

The Daily COVID-19 Literature Surveillance Summary

December 11, 2020



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COVID-19 Daily Literature Surveillance

COVID19LST



Bringing you real time, distilled information for guiding best practices during the COVID-19 pandemic

LEVEL OF EVIDENCE

Oxford Centre for Evidence-Based Medicine 2011 Levels of Evidence

Question	Step 1 (Level 1*)	Step 2 (Level 2*)	Step 3 (Level 3*)	Step 4 (Level 4*)	Step 5 (Level 5)
How common is the problem?	Local and current random sample surveys (or censuses)	Systematic review of surveys that allow matching to local circumstances**	Local non-random sample**	Case-series**	n/a
Is this diagnostic or monitoring test accurate? (Diagnosis)	Systematic review of cross sectional studies with consistently applied reference standard and blinding	Individual cross sectional studies with consistently applied reference standard and blinding	Non-consecutive studies, or studies without consistently applied reference standards**	Case-control studies, or "poor or non-independent reference standard"**	Mechanism-based reasoning
What will happen if we do not add a therapy? (Prognosis)	Systematic review of inception cohort studies	Inception cohort studies	Cohort study or control arm of randomized trial*	Case-series or case-control studies, or poor quality prognostic cohort study**	n/a
Does this intervention help? (Treatment Benefits)	Systematic review of randomized trials or n-of-1 trials	Randomized trial or observational study with dramatic effect	Non-randomized controlled cohort/follow-up study**	Case-series, case-control studies, or historically controlled studies**	Mechanism-based reasoning
What are the COMMON harms? (Treatment Harms)	Systematic review of randomized trials, systematic review of nested case-control studies, n-of-1 trial with the patient you are raising the question about, or observational study with dramatic effect	Individual randomized trial or (exceptionally) observational study with dramatic effect	Non-randomized controlled cohort/follow-up study (post-marketing surveillance) provided there are sufficient numbers to rule out a common harm. (For long-term harms the duration of follow-up must be sufficient.)*	Case-series, case-control, or historically controlled studies**	Mechanism-based reasoning
What are the RARE harms? (Treatment Harms)	Systematic review of randomized trials or n-of-1 trial	Randomized trial or (exceptionally) observational study with dramatic effect			
Is this (early detection) test worthwhile? (Screening)	Systematic review of randomized trials	Randomized trial	Non-randomized controlled cohort/follow-up study**	Case-series, case-control, or historically controlled studies**	Mechanism-based reasoning

* Level may be graded down on the basis of study quality, imprecision, indirectness (study PICO does not match questions PICO), because of inconsistency between studies, or because the absolute effect size is very small; Level may be graded up if there is a large or very large effect size.

** As always, a systematic review is generally better than an individual study.

How to cite the Levels of Evidence Table

OCEBM Levels of Evidence Working Group*. "The Oxford 2011 Levels of Evidence". Oxford Centre for Evidence-Based Medicine. <http://www.cebm.net/index.aspx?o=5653>

* OCEBM Table of Evidence Working Group = Jeremy Howick, Iain Chalmers (James Lind Library), Paul Glasziou, Trish Greenhalgh, Carl Heneghan, Alessandro Liberati, Ivan Moschetti, Bob Phillips, Hazel Thornton, Olive Goddard and Mary Hodgkinson

EXECUTIVE SUMMARY

Climate

- The editorial team at JAMA [explains their decision to publish the Lenze et al study](#) "Fluvoxamine vs placebo and clinical deterioration in outpatients with symptomatic COVID-19: a randomized clinical trial." While it is a preliminary study with limitations, authors argue it warranted publication due to its design as a placebo-controlled, double-blind randomized clinical trial and the authors' methodological adjustments to conduct study activities without physical contact, such as arranging oxygen and medications deliveries for self-quarantining patients and obtaining consent virtually. The team suggests that the Lenze et al pilot study provides an excellent example of pandemic-appropriate research methods and lays the groundwork for research into preventing outpatients with COVID-19 from deteriorating into a more severe disease status.

Epidemiology

- A retrospective cohort study that analyzed pediatric electronic health record data from PEDSnet (n=135,794 patients <25 years) found [4% of patients were infected with SARS-CoV-2 \(n=5,374\), while Kawasaki Disease prevalence decreased in 2020](#) compared to 2018 or 2019*. Risk factors for SARS-CoV-2 infection included preexisting chronic disease and increased age. Additionally, subjects of Hispanic, Black, or Asian race/ethnicity had a higher likelihood of positive test result compared to White race/ethnicity, though they received fewer tests per population. These results provide large-scale data on pediatric risk factors, which authors hope will add to the growing knowledge on SARS-CoV-2 and host biology in pediatric populations.
- Leaders in the United Kingdom's Department of Health conducted a systematic review and narrative synthesis of literature on SARS-CoV-2 infection in children published through March 9, 2020 (24 articles) and found that children under age 10 had similar infection rates to adults, but the observed rate of infection in children was perceived as lower since [children often had undetected milder infections](#), and transmission usually occurred within familial clusters when children were exposed to an infected adult. Authors suggest further data collection is necessary to understand child to child transmission of SARS-CoV-2 and to detail the clinical course of COVID-19 in children.

Understanding the Pathology

- A cohort-control study by pediatric infectious disease specialist in Ankara, Turkey evaluated cytokine and chemokine levels from serum samples of 60 COVID-19 positive patients (30 pediatric, 30 adult) and found that [COVID-19 patients had higher levels of IP10 and MIP-3B](#) compared to 30 healthy controls (15 pediatric, 15 adult; p<0.001), while IP-10 was a predictor for disease severity in children and IL-6 a predictor for disease severity in adults. They suggest these findings provide insight into potential treatment targets for COVID-19 and further elucidate potential uses for inflammatory markers as prognostic indicators for pediatric and adult COVID-19 severity.

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CLIMATE

AFFECTING THE HEALTHCARE WORKFORCE

COVID-19 INFECTION-PREVENTING CLINICAL DETERIORATION

Seymour CW, Bauchner H, Golub RM.. JAMA. 2020 Dec 8;324(22):2300. doi: 10.1001/jama.2020.21720.

Level of Evidence: 5 - Expert Opinion

BLUF

Members of the editorial team at JAMA explained their decision to publish the Lenze et al study "Fluvoxamine vs placebo and clinical deterioration in outpatients with symptomatic COVID-19: a randomized clinical trial." While it is a preliminary study with limitations, authors argue it warranted publication due to its design as a placebo-controlled, double-blind randomized clinical trial and the authors' methodological adjustments to conduct study activities without physical contact, such as arranging oxygen and medications deliveries for self-quarantining patients and obtaining consent virtually. The team suggests that the Lenze et al pilot study provides an excellent example of pandemic-appropriate research methods and lays the groundwork for research into preventing outpatients with COVID-19 from deteriorating into a more severe disease status.

EPIDEMIOLOGY

SYMPTOMS AND CLINICAL PRESENTATION

PEDIATRICS

ASSESSMENT OF 135 794 PEDIATRIC PATIENTS TESTED FOR SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2 ACROSS THE UNITED STATES

Bailey LC, Razzaghi H, Burrows EK, Bunnell HT, Camacho PEF, Christakis DA, Eckrich D, Kitzmiller M, Lin SM, Magnusen BC, Newland J, Pajor NM, Ranade D, Rao S, Sofela O, Zahner J, Bruno C, Forrest CB.. JAMA Pediatr. 2020 Nov 23. doi: 10.1001/jamapediatrics.2020.5052. Online ahead of print.

Level of Evidence: 3 - Local non-random sample

BLUF

A retrospective cohort study which analyzed pediatric electronic health record data from PEDSnet (n=135,794 patients <25 years) from January 1 to September 8, 2020 and found 4% of patients were infected with SARS-CoV-2 (n=5,374; Table 1), while Kawasaki Disease prevalence decreased in 2020 compared to 2018 or 2019*. Risk factors for SARS-CoV-2 infection included preexisting chronic disease (Figure 1) and increased age. Additionally, subjects of Hispanic, Black, or Asian race/ethnicity had a higher likelihood of positive test result compared to White race/ethnicity, though they received fewer tests per population (Table 3). These results provide large-scale data on pediatric risk factors, which authors hope will add to the growing knowledge on SARS-CoV-2 and host biology in pediatric populations.

SUMMARY

* There was a decrease in Kawasaki Disease (KD) prevalence in 2020 compared to 2018 or 2019, potentially due to lower overall health care utilization during the pandemic or lessening prevalence of other pathogens due to preventative measures for SARS-CoV-2. However, KD is not an effective proxy for multisystem inflammatory syndrome of childhood.

ABSTRACT

Importance: There is limited information on severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) testing and infection among pediatric patients across the United States. **Objective:** To describe testing for SARS-CoV-2 and the epidemiology of infected patients. **Design, Setting, and Participants:** A retrospective cohort study was conducted using electronic health record data from 135 794 patients younger than 25 years who were tested for SARS-CoV-2 from January 1 through September 8, 2020. Data were from PEDSnet, a network of 7 US pediatric health systems, comprising 6.5 million patients primarily from 11 states. Data analysis was performed from September 8 to 24, 2020. **Exposure:** Testing for SARS-CoV-2. **Main Outcomes and Measures:** SARS-CoV-2 infection and coronavirus disease 2019 (COVID-19) illness. **Results:** A total of 135 794 pediatric patients (53% male; mean [SD] age, 8.8 [6.7] years; 3% Asian patients, 15% Black patients, 11% Hispanic patients, and 59% White patients; 290 per 10 000 population [range, 155-395 per 10 000 population across health systems]) were tested for SARS-CoV-2, and 5374 (4%) were infected with the virus (12 per 10 000 population [range, 7-16 per 10 000 population]). Compared with White patients, those of Black, Hispanic, and Asian race/ethnicity had lower rates of testing (Black: odds ratio [OR], 0.70 [95% CI, 0.68-0.72]; Hispanic: OR, 0.65 [95% CI, 0.63-0.67]; Asian: OR, 0.60 [95% CI, 0.57-0.63]); however, they were significantly more likely to have positive test results (Black: OR, 2.66 [95% CI, 2.43-2.90]; Hispanic: OR, 3.75 [95% CI, 3.39-4.15]; Asian: OR, 2.04 [95% CI, 1.69-2.48]). Older age (5-11 years: OR, 1.25 [95% CI, 1.13-1.38]; 12-17 years: OR, 1.92 [95% CI, 1.73-2.12]; 18-24 years: OR, 3.51 [95% CI, 3.11-3.97]), public payer (OR, 1.43 [95% CI, 1.31-1.57]), outpatient testing (OR, 2.13 [1.86-2.44]), and emergency department testing (OR, 3.16 [95% CI, 2.72-3.67]) were also associated with increased risk of infection. In univariate analyses, nonmalignant chronic disease was associated with lower likelihood of testing, and preexisting respiratory conditions were associated with lower risk of positive test results (standardized ratio [SR], 0.78 [95% CI, 0.73-0.84]). However, several other diagnosis groups were associated with a higher risk of positive test results: malignant disorders (SR, 1.54 [95% CI, 1.19-1.93]), cardiac disorders (SR, 1.18 [95% CI, 1.05-1.32]), endocrinologic disorders (SR, 1.52 [95% CI, 1.31-1.75]), gastrointestinal disorders (SR, 2.00 [95% CI, 1.04-1.38]), genetic disorders (SR, 1.19 [95% CI, 1.00-1.40]), hematologic disorders (SR, 1.26 [95% CI, 1.06-1.47]), musculoskeletal disorders (SR, 1.18 [95% CI, 1.07-1.30]), mental health disorders (SR, 1.20 [95% CI, 1.10-1.30]), and metabolic disorders (SR, 1.42 [95% CI, 1.24-1.61]). Among the 5374 patients with positive test results, 359 (7%) were hospitalized for respiratory, hypotensive, or COVID-19-specific illness. Of these, 99 (28%) required intensive care unit services, and 33 (9%) required mechanical ventilation. The case fatality rate was 0.2% (8 of 5374). The number of patients with a diagnosis of Kawasaki

disease in early 2020 was 40% lower (259 vs 433 and 430) than in 2018 or 2019. Conclusions and Relevance: In this large cohort study of US pediatric patients, SARS-CoV-2 infection rates were low, and clinical manifestations were typically mild. Black, Hispanic, and Asian race/ethnicity; adolescence and young adulthood; and nonrespiratory chronic medical conditions were associated with identified infection. Kawasaki disease diagnosis is not an effective proxy for multisystem inflammatory syndrome of childhood.

FIGURES

Table 1. SARS-CoV-2 Testing Patterns by Health System

Characteristic	Overall	Health system ^a							
		A	B	C ^b	D	E	F	G	H
Recent patients, No. ^c	2 425 942	225 762	537 652	198 332	331 408	351 973	311 441	197 848	271 526
Patients tested, No. (%)									
Recurring ^d	111 785 (82)	9872 (93)	25 513 (93)	8657 (61)	22 920 (82)	10 594 (86)	15 684 (82)	11 929 (71)	6616 (95)
Nonrecurring	24 009 (18)	798 (7)	1961 (7)	5555 (39)	5043 (18)	1783 (14)	3539 (18)	4982 (29)	348 (5)
Test result, No. (%)									
Positive	5374 (4)	425 (4)	1152 (4)	952 (7)	1046 (4)	751 (6)	503 (3)	250 (1)	295 (4)
Negative	130 420 (96)	10 245 (96)	26 322 (96)	13 260 (93)	26 917 (96)	11 626 (94)	18 720 (97)	16 661 (99)	6669 (96)
No. tested per 10 000 recent patients	338	314	375	239	555	235	341	426	161
No. of cases of SARS-CoV-2 infection per 10 000 recent patients	13	13	16	11	17	15	10	6	8

Abbreviation: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

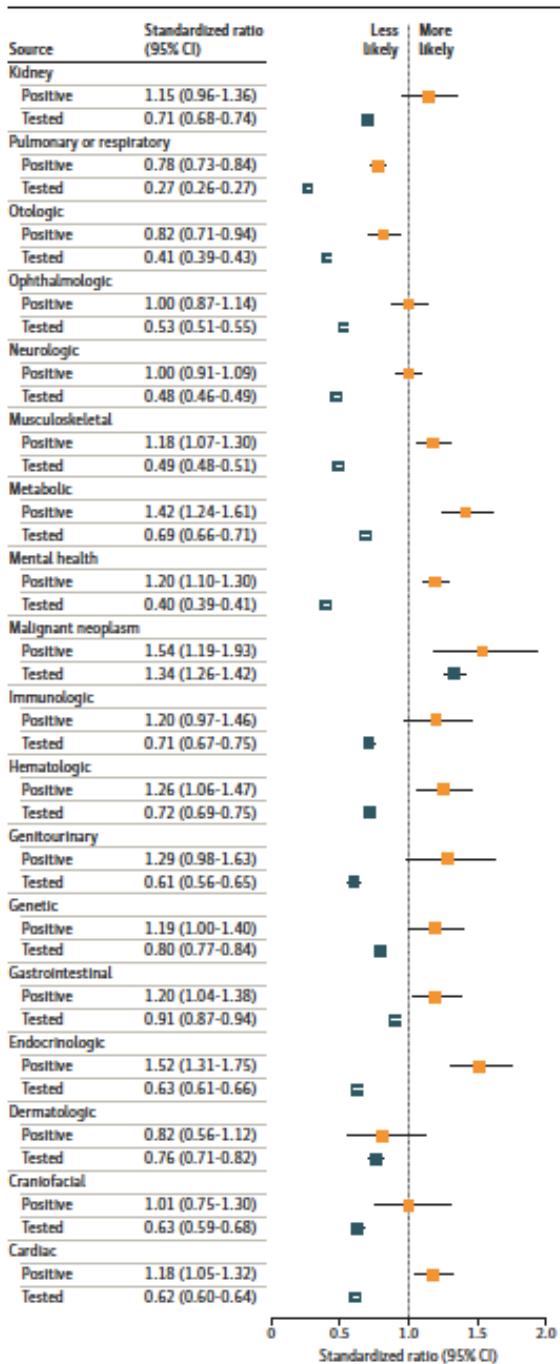
^a The Delaware and Florida sites in the Nemours Children's Health System are represented separately.

^b Patients 18 years or older were removed owing to the presence in this health system's data of test information from adults seen at affiliated institutions.

^c Patients younger than 25 years with at least 1 physician visit with a recorded diagnosis from July 2018 to December 2019.

^d Patients with at least 2 in-person visits in the 3 years prior to testing, or to March 1, 2020, if not tested.

Figure 1. Standardized Ratios for Chronic Conditions Among Pediatric Patients With Severe Coronavirus Disease 2019 Illness



Ratios were the quotient of observed number of patients with at least 1 condition in body system category and expected number. Expected values were obtained by computing for each chronic condition category the proportion of patients seen from March 1 to May 15 in 2018 and 2019 and having an inclusion diagnosis, and then multiplying these proportions by the total number of patients in the 2020 cohort (testing outcome) or undergoing testing (positive result outcome). A vertical line is placed at 1.0 for reference.

Table 3. Logistic Regression of SARS-CoV-2 Test Use and Positivity for Recurring Patients

Characteristic ^a	Adjusted odds ratio (95% CI)	
	Test performed ^b (n = 218 537)	Positive test result (n = 102 919)
Age, y		
<1	1.54 (1.49-1.59)	1.17 (1.03-1.33)
1-4	1 [Reference]	1 [Reference]
5-11	1.08 (1.05-1.11)	1.25 (1.13-1.38)
12-17	2.20 (2.13-2.26)	1.92 (1.73-2.12)
18-24	3.40 (3.24-3.57)	3.51 (3.11-3.97)
Sex		
Male	1.13(1.11-1.16)	0.97 (0.91-1.04)
Female	1 [Reference]	1 [Reference]
Race/ethnicity		
Hispanic	0.65 (0.63-0.67)	3.75 (3.39-4.15)
Asian or Pacific Islander	0.60 (0.57-0.63)	2.04 (1.69-2.48)
Black or African American	0.70 (0.68-0.72)	2.66 (2.43-2.90)
White	1 [Reference]	1 [Reference]
Payer ^c		
Commercial	1 [Reference]	1 [Reference]
Public	0.95 (0.93-0.97)	1.43 (1.31-1.57)
Testing location	Not applicable	
Outpatient	Not applicable	2.13 (1.86-2.44)
Emergency department	Not applicable	3.16 (2.72-3.67)
Inpatient	Not applicable	1 [Reference]

Abbreviation: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

^a Regression models also adjusted for health system and chronic condition body systems as fixed effects. They also included an "other" category for observations with missing data for race/ethnicity, payer, and testing location. The 95% CIs were generated using the profile likelihood method for generalized linear models.

^b These analyses excluded data from 1 PEDSnet health system because information about recent patients was not available.

SARS-COV-2 (COVID-19): WHAT DO WE KNOW ABOUT CHILDREN? A SYSTEMATIC REVIEW

Mehta NS, Mytton OT, Mullins EWS, Fowler TA, Falconer CL, Murphy OB, Langenberg C, Jayatunga WJP, Eddy DH, Nguyen-Van-Tam JS.. Clin Infect Dis. 2020 Dec 3;71(9):2469-2479. doi: 10.1093/cid/ciaa556.

Level of Evidence: 5 - Review / Literature Review

BLUF

Leaders in the United Kingdom's Department of Health conducted a systematic review and narrative synthesis of literature on SARS-CoV-2 infection in children published through March 9, 2020 (Figure 1), ultimately including 24 articles. Most reports were from China and showed children under age 10 had similar infection rates to adults, but the observed rate of infection in children was perceived as lower since children often had undetected milder infections (Table 1). Transmission usually occurred within familial clusters when children were exposed to an infected adult. Authors suggest further data collection is necessary to understand child to child transmission of SARS-CoV-2 and to detail the clinical course of COVID-19 in children.

ABSTRACT

BACKGROUND: Few paediatric cases of COVID-19 have been reported and we know little about the epidemiology in children, though more is known about other coronaviruses. We aimed to understand the infection rate, clinical presentation, clinical outcomes and transmission dynamics for SARS-CoV-2, in order to inform clinical and public health measures. **METHODS:** We undertook a rapid systematic review and narrative synthesis of all literature relating to SARS-CoV-2 in paediatric populations. The search terms also included SARS-CoV and MERS-CoV. We searched three databases and the COVID-19 resource centres of eleven major journals and publishers. English abstracts of Chinese language papers were included. Data were extracted and narrative syntheses conducted. **RESULTS:** 24 studies relating to COVID-19 were included in the review. Children appear to be less affected by COVID-19 than adults by observed rate of cases in large epidemiological studies. Limited data on attack rate

indicate that children are just as susceptible to infection. Data on clinical outcomes are scarce but include several reports of asymptomatic infection and a milder course of disease in young children, though radiological abnormalities are noted. Severe cases are not reported in detail and there are little data relating to transmission. CONCLUSIONS: Children appear to have a low observed case rate of COVID-19 but may have similar rates to adults of infection with SARS-CoV-2. This discrepancy may be because children are asymptomatic or too mildly infected to draw medical attention, be tested and counted in observed cases of COVID-19.

FIGURES

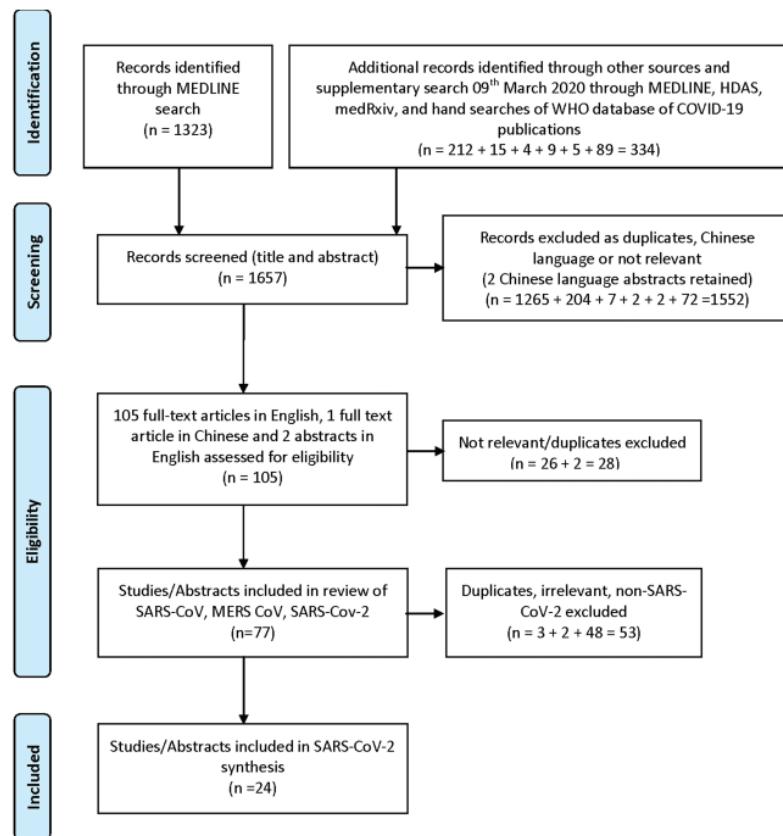


Figure 1. PRISMA 2009 flow diagram [13]. Abbreviations: COVID-19, coronavirus disease 2019; HDAS, Healthcare Databases Advanced Search; MERS-CoV, Middle East respiratory syndrome coronavirus; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; SARS-CoV, severe acute respiratory syndrome coronavirus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; WHO, World Health Organization.

Table 1. Summary of Reviewed Papers

Date/Title of Paper, Authors	Study Type and Synopsis	What Does the Paper Tell Us?
31 January 2020 Diagnosis, treatment, and prevention of 2019 novel coronavirus infection in children: experts' consensus statement, Shen E et al	Expert consensus statement. Review reported (World Journal of Pediatrics). Development of expert consensus statement on the diagnosis, treatment and prevention of COVID-19 infection in children.	Evidence of infection: Since the outbreak of 2019 novel coronavirus infection (COVID-19) in Wuhan City, China, by 30 January 2020, a total of 6062 cases and 136 deaths were reported. Of these, 2883 cases had been cured and 238 deaths had been reported. They have no fever or symptoms of pneumonia with a mild clinical presentation. Clinical presentation: Most identified infected children have had mild clinical presentations. They have no fever or symptoms of pneumonia with a mild clinical presentation. Some children have had "closed" asymptomatic. This paper groups "latent infection" as those individuals who test positive for coronavirus but have no symptoms.
2 [Date unpecified] February 2020; Impact assessment of non-pharmaceutical interventions against COVID-19 using influenza transmission as proxy in Hong Kong. February 2020 an observational study; Cowling BJ et al	Observational modeling study. Non-peer reviewed (arXiv:2002.02020). Assess the impact of non-pharmaceutical interventions on control measures and changes in population behaviors in Hong Kong in late January 2020 as a proxy for COVID-19.	Likelihood of infection: No direct evidence of infection in children. Clinical presentation: Original clinical outcomes reported.
2 February 2020 (Epub ahead of print) on:cn2020/2020). Diagnosis and treatment recommendations for pediatric respiratory infection caused by the 2019 novel coronavirus. Chen Z et al	Consensus guidelines. Review reported (Journal of Pediatrics). Expert consensus guidelines developed to standardize the practice for respiratory infection in children caused by COVID-19.	Evidence of infection: Between December 1st-January 29th 2020, 100 cases of COVID-19 have been reported in China including 10 children. Province: Jiangxi.
5 February 2020. Diagnosis and treatment of 2019 novel coronavirus infection in children: a pressing issue. Shen KC, Ying YH	Opinion piece. Peer reviewed (World Journal of Pediatrics). Author(s) on initial 26 pediatric cases reported.	Clinical presentation: At the onset of the disease, infected children may present with fever, dry cough, runny nose, expectoration, diarrhea and headache. Most children have fever or no fever. Most have good prognosis and no illness recover 1-2 weeks after onset. Some children have severe complications. These symptoms can occur in the condition progress, usually after 1 week of the onset. Accompanying systemic toxic symptoms such as malaise, rashes, headache, conjunctival injection, etc.
7 February 2020. A contingency plan for the management of the 2019 novel coronavirus outbreak in non-intensive care units, Wang J et al	Opinion piece. Peer reviewed (Lancet Child and Adolescent Health). Expert consensus of a contingency plan for the COVID-19 outbreak in non-intensive care units focused on diagnostic and discharge criteria, treatment, prevention, and control strategies.	Transmission: No data reported on transmission. Children mainly belong to the community.
14 February 2020. Clinical and CT features in pediatric patients with COVID-19 infection: different patterns from adults. Xie W et al	Case series. Peer reviewed (Pediatric Pulmonology).	Evidence of infection: From January 2020, the number of confirmed cases exceeded 20,000. About 100 children were affected, with the youngest being 10 years old.
15 26 February 2020 Report of the WHO-China Joint Mission on Coronavirus Disease 2019 (COVID-19) - 26 February 2020 WHO	Consensus report. Unclear whether peer reviewed.	Clinical presentation: Most adults or children presented with cold/flu symptoms. Disease severity: Concerned about neurons might be more susceptible than the lung to immune system attacks. Admits infectious routes between humans.
1 March 2020 Coronavirus disease (COVID-19) and neonate: what neonatologists need to know. Liou GL, SINY	Expert review. Peer reviewed (Journal of Medical Virology). Review of reported case characteristics.	Transmission: No direct evidence of transmission. All cases were part of family clusters or close contact networks.
17 2 March 2020. Clinical analysis of 31 cases of 2019 novel coronavirus infection in children from six provinces (autonomous region) of northern China; Wang D et al	Case series. Unclear whether peer reviewed. Under 3 years old aged 1 month to 17 years old. Case report. Diagnoses: epigastric pain, diarrhea, epigastric pain, history, clinical manifestations, treatment, and the short term prognosis.	Evidence of infection: From January 2020 to February 2020, a total of 200 COVID-19 cases in children (158 boys) have been reported. In the absence of serological studies, it is not possible to determine the precise age distribution of children with COVID-19. The clinical presentation was similar to that of adults, with children who were identified through household contact tracing of adults.
18 3 March 2020. Coronavirus disease 19 among children outside Wuhan, China; Chomeng C et al	Case series. Non-peer reviewed (Unfinished draft). Prospective cohort of 31 confirmed cases (>8 years of age) from 10 provinces (autonomous regions) of China. The recruitment period between 16 January and 19 February 2020.	Clinical presentation: SARS-CoV-2 infection can range from asymptomatic to severe respiratory distress in neonates and children. Respiratory distress: Children with COVID-19 have a higher rate of respiratory distress than adults. The 3 newborns identified had short breath, running of milk, cough, and fever. Signs of illness: There was no clear evidence that SARS-CoV-2 can be transmitted transplacentally from mother to the newborn.
19 26 March 2020. Clinical features of 317 (9%) cases of 2019 novel coronavirus infection in children from 6 provinces (autonomous regions) of northern China; Wang D et al	Case series. Unclear whether peer reviewed. Under 3 years old aged 1 month to 17 years old. Case report. Diagnoses: epigastric pain, diarrhea, epigastric pain, history, clinical manifestations, treatment, and the short term prognosis.	Evidence of infection: From January 2020 to February 2020, a total of 200 COVID-19 cases in children (158 boys) were family clusters. Case numbers: 21 cases had contact with confirmed infected adults. One child (3%) had contact with asymptomatic carriers from abroad.
20 26 March 2020. Clinical features of 317 (9%) cases of 2019 novel coronavirus infection in children from 6 provinces (autonomous regions) of northern China; Wang D et al	Case series. Unclear whether peer reviewed. Under 3 years old aged 1 month to 17 years old. Case report. Diagnoses: epigastric pain, diarrhea, epigastric pain, history, clinical manifestations, treatment, and the short term prognosis.	Clinical presentation: Common symptoms were fever in 20%, including 1 case (3%) with fever >39°C. The mean age of onset was 5.9 days. The mean time from symptom onset to hospital admission was 9.7 days. The mean of 15 cases lasted for 3 days, while in the other 5 cases lasted >3 days. Other symptoms included cough, in 14, 45%. Fatigue in 10 (30%). Headache in 1 (3%). Diarrhea in 1 (3%). Epigastric pain in 1 (3%). Vomiting was rare. The typical diabetics were asymptomatic in 4 cases (13%). The 3 newborns identified had short breath, running of milk, cough, and fever. Critical types were identified. Among them, 24 children (77%) recovered and were discharged from hospital. If remaining 3 were affected at the time of publication, they were still hospitalized.
21 26 March 2020. Coronavirus disease 19 among children outside Wuhan, China; Chomeng C et al	Case series. Non-peer reviewed (Unfinished draft). Prospective cohort of 31 confirmed cases (>8 years of age) from 10 provinces (autonomous regions) of China. The recruitment period between 16 January and 19 February 2020.	Transmission: None reported.

Table 1. Continued

Table 1. Continued

Date / Title / Paper Author(s)	Study Type & Synopsis	What Does the Paper Tell Us?
3 March 2020. Epidemiology and transmission of COVID-19 in Shenzhen: Case analysis of 391 cases and 76 of their close contacts. Bi S et al	Observational study—first test only available in Chinese. Not peer-reviewed (preprint).	Evidence of infection: The household secondary attack rate was 15%, and children were as likely to be infected as adults. Children reported to be similar risk of infection as adults.
4 March 2020. Clinical and CT imaging features of the COVID-19 pneumonia: focus on pregnant women and children. Li L et al	Case reports. Not peer-reviewed (preprint).	Observation: 20 COVID-19 cases had COVID-19 from January 20 to February 2020 and 136 close contacts. Cases identified through systematic surveillance were compared to those without active monitoring.
4 March 2020. Clinical and CT imaging features of the COVID-19 pneumonia: focus on pregnant women and children. Li L et al	Case reports. Not peer-reviewed (preprint).	Evidence of infection: Four laboratory-confirmed cases in children.
5 March 2020. Clinical characteristics of COVID-19 in children compared with adults outside of Hubei Province in China. Du W et al	Case report. Not peer-reviewed (unpublished draft).	Clinical presentation: No report.
6 March 2020. Influv-EV preprint. Preliminary epidemiological investigation of COVID-19 cases with novel coronavirus disease 2019 outside Hubei Province, China: An observational study during cross-border travel. He Y, Wang J, Samore M, Huo B, et al	Observational study.	Evidence of infection: There were 14 children confirmed cases among the 67 cases, with a median age of 6.3 years (range, 0–14 years).
6 March 2020. Influv-EV preprint. An observational study utilizing crowdsourced data outside Hubei Province, China: An observational study during cross-border travel. He Y, Wang J, Samore M, Huo B, et al	Observational study (preprint).	Observation: 21 cases (32%) of the mild type and 11 cases (78%) of the conventional type. No severe or critical cases. Diagnostic criteria for mild cases: no radiographic findings of pneumonia. Diagnostic criteria for common cases: fever, respiratory symptoms, and radiographic manifestations of pneumonia.
6 March 2020. Influv-EV preprint. An observational study utilizing crowdsourced data outside Hubei Province, China: An observational study during cross-border travel. He Y, Wang J, Samore M, Huo B, et al	Observational study.	Transmission: All of the cases in children were family clusters.
6 March 2020. Influv-EV preprint. An observational study utilizing crowdsourced data outside Hubei Province, China: An observational study during cross-border travel. He Y, Wang J, Samore M, Huo B, et al	Observational study (preprint).	Evidence of infection: A total of 82 patients were included. Fifty-four (66%) patients were children and 27 (33%) patients were between 13–19 years. Limited evidence available.
6 March 2020. Influv-EV preprint. An observational study utilizing crowdsourced data outside Hubei Province, China: An observational study during cross-border travel. He Y, Wang J, Samore M, Huo B, et al	Observational study.	Clinical presentation: All of the cases had fever. The most common symptom was fever (88%) followed by cough (64%). Two (8%) were asymptomatic.
6 March 2020. Influv-EV preprint. An observational study utilizing crowdsourced data outside Hubei Province, China: An observational study during cross-border travel. He Y, Wang J, Samore M, Huo B, et al	Observational study.	Transmission: A total of 23 (28%) patients were noted to have an infected family member.
6 March 2020. Influv-EV preprint. An observational study utilizing crowdsourced data outside Hubei Province, China: An observational study during cross-border travel. He Y, Wang J, Samore M, Huo B, et al	Observational study (preprint).	Evidence of infection: 3 children older than the 10-year confirmed cases.
6 March 2020. Influv-EV preprint. An observational study utilizing crowdsourced data outside Hubei Province, China: An observational study during cross-border travel. He Y, Wang J, Samore M, Huo B, et al	Observational study.	Transmission: No report.
6 March 2020. Influv-EV preprint. An observational study utilizing crowdsourced data outside Hubei Province, China: An observational study during cross-border travel. He Y, Wang J, Samore M, Huo B, et al	Observational study.	Observation: Asymptomatic transmission was the best example given was a woman who traveled to Wuhan and then to Beijing with her son. Both were asymptomatic patients, with transmission among 2 generations within the family through direct transmission of not specified.
6 March 2020. Influv-EV preprint. An observational study utilizing crowdsourced data outside Hubei Province, China: An observational study during cross-border travel. He Y, Wang J, Samore M, Huo B, et al	Observational study.	Evidence of infection: No report.
6 March 2020. Influv-EV preprint. An observational study utilizing crowdsourced data outside Hubei Province, China: An observational study during cross-border travel. He Y, Wang J, Samore M, Huo B, et al	Observational study (preprint).	Clinical presentation: Of the 556, 68 were male and 478 were female. Clinical symptoms including shortness of breath, asthenia, respiratory distress, apnea, cyanosis, dyspnea, tachypnea, and tachycardia. The percentage of patients with fever was 53.8%. Of the 103 cases, 8 were critically ill.
6 March 2020. Influv-EV preprint. An observational study utilizing crowdsourced data outside Hubei Province, China: An observational study during cross-border travel. He Y, Wang J, Samore M, Huo B, et al	Observational study.	Transmission: No report.
10 March 2020. Influv-EV preprint. Data analysis of diversity of clinical presentation for severity detection in COVID-19 pediatric cases. Yu H et al	Case series.	
10 March 2020. Influv-EV preprint. Data analysis of diversity of clinical presentation for severity detection in COVID-19 pediatric cases. Yu H et al	Not peer-reviewed (preprint).	
10 March 2020. Influv-EV preprint. Data analysis of diversity of clinical presentation for severity detection in COVID-19 pediatric cases. Yu H et al	Case report.	
10 March 2020. Influv-EV preprint. Data analysis of diversity of clinical presentation for severity detection in COVID-19 pediatric cases. Yu H et al	Not peer-reviewed (preprint).	
10 March 2020. Influv-EV preprint. Data analysis of diversity of clinical presentation for severity detection in COVID-19 pediatric cases. Yu H et al	Analysis of 105 cases of COVID-19 in children diagnosed between January 2020 and February 2020 at Tongji Hospital, Wuhan Children's Hospital, Tongji Medical School, Huazhong University of Science and Technology. The study designated hospital in Wuhan by COVID-19.	

Abbreviations: CDC, Centers for Disease Control and Prevention; CI, confidence interval; COVID-19, coronavirus disease 2019; CT, computed tomography; CRB, chest X-ray; ICU, intensive care unit; MERS, Middle East respiratory syndrome; SARS, severe acute respiratory syndrome; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; WHO, World Health Organization.

UNDERSTANDING THE PATHOLOGY

PREDICTIVE VALUE OF CYTOKINE/CHEMOKINE RESPONSES FOR THE DISEASE SEVERITY AND MANAGEMENT IN CHILDREN AND ADULT CASES WITH COVID-19

Ozserekci Y, Aykac K, Er AG, Halacli B, Arasli M, Oygar PD, Gürlevik S, Cura Yayla BC, Karakaya J, Alp A, Topeli A, Cengiz AB, Akova M, Ceyhan M.. J Med Virol. 2020 Nov 23. doi: 10.1002/jmv.26683. Online ahead of print.

Level of Evidence: 3 - Non -randomized controlled cohort/follow-up study

BLUF

A cohort-control study by pediatric infectious disease specialist in Ankara, Turkey evaluated cytokine and chemokine levels from serum samples of 60 COVID-19 positive patients (30 pediatric, 30 adult; Table 1, see summary). Authors found that COVID-19 patients had higher levels of IP10 and MIP-3B compared to 30 healthy controls (15 pediatric, 15 adult; $p < 0.001$), while IP-10 was a predictor for disease severity in children and IL-6 a predictor for disease severity in adults (Table 2). They suggest these findings provide insight into potential treatment targets for COVID-19 and further elucidate potential uses for inflammatory markers as prognostic indicators for pediatric and adult COVID-19 severity.

SUMMARY

The acute phase serum levels of cytokine and chemokine levels of the following were measured:
IFN- γ , IL1 α , IL-1 β , IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, IL-17A, IL-27, LL-37, IL-33, IP10, MIP-1 β , IL-1 β , TNF- α , 6CKine, interferon-inducible T-cell alpha chemoattractant (I-TAC), chemokine (C-C motif) ligand 2 (CCL2; MCP-1), CCL3 (MIP-1 α), MIP-3 β , and macrophage-derived chemokine (CCL22; MDC)

ABSTRACT

BACKGROUND: The disease course of children with COVID-19 seems milder as compared with adults, however, actual reason of the pathogenesis still remains unclear. There is a growing interest on possible relationship between pathogenicity or disease severity and biomarkers including cytokines/chemokines. **OBJECTIVES:** We wondered whether these biomarkers could be used for the prediction of the prognosis of COVID-19 and improving our understanding on the variations between pediatric and adult cases with COVID-19. **STUDY DESIGN:** The acute phase serum levels of 25 cytokines and chemokines in the serum samples from 60 COVID-19 pediatric ($n=30$) and adult cases ($n=30$) including 20 severe/critically ill, 25 moderate and 15 mild patients and 30 healthy pediatric ($n=15$) and adult ($n=15$) volunteers were measured using commercially available fluorescent bead immunoassay and analyzed in combination with clinical data. **RESULTS:** Interferon gamma-induced protein 10 (IP-10) and macrophage inflammatory protein (MIP)-3beta levels were significantly higher in patient cohort including pediatric and adult cases with COVID-19 when compared with all healthy volunteers ($p = <0.001$ in each) and whereas IP-10 levels were significantly higher in both pediatric and adult cases with severe disease course, MIP-3beta were significantly lower in healthy controls. Additionally, IP-10 is an independent predictor for disease severity, particularly in children and IL-6 seems a relatively good predictor for disease severity in adults. **CONCLUSION:** IP-10 and MIP-3beta seem good research candidates to understand severity of COVID-19 in both pediatric and adult population and to investigate possible pathophysiological mechanism of COVID-19. This article is protected by copyright. All rights reserved.

FIGURES

Characteristics	Children with COVID-19 (n = 30)	Adults with COVID-19 (n = 30)	<i>p</i> Value
Age (years; median [min-max])	10.5 (0–17)	62.5 (48–77)	NA
Sex (n, %)			1.0
Male	14 (46.7%)	14 (46.7%)	
Female	16 (53.3%)	16 (53.3%)	
Underlying diseases (n, %)	7 (23.3%)	15 (50%)	.032 ^a
Intensive care unit (ICU)/pediatric ICU (PICU) admission	4 (13.3%)	7 (23.3%)	.317
Mechanical ventilation	5 (16.7%)	10 (33.3%)	.136
Extracorporeal membrane oxygenation (ECMO)	2 (6.7%)	0 (0)	.492
Laboratory parameters (median [min-max])			
White blood cells (WBC) (/ml)	8470 (4000–11600)	4400 (2100–7700)	<.001 ^a
Lymphocytes (/ml)	1300 (420–6100)	740 (200–2400)	<.001 ^a
Neutrophils (/ml)	4300 (600–9200)	2390 (1000–7040)	.026 ^a
Platelets (/ml)	209 × 10 ³ (153–344 × 10 ³)	168 × 10 ³ (63–252 × 10 ³)	.001 ^a
C-reactive protein (CRP) (mg/dl)	1.16 (0.08–12.36)	2.69 (0.08–31.7)	.007 ^a
Procalcitonin (PCT) (ng/ml)	0.09 (0.01–7.9)	0.03 (0.01–12.75)	.015 ^a
<i>d</i> -dimer (mg/L)	1.0 (0.19–6.65)	0.38 (0.03–80.0)	.055
Ferritin	63.2 (4–1967)	124 (12.9–1648)	.705
Treatment (n, %)			
Antiviral	7 (23.3%)	29 (96.7%)	<.001 ^a
Anticoagulant	1 (3.3%)	29 (96.7%)	<.001 ^a
Corticosteroids	2 (6.7%)	0	.492
IVIG	4 (13.3%)	0	.112
Antibacterial	18 (60%)	23 (76.7%)	.165
Outcome (n, %)			
Death	2 (6.7%)	1 (3.3%)	1.0

Abbreviations: COVID-19, coronavirus disease 2019; IVIG, intravenous immunoglobulin.

^aStatistically significant.

Table 1. Demographic and clinical characteristics of patients with COVID-19

TABLE 2 Cytokine or chemokine levels in patients and control groups

Cytokine/chemokine levels (pg/ml)	Group 1 (Children with COVID-19) (n = 30)	Group 2 (Adults with COVID-19) (n = 30)	Group 3 (Healthy children) (n = 15)	Group 4 (Healthy adults) (n = 15)	p Value
Chemokine levels					
I-TAC	118.7 (0-749.7)	0 (0-1382.4)	0 (0-615.3)	0 (0-0)	.071 ^a .162 ^b .005 ^c
MDC	27686.7 (4900.3-102883.8)	16488.2 (2162.4-46068.7)	31668 (8923.3-73679.6)	17384.4 (0-38555.2)	.001 ^a 523 ^b .923 ^c
MIP-1 α	1934.9 (0-21283.6)	11.5 (0-3997.4)	3201.5 (0-33177.7)	0.9 (0-6142.5)	.045 ^a 202 ^b .202 ^c
IP-10	380.2 (158.4-4115.4)	525.2 (21.5-1635.1)	176.3 (10.1-889.4)	26.2 (2.3-386.6)	.183 ^a .005 ^b .001 ^c
MIP-3 β	1504.2 (552.1-3588.6)	1051.9 (17.8-3159.9)	684.8 (0-3235.4)	561.3 (0-3017.2)	.003 ^a <.001 ^b .003 ^c
MIP-1 β	697.9 (0-14573.1)	624.4 (68.0-2684.7)	2049.3 (170.8-12969.2)	587.4 (0-1650.2)	.579 ^a .006 ^b .42 ^c
Cytokine Level					
IL-4	2086.6 (0-6803.2)	1668.6 (0-19131.5)	1653.9 (0-3729.2)	1545.1 (0-3184.9)	.146 ^a 455 ^b .346 ^c
TNF- α	1283.9 (0-3760.7)	713.4 (0-3930.9)	1285.2 (0-2875.4)	435.6 (0-1520.5)	.125 ^a 579 ^b .183 ^c
IL-17A	1794.3 (0-10495.1)	1335.3 (0-10721.5)	1525.5 (864.0-2561.9)	1235.1 (0-2059.7)	.031 ^a 531 ^b .621 ^c
IL-13	52.2 (0-210.6)	43.4 (0-232.8)	75.8 (0-153.3)	0 (0-102.6)	.471 ^a 149 ^b .179 ^c
MCP-1	0 (0-29634.3)	0 (0-6410.9)	0 (0-2127.9)	0 (0-0)	.512 ^a .175 ^b .045 ^c
IL-1 β	100.2 (0-151.02)	93.4 (0-199.4)	111.6 (46.9-18636.3)	73.6 (0-121.6)	.191 ^a .051 ^b .123 ^c
IL-10	3777.8 (0-10846.3)	2850.2 (0-23439.2)	3633.1 (1171.3-6114.7)	2361.9 (0-4100.5)	.056 ^a 295 ^b .018 ^c
IL-6	1922.4 (0-56607.6)	2124.8 (1058.1-4358.2)	2103.5 (899.0-59838.3)	1107.9 (0-2357.9)	.416 ^a 531 ^b <.001 ^c
IL-1 α	20.2 (0-185.2)	13.2 (0-412.3)	7.6 (0-92.8)	0 (0-28.1)	.685 ^a 532 ^b .015 ^c

COVID-19 ASSOCIATED THROMBOSIS AND COAGULOPATHY: REVIEW OF THE PATHOPHYSIOLOGY AND IMPLICATIONS FOR ANTITHROMBOTIC MANAGEMENT

Ortega-Paz L, Capodanno D, Montalescot G, Angiolillo DJ.. J Am Heart Assoc. 2020 Nov 24:e019650. doi:

10.1161/JAH.120.019650. Online ahead of print.

Level of Evidence: 5 - Review / Literature Review

BLUF

This literature review of 138 publications by an international group of cardiologists describes pathophysiology of SARS-CoV-2 in the cardiovascular system, reporting that it binds ACE2 receptors leading to apoptosis, inducing a pro-inflammatory and prothrombotic state, while causing myocardial and lung tissue injury, likely via cytokine storm. Authors report the resultant coagulation dysregulation may lead to thrombi and emboli in COVID-19 patients (Figures 1,2), advocating for further research into antithrombotic therapy to better understand management of complicated SARS-CoV-2 infections.

ABSTRACT

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) which has posed a significant threat to global health. Although the infection is frequently asymptomatic or associated with mild symptoms, in a small proportion of patients it can produce an intense inflammatory and prothrombotic state that can lead to acute respiratory distress syndrome, multiple organ failure, and death. Angiotensin-converting enzyme 2

(ACE2), highly expressed in the respiratory system, has been identified as a functional receptor for SARS-CoV-2. Notably, ACE2 is also expressed in the cardiovascular system and there are multiple cardiovascular implications of COVID-19. Cardiovascular risk factors and cardiovascular disease have been associated with severe manifestations and poor prognosis in patients with COVID-19. Importantly, patients with COVID-19 may have thrombotic and coagulation abnormalities promoting a hypercoagulable state, resulting in an increased rate of thrombotic and thromboembolic events. This review will describe the pathophysiology of the cardiovascular involvement following infection by SARS-CoV-2, with a focus on thrombotic and thromboembolic manifestations and implications for antithrombotic management.

FIGURES

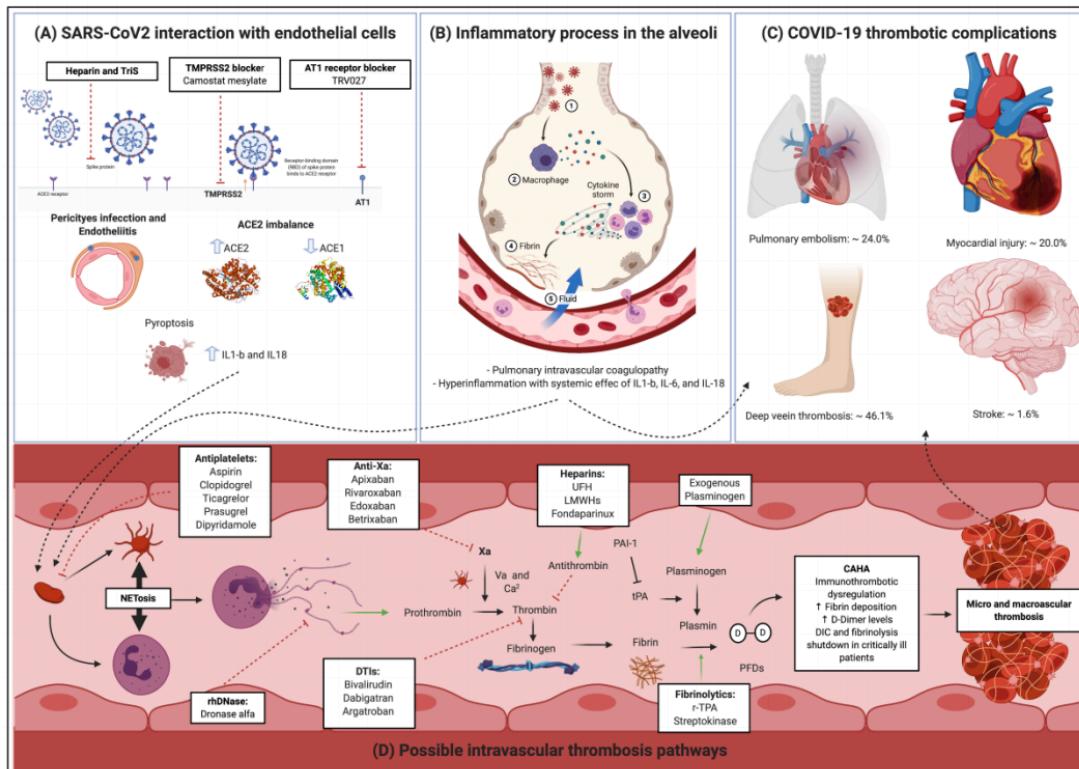


Figure 1. Pathophysiological mechanism related to COVID-19 associated thrombosis and coagulopathy. A: The interaction of the SARS-CoV2 with endothelial cells (type II pneumocytes, glomerular capillary loops, small intestine capillaries, etc.) ACE2 imbalance may promote susceptibility to the SARS-CoV2 infection of these cell types. Furthermore, cell infection and induced inflammation in pericytes and endothelial cells may promote local apoptosis and potent inflammatory cytokines. B: Inflammatory process in the pulmonary alveoli leading to pulmonary tissue edema and intravascular coagulopathy. C: Selection of thrombotic complications in COVID-19 and their approximate frequency. D: Proposed intravascular thrombosis pathways leading to micro and macrovascular thrombosis complications. Due to the potent local and systemic cytokines production, the platelet platelets are activated and interact with neutrophils. The NETosis process may also stimulate thrombin production and fibrin deposition. The excess of fibrin deposition and fibrinolysis shutdown leads to intravascular thrombosis, and finally, to clinical thromboembolic complications. The pointed black and continued black lines denote pathway connections, pointed red lines denote inhibition, and green arrows denote agonism. SARS-CoV2: severe acute respiratory syndrome coronavirus-2; COVID-19: Coronavirus Disease 2019; IL: interleukin; TriS: synthesized trisulfated heparin; TMPRSS2: transmembrane protease serine 2; AT1: angiotensin II receptor type 1; ACE2: angiotensin-converting enzyme 2; ACE-I: angiotensin-converting-enzyme inhibitors; NETs: neutrophil extracellular traps; Anti-Xa: antifactor Xa; UFH: unfractionated Heparin; LMWH: low-molecular weight heparin; PAI-1: plasminogen activator inhibitor I; tPA: tissue plasminogen activator; r-tPA: recombinant-tissue plasminogen activator; DTIs: direct thrombin inhibitors; PDFs: fibrin degradation products; D-D: D-dimer; CAHA: COVID-19-associated hemostatic abnormalities. Data derived and visual presentation modeled from Bikdelli et al.91

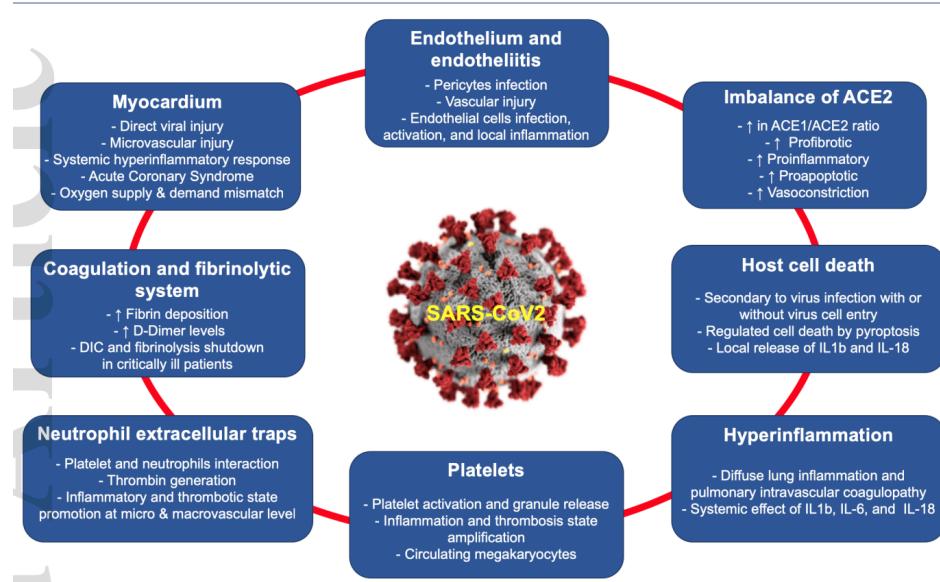


Figure 2. Effects of COVID-19 on the cardiovascular and coagulation system. COVID-19: coronavirus disease 2019; ACE2: Angiotensin-converting enzyme 2; ACE1: Angiotensinconverting enzyme 1; IL: interleukin.

IN VITRO

SARS-COV-2-TRIGGERED NEUTROPHIL EXTRACELLULAR TRAPS MEDIATE COVID-19 PATHOLOGY

Veras FP, Pontelli MC, Silva CM, Toller-Kawahisa JE, de Lima M, Nascimento DC, Schneider AH, Caeté D, Tavares LA, Paiva IM, Rosales R, Colón D, Martins R, Castro IA, Almeida GM, Lopes MIF, Benatti MN, Bonjorno LP, Giannini MC, Luppino-Assad R, Almeida SL, Vilar F, Santana R, Bollela VR, Auxiliadora-Martins M, Borges M, Miranda CH, Pazin-Filho A, da Silva LLP, Cunha LD, Zamboni DS, Dal-Pizzol F, Leiria LO, Siyuan L, Batah S, Fabro A, Mauad T, Dolhnikoff M, Duarte-Neto A, Saldiva P, Cunha TM, Alves-Filho JC, Arruda E, Louzada-Junior P, Oliveira RD, Cunha FQ.. J Exp Med. 2020 Dec 7;217(12):e20201129. doi: 10.1084/jem.20201129.

Level of Evidence: 3 - Local non-random sample

BLUF

Pharmacists and biochemists from Ribeirão Preto Medical School in São Paulo, Brazil analyzed samples (tracheal aspirate, lung autopsy tissue, and plasma) from 32 patients hospitalized with COVID-19. They found that the concentration of neutrophil-derived extra cellular traps (NETs) was higher in COVID-19 patients compared to controls (Figure 1) and that SARS-CoV-2 directly induced NET release in vitro (Figure 3). In vitro studies also showed NETs utilize the ACE-2 and serine protease TMPRSS2 pathway (Figure 4). Because authors found NETs were associated with lung damage on histopathological examination, they suggest NETs contribute to lung epithelial cell damage in COVID-19 and therapies inhibiting NET formation may offer a therapeutic target.

ABSTRACT

Severe COVID-19 patients develop acute respiratory distress syndrome that may progress to cytokine storm syndrome, organ dysfunction, and death. Considering that neutrophil extracellular traps (NETs) have been described as important mediators of tissue damage in inflammatory diseases, we investigated whether NETs would be involved in COVID-19 pathophysiology. A cohort of 32 hospitalized patients with a confirmed diagnosis of COVID-19 and healthy controls were enrolled. The concentration of NETs was augmented in plasma, tracheal aspirate, and lung autopsies tissues from COVID-19 patients, and their neutrophils released higher levels of NETs. Notably, we found that viable SARS-CoV-2 can directly induce the release of NETs by healthy neutrophils. Mechanistically, NETs triggered by SARS-CoV-2 depend on angiotensin-converting enzyme 2, serine protease, virus replication, and PAD-4. Finally, NETs released by SARS-CoV-2-activated neutrophils promote lung epithelial cell death in vitro. These results unravel a possible detrimental role of NETs in the pathophysiology of COVID-19. Therefore, the inhibition of NETs represents a potential therapeutic target for COVID-19.

FIGURES

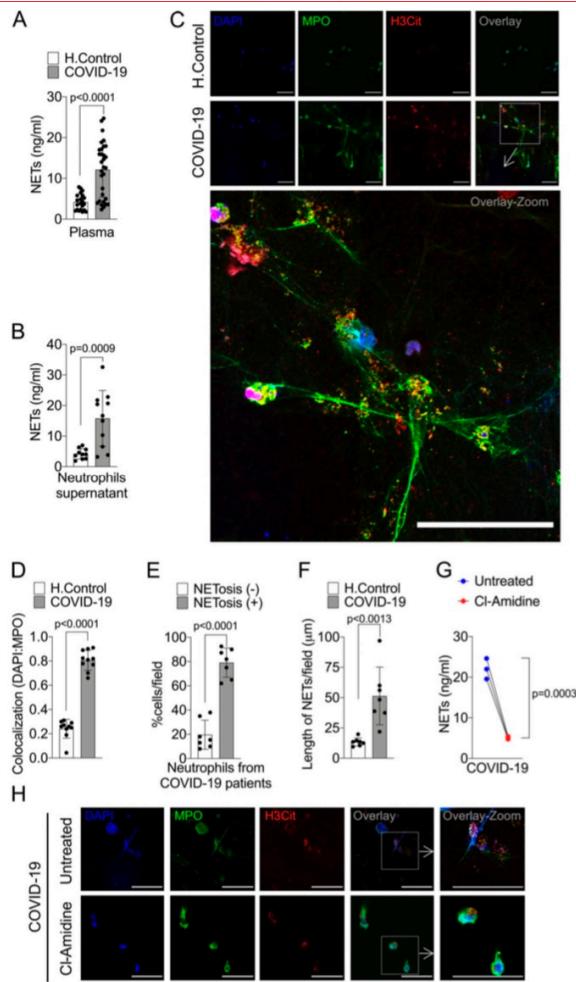


Figure 1. COVID-19 patients produces high concentrations of NETs. Plasma and neutrophils were isolated from healthy controls and COVID-19 patients. **(A)** NET quantification by MPO-DNA PicoGreen assay in plasma from healthy controls (H.Control; $n = 21$) or COVID-19 patients ($n = 32$). **(B)** Supernatants from cultures of blood isolated neutrophils from healthy controls ($n = 10$) or COVID-19 patients ($n = 11$). NET quantification was performed using MPO-DNA PicoGreen assay. **(C)** Representative confocal analysis of NETs release by neutrophils isolated from healthy controls ($n = 10$) or COVID-19 patients ($n = 11$), cultured for 4 h at 37°C. Cells were stained for nuclei (DAPI, blue), MPO (green), and H3Cit (red). Scale bar indicates 50 μm. **(D)** Colocalization of DAPI and MPO between healthy controls ($n = 10$) and COVID-19 ($n = 10$). The data depicts Pearson's correlation coefficient assessed by Fiji/ImageJ software. **(E)** Percentage of NETosis in neutrophil from COVID-19 patients ($n = 7$). **(F)** NET length quantification. **(G)** NET quantification by MPO-DNA PicoGreen assay in the supernatants of blood-isolated neutrophils from COVID-19 patients ($n = 3$) preincubated, or not, with PAD-4 inhibitor (Cl-Amidine; 200 μM) for 4 h at 37°C. **(H)** Representative confocal images showing the presence of NETs in isolated neutrophils from COVID-19 patients, treated or not, with Cl-Amidine (200 μM). Cells were stained for nuclei (DAPI, blue), MPO (green), and H3Cit (red). Scale bar indicates 50 μm. Data are representative of at least two independent experiments and are shown as mean ± SEM. P value were determined by two-tailed unpaired (A, B, and D-F) or paired (G) Student t test.

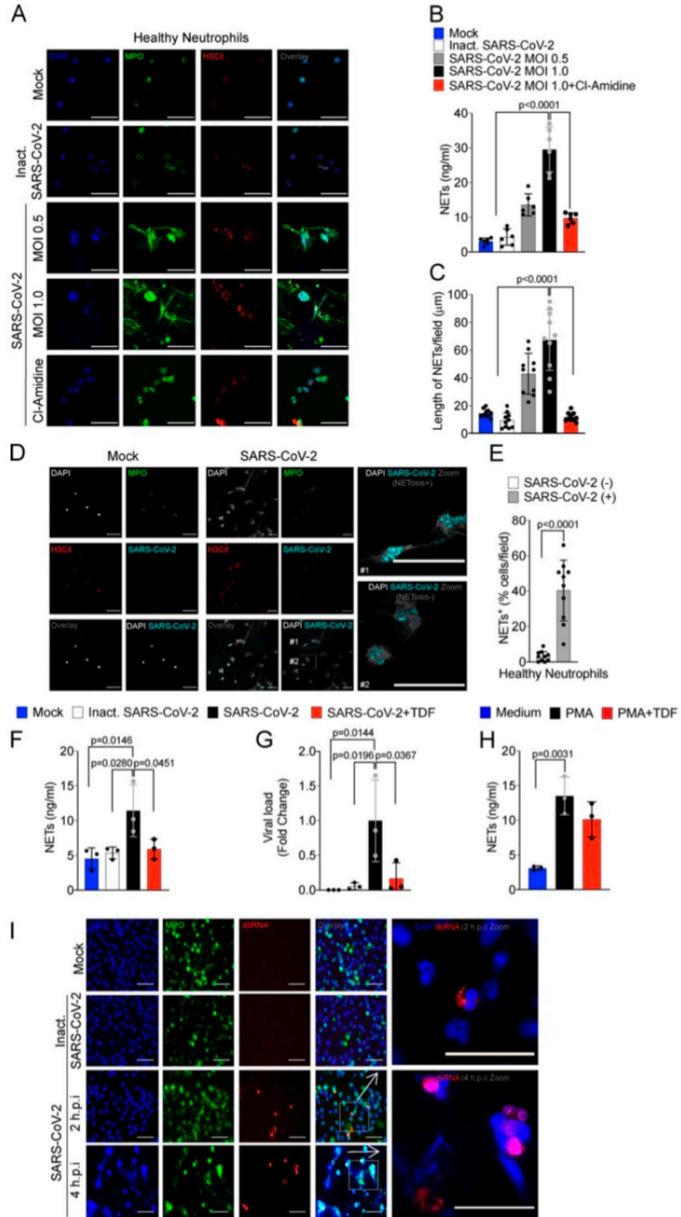


Figure 3. SARS-CoV-2 induces the release of NETs by healthy neutrophils. Neutrophils were isolated from healthy controls and incubated with Mock, inactivated SARS-CoV-2, or SARS-CoV-2 (MOI = 0.5 or 1.0). One group of cells incubated with SARS-CoV-2 MOI = 1.0 was pretreated with a PAD-4 inhibitor (Cl-Amidine, 200 μM). **(A)** Representative images of NET release. Cells were stained for nuclei (DAPI, blue), MPO (green), and H3Cit (red). Scale bar indicates 50 μm. **(B and C)** NET quantification by MPO-DNA PicoGreen assay (B) and quantification of NETs length in these neutrophils supernatants (C; n = 6). **(D)** Representative images showing immunostaining for nuclei (DAPI, white), MPO (green), H3Cit (red), and SARS-CoV-2 (cyan) in neutrophils incubated with Mock or SARS-CoV-2 (MOI = 1.0). Scale bar indicates 50 μm. **(E)** Percentage of NETs positive cells stained, or not, for SARS-CoV-2 antigens (10 fields were analyzed). SARS-CoV-2-infected neutrophils (MOI = 1.0, n = 3) were pretreated with 10 μM TDF, an RNA polymerase inhibitor. **(F and G)** NETs quantification by MPO-DNA PicoGreen assay (F) and SARS-CoV-2 viral load detection in neutrophil cell pellet by RT-PCR, 4 h after infection (G). Fold change relative to SARS-CoV-2 group was used. **(H)** Neutrophils from healthy controls (n = 3) were stimulated with PMA pre-treated or not with 10 μM TDF. NET quantification was assessed by MPO-DNA PicoGreen assay in neutrophil supernatants after 4 h incubation. **(I)** Detection of replication by immunostaining for dsRNA (red) 2 and 4 h after infection. Nuclei (DAPI, blue) and MPO (green) were used as control of neutrophil staining. Scale bar indicates 50 μm. Data are representative of at least two independent experiments and are shown as mean ± SEM. P values were determined by one-way ANOVA followed by Bonferroni's post hoc test (B, C, and E–H).

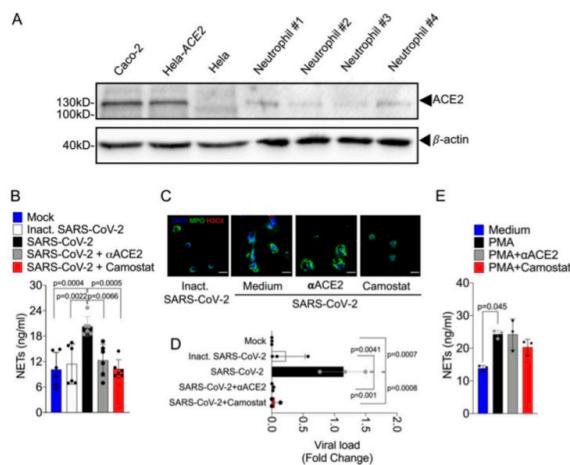


Figure 4. SARS-CoV-2 infection in neutrophils depends on ACE2 and serine protease TMPRSS2 pathway for the NETs formation. **(A)** Expression of ACE2 was assessed by Western blot (A) in Caco-2, HeLa cells transduced with hACE2 (HeLa-ACE2), HeLa cells, and isolated neutrophils from healthy controls. β-Actin expression was used as load control for protein expression. SARS-CoV-2-infected neutrophils (MOI = 1.0) were pretreated with neutralizing anti-ACE2 antibody (αACE2, 0.5 μg/ml) and camostat (10 μM), a serine protease TMPRSS2 inhibitor. **(B)** NETs quantification by MPO-DNA PicoGreen assay in these neutrophils supernatants (n = 6). **(C)** Immunostaining for nuclei (DAPI, blue), MPO (green), and H3Cit (red). Scale bar indicates 50 μm. **(D)** SARS-CoV-2 viral load detection in neutrophil cell pellet (n = 3) by RT-PCR 4 h after infection. Fold change relative to SARS-CoV-2 group. **(E)** PMA-stimulated neutrophils from healthy controls (n = 3) were pre-treated or not with 0.5 μg/ml αACE2 and 10 μM camostat. NET quantification was assessed by MPO-DNA PicoGreen assay in neutrophils supernatants after 4 h of PMA stimulation. Data are representative of at least two independent experiments and are shown as mean ± SEM. P values were determined by one-way ANOVA followed by Bonferroni's post hoc test (B, D, and E).

ACKNOWLEDGEMENTS

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