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.
Human Leukocyte Transcriptional Response to SARS-CoV-2 Infection

Sandra Elisabete Vieira, Silvia Yumi Bando, Gerhard da Paz Lauterbach, Carlos Alberto Moreira-Filho.*

*Corresponding author. E-mail: carlos.moreira@hc.fm.usp.br

Vieira SE, Bando SY, Lauterbach GP, Moreira-Filho CA. Human Leukocyte Transcriptional Response to SARS-CoV-2 Infection. Clinics. 2020;75:e2078

It is necessary to elucidate why in the present coronavirus disease-19 (COVID-19) global pandemic infection by SARS-CoV-2 affects children, adults, and elderly people differently. Epidemiological and clinical data have shown that most children who test positive for SARS-CoV-2 are either asymptomatic or develop mild symptoms (1-3). Several hypotheses have been suggested to explain these findings: differences in infection of the immune system of adults and children (and between very young children, pre-schooling children, and adolescents); competition with other respiratory viruses in the respiratory mucosa and lungs influencing the viral load; and differences between adults and children in the expression of the ACE2 protein, the SARS-CoV-2 receptor (1). It was recently described that the hypomethylation and subsequent hyperexpression of the ACE2 gene increased susceptibility for developing COVID-19 (4).

In severe COVID-19 cases, the viral and pulmonary phases are followed by a final hyperinflammatory phase, which can lead to severe acute respiratory distress syndrome (ARDS), often with a fatal outcome. Here, it should be noted that children are quite susceptible to H1N1-related ARDS (5) and that the 2003 coronavirus SARS pandemic affected patients of all ages (6). Therefore, despite the relatively small number of reported COVID-19 cases in children and the scarce information on these cases, it is not possible to assume that all pediatric COVID-19 cases will follow a mild course (7).

The study of the differences between children and adults with COVID-19 regarding the immune response and disease course represents a unique opportunity for developing new therapies (8), which is demanded (9) to avoid the collapse of health systems now and in the immediate post-pandemic period (2022-2024), as shown by recent epidemiological studies (10). Consequently, we decided to investigate the genomic basis of those differences through a comparative study of the transcriptional responses of human leukocytes to SARS-CoV-2 infection in children and adults, also focusing on the differences between oligosymptomatic and severe cases, as further described in the following paragraphs.

Severe COVID-19 cases are characterized by a “cytokine storm” (hypercytokinemia) that promotes hyperinflammation and ARDS (11,12), which is not observed in oligosymptomatic cases (13). The inflammatory responses in adults and children vary with age, with a progressive increase in inflammatory cytokines and neutrophil activity, which correlates with the augmented severity of ARDS in elderly people. Even in pediatric septic shock, the vast majority of genes with altered expression profiles are in neutrophils (69%) and monocytes (28%), and just a small minority are in lymphocytes (14). Therefore, it is quite probable that in circulating leukocytes distinct transcriptional modules (see below) are associated with different responses to SARS-CoV-2 in COVID-19 patients, thus allowing us to delineate adult and child responses and, in these two groups, the oligosymptomatic and severe case subgroups. The functional analysis of these transcriptional modules will allow, as commented on below, a better understanding of the pathogenic mechanisms triggered by SARS-CoV-2 and eventually, the identification of new therapeutic targets.

The availability of platforms for large scale gene expression analysis—mainly DNA microarrays and next generation sequencing (NGS)—has made it possible for immune response studies to migrate from a reductionist approach to one of systems biology (15), enabling a global perception of the molecular, cellular, and tissue events involved in the different types of immune response (16). Studies at this new global transcriptome scale have permitted, for instance, a better understanding of the innate and adaptive immune responses, of the defense mechanisms against different pathogens, and the evaluation of the responses to vaccination (17-21).

An initial major hurdle in global transcriptome studies was how to analyze and interpret the enormously large gene expression datasets obtained through DNA microarrays or NGS platforms. The development of statistical and computational tools for the analysis of gene co-expression networks helped to overcome this limitation (22,23). These tools are presently used for associating genes and gene expression profiles with biological processes and for finding potential therapeutic targets (24-25). Clustering techniques have been employed to find genes with similar expression patterns in multiple samples, thus identifying modules (26,27).
Transcriptional modules often represent biological processes and can be phenotype specific (25). The functional enrichment among the genes within a module is widely used for disclosing its biological meaning (25). Moreover, it was found that in gene co-expression networks, the highly connected genes hold the whole transcriptional network together and are either associated with specific cellular processes or link different biological processes (23). Connectivity measures are currently used for the hierarchical categorization of genes in transcriptional modules highly correlated with at least one trait of interest (gender, age, disease features, etc.), helping to find genes that are highly significant for a certain trait or that link molecular pathways in a cell (25).

The development of mathematical and computational methods for analyzing modular transcriptional repertoires has been essential for unraveling the human immune defense mechanisms associated with good and bad responses to respiratory viruses (17,28-30). Our group, at the Department of Pediatrics, FMUSP, has tackled this approach for investigating the genomic mechanisms associated with the development, maturation, and decline of the immune system in health and disease (31-33). Recently, studying children under six months of age hospitalized with acute viral bronchiolitis, we were able to show that in peripheral blood mononuclear cells (PBMC) there are distinct transcriptional modules associated either with responses to syncytial respiratory virus (HRSV) or rhinovirus (HRV) (20). We also identified host-response molecular markers that could be used for etiopathogenic diagnosis. The finding of distinct transcriptional profiles associated with specific host responses to HRSS or HRV may contribute to unraveling the pathogenic mechanisms triggered by different respiratory viruses that are indistinguishable by clinical presentation, paving the way for new, specific therapeutic strategies.

The experimental approach first adopted for studying the PBMC response to HRSS and HRV is now being used in our laboratory to identify the transcriptional responses of human peripheral blood leukocytes to SARS-CoV-2 following respiratory tract infection. Relevant knowledge on this subject has been newly published. Transcriptome characteristics of the bronchoalveolar lavage fluid and peripheral PBMC of COVID-19 patients revealed distinct host inflammatory cytokine profiles and the association between COVID-19 pathogenesis and excessive cytokine release (34). Compared to other respiratory viruses, SARS-CoV-2 drives a lower antiviral transcriptional response—low IFN-I and IFN-III levels and elevated chemokine expression—in accordance with the pro-inflammatory disease state associated with COVID-19 (35). In a complementary line of work, COVID-19 patients were compared to recovered and healthy subjects through high dimensional cytometry, and the subsequent integration of immune and clinical data revealed different immunotypes related to poor clinical course versus improving health (36). Returning to our approach, it aims to identify distinctive transcriptional modules in the response of human leukocytes to SARS-CoV-2 infection in children and adults, and between oligosymptomatic and severe cases in both groups. The transcriptomic data thus obtained—distinctive transcriptional modules and their associated biological functions, highly connected and high significance genes, etc.—will be integrated with clinical and demographic data in order to gain a better understanding of the molecular mechanisms involved in the immune response to SARS-CoV-2 and, eventually, for identifying host-response predictors and potential therapeutic targets for drugs and vaccines.

ACKNOWLEDGMENTS
This work was funded by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) grants no. 2015/22308-2 and 2020/06160-3 (“Acordos de Cooperação Covid-19”), and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) grant no. 307626/2011-4.

AUTHOR CONTRIBUTIONS
All the authors contributed equally to this study and have read and approved the final manuscript.

REFERENCES
1. Buxton PW. Why is COVID-19 so mild in children? Acta Paediatr. 2020;109(6):1082-3. https://doi.org/10.1111/apa.15271
2. Dong Y, Mo X, Hu Y, Qi J, Jiang F, Jiang Z, et al. Epidemiology of COVID-19 Among Children in China. Pediatrics. 2020;145(6):e20200702. https://doi.org/10.1542/peds.2020-0702
3. Ludvigsson JF. Systematic review of COVID-19 in children shows milder cases and a better prognosis than adults. Acta Paediatr. 2020;109(6):1088-95. https://doi.org/10.1111/apa.15279
4. Sawalha AH, Zhao M, Corl F, Lu Q. Epigenetic dysregulation of ACE2 and interferon-regulated genes might suggest increased COVID-19 susceptibility and severity in lupus patients. Clin Immunol. 2020;215:108410. https://doi.org/10.1016/j.clim.2020.108410
5. Nye S, Whitley RJ, Kong M. Viral Infection in the Development and Progression of Pediatric Acute Respiratory Distress Syndrome. Front Pediatr. 2016;4:128. https://doi.org/10.3389/fped.2016.00128
6. Perissis JS, Yuen KY, Osterhaus AD, Störk K. The severe acute respiratory syndrome. N Engl J Med. 2003;349(25):2431-41. https://doi.org/10.1056/NEJMra032498
7. Shen K, Yang Y, Wang T, Zhao D, Jiang Y, Jin R, et al. Diagnosis, treatment, and prevention of 2019 novel coronavirus infection in children: experts’ consensus statement. World J Pediatr. 2020. https://doi.org/10.1007/s12519-020-00343-7
8. Yongker LM, Shen K, Kirane TB. Lessons unfolding from pediatric cases of COVID-19 disease caused by SARS-CoV-2 infection. Pediatr Pulmonol. 2020;55(5):1085-6. https://doi.org/10.1002/ppul.24748
9. Sanders JM, Monogue ML, Jodlowski TZ, Caturell JB. Pharmacologic Treatments for Coronavirus Disease 2019 (COVID-19): A Review. JAMA. 2020. https://doi.org/10.1001/jama.2020.6019
10. Kiosler SM, Tedijanto C, Goldstein E, Grad YH, Lipshitz M. Projecting the transmission dynamics of SARS-CoV-2 through the post-pandemic period. Science. 2020;368(6493):860-8. https://doi.org/10.1126/science.abb5793
11. Miller P, McAuley DF, Brown M, Sanchez E, Zattarriás RS, Mansor J, et al. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet. 2020;395(10229):1033-4. https://doi.org/10.1016/S0140-6736(20)30628-9
12. Ye Q, Wang B, Mao J. The pathogenesis and treatment of the ‘Cytokine Storm’ in COVID-19. J Infect. 2020;80(6):607-13. https://doi.org/10.1161/jinf.2020.03.037
13. Ruan Q, Yang K, Wang W, Jiang L, Song J. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. Intensive Care Med. 2020;46(5):846-8. https://doi.org/10.1007/s00134-020-05991-x
14. Wong HR, Ciyvanovich NZ, Allen GL, Thomas NJ, Freishtat RJ, Anas N, et al. Validation of a gene expression-based subclassification strategy for pediatric septic shock. Crit Care Med. 2011;39(11):2531-7. https://doi.org/10.1097/CCM.0b013e3182257675
15. Villani AC, Sarkozova S, Hacohen N. Systems Immunology: Learning the Rules of the Immune System. Annu Rev Immunol. 2018;36:813-42. https://doi.org/10.1146/annurev-immunol-042617-053035
16. Zeller B, SCIENII CHI. The role of the Innate Immune System. In: Kassab M, editor. 2010. doi:10.1016/j.cell.2018.06.045
17. Tikhonova EA, Egorova A, Pravdivets A, Oleszko R, Brion M, et al. Systematic immunology: learning the rules of the immune system. Immunity. 2011;34:863-74. https://doi.org/10.1016/j.immuni.2011.01.014
18. Bando SY, Iamasita P, Silva FN, Costa LDF, Abe CM, Bertonha FB, et al. Dynamic Gene Network Analysis of Caco-2 Cell Response to Shiga Toxin-Producing Escherichia coli-Associated Hemolytic-Uremic Syndrome. Microorganisms. 2019;7(11):195. https://doi.org/10.3390/microorganisms7110195
19. Parvandeh S, Poland GA, Kennedy RB, McKinney BA. Multi-Level Model to Predict Antibody Response to Influenza Vaccine Using Gene Expression Interaction Network Feature Selection. Microorganisms. 2019;7(3):79. https://doi.org/10.3390/microorganisms7030079

20. Vieira SE, Bando SY, de Paulis M, Oliveira DBL, Thomazelli LM, Durigon EL, et al. Distinct transcriptional modules in the peripheral blood mononuclear cells responsive to human respiratory syncytial virus or human rhinovirus in hospitalized infants with bronchiolitis. PLoS One. 2019;14(3):e0213501. https://doi.org/10.1371/journal.pone.0213501

21. Rinchai D, Altman MB, Konza O, Haessler S, Martina F, Toufiq M, et al. Identification of erythroid cell positive blood transcriptome phenotypes associated with severe respiratory syncytial virus infection. bioRxiv. https://doi.org/10.1101/527812

22. Zhu X, Gerstein M, Snyder M. Getting connected: analysis and principles of biological networks. Genes Dev. 2007;21(9):1010-24. https://doi.org/10.1101/gad.1528707

23. Barabási AL, Gulbahce N, Loscalzo J. Network medicine: a network-based approach to human disease. Nat Rev Genet. 2011;12(1):56-68. https://doi.org/10.1038/nrg2918

24. Moreira-Filho CA, Bando SY, Bertonha FB, Silva FN, Costa Lda F. Methods for gene coexpression network visualization and analysis. In: Passos GA, editor. Transcriptionomics in Health and Disease. Cham (ZG), Switzerland: Springer International Publishing AG. pp. 79-94(2014).

25. van Dam S, Võsa U, van der Graaf A, Franke L, de Magalhães JP. Gene coexpression analysis for functional classification and gene-disease predictions. Brief Bioinform. 2018;19(4):575-92. https://doi.org/10.1093/bib/bby139

26. Langfelder P, Horvath S. WGCNA: an R package for weighted correlation network analysis. BMC Bioinformatics. 2008;9:559. https://doi.org/10.1186/1471-2105-9-559

27. Moreira-Filho CA, Bando SY, Bertonha FB, Lamachita P, Silva FN, Costa Lda F, et al. Community structure analysis of transcriptional networks reveals distinct molecular pathways for early- and late-onset temporal lobe epilepsy with childhood febrile seizures. PLoS One. 2015;10(5):e0128174. https://doi.org/10.1371/journal.pone.0128174

28. Chaussabel D, Baldwin N. Democratizing systems immunology with modular transcriptional repertoire analyses. Nat Rev Immunol. 2014;14(4):271-80. https://doi.org/10.1038/nri3642

29. de Steenhuijzen Piters WA, Heinonen S, Hasrat R, Bunsow E, Smith B, Suarez-Arrabal MC, et al. Nasopharyngeal Microbiota, Host Transcriptome, and Disease Severity in Children with Respiratory Syncytial Virus Infection. Am J Respir Crit Care Med. 2016;194(9):1104-15. https://doi.org/10.1164/ajcc.201602-0220OC

30. Bougarn S, Boughorbel S, Chaussabel D, Marr N. A curated transcriptome dataset collection to investigate the blood transcriptional response to viral respiratory tract infection and vaccination. F1000Res. 2019;8:284. https://doi.org/10.12688/f1000research.18533.1

31. Moreira-Filho CA, Bando SY, Bertonha FB, Silva FN, Costa Lda F, Ferreira LR, et al. Modular transcriptional repertoire and MicroRNA target analyses characterize genomic dysregulation in the thymus of Down syndrome infants. Oncotarget. 2016;7(7):7497-533. https://doi.org/10.18632/oncotarget.7120

32. Moreira-Filho CA, Bando SY, Bertonha FB, Ferreira LR, Vinhas CF, Oliveira LHB, et al. Minipuberty and Sexual Dimorphism in the Infant Human Thymus. Sci Rep. 2016;6(1):3169. https://doi.org/10.1038/srep31693

33. Bertonha FB, Bando SY, Ferreira LR, Chaccur P, Vinhas C, Zerbini MCN, et al. Age-related transcriptional modules and TF-miRNA-mRNA interactions in neonatal and infant human thymus. PLoS One. 2020;15(4):e0227547. https://doi.org/10.1371/journal.pone.0227547

34. Xiong Y, Liu Y, Cao L, Wang D, Guo M, Jiang A, et al. Transcriptomic characteristics of bronchoalveolar lavage fluid and peripheral blood mononuclear cells in COVID-19 patients. Emerg Microbes Infect. 2020;9(1):761-70. https://doi.org/10.1080/22221751.2020.1747363

35. Blanco-Melo D, Nilsson-Payant BE, Liu WC, Uhl S, Hoagland D, Moller R, et al. Imbalanced Host Response to SARS-CoV-2 Drives Development of COVID-19. Cell. 2020;181(5):1036-1045.e9. https://doi.org/10.1016/j.cell.2020.04.026

36. Mathew D, Giles JR, Baxter AE, Greenplate AR, Wu JE, Alanio C, et al. Deep immune profiling of COVID-19 patients reveals patient heterogeneity and distinct immunotypes with implications for therapeutic interventions. bioRxiv. 2020. https://doi.org/10.1101/2020.05.20.106401