Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
COVID-19 and hypertension: Is there a role for dsRNA and activation of Toll-like receptor 3?

Vanessa Dela Justina a,*, Fernanda R. Giachini a, b, Fernanda Priviero c, R. Clinton Webb c

a Graduate Program in Biological Sciences, Federal University of Goiás, Goiânia, Brazil
b Institute of Health Sciences and Health, Universidade Federal De Mato Grosso, Barra Do Garças, Brazil
c Cardiovascular Translational Research Center - School of Medicine, University of South Carolina, Columbia, SC, United States

ARTICLE INFO

Keywords:
COVID-19
dsRNA
TLR3
Hypertension

ABSTRACT

The virus responsible for the coronavirus disease of 2019 (COVID-19) is the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Evidences suggest that COVID-19 could trigger cardiovascular complications in apparently healthy patients. Coronaviruses are enveloped positive-strand RNA viruses acting as a pathogen-associated molecular pattern (PAMP)/ danger-associated molecular patterns (DAMP). Interestingly, Toll-like receptor (TLR) 3 recognize both PAMPs DAMPs and is activated by viral double-stranded RNA (dsRNA) leading to activation of TIR receptor domain-containing adaptor inducing IFN-β (TRIF) dependent pathway. New evidence has shown a link between virus dsRNA and increased BP. Hence, we hypothesize that COVID-19 infection may be over activating the TLR3 through dsRNA, evoking further damage to the patients, leading to vascular inflammation and increased blood pressure, favoring the development of several cardiovascular complications, including hypertension.

The link between COVID-19 and cardiovascular complications could be due to the interaction of SARS-CoV-2 with one or more crucial pathway(s) controlling the cardiovascular system [7]. Indeed, recent studies have shown the role played by the renin-angiotensin-aldosterone system (RAAS) in COVID-19, is integral for recognition of the SARS-CoV-2 by the virus binding to angiotensin converting enzyme 2 (ACE2) on the surface of epithelial cells [8]. Interestingly, both reduced or increased function of ACE2 can induce systemic and pulmonary hypertension, heart failure, myocardial infarction, and diabetic cardiovascular complications [9]. As a result, coronavirus entry into susceptible cells is a complex process that requires the concerted action of receptor-binding and proteolytic processing of the transmembrane spike (S) protein to promote virus-cell fusion [10]. The loss of ACE2, caused by binding SARS-CoV-2, may shift the system to an overall higher angiotensin II and lower angiotensin-(1-7) tone, promoting vasoconstriction, sodium retention, oxidative stress and inflammation, among other consequences [11].

In addition to this direct mechanism of SARS-CoV-2 in the RAAS, the coronavirus might be affecting the cardiovascular system through the excessive activation of the innate immune system. Coronavirus infections are enveloped positive-strand RNA viruses with the largest known RNA genomes (30–32 kb) that infect humans and a wide range of animals [12]. Double-stranded RNA (dsRNA), which is produced by most viruses

* Corresponding author at: Federal University of Goiás, Av. Esperança, s/n - Chácaras de Recreio Samambaia, Goiânia, GO 74690-900, Brazil.
E-mail address: vane_cessa@hotmail.com (V. Dela Justina).

https://doi.org/10.1016/j.vph.2021.106861
Received 25 January 2021; Received in revised form 15 March 2021; Accepted 6 April 2021
Available online 22 April 2021
1537-1891/© 2021 Elsevier Inc. All rights reserved.
at the replication stage, can act as a pathogen-associated molecular pattern (PAMP)/danger-associated molecular patterns (DAMP), leading to activation of several antiviral pathways critical for early defense against viral invasion, including toll-like receptor (TLR)3 [13]. The TLR3 downstream signaling includes the activation of dsRNA-dependent protein kinase (PKR), production of type I interferon (IFN), and RNA degradation [14].

Increased PKR activity by dsRNA sensing is reported to induce inflammation, oxidative stress, and apoptotic death, implicated in the pathogenesis of vascular diseases [15]. In fact, vascular smooth muscle cells (VSMCs) from the thoracic aorta of rats treated with high glucose, a well-known source for vascular dysfunction, showed increased PKR expression, accompanied by increased gene-level markers of inflammation, oxidative stress, and apoptosis. Incubation with selective PKR inhibitor, imoxin (C16), attenuated all these effects elicited by high glucose [16]. Moreover, increased PKR expression followed by vascular impairment and augmented inflammatory markers is observed in an experimental model of hypertension induced by L-NAME treatment [17]. These data suggested that PKR pathway activation by dsRNA through TLR3 [18] leads to vascular dysfunction and inflammation, possibly progressing to increased blood pressure and hypertension. Of importance, the components of the vascular wall are critical for regulating blood pressure and blood flow throughout the body. In this way, chronic inflammation in vessel structure is a pathological process that acts as a crucial contributor to the development of hypertension [19–21]. Investigations have reported that hypertension, diabetes, and cardiovascular diseases were the most prevalent comorbidities among patients with COVID-19, in which hypertension appeared consistently as the most prevalent risk factor [22–25].

Interestingly, several viral infections are associated with hypertension or increased blood pressure, including human herpesvirus 8, cytomegalovirus and human immunodeficiency virus 1 in primary pulmonary hypertension [26–29]. However, the mechanisms underlying viral infection and their contribution to hypertension are not fully understood, and dsRNA might be the link since TLR3 recognizes dsRNA produced by most viruses at the replication stage [13]. Likewise, this could also be one of the links between COVID-19 and its complications. As evidence, nasal instillation of Poly I:C, a synthetic dsRNA that activates TLR3, led to the development of the acute respiratory distress syndrome and a cytokine storm [30], both symptoms often observed in severe COVID-19 patients. Interestingly, TLR3-deficient mice (Tlr3−/−) did not develop increased blood pressure in response to angiotensin II infusion, suggesting that hypertension is dependent on activation of the TLR3-TRIF [31]. Another study reported that primary nasal epithelial cells infected by SARS-CoV-2 induced the expression of pluripotent stem cell (iPSC)-derived alveolar type 2 cells (iAT2) and iPSC-derived cardiomyocytes (iCM), which together, represent the host tissues likely

**Fig. 1.** The schematic hypothesis of the proposed mechanisms of TLR3 derived from SARS-CoV-2 leading to hypertension. SARS Cov-2 cellular entry and subsequent replication may trigger the immune system. dsRNA derived from SARS-CoV-2 nucleic acids trigs TLR3 leading to IRAK-dependent and -independent TLR3-mediated pathway, including PKR activation. Further, an inflammatory cascade is formed in response to this signaling which results in vascular damage, augmented blood pressure and hypertension. In this way, Sars-CoV-2 dsRNA may contribute to hypertension through the above pathway (dsRNA/TLR3/PKR/vascular inflammation/increased blood pressure). Key references: [13, 18, 39].

### Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| ACE2 | Angiotensin converting enzyme 2 |
| CMR | Cardiovascular magnetic resonance |
| COVID-19 | Coronavirus disease of 2019 |
| DAMP | Danger-associated molecular patterns |
| dsRNA | Double-stranded RNA |
| PKR | dsRNA-dependent protein kinase |
| iAT2 | Pluripotent stem cell (iPSC)-derived alveolar type 2 cells |
| IFN | Production of type I interferon |
| RAAS | Renin-angiotensin-aldosterone system |
| SARS-CoV-2 | Severe acute respiratory syndrome coronavirus 2 |
| TLR | Toll-like receptor |
| VSMCs | Vascular smooth muscle cells |

| Abbreviation | Description |
|--------------|-------------|
| ACE2 | Angiotensin converting enzyme 2 |
| CMR | Cardiovascular magnetic resonance |
| COVID-19 | Coronavirus disease of 2019 |
| DAMP | Danger-associated molecular patterns |
| dsRNA | Double-stranded RNA |
| PKR | dsRNA-dependent protein kinase |
| iAT2 | Pluripotent stem cell (iPSC)-derived alveolar type 2 cells |
| IFN | Production of type I interferon |
| RAAS | Renin-angiotensin-aldosterone system |
| SARS-CoV-2 | Severe acute respiratory syndrome coronavirus 2 |
| TLR | Toll-like receptor |
| VSMCs | Vascular smooth muscle cells |
affected by clinical SARS-CoV-2 infection, leading to PKR activation as well as viral dsRNA localized to perinuclear foci [32]. A case report with a 60-year-old man showed unusually rapid development of pulmonary hypertension and right ventricular failure after recent severe COVID-19 pneumonia with cytokine release syndrome [33]. Furthermore, pulmonary hypertension and right ventricular failure are common complications observed among patients aged under 55 years and displaying COVID-19 infections in a moderate pattern [34]. As COVID-19 infection is increasingly being associated with systemic and multi-organ involvement, encompassing cytokine release syndrome and thromboembolic, vascular and cardiac events, it could be a consequence of TLR3 activation, which is known to play a role in the development of preclampsia [35] and also to induce endothelial dysfunction and oxidative stress [36].

In this way, TLR3 activation through dsRNA increases blood pressure associated with several pathological conditions, as mentioned above. Due to the deleterious effects of this response on virus survival, coronaviruses seem to be adept at evading host antiviral pathways induced by viral dsRNA-induced pathways that are essential components of the host innate immune response [32,37]. Therefore, our hypothesis is that the influx of SARS-CoV-2 to the cells [38] may be over activating the TLR3-related pathway, evoking further damage to the patients, favoring the development of several cardiovascular complications, including hypertension (Fig. 1).

COVID-19 has been a challenging disease for the entire world. On a few occasions, humanity has addressed the same target efforts, aiming to reveal a relatively robust body of evidence in a minimal period. In the future, it will be incredibly challenging to investigate if, indeed, TLR3 may be a target for SARS-CoV-2 infection and which pathways are being activated during this phenomenon, unveiling whether COVID-19 compromises elements of the innate immune system to further damage the cardiovascular system.

Author statement
Vanessa Dela Justina and R. Clinton Webb designed the hypothesis; R. Clinton Webb revised the paper; Vanessa Dela Justina performed research; Vanessa Dela Justina, Fernanda Priviero and Fernanda Giachini wrote the paper.

Declaration of competing interest
The authors declare that they have no conflict of interest.

Acknowledgments
Financial support for this work was provided by the NIDDK Diabetic Complications Consortium (DK706169 and DK115255 to RCW); the National Institutes of Health (HL-134604 to RCW); Fundação de Amparo à Pesquisa do Estado de Mato Grosso (FAPMAT, 0324552/2018 to F.R.G.); Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, 306166/2019-4 to F.R.G); Coordenaçôâ'¬¬ G.); Conselho Nacional de Desenvolvimento Científico e Tecnologia do Estado de Mato Grosso (FAPEMAT, 0324552/2018 to F.R.G); the Complications Consortium (DK076169 and DK115255 to RCW); the Prevalence of comorbidities and its effects in coronavirus disease 2019 patients: a systematic review and meta-analysis, Int. J. Infect. Dis. 94 (2020) 91–294, https://doi.org/10.1016/j.ijid.2020.03.017.

References
[1] N. Zhu, D. Zhang, W. Wang, X. Li, B. Yang, J. Song, X. Zhao, B. Huang, W. Shi, R. Lu, P. Niu, F. Zhan, X. Ma, D. Wang, W. Xu, G. Wu, G.F. Gao, W. Tan, A novel coronavirus from patients with pneumonia in China, 2019. Engl. J. Med. 382 (2020) 727–733, https://doi.org/10.1056/nejmoa2001071.
[2] J. Yang, Y. Zheng, X. Gou, K. Pu, Z. Chen, Q. Guo, R. Ji, H. Wang, Y. Wang, Y. Zhou, Prevalence of comorbidities and its effects in coronavirus disease 2019 patients: a systematic review and meta-analysis, Int. J. Infect. Dis. 94 (2020) 91–95, https://doi.org/10.1016/j.ijid.2020.03.017.
[3] J. Zhang, Y. Wan, C. Luo, G. Ye, Q. Geng, A. Auerbach, F. Li, Cell entry mechanisms of SARS-CoV-2, Proc. Natl. Acad. Sci. U. S. A. 117 (2020) 1–8, https://doi.org/10.1073/pnas.200138117.

[23] W.J. Tu, J. Cao, L. Yu, X. Hu, Q. Liu, Clinicolaboratory study of 25 fatal cases of COVID-19 in Wuhan, Intensive Care Med. 46 (2020) 1117–1120, https://doi.org/10.1007/s00134-020-06023-4.

[24] W. Guan, W. Liang, Y. Zhao, H. Liang, Z. Chen, Y. Li, X. Liu, R. Chen, C. Tang, T. Wang, C. Ou, L. Li, L. Sang Ping-yan Chen, W. Wang, J. Li, C. Li, L. Ou, B. Cheng, N.Z. Shan, J. He, Comorbidity and its impact on 1590 patients with COVID-19 in China: a nationwide analysis, Eur Respir J. 55 (2020) 1–14, https://doi.org/10.1183/13993003.00547-2020.

[25] X. Li, S. Xu, M. Yu, K. Wang, Y. Tao, Y. Zhou, J. Shi, M. Zhou, B. Wu, Z. Yang, C. Zhang, J. Yue, Z. Zhang, H. Renz, X. Liu, J. Xie, M. Xie, Jiaping Zhao, Risk factors for severity and mortality in adult COVID-19 inpatients in Wuhan, J. Allergy Clin. Immunol. 146 (2020) 110–118.

[26] V.K. S., F. Nyberg, P. Paatero, A. Peters, M. Mittleman, J. Samet, S.S.C. Jr, Herpesvirus-associated pulmonary hypertension? Am. J. Respir. Crit. Care Med. 172 (2005) 1–2, https://doi.org/10.1164/rccm.2508005.

[27] E. Daley, C. Emson, C. Guignabert, R.D.W. Malefyt, J. Louten, V.P. Kurup, C. Hogaboam, L. Taraseviciene-stewart, N.F. Voelkel, M. Rabinovitch, E. Grunig, G. Grunig, Pulmonary arterial remodeling induced by a Th2 immune response, JEM 205 (2008) 361–372, https://doi.org/10.1084/jem.20071108.

[28] J. Cheng, Q. Ke, Z. Jin, H. Wang, O. Kocher, J.P. Morgan, C.S. Crumpacker, Cytomegalovirus infection causes an increase of arterial blood pressure, PLoS Pathog. 5 (2009) 1–14, https://doi.org/10.1371/journal.ppat.1000427.

[29] P.M. George, R. Badiger, D. Shao, M.R. Edwards, S.J. Wort, M.J. Paul-Clark, J. A. Mitchell, Viral Toll-like receptor activation of pulmonary vascular smooth muscle cells results in endothelin-1 generation: relevance to pathogenesis of pulmonary arterial hypertension, Biochem. Biophys. Res. Commun. 426 (2012) 486–491.

[30] H. Khodadadi, ´E.L. Salles, A. Jarrahi, F. Chibane, V. Costigliola, J.C. Yu, K. Vaibhav, D.C. Hess, K.M. Dhandapani, B. Baban, Cannabidiol modulates cytokine storm in acute respiratory distress syndrome induced by simulated viral infection using synthetic RNA, Cannabis Cannabinoid Res. 5 (2020) 197–201, https://doi.org/10.1089/can.2020.0043.

[31] X.M.V. Singh, M.Z. Cicha, S. Nunez, X.D.K. Meyerholz, M.W. Chapleau, F. M. Abboud, Angiotensin II-induced hypertension and cardiac hypertrophy are differentially mediated by TLR3- and TLR4-dependent pathways, Am J Physiol. Hear. Circ. Physiol. 316 (2019) H1027–H1038, https://doi.org/10.1152/ajpheart.00977.2018.

[32] Y. Li, D.M. Renner, C.E. Comar, J.N. Whelan, H.M. Reyes, F.L. Cardenas-Diaz, R. Truitt, L.H. Tan, B. Dong, K.D. Aysandratos, J. Huang, J.N. Palmer, N. D. Adappa, M.A. Kohanski, D.N. Kotton, R.H. Silverman, W. Yang, E. Morrissey, N. A. Cohen, S.R. Weiss, SARS-CoV-2 induces double-stranded RNA-mediated innate immune responses in respiratory epithelial derived cells and cardiomyocytes, BioRxiv Prepr. Serv. Biol. (2020), https://doi.org/10.1101/2020.09.24.312553.

[33] C.M. van Dongen, M.T. Janssen, R.P. van der Horns, D.J. van Kraaij, R.H. Peeters, L. M. van den Toorn, R.L. Mostard, Unusually rapid development of pulmonary hypertension and right ventricular failure after COVID-19 pneumonia, Eur. J. Case Reports Intern. Med. 7 (2020) 001784, https://doi.org/10.12890/2020_001784.

[34] C. Tudoran, M. Tudoran, V.E. Lazureanu, A.R. Marinescu, G.N. Pop, A.S. Pescaru, A. Enache, T.G. Cut, Evidence of pulmonary hypertension after SARS-CoV-2 infection in subjects without previous significant cardiovascular pathology, J. Clin. Med. 10 (2021) 199, https://doi.org/10.3390/jcm10020199.

[35] P. Chatterjee, V.L. Chiasson, G. Seerangan, R.P. Tobin, S.E. Kopriva, M.K. Newell-Rogers, B.M. Mitchell, Cotreatment with interleukin 4 and interleukin 10 modulates immune cells and prevents hypertension in pregnant mice, Am. J. Hypertens. 28 (2015) 135–142, https://doi.org/10.1093/ajh/hpu106.

[36] S. Zimmer, M. Steinmetz, T. Asdonk, I. Motz, C. Coch, E. Hartmann, W. Barchet, S. Wassmann, G. Hartmann, G. Nickenig, Activation of endothelial toll-like receptor 3 impairs endothelial function, Circ. Res. 108 (2011) 1358–1366, https://doi.org/10.1161/CIRCRESAHA.111.243246.

[37] J.L.G. Cruz, I. Sola, M. Becares, B. Alberca, J. Plana, L. Enjuanes, S. Zuñiga, Coronavirus gene 7 counteracts host defenses and modulates virus virulence, PLoS Pathog. 7 (2011), https://doi.org/10.1371/journal.ppat.1002090.

[38] N.A. Wong, M.H. Saier, The sars-coronavirus infection cycle: a survey of viral membrane proteins, their functional interactions and pathogenesis, Int. J. Mol. Sci. 22 (2021) 1–63, https://doi.org/10.3390/ijms22031308.

[39] A. Fara, Z. Mitrev, R.A. Rosalia, B.M. Assas, Cytokine storm and COVID-19: a chronicle of pro-inflammatory cytokines: cytokine storm: the elements of rage!, Open Biol. 10 (2020), https://doi.org/10.1098/rsob.200164.