

SARS-CoV-2 infection and children: Insights from the 6th Workshop on Paediatric Virology (Review)

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Abstract. The present article provides an overview of the key messages of the plenary lectures on severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) infection in children, which were presented at the '6th Workshop on Paediatric Virology' organised by the Institute of Paediatric Virology on October 24, 2020. SARS-CoV-2 is a novel

human coronavirus with a positive-sense, monopartite mRNA genome, 26-32 kilobases in length, causing mild to severe respiratory infection in humans, including children. The spike (S) protein of SARS-CoV-2 is the key determinant of host/cell tropism and capacity for transmission, mediating receptor binding and membrane fusion. SARS-CoV-2 is predominantly transmitted via the respiratory route. The effects of SARS-CoV-2 infection occurring early during pregnancy remain unknown. Newborns rarely experience significant morbidity and mortality. In children, SARS-CoV-2 severe symptomatic respiratory infection has been reported much less frequently than in adults. In addition, there have been rare cases of the newly reported multisystemic inflammatory syndrome in children (MIS-C), which has similarities with Kawasaki disease. Several therapeutic agents have been evaluated for the treatment of SARS-CoV-2 infection; however, few have been shown to be truly efficacious. On the whole, further research, both basic and clinical, on SARS-CoV-2 is essential, as without a thorough knowledge of the molecular virology of SARS-CoV-2, effective preventive and treatment

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modalities, which are so urgently required, cannot be designed and implemented.

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1. Introduction

The present review article discusses the novel coronavirus, known as severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) causing coronavirus disease 2019 (COVID-19), aiming to shed insight into unanswered questions such as: Whether SARS-CoV-2 can be transmitted to newborn neonates; what are the signs and symptoms that SARS-CoV-2 causes in children; what are the current therapeutic perspectives for the management of SARS-CoV-2 in children; what are the recent research data on vaccine candidates against SARS-CoV-2; how has medical education changed due to SARS-CoV-2; and what are the perspectives and challenges of the current global pandemic threat due to SARS-CoV-2. These were the questions, which the presenters attempted to answer during a separate session on SARS-CoV-2 in the context of the '6th Workshop on Paediatric Virology' organised virtually by the Institute of Paediatric Virology (1) on October 24, 2020. The workshop was chaired by Professor Anne Greenough, Professor of Neonatology and Clinical Respiratory Physiology at the King's College London (London, UK) and President of the Academic Board of the Institute of Paediatric Virology. The workshop was also attended by Nobel prize winner Professor of Virology Harald zur Hausen, Professor Emeritus of Virology at the German Cancer Research Center (Deutsches Krebsforschungszentrum) in Heidelberg, Germany, who also presented the closing lecture of the event focusing on novel developments in the identification of causes of common human cancers.

The workshop was held under the auspices of the World Academy of Sciences (WAS) and was supported by the Laboratory of Clinical Virology of the University of Crete School of Medicine, the First Department of Paediatrics of the University of Athens School of Medicine and the 'Penteli' Children's Hospital, which was the guest hospital of the event. In the context of the workshop, Dr Nikolaos Myriokefalitakis, former Clinical Director and Consultant Paediatrician at the 'Penteli' Children's Hospital in Athens, Greece, was awarded with the '2020 George N. Papanicolaou Humanitarian Award' for his exceptional clinical, teaching, publishing and academic contribution on Hellenic Paediatrics and the wealth, health,

and future of humanity (2). Professor Vana Papaevangelou, Professor of Paediatrics at the University of Athens School of Medicine (Athens, Greece), was also awarded with the '2020 Paediatric Virology Award' for her exceptional clinical, research, teaching, publishing and academic contribution in the field of paediatric viral infectious diseases (3). The workshop was organised in parallel with the 'Official Opening of the Institute of Paediatric Virology' (1), which is based on the island of Euboea (Greece); for further information about the Institute of Paediatric Virology please visit its official website <https://paediatricvirology.org>.

The present review article provides an overview of the key messages of the plenary lectures on SARS-CoV-2 in children presented at the '6th Workshop on Paediatric Virology', 12 months after the first case of pneumonia caused by this novel coronavirus, which was reported for the first time in Wuhan, China in December, 2019. Data up to December 10, 2020 were included. Since then, there has been an exponential growth of knowledge in the field of SARS-CoV-2 and COVID-19, which has caused an unprecedented pandemic viral threat globally, the largest after the 1918 Spanish flu outbreak.

2. Molecular virology and the pathogenesis of SARS-CoV-2

Structure of coronaviruses. Coronaviruses, which infect vertebrates, are spherical in shape, with club-like projections on their envelope, responsible for their 'crown' appearance (4). The positive-sense, monopartite mRNA genome, 26-32 kilobases (kb) in length, consists of five open reading frames (ORFs) with a genome organization from 5' to 3' as follows: ORF1a and ORF1b: polyprotein, S: Spike (S) protein, E: Envelope (E) protein, M: Membrane (M) protein, N: Nucleocapsid proteins and 5' and 3' terminal sequences typical of betacoronaviruses, with 265 and 225 nt, respectively. The coronavirus genomic RNA is transcribed non-contiguously into subgenomic mRNAs when the RNA-dependent-RNA-polymerase jumps from one part of the genome to another. This results in a high rate of recombination, enhancing evolution and facilitating inter-species transmission. Coronaviruses are transmitted via the respiratory route but can also survive for a short period outside the body. The Spike (S) protein is the key determinant of host/cell tropism and capacity for transmission, as it mediates receptor binding and membrane fusion via the human angiotensin-converting enzyme 2 (ACE-2).

Classification of SARS-CoV-2. According to the Coronaviridae Study Group of the International Committee on Taxonomy of Viruses, SARS-CoV-2 belongs to the subgenus of Sarbecovirus, the genus *Betacoronaviridae* and the family of *Coronaviridae* (5). Members of the family *Coronaviridae* are viruses infecting vertebrates, characterised by club-like projections on their envelope, responsible for their characteristic 'crown' appearance. The virus is spherical in shape (60-140 nm in diameter) with distinctive spikes of 8 to 12 nm in length (6).

In total, seven coronaviruses, with zoonotic origins are responsible for respiratory infections in humans, ranging from asymptomatic infections to acute respiratory disease and pneumonia. The following strains: HCoV-229E, HCoV-OC43, HCoV-NL63 and HCoV-HKU1 have been infecting humans

for >800 years and cause mild, self-limiting symptoms. The other three cause more severe clinical manifestations, with outbreaks occurring approximately every 10 years during the 21st century. In 2002/2003, SARS-CoV caused an epidemic in China and in 2012, middle eastern respiratory syndrome coronavirus (MERS-CoV) was responsible for an epidemic in Saudi Arabia, with a fatality rate of 9.6 and 34%, respectively. From early estimates, SARS-CoV-2 was shown to have a mortality of 0.2% among young individuals, but 14.8% in those >80 years with concomitant co-morbidities (7). However, this is being adjusted as the landscape of the disease changes with improved detection, treatment and preventive options.

Compared to SARS-CoV, SARS-CoV-2 seems to have a lower pathogenicity and mortality. However, it is more contagious, more transmissible and has spread very rapidly across communities and continents. It has a broad range of clinical manifestations, ranging from asymptomatic infection, mild upper respiratory tract illness, severe viral pneumonia with respiratory failure, multisystem inflammatory syndrome in adults (MIS-A) and in children (MIS-C), and death. It is also more effective at the evasion of immune surveillance, with patients developing low-titre neutralizing antibodies and suffering from prolonged illness (8).

Viral structure of SARS-CoV-2. Similar to all coronaviruses, SARS-CoV-2 has a positive-sense, monopartite mRNA genome, of 26-32 kb in length, consisting of five ORFs with a genome organization from 5' to 3' as follows: ORF1a, ORF1b, S, E, M, N, and 5' and 3' terminal sequences typical of betacoronaviruses, with 265 and 225 nt, respectively (9). The SARS-CoV-2 RNA is transcribed into a set of subgenomic mRNAs in a non-contiguous fashion, as a result of the RNA-dependent-RNA-polymerase (RdRp) jumping from one part of the genome to another. A leader sequence from the 5' end of the genome as well at the 3' end is found on each of the subgenomic mRNAs (10,11). The ORFs of the SARS-CoV-2 genome code for structural and non-structural proteins (12) (Fig. 1).

Host cell receptor binding of SARS-CoV-2. The Spike (S) protein is the key determinant of host/cell tropism and capacity for transmission, as it mediates receptor binding and membrane fusion. Using computational modelling, biochemical interaction studies, crystal structural analysis and cryo-electron microscopy, the S proteins of SARS-CoV and SARS-CoV-2 have been shown to have almost identical 3-dimensional structures, and both to bind to human ACE-2 (13-15). ACE2 is expressed by alveolar epithelial type II cells (AECII) in the lungs (16) and its primary physiological role is in the maturation of angiotensin, a peptide hormone controlling vasoconstriction and blood pressure (13). After the receptor binding domain (RBD) of the S protein of SARS-CoV-2 binds to the ACE-2 receptor, it needs to be proteolytically activated by the transmembrane protease/serine 2 (TMPRSS2) (17). This leads to the fusion of the viral envelope to the cell membrane, the release of the genome into the cytosol and the initiation of viral replication (18). Host or cell susceptibility to SARS-CoV-2 requires the cells to express both the ACE-2 receptor and TMPRSS2. Soon after the publication of the first sequence of SARS-CoV-2, bioinformatic analysis

demonstrated that the spike protein of SARS-CoV-2 contains a furin-like cleavage site absent in SARS-CoV, but present in MERS-CoV (19). Thus, for SARS-CoV-2, spike protein activation is achieved via initial cleavage by proprotein convertase furin, followed by TMPRSS2 cleavage (20).

Even though phylogenetically SARS-CoV-2 clusters are closer to bat-CoV and pangolin CoV than to either SARS-CoV or MERS-CoV (6,9,21-23), and shares five key amino acids in the RBD with pangolin-CoV, the animal coronaviruses, similar to SARS-CoV, lack the furin [proline-arginine-arginine-alanine (PRRA)] cleavage site (24). Wong *et al* (23) postulated that the acquisition of the PRRA insertion in SARS-CoV-2 may allow the virus to adapt to the human host and lead to increased replication and pathogenicity. They hypothesised that following zoonotic transmission, PPRA-positive SARS-CoV-2 may be selected from a PPRA-negative strain to prevail in both inter- and intra-host quasispecies in humans.

The RBD of the S protein alternates between a 'lying down' and a 'standing up' position, with the former being associated with lower binding affinity and higher immune evasion relative to the 'standing up' position (8,20). In the case of SARS-CoV the RBD is mostly 'standing up', whereas in SARS-CoV-2 it is mostly 'lying down' resulting in poor host receptor recognition and inefficient entry (20). In order to overcome this disadvantage SARS-CoV-2 has evolved a RBD with high hACE2 binding affinity and the PPRA furin motif that allows the S protein to be pre-activated.

Superantigenic insert in SARS-CoV-2 S protein and MIS-C. Using structure-based computational models, Cheng *et al* (25) were able to demonstrate that the PRRA insert in the SARS-CoV-2 spike protein exhibits sequence and structural characteristics similar to those of the bacterial superantigen staphylococcal enterotoxin B. The superantigenic insert may directly bind T-cell receptors, disrupting the T-cell repertoire, and thus causing the severe hyperinflammation observed in patients with COVID-19. The absence of this motif in SARS-CoV may explain the unique potential for SARS-CoV-2 to cause both MIS-C and the cytokine storm observed in adult COVID-19.

The SARS-CoV-2 nucleocapsid and mode of transmission. In previous research, using the amino acid sequence of a query protein as input, predicted intrinsic disorder (PID) was calculated with a model using artificial intelligence (AI) tools (26). Coronaviruses with a higher PID in the nucleocapsid were associated with higher levels of respiratory transmission and lower levels of faecal-oral transmission. By contrast, coronaviruses with the more ordered N proteins (lower PID) were more likely to be transmitted by the faecal-oral route rather than the respiratory one. Coronaviruses, with intermediate PIDs, may be transmitted by both routes, at intermediate levels (26,27). When the model was applied to SARS-CoV-2, it predicted that the respiratory route could efficiently spread the virus, as is the case for SARS-CoV, but probably has the potential to survive outside the body longer than SARS-CoV and perhaps MERS-CoV, and would thus have the potential to be transmitted via the faecal-oral route. It has been postulated that these properties of SARS-CoV-2 are responsible for its high contagiousness and its ability to spread

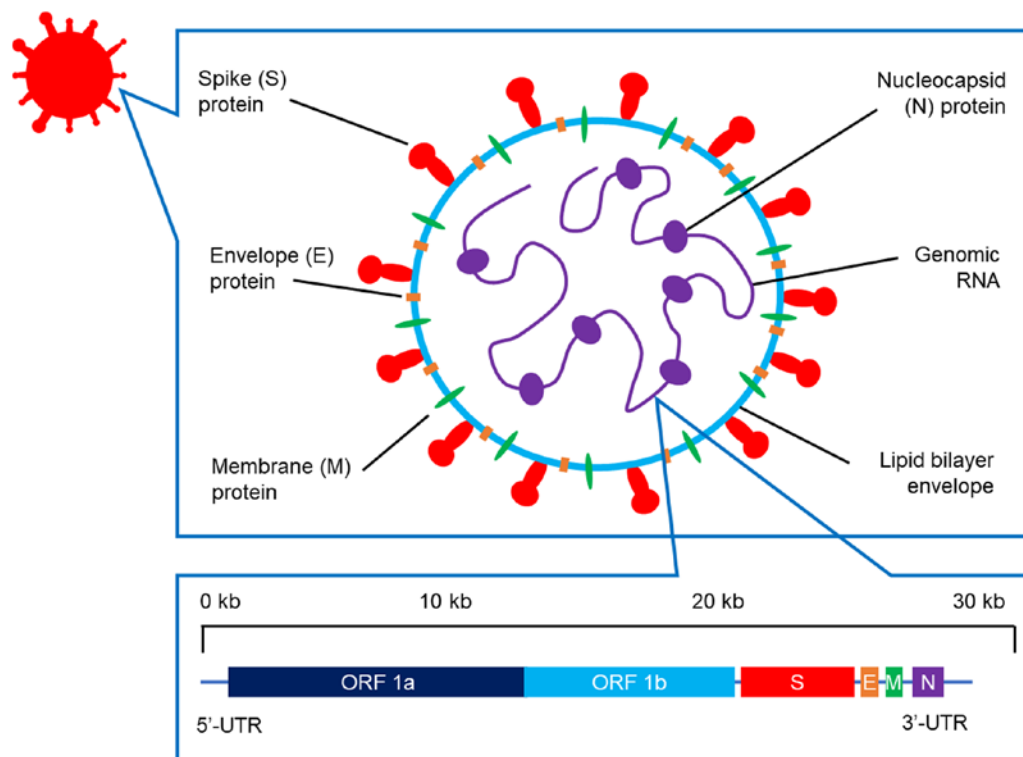


Figure 1. Schematic structure of the SARS-CoV-2 virion showing major structural proteins and genomic organization ORFs coding for structural proteins, including spike (S), envelope (E), membrane (M) and nucleocapsid (N) proteins, as well as non-structural proteins translated from ORF 1a and ORF 1b. There are two untranslated regions, 5'-UTR, at the N- and C-terminal regions, respectively. The figure has been adapted from the study by Wang *et al* (94), licensed under <https://creativecommons.org/licenses/by/4.0/>. kb, kilobase pair; ORFs, open reading frames; RNA, ribonucleic acid; UTR, untranslated region.

from asymptomatic carriers (27). Although further research is required, evidence is mounting that although SARS-CoV-2 is mainly a respiratory virus, it may also be transmitted by the faecal-oral route (28). This knowledge will aid the implementation public health measures necessary to prevent the spread of the virus.

3. Vertical transmission of SARS-CoV-2

To date, at least to the best of our knowledge, there have been only a limited number of published reports of COVID-19 in pregnant women and neonates born to mothers with confirmed COVID-19 (29-34). Vertical transmission can be antenatal or peri-partum, although perinatal or postnatal transmission can also have consequences (29). The perinatal transmission of SARS-CoV-2 in newborns of mothers with confirmed COVID-19 infection during the perinatal period, has been well documented, since neonates are at risk of transmission through maternal respiratory secretions. Additionally, horizontal transmission may occur from other household members during the neonatal period. Although there have been some case reports describing severe disease among newborns, the majority have had mild disease with a favourable outcome. Signs and symptoms include fever, lethargy, rhinorrhoea, cough, tachypnoea, increased work of breathing, vomiting, diarrhoea and feeding intolerance or decreased intake (30).

During the antenatal period, the transplacental transmission of pathogens usually increases with an advancing gestational age, while the severity of foetal injuries decreases. For any virus, to be transmitted transplacentally, viremia needs

to exist. In particular, for SARS-CoV-2, the ACE receptor, the receptor on human cells by which this virus enters, is expressed at the maternal-foetal interface in the placenta. Initially, intrauterine vertical transmission was considered unlikely based on early findings suggesting that viremia is rather rare and transient, while the SARS-CoV-2 receptor appears in very low numbers in trophoblasts during the first trimester, reducing the possibility of the mother-to-foetus transmission of SARS-CoV-2 (31). However, over the past months, SARS-CoV-2 RNA has been detected in the placentas of COVID-19-infected mothers, suggesting possible placental infection; thus, the question of transplacental transmission remains unanswered. Moreover, a recent study indicated the strong and diffuse membranous expression of ACE receptors in cytotrophoblast and syncytiotrophoblast cells of placental villi, which was present consistently and throughout pregnancy, regardless of the COVID-19 status (32). Recently, a systematic review and meta-analysis revealed that, based on the detection of SARS-CoV-2 RNA in nasopharyngeal swabs of newborns, vertical transmission occurs in 3.2% (33).

On the other hand, it is important to consider that maternal infection may have multiple harmful effects on the foetus. SARS-CoV-2 virus activates endothelial damage pathways, predisposing the host to the development of hypertensive disorders of pregnancy potentially associated with adverse maternal and neonatal outcomes, such as prematurity and growth restriction. Additionally, prolonged and severe hypoxia may potentially have severe consequences to the foetus, resulting in the need of intensive and prolonged resuscitation and ventilation. Finally, a UK study (34), revealed that during

this pandemic, there was a significant increase in the number of stillbirths. Of note, none of the stillbirths occurred in COVID-19-infected woman; however, this increase was rather due to indirect effects, such as the reluctance of pregnant women to visit health services due to the fear of COVID-19 infection and potentially, changes in obstetric services. Thus, SARS-CoV-2 can be transmitted transplacentally, although it appears to be a rare phenomenon. However, the effects of COVID-19 infection occurring early in pregnancy remain unknown. Newborns rarely experience significant morbidity and mortality.

4. Clinical manifestations of SARS-CoV-2 infection in children

To date, children with SARS-CoV-2 infection have been shown to present milder clinical manifestations compared with adult patients (35); the majority of infected children remain asymptomatic or develop only mild symptoms. Different theories have been reported for the low prevalence of severe disease among children, including lower exposure to the virus compared with adults, less international traveling and less outdoor activities, as well as less exposure to smoke and air pollution (36). Furthermore, children possess a more active innate immune response and healthier respiratory tracts (30,37). Previous studies have demonstrated a positive association between the exposure rate and the population in public areas (30,38,39). Others have suggested that it may be the result of the immaturity of ACE2, which is the binding site for SARS-CoV-2. The difference in the distribution, maturation and functioning of viral receptors in children has been referred/cited as the most common reason for the clinical differences found in children compared with adults (39).

Recently published data of 38 clinical studies have provided evidence that 14.2% of paediatric cases were asymptomatic, 36.3% had mild clinical features, 46% were moderate, 2.1% were severely ill and 13% were in critical condition (35). SARS-CoV-2 symptoms commonly appear 2-10 days following exposure and include fever, cough, shortness of breath, myalgia, a sore throat, loss of taste or smell, headache and diarrhoea. According to the systematic review by de Souza *et al* (40), the most common clinical signs presented upon admission were pharyngeal erythema, tachycardia and tachypnoea. In addition, some paediatric patients presented with an atypical skin rash (41). In a cohort study of 624 paediatric cases by Henry *et al* (42), the most common hematologic abnormality in mild infections was the decreased neutrophil count with pooled prevalence estimates of 38%, and a 95% confidence interval of 19-60%.

In severe SARS-CoV-2 infection, the liver and muscle enzyme and D-dimer levels may increase (43). It has been revealed that the number of lymphocytes in the blood of infected children is higher in comparison with that of adults (44). As a result of the higher level of lymphocytes in children, particularly natural killer cells, it has been observed that adults present with lymphocytopenia, while infected children are more likely to exhibit normal lymphocyte levels (45). As the disease progresses, within an average of 7 days, patients can become dyspnoeic and cyanotic accompanied by systemic manifestations, such as restlessness, poor feeding, loss of

appetite and decreased activity. In some cases, COVID-19 can progress to pneumonia and can rapidly lead to acute respiratory distress syndrome (46).

Acute presentation is usually considerably less severe than in adults; however, there are multiple reports of paediatric cases requiring intensive care. Lately, it has been observed that there is a link between SARS-CoV-2 and the manifestation of MIS-C (in children and adolescents). However, available data are insufficient to clarify the exact risk factors for MIS-C. Some of these factors include severe immunocompromise, co-infection with another respiratory virus (e.g., influenza), obesity, diabetes, hypertension and cardiopulmonary comorbidities, such as congenital heart diseases. In a previously published study, this hyperinflammatory syndrome was shown to cause a clinical picture similar to the one found in Kawasaki disease, including fever and mucocutaneous manifestations (47). Furthermore, some patients exhibit features of toxic shock syndrome and secondary hemophagocytic lymphohistiocytosis (48,49). Both Kawasaki disease and MIS-C have a wide range of clinical signs, and lack pathognomonic findings and accurate diagnostic criteria. However, MIS-C has been observed to affect children >5 years of age more commonly, and to result in a high incidence of cardiovascular compromise (50,51).

Recently, Feldstein *et al* (52) provided data on MIS-C in 26 US states. There were 186 patients with MIS-C; 62% were male, 73% had positive antibody or RT-PCR tests, and the majority required hospitalization. The most commonly involved systems were gastrointestinal and cardiovascular, and the average duration of hospitalization was 1 week. Approximately 80% of the patients were admitted in the intensive care unit (ICU) and 20% required intubation. Kawasaki disease-like features were present in 40% of all the patients with MIS-C and 15 cases of coronary artery aneurysms were documented.

5. Therapeutics for SARS-CoV-2 infection

An overview of clinical trials. Therapeutics against SARS-CoV-2 remain the main challenge of the current global COVID-19 pandemic. At the beginning of the pandemic, hydroxychloroquine (HCQ) and chloroquine (CQ) were proposed as treatments for COVID-19 based on *in vitro* activity and data from uncontrolled studies and small, randomized trials (53-58). The large, controlled, open-label randomized evaluation of COVID-19 therapy (RECOVERY) trial subsequently investigated this in patients hospitalized with COVID-19 (56). A total of 1,561 patients were randomly assigned to receive HCQ and 3,155 to receive usual care. Patients in the HCQ group were less likely to be discharged from the hospital alive within 28 days than those in the usual-care group. HCQ is no longer recommended for the treatment of COVID-19.

In a controlled, open-label trial comparing a range of possible treatments in patients, who were hospitalized with COVID-19 (UK RECOVERY trial), 2,104 patients were randomly assigned to receive oral/intravenous dexamethasone (at a dose of 6 mg once daily) for up to 10 days or to receive usual care alone. In a preliminary report, the use of dexamethasone resulted in a lower 28-day mortality, namely by 35% in ventilated patients and by 20% in patients receiving

oxygen only compared with standard of care (n=4,321). No benefit was observed in patients who did not require respiratory intervention (58).

In a period of only 6 months, the world's largest randomized control trial on COVID-19 therapeutics generated conclusive evidence on the effectiveness of repurposed drugs for the treatment of COVID-19. The WHO in the interim results from the SOLIDARITY therapeutics trial indicated that the remdesivir, an anti-viral medication, HCQ, lopinavir/ritonavir and interferon regimens appeared to have 'little or no effect' on 28-day mortality or the in-hospital course of COVID-19 among hospitalized patients. The study, which enrolled 11,266 patients in 405 hospitals in 30 countries, examined the effects of these treatments on overall mortality, initiation of ventilation, and the duration of hospital stay (59). Contrary to the remdesivir result, a double-blind, randomized, placebo-controlled trial (sponsored by the company who developed the drug) of 10 days of intravenous remdesivir in adults who were hospitalized with COVID-19, remdesivir was superior to placebo in shortening the time to recovery (57).

In May 2020, the US Food and Drug Administration (FDA) granted emergency use authorizations (EUAs) for some medicines to be used for certain patients when hospitalized with COVID-19 disease, including dexamethasone, remdesivir and convalescent plasma. Nevertheless, there is still the need for the identification of more promising effective candidates and thus further well designed, large, high priority randomized controlled trials, such as the RECOVERY (58) and SOLIDARITY trials (59), need to be conducted. A phase 3 trial evaluating a combination of anti-coronavirus hyperimmune intravenous immunoglobulin (hIVIG) plus remdesivir for the treatment of COVID-19 is a multicentre, randomized, double-blind, placebo-controlled ITAC trial that will assess the safety, tolerability and efficacy in hospitalized adults >18 years (n=500). Patients will be randomized to receive infusions of either hIVIG or placebo, in addition to remdesivir. The primary objective of the trial is to compare the clinical status of patients in each group on day 7 of follow-up. The trial will follow patients for 28 days (60).

The FDA has created a special emergency program for possible coronavirus therapies, the Coronavirus Treatment Acceleration Program (CTAP) (61). The program uses every available method to move new treatments to patients as rapidly as possible and has granted EUAs for some medicines to be used for certain patients when hospitalized with COVID-19 disease, taking also into consideration the Hippocratic Oath and one of the promises within that oath 'to do good, or to do no harm' (62). The National Institute of Allergy and Infectious Diseases (NIAID) launched a study designed to determine whether certain approved therapies or investigational drugs in late-stage clinical development show promise against COVID-19 and merit advancement into larger clinical trials. The ACTIV-5 Big Effect Trial will enrol adult volunteers hospitalized with COVID-19 at 40 US sites (63). There are ongoing clinical trials on the potential of other therapeutic modalities, such as monoclonal antibodies, convalescent plasma, and cell therapy. In addition, vaccines against SARS-CoV-2 are being developed and tested which can modify the natural course of the pandemic, but definitive and conclusive results are yet to be obtained (64). As of September, 2020, there were >550 trials

Table I. Type of treatment against SARS-CoV-2 infections being studied (FDA, CTAP program).

Treatment against SARS-CoV-2	No. of trials
Single agent treatments	
Antivirals	30+
Cell and gene therapies	30+
Immunomodulators	110+
Neutralizing antibodies	40+
Other	80+
Combinations (including INDs with more than one product)	20+

SARS-CoV-2, severe acute respiratory syndrome coronavirus type 2; FDA, US Food and Drug Administration; CTAP, Coronavirus Treatment Acceleration Program; INDs, investigational new drugs.

in the drug development planning stages, >350 trials reviewed by the FDA, five treatments authorized for emergency use, and no treatments currently approved by the FDA. The types of COVID-19 treatment being studied thus far are presented in Table I (61). This limited evidence on therapeutic options against SARS-CoV-2 infection in children highlights the necessity for further research.

SARS-CoV-2 infection and remdesivir. Remdesivir is a prodrug of nucleoside triphosphate, which inhibits RNA dependent RNA polymerization and therefore, viral RNA replication in infected cells (65). As a result, it effectively inhibits the replication viral genomes and therefore is a wide-spectrum anti-viral agent *in vitro*. Remdesivir was developed by the company, Gilead Sciences, as a therapeutic agent against hepatitis C virus, against which it did not exhibit a good therapeutic effect. Subsequently Gilead Sciences, the US Centres for Disease Control and Prevention (CDC) and the US Army Medical Research Institute of Infectious Diseases (USAMRIID) collaborated to identify anti-virals targeting RNA viruses with pandemic potential. Remdesivir (then known as GS-5734) was found to be effective *in vitro* against several such viruses, including yellow fever virus, Dengue virus type 2, influenza A, parainfluenza 3, SARS, MERS, zoonotic coronaviruses, as well as the circulating human coronaviruses HCoV-OC43 and HCoV-229E (65-68).

Upon diffusion into the cell, remdesivir is metabolised into the nucleoside monophosphate form via a sequence of steps that are presumably initiated by esterase-mediated hydrolysis of the amino acid ester that liberates a carboxylate, which cyclizes on to the phosphorus displacing the phenoxide. The unstable cyclic anhydride is hydrolysed by water to the alanine metabolite, GS-704277, whose P-N bond is hydrolysed by phosphoramidase-type enzymes to liberate the nucleoside monophosphate or nucleotide analogue. The artificial nucleoside monophosphate is routed to further phosphorylation events (hijacking the endogenous phosphorylation pathway) yielding the active nucleoside triphosphate analogue form that is utilized by the viral RNA-dependent RNA polymerase (RdRp). The utilisation of the GS-441524 nucleoside triphosphate analogue by RdRp inhibits viral replication (67,68).

Evidence is lacking for the safety and efficacy of remdesivir in children as it has not been evaluated in clinical trials that include children with COVID-19. The FDA has granted emergency use authorisation for remdesivir for the treatment of suspected or laboratory confirmed COVID-19 in hospitalized adult and paediatric patients (>12 years and >40 kg) to be used as follows: Initial loading dose of 5 mg/kg on day 1 followed by 2.5 mg/kg once daily from day 2 for totally 5 days and a further 5 days if necessary. Adverse events will be monitored closely (69). The National Institute for Health and Care Excellence (NICE) conducted an evidence review on remdesivir and concluded that the included studies in that review suggested some benefit, with remdesivir compared with the placebo for reducing supportive measures including mechanical ventilation and time to recovery in patients with mild or moderate, or severe COVID-19 disease who are on supplemental oxygen treatment (70). However, no statistically significant differences were found for mortality and serious adverse events (fewer reported with remdesivir compared with placebo).

On October 22, 2020, the FDA approved remdesivir for the treatment of patients with COVID-19. The WHO SOLIDARITY trial concluded that remdesivir, HCQ, lopinavir and interferon regimens appeared to have a minimal or no effect on hospitalized patients with COVID-19, as indicated by overall mortality, initiation of ventilation and duration of hospital stay (59). The mortality findings contain most of the randomized evidence on remdesivir and interferon, and are consistent with meta-analyses of mortality in all major trials. The CARAVAN trial to evaluate the safety, tolerability, pharmacokinetics and efficacy of remdesivir (GS-5734™) in participants from birth to <18 years of age with COVID-19 specifically included children (71).

SARS-CoV-2 infection and glucocorticoid therapy. One of the most effective anti-inflammatory medications is the natural corticosteroid cortisol (hydrocortisone) and its synthetic analogues, such as methylprednisolone and dexamethasone (72,73). In the recent report of the RECOVERY trial, dexamethasone decreased the 28-day mortality of patients with critical COVID-19 by ~30% (58). Therefore, glucocorticoids are currently the only effective medication as far as mortality is concerned. Of course, with the accumulating experience that is gradually being acquired and the progressively improving therapeutic management of COVID-19 with anti-viral, anti-inflammatory and anti-coagulant agents, the morbidity and mortality curves of the pandemic have diverged, with the former increasing much rapidly than the latter.

Patients critically ill with COVID-19 have markedly elevated circulating levels of cortisol, a biomarker of high stress and poor prognosis (74). Glucocorticoids, regardless of the cause of critical illness, are involved in the preparation, onset, development and healing processes that take place serially in this state (75). Their actions include the preparation and empowerment of innate immunity, the suppression of inflammation and the re-establishment of the anatomy and function of the affected tissues. Cortisol regulates ~20% of the human genome and functions as a natural rheostat of the serial disturbances of homeostasis and homeostatic corrections that take place in the evolving process of critical illness. The rheo-

static function of glucocorticoids that concerns the immune and inflammatory reaction is opposed by the innate immunity network orchestrated by NF- κ B, a key transcription factor that regulates the activity of another large set of genes throughout the brain and body.

In the critical phase of systemic COVID-19, there are three main issues that require attention (75). First, the relative inability of endogenous cortisol to control the profound inflammation and hemodynamic instability that accompanies the disease. This phenomenon is termed 'Critical illness-related Corticosteroid Insufficiency' (CIRCI) and is due to either the inability to produce sufficient amounts of cortisol, or the resistance of the tissues to its actions, or both. Second, damage and dysfunction of the mitochondria leading to necrosis or apoptosis, and, third, the relative insufficiency of several micronutrients, such as vitamins B1 (thiamine), C (ascorbic acid) and D that are involved in various adaptive processes. In combination, these three situations constitute a potent anti-homeostasis threat, which in the pre-ICU era frequently led to death. The subjects that are most vulnerable to severe or critical COVID-19 are those whose organs have suffered the ravages of chronic stress and inflammation.

6. Prevention against SARS-CoV-2 infection

Since SARS-CoV-2 was initially identified in December, 2019, the search to develop a vaccine to prevent COVID-19 has been undertaken at an unprecedented pace. After merely 11 months, according to the WHO draft landscape of COVID-19 vaccine candidates (76), there were (up to December 10, 2020) 52 vaccines in clinical evaluation. Of these, 13 are in phase 3 clinical trials, four are in phase 2, 12 in phase 1/2 and 23 in phase 1. There are a further 162 vaccines in preclinical evaluation. The types of vaccine candidates under evaluation vary and include inactivated, viral-vectored, RNA, DNA, virus-like particle, protein subunit and live-attenuated vaccines. While the route of administration for the majority of vaccines in clinical evaluation is intramuscular, other routes under investigation include oral, subcutaneous and intradermal. Two initial doses of a vaccine are expected to be required to provide adequate protection for the majority of vaccines in development.

Preliminary data published from the earlier phases of later stage trials have not highlighted any significant safety signals, such as antibody enhanced disease, and have revealed very encouraging immunogenicity results (77-79). Three of the phase 3 trials have published interim analysis results, with all three demonstrating an excellent safety profile and high efficacy, ranging from 62-95% against symptomatic SARS-CoV-2 infection (80-82) (Table II). One of these vaccines (Pfizer/BioNTech) has been granted emergency use in several countries worldwide with the United Kingdom being the first country to begin vaccinations against SARS-CoV-2 as part of a national programme on December 8, 2020, just 342 days after SARS-CoV-2 was officially recognised. By December 10, 2020, the other two vaccines (Oxford/AstraZeneca and Moderna), which had demonstrated efficacy in late-stage trials, were being assessed by regulatory agencies worldwide for emergency use. Of note, there were no published data from COVID-19 vaccine trials

Table II. Summary of the characteristics of the three leading COVID-19 vaccine candidates.

Company	Type of vaccine	Number of doses	Efficacy (from preliminary analyses) (%)	Storage
Pfizer-BioNTech	mRNA	2x intramuscular injections	95	-70°C
Oxford-AstraZeneca	Viral-vectored	2x intramuscular injections	62-90	2-8°C
Moderna	mRNA	2x intramuscular injections	95	-20°C

in children or pregnant women at that time, although several trials were in development or underway.

One of the numerous ethical questions that have arisen during the COVID-19 pandemic is of how to maintain fair and equitable access to COVID-19 vaccines. A number of high-income countries have 'pre-ordered' several tens of millions of doses of various vaccines to ensure their population has access to these vaccines as soon as possible. However, there are a number of countries globally, which cannot afford, and are not able, to do this. The Global Alliance for Vaccines and Immunisations (GAVI) is co-leading COVAX, the vaccines pillar of the Access to COVID-19 Tools (ACT) Accelerator. This involves coordinating the COVAX Facility, a global risk-sharing mechanism for the pooled procurement and equitable distribution of COVID-19 vaccines (for further information please visit <https://www.gavi.org/covax-facility>). Ensuring vaccines can be distributed equally to all countries, not only high-income countries, in a timely manner, is likely to be the only strategy which can be used to effectively combat the current global pandemic.

7. SARS-CoV-2 and medical education

The COVID-19 pandemic has brought numerous challenges regarding education and training, with the majority of students being taught 'off-campus' and the majority of education being delivered virtually. Academics have risen to this challenge with amazing speed, resulting in reflection in what is required to deliver teaching. Focus needs to be on value-based health care and quality improvement. Students and staff should be upskilled in digital learning, virtual simulation, artificial intelligence and innovative methods. The growing population of patients with multi-morbidities means learning to improve adherence to treatments is increasingly important. It is also essential that the research capacity is increased. During the COVID-19 pandemic, it is important that staff and students, who are redeployed, are upskilled rapidly; in one excellent example, this was undertaken by the King's Health Partner's Learning Hub and using the King's Health Partners' global clinical fora; for further information please visit <https://learninghub.kingshealthpartners.org/product?catalog=khp1134c>. Medical education, including undergraduate and post-graduate medical training in paediatrics as well as the process of continuing professional development, is expected to be different during the post-COVID-19 era.

8. Conclusions and future perspectives

SARS-CoV-2, the novel virus that has caused COVID-19, is an emerging, rapidly evolving global threat. Healthcare systems

worldwide are trying concurrently to treat adults and children with COVID-19, to be prepared for its long-term effects, and treat patients with other common diseases. Infection with SARS-CoV-2 can, in a small percentage of patients, lead to severe disease and mortality. Repurposed and new medications, have been tried in patients with critical COVID-19, with equivocal results. Critical COVID-19 infection is characterized by intense inflammation and the activation of the haemostatic pathway, biological phenomena that can lead to mortality. The urgency of the COVID-19 pandemic markedly accelerated the mechanisms to design, approve, fund and execute important research, and led to the widespread use of unproven treatments, supported largely by small observational studies. Although several therapeutic agents have been evaluated for the treatment of COVID-19, only a few have been shown to be efficacious. By December 10, 2020, there were no therapeutics approved by the FDA for the treatment of COVID-19. Nevertheless, there is a need for the identification of promising effective candidates and conduct well designed, large, high priority randomized controlled trials.

Further research, both basic and clinical, on SARS-CoV-2 is essential. It is crucial to fully understand its origin, evolution, transmission and pathogenesis. Without a thorough, exact, evidence-based, non-theoretical, but experimental knowledge of the molecular virology of SARS-CoV-2, effective preventive and treatment modalities, which are so urgently required, cannot be designed and implemented. Coordinated clinical and research efforts constitute an important step in limiting SARS-CoV-2 global predominance, improving epidemiological surveillance, exploring new therapeutic and prevention strategies and advancing neonatal and paediatric care. Medical education, even under pressure conditions, such as the current SARS-CoV-2 crisis, should continue to be our priority.

9. Looking back one year later: Developments since the workshop

Looking back, 14 months after the '6th Workshop on Paediatric Virology', the volume of literature and data on SARS-CoV-2 and COVID-19 being published daily by the world scientific community is impressive (83-93). Since the workshop, as expected for a RNA virus, multiple variants of SARS-CoV-2 have emerged. Several of these are considered variants of concern (VOCs) due to increased transmissibility and/or virulence; ability to escape antibody neutralization leading to immune-, vaccine- or detection-escape; and resistance to treatments. To date, SARS-CoV-2 VOCs, which have been named using letters of the Greek alphabet and initially identified in various countries include Alpha (B.1.1.7 lineage/United Kingdom), Beta (B.1.351 lineage/South

Africa), Gamma (P.1 lineage/Brazil), Delta (B.1.617.2 lineage/India) and Omicron (B.1.1.529 lineage/South Africa). Other variants, which have not spread extensively are variants of interest (VOIs) and include Epsilon (B.1.427 and B.1.429); Zeta (P.2); Eta (B.1.525); Theta (P.3); Iota (B.1.526); Kappa (B.1.617.1); Lambda (C.37) and Mu (B.1.621). During 2021, the world witnessed the implementation of the largest vaccination programme in history, with ten billion doses of COVID-19 vaccines administered globally. There are however several inequities as regards vaccine access, with only 5.5% of individuals in low-income nations having received two doses (86). The armamentarium of therapeutic options for COVID-19 has grown and includes anti-viral drugs (e.g., molnupiravir, paxlovid, remdesivir), anti-SARS-CoV-2 monoclonal antibodies (e.g., bamlanivimab/etesevimab, casirivimab/imdevimab), anti-inflammatory drugs (e.g., dexamethasone), immunomodulatory agents (e.g., baricitinib, tocilizumab) amongst others (87). NHS England has approved the use of Sotrovimab for high-risk children (aged 12 years and above) with COVID-19 (88). Pregnancy does not appear to exacerbate the course or mortality of COVID-19 pneumonia significantly (89). Additional research has confirmed that vertical transmission is rare, with perinatal transmission occurring in some instances (90). A shorter interval between maternal COVID-19 symptoms and delivery is a risk factor for transmission (91). However, there is no increased risk for neonates apart from abnormal ophthalmologic outcomes identified in 15% of cases in a previous study (92). The absence of the co-expression of TMPRSS2 and ACE-2 in the placenta across the second trimester and at term, as well as the absence of ACE2 in the foetal lung help explain the rarity of vertical transmission (93). Fortunately, COVID-19 symptoms in children tend to be milder and slightly different from those in adults (89). Both vaccination schedules and treatment algorithms need to be further tested and optimized for children and infants.

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