially active against this virus have been introduced in the clinical practice as well as a few vaccines have been urgently approved and a vaccination program has been

begun worldwide [3]. Unfortunately, although promising data concerning the vaccines that have been reported, this strategy is in its early phase and no consolidated data on its usefulness are available [4]. Therefore, novel diagnostic and therapeutic strategies are urgently requested for the management of patients with COVID-19, to counteract this emergency. However, the reaching of this crucial end-point must rely on a comprehensive and global approach. This strategy must take into account a wide spectrum of factors, concerning not only the viral characteristics, including the epidemiology, the structure, the route of infection and the pathogenetic mechanisms of SARS-CoV-2, but also the host's features, such as the age, the comorbidities, the reactivity of the immune system and the type of immune response as well as several critical physical quantities such as climate, temperature, humidity and the duration of sunshine of the geographical regions where the pandemics spreads. This kind of approach may promote an effective improvement of our knowledge concerning SARS-CoV-2 and, consequently, its management. With this aim, we have examined in brief the current scientific literature, concerning the epidemiological, pathological and clinical features of SARS-CoV-2. Accordingly, we have analyzed the features of some RNA viruses, widely spread in mankind worldwide for several decades and responsible for a high burden of morbidity and mortality, with the purpose to search possible analogies in the structure, mechanisms of pathogenicity and clinical course among these pathogens. In particular, we have decided to consider the following RNA viruses in our paper: Human Immunodeficiency Virus (HIV), Hepatitis C Virus (HCV) and Influenza viruses. Several reasons have induced us to make this choice. In particular, we considered Influenza viruses, as these RNA pathogens have been recognized as the etiological agents of the main previous pandemics in mankind.

Furthermore, we have focused our attention on HIV and HCV as these pathogens also show some potential similarities with the SARS-CoV-2, even if they differ widely from the etiological agent of COVID-19 in several structural elements and pathological features, mainly for their mode of infection, propagation and circulation in the human population, as well as in their ability to

originate chronic infections (HCV and HIV). In particular, all these viruses infect some classes of immune cells (mainly T cells and macrophages) using them as carriers. In this way, they are able to spread into the body via blood circulation. Two very important points have to be considered: the epidemiological factors and the mechanisms involved in the ability of Influenza virus, HCV and HIV in inducing infectious diseases in mankind. These pathogens have been studied longer than SARS-CoV-2 and a better comprehension, concerning their biological behavior in human pathology has been reached. Taking advantage from the data available in the literature, our aim has been to get useful lessons and insights through the analysis of SARS-CoV-2 and the above-mentioned RNA viruses. This approach may contribute to give us the opportunity to exploit the historical knowledge, mainly with regard to the long-term effects of viral infections, which have caused pandemics in the past. Furthermore, this strategy may also improve the understanding of the biological behaviour of SARS-CoV-2, by identifying possible common viral characteristics and pathogenic mechanisms of the pathogens described above [5]**.** Lastly, we have analysed and discussed the following points: 1) the biology and the pathogenesis of SARS-CoV-2 and of the above-mentioned RNA viruses in mankind. We have included a brief description of: a) the biology and of the structure concerning HIV, HCV and Influenza virus, b) the clinical manifestations and the pathogenetic mechanisms detectable during both the acute- and post acute-phases of the infection associated with these pathogens in a variable period of time ranging from weeks, to months and to years after acute infection; 2) the onset and spreading of pandemics caused by respiratory viruses according to a perspective historical point of view; 3) the possible development of a persistent SARS-CoV-2 reservoir worldwide; 4) the possibility of SARS-CoV-2 reinfection/recurrence; 5) the possible involvement of climate factors in increasing the risk of SARS-CoV-2 spreading.

1.Biology and pathogenesis of SARS-CoV-2, HIV, HCV and Influenza viruses in mankind

SARS-CoV-2 structure and pathogenesis

SARS-CoV-2 is an enveloped non-segmented virus, with a spherical-shape [6]. The envelope is composed of a lipid bilayer, with spike proteins, emerging from the surface of the viral particles. These structures confer to the virions a crown-like morphology under electronic microscopy. Each virion consists of a positive 5'-capped and 3'-polyadenylated single-stranded RNA. The viral genome sequence is approximately 30,000 bases in length (Figure 1) [7] and includes 14 ORFs. OFR1a and OFR1b genes are located on the 5′-end and codify two long polypeptides, defined as pp1a and pp1ab respectively. They are cleaved into 16 nonstructural proteins (Nsps). To date, the functions of Nsps are not fully understood, however, according to the current evidence, the identified activities of these molecules are the following: 1) Nsp1 (modulation of viral RNA replication and inhibition of protein translation within the infected cells); 2) Nsp2 (regulation of cell signalling cascades, controlling the survival of infected cells); 3) Nsp3 (a protease cleaving the translated polyprotein into its different components); 4) Nsp4 (a protein including the transmembrane domain 2, probably anchoring a structure involved in viral transcription and replication to endoplasmic reticulum membranes); 5) Nsp5 (a proteinase, contributing to viral replication via polyprotein processing); 6) Nsp6 (a transmembrane domain, exerting a role in the early activation of autophagosomes from endoplasmic reticulum of infected cells); 7) Nsp7 (it is a RNA-dependent RNA polymerase, with a hexadecameric structure, forming a complex with nsp8, modulating viral replication); 8) Nsp8 (a RNA polymerase/replicase with a hexadecameric structure, forming a-complex with nsp7, regulating viral replication; 9) Nsp9 (it is a viral protein binding to single-stranded SARS-CoV-2 RNA. It is involved in viral replication by functioning as an ssRNA-binding protein); 10) Nsp10 (it is a Growth-factor-like protein, including two zinc-binding motifs. It stimulates both nsp14 3′-5′ exoribonuclease and nsp16 2′-*O*-methyltransferase activities, during viral transcription. It exerts an essential role in viral mRNAs cap methylation); 11) Nsp11 (unknown activity); 12) Nsp 12 (it is a RNA-dependent RNA polymerase (Pol/RdRp), ~~it~~ is

requested for ~~an~~ efficient replication and transcription of the viral RNA genome; 13) Nsp13 (it is an RNA 5′-triphosphatase and includes a Zinc-binding domain, regulating replication and transcription and an NTPase/helicase domain, which binds ATP); 14) Nsp14 (It includes a proofreading Exoribonuclease domain-ExoN/nsp14 and exerts an Exoribonuclease activity, functioning in a 3′ to 5′ direction and N7-guanine methyltransferase activity); 15) Nsp 15 (it is an EndoRNAse; with Mn(2+)-dependent endoribonuclease function); 16) Nsp16 (it is a methyltransferase, modulating methylation of mRNA cap 2′-O-ribose to the 5′-cap structure of viral mRNAs) [8]**.** The 3' terminus of the viral genome includes 4 genes, encoding 4 structural proteins such as the nucleocapsid (N) protein, the matrix (M) protein, the small envelope (E) protein and the spike (S) glycoprotein as well as genes encoding accessory proteins. The N protein is enclosed in the core of the SARS-CoV-2 particle. It interacts with the viral RNA and exerts a key role in the transcription of SARS-CoV-2 genome and in nucleocapsid assembly to produce mature virions [9]**.** The S protein is composed of glycosylated proteins, forming spikes on the surface of viral particles and regulating the viral entry into the host's cells [10]**.** The accessory genes are represented by the following: 3a, 3b, p6, 7a, 7b, 8b, 9b and 10 (**Figure 1**). These proteins inhibit type I interferon synthesis and type I signaling pathways [11]. The structural and accessory proteins are generated from viral subgenomic RNAs (sgRNAs) [12]. The SARS-CoV-2 enters the human body via the airway tract, infecting both the epithelial cells of the trachea, bronchi, bronchioles and lungs [6], as well as resident, infiltrating and circulating cells of the immune system.

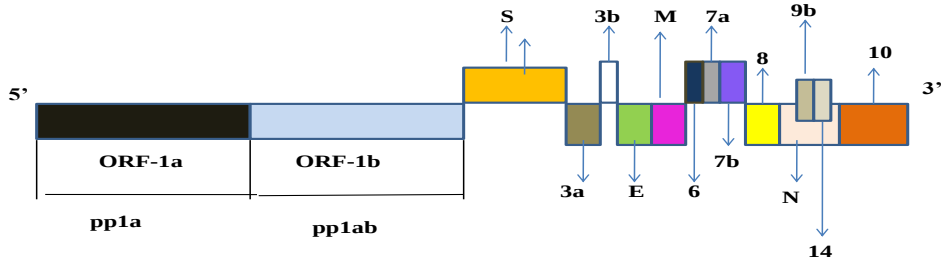

Figure 1. SARS-CoV-2 genome consists of a positive 5'-capped and 3'-polyadenylated single-stranded RNA. The viral genome sequence is approximately 30,000 bases in length. OFR1a and OFR1b genes are located on the 5′-end and codify two long polypeptides, defined as pp1a and pp1ab respectively. These polypeptides are cleaved into 16 nonstructural proteins (Nsps)(not shown), from Nsps 1 to Nsps 16, such as some transmembrane domains, including Nsp4 and Nsp6 (see text) as well as Nsp12 RNA-dependent RNA polymerase (Pol/RdRp). This enzyme is needed for efficient replication and transcription of the viral RNA genome. Furthermore, SARS-CoV-2 genome on 3' terminus encodes 4 structural proteins, the nucleocapsid (N) protein, the matrix (M) protein, the small envelope (E) protein and the spike (S) glycoprotein and also some accessory proteins, like ORF 3a, 3 b, 6, 7a, 7b, 8, 9b, 10 and 14.

Furthermore, this pathogen may directly invade the epithelial cells of the renal distal tubules, the cells of the mucosa of the intestine, the liver and the pancreas [13,14], as well as, it may reach the neurons of the brain, the heart, the spleen, the lymph nodes and other lymphoid tissues [15]. In particular, SARS-CoV-2 infects both host's lymphocytes and macrophages, causing both their death through apoptosis and subsequent severe lymphopenia [16], and afterwards, making them very efficacious carriers for its spreading [17]. As consequence, this virus reaches the Mucosa-associated lymphoid tissue (MALT), Bronchus-associated lymphoid tissue (BALT) and Nasopharynx

associated lymphoid tissue (NALT). On the basis of the results emerging from clinical studies, SARS-CoV-2 may induce a systemic involvement with different clinical courses. According to current knowledge, the genome of SARS-CoV-2 may be detected in distinct sites of infected human hosts, including nasal cavities, pharynx, bronchoalveolar lavage, faeces and blood [18]. This observation suggests that the live virus may be transmitted via the fecal route. In a small percentage of subjects, SARS-CoV-2 is sometimes detectable in blood samples positive by PCR tests, these results suggest a systemic infection [19,20]. On the other hands, a large part of patients may be asymptomatic or develop mild or moderate symptoms [21]. Nevertheless, some of these subjects may develop ~~a~~ severe disease, potentially leading to progressive respiratory insufficiency and requiring mechanical ventilation. Therefore, these individuals may experiment with a sudden deterioration of their clinical status with the onset of multiorgan dysfunction syndrome (MODS) with the subsequent admission to the intensive care units. This specific condition is associated with ~~an~~ elevated mortality. In particular, aged individuals with chronic pathologies have often a compromised function of the immune system, develop more severe clinical forms and are at higher risk of death in comparison with younger and healthier subjects [22]. The coordinated and tightly regulated activity of innate and adaptive arms of the immune system is a crucial factor for the development of a protective response against viral pathogens invading the human body. Our knowledge concerning the dynamic changes of immune cells and of cytokines/chemokines patterns in patients with COVID-19 is still limited [23,24]**.** However, the available studies have suggested that in the early phases of this disease, when the activation of the innate arm of the immune system occurs, there is generally a suboptimal expression of ~~the~~ type I and III antiviral Interferons and an increased release of other inflammatory cytokines, including mainly Interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP1), chemokine (C-X-C motif)-ligand-1 (CXCL1), chemokine (C-X-C motif)-ligand-1 (CXCL5), and C-X-C motif chemokine ligand 10 (CXCL10) also known as Interferon gamma-induced protein 10 (CXCL10/IP10) and tumo~~ur~~ necrosis factor-α (TNF- α) **[25].** These mediators attract several immune cells, including polymorphonuclear leukocytes, monocytes, NK cells, dendritic cells (DCs), which also release further chemokines and generate the inflammatory process. DCs are antigen presenting cells (APCs) and represent a link between the innate and adaptive responses of the immune system**.** These cells present SARS-CoV-2 antigens to specific T lymphocytes [26]. In particular, a recent study has shown that CD4+ and CD8+ T lymphocytes recognize multiple regions of the N protein in subjects convalescing from COVID-19. Furthermore, in individuals who were affected by SARS-CoV infection in 2003 a robust CD4 and CD8 T cell's response against epitopes of N protein of SARS-CoV was still detectable even 17

years later. These lymphocytes exhibited strong cross-reactivity towards the regions of N protein of SARS-CoV-2 [27]. The involvement of lymphocytes in immune response marks the transition from the innate to the adaptive immune response. This step represents a key event in determining the course and the clinical outcomes of patients with COVID-19 [28]. In particular, it has been suggested that in this phase a favourable course of SARS-CoV-2 related infection depends on an efficacious activation of regulatory components of the immune system, mainly mediated by specific CD4 and CD8 positive T cells and by B cells. Under proper conditions, the development of plasmacells, releasing specific and protective antibodies against the etiological agent of COVID-19 occur [29]. On the other hand, an inappropriate and uncoordinated activity of the immune system generates a robust inflammatory response, resulting in the cytokine release syndrome [30]. This clinical condition is characterized by severe clinical manifestations, including acute respiratory distress syndrome (ARDS) and disseminated intravascular coagulation (DIC) [31]. In particular, the elderly patients present the most serious forms of SARS-CoV-2 related infections, where may be observed impairment in crucial activities of the immune system, mainly the T cell dependent responses [30,32]. Furthermore, recent studies have shown that elevated viral loads of this pathogen, assessed via the nasopharyngeal swab samples by means of the real time RT-PCR, have been independently associated with higher mortality rates in comparison with individuals with lower SARS-CoV-2 levels [33,34]. An excessive pro-inflammatory Th1 and Th17 mediated response has been reported in patients with SARS-CoV-2 infection, with increased concentrations of IFN-γ, TNF-α, IL-1β, IL-6, IL-15 and IL-17 [32]. In humans, Th17 cells (T-Helper 17) are stimulated by IL-6 and IL-1β [35]. Experimental and in “in vivo” studies have suggested the potential pro-inflammatory role of some among them in the pathogenesis of SARS- CoV-2. Particular regions in nucleocapsid (N) and spike (S) proteins of this pathogen are able to directly bind several specific DNA sequences, detectable in the promoter region of a wide series of interleukins and cytokines[36,37], promoting the production and release of these mediators [17,22]. SARS-CoV-2 induced disease with severe clinical courses and with a fatal outcome is characterized by a massive release of a wide spectrum of cytokines. This event is defined as “cytokine storm” and leads to the life-threatening condition, known as “Cytokine Release Syndrome” [38]. The cells (macrophages, monocytes, lymphocytes and dendritic cells) [39], mediators, costimulatory-, inhibitory-molecules and mechanisms involved in this complex process have been widely described in previous recent studies [17,22]. This topic is beyond the purpose of this paper and it will be not discussed further.

HIV structure and pathogenesis

HIV is a spherical-shaped non-segmented virus with a diameter of about 120 nm. It consists of two copies of positive-sense single-stranded RNA, housed in a conical capsid. The viral genome sequence is approximately 9,200-9,600 bases in length [40]**.** On the basis of the phylogenetic analysis, HIV is subdivided into two major types, HIV type 1 (HIV-1) and HIV type 2 (HIV-2). HIV-1 is distinct into four different groups: M (major), O (outlier), N (non-M, non-O), and P (pending) [41]. The viruses belonging to Group M have been recognized as the cause of the AIDS pandemic worldwide and have been subdivided into nine subtypes, designated by the letters A-D, F-H, J and K [42]. The conical capsid structure is surrounded by a matrix and by a more external element, represented by a viral envelope. Both elements together contribute to the maintenance of the virion integrity. The viral envelope is composed of a lipid bilayer, derived from the plasmatic membrane of infected cells when the virions bud from them [43]**.** A complete discussion about the HIV structure, organization and viral cell cycle is beyond the purpose of this paper. Therefore, these aspects will be discussed only in brief in this paragraph. HIV genome includes at least nine genes, known as *gag, pol, env, tat, rev, nef, vif, vpr, vpu* and codifying 19 proteins and two Long Terminal Repeat regions (LTRs) at its 5'and 3' ends. *Gag, pol and env* encode structural proteins, which are incorporated into the new virions [44]. *Env* codifies a glycoprotein (gp)160, which is cleaved into two parts, defined gp120 and gp41, by a cellular protease Three molecules of gp120 and three of gp41 form a cap and a stem respectively, generating a structure anchoring to viral envelope. This apparatus allows gp120 to interact with specific receptors on the target cells, such as CD4 [45]. Following this event, changes in gp120 and gp41structural conformation occur with the reorganization of their spatial disposition and exposition of coreceptor binding domains. The physical interaction between HIV and specific cell receptors induces the fusion of the viral envelope with the plasmatic membrane of target cells. The content of HIV virions enters the host's cells, initiating a productive infectious cycle. *Gag* (Group-specific antigen) gene encodes the information for the synthesis of a 55 kDa polyprotein in the cytoplasm of infected cells. This molecule is cut into several proteins after viral budding, including Matrix (MA), Capsid (CA), Nucleocapsid (NC), spacer p1, spacer p2 and p6 domain. About 1500 copies of the HIV protein CA monomers contribute to form the structure of the viral conical capsid, whereas the matrix surrounding it consists of MA elements. A ribonucleoprotein containing NC complexed to genomic RNA in association with the replicative enzymes forms the inner component of each virion. The *pol* gene encodes the enzymatic proteins protease (PR), reverse transcriptase (RT) and integrase (IN). HIV complete virions, causing a productive infection in the target cells, include these three enzymes. Viral RNA is converted into DNA by RT and inserted into the DNA of the host's cell

nucleus, where it may either remain in a latent status or it may undergo the process of transcription into messenger RNA and generate the viral polyprotein. Some complex events characterize HIV replication. In particular, the mature PR cut this polyprotein into the prior indicated proteins. The PR precursor generates its functional form from the polyprotein by means of a mechanism defined auto-processing. This event consists of a double cleavage involving both its N- and C-terminus [46-48]**.** *Tat, rev, nef, vif, vpr, vpu* represent regulatory genes and express proteins, modulating and regulating viral infectivity and replication and affecting the HIV ability to induce disease in the infected host [49]. In particular, *Tat* is a multifunctional protein, expressed into two forms (p16 and p14) and synthesized during the early phases of the viral cycle. *Tat* is involved in chromatin remodelling, in phosphorylation of RNA polymerase II, modulating the transcription of the full-length viral mRNAs, in transactivation of viral genes and in interacting with specific sites of HIV-1 mRNAs. *Tat* proteins considerably increase the efficiency of HIV replication [50]**.** In the cells the *rev* protein (p19) is involved in a crucial process of HIV replication, as it regulates the export of unspliced RNA messengers from the nucleus to the cytoplasm in the infected cells. The lack of *Rev* protein is associated with the retention in the cell nucleus of RNA messengers codifying structural viral genes (*Gag* and *Env*). The *nef* Negative Regulatory Factor physically binds to a wide series of the components belonging to the host's molecular machinery in the cytoplasm. Nef controls protein trafficking paths and promotes the release of virions and increases their infectious ability. The *vif* (virion infectivity factor) (p23) is a protein blocking a cellular cytosine deaminase, known as APOBEC3G. This enzyme represents a constitutive cell defense, converting cytidine into uridine, causing alterations in the viral genome, during reverse transcription. Viral particles in cells expressing APOBEC3G will consequently not be able to replicate. The *vif* protein interacts with APOBEC3G and form complex, binding ubiquitin, thereby causing the proteolytic degradation of this enzyme. It is therefore a system to neutralize one of the organism's innate defenses against viruses. The *vpr* protein or viral protein r (p14) modulates the entry of the HIV-1 pre-integration complex into the nucleus and regulates its replication in non-dividing cells including macrophages and causes the cell cycle arrest in G2 phase and apoptosis in proliferating cells. The *vpu* protein or viral protein u (p16) promotes the degradation of CD4 receptor in the endoplasmic reticulum and increases the release of HIV virions [49]**.** The organization of HIV genome is shown in Figure 2.

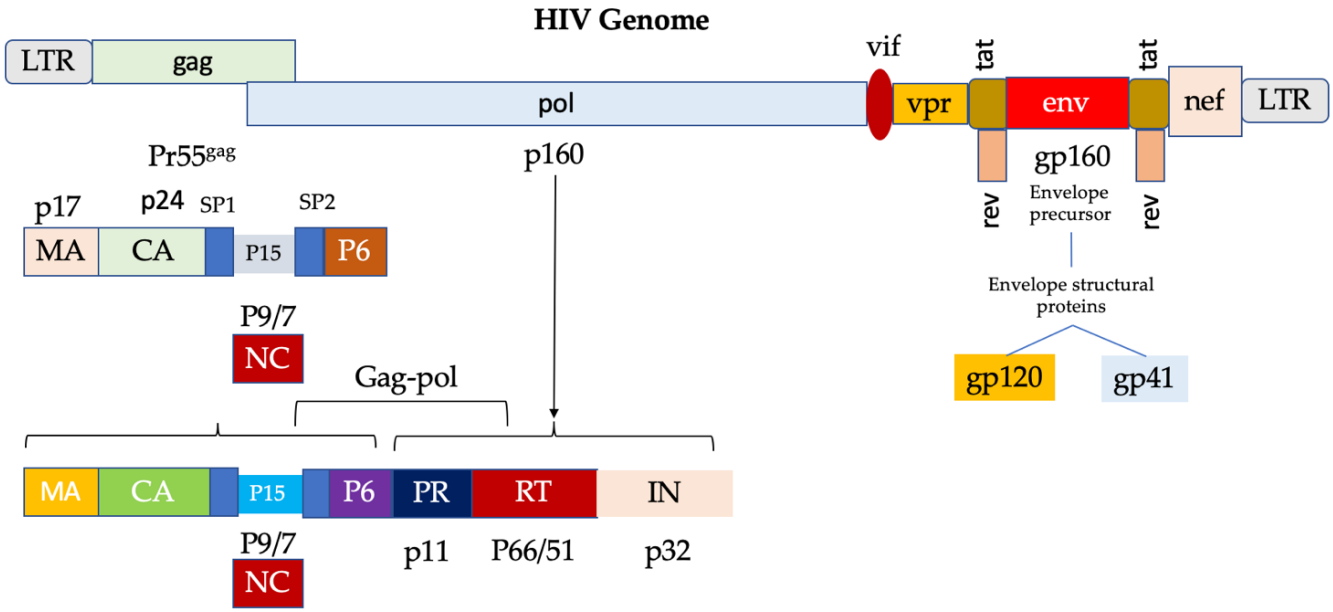

Figure 2 HIV consists of two copies of positive-sense single-stranded RNA, housed in a conical capsid. HIV genome is composed by at least nine genes, defined *gag, pol, env, tat, rev, nef, vif, vpr, vpu,* encoding 19 proteins and two Long Terminal Repeat regions (LTRs) at their 5'and 3' ends. *Gag, pol and env* codify structural proteins, which are incorporated into the new virions. *Env* encodes a precursor glycoprotein (gp160), which is cleaved into two parts, defined gp120 and gp41, by a cellular protease. Three molecules of gp120 and three of gp41 form a structure anchoring to the viral envelope. This apparatus allows gp120 to interact with specific receptors on target cells, such as CD4. *Gag* (Group-specific antigen) gene encodes the information for the synthesis of a 55 kDa polyprotein. This molecule is cut into several proteins after viral budding, including Matrix (MA), Capsid (CA), Nucleocapsid (NC), spacer p1, spacer p2 and p6 domain. The *pol* gene encodes the enzymatic proteins protease (PR), reverse transcriptase (RT) and integrase (IN). *Tat, rev, nef, vif, vpr, vpu* represent regulatory genes and express proteins, modulating and regulating viral infectivity and replication and affecting the HIV ability to induce disease in the infected host (see text).

HIV infects cells expressing the CD4 receptor and the chemokine receptors CCR5 and CXCR4, including: 1) CD4+ T cells; 2) Monocytes and macrophages (CD68+), detectable in lymph nodes, spleen, liver, brain, lung, bone marrow; 3) Dendritic cells in lymphoid germinal components and in lymphoepithelial structures, detectable in vagina, tonsils, and rectum. Furthermore, HIV may infect cells in a wide spectrum of human tissues through an independent CD4 receptors, including kidney tubules, astrocytes, cardiac muscle, enterocytes, endothelial cells. This pattern of cells, which may be infected by this infection, have a strong impact on the understanding of HIV pathogenesis and its related diseases [51,52]. Since several years ago, the availability of more sophisticated and advanced diagnostic techniques has made it possible to identify patients during the first weeks after HIV acute infection. Therefore, this opportunity has contributed to improving our knowledge of the earliest phases of the host's immune system activation against this pathogen and before a stable viraemia has been established. This event is detectable about 3-6 months after acute infection. On the basis of the current evidence concerning acute HIV infection, host's immune system activation is characterized by the increase of a wide spectrum of plasma cytokines and chemokines [53]. Stacey et al have studied the dynamic pattern of these mediators in samples obtained from patients with acute HIV-, HBV- and HCV-infections, during the earliest phases of exponential viraemia increase. They have shown an elevation in the levels of these mediators in all groups, but with distinct hierarchy and kinetics, depending on the different viruses. In particular, the viral expansion, in subjects with acute HIV-1 infection, has been characterized by dynamic changes in plasma levels of several cytokines/chemokines, such as the fast and transient increase in interleukin-15 and interferon-α (IFN-α), the long-lasting elevations in inducible protein 10 (IP-10), in tumor necrosis factor-α (TNF-α) and in monocyte chemotactic protein 1 (MCP-1) as well as the slow rise of IL-6, IL-8, IL-18, IFN-γ and IL-10. The rapid activation of the cytokine pathway, observed in this pathological condition, originates the process known as a cytokine storm. However, the magnitude of this strong systemic cytokine release, is unable to control HIV infection and to prevent its persistence [54].

Similar results have been obtained in a research by Muema's, who has investigated the kinetics of plasma cytokines, immune cell dynamics and viral replicative ability in a cohort of patients with hyperacute HIV infection, before the viral expansion has reached its peak. This study has confirmed the increase of plasma IP-10, MIG, IFN-γ, IFN-α, MCP-1, IL-12, soluble IL-2 receptor, IL-1RA, IL-8, CXCL13 and soluble CD14 in untreated individuals. In these subjects, acute HIV infection is associated with the development of cytokine storm, with lymphocytes-, eosinophils-, and basophils-depletion with a rise in the monocytes of peripheral blood [55].

HCV structure and pathogenesis

HCV is a small circular enveloped positive-sense, single-stranded non-segmented RNA genome virus, approximately 9,600 bases in length [56]. Viral particles are about 50-70 nm in diameter [57]. HCV belongs to genus Hepacivirus and family Flaviviridae [58]. Its genome includes a single open reading frame (ORF) that codifies a polyprotein of about 3,010 aminoacids in length and 5' and 3'untranslated regions (UTRs) at both ends (Figure 3).

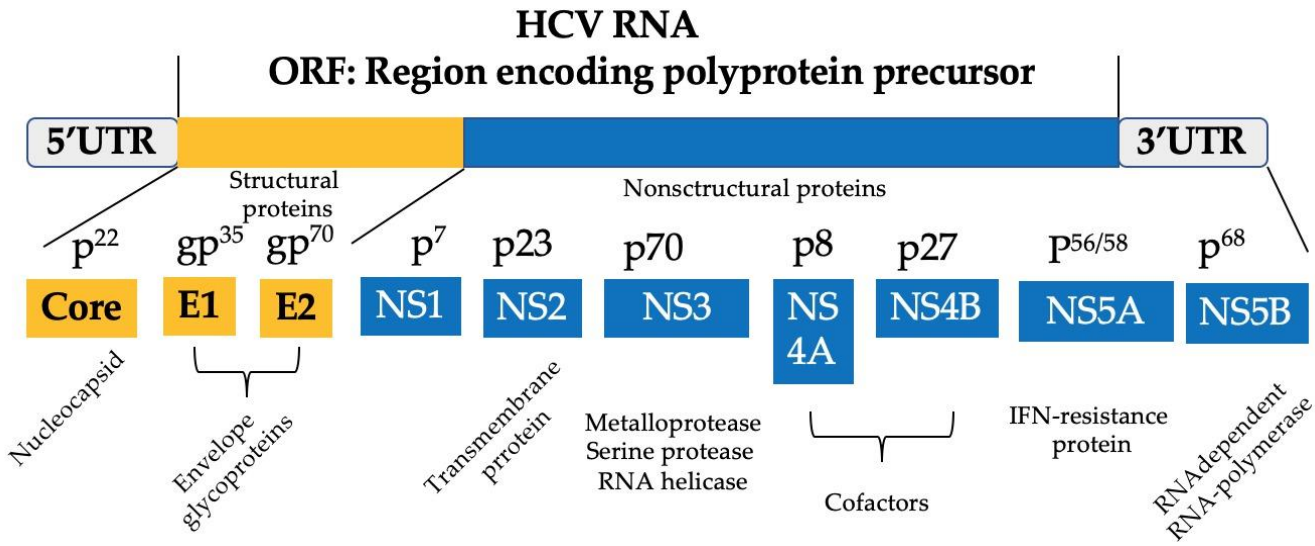

Figure 3 HCV consists of a single-stranded RNA genome, approximately 9.6 kb in length. The viral genome includes a single open reading frame (ORF) codifyng a polyprotein precursor of about 3,010 aminoacids in length and 5' and 3'untranslated regions (UTRs) at both ends.

Polyprotein cleavage generates mature structural- (core, E1 and E2) and non-structural (NS1 or p7, NS2, NS3, NS4A, NS4B, NS5A, NS5B) proteins. Core (C) protein is involved in the development of viral nucleocapsid structure and by the envelope glycoproteins; E1 and E2 are detectable on cell membrane; NS1or p7 is a short membrane protein; NS2 is a transmembrane protein; NS3 protein has serine protease- and NTPase/helicase-activity; NS4A protein has a double function: a) cofactor for NS3 protein, its binding to NS3 increase its activity with a more efficient process of cleavage, b) modulation of NS5A phosphorylation; NS5A with unknown function; NS5B is the RNA-dependent RNA polymerase (RdRp)

These UTRs are highly conserved key RNA elements for a proper viral genome replication and protein translation. A 340-nucleotide long sequence at 5' non translated region (NTR) functions as an internal ribosome entry site. When this zone in HCV genome binds the 40 S ribosome subunit, the process of the polyprotein synthesis in host's cells begins [59]. Cell- and virus-associated proteases cleave HCV polyprotein at the endoplasmic reticulum (ER) membrane, generating 10 mature structural and non-structural proteins. The former group of proteins is represented by the core (C), which is involved in the development of viral nucleocapsid structure and by the envelope glycoproteins E1 and E2. These proteins are detectable on the surface of virions and are considered essential in mediating the HCV entry into the host's susceptible cells by means of the binding to specific cell membrane receptors. The latter group includes: 1) a short membrane protein, defined as peptide p7 (or NS1), probably a viroporin; 2) the NS2 protein, a transmembrane protein; 3) the NS3 protein, with serine protease activity at its N-terminal and an NTPase/helicase activity at its C-terminal; 4) NS4A protein, it is a cofactor for NS3 protein, NS4A binding to NS3 increases its activity and promotes a more efficient process of cleavage, furthermore NS4A modulates the phosphorylation of NS5A; 5) NS5A is a polyphosphorylated protein with unknown function; 6) NS5B is the RNA-dependent RNA polymerase (RdRp) [59]**.** NS3 serine-like protease and the RNA-

dependent RNA polymerase (RdRp) are believed to be components of the HCV replication complex and are required for efficient viral maturation and replication. In particular, HCV core proteins interact with viral RNA and generate nucleocapsid particles on the membrane of the endoplasmic reticulum (ER) in the host's cells. Then, these particles associate with viral envelope proteins that are embedded in the ER membrane, generating complete enveloped virions by budding into the ER lumen. RdRp is lacking proofreading activity, therefore HCV genome is characterized by remarkable sequence variation. To date, 7 HCV genotypes have been described with a wide number of subtypes [60]**.** Furthermore, HCV generates within the infected hosts a pool of genetically different variants, although closely related. They are known as "quasispecies." HCV virions are detectable in different forms in the serum of human carriers. In particular, viral particles may either bind to: a) low-density and very low-density lipoproteins, b) or to immunoglobulins [61]. Furthermore, HCV may: c) circulate in the blood in the form of free virions [62]**;** d) infect not only liver cells~~,~~ but also peripheral blood mononuclear cells (PBMNC), including T-, B-~~,~~ monocyte and macrophage populations [63]**;** and even cells of extrahepatic tissues, such as heart, kidney, pancreas, intestine, adrenal, lymph node, and gallbladder. HCV antigens, HCV-RNA and its intermediate forms have been detected in the cells of all these organs, suggesting that these sites can act as a reservoir for this pathogen and may host its replication [64]. A large series of studies concerning HCV acute infection in humans and chimpanzees have shown that this condition may be followed by a double outcome: either the spontaneous clearance of this pathogen or its persistence in the host. The former event is characterized by the generation of a pool of HCV-specific CD4 and CD8 T cells [65]**.** These T cells release a wide spectrum of cytokines and exert strong and sustained effector polyfunctional activities against multiple epitopes within the different proteins of this pathogen both in intrahepatic and peripheral sites [66]**.** This type of immune response leads to the control of HCV and the development of specific memory T cells, which express the IL-7 receptor CD127 on plasmatic membrane [67]**.** If the immune system fails in controlling HCV infection, specific CD8 T cells

undergo exhaustion and increase the expression of some specific markers, including programmed death 1 (PD1), T-cell immunoglobulin and mucin domain-containing-3 (Tim-3), cytotoxic T-lymphocyte protein 4 (CTLA4), 2B4, CD160, KLRG1, T-cell immunoreceptor with Ig and ITIM domains (TIGIT), and CD39 [68]**.** Furthermore, the production of early neutralizing antibodies can have a favorable impact on the outcome of the acute infection [69]**.** According to the results of Stacey's study (see paragraph concerning HIV), a rapid increase in plasma concentration of pro-inflammatory cytokines and chemokines, including TNF-α, IFN- γ, IL-6, IL-10, IL-18, IP-10, MIP-1 β and RANTES, was observed in patients with acute HCV-infection during the earliest phases of the disease. However, in this group the production and release of the above mentioned mediators have been delayed and of lower magnitude in comparison with ones observed in individuals with HIV-related acute infection. Furthermore, substantial differences have been observed in the hierarchy of cytokines/chemokines elevation, detectable in acutely HIV- and HCV-infected patients [54,70]**.** According to Stacey's conclusions, two important points have to be underlined: 1) the activation of the cytokine cascade in subjects with acute HCV infection does not reach such a magnitude as to originate the process known as a cytokine storm, which has been described in patients with HIV infection; 2) however, even if some of these cytokines/chemokines may have a role in the control of HCV replication, an excessive and uncoordinated release of these mediators ~~is~~ are not protective and curative for the infected host. This event is rather associated with both the well-known acute and the long lasting immune-pathological consequences, deriving from this infection.

Influenza virus structure and pathogenesis

Influenza viruses belong to the family of *Orthomyxoviridae*, which includes four genera able to infect vertebrates: *Alphainfluenzavirus* (type A), *Betainfluenzavirus* (type B), *Gammainfluenzavirus* (type C) and *Deltainfluenzavirus* (type D) [71]**.** Influenza viruses type A, B and C possess the capacity

to infect mankind, but only the A and B ones cause important clinical manifestations in humans [71]. All these types of Influenza viruses consist of a single-stranded RNA with negative polarity ((-) ssRNA), but they differ in their organization. Therefore, we will consider the type A as a paradigm [71]. By electron microscopy, influenza A and B viruses appear as spherical or filamentous forms, about 100 nm in diameter and 300 nm in length, respectively. The genome of influenza virus A and B is about 13,500 bases in length and includes eight single-stranded RNA segments. Each of these elements is separately enclosed in viral ribonucleoprotein complexes (vRNPs). Within these structures, the 5′ and 3′ ends of viral RNA are associated with RNA-dependent RNA polymerase (RdRP) with manifold copies of nucleoprotein (NP). The vRNPs regulate the viral life cycle, modulating transcription and replication of this pathogen in infected cells [72]. The influenza virus genome encodes 11 proteins (HA, NA, NP, M1, M2, NS1, NS2 or NEP, PA, PB1, PB1-F2, PB2) [73] **(Figure 4).**

The RNA polymerase of the influenza virus is composed of three subunits, such as polymerase basic 1 (PB1), PB2 and polymerase acidic (PA) in influenza A and B viruses. A matrix consisting of M1 protein incorporates the virion core and it is surrounded by an external lipid envelope, where three integral glycoproteins, such as M2 (an ion channel), haemagglutinin (HA) and neuraminidase (NA), are embedded [74]. The several subtypes are indicated according to an H number (for the type of hemagglutinin) and an N number (for the type of neuraminidase). To date, 18 different H antigens (from H1 to H18) and 11 distinct N antigens (from N1 to N11) have been identified [71]. HA is a lectin mediating the entry of the viral genome into target cells, while NA is an enzyme involved in the release of the virions from infected cells [75]. These two glycoproteins are both a target for antiviral drugs [76] and for specific antibodies.

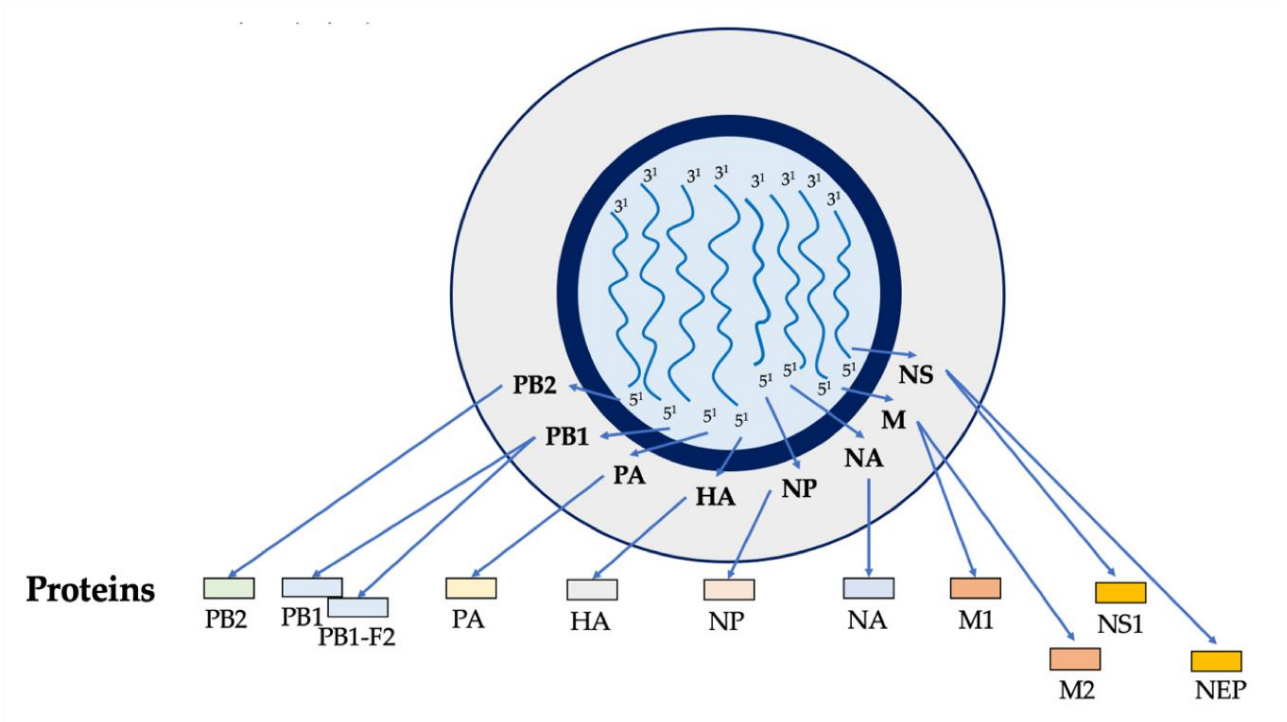

Figure 4. Representation of Influenza virus genome. Humans may be infected by Influenza viruses type A, B and C. They differ in their organization, but only the A and B ones cause important clinical manifestations in mankind. Type A is considered as a paradigm. Influenza virus genome is about 13.5 kb in length, consists of a single-stranded RNA with negative polarity ((-) ssRNA and includes eight single-stranded RNA segments. Each of these elements is separately enclosed in viral ribonucleoprotein complexes (vRNPs). The 5′ and 3′ termini of viral RNA are associated with RNA-dependent RNA polymerase (RdRP) with multiple copies of nucleoprotein (NP). The **influenza virus genome is composed of 11 proteins (HA, NA, NP, M1, M2, NS1, NS2 or NEP, PA, PB1, PB1-F2, PB2). The RNA polymerase of the influenza virus consists of three subunits, such as polymerase basic 1 (PB1), PB2 and polymerase acidic (PA), codified by three genes in influenza A and B viruses. A matrix consisting of M1 protein includes the virion core and it is surrounded by an external lipid envelope, where three integral glycoproteins, such as M2 (an ion channel), haemagglutinin (HA) and neuraminidase (NA) are embedded. HA and NA are used to classify the different**

serotypes of influenza A viruses. Additional Influenza virus proteins include the accessory protein PB1-F2, displaying pro-apoptotic activity, NS1 protein with anti IFN-β function and promoting of viral replication, NEP (or NS2) nuclear export of viral ribonucleoproteins.

Therefore, they are used to classify the different serotypes of influenza A viruses (16 HA and 9 NA) [71,77]. The isolated influenza strains are identified through a standard nomenclature which indicates the type of virus, the geographical location where it was first isolated, the sequential number of isolation, the year of isolation and the HA and NA subtypes [71]**.** Additional Influenza virus proteins are represented by the accessory protein PB1-F2, with pro-apoptotic activity, by NS1 protein with anti IFN-β function and with the promotion of viral replication as well as by NEP (or NS2) nuclear export of viral ribonucleoproteins. Influenza viruses are mainly transmitted via the air and spread very easily through the droplets of saliva that the patient produces by coughing, sneezing or simply talking, especially in crowded and closed environments [78]. New influenza viruses constantly emerge by mutations or reassortment in their genome, leading to the events respectively known as “antigenic drift” with the generation of "strain variants" and as “antigenic shift”, when the virus acquires completely new antigens, by recombination between avian- and human-viral forms. One of these variants may, eventually, achieve a greater virulence becoming dominant and rapidly spreading throughout the population and often causing a pandemic [79,80]**.** According to an alternative model, periodic pandemics are produced by the interaction between a fixed set of viral strains and a human population with a constantly evolving immune response against these pathogens [72,80-82]**.** High levels of several pro-inflammatory mediators including interferons (IFNs), tumor necrosis factor-α and IL-1β, IL-6, IL-8, IL-10, MCP-1, IP-10 and monokines induced by IFN-γ (MIG) have been observed in patients admitted to Hospitals with severe forms of influenza virus infection (2009H1N1 and H5N1) and hypothesized in subjects, who have suffered from H1N1-related disease in 1918 [83,84]**.** The dysregulation in the production and release of these

Cytokines/Chemokines, known as the cytokine storm, has been described also in the patients who are infected by these pathogens [85,86]. Elevated plasma amounts of these interleukins/cytokines are strongly associated with the severity of the disease [87]. Furthermore, cytokine storm has been reported to exert a direct role in the morbidity and mortality, during the acute phases of influenza virus infection [88].

2.Considerations on the onset and spreading of pandemics caused by respiratory viruses according to a historical perspective

On the basis of a historical perspective, since the 19th century onwards several major viral pandemics, almost exclusively caused by influenza viruses, have affected mankind, spreading across the world. We have examined the features of all these infectious events with the aim to get lessons potentially useful in effectively managing and counteracting the current SARS-CoV-2 related pandemic. The knowledge and the experience deriving from History of Medicine indicates that the infectious diseases caused by respiratory viruses generally develop during the autumn and winter months [89-91]. In this period of the year, cold, decreased hours of sunshine, elevated levels of humidity and crowding of individuals in closed spaces are optimal conditions to promote the spread of epidemics associated with respiratory pathogens. Furthermore, the major viral pandemics generally start in Eastern or Southern Hemisphere nations and then tend to disseminate to Europe and America [92]. During the course of all these infectious events, the number of affected individuals and of deaths has been characterized by multiple well-separated temporal peaks with a time-scale months [93]. Each of these epidemics had its own features, such as the type of influenza viruses involved, although at least one study has suggested that the etiological agent of the Russian pandemic in 1889 may have been the human Coronavirus 043 and not an influenza virus, their high transmissibility, the geographical area of onset, the clinical impact in distinct regions of the globe as well as the death rate in the general population or in the different age-classes. Overall, all these

infectious events occurred with different waves over a duration of 2-3 years. The main characteristics of each epidemic are shown in **Table 1** [94-106]. To date, the mechanisms causing single or multiple waves during pandemics are not definitively clarified. However, some researchers have focused their interest on the study of this complex problem. Therefore, they have proposed some mathematical models with the purpose of reproducing in a quantitative way the pattern of the waves and with the aim to recreate the course of the outbreaks, during the pandemics [107]**.** One study has examined the conditions contributing to generate two-waves shaped curves via the design and the analysis of five possible mathematical models. This strategy simulates the development and the course of acute infectious diseases as observed in the actual clinical practice [107]. In this paragrapher of our paper, we have considered as a paradigm the pandemics, which have affected mankind, starting from the one occurring in 1889 and known as the "Russian pandemic influenza". The main features of each pandemic, including the period of onset, the virus strains, the estimated world population and the estimated infected people, the number of waves, the case fatality rates and the number of deaths are described in Table 1. The "Russian pandemic influenza" began in October 1889 and reached Europe and North America in 1890 [104]**,** with distinct recurrences in March - June 1891, November 1891 - June 1892, winter 1893-1894. The second wave during the spring of 1891 and the third one in the winter of 1892 resulted more lethal than the first one. It has been estimated that about 1 million of people died. Most of the deaths were caused by respiratory forms, mainly in the middle age patients [92,100]. It is still uncertain what pathogen has caused the "Russian flu" pandemic. According to available studies, three different viral strains have been proposed as a possible agent responsible for this infectious event, including Influenza A virus subtype H2N2 or subtype H3N8 [104] or the Human Coronavirus OC43 [106]**.** Also during the Spanish epidemic also 4 waves were observed [98,103]. the first in March 1918, the second in the half of August 1918, while World War I was ongoing, the third and the fourth in the period January-June 1919 and January–April 1920 respectively [108]. Even during this infectious outbreak, the second and third waves

resulted more lethal than the first one [109]. Although the number of deaths that occurred during this pandemic remains uncertain, it has been calculated that 500 million- 1 billion of people became infected and about 17-50 million individuals died [89]. The agent responsible for this infectious event is represented by the Influenza A virus subtype H1N1. Mortality rates were elevated in individuals younger than 5 years old as well as in subjects 20-40 years old and 65 years and older. The high mortality observed in the healthy population, such as people in the 20-40-year age class, represented a unique feature of this pandemic [103,110]**.** The Asian influenza pandemic onset occurred in the early 1956 or 1957, probably in Guizhou, where the first cases were observed [105]**.** Then it spread to Yunnan province and to Hong-Kong on April 1957, reaching Singapore, Taiwan, Japan and India in May. In June 1957 the epidemic involved the United Kingdom and the United States of America (USA). It has to be underlined that in the USA, in the first phase, the outbreak mainly affected children, returning to school after the summer break in 1957 [102]. This circumstance probably provided the opportunity for the pandemic spread in this group of patients with the development of high morbidity rates and a relative excess of mortality. In the following period (January-March 1958) the mortality rates of children decreased, whereas they remained elevated in the age group of 45-74 years old or in the age over 75 years [111]**.** During the second wave, the pandemic spread across the globe and it has been calculated that at least 500 million of individuals were infected worldwide. This infectious event caused more than 1 million of deaths. The etiological cause of the Asian influenza pandemic was represented by influenza A subtype H2N2, which has been generated by the reassortant of avian and human influenza viruses. After the first two waves, two major recurrences of the epidemic, associated with the human H2N2 strain, occurred in January-March 1960 and January-March 1963. These periodic outbreaks were caused by H2N2 viral subtypes, progressively acquiring minor genetic modifications (an event known as antigenic drift). Then, after a decade, a new influenza A subtype emerged (H3N2 strain) and was associated with the development of the Hong Kong pandemic in 1968 (an event known as antigenic shift) [96,112]. The

genome of this pathogen included two genes, originating from an avian influenza virus and from six genes from the A (H2N2) virus, which had spread across the world during 1957 infectious event [113]. The 1968 pandemic has been characterized by two waves, the first in 1968/1969, the second in 1969/1970. The last outbreak was characterized by a drift in the neuraminidase antigen [114].

Although it has been hypothesized that the onset of the 1968 pandemic have occurred in Mainland China, the first case was observed on 13 July 1968 in Hong Kong. At the end of the month, the virus has reached Vietnam and Singapore, and, in September, the Philippines, northern Australia, and Europe were involved. Furthermore, in December 1968 the pandemic has spread in the USA and, in 1969, in Japan, Africa, and South America. A peak in the number of deaths was reached in December 1968 and January 1969 across the globe [94,97]. It has to be underlined that most of the deaths associated with this infectious event (about 70%) were observed in 1968/1969 in North America (USA and Canada). On the other hand, in Europe, Japan and Australia the majority of the subjects (70%) died during the second wave of the epidemic in 1969-1970. The causes of this difference in mortality rates detected in North America and in Europe, Japan and Australia are not well understood. However, a phylogenetic analysis of the virus found a drift in the neuraminidase antigen, whereas the hemagglutinin antigen had not [114].Lindstrom has shown that 2 distinct neuraminidase lineages have identified during the1968/1969 and 1969/1970 pandemic and suggested that this genetic diversity could have been caused by several reassortments occurring in A/H2N2 viruses. These evidence might contribute to explain the differences in disease severity and clinical outcomes observed in distinct geographical areas in the two considered periods (1968/1969 and 1969/1970) [115]. It has been calculated that at least 500 million of individuals were infected worldwide and more than 1 million deaths occurred [116]. The 2009 Swine influenza virus is an H1N1 strain, defined as H1N1/09. It has originated from the recombination of genomic material from a bird, swine, and human flu viruses with a Eurasian pig flu virus. The epidemic developed between January 2009 and August 2010, starting from Mexico, then it spread worldwide [101]. This pandemic

was characterized by at least two waves, although some Researchers have reported the third one. The first infectious event occurred in April -July 2009 and, according to research carried out in the United Kingdom (UK), it quickly subsided at the onset of the summer closing period for schools. The second wave appeared in August 2009 when the students resumed school lessons and lasted until March 2010 [95]. A third outbreak occurred between December 2010–February 2011 and was described in several countries, including Denmark [117] and the UK, where this infectious event was associated with an atypically cold period with dry weather [95]. Both the second and the third waves were characterized by a more severe clinical course in the affected patients in comparison with the first one [118]**.** However, to date, it has not been demonstrated that the potential increased virulence of this virus, as it has been observed in 2010 and in 2011, is correlated with the emergence of viral clusters which have undergone to the event known as antigenic drift in viral 2009 progenitor or with the development of further mutations in its genome [99]**.** Overall, near 80 percent of deaths associated with the Swine influenza virus were observed in people younger than 65 years of age. According to CDC estimates it has been suggested that about 151,700-575,400 people across the globe have died from the H1N1/09 related infection, during the first year of the pandemic [119,120]. Overall, the available historical descriptions as well as the results of the more recent studies focusing on the previous viral epidemics (Russian-, Spanish-, Asian-, Hong-Kong-, Swine Flu- epidemics) teach to us that each of these infectious events consists of peculiar signs in comparison with typical seasonal flu outbreaks as it is generally associated with at least two or three waves, with a more elevated patients' morbidity and mortality during the second and third waves in comparison with the first, as well as with possible recurrences associated with the event known as antigenic drift [96,101,104]**.** Therefore, these observations must be taken into strict consideration by the Governments and Health Services of each Nation across the world and should suggest to planning preventive programs with the purpose to hamper the deleterious effects of the current and of the possible future SARS-CoV-2 related waves. These pandemics have presented peculiar signs in comparison with

typical seasonal flu outbreaks, such as numbers of waves, generally more than two/three, morbidity, and mortality rates.

Name of pandemic	**Virus strains**	**Date of onset/ duration**	**Number of waves/ Period of onset**	**Suspected origin of epidemic**	**Estimated world population**	**Estimated infected population**	**Number of deaths in the world**	**Case fatality rates**
"Russian" pandemic influenza [100,104,106]	Likely H3N8 or H2N2 Or Corona-virus OC43	1889 1889-1894	4 First: October 1889-December 1890; Second: March-June 1891; Third: November 1891-June 1892; Fourth: winter 1893-1894	Russia	1,5 billion	300-900 million	1 million	0.1%–0.3%
"Spanish" pandemic influenza [96,98,103]	H1N1	1918 1918-1920	4 First: March 1918 Second: second half of August 1918 Third: January-June 1919 Fourth: January–April 1920	China	1.8 billion	500 million- 1 billion	17-50 million	2-3% Until 10%
Asian influenza [95,102,105]	H2N2	1957 1957-1958	2 First: October-December 1957 Second: January-March 1958 Recurrences of H2N2 virus associated pandemic in: January-March 1960 January-March 1963	China	3 billion	about 500 million	1.1 million	0.2 %-0.3%
Hong-Kong	H3N2	1968	2 waves	Hong Kong	3.5 billion	about 500 million	1-4 million	0.1%-0.3%

influenza [94,97]		1968-1970	First: July 1968-April 1969 Second: November 1969-March 1970					
Swine influenza [99,101]	H1N1/09	2009 2009–2010	2/3 waves First: April to July 2009 Second: August 2009 to March 2010. Third: November/December 2010–February 2011	Mexico	6.8 billion	About 700 million-1,4 billion	151,000-575,000	0.01%

Table 1. Main characteristics of Russian-, Spanish-, Asian-, Hong-Kong-, Swine influenza pandemics, including the virus strain involved, the geographical area of onset, the clinical impact in distinct regions of the globe and the death rate in the general population or in the different age-classes.

3.Considerations on the possible establishment of a persistent SARS-CoV-2 reservoir worldwide and on the potential risk of developing malignant and non malignant-conditions

On the basis of current epidemiological data, the number of patients with SARS-CoV-2 related infection is quickly increasing. To date, it is thought that at least 96,000,000 people in the world have come into contact with the virus with about 2,08 million of deaths (WHO Coronavirus Disease COVID-19 Dashboard, accessed on 22/1/2021), but it is very probable that this figure is underestimated and a more elevated number of individuals have got the infection. The possibility of understanding when a patient with a proven positivity to specific tests for detecting the presence of a virus has cleared the pathogen represents a crucial element for the control of infectious diseases. According to the initial WHO's recommendations, subjects with a previous diagnosis of SARS-CoV-2 related infection is confirmed to have cleared the virus and may be discharged from

isolation, when they are clinically recovered and have two negative RT-PCR results on sequential samples taken at least 24 hours apart [121]. RT-PCR test is currently considered the gold standard for the diagnosis of SARS-CoV-2 infection. An update of the criteria for the conclusion of the isolation as part of the clinical care in patients with SARS-CoV-2 related infection has been proposed on May 27, 2020, with the introduction of an interim guide for the clinical management of COVID-19. The application of these recommendations concerns all patients suffering from this disease irrespective of the affected areas worldwide or of the disease severity [121]. Criteria for the discharge of the subjects from isolation without requiring retesting: a) In symptomatic patients, 10 days following the development of symptoms plus at least 3 additional days without symptoms (in absence of fever and without respiratory symptoms); b) in asymptomatic patients, 10 days following positive tests for SARS-CoV-2 [121]. Although on the basis of these recommendations, subjects with the previous contact with this pathogen are considered to have cleared SARS-CoV-2, there is the possibility that this pathogen may constitute a persistent reservoir at least in a part of the very large number of people who had a contact with this pathogen worldwide. This potential biological behavior for SARS-CoV-2 is not surprising, as it has been observed for other viruses-related diseases, not only in subjects receiving chemotherapy or immunosuppressive therapy, but also in apparently immune competent individuals, although at lower rates throughout the patient's lifetime. It has been shown that both RNA- and DNA-viruses have elaborated mechanisms to establish latent or occult infections in human hosts. These pathogens are represented by HIV, HCV, Respiratory syncytial virus (RSV), Measles virus, West Nile virus, Zika virus, Enterovirus and even Ebola virus [122]. Furthermore, this condition of persistence is associated with a risk of viral reactivation. RNA viruses, such as HIV [123,124] and HCV [125] exhibit this biological behavior. Although the contribution of Respiratory syncytial virus (RSV), Measles virus, West Nile virus, Zika virus, Enterovirus and even Ebola virus to the long-term human pathology has not yet been defined, some patients who were infected by these pathogens experienced complications or diseases lasting for weeks or

months after the acute phase of infection and the genome of these pathogens was detectable for the same period of time [122]. Furthermore, DNA viruses, such as Herpesviruses, Adenoviruses, Polyomaviruses and HBV are able to establish lifelong persistence infections. Among these pathogens, HBV and Herpesviruses, are is able to induce the development of severe sequelae in a significant percentage of infected individuals [126-128] and Herpesvirus Family present this biological behaviour. It is now well known that HCV-RNA and HBV-DNA can be present and detectable in the liver and/or cells of different tissues, such as immune-, pancreatic- and renal-cells, even in subjects without a well-recognized and well-recorded contact with these pathogens or in individuals who have reached the apparent eradication of these viruses, after their spontaneous or therapy-related clearance. This condition is known as occult infection and it has been described for HCV as well as for HBV [127,129]**.** HIV also displays strategies to induce its persistence in human hosts, such as promoting a latent stable reservoir, in cells expressing the CD4 receptor, such as resting memory CD4(+) T cells, monocytes and macrophages (CD68+) as well as dendritic and also in cardiac muscle-, endothelial-, kidney tubules-cells, astrocytes and enterocytes [51]. This condition contributes to facilitating its persistence in infected subjects and to promote its transmission to other individuals [124,130]**.** Several sites of latency have been also described for the members of the Herpesvirus Family. EBV infects human lymphocytes and oropharyngeal epithelial cells, whereas Herpesvirus-8 colonizes monocytes as well as endothelial- and B-cells. Furthermore, Primary varicella-zoster virus establishes a lifelong latent infection in ganglionic neurons. Its reactivation in infected individuals induce a disease known as Herpes Zoster, associated with neurological complications [131-133]. On the basis of these data, it has to be taken into the due account the possibility that even SARS-CoV-2 may become persistent in infected cells. This aspect will be of great relevance in clinical practice and it will have to be investigated in the future to clarify the possible carcinogenic properties of this virus and its potential impact in human non-malignant as well as malignant pathologies. This assessment will be important also in patients, who have been reported to have

cleared SARS-CoV-2 and to have obtained a complete recovery. HCV and HIV, as well as HBV are oncogenic pathogens and they have been associated with the development of non-cancerous diseases as well as with several types of cancers. A broad spectrum of long-term HCV-related hepatic and extrahepatic manifestations, both malignant and non-malignant ones, has been described in the literature. Several organs are involved by this pathogen [134,135]. HCV persistent infection is characterized not only by mild to severe forms of liver damage, but also by a wide spectrum of extrahepatic manifestations (EMs), ranging from conditions with subclinical expression to ones with very serious clinical presentation. In particular, it is well-known that patients with HCV chronic infection may develop over time both non-malignant, or malignant diseases. In particular, non neoplastic pathological conditions include insulin resistance, type 2 diabetes, myocarditis, cardiomyopathies, cardiovascular diseases (i.e., Stroke, ischemic heart disease), chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis, asthma, interstitial lung diseases, neurological and hematological disorders. Furthermore, the identification in clinical practice of subjects with occult HCV infection, both naïve untreated individuals and patients with sustained virological response to direct-acting antiviral agents, contributes to making even more complex the understanding of the immunopathogenesis concerning this virus [136]. Hepatic (liver and cholangiocarcinoma cancers) and extrahepatic tumors (distinct subtypes of B-cell Non-Hodgkin lymphomas, pancreatic-cancer and probably breast-, thyroid-, ovarian- and renal-neoplasms) have been reported in patients with HCV-related previous infections [137-139]. On the basis of all the available data and results, chronic HCV infection is characterized by several significant EMs. Therefore, it must be considered not as a liver disease, but rather as a systemic pathological condition [140]. Patients with HIV-related and not treated infections undergo a state of immune dysregulation and immunodeficiency with the impairment of the host's immune system function. Therefore, these subjects also present an increased risk of developing several non-malignant or cancerous diseases. Before the introduction of effective anti-retroviral therapy, the most important

pathological burden associated with this pathogen was due to the emergence of opportunistic infections (OIs) [141]. These pathological conditions emerge with the progressive development of the host's CD4 T lymphocytes serum level. It is known that patients

with a count of these immune cells below 200/mm3 present an increased risk of OIs. However also patients with more elevated levels of CD 4 T lymphocytes (about 500/mm3) maintain a higher incidence of Ois [142]. The decrease in the number of these immune cells below a critical count causes a global deficit of the immune system. These opportunistic infections are generally characterized by an aggressive clinical course, resistance to treatment and an elevated rate of relapse. Protozoal- (Pneumocystis carinii pneumonia, Cryptosporidium, Toxoplasma gondii, Isospora belli), Fungal- (Candida, Cryptococcus), Bacterial- (Mycobacterium Tuberculosis, Mycobacterium Avium-Intracellulare, Salmonella), Viral- (Herpes Simplex Virus, Herpes Zoster, Cytomegalovirus) are the most common pathogens associated with HIV infection. However, even after the advent of effective antiviral treatment, OIs are still observed effective antiretroviral therapy (ART), mainly in subjects who have not yet been diagnosed with HIV and in individuals not receiving therapy [141]. The introduction of ART has increased the life-expectancy of HIV-infected patients, but this condition is associated with a higher risk to develop a large spectrum of pathological conditions, including: A) autoimmune diseases involving lungs (sarcoidosis), thyroid gland (Graves' disease), liver (autoimmune hepatitis), connective tissue (systemic lupus erythematosus, rheumatoid arthritis, polyarteritis nodosa and other types of vasculitis, antiphospholipid syndrome) or hematopoietic system (autoimmune cytopenias) [143]; B) neurological diseases, such as cognitive impairment, cerebrovascular disease, and peripheral neuropathy [144]; C) cardiovascular diseases, such as heart failure, stroke, and arrhythmias [145]; D) insulin resistance, as well as metabolic derangement and the occurrence of type 2 diabetes associated with ART [146]; E) renal injury through direct HIV-related cytotoxicity or immune complex-mediated glomerulonephritis as well as nephrotoxicity and co-morbidities associated with ART for a long-last period [147]. Furthermore, HIV infection correlates

with an increased risk of developing some neoplasms. The term “HIV-associated cancers” indicates a wide spectrum of malignancies occurring in these subjects, such as non-Hodgkin lymphomas, Kaposi's sarcoma, invasive cervical cancer (known as AIDS-defining cancers) and lung-, anal-, vulvar-, penile-, hepatocellular-cancers, Hodgkin’s lymphoma, oropharyngeal cancer and squamous-cell skin cancer, Merkel-cell carcinoma, the myelodysplastic syndrome, polycythemia vera, and (mainly in sub-Saharan Africa) squamous-cell carcinoma of the conjunctiva (known as non-AIDS-defining cancers) [148,149]. To date, also viruses with DNA genomes, such as HBV and some members of the Herpesvirus Family, including Epstein Barr virus (EBV) and Herpesvirus-8 are recognized as oncogenic agents. EBV infection is associated with an increased risk of non-Hodgkin’s and Hodgkin’s lymphomas, nasopharyngeal carcinoma and gastric cancer. A large spectrum of EBV latent genes is induced during the course of infection and it is mainly expressed during its later stages. This biological behaviour allows the survival of the viral genome and prevents the protective activity of the host's immune response by means of a limited expression of the viral genome and proteins. Interaction between cell oncogenes and EBV latent genes causes the perturbances of some steps of the host’s cell cycle, such as the transition from G1 to S phase and the inhibition of cell apoptosis [133,150]. Furthermore, Herpesvirus-8 has been associated with the development of three malignancies: Kaposi sarcoma, a plasmablastic form of multicentric Castleman disease (KSHV-MCD) and primary effusion lymphoma. These tumors affect subjects with acquired immunodeficiency syndrome, iatrogenic immunosuppression including organ transplantation and elderly people [131]. Furthermore, although it is still unknown whether the influenza virus, causing the Spanish flu pandemic, may have had a significant impact on the oncogenic risk in patients infected with this pathogen in the years after 1918, some studies are investigating this problem. It has been observed that the subjects who acquired this infection had a more elevated old-age mortality due to cancerous and non-cancerous causes in comparison with individuals who had no contact with this virus. Patients with low non-cancerous mortality had high

cancer mortality and vice versa [151]. These investigations should be aimed to understand the clinical significance of this event. The data available on the possibility of SARS-CoV-2 recurrence/redetection will be discussed in the next paragraph.

4.Considerations on the SARS-CoV-2 re-detection in patients with previous contact with this pathogen.

One of the most important concerns associated with SARS-CoV-2 pandemic is represented by patients with an infection related to this virus and previously confirmed at laboratory tests who remain or become again RT-PCR positive for viral RNA search at nasopharyngeal swab even after a prolonged period of time or after their clinical recovery [152,153].

To date, the re-detection of this pathogen is observed with increasing frequency in patients who have been thought to have cleared the virus, but the clinical significance of this observation, as well as the reasons of this event, remain uncertain and poorly known [154,155]. However, the interest in clarifying this topic is progressively increasing worldwide and some Authors are investigating this issue and searching for the possible causes of this event [156]. The lack of definitive results depends on several reasons, such as the limited knowledge concerning the biological behavior of this virus, due to its recent identification, as well as the heterogeneity of the available studies, such as their design, the criteria for patients' inclusion, the different diagnostic tools used to detect the presence of this pathogen, the considered period of follow-up and the end-points. It is possible that SARS-CoV-2 re-apparence is due to re-infection (defined as a new infection by the etiological agent of COVID-19 after its clearance following the previous contact with this pathogen) or to recurrence (defined as reactivation of SARS-CoV-2, after the apparent control of the virus by the host's antiviral immune response). Some reports worldwide have shown cases of reinfection by phylogenetically distinct clades of this pathogen, proved by means of the entire viral genome sequencing [157-159]. Wide variations in viral loads, clinical manifestations and periods of the time

elapsing between the infectious episodes have been described in these patients. These individuals were either asymptomatic or experienced mild/moderate symptoms [158,159]. However, other studies have suggested that some subjects, who had apparently recovered from the SARS-CoV-2-related infection and have become positive again at a successive control by means of RT-PCR-test at nasopharyngeal swab even several days after the first negativization, may have experienced a reactivation of this pathogen, rather than a reinfection [160]. Most of these studies have included a limited number of patients with a short-lasting period of follow-up [161-163]. According to the current pieces of evidence, the number of reports, describing subjects with possible SARS-CoV-2 redetection is progressively increasing. A recent large cohort study enrolled 1,146 patients with an infection associated with the etiological agent of COVID-19. These subjects were admitted to Hospital and then discharged, after their clinical recovery. Among these individuals, 125 presented a new positive nasopharyngeal swab after two negative tests, during the course of follow-up. It may be hypothesized that these patients have experienced a reactivation of SARS-CoV-2, although it is not possible to exclude a reinfection [152]. Similar results have been obtained in further small-sized studies [163,164] as well as in a Chinese retrospective research, including 758 individuals with SARS-CoV-2 infection. Fifty-nine of them presented recurrent positive RT-PCR after their clinical recovery [165]. According to available data, resulting from about 80 studies, Gidari has reported that from January to September 1, 2020, 1,350 patients, who have recovered from the COVID-19, presented again a positive RT-PCR at nasopharyngeal swab worldwide. New positive respiratory tests were observed in mean 34.5 days after the second negative sample had been observed. However, the data was available for only 123 subjects [166]. Similar results have been reported in a further systematic review by Dao et al. Overall, the percentage of re-positive subjects among discharged individuals with SARS-CoV-2 infection ranged from 2.4 to 69.2% in the considered studies, but in most of them was equal to 10-11%. The time elapsing from patients' discharge to the first re-detection test varied between 1 and 35 days [167]. The reactivation of SARS-CoV-2 replication

has been recently described in one patient suffering from meningoencephalitis with unexpected severe clinical manifestations even after the apparent resolution of the infection [168] as well as in one subject who has undergone immunosuppression, following the treatment for B cell acute lymphoblastic leukemia [169]. It may be hypothesized that both cases are associated with a reactivation of SARS-CoV-2, persisting in a latent status in the previously infected host rather than ~~a~~ reinfection. On the basis of these consideration and taking into the due account the biological behavio~~u~~r of other viruses such as HIV, HCV (see prior paragraph) we have to consider that SARS-CoV-2 might persist at very low or undetectable titers with the common detection tests in the so-called sanctuary organs and periodically reactivate and resume its replication. The relevance of this issue may have a double effect: 1) the reactivation of viral replication in different anatomic sites in some individuals, with the generation of significant viral loads in these carriers, these individuals may remain asymptomatic or become symptomatic and they might theoretically contribute to spread the virus; 2) the lack of the complete eradication of this virus and its persistence at undetectable titers in the host may correlate with the re-emergence of its replication and cause unexpected severe clinical manifestations. These events may occur:

a) in a relatively short-lasting time, as it has already showed in previous case reports, describing immunocompromised patients [168,169] as well as in subjects with persistent severe clinical manifestations after the resolution of SARS-CoV-2 infection. Some pneumological- [170], neurological- (Cerebrovascular accident such as acute hemorrhagic necrotizing encephalopathy, Guillian Barre syndrome, acute transverse myelitis, epilepsy and acute encephalitis [171,172] and cardiovascular- (acute coronary syndrome, myocarditis, stress-cardiomyopathy, arrhythmias, heart failure and venous thromboembolism) complications [173,174] have been described to occur in patients with COVID-19. The real impact of these clinical manifestations on the public Health will have to be carefully assessed in the next future and in the long term with well-designed and well-sized studies. b) In a long-lasting time, with an increased risk of developing progressively adverse

pneumological-, cardiovascular- and neurological-outcomes [175-177] or malignancies, as observed for other oncogenic viruses that cause latent infections (HCV, HIV) in carriers **(see prior paragraph)**. This possibility should be considered in the next years or decades.

Overall, all these considerations underline the importance of understanding whether the patients with SARS-CoV-2 infections may experience both reinfection and reactivation, as the clarification of this topic may have a strong impact on clinical practice. The identification of individuals at high risk of SARS-CoV-2 recurrence/reactivation may represent a fundamental measure for the control of the pandemic spread of this pathogen.

5.**Considerations on the possible involvement of climatic factors in increasing the risk of SARS-CoV-2 spreading**

Since several decades ago, climatic factors have been reported to influence strongly the transmission of several infectious diseases and to have an impact in their geographical and seasonal distributions, mainly in the winter in temperate regions, as well as in long-lasting trends [178]**.** Current evidence has underlined the effects of the environmental factors, such as temperature and humidity, in influencing innate, and adaptive immune responses of the host to viral infections involving the respiratory tract [179]. Taking advantage of all this evidence, several Authors have investigated the impact of some climatic factors, such as air temperature and humidity, in the transmission of the SARS-CoV-2, with discordant results. High levels of these two physical parameters have been reported to promote the transmission of this virus by some studies [180]**.** On the other hand, further trials have not confirmed these results, suggesting that high temperatures are able to prevent the diffusion of this pathogen in different geographical areas, with a negative association between the average temperature and the number of cases of SARS-CoV-2 infections [181,182]. This conclusion agrees with the results of the studies on the survival of SARS-CoV-2 in nasal mucus or sputum as

well as in faeces obtained from patients with the infection caused by this pathogen. Higher stability of this virus was observed when the specimens were maintained at low-temperature and low-humidity conditions. On the other hand, a hotter temperature and a more elevated humidity decreased its half-life (when the temperature ranged from 4 to 27 °C) [183,184]. Further investigations have confirmed that mean low temperatures ranging from 5 to 11 °C, low specific humidity (3-6 g/kg) and low absolute humidity (4-7 g/m3) correlate with a more elevated SARS-CoV-2 ability to spread in different geographical areas worldwide [185]. A large series of reviews, including in-vitro data about several coronavirus types have reported that the increase of temperature decreases the virus viability on different materials (including SARS-Cov-2) [183,186]. Other studies have examined the overall effects of several factors, including temperature, humidity and rainfall, but the results of these studies are still controversial, and no definitive conclusions have been reached about the transmission of respiratory viruses, when these environmental conditions are considered together [187]. In particular, air relative humidity (RH) has resulted as a physical parameter of great interest in some studies, as high values of this factor are associated with inhibitory effects for SARS Coronavirus-2, MERS-CoV, and Influenza A. Nevertheless, the impact of RH in influencing SARS-CoV-2 activity is less pronounced in comparison with the parameter represented by the temperature, although no definitive results have been obtained. A specific study highlights that both high (>85%) and low (<60%) relative humidity determine significantly decrease of infectivity in a model surrogate for influenza virus and SARS coronavirus [188]. Furthermore, low absolute humidity (4-7 g/m3 humid air) and specific humidity (3-6 g/kg dry air) turn out to characterize the areas in the world with a high spread of SARS-Cov-2, whereas the relative humidity levels of the same impacted areas are not discriminating against poorly affected territories across the northern hemisphere [185]. A different result arises from selected cities in Brasil [180] where higher relative humidity has been found to favor the COVID-19 diffusion, on the contrary, a tendency for a decrease of cases with the increasing relative humidity is described in the New South Wales [189]

during the falling phase of the epidemic. Such contradictory shreds of evidence could convince that the water vapor is a weak forcing factor for SARS-Cov-2 transmission, but not to be theoretically neglected, as the European Commission conceives the absolute humidity among the few possibly significant environmental claims in this context. The sun UV radiation represents a more definite and characterized environmental variable on the virus survival. Only the sun radiation in the range of the UV-A (400-315 nm) and UV-B (315-280 nm) reaches the earth surface, whereas the UV-C component (< 280 nm) is absorbed by the atmosphere. The UV-C radiation exerts antiviral and antibacterial activities, and it is commonly used as germicidal tool against viral aerosols with very promising results [190], however, UV-C cannot be considered as an outdoor environmental factor [191]. Nevertheless, the effectiveness of solar radiation in reducing the viability of the influenza virus under laboratory conditions is known [192]. Also for SARS-COV-2 laboratory data on anhydrous surfaces and culture media show similar results and significant differences in the virus activity are observed comparing the sunlight conditions during the winter, the middle season and the summer at mid latitudes [193]. These laboratory results agree with theoretical computations [194] and with ecological studies, which suggest a correlation between lower COVID-19-infection rates with higher solar radiation [195]. A wide population study has considered approximately 18000 cases of COVID-19 in China [196] with apparently contradictory results, deriving from the ecological approach: on one hand the solar radiation is still considered to exert an effective contrast against the virus survival (based on the laboratory results on influenza by Ianevski et al [192]), on the other hand, sunshine days induce higher sociality and a consequently higher probability of transmission. The efficacy of the UV solar radiation has been considered [197] also as a therapy after effective use of this tool had been observed in the 1930s and 1940s for a variety of diseases. Biological adverse or beneficial effects of the UV-B radiation have been reported by several studies. In brief, UV-B radiation may either induce the well-known damage of human skin [198] or presenting a possible activating factor for the dormant HIV-1 genome, at appropriate doses [199]. However, UV-B rays are

involved in the activation of vitamin D in the human skin. The overall effect of exposure to sunlight seems very beneficial against the influenza virus as well as against other viruses. This effect has been correlated with the increased amounts of activated forms of vitamin D, induced by UV-B rays [200]. Exposure to sun radiation has been associated with a significant increase of the total vitamin D concentration in a cohort of Norwegian women [201] and with the reduced risk of influenza virus infection. Even in HCV patients the hours of exposure to sunlight been demonstrated to correlate with a better response to a therapy based on the administration of peginterferon/ribavirin and boceprevir/ telaprevir in a cohort of patients with HCV chronic hepatitis, who were treated in the summer in comparison with a group of HCV positive subjects with persistent liver disease with similar baseline characteristics, who have been treated in winter months [202]. The production of vitamin D stimulated by sun radiation seems very effective in reducing viral infectivity and in preventing the development of more severe forms of viral related infections. herefore, its use has been proposed for several months ago with a therapeutic or preventive role in patients at higher risk of severe clinical manifestations of SARS-CoV-2 related infection, such as aged-people or for the treatment of these individuals [17,22]. Some interesting studies have been published [203-205]**.** but further studies are needed to clarify these preliminary very promising results. To date, 8 trials investigating the use of vitamin D in patients with COVID-19 have been proposed. Whether solar radiation can be effective for viruses inactivation and vitamin D production, air pollution could severely prevent such beneficial effects because airborne particles shield solar radiation up to significant levels [206]. Moreover, the particulate matter (PM) is mentioned as an additional risk factor, because it is associated with cardiovascular and respiratory diseases [207]**.** These pathological conditions can increase the susceptibility to respiratory virus infections [208]. The effect of air pollution in increasing the lethality of COVID-19 has been suggested for high polluted areas in Italy [209] and similar results have been obtained for China and USA both for SARS-COV-1 and SARS-COV-2 [210]. An additional point is whether air particulate matter can transport viruses. Genetic material from the SARS-C0V-2

virus has been detected on PM10 samples have been obtained from the city of Bergamo (Italy). Bergamo has been severely hit by SARS-C0V-2 infection [211]. Further data, collected from other Italian areas, during the COVID-19 pandemic [212], show that in crowded sites SARS-CoV-2 is detectable in the PM10, whereas the virus is absent in the particulate in non-polluted regions. A very recent nationwide observational study, carried out in Italy, has shown that a significant positive association exists between COVID-19 incidence rates and levels of PM2.5 and NO_2, when the period 2016-2020 and the months of the epidemic were considered [213]. However, currently, a definitive conclusion, concerning this important problem, is not yet possible, however the association of viruses on colloidal and particulate materials are well known in aqueous medium and can enhance the transport of viruses to long distances [214]. Therefore, the identification of particulate composition can be a critical factor in the control of infectivity, mainly for airborne particles, because despite viruses can be light-shielded during UV irradiation, they can be inactivated by Fenton-like processes, triggered by iron atoms at the particulate surface (Nieto-Juarez and Kohn, 2013). These very interesting observations need further well-designed studies to clarify definitively all these points [215].

Discussion

The current pandemic caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) represents a formidable public health concern worldwide as it is associated with elevated morbidity, mortality and lethality rates in the general population. To date, the seriousness of the situation has prompted the implementation of considerable efforts to counteract the spread of this pathogen. Therefore, a broad series of studies have been performed in a short period of time or are currently in progress with the purpose to clarify quickly the molecular structure of SARS-CoV-2, its epidemiology, its biological behavior, its dynamics of spreading as well as the pathogenetic mechanisms involved in its development and detectable both during the early phases of the host's

infection and during its course both in symptomatic and in asymptomatic subjects. This approach has lead to suggest the adoption of: a) preventive strategies, such as the very recent introduction in clinical practice of vaccines or other control measures, including the implementation of health-behaviours, such as the social distancing, the use of facial masks and the recommendation of more strict hygiene procedures in everyday life; b) treatments with potential activities against SARS-CoV-2, including antiviral drugs or as well as therapy with immune-modulatory effects, with the aim to attenuate the excessive immune response, detectable in patients with the most severe forms of SARS-CoV-2 related infections, and leading to deleterious clinical manifestations. Unfortunately, despite all these efforts the knowledge of epidemiology and pathogenesis of SARS-CoV-2 is still limited. The lack of definitive results depend on several reasons, such as: 1) its recent identification, 2) the restricted period of follow-up available for the subjects, who suffered SARS-CoV-2 related infection and who either experienced clinical manifestations of different severity or who remained asymptomatic and 3) the heterogeneity of the available studies, including their design, the criteria for patients' inclusion, the different diagnostic tools used to detect the presence of this pathogen, the considered period of follow-up and the end-points. We have performed an analysis between the structural and the biological SARS-CoV-2 characteristics and those of other well-known RNA viruses (HIV, HCV, IVs), with the purpose to identify possible similarities and analogies between all these pathogens. HIV, HCV and IVs have been widely studied since several years ago and a large series of reports, about their structural and biological features, are available in the literature. Therefore, we have collected historical data, clinical manifestations and pathogenetic mechanisms associated with these infectious agents. Taking advantage from the results of our research, we have prepared this narrative review, with the aim to get useful insights and lessons from HIV, HCV and IVs characteristics and to transfer the experience arising from prior pandemics as well as from the knowledge of the structure, pathogenesis and of symptoms and signs correlated with these infectious agents to the study of SARS-CoV-2 biology. This strategy may contribute to

predict the SARS-CoV-2 behaviour and to Several differences exist among all these pathogens. In particular, they present a distinct mode of transmission, as SARS-CoV-2 and Influenza viruses are airborne pathogens, whereas HIV and HCV are bloodborne infectious agents. However, these viruses exhibit some potential common clinical manifestations and pathogenetic mechanisms and their understanding may contribute to establishing preventive measures and new therapies against SARS-CoV-2. Therefore, we have discussed the following points:

1) **The genome organization of HIV, HCV, IVs and SARS-CoV-2, the existence of distinct genotypes, quasispecies and the processes of viral reassortment and recombination during the replication of these pathogens.** Furthermore, we have briefly discussed the impact of all these elements in determining the virulence of these viruses and their ability to adapt to the changing environmental conditions as well as the possible repercussions on the preventive and therapeutic strategies against SARS-CoV-2 infection. HIV, HCV, IVs and SARS-CoV-2 are RNA viruses. Most of the RNA viruses are characterized by some common elements: a small-size genome, about 10,000-15,000 bases, and an elevated mutation rate (around 10^{-3} and 10^{-5} errors per nucleotide and replication cycle), short replication time and large progeny [216]. These elements contribute to explain some very important features exhibited by these pathogens. In particular, it has been suggested that the small length of their RNA is in relationship with their high mutation rates as well as with the presence of compression and of overlapping genes in their genome [217]**.** HIV, HCV and IVs also consist of a short genome, approximately 9,200-9,600 [218]**,** 9,600 and 13,500 bases in length respectively [72]. RNA [41,219,220] as well as DNA [221-223] viruses are classified into distinct genotypes and subtypes, depending on the nucleic sequence identity worldwide. In general, the distribution of genotypes for each virus varies on the basis of the geographical areas considered across the globe. A large series of studies have reported the existence of distinct genotypes also for HIV, HCV and IVs. Furthermore, it is well known that RNA viruses generally present high mutation rates and exhibit error-prone genomic replication because their RNA polymerase lacks a proofreading

activity or postreplicative RNA repair mechanisms [224]. Therefore, during viral replication, nucleotide random substitutions are incorporated into the nascent genome. The poor fidelity of viral RNA polymerases in mediating this process is considered as the primary contributing factor for the variability in the genomes of RNA viruses. The increase in the mutation frequency in patients suffering from infections associated with these pathogens may have double effects, it may: a) either force the virus beyond a tolerable limit and results into the "error catastrophe" with the consequent reduction in its fitness, its impossibility to replicate and its extinction or b) promote the emergences of variants escaping from the pressure due to the immune system, to specific pharmacological treatments and to vaccines [225]. The possible occurrence of these events has been described also in patients with HIV-, HCV- and IVs-related infections. In particular, the Reverse Transcriptase codified by the *pol* gene of HIV [226] as well as the HCV and IVs RNA-polymerases encoded by the NS5B gene and by the PB1, PB2 and PA genes respectively lack the proofreading capacity and this enzymatic characteristic causes a low replication fidelity of these pathogens [227,228]. Therefore, the production of large amounts of viral genomes, the elevated number of host's infected cells and the high mutation rates detectable daily, lead to the generation of different but closely related variants, known as quasispecies. These populations are characterized by a large intra-host and inter-host genetic plasticity and heterogeneity and may harbor both dangerous and beneficial base substitutions, leading either to nonviable forms or to strains with more elevated replication fitness. A natural selection acts on all these viral strains, which are continously induced to adapt to the changing environment [229]. A large series of studies have investigated the generation, transmission, evolution and fitness of HIV, HCV and IVs quasispecies [230-233]. Furthermore, RNA viruses possess the capacity to exchange genetic material with each another. Two distinct mechanisms contribute to generating continuously new viral variants, during the infections associated with RNA viruses. They are represented by recombination and reassortment. Gene recombination occurs in both the segmented and non-segmented RNA virus families and it is

characterized by the mixing of gene segments, belonging to two different parental genomes and generating a viral progeny with new combinations of genetic material [234]. On the other hand, gene reassortment is the exchange of genome fragments between two or more viruses and it is detectable only in segmented viruses, such as influenza viruses, infecting the cells of the same host [235]. Both mechanisms also contribute to increase genetic variability of the viruses, erasing the non-viable variants, selecting strains with biological characteristics useful to their spread and with greater adaptability to the environmental conditions [236]. Therefore, the resulting biological behavior of these RNA viruses depend on the combined interaction between the heterogeneity, complexity, epistasis, and mutational robustness of their genetic material. Unlike HIV, HCV and IVs, SARS-CoV-2 and the other Coronaviruses (CoVs) exhibit more lenght genomes (about 30,000 bases) [7]. It has been suggested that this viral characteristic is linked to a lower mutation rate among the members of the Coronaviridae Family (CoVs), including SARS-CoV-2, in comparison with other small RNA viruses. This peculiar feature has been associated with the capacity of CoVs to produce a proofreading exoribonuclease (ExoN), known as nsp14, that mediates a high-fidelity in RNA genome replication [237]. In particular, the process of SARS-CoV-2 replication is mediated by a polymerase complex, composed of multiple subunits. It has suggested that SARS-CoV-2 replicative machinery, as observed for SARS-CoV or for other CoVs, consists of a catalytic subunit (nsp12), exerting the RNA-dependent RNA polymerase (RdRp) activity. Nsp12 displays a poor efficiency in the processivity of RNA synthesis in vitro. Nsp7 and nsp8 are crucial co-factors for nsp12, stimulating its polymerase function. In particular, nsp8 is critical for the enzymatic activity of this protein complex [238]. Together all these elements constitute (nsp7, nsp8 and nsp12) the core polymerase complex**.** The nsp7/nsp8/nsp12 structure displays the capacity to bind with nsp14, a 3'-5' exoribonuclease. This enzyme exerts a proofreading function with error-correcting capability during viral transcription and RNA cap N7-guanine methyltransferase activity [128]. Therefore, nsp14 regulates replication fidelity as well as it controls 5'-RNA capping [239,240]. The crucial role of nsp14

protein is shown by recent studies, reporting that its genetic inactivation leads to a 21-fold decrease in replication fidelity compared to wild type SARS-CoV [241]. However, although the mutation rate for SARS-CoV-2 is currently believed to be lower in comparison with the other small RNA viruses, the genome heterogeneity of this virus is elevated. To date, six major subtypes have been identified in the global population [242]**,** Furthermore, SARS-CoV-2 quasispecies have been isolated in "in vitro" studies as well as the evolution of these variants has been described also in "in vivo" investigations. This pathogen displays a high intra-host genomic plasticity [243,244]. In clinical practice, SARS-CoV-2 quasispecies have been shown to differ dynamically day to day and to have variable distributions in the distinct anatomical sites of the infected individuals, during the course of the disease [243]. Similarly to other RNA viruses, SARS-CoV-2 also exhibit random mutations and recombination and these events, which contribute to generating genetic diversity, have been described in some studies [245]. A large series of missense, synonymous and non-coding mutations, emerging in SARS-CoV-2 genome, have been reported and their number is continuously increasing. Changes in nucleotide sequences involve all viral genome, however, most of them have been described in the S gene, as its crucial role in SARS-CoV-2 transmission has attracted a wide broad of studies. The recombination has been reported to involve 9 areas in SARS-CoV-2 genome. In particular, this event has been detected in 6 key regions in the S gene as well as in 1 region in the RNA polymerase, nsp13 and ORF3a genes [246]. Additional investigations are needed to confirm these early observations and to clarify the real impact of the viral characteristics in human pathologies. However, all these studies underline the importance of improving our knowledge concerning the molecular mechanisms by which SARS-CoV-2 modulate its replication fidelity. It has to be considered that these aspects have a crucial impact in the development of anti-CoV-2 strategies, including the development of effective drugs or vaccines or in the planning of surveillance strategies to counteract the spreading of this pathogen worldwide. The poor fidelity of viral RNA polymerases in incorporating promiscuous nucleotide substrates into the nascent RNA

genome has been used as an effective therapeutic strategy. Since years ago several nucleoside analogs have been introduced in clinical practice as treatment of patients with some RNA-related viral infections. Ribavirin is one of these compounds and displays its antiviral action via some distinct mechanisms. Following its inclusion into the viral genome, the 5′-triphosphate form of this drug, induces lethal mutagenesis [46]. Furthermore, Ribavirin 5′-monophosphate exerts inhibitory activity on the cellular enzyme inosine monophosphate dehydrogenase (IPDH). Therefore this event causes ~~the~~ a decrease in the amounts of cytoplasmic guanosine triphosphate and promotes the preferential incorporation of Ribavirin into the viral RNA genome [247]. Although the efficacy of this drug has been demonstrated for the treatment of HCV, of respiratory syncytial virus and of Lassa fever infections [248], CoVs are unresponsive to this guanosine analog. It has been shown that Ribavirin monophosphate is actively incorporated at the 3' terminus of the CoVs genome by the nsp12 component of the replication machinery, but it is then removed by the ExoN proofreading activity of the nsp14 [240,249]. Taking advantage of these observations, several Researchers are studying drugs with possible effective activity against SARS-CoV-2. Remdesevir is one of these compounds, it is an adenosine nucleotide analog, and it has been reported to compete with ATP for binding to RNA polymerase and for being incorporated into the nascent viral genome, causing its termination, after the additional insertion of up to three further nucleotides into the RNA strand [250]. It has been suggested that a steric hindrance prevents the RNA polymerase to continue its activity and may inhibit the proofreading action of nsp14 component and hinder the excision of the analog from the viral chain. Nevertheless, it is probable that a residual exonuclease function of nsp14 persists. Studies in vitro have shown that the absence of proofreading activity in mutant viral strains is associated with a more elevated remdesevir-associated inhibition of replication in comparison with wild-type ones [251]. A careful examination of all these data from the above-mentioned studies should help in the searching for antiviral drugs effective against SARS-CoV-2 and in overcoming the problems, which are associated with the resistance to these compounds. Some additional

treatments have been proposed for the treatment of patients with COVID-19 [252]. Furthermore, HIV, HCV, and IVs, as well as SARS-CoV-2, include in their genomic structure, some genes encoding proteins with crucial antiviral activity. Some of these molecules affect the host's innate mechanisms, such as IFN system. In particular, these proteins are able to interact with several cell pathways and influence their functions. During HIV infection, the products of Vpu and Nef genes, strongly antagonize IFN induction. The production of these proteins leads to loss of expression of the innate immune viral RNA sensing adaptor proteins in T cells [253]. HCV NS3, NS4A and NS5A genes code proteins decreasing IFN-α productions by several immune cells and influencing their antiviral activities [254] NS1 is a small multifunctional protein counteracting both the innate and adaptive immune response in patients with IVs infection. In particular, it acts as an interferon antagonist and functions by inhibiting the type I interferon system at multiple steps during influenza virus infection [255,256]. Additional investigations are needed to confirm these early observations and to clarify the real impact of all these viral characteristics in human pathologies. However, all these studies underline the importance of improving our knowledge concerning the molecular mechanisms by which SARS-CoV-2 modulate its replication fidelity. It has to be considered that these aspects have important repercussions in the searching of anti-CoV-2 strategies, including the development of effective drugs or vaccines and in the planning of surveillance measures to counteract the spreading of this pathogen worldwide. In particular, in the next months, the studies will have to elucidate the possible impact of the emerging SARS-CoV-2 strains with mutations in their genome on the currently made available vaccines. However, although these recently identified viral strains exhibit a more elevated infectivity in comparison with wild-type populations, preliminary reports suggest that the ability of these vaccines to confer an effective protection against these mutated viral strains remains unchanged [257].2) **The onset and spreading of pandemics caused by respiratory viruses according to a perspective historical point of view.** According to the reports available in literature, the pandemics listed above (see table 1) and the

spreading around the world from 1889 onwards had been caused by IVs. However, although the etiological agent of the current infectious disease is represented by a Coronavirus with a different structural organization and apparently distinct biological behaviour in comparison with IVs, some common characteristics and potentially useful insights may be obtained by the careful examination of the beginning, development, duration and course of the present viral outbreak. Since their onset, all these pandemics have lasted at least two years, with a number of waves ranging from 2 and 4 (Table 1) during their course. This is a very important point, it is conceivable that even the current SARS-CoV-2 associated pandemic will be characterized by some waves with the second and the third exhibiting a higher mortality than the first, as reported for the previous pandemics. The reasons for the greater severity of the second and third waves than the first during the pandemics occurred in the past are unknown [107,109]. It can be hypothesized that a broad series of elements have to be considered to explain this observation, including the development of bacterial pneumonia sovrainfection involving the respiratory tract after viral infection onset, the progressively increasing rate of viral mutations and the emergence of variants with greater infectivity and virulence associated with the spreading of these viruses in the general population and the quality of population's life. Some mathematical models have been proposed to describe quantitatively the causes and mechanisms that may promote multiple waves during pandemics [107].

The onset of these infectious outbreaks has been observed in different periods of the year, either at the end of the winter (Spanish influenza), or at the end of autumn (Russian and Asian influenza), or in the spring (Swine influenza) or in the summer (Hong-Kong influenza) (Table 1). The influenzavirus outbreak in 1957 mainly affected children, returning to school after the summer holidays. It has been suggested that the fewer incidence of infections during the period of school closing has been due to a lower number of contacts among children and people in comparison to one occurring in the frame time of school opening. Nevertheless, this event has not been observed during other pandemics. In 1968, the onset of second wave was in November, when the children

had already started school about two months before [107]. During these infectious outbreaks Doctor Maurice Hilleman developed a vaccine against Asian flu within 4 months in USA and contributed to decrease its impact [258]. Furthermore, the spectrum of age-groups, who were hit by distinct influenza virus subtypes in the course of the past pandemics, differs broadly [84]. For example, the Spanish flu has caused more elevated fatality rates in healthy young adults in comparison with older people. On the other hand, the past pandemics and the interpandemic seasonal influenza cases have been characterized by higher mortality in individuals suffering from chronic diseases as well mainly in children and in older people [259]. During Spanish Influenza pandemic, about 99% of excess influenza related deaths were observed in subjects with an age under 65 years. In particular, the most severely affected individuals had an age ranging from 20 to 40 years, whereas the excess deaths caused by influenza viruses in individuals less than 65 years old was respectively equal to 36% and 48% in 1957 and 1968 pandemics [260,261]**.** The reasons for the elevated pathogenicity of the H1N1 virus, causing Spanish influenza, as well as its higher virulence in young people are not fully understood. However, some studies in animals have shown that HA, NA and PB1 genes of the 1918 H1N1 virus may have exerted a crucial role in its replication and in its extraordinary pathogenecity, during the development of the pandemic [262]**.** Furthermore, Ahmed has reported that NS1 protein of the 1918 viral strain has contributed to the virulence of this pathogen, due to its key immunomodulatory function. In particular, NS1 is an interferon-α antagonist and it is able to effectively downregulate the host's innate immune response. Reverse genetic techniques have been used to generate influenza viruses with the eight gene segments of the 1918 H1N1 virus with the purpose to investigate the causes associated with the elevated virulence of this pathogen. According to Ahmed's results, the 1918 virus caused a severe respiratory insufficiency, when it was inoculated into the cynomolgus macaques (*Macaca fascicularis*). Furthermore, elevated plasma levels of interleukin-6 (IL-6), IL-8 and the chemokines CCL2 (monocyte chemotactic protein 1) and CCL5 (RANTES) were detected in these animals [263]. The coordinated activity of the 1918 virus genes has

probably conferred the unique high-virulence phenotype observed with this pandemic virus [264]. Similar viral genes, producing proteins with anti-IFN-α activities, have been demonstrated also in HIV, HCV as well as in SARS-CoV-2. The study of the combined interaction of NS1 with all the other genes of SARS-CoV-2 and vice versa as well as the comparative analysis of the excessive immune response detectable in the acute phase of HIV and IVs infection, resembling the cytokine storm observed during the early step of COVID-19 development, will be able to provide useful insights about the virulence of SARS-CoV-2 [54].

3) **The possible emergence of a persistent SARS-CoV-2 reservoir worldwide, the possibility of SARS-CoV-2 reinfection/recurrence and the potential development of long-term clinical manifestations**. The broad diffusion of SARS-CoV-2 worldwide raises the possibility that this pathogen has now become endemic in human populations. As reported in paragraph 3, it is well-known that RNA- [122] as well as DNA-viruses [265] are able to induce a status of persistence in patients who are infected by them, with very variable effects. These viruses may remain in the host's cells, alternating periods of latency to periods of reactivation with more or less active replication. The duration of these phases as well as the mechanisms of persistence and the possible pathological consequences associated with these infections may depend on host's characteristics, such as the age and sex of the affected patients, the coexistence of pathologies, the activity of the immune system both in its innate and adaptive arms and on viral factors, such as their error prone RNA-polymerases, the development of specific immune evasion immune mechanisms, their poor immunogenicity and the existence in the body of "sanctuary organs" [266,267]. Furthermore, IVs are not considered pathogens able to cause persistent infections in humans and few data are available on the long-term impact of the Spanish flu pandemic on public health. However, as reported in paragraph 3, A higher rate of deaths, due not only to malignant but also to non-malignant causes, was observed in individuals with early life exposure to the 1918 influenza outbreak, in comparison with subjects who were not. Respiratory and cardiovascular diseases were the most important

contributors [151]. Furthermore, this pandemic has been characterized by the appearance of neurological manifestation in some infected individuals. One of these is known as von Economo's encephalitis lethargica. Although definitive conclusions about a causal relationship between 1918 Influenza virus and this pathological condition has not been definitively reached, this association has been proposed [109,268,269].

To date, it is not known if the etiological agent of COVID-19 may establish a persistent infection in the host. However, on the basis of the data currently available on HCV, HIV, HBV and Herpesviruses as well as on the reports, concerning the other RNA and DNA viruses, it has to be taken into account the possibility that SARS-CoV-2 also may induce latent infections. Some studies are investigating the possible persistence of symptoms in patients recovered from COVID-19. A recent research has shown that about 87% of 143 patients suffering from this disease had the persistence of at least one symptom (mainly dyspnea and fatigue) about 60 days after the discharge from the Hospital [175]. These results have been confirmed by a large survey including 112 hospitalized and 2001 non-hospitalized patients (confirmed COVID-19, n=345; symptom-based COVID-19, n=882; and suspected COVID-19, n=774). It has reported that multiple symptoms are still detectable 3 months after the onset of SARS-CoV-2 associated infection in these individuals and only a small percentage of them are free of symptoms. The Authors concluded that in these patients a “post-COVID-19 syndrome” may be hypothesized and this condition underlines healthcare need for proper clinical management of subjects, after the acute phase of this infection [176]. Furthermore, a very important question remains open, it may be possible that patients may undergo either to a reinfection or to a reactivation. In both conditions these individuals may be a potential source of transmission. Currently, the re-detection of SARS-CoV-2 in patients with previous contact with this pathogen has been determined only by means of RT-PCR tests in distinct samples [167]. Nevertheless, this diagnostic technique is unable to differentiate the active from the inactive forms of this virus. A recently published Korean study analyzed the recurrence of SARS-

CoV-2 in patients with previous COVID-19, showing that no viral genome with infecting ability was detectable in samples collected from these subjects [270]. On the basis of these results, further studies are needed to clarify whether individuals with previous contact with this pathogen may become “carriers”, in what percentage of patients this event might occur and possibly to establish whether in these individuals SARS-CoV-2 replication may recur throughout the patient's lifetime, as it has been described in patients with HIV, HCV, HBV and Herpesvirus infection. Studies focused on the searching SARS-CoV-2 genoma by means of quantitative PCR-tests on distinct samples and at prefined steps, during a period of some months (at least one year), might be an appropriate strategy to clarify this point. The study population should involve subjects with long-term clinical manifestations or long-lasting persistence of serum immunoglobulins M against this pathogen. It is not yet known whether the subjects with previous SARS-CoV-2 infection will experiment a significant pathological burden in the future and its possible impact on the health worldwide. The present paper should provide a useful synthesis, concerning the body of data and evidences, concerning lessons with the purpose to counteract the pandemic caused by SARS-CoV-2 by planning effective preventive and therapeutic strategies. This approach should finally allow us to precede this virus, preventing its hamper effects and not to chase it.

Conclusions

In this narrative review, we have tried to analyze the various features of SARS-CoV-2 with those of other RNA viruses (Human Immunodeficiency Virus, Hepatitis C Virus, Influenza viruses), in order to underline similarities and differences, with the purpose to hypothesize a behavior model of this virus. The data that have emerged, describing the history and management of this topic, offer a broad perspective for discussion. SARS-Cov-2 is a recently identified virus and its structure and biological behavior are not fully understood, therefore some open questions remain: a) what will be

the duration of this pandemic? b) how many waves will develop during this pandemic? c) is it possible that this virus causes a persistent infection? d) what is the risk of reactivation or reinfection? d) Will patients with previous SARS infection develop persistent organ damage and clinical manifestations? Could SARS-CoV-2 cause long-term pathological sequelae in the clinical history of infected patients? e) How will we possibly manage them? f) what are the possible environmental effects (temperature, humidity, atmospheric pollution, substances with potential antiviral activity?).

Take home messages

1) SARS-CoV-2 represents a formidable public health problem worldwide, due to its high morbidity, mortality and lethalithy rates in human populations, but the preventive measures and therapeutic strategies currently adopted have not yet allowed its control.

2) The purpose of this narrative review has been: a) to examine in brief the current scientific literature, concerning the epidemiological, pathological, structural and clinical characteristics of some well-known RNA viruses (HIV, HCV and IVs), widely spread in mankind worldwide for several decades and responsible for a high burden of morbidity and mortality, b) to identify possible similarities between all these pathogens, c) to analyze the data obtained and to get useful lessons for the study of SARS-CoV-2 biology.

3) Several differences exist among all these pathogens, including a distinct mode of transmission, as SARS-CoV-2 and Influenza viruses are airborne pathogens, whereas HIV and HCV are bloodborne infectious agents. However, these viruses exhibit some potential common clinical manifestations and pathogenetic mechanisms and their understanding may contribute to establish preventive measures and new therapies against SARS-CoV-2.

4) HIV, HCV, and IVs possess small-size genome, about 10,000-15,000 bases and are characterized by high mutation rates due to the error-prone genomic replication of their RNA polymerase, as this enzyme lacks a proofreading activity. SARS-CoV-2 has a longer genome (about 30,000 bases) in comparison with the other RNA viruses. SARS-CoV-2 replicative machinery consists of a catalytic subunit (nsp12), exerting the RNA polymerase activity. Nsp12 also is error-prone during viral genome replication, however, the high rate of mutations detectable in the course of this process is attenuated by the existence of an additional subunit, known as nsp14. It exerts a proofreading exoribonuclease (ExoN) activity with error-correcting capability. This structure mediates a higher fidelity in RNA genome replication.

5) The poor fidelity of viral RNA polymerases in incorporating promiscuous nucleotide/nucleoside substrates into the nascent RNA genome has been used as an effective therapeutic strategy. Some analogs, such as Ribavirin have been approved for the treatment of some small-sized RNA viruses, such as HCV. After its insertion into the nascent viral genome this drug blocks this process. Ribavirin is not effective for the therapy of SARS-CoV2, due to the capability of nsp14 to remove this drug from the nascent viral genome, after its incorporation mediated by nsp12. All these elements are crucial for the searching and discovering of effective antiviral drugs for SARS-CoV-2 treatment.

6) The poor fidelity of viral RNA polymerases in mediating this process is considered as the primary contributing factor for the variability in the genomes of RNA viruses. SARS-CoV-2 also is characterized by a wide variability due to: a) the existence of several genotypes and subtypes, b) the elevated daily mutation rates (missense, synonymous and non-coding mutations), leading to the generation of different but closely related variants, known as quasispecies. c) the capacity to exchange genetic material among viral genomes, during viral replication. This event is defined gene

recombination and it is characterized by the mixing of gene segments, belonging to two different parental genomes, producing a viral progeny with new combinations of genetic material.

7) The increase in the mutation frequency in patients suffering from infections associated with these pathogens may have a double effect: a) either forcing the viruses beyond a tolerable limit and results into the “error catastrophe” with the consequent reduction in its fitness, its impossibility to replicate and its extinction b) or promoting the emergences of variants escaping from the pressure due to the immune system, to specific pharmacological treatments and to vaccine.

8) Several IVs-pandemics have been described worldwide, since 1889, all these pandemics have lasted at least two years, with a number of waves ranging from 2 and 4 (Table 1) during their course, with the second and the third generally exhibiting a higher mortality rate than the first.

9). Both DNA as well as RNA visues are able to induce a status of persistence in patients who are infected by them. To date, it is well-known that these pathogens, in particular HIV, HCV and IVs, may cause variable short- and long-term effects, promoting the development of malignant and/or non-malignant diseases, involving different organs.

10) The large diffusion of SARS-CoV-2 worldwide raises the possibility that this pathogen has now become endemic in human population. Currently, it is not known if SARS-CoV-2 may establish a long-lasting infection in the host. However, this possibility has to be taken into account. Some studies have reported the persistence of symptoms in patients recovered from COVID-19 until 3 months after the onset of SARS-CoV-2 associated infection, but, to date, viable forms of this virus have not been demonstrated in human samples in these subjects.

11) To date, re-detection of SARS-CoV-2 is observed with increasing frequency in patients who have been thought to have cleared this pathogen, but the clinical significance of this observation, as well as the reasons of this event, remain uncertain and poorly known. Whereas some studies have

reported cases of individuals with well-documented SARS-CoV-2 reinfection, no data are available concerning the possibility of its reactivation, as observed for HIV and HCV. Further studies are requested to clarify this point.

12) Since several decades ago, climatic factors have been reported to influence strongly the transmission of several infectious diseases. mean low temperatures ranging from 5 to 11 °C, low specific humidity (3-6 g/kg) and low absolute humidity (4-7 g/m3) correlate with a more elevated SARS-CoV-2 ability to spread in different geographical areas worldwide. In addition, both high (>85%) and low (<60%) relative humidity determine a significant decrease of infectivity in a model surrogate for influenza virus and SARS coronavirus. The increase of temperature causes the reduction of virus viability on different materials (including SARS-Cov-2).

13) The sun UV radiation represents a more definite environmental variable on the virus survival. Theoretical computations and ecological studies suggest a correlation between lower COVID-19-infection rates and higher solar radiation. In particular UV-B rays are involved in the activation of vitamin D in the human skin. The overall effect of exposure to sunlight seems very beneficial against the influenza virus as well as against other viruses. This effect has been correlated with the increased amounts of activated forms of vitamin D, induced by UV-B rays and several beneficial immunomodulatory and antiviral activities against SARS-CoV-2 have been proposed also for this compound. Some trials on the potentisl preventive or therapeutic role of this fat-soluble vitamin to counteract this pathogen are in progress.

14) The particulate matter (PM) is also an additional risk factor for SARS-CoV-2 infection. Iy has been suggested that a significant positive association exists between COVID-19 incidence rates and levels of PM 2.5 and PM 10.

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