INTERNATIONAL JOURNAL OF HYPERTHERMIA
2021, VOL. 38, NO. 1, 202–212
https://doi.org/10.1080/02656736.2021.1883127



# Exploring the rationale for thermotherapy in COVID-19

Javier Mancilla-Galindo[a] and Norma Galindo-Sevilla[b]

[a]Facultad de Medicina, División de Investigación, Unidad de Investigación UNAM-INC, Instituto Nacional de Cardiología Ignacio Chávez, Mexico City, Mexico; [b]Departamento de Infectología e Inmunología, Instituto Nacional de Perinatología, Mexico City, Mexico

**ABSTRACT**

Increased transmissibility of the pandemic severe acute respiratory coronavirus 2 (SARS-CoV-2) has been noted to occur at lower ambient temperatures. This is seemingly related to a better replication of most respiratory viruses, including SARS-CoV-2, at lower-than-core body temperatures (i.e., 33 °C vs 37 °C). Also, intrinsic characteristics of SARS-CoV-2 make it a heat-susceptible pathogen. Thermotherapy has successfully been used to combat viral infections in plants which could otherwise result in great economic losses; 90% of viruses causing infections in plants are positive-sense single-stranded ribonucleic acid (+ssRNA) viruses, a characteristic shared by SARS-CoV-2. Thus, it is possible to envision the use of heat-based interventions (thermotherapy or mild-temperature hyperthermia) in patients with COVID-19 for which moderate cycles (every 8–12 h) of mild-temperature hyperthermia (1–2 h) have been proposed. However, there are potential safety and mechanistic concerns which could limit the use of thermotherapy only to patients with mild-to-moderate COVID-19 to prevent disease progression rather than to treat patients who have already progressed to severe-to-critical COVID-19. Here, we review the characteristics of SARS-CoV-2 which make it a heat-susceptible virus, potential host mechanisms which could be enhanced at higher temperatures to aid viral clearance, and how thermotherapy could be investigated as a modality of treatment in patients with COVID-19 while taking into consideration potential risks.

**ARTICLE HISTORY**
Received 10 August 2020
Revised 14 January 2021
Accepted 22 January 2021

**KEYWORDS**
Thermotherapy; non-oncologic applications of hyperthermia; COVID-19; SARS-CoV-2; RNA interference

Susceptibility to heat of the pandemic severe acute respiratory coronavirus 2 (SARS-CoV-2) has been a matter of debate since the World Health Organization (WHO) declared coronavirus disease (COVID-19) as a pandemic on 11 March 2020. Low ambient temperature and humidity are thought to favor transmission of the virus. One early report found no association between increased transmissibility of SARS-CoV-2 and lower ambient temperatures and humidity in Chinese cities [1]; however, reanalysis of extended data suggested temperature could be related to increased transmissibility [2]. Increasing numbers of research items are pointing to a reduced transmission of the virus with increasing ambient temperatures and humidity worldwide [3,4]. The effectiveness of face masks has even been thought to be partly due to increased temperature and humidity in the upper respiratory tract [5].

Susceptibility of SARS-CoV-2 to higher temperatures could have implications extending beyond transmission dynamics. In this sense, early application of heat-based interventions in specific groups of patients with COVID-19 could possibly reduce progression to severe forms of the disease, thereby aiding to combat this pandemic for which no sufficiently proven safe and effective medications or vaccines exist yet. Developing simple, low-cost, and accessible therapeutics for COVID-19 should be a priority, since disadvantaged populations and those living in poverty could be the last to be benefited from pharmaceuticals [6].

In the following subheadings, we will describe knowledge pointing toward increased susceptibility to heat by SARS-CoV-2 and other respiratory viruses, mechanisms by which application of heat could aid clearance of the virus and detain disease progression, and ways thermotherapy could be implemented in patients with COVID-19 while considering potential risks.

## Characteristics of SARS-CoV-2 and susceptibility to heat

The species SARS-CoV-2 belongs to the family *Coronaviridae*, genus *Betacoronavirus* [7]. Coronaviruses possess a positive-sense single-stranded ribonucleic acid (+ssRNA) genome of approximately 30 kilobases (kb), which is nonsegmented, 3′ polyadenylated and with a 5′-cap initiator fragment [8]. One of the first sequenced genomes of this virus consisted of six mayor open reading frames (ORFs) [9], corresponding to ORF1a and ORF1b which are typically found in coronaviruses [8] encoding 16 nonstructural proteins (nsp) involved mainly in viral RNA processing and replication (the process leading to more copies of the viral RNA genome), and the ORFs for the four main structural proteins which mainly give the virus

CONTACT Norma Galindo-Sevilla ngalindosevilla@hotmail.com Departamento de Infectología e Inmunología, Instituto Nacional de Perinatología, Mexico City, Mexico

its shape and guide entry to the cell: spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins. Additional ORFs in the SARS-CoV-2 genome are: 3a, 6, 7a, 7 b, and 8 [9].

The N protein is an RNA-binding protein which plays a key role in maintaining a suitable RNA conformation for viral RNA replication and translation (synthesis of viral proteins) [10]. The N protein of SARS-CoV, which shares 90.3% homology with the SARS-CoV-2 N protein [11], starts unfolding at 35 °C and reaches complete denaturation at 55 °C (melting temperature [$T_m$] = 43 °C) [12]. Both SARS-CoV and SARS-CoV-2 are known to be heat susceptible in a temperature-dependent manner and completely lose their infective potential when incubated at 56 °C for at least 10 min [13,14], which is only 1° C higher than the denaturation temperature of the N protein. More recently, the nsp8 and nsp12 subunits of the SARS-CoV-2 RNA polymerase were shown to have lower $T_m$ (45.4 and 43.6 °C, respectively) than their counterparts in SARS-CoV (48.7 and 45.2 °C, respectively) [15], which could represent an adaptation for greater infectivity in humans while also conferring less thermostability. Furthermore, a predictive model of thermal inactivation of coronaviruses supports that inactivation follows protein denaturation, while the time required for 3-log inactivation of the virus due to thermal denaturation outside hosts was predicted to be 9.4 h at 35 °C and 4 h at 40 °C [16].

Most respiratory viruses are particularly heat-sensitive and have greater replication and infectivity at the colder temperatures found in the upper respiratory tract as compared to the lower respiratory tract (i.e., 33 °C versus 37 °C) [17,18]. This is seemingly the case for SARS-CoV-2, which replicates more efficiently at 33 °C compared to 37 °C in human airway epithelial cells (AECs) [19]. For instance, incorporation of the SARS-CoV-2 S protein into pseudoviral particles is diminished at 37 °C with respect to 33 °C [20]. Increased temperature is known to suppress rhinovirus replication through increased expression of antiviral defense response genes in AECs and higher levels of type I and III interferons (IFN) [17], and through IFN-independent mechanisms relying on viral double-stranded RNA (dsRNA) intermediates inducing earlier apoptosis of infected cells and enhanced ribonuclease L (RNAseL) activity (independent of its known increased expression by IFN) [21]. RNA interference (RNAi) could be a third dsRNA-dependent mechanism being enhanced at higher temperatures, which is both IFN and non-IFN dependent, and possibly relevant for SARS-CoV-2 infection; we discuss on this in a different subheading.

Uncharacterized thermal inactivation of coronaviruses in hosts notwithstanding, it is unlikely that sterilization temperatures for prolonged periods could be reached in humans without causing damage. Thus, inactivation of SARS-CoV-2 in hosts through mere thermodynamic effects would unlikely be possible. However, mild thermodynamic effects could act synergistically with the known biological effects of increased temperature in the clearance of respiratory viruses, particularly if done promptly since low early type I and III IFN responses are associated with disease progression in COVID-19 [22,23].

## Effects of temperature in protein synthesis and organelle trafficking

Cells are adapted to perform their functions most efficiently at a specific temperature. For instance, testicles in mice and humans are exposed to lower temperatures than core body temperature (i.e., 34 °C vs 37 °C). After exposure of mice spermatids to 34 °C, 37 °C, and 40 °C for 1 h, protein synthesis decreased in approximately 25% at 37 °C and 50% at 40 °C when compared to 34 °C [24]. RNA templates called messenger RNA (mRNA) are normally used by cell ribosomes to assemble amino acids into proteins, ribosomes can group into polyribosomes to enhance protein synthesis. In the study by Nakamura and Hall, temperature-dependent reductions in polyribosomes were associated to decreased protein synthesis. HeLa cells exposed to 42 °C also had disaggregation of polyribosomes leading to lower protein synthesis [25].

Ribosomes are composed of ribosomal RNA (rRNA) and proteins. Cells exposed to 42 °C characteristically almost cease production of rRNA to only 3% after 1 h, through diverse mechanisms [26,27]. This significantly reduces translation of proteins to preserve energy. Other mechanisms being altered at 42 °C are the adequate processing of certain mRNAs [28] and the inhibition of polyadenylation (a process which adds a poly(A) tail to RNA, leading to more stable and efficient lecture of RNA) [29]. Polyadenylation of the coronavirus RNA genome is known to promote virus survival through enhanced translation and replication [30]. Taken together, inhibition of protein synthesis and polyadenylation of viral RNA could potentially and significantly dampen SARS-CoV-2 infectivity.

As part of its pathogenesis, cellular organelles are remodeled in SARS-CoV-2 infected cells; inhibition of cytoskeletal rearrangements in these cells has been shown to suppress production of viral particles [31]. Since both hyperthermia [32] and radiotherapy [33] have the potential to alter cytoskeleton dynamics, their joint use could potentially decrease SARS-CoV-2 infectivity.

Furthermore, SARS-CoV-2 alters lysosomal trafficking since this is the way new virions are released. By deacidifying lysosomes, which results in inactivation of lysosomal enzymes, SARS-CoV-2 infection leads to impaired antigen processing and presentation [34]. Acidification of lysosomes occurs with increasing temperatures [35], thus thermotherapy could limit the release of newly assembled viral particles and enhance antigen recognition by cells of the immune system.

## Hyperthermia and the immune response

The immune system is characterized for being inducible by diverse stimuli, with several possible routes of response according to individual factors which have not been fully characterized yet. Fever-range temperatures (38–41 °C) are able to alter the immune response at diverse levels, including both the innate and adaptive immune responses [36–38].

Upon increased temperatures (i.e., 42 °C) or other inducers of cell stress, dramatic changes in gene expression profiles

are seen, with increases in stress response proteins (also known as heat-shock proteins [HSPs] after their discovery in cells accidentally exposed to higher temperatures). HSPs confer protection to cells *via* immunomodulation as one of the known mechanisms. Enhancing the stress response is thought to limit progression to acute lung injury after sepsis [39] with the HSP70 pathway being one of the main mechanisms that could dampen hyperinflammation after SARS-CoV-2 infection [40]. While some viruses use HSPs to achieve greater infectivity (i.e., human papillomavirus [HPV], adenovirus, and dengue virus), others halt with higher levels of HSPs (i.e., influenza virus and human immunodeficiency virus [HIV]) [41]. SARS-CoV has sophisticated mechanisms to modulate the stress response through its S protein [42] and E protein [43]. Removing the E protein from SARS-CoV resulted in less infectivity. Thus, the stress response would mostly affect SARS-CoV infectivity rather than promoting it, which would explain why the virus has evolved mechanisms that modulate the stress response.

As previously mentioned, SARS-CoV-2 alters immune cell processing of antigens through disruption of organelle trafficking. Another pathophysiological feature of SARS-CoV-2 is that it enhances sphingomyelinase activity, thereby increasing ceramide-rich membrane domains which serve as anchors for enzymes that promote viral infectivity (cathepsin D, protein phosphatases and kinases) [44]. During mild hyperthermia, HSPs are transported and anchored to ceramide-rich domains to stabilize the cell membrane in response to heat; the superficial exposure of HSP70 allows cells to be recognized by macrophages and natural killer (NK) cells [45], thereby constituting a second mechanism by which thermotherapy could promote antigen presentation. Cytotoxicity of NK and T $CD8^+$ MHC Class I restricted cells is also increased under mild hyperthermia [46,47].

Fever-range temperatures also enhance immune cell trafficking, thereby improving immune surveillance during infection [38]. Dendritic cells (DCs) display increased migration and recruitment to secondary lymphoid organs with improved ability of antigen presentation to naive CD8+ T lymphocytes and increased cytokine production, particularly TNF-α [48]. Under these temperatures, gene expression is modified in DCs, with upregulation of 43 genes and downregulation of 24 genes involved in post-transcriptional modifications, protein folding, cell death/survival, and migration [49], as well as interleukin (IL)-12 production [50], which could bias the immune response toward a Th1 phenotype [51,52]. An early Th1 immune response is important since Th1 cells are able to produce cytokines and recruit innate immune cells [53], which has been associated with rapid viral clearance and mild disease in COVD-19 [54]. Notably, children who have milder disease after SARS-CoV-2 infection have predominantly increased levels of IL-17A and IFN-γ, whereas adults typically do not, possibly reflecting a protective role for Th17 cells [55]. The capacity to produce IL-22 could be important for tissue repair after SARS-CoV-2 infection [53]. Thus, thermotherapy could be particularly useful in adults with COVID-19 who do not express a Th17-compatible response after infection since temperatures between 38.5 and 39.5 °C promote Th17 differentiation *in vitro*, with increased production of IL-17 and IL-22 [56].

One of the hallmarks of severe COVID-19 is that patients who progress to severe forms of the disease develop a hyperinflammatory state (also known as cytokine storm), characterized by increased serum levels of IL-1β, IL-6, IL-8, and TNF-α, which have been associated with decreased survival [57,58]. Furthermore, pro-inflammatory cytokines contribute to thrombosis by increasing fibrinogen production (*via* IL-6) and promoting rapid clot formation (*via* IL-8, IL-1β, and IL-6) [59,60], a reason which has led to identify hypercoagulability in COVID-19 as 'thromboinflammation' [61]. In this sense, thermotherapy could be of use since downregulation of pro-inflammatory gene expression through exposition of cells to heat (41 °C for as short as 15 min) results in inhibition of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and mitogen-activated protein kinase (MAPK) p38 for up to 20 h [62]. Furthermore, after exposition of mononuclear blood cells from healthy donors to 39 °C, lymphocytes expand in number with no predilection of lymphocyte subtype as determined through flow cytometry, and levels of IL-1β, IL-6, and IFN-γ are decreased [63].

The integrated cellular mechanisms discussed so far by which thermotherapy could alter SARS-CoV-2 pathogenesis are shown in Figure 1.

## RNA interference (RNAi) in plants and mammals

Plants and invertebrates have evolved diverse mechanisms of protection against viruses; ssRNA viruses cause 90% of infections in plants [64]. Even though plants do not rely on antibodies and a robust and complex cellular immune system such as higher vertebrates, plants have developed an adaptive response to viral infections which mostly uses RNA molecules as effectors [64,65].

Two main antiviral activities for RNAi are recognized: 1. processing of viral dsRNA products into ∼22-nucleotide-long virus-derived small interfering RNAs (vsiRNAs) impedes viral replication, and 2. vsiRNAs recruited to complementary viral RNAs through the RNAi machinery promote cleavage of viral transcripts, thereby limiting the production of viral proteins [66]. RNAi mechanisms are present in most eukaryotes and are evolutionary deemed as early defense elements against viruses and transposable elements, which were likely present in the last common ancestor of modern eukaryotes [67]. Nonetheless, it is possible that their role in different eukaryotes could have diverged later in evolution [68].

Whether similar mechanisms of defense against RNA viruses exist and are of relevance in mammals (due to the predominant role of IFN responses) has long been controversial. Numerous viral proteins acting as viral supressors of RNAi (VSRs) have been identified in pathogenic viruses (Table 1). The presence of VSRs results in greater infectivity when compared to viruses deficient in these proteins. Initial debate on antiviral RNAi in mammals resulted from inconsistent findings regarding vsiRNAs, and some thought these could be breakdown products of viral RNA. However, vsiRNAs were clearly differentiated from degradation

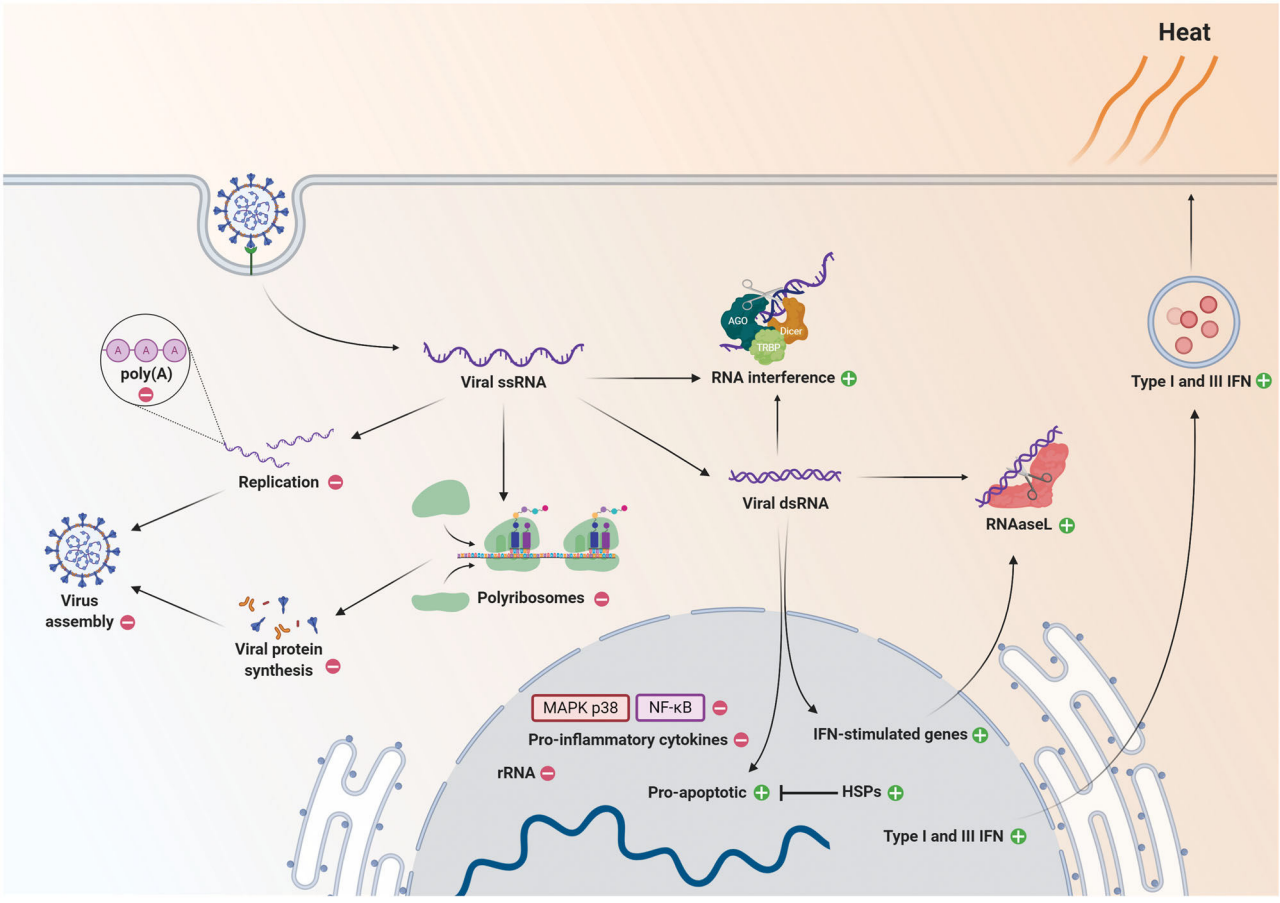


Figure 1. Potential effects of thermotherapy at the cellular level after infection by a single-stranded RNA (ssRNA) virus. After entry to the cell, viral particles are unassembled, leaving single-stranded RNA (ssRNA) free to lead viral protein synthesis and replication. Double-stranded RNA (dsRNA) intermediates are formed, which are used both by the RNA interference machinery and ribonuclease L (RNAseL) to degrade viral RNA; dsRNA promotes the expression of pro-apoptotic and interferon (IFN)-stimulated genes. Exposition to heat could potentially increase (green circle with + sign) viral RNA degradation and expression of heat-shock proteins (HSPs), IFN-stimulated genes, type I and III IFN release, and proapoptotic mechanisms of infected cells. Higher temperatures would inhibit (red circle with - sign) viral replication, viral RNA polyadenylation (poly[A]), ribosomal RNA (rRNA) synthesis, polyribosome formation, viral assembly, and expression of pro-inflammatory cytokines through inhibition of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and mitogen-activated protein kinase (MAPK) p38.

Table 1. Viral proteins with roles as viral suppressors of RNA interference (VSRs)[a].

| Virus | Genus | Protein | Reference |
|---|---|---|---|
| Semliki Forest Virus | *Alphavirus* | Capsid (C) | [115] |
| Dengue Virus | *Flavivirus* | Nonstructural protein 2A (NS2A) | [116] |
| SARS-CoV | *Betacoronavirus* | Nucleocapsid (N) | [117] |
| | | 7a | [118] |
| SARS-CoV-2 | *Betacoronavirus* | Nucleocapsid (N) | [119] |

[a]A comprehensive review of VSRs has been done by Maillard et al. [79]. We have only included studies not reviewed by Maillard et al. which were published afterwards, as well as studies involving coronavirus VSRs.

products when studying their production in the absence of the VSR B2 protein of Nodamura Virus (NoV) infecting mouse embryonic stem cells (mESCs) and baby hamster kidney 21 (BHK-21) cells [69,70].

Argonaute proteins (AGO) are key effectors of antiviral defense *via* RNAi in plants and invertebrates [64]. Of the four AGO encoded in mice and humans, AGO2 was initially most studied as the candidate for antiviral RNAi in mammals, due to its proven catalytic activity *in vitro* and better characterization, with conflicting results. Successful identification of vsiRNAs was possible after NoV infection in mESCs depleted of all four AGOs and an ectopically expressed inducible human AGO2, which resulted in decreased viral titers [70]. A subsequent study by this group suggested that robust IFN responses in mammals could mask antiviral RNAi; this was done in mouse embryonic fibroblasts (MEFs) which respond efficiently to IFN, contrary to mESCs [71].

These findings were challenged by two studies using HeLa cells engineered by CRISPR/Cas9 to be deficient in RIG-1 and MDA5 – two pattern receptors which induce IFN expression – [72] and AGO2 [73]. After infection with Sindbis virus, YFV17D, and coxsackie virus B3 (encephalomyocarditis virus [EMCV] was also included in the latter study), no detectable vsiRNAs nor a role for human AGO2 in antiviral activity *via* RNAi were identified. Adding to evidence against RNAi in mammals, phylogenetic comparisons of AGOs in different taxa suggest that an RNAi antiviral function is lacking in vertebrates, since only-miRNA-class AGOs were identified by this approach [74]. However, phylogenetic comparison of human AGOs has shown that functional adaptations in AGOs do not directly correlate with evolutionary distancing [75].

Recently, AGO4 has been proposed as the argonaute involved in antiviral RNAi in mammals. Somatic cell lines (i.e., HeLa) are known for low expression on AGO4 [76], which is

highly expressed in male mouse germlines [77] and IFN-producing innate immune mouse cells [78]. Of note, AGO4 has at least moderate expression in human airway epithelial cells infected with SARS-CoV-2, and certain peripheral blood cells from patients with COVID-19 (Figure 2). In the study by Adiliaghdam et al. AGO4 was found to possess both IFN-dependent and IFN-independent mechanisms of antiviral protection. After infection with influenza A virus (FLUAV), EMCV and Vesicular Stomatitis Virus (VSV), higher viral titers and viral RNA were detected in AGO4 knockout macrophages, dendritic cells, and MEFs. Lower levels of IFN-β were detected after viral infection in AGO4-deficient macrophages, and vsiRNAs were detected after infection with FLUAV, with higher levels of vsiRNAs after infection with NS1-deficient FLUAV resulting in higher viral loads. AGO4-deficient mice had greater viral titers and virus-induced pathology after being infected with FLUAV.

Evidence of the importance of RNAi in mammals has been extensively reviewed elsewhere [66,79]. It is currently not possible either to discard or confirm the presence of antiviral RNAi in mammals. However, the identification of the SARS-CoV-2 N protein, as well as the SARS-CoV N and 7a proteins as potential VSRs, warrants further investigation of their potential role in the pathogenesis of SARS-CoV-2 infection. A more through overview of the potential importance of RNAi in the pathogenesis of SARS-CoV-2 has been done by Karjee S., *et al.* [80]. Studies addressing antiviral RNAi in cells known to be infected by SARS-CoV-2 early in the disease, such as AECs, type 1 and 2 pneumocytes, and alveolar macrophages could be of great relevance. Recently, through immunostaining of the trachea of one patient who died after developing critical COVID-19, co-localization of SARS-CoV-2 with lymphocytes was found, which could translate that SARS-CoV-2 is able to decimate lymphocytes through direct mechanisms. As highlighted in Figure 2, lymphocytes characteristically do not have an important expression of AGO4. Obtaining greater insight into the possible role of RNAi in SARS-CoV-2 infection would be important toward developing heat-based therapeutics in COVID-19 since thermotherapy is known to enhance antiviral RNAi in plants.

Plant cells exposed to higher temperatures have been observed to be more resistant to ssRNA viral infections, mainly through enhanced RNA silencing mechanisms [81,82]. In Apple stem grooving virus (ssRNA + virus)-infected plant *in vitro* cultures, thermotherapy was shown to up-regulate the expression of genes in the RNA silencing pathways, increase the production of vsiRNAs, and inhibit viral RNA accumulation [83]. Other proposed mechanisms for the antiviral activity of thermotherapy are inhibition of viral replication, viral RNA degradation and micro RNA (miRNA)-mediated regulation of key genes involved in resistance to viral infections [84].

## Thermotherapy in humans

Thermotherapy can be defined as variations in temperature distribution in a region of the body or whole body due to heat transfer, during a determined period, which causes a desired physiological effect and/or promotes pathogen or abnormal cell elimination without damaging the host. We refer to the effects of mild increases in temperature rather than decreases in temperature (i.e., cryotherapy). In this

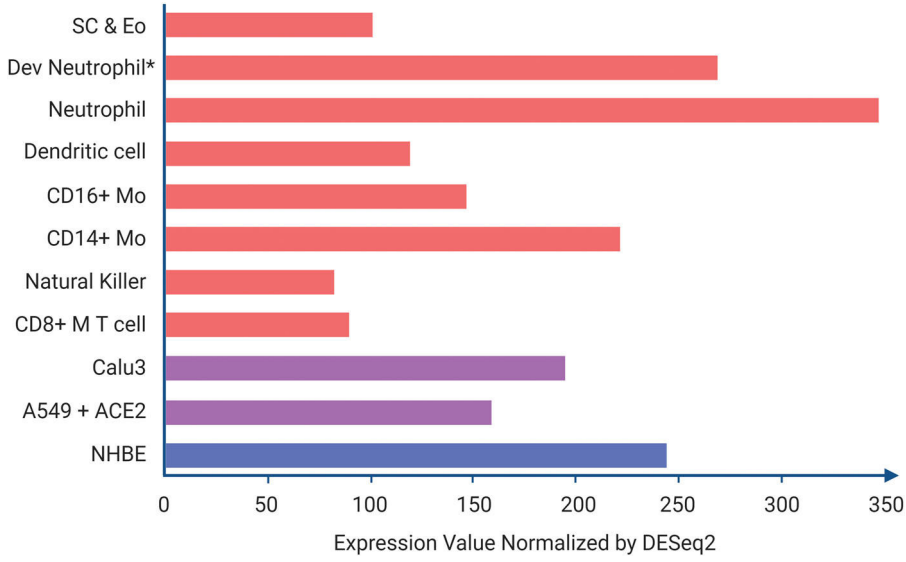


Figure 2. Expression of argonaute 4 (AGO4) in human airway epithelial cells infected with SARS-CoV-2, and peripheral blood cells of patients with COVID-19. Cells with at least moderate expression of AGO4 (defined as an expression value normalized by DESeq2 greater than or equal to 80) after *in vitro* infection of human bronchial epithelial cells (blue bar) and human airway epithelial cell lines (purple bars) with SARS-CoV-2 [22], and from peripheral blood cells (red bars) recovered from patients with COVID-19 [96]. Expression levels were obtained from immgen.org.
Abbreviations: alveolar basal lung adenocarcinoma-derived human airway epithelial cell line modified to express the angiotensin-converting enzyme 2 receptor (A549 + ACE2), lung adenocarcinoma-derived human airway epithelial cell line (Calu3), cluster of differentiation 8 positive memory T cell (CD8+ M T cell), cluster of differentiation 14 positive monocyte (CD14+ Mo), cluster of differentiation 16 positive monocyte (CD16+ Mo), developing neutrophil (Dev neutrophil), eosinophil (Eo), normal human bronchial epithelial cell (NHBE), stem cell (SC).
*Developing neutrophils are peripheral immune cells expressing neutrophil granule proteins (i.e., *ELANE*, *MPO*, *LTF*, *CTSG*, *LCN2*, and *MMP8*), but not canonical neutrophil markers (i.e., *FCGR3B* and *CXCR2*).

sense, thermotherapy could be used interchangeably with mild-temperature hyperthermia (39 to 42 °C); the choice to use the term thermotherapy rather than hyperthermia is for consistency with terminology used to treat viral diseases in plants and to highlight the importance of avoiding damage to cells, which is often the desired effect of hyperthermia in cancer therapy (≥43 °C).

Hyperthermia has been widely studied for its use in the treatment of proliferative lesions (i.e., cancer and other non-cancerous tumors), and the habitual desired heat doses – cumulative equivalent minutes at 43 °C ($CEM_{43}$) or thermal ablation (>45 °C) [85] – to kill these cells are usually high enough to cause significant damage to otherwise normal tissues [86]. Thermal effects favoring immune responses occur in the fever-range temperatures (38–41 °C) [87,88], whereas the stress response and enhanced immunogenicity of cancer cells occurs at heat-shock temperatures (41–43 °C). Increased cytotoxic effects occur at nonphysiological temperatures (≥43 °C) [89,90].

Local thermotherapy has been used to treat rheumatic diseases. While thermotherapy does not modify objective measures of disease activity in rheumatoid arthritis (joint swelling, pain, medication intake, range of motion, grip strength, and hand function), it is still recommended as an adjuvant measure for its safety profile (does not cause harm to patients) [91]. Cryotherapy in osteoarthritis has been mostly studied as a beneficial therapy modality for patients, whereas heat-based thermotherapy is not supported by the small clinical studies done; clinical studies of thermotherapy in osteoarthritis are scarce [92]. Local thermotherapy could be particularly useful for patients with viral arthritis since higher temperatures enhance type I IFN responses in the affected joint [93]; clinical studies in humans are needed to address this hypothesis. Local thermotherapy could have an important role in the treatment of viral diseases for its ability to increase type I and II IFN and IFN-dependent signaling pathways [94].

## Thermotherapy in COVID-19

The determinants of disease progression in COVID-19 have not been completely characterized yet. However, we have learned that individuals with increasing ages, comorbidities, male sex, deprivation (a correlate of poverty), and certain ethnic groups are at a higher risk of having a fatal outcome [95]. From a biological perspective, greater viral loads and differential host responses (lower type I and III IFN, and increased production of pro-inflammatory cytokines and chemokines) are the hallmarks of severe and critical COVID-19 [22,23,96].

According to clinical parameters, patients with COVID-19 can be classified as: asymptomatic or pre-symptomatic (no symptoms), mild (symptoms of the disease, may or may not have pneumonia, and no need of supplementary oxygen at evaluation), moderate (pneumonia, shortness of breath, risk factors for progression, and no need of supplementary oxygen at evaluation), severe (pneumonia which affects greater than 50% of the lungs, accelerated breathing, or other clinical evidence of requiring supplementary oxygen), and critical (acute respiratory distress syndrome [ARDS], acute lung injury (ALI), septic shock, or multiorgan failure) [97,98]. Not all patients with COVID-19 will progress through each of these stages, and clinical presentations and courses of individual patients are highly variable. A scheme of these stages as a continuum of disease progression by using the median days from symptom onset to the development of an outcome of interest is provided in Figure 3; this conceptualization could be useful to design interventional studies.

Treatments with an antiviral mechanism of action would have the most impact when administered early on the disease, while interventions to prevent complications (i.e., multiorgan failure and thromboembolic events) could still be effective in less early stages [99]. Thus, early interventions should be started prior to exposition to the virus (prophylactic o preventive), after known or potential exposition to the virus (pre-symptomatic period), or soon after symptom onset – preferably in patients with mild-to-moderate COVID-19 with less than 5 days from symptom onset – [100]. Most patients seek first medical attention at an early stage (~2 days after symptom onset), but patients are frequently sent home with a prescription of symptomatic treatment, with the utmost intention of preventing spread of the disease. The majority of clinical studies are being focused at studying potential interventions in hospitalized patients with severe-to-critical COVID-19, and we could be losing a great opportunity to deliver such interventions to the patients who might benefit the most.

The potential antiviral effects of thermotherapy are likely to be beneficial for patients during early stages of COVID-19 (mild-to-moderate disease). Furthermore, there are potential risks to administering heat-based interventions in patients with severe-to-critical COVID-19 since being admitted to hospital with temperatures lower than 36 °C or higher than 39.5 °C has been associated to higher mortality [101], strict control of core body temperature is especially important in critically ill patients [102], and fever-range hyperthermia has been found to increase lung injury and mortality in animal models of bacterial pneumonia [103]. Early timing of hyperthermia could be the key to balance the desired immune effects while avoiding potential risks [104]. Furthermore, moderate cycles (every 8 to 12 h) with short times (1–2 h) of effective mild hyperthermia could be sufficient to reach the desired effects on the immune system while also limiting potential adverse events since these have been hypothesized to be the optimal times to maximize the benefits of hyperthermia in patients with COVID-19 [105]. However, this hypothesis remains to be tested in clinical studies.

## Thermotherapy plus radiotherapy in COVID-19

As mentioned earlier, thermotherapy and radiotherapy could have synergistic effects to tackle SARS-CoV-2 infection since many of the mechanisms by which they achieve their therapeutic effects are similar or potentiate each other. Hyperthermia has largely been studied as an adjunct therapy for cancer alongside radiotherapy and chemotherapy

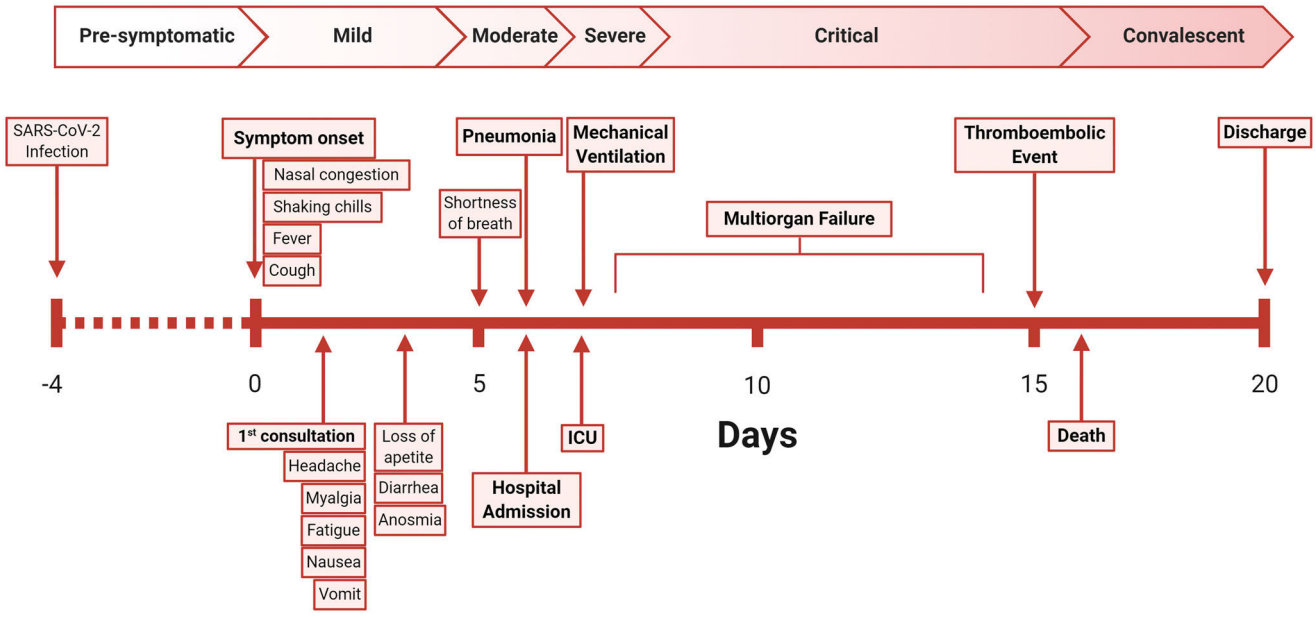


Figure 3. Events in the sequence of progression of patients with symptomatic COVID-19[a]. Median number of days (horizontal line) from symptom onset (day 0) to the first appearance of an event of interest (boxes) in the progression sequence of an average patient who progresses to critical COVID-19 with one of two outcomes: death or recovery. The median incubation period (time from infection to symptom onset, dashed line) is 4 days [112], which corresponds to the pre-symptomatic period (day -4 to 0, horizontal arrow). The hypothetical sequence of progression through each of the clinical stages with approximate median days to onset are represented as horizontal arrows with a red gradient: mild (onset: day 0), moderate (onset: day 5), severe (onset: day 6), critical disease, and the convalescent or recovery period (onset: day 16).
[a]Not all patients will progress or go through each of these clinical stages. The type of symptoms and days from onset at the time of medical evaluation are also highly variable as outlined by the wide interquartile ranges in descriptive clinical studies. Data of median days to individual symptom onset, pneumonia, first consultation, hospital admission, and discharge are from patients classified as having mild or severe COVID-19 [113]. Median days to mechanical ventilation, intensive care unit (ICU) admission, multiorgan failure, thromboembolic event, and death are from patients who progressed to critical COVID-19 [114].

[106,107]. Other authors have thoroughly reviewed the rationale for radiotherapy in COVID-19 [108,109]. Preliminary reports have found that low-dose radiotherapy is safe for patients with COVID-19 and could be a useful therapeutic modality [110,111]. The combination of mild hyperthermia with low-dose radiotherapy could be a potentially beneficial therapy for patients with COVID-19.

## Conclusions and perspectives

There is evidence that certain viruses that infect different species are susceptible to heat, and that infected hosts can enhance viral clearance through increases in temperature. This has been studied for respiratory viruses which typically infect humans, and there are reasons to think that SARS-CoV-2 is especially susceptible to heat; to what extent remains to be studied. Increasing the temperature in certain cells could aid viral clearance and possibly detain disease progression. However, this hypothesis has not been explored yet in preclinical or clinical studies. Addressing these issues could expand our knowledge of the mechanisms of disease in COVID-19 and could possibly allow us to develop heat-based therapeutics to prevent or treat viral diseases. If thermotherapy is found to be a successful intervention in viral respiratory diseases, we would not only have found a way to reduce the impact of the current COVID-19 pandemic, but we would have found an important tool which could in future outbreaks of emerging diseases.

Unfortunately, until the date of writing this manuscript there is only one published study comparing replication dynamics of SARS-CoV-2 at different physiological temperatures (33 °C and 37 °C). Thus, future studies could address whether SARS-CoV-2 replication and infectivity are effectively diminished at higher physiological and tolerable temperatures (i.e., incubation of infected cells at 33 °C and temperatures between 37 and 42 °C, with a 1 °C gradient), as well as specific processes being enhanced or inhibited at these temperatures in infected cells (gene expression, type I and III IFN production, pro-inflammatory cytokine production, RNA interference, viral protein synthesis, viral RNA replication). Time of exposition to heat and frequency of exposition to observe a significant effect would be important toward establishing an adequate temperature dose and frequency to be applied in animal models, and eventually, human patients. Even when answers to these questions in pre-clinical models could take long, it would be important to address them since thermotherapy could potentially be effective to treat diverse viral diseases, including possible future outbreaks of novel viruses.

In the absence of clear answers from preclinical studies which could take long, thermotherapy could still be attempted by using low-risk and low-cost devices. Heating pads are characterized by their safety and inexpensiveness. However, even when temperatures up to 43 °C can be reached at the contact interface between the skin and pad, heat penetration to the cells of interest could be low or negligible. Whether significant systemic effects could be achieved by increasing local temperature of skin cells and

vasculature should be investigated. Also, better characterizations of heat penetrance in tissues *in vivo* could be done through methods such as magnetic resonance spectroscopy (MRS) and imaging (MRI) to evaluate if significant changes in the temperature of deep organs is achievable through local thermotherapy.

Clinical studies using heat pads to deliver local thermotherapy could be attempted in patients with recent exposition to the virus or seeking medical attention early on the disease with mild symptoms. Such studies could evaluate disease progression as the main outcome. In the absence of clear preclinical studies to guide the optimal way of delivering thermotherapy, the established dose and frequency should balance greater exposition to heat with patient tolerability and attachment to the intervention. Steamers are other low-risk and low-cost devices which could also be studied under these considerations.

Thermotherapy in patients with severe COVID-19 could be studied only occur under strict clinical monitorization and after an institutional ethics and safety board evaluation. This also applies to any potential evaluation in patients with mild-to-moderate disease, notwithstanding lesser potential risks. However, hospitalization of these patients might not be possible in regions highly affected by the pandemic with high hospital occupancies. Clinical studies in the ambulatory setting to prevent disease progression could still be possible under these conditions and could provide evidence of greater clinical relevance and applicability. Lastly, thermotherapy should generally not be attempted in critically ill patients, although extracorporeal whole-body hyperthermia could eventually be carefully investigated as long as no other therapeutic alternatives exist por patients with critical COVID-19.

## Acknowledgments

J.M.G. would like to thank 'Dirección General de Calidad y Educación en Salud' for supporting his participation in 'Programa Nacional de Servicio Social en Investigación en Salud'. This work benefitted from data assembled by the ImmGen consortium. Figures were created with BioRender.com.

## Disclosure statement

No potential conflict of interest was reported by the author(s).

## Funding

This work was supported by Instituto Nacional de Perinatología 'Isidro Espinosa de los Reyes' under Grant INPer 2020-1-19 awarded to N.G.S.

## ORCID

Javier Mancilla-Galindo http://orcid.org/0000-0002-0718-467X
Norma Galindo-Sevilla http://orcid.org/0000-0002-6639-8475

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