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REVIEW

COVID-19: a user's guide, status of the art and an original proposal to terminate viral recurrence

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ABSTRACT

The world is now entering its 9th month of combat against a pandemic of deadly pneumonia. Started out from China in December 2019, the disease has been declared as caused by infection with a so far unknown RNA Coronavirus of the respiratory family, then named severe acute respiratory syndrome coronavirus SARS-CoV-2. In the absence of a vaccine, and with scientists still struggling for an effective therapy, COVID-19 (the SARS-dependent syndrome) carries up to now, a death toll of more than 590,000 (July 18,2020) undermining jobs and finance of contemporary society in all continents. Social distancing, the only measure hitherto shown to restrain virus spread, has been progressively loosened from May 2020 in some countries, leaving us in the fear of repeat attacks from the unchecked virus. We discuss the problem and propose to tentatively boost the antivirus cell machinery by using lab-made viral mimics to engage cell receptors.

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KEY WORDS: Severe acute respiratory syndrome coronavirus 2; COVID-19; Poly I-C; Poly ICLC;

Clinical facts and epidemiologic figures

At the end of December 2019, an unusual concentration of cases of severe pneumonia in the Chinese region of Hubei (and Wuhan as the main city) aroused investigators' and physicians' attention towards the possible breaking-in of a novel pathogen.[1] Soon, relevant cases invaded mainland China and several countries abroad, leading International Health Authorities to classify it as a pandemic a few weeks following the initial claims.[2] As of today (July 18, 2020), media information is reckoning worldwide figures of:1)14,104,033, and 597,36; 2) 2.062,189 and 77,851; 3) 3,650,962 and 161,163 respectively, of infected people and fatalities, worldwide, in Brazil, and in the USA.[3] Worst hit areas localize to West-Central Europe and the USA. Social distancing, the only form of policy commonly activated in all countries, is now yielding mixed results probably as a function of timing of its implementation and people adherence. Italy, France, Spain and Germany which were involved first, seem now to begin the plateau phase hopefully heralding phases 2 then 3 of descent and resolution; in contrast, in the USA, number of cases is still on the rise. In the China province of Hubei, where possibly everything had begun (probably earlier than initially believed), data are variable: whilst contagion and disease figures seem to decay in the initial infection sparkle of Hubei, so-called return infections are threatening to invert the trend. Recently noted infection spots at the Russian border are now setting up a Russian epidemic. The Spanish province of Cataluña is

now facing a new lockdown, owing to heavy viral return.

Worldwide data and trends gathered in the past few months show that the viral infection is not dependent on season or race, and urgently warrants antiviral solutions.

The virus

The recognized agent of the syndrome (as described below) belongs to the Coronaviridae family (now officially filed as severe acute respiratory syndrome coronavirus-CoV-2 [SARS-CoV-2]); it is a single-stranded/positive stranded RNA entity. The genome is 26,000 to 32,000 bases long and is organized and wrapped into a nucleocapsid, making this virus the largest among the known RNA viruses. Two thirds of the sequence encode for the functional proteins of transcriptase/helicase; the remaining 1/3 relates to the transcription of 4 structural proteins: spike; envelope; membrane and nucleocapsid. The mature viral particles have a dimension of 70-90 nm.[4] Among the many host species that can potentially be infected by coronaviruses, birds and humans are at the top of the list. In such categories SARS-CoV-2 may induce an array of disturbances ranging from mild-to-severe respiratory disease, to gastrointestinal malfunction.[5-8]

Clinical manifestations

SARS-CoV-2-related infection may be confusing and deceptive at presentation and at resolution. By and large, the disease (named COVID 19) development may include two phases.[9] In the initial phase, when the viral effects predominate, respiratory signs/symptoms are seen; later on, the virus' role may become less prominent and inflammation leading the well-known cytokine storm prevail. To this regard, treatment should mostly rely on antivirals in initial phases while immune modulation with steroids and/or anti-inflammatory monoclonals will be mostly indicated for the second phase. Among the Coronaviridae, this virus seems to be the most apt to spill from the upper airways, thus creating the condition for an early contagion; a significant proportion of so-called healthy carriers are suspected to be infectious. According to Chinese data, approximately 82% of COVID-19 patients may present with mild symptoms and prompt recovery; in a series of severe hospitalized patients in Guangdong (China), 26% recovered, and 13% died. In other series, mortality in the intensive care unit (ICU) was 26%.[10] Study of patient's characteristics may yield prognostic clues. Elderly male subjects with co-morbidities have been found to be the most susceptible. Frailty, as in case of diabetes, hypertension, and stenotic cardiomyopathy can play a negative role,[10, 11] and sexual hormones and their age-driven fluctuations may also contribute to disease severity. Studies of influenza vaccination have suggested that women are more responsive to vaccination than men, insofar by reporting more local and systemic reactions to flu vaccine than men, when questioned in surveys.[12] This is due to a clear immune suppressive effect of male testosterone witnessed by a negative post-vaccination antibody regulation in males with regular male hormone secretion (of great interest for the search for prognostic markers of the COVID-19 syndrome).[13] The opposite is clearly true: elderly males with diminished testosterone levels have higher levels of circulating proinflammatory cytokines.[14] Such differences are also mirrored by clinical evidence: epidemiological data for 2004-2010 flu epidemics in Hong-Kong showed a higher resort to hospitalization for men than women.[15] Finally, of top relevance is the finding of a heightened susceptibility for fatal complications in middle-aged men hospitalized for acute respiratory disorders,[16] hence confirming an important role played by patient variability. Regarding the modalities of infection, first-hand experience indicates that the health-care staff is more often hit by a severest disease grade, implying that a serious causative role is played by the higher viral loads encountered by the workers in the hospital setting.[17]

Underlying physiopathological circuits mirrored in specific clinical pictures

Many infectious (like COVID-19) and non-infectious conditions (for example, autoimmu-

nity), thanks to their altered inflammatory circuits, can impact on the coagulation pathways. Specifically, the COVID-19 syndrome has been described in conjunction with severe veno-occlusive disease.[18] Most recently, and particularly alarming for pediatricians, is the description of an unexpected prevalence of Kawasaki disease (inflammatory thrombotic disease of cardiac vessels) involving infants from a restricted area of Lombardy (Italy).[19] To this regard, current breaking news have revealed that COVID-19-related veno-occlusive disease may cause severe limb ischemia, requiring urgent amputation.[20]

Uncommon clinical presentation

Up to 50% of all infections by SARS-CoV-2 may present exclusively with gastrointestinal symptoms, as their gut microbiota might have been upset by interferon (IFN) released during the viral lung attack.[21] Furthermore, gut permeability, that plays a crucial role in health as well as in several diseases,[22] could be altered by this infection. This special COVID-19 subset may run a prolonged disease course, with diarrhea and abdominal pain and SARS-CoV-2 viral elements found in the feces.[23]

Real time notes

The Doctor's Guide Bulletin is reporting on European cases of a non-better characterized Pediatric Inflammatory Multi-System Syndrome, marked by fever, abdominal pain, and cardiac involvement. Clinically, the picture may fall half-way between Kawasaki disease and toxic shock syndrome. A temporal association between this syndrome and SARS-CoV-2 must not be neglected, but no official position has been taken until further data come to the Regulatory Authority.[24]

Treatment

Data reported hitherto suggest that SARS-CoV-2-induced infection should be regarded more than a "tenacious flu," due to its ability to induce severe respiratory illnesses leading to death in the most susceptible individuals.[25, 26]

Thus, the USA National Institutes of Health (NIH) gathered several experts in order to provide the available therapeutic options for COVID-19. This document, entitled "Treatment Guidelines for COVID-19" was released April 21, 2020 and is synthesized below.[27]

- There are no sufficient data to advise or discourage the use of the following measures:
  - chloroquine (recently deleted because of conflicting results);
  - Remdesivir (on May 1st, the regulatory authority has reckoned that, despite the absence of a formal proof of effectiveness, Remdesivir [Gilead Sciences, Foster City, CA, USA] seems to shorten disease course in hospital; hence, a "emergency use authorization" has been issued under this specific indication;
  - convalescent plasma;
  - interleukin (IL)-1 or IL-6 inhibitors;
  - interferons or Janus Kinase inhibitors may be allowed only in clinical trials.
- Concomitant medications:
  - angiotensin-converting enzyme (ACE) inhibitors are discouraged outside a trial context;
  - systemic corticosteroids are discouraged in mechanically ventilated subjects with or without acute respiratory distress syndrome (ARDS). These medications should not be routinely prescribed to patients affected by SARS-CoV-2 in Intensive Care Unit (ICU);
  - pre-existing prescriptions of oral/inhaled corticosteroids should be continued for the usual indications. Betamethasone and dexamethasone (crossing the placenta) should be prescribed only for strict fetal benefit; the other steroids (not passing the placenta) may not be restricted. The offering of antenatal steroids is not encouraged and will be subject to the guidelines of the relevant American Associations;
  - statins or non-steroidal anti-inflammatory drug (NSAIDs) should be continued if prescribed previously for comorbid condition;
  - a positive or negative SARS-CoV-2 condition should not influence choice of an antipyretic therapy (NSAID or paracetamol);
  - an excessive prevalence of severe venous thromboembolic accidents has been reported in a number of patients affected by SARS-CoV-2 in the ICU.[18] Whether this new observation will entail modification of current heparin trials will have to be decided.

Most recently, an updated reappraisal of the treatments and strategies for the syndrome of COVID-19 has been published.[28]

Two issues that relevantly impact on the management of COVID-19 syndrome

The cytokine storm (or syndrome)

The term "cytokine storm" was first used in 1993, alluding to a hitherto undescribed picture of uncontrolled immune activation, usually following cluster of differentiation (CD)3 maneuvers to modulate transplant and graft-*versus*-host disease. The syndrome included clinical features of immune activation (fever and generalized malaise), as well as accumulation of proinflammatory cytokines. The study on severe acute respiratory syndrome related to SARS-CoV in mice has been seminal in this respect. The rapid replication of SARS-CoV in BALB/c mice induced the delayed release of IFN-α/β, which was accompanied by the influx of many pathogenic inflammatory mononuclear macrophages. These cells were activated through the IFN-α/β receptors on their surface and produce more monocyte chemoattractants (such as CCL2, CCL7, and CCL12), resulting in further accumulation of mononuclear macrophages and production of elevated levels of proinflammatory cytokines (tumor necrosis factor [TNF]), IL-6, IL1-β, and inducible nitric oxide synthase), thereby increasing the disease severity. Interestingly, depleting the inflammatory cytokine TNF, protected mice from the fatal SARS-CoV infection. IFN-α/β or mononuclear macrophage-derived proinflammatory cytokines also induced the apoptosis of T cells, which further hindered viral clearance. Another consequence of rapid viral replication and vigorous proinflammatory cytokine/chemokine response is the induction of apoptosis in lung epithelial and endothelial cells. Indeed, apoptosis of alveolar and epithelial cells, completed by vascular and generalized organ damage can form the basis for the genesis of the ARDS, a leading cause of death in these SARS-CoV-2-infected patients. Thus, treatment of this cytokine storm might be preferentially based on immune modulators.

Therapy options

Usual corticosteroids (intravenous methylprednisolone 1 mg/Kg) can control the inflammation cascade in these patients, from initial tissue infiltration to full ARDS. Timely choices from experienced clinicians are of course required, as excessive or too early doses can be detrimental if fostering viral replication. The variegated steroid toxicity must equally be monitored. TNF blockers can be anticipated to be effective, but specific experience with their use is null or limited.[29, 30]

According to the available literature, convalescent plasma has been infused to patients affected by SARS-CoV-2 in some cases.[31] The reportedly good results are in keeping with the simple but robust fact that convalescent plasma must necessarily contain the relevant healing antibody. Given the cost-effectiveness and relative safety, the pharmacological profile of COVID-19-convalescent plasma should be regarded more closely.[32] The plasma of patients discharged after recovery from COVID-19 should be bio-banked for further studies.

The need to protect large masses of people infected or re-infected by respiratory viruses including SARS-CoV-2

Historical background

At the turning of the 9th month of the COVID-19 pandemic, most countries around the world are facing the challenge of gradually loosening the rules of social distancing, as a prerequisite to slowly return to a "normal" life.[26] This might be risky in the absence of a vaccine and of any approved anti-SARS-CoV-2 drug. A reactivation of the pandemic during the downgrading of the restriction measures would have mass psychologic and medical consequences with a difficult-to-see outlook.[33] A possible response to the challenge may include: harnessing the innate immune system to non-specifically respond to viral infection, creating a continuous antivirus shield. This strategy would be successful despite any viral mutation.[34] To succinctly but precisely illustrate the essential steps, the following is needed: 1) using its cell effectors natural killer (NK) and natural t-killer cells (NKT) bearing pathogen recognition receptors (PRR), the innate immune system

recognizes and responds to specific ligands on the pathogen surface termed pathogen associated molecular patterns (PAMPs). This is then followed by release of cytokines to mount an effective immune response. In humans, the invader antigens are recognized by detectors Toll-like receptors (TLR) belonging to the PRR family. All TLR basically include a membrane leucine-rich motif (antigen-sensing) and an effector cytoplasmic tail that conveys proinflammatory signals to the cell machinery. Antigen categories recognize specific TLR. The double-strand RNA of the respiratory viruses like SARS-CoV-2 is recognized by TLR-3.[35] Thus, TLR3 is the cell sensor that has to be targeted if projecting maneuvers to arouse a response to an experimental or spontaneous SARS-CoV-2 attack. Located at the cell membrane of nasal, alveolar, bronchial epithelial cells, and ready to respond to any sub-cellularly localized RNA, TLR3 constitutes a prompt nonspecific antiviral weapon. Its ligands are generally named TLR3 agonists. At this point, we should ask whether the Coronaviridae can be included in this strategy, which double strain (ds) RNA mimicry molecules are best candidates as the drugs for this project, and how feasible is all this in humans.

The virus and its candidacy for prevention with synthetic dsRNAs

SARS-CoV-2 can be expected to engage regular forms of TLR3. There is an emphasis in the literature on the Sars-CoV-2 propensity to induce multiple reactivations with time, probably due to a high mutation frequency.[36] In T-cell immunodeficient hosts, this virus tends to provoke cytokine storms, rendering treatment even more demanding.

Various TL3-agonists exhibit the structure of dsRNAs

Synthetic dsRNAs, at least 40-50 base pairs in length, including polyinosinic:polycytidylic acid (poly(I:C)) alone or stabilized with poly-L-lysine and carboxymethyl cellulose (Poly-ICLC) and polyadenosinic-polyuridylic acid (poly [AU]) have been used as molecular mimics for viral dsRNA.[37] Such compounds have been shown to potently induce IFN and down-regulate IL-2, IL-4 and IL-5 in lung tissue. Given prophylactically, poly(I:C) abolished the replicative competence of many viruses, including influenza, herpes simplex, and yellow fever.

For clinical use, poly(I:C) may be one of the most appropriate agents to generate stable mature dendritic cells (DCs). These mature DCs might generate effective immune responses *in vivo* after injection, as they retain the ability to secrete bioactive IL-12 after CD40 ligation.

Toxicity

In preliminary studies, poly(I:C) and derivatives have consistently shown varying degrees of intrinsic toxicity, a major obstacle hindering their release as regular drugs. TLR3 activation was used to detect toxicity signs. Essentially, two toxicity patterns were described in mice. Repeated intranasal administrations were followed by lung inflammation including edema and cell infiltration; several administration routes in autoimmunity prone animals mainly resulted in pancreatitis or worsening of nephritis.[38, 39] Attempts to characterize the consequence of diversifying routes of administration yielded conflicting results depending on the animal species. Attempts were made using lower doses in adjuvant designs, masquerading poly IC under liposomes shells, co-administering steroids, but albeit promising these yielded mixed results. Specific thiolation of the cytosine residues on the RNA chain has also attracted attention.[40]

Where are we now?

Today's reappraisals indicate the following. Poly-ICLC is now dispensed as a regular drug under the registered label HILTONOL® (Oncovir Inc., Washington DC, VA, USA). HILTONOL® is now employed in three investigational settings: 1) as an adjuvant in antitumor therapeutics; 2) as a TLR 3 agonists in viral diseases; 3) as a drug included in antihuman immunodeficiency virus (HIV) trials. In the latter ones, it is at the I/II dose-finding levels.

A work published in 2017[41] summarizes the state of the art reached with the experimental use of poly(I:C) against respiratory viruses in pre-

clinical studies. Briefly, pre-emptive intranasal doses from 0.25 to 5 mg/Kg/day significantly protected mice from lethal units of SARS-CoV; interestingly, poly(I:C) exerted 100% protection if administered up to 8-hours postviral exposure. Literally citing the authors: "host-targeted therapeutics, such as HILTONOL®, that activate innate immunity and provide immediate broad spectrum resistance, could fill the gap in protection by allowing time for more specific vaccination strategies to take effect, and could thus become an important element of the rapid response to a bioterror attack or pandemic outbreak. The demonstrated clinical safety, its immediate induction of an innate immune persistent broad spectrum antiviral state, its relatively low cost, its storage stability and relative ease of use, make it a potentially very valuable agent for containment of epidemics caused by respiratory pathogens."[41]

Conclusions

At the beginning of this year, an unexpected combat began (and there are no clues on when it will end) between mankind (ourselves) and a supposedly unknown respiratory virus labelled as SARS-CoV-2. Both contestants do manifest a few intriguing yet worrying features. The virus is reported to easily grow in the upper airways, hence exhaled from breath droplets for a maximum contagion; serology or molecular screening have often failed to confirm eradication; thus, this pathogen can undermine the social, productive, and private lives of people. As the targets of this attack, we have not fared well so far. Lost in debates about the excessive sophistication of our lifestyles after 30 years of (apparent) peace,[42] we were caught unprepared. So far, propelled by the SARS syndrome (a deadly pneumonia killing anyone without notice) and by the consequences of social distancing (bankruptcy due to society freezing), most of us have submissively witnessed destruction of life which we were used to.[43, 44] Last but not least, mixed information echoed by media seems to rather launch a shadow of doubt on health authority: despite the daily news on progresses for a vaccine production, it is unclear the time needed for its availability, antivirals are complex and expensive, and subject to virus mutation. Looking at the frustration of people trying to use an escalator or entering a shop, yet struggling to maintain safety distances, we thought it worth inaugurating a strategy to forget about the virus and concentrate on shielding every individual: if it is raining and you cannot push the clouds away, you can still cover people's shoulders with a raincoat, taking them to a sheltered place: clearly a periphrasis for "keep momentarily safe until a vaccine comes out."[45] The recent announcement (August 11, 2020) of the availability of a Russian vaccine has raised perplexities due to the lack of large trials aiming to test vaccine safety.[46]

We chose to concentrate on poly(I:C) as a means to "taxi" virus-exposed people to a virus-free shelter, acknowledging that this is not the only way. Of course, we have no conflict of interest whatsoever, we are simply committed to the idea. Neither are we planning to press regulatory authority to release an emergency use authorization (EUA). On the other hand, nine months after the pandemic outbreak, there is a need for a factual (though temporary) action, to restore some ease of mind for people battered by unmet anxiety and scare.

Current virus-host co-existence in the absence of a strategy based on poly:IC

The long delay in the implementation of an antivirus strategy using TLR3 agonists, has led SARS-CoV-2 infection to become a pandemic. SARS-CoV-2 will integrate in our society, establishing a mutual co-existence balance with mild diseases, that unfortunately will reactivate in permissive conditions, with erratic re-appearance of symptoms. Spontaneous down-grading of virus power coupled with our improved skills to control disease manifestations will hopefully achieve an acceptable co-existence. If the virus elude control, there will be the risk of renovating the core presentation with cytokine storm and lung disease requiring admission to intensive care. At that point, the threat of massive admissions with collapse of resuscitation facilities, will renovate the need for social distancing culminating in the well-known lock-down and its unavoided implications.[26] Certain negative

TABLE I.—*Certainties and novelties of SARS-CoV-2 infection.*

Certainties of SARS-CoV-2 infection
Seeking vaccine/antivirals represents the correct strategy; but at the moment this strategy is inadequate, in terms of immediacy, effectiveness, and people's consensus
Four months of physical distancing have produced some results, but the pay back (under everybody's eyes) is emerging as job loss and unexpected poverty, financial and family breakdown
Novelties of SARS-CoV-2 infection
We feel urged to justify our decision to include a proposal for an alternative strategy to arrest the viral diffusion
We propose to try an artificial nucleic acid to arouse the cell non-specific antiviral state, a strategy that is not unknown in other fields

consequences will be hardly foreseeable. For example, strict reinforcement of safety measures in all medical treatment including day-hospital mini-surgery will force the use of disposable sheaths, gowns, instruments, increasing costs, and heavily polluting the environment with tons of discarded materials. In brief, this unfortunate encounter with SARS-CoV-2 has subverted our "normal" scale of values.

There are now indications that the virus is loosening its strength. In fact, current search has failed to detect mutated viral strains with less infectivity. On the same line, the hottest temperatures now being attained in infected Middle East countries do not seem to weaken disease diffusion.

This pandemic is different. For the minor part, the difference is that SARS-CoV-2 is highly infectious and diffusive; for the major part, we ourselves did react in a fully different fashion (Table I).[47-50]

Among several research strategies so-called defective viral particles (DVP) could deserve attention as viral modulators. DVP are viral particles often lacking part of the genome; unable to replicate, they can behave as companions of the main helper virus, which in turn can support replication.[48] Interestingly, DVP can act as viral modulators or inhibitors, either directly, or by enhancing innate immunity mechanisms and potentiating dendritic cells to better present the viral antigen. Intriguingly in this case, DVP could recall the functions of the poly:IC as dealt with in our proposal. A research group based in Holland is describing the enhancement of innate immunity by education through injections of antitubercular vaccines,[51] clearly confirming the data presented in this manuscript with poly:IC. This data, mixed with the results described above with DVP, witness the spreading of an internationally agreed planning to enhance natural immunity pathways as a way to smart control of pathogens before the full definition and proof of efficacy of vaccines.

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