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Letter From The Editors

Perspectives

Original Science

Reviews

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JOURNAL of SCIENCE & MEDICINE

Dear Readers,

The year 2020 is etched in our minds as a year marked in large part by fear, loss, prejudice, and adversity. In the face of the challenges presented by the pandemic, we are proud our institution responded with courage, teamwork, and leadership for our community.

Ultimately, our collective experience will direct and make us better equipped for the unseen challenges that certainly stand waiting on the horizon. In response to the unique challenges introduced during the COVID-19 pandemic, the *Wake Forest Journal of Science and Medicine* sought to offer a platform for members of the Wake Forest and surrounding community to share pandemic-related scientific and medical research and healthcare perspectives through a COVID-19 special issue. Our primary goals were to 1) disseminate peer-reviewed literature, 2) cultivate open dialogue among community members, and 3) facilitate student engagement in research during a year when typical research programs, including the medical student research program, were suspended by social distancing.

The manuscripts accepted for publication in this special issue offer insight into the medical and scientific advances made in response to the COVID-19 pandemic, as well as the pandemic's profound personal impact. The perspective articles herein were contributed by a diverse group of authors, such as our administrative leadership, frontline healthcare workers, and an expecting mother; topics including mental health challenges and the resiliency to overcome COVID-related stress and anxiety, racial disparities in local public health, and policies aimed at reforming these inequalities are explored. Also included in this edition are student-led, faculty-mentored review articles that focus on an array of basic science and clinical topics central to the COVID-19 pandemic, such as immunology and epidemiology; diagnostic, therapeutic, and preventive measures; clinical presentation, specifically cutaneous manifestations; as well as original science investigating the COVID-19 exposure locally.

We would like to express our gratitude to the authors for contributing their work and to the multitude of people who selflessly volunteered their time and effort towards making this special issue come to fruition. From the field experts who critically reviewed the manuscripts and the faculty who served as mentors for the student-led reviews, to the medical and graduate student volunteers that facilitated the peer-review and editorial process, the collaborative spirit that embodies the Wake Forest community was inspiring to witness on full display. Additionally, we cannot overstate our appreciation for the unprecedented effort of the staff at Creative Communications and in particular Tiffany Montgomery, who managed the publication of this special issue. Lastly, on behalf of the Editors-in-Chief, we thank Adam Jorgensen for his service and leadership as Guest Editor of this COVID-19 special issue of the *Wake Forest Journal of Science and Medicine*. While publishing this edition needed a village, Adam's relentless effort was singularly critical to its success.

Sincerely,

Adam Jorgensen, Guest Editor
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Perspectives

This Could Be A Chance: Leading through COVID-19

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I never thought I would lead through a pandemic.

As a vascular surgeon, I'm used to treating problems quickly and methodically with a clamp or suture. And as one of just a handful of female health system CEOs and Deans across the country, I've been in my fair share of uphill battles.

But I never imagined I would lead through a pandemic.

COVID-19 came on abruptly, upending our normal patterns and practices overnight. There was fear of the disease, uncertainty about what lay ahead, and doubts of whether we were brave and strong enough to move forward. But there, in the middle of it all, has been opportunity, and I realized this could be a chance — to unite, grow, and become even more.

Flexibility in the Face of Uncertainty

I am an optimist by nature, but COVID-19 has been a challenge unlike any other. It has underscored the resilience, courage, and strength that define our Wake Forest Baptist team and solidified how we rally against the unknown.

Over the past months, we have come together in innovative ways to keep our patients, community, and health system safe. At the onset, we moved quickly but thoughtfully. We opened an Incident Command Center to monitor staffing, patient flow, and issues occurring locally, regionally and statewide, 24 hours a day, seven days a week. We restricted visitors, stopped non-essential surgeries, and worked with our supply chain to ensure frontline caregivers had continued access to personal protective equipment. We paused research, transitioned students to distance learning, and sent as many corporate teams that we could home to work remotely.

When we didn't have the answers, we shared what we knew. We were transparent, communicating through weekly videos, a daily "Need to Know" newsletter to all employees, and podcasts and videos from our public health and infectious diseases experts.

The changes we have been through have been difficult, unsettling, and required flexibility in the face of great uncertainty. Academics were restructured, schedules were flexed, and as fewer patients came to the hospital, finances tightened and we made sacrifices. We balanced these changes with new tugs in our personal and home lives.

Our New World

As the weeks passed, we have learned to work and live alongside COVID-19, becoming more creative in our new world. Through telehealth, we continued caring for patients, flipping primary care and other services virtually. This has been an opportunity to learn new skillsets, and while patients have safely returned to the hospital, virtual health services continue to shape our practices.

We have become even more active partners in our community, coming together with local organizations through the "Mask the City" initiative to provide every person in Winston-Salem with a mask.[1] Being part of this city-wide collaboration to support community health has been powerful. Our physicians and researchers designed the prototype of the Nightingale mask, which was manufactured by Renfro Corporation and distributed across the city by numerous organizations. We have given more than 60,000 Wake Forest Baptist Health branded masks to our employees and their family members, and it gives me great pride to see them — both at our facilities and in the community — doing their part to keep us safe wearing their black and gold.

As we continue to face the great challenge of COVID-19 and take steps toward recovery, we are pivoting, using our collaborative energies in new ways and celebrating our victories, large and small.

In May, we celebrated our medical school and physician assistant Class of 2020 graduates virtually, with students sharing footage of hoodings from afar. Although it was different, we experienced the same sense of community and class pride. We have seen countless acts of compassion across our health system, from caregivers finding innovative ways to connect patients with loved ones to critical care teams singing "Happy Birthday" virtually to COVID-19-positive patients in isolation.

This is our new world right now, and we are learning and juggling through the unknown. It takes flexibility, bravery, resilience, and trust. It also takes a shift in actions and attitude.

Listening, Learning, and Sharing

This shift is increasingly important in our response not only to COVID-19, but also to the reality of racism and racial injustices that are increasingly visible across our country. We are taking a hard look at our institutional processes and how we can bring about lasting change.

Our new fiscal year organizational theme is "Community," and while we're listening, learning and sharing — we're also taking action. Last year, we formed several affinity groups, including our Black/African American Allies group. Recently, we added White Allies for Human Equity. These groups come together under common identities and experiences to build connections and understanding. We launched a racial equity task force to champion the needs of traditionally underrepresented groups, and we are appointing diversity and inclusion liaisons among our staff. We offer weekly engagement sessions on cultivating inclusive spaces as well as implicit bias and active bystander trainings.

We have the opportunity to open our circles wider, and I'm proud to stand with my team in our commitment to delivering equitable care and treatment to all in our communities.

Leading Forward, Together

COVID-19 changed modern history as we know it, affecting the world in countless ways and illuminating disparities in how underrepresented communities suffer.[2,3] We've had to admit we couldn't foresee the severity of its consequences, and while we've slowed down and listened, we know we have more work to do.

Now, there will be paradoxes to manage as we move toward possibility — guilt versus relief and reality versus hope. Staying safe and healthy, being smart and efficient, and empowering and innovating will be required.

No, I never thought I'd lead through a pandemic.

But with my team by my side and the community surrounding me, I believe this is a chance. We have an opportunity, as a Wake Forest Baptist family, to unite and come out of this even stronger for our patients, community, and each other.

Disclosures

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Perspectives

Doing Our Jobs: COVID-19 from an Emergency Medicine Resident Perspective

Blake Briggs, M.D.[1]

I am not a Patriots fan. However, I enjoy watching football, and well-coached football is even better. There is a leadership mantra in Boston that has made the rounds nationally since the 90's: "do your job." Multiple sports analysts, life coaches, and even authors have pursued this phrase relentlessly in the hopes of capturing its essence. In the world of medicine, it has significant meaning. Former Patriots linebacker Willie McGinest once provided some context to this storied phrase. "For us as players, we didn't want to let each other down. We didn't want to be the weak link. We didn't want to be the cause of something not going the way it was supposed to go."[1]

Doing your job means responsibility to your team, your coworkers. It means much more than simply the popular label of #healthcarehero. It means showing up to a full waiting room in the ED and realizing it will be a tough shift, but you owe it to your teammates to hold your ground and work hard. A waiting room of 35 people at the beginning of a shift is like starting the first quarter in football down 35 points. From experience, there is nothing more demoralizing than starting the shift (e.g. game), down 35.

When the ED team is at peak efficiency, nurses and doctors execute coordinated plans and move room to room, separating life-threatening from "just a scratch," bedding patients in the hallways to save space, and working to clear the mighty waiting room. While this is happening, team members still make time to sit down and holding the hand of a grieving family member.

Doing your job means that when a pandemic hits and emergency departments are being overwhelmed across the country, you still come to work and embrace the uncertainty of the frontline. The emergency department frontline continues to catch everyone- the young, the old, the healthy, the dying, the uninsured, and the undocumented, yet the rules of the game changed. It's no longer the "regular season;" this is the COVID-IXX Super Bowl! The stakes are higher. Team dynamics and "doing your job" seem to be more important than ever.

Instead of asking who qualifies as a #healthcarehero, the real question is: does working on the frontline in the ED during a medical crisis of global proportions truly make one a hero? Or, better yet, are you just human? Perhaps it's more about doing your job, fulfilling the oath we swore first year of medical school.

Doing your job means not posing for social media selfies in your PPE and tagging #healthcarehero. It means not giving into the hype surrounding COVID-19 with the sole purpose of seeking attention for oneself and promoting your status as a #healthcarehero. It means not succumbing to the national spotlight and sacrificing

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the team's performance to promote yourself to get likes and followers. It's more about the time you put in that defines how you contribute to medicine, to humanity. Raising awareness for the deadliness of disease and the need for PPE? Makes sense. Posting a selfie to show off your mask with #healthcarehero, not so much. In football, each week the media and certain players seem to focus on the "hype," whether it be flashy posts on social media, or rumors about certain gossip on teams. You can always separate the players who buy into the hype from those who simply show up and work hard, doing their job for their team. It's much the same in healthcare.

I look at my selfless colleagues showing up to work, the ones my age, and the ones much older and at risk for COVID-19. I wonder how much they contemplate about leaving their loved ones at home and debate coming to work. Is there more fear about letting their loved ones down, of being the weak link at work? I'm sure they would say something eerily similar to Willie McGinest: "for us as colleagues, we didn't want to let each other down. We didn't want to be the weak link." If you could walk up to each of these physicians, nurses, therapists, and environmental service staff, you would get the usual response of why they showed up to work: "just doing my job."

Perhaps the worst aspect of the #healthcarehero trend is that in the end, it measures to be quite shallow. As the nation "moves on" from COVID-19, the news and social media will find new, more flashy items to discuss, and #healthcarehero syndrome will die long before COVID-19 is defeated. What's left for us in healthcare is what has always been present since the dawn of illness: doing our job in a never-ending struggle against a relentless opponent. The opponent might be influenza in the winter, trauma in the summer, or COVID-19 in 2020, but the team dynamics should never change. The stakes may be higher as the nation is restless with uncertainty and anxiety, but the practice of medicine has not changed: just do your job.

Forgetting football, medicine might be the greatest saga of all time. The stakes are higher, the opponent (illness) almost always has an advantage even before the teams take the field. The emergency department is a recurring battle royale in the ongoing struggle between life and death, knowledge versus ignorance, comradery versus divisiveness. It is up to us to choose how we perform in these events. We can focus on the flashy, fleeting aspects of healthcare, the hype, or we can come to work, no matter how stacked the waiting room is, and deliver excellent care to our patients. We can just do our jobs.

After I walk into the ED I look at the waiting room swarming in high volume. The face-shield is properly tightened on my forehead, "Time to do my job- as part of a big team, coordinated for victory."

Disclosures

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Perspectives

New Opportunities for the Expansion of Telemedicine during the COVID-19 Pandemic

Bryan T. Arkwright, M.H.A.[1]

Abstract

Telehealth / Telemedicine has realized extensive growth through the global presence of COVID-19. The Centers for Medicare and Medicaid Services (CMS) and other federal and state agencies have lifted restrictions as well as enacting both permanent and temporary changes, creating a thriving and more accessible market of telehealth / telemedicine activities, innovations, and advances in care. Healthcare and the world are now amidst a digital health revolution; exploring the opportunities, planning for growth, and applying technology to care in new and exciting ways. This article compares industrial revolution elements and outlines the popular COVID-19 telehealth use cases in the market today as well as emerging future trends and opportunities.

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Introduction

The profound impact COVID-19 has on the world is undeniable and will be measured well into the future, we are only beginning to understand many of the effects and others are still yet to be discovered.

Perhaps one silver lining is the digital health revolution and the new and plentiful opportunities related to the expansion of Telehealth / Telemedicine during the COVID-19 Pandemic.

Merriam Webster defines revolution in a variety of ways; several revolution definitions are related to a celestial body and orbiting, periods of time and motion, and finally; change. The definitions related to change are a great layering of explanation as it pertains to the digital health revolution and emerging opportunities.

Specifically;

"Revolution –

- *2a: a sudden, radical, or complete change*
- *2c: activity or movement designed to effect fundamental changes in the socioeconomic situation*
- *2d: a fundamental change in the way of thinking about or visualizing something: a change of paradigm*
- *2e: a changeover in use or preference especially in technology."*[1]

Each of the change definitions describe to an extent the experience that healthcare providers, leaders, and patients are realizing as telehealth reaches new levels of high

utilization, both in the access and delivery of healthcare in the US and globally.

These straightforward definitions set the stage of understanding as one begins to analyze the recent adoption and utilization of telehealth throughout the pandemic. The significant growth of telehealth during the pandemic is also a product of a series of coverage policies and restrictions being temporarily lifted or enacted by the Centers for Medicare and Medicaid (CMS).[3]

The Fourth Industrial Revolution

Klaus Schwab's 2017 book, "The Fourth Industrial Revolution", describes three reasons that fortifies his belief that we are in fact in a fourth and recognizable industrial revolution. Each of the three reasons describes well the types of impact telehealth has had during the pandemic, and will continue to have.

1. *"Velocity: Contrary to the previous industrial revolutions, this one is evolving at an exponential rather than linear pace. This is the result of the multifaceted, deeply interconnected world we live in and the fact that new technology begets newer and ever more capable technology."*
2. *"Breadth and Depth: It builds on the digital revolution and combines multiple technologies that are leading to unprecedented paradigm shifts in the economy, business, society, and individually. It is not only changing the "what" and the "how" of doing things but also the "who" we are."*
3. *"Systems Impact: It involves the transformation of entire systems, across (and within) countries, companies, industries and society as a whole."*

"In all case, particularly with artificial intelligence, genetic *engineering and other technologies that could conceivably escape our control, we need to take care in building systems that minimize risks and improve the human condition."*[4]

Schwab concludes his explanation of the three reasons with a challenge to society of taking great care in our handling of the decisions to expand, manage, and regulate new technologies like telehealth; holding priority in minimizing risks and improving the human condition.

COVID-19 Telehealth Use Cases in Market Today

The spring of 2020 saw hospitals and health systems who were in a state of zero or limited digital health or telehealth activities aggressively launch and scale telehealth business models and use cases. Opportunity was not always the listed reason for implementation when speaking with healthcare leaders, more often it was in a manner of desperation and need to contain, isolate, and maintain safe access to care due to COVID-19.

The most popular telehealth use cases through COVID-19 include:

- "A patient with mild respiratory symptoms needs evaluation, but has been told not to go to the emergency room;
- A patient has no symptoms of COVID-19 but had contact with someone infected by the novel coronavirus and wants to be evaluated;
- A patient needs care of an unrelated reason (e.g. management of chronic health condition), but cannot go in-person due to clinic closure or fear of coronavirus exposure;
- A provider has been quarantined due to COVID-19, but can continue to see patients from their home via virtual visits; and,
- A patient with severe symptoms of COVID-19 is hospitalized, and needs a specialty consult with an infectious disease doctor in a remote location."[5]

Patients leveraging these popular COVID-19 telehealth use cases were accessing private practice providers, for-profit telehealth provider companies and hospitals and health systems who were offering telehealth services. Providers themselves were learning to prioritize care by any and all means possible, working to deliver care in both an efficient and high-quality, safe manner. Patients have also benefitted beyond the safety and quality of efficient telehealth; now awakened to convenience and new access to their provider many did not have prior, patient centered care is not only a perception but a reality associated with telehealth as shown by satisfaction scores and surveys from COVID-19.[6]

Emerging Telehealth Use Cases in Market

The complexity of these use cases and operating an overall telehealth program are not to be overlooked as these programs and use cases all have unique operations, clinical, technical, and financial elements to them.[5] Taking an active management approach to the operations, clinical, technical, and financial has never been more important to ensuring a program's or service's sustainability and ability to operate within the ever changing and maturing policy and regulatory environment.[7]

The opportunities for telehealth during the COVID-19 pandemic are well documented and proving essential as the US and global population attempts to control and mitigate the spread of COVID-19. However, the near-term future is bright for telehealth as patients, providers, and the healthcare market in general has a newfound confidence in digital health and telehealth.[8] Emerging opportunities the market will see an increase in include but are not limited to:

- Virtual Care Centers or large provider (nurse, nurse practitioner, physician assistant, and physician) staffed call centers with advanced communications technologies;
- Remote Patient Monitoring; large scale population health management of chronic condition patients using telehealth synchronous and asynchronous technologies;
- Behavioral Health and Mental Health applications, services, and networks; while already a well-documented and utilized telehealth use case before and during COVID, the market will see a rise in both the demand and inventory of behavioral and mental health offerings as a result of social distancing, isolation, and long term effects on front line healthcare workers.

Whatever role one plays in the healthcare market, clench the opportunities with telehealth while recognizing and respecting how it links to the US and global economy entering and thriving in the fourth industrial revolution.

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Perspectives

A Rapidly Scalable COVID-19 Surveillance Program Using Real-Time Syndromic Surveillance, In-home Serologic Testing, and Electronic Medical Records

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Introduction

The COVID-19 pandemic has created a worldwide clinical, public health, and economic crisis. In most countries, initial efforts have addressed the clinical challenges of diagnosis and treatment, reconfiguring and supplying healthcare systems to manage surges in clinical volume, and implementing temporary public health strategies to suppress community transmission. However, the next major challenge is to launch population-wide surveillance to further inform clinical preparedness planning and public health interventions[1], and to address major gaps in knowledge about risk factors such as socio-demographic, behavioral and clinical factors[2], transmission dynamics[3,4], seasonal and geographic patterns, correlations between immune markers and reinfection[5], and the long-term immunologic, physiologic and clinical sequelae of infection including mortality.[6]

COVID-19 Surveillance Strategies

A unique dimension of the COVID-19 pandemic from a surveillance perspective is the high percentage of infected individuals lacking symptoms to require clinical care. Current monitoring of clinical volumes and registries of clinically documented cases, while extremely helpful, completely miss this large reservoir of infected individuals that may be driving transmission rates[7,8] and that will be the leading edge of changes in the epidemic. The purpose of this report is to outline key elements of a population surveillance strategy and to describe one currently operational approach that can be rapidly implemented by healthcare systems nationwide.

Effective population surveillance requires sensitive and specific measures of current and previous infection. This has fueled vigorous efforts to develop and validate assays to detect an immunologic response to the virus. These efforts have been challenged by uncertainties concerning the antigen targets, assay platforms, and availability of supply chains needed to support widespread testing. Fortunately, rapid progress is being made and these limitations are being addressed.[9] An early cautionary signal that many true positive cases may not produce typical immunologic responses[10] suggests that other serologic markers and/or other classes of biomarkers may also be important. The projected cost of serosurveillance is another important limitation for a large scale population-based strategy. Due to the uncertainties and costs of serosurveillance, and in light of the large numbers of cases escaping clinical detection, a two-pronged

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approach using simple syndromic surveillance to augment a serosurveillance strategy is warranted. While too nonspecific in isolation, when combined with serosurveillance, syndromic surveillance can expand the reach of a surveillance program to a considerably larger population and reinforce signals or clarify patterns emerging from serosurveillance.

Surveillance Program Requirements

The other requirement for effective surveillance is an implementation strategy to deploy these tests widely and in a manner that yields valid estimates of the population prevalence and incidence of the infection overall, and in important subgroups. This requires thoughtful attention to sampling strategy, participant engagement, data dissemination and other factors that will be necessary elements of a successful COVID-19 surveillance program. There are several desirable features to consider for such an implementation strategy including populations to enroll, participant engagement and testing, use of electronic medical records, real-time analysis, flexible surveillance models, and ease and speed of implementation (Table 1).

The NIH, several medical centers, and other organizations have already launched a variety of population-based surveillance initiatives.[11-16] Collectively, these efforts span a wide range of sample sizes, population types, measures of disease, and participant engagement strategies. Each of these efforts will add important data to the emerging body of evidence about this pandemic. One such program has been implemented at Wake Forest Baptist Health and shortly will be activated at our large regional partner, Atrium Health, with a collective patient population of approximately 12 million people. Several other medical centers in the Mid-Atlantic, South, and the West Coast are making plans to adopt this model as described on the following page.

Table 1. Desirable features of a COVID-19 surveillance strategy

1. Broad Representation	Enroll across all socio-demographic groups, geographic regions, etc. but also with over- sampling of important subgroups that are especially important for community-wide transmission (e.g. children) or at greatest risk for infection or significant morbidity and mortality (e.g. health-care workers, minority populations, the elderly).
2. Remote Participant Engagement and Testing	Use e-informed consent, electronic/mobile technologies and in-home sample collection and testing to avoid the need for face-to-face encounters.
3. Integration of Participant Electronic Medical Record Data	Capture data on confirmatory laboratory testing for SARS-CoV-2 infection, comorbid conditions, concomitant medications, incident hospitalizations, and other important clinical information and outcomes.
4. Real-Time Analytics	Rapidly disseminate information to the CDC, other federal agencies, state and local public health officials and individual healthcare systems.
5. Standardized but Flexible Models	Capable of adjusting surveillance frequency and content in response to new data or research priorities, advances in serologic testing, or changes in public health interventions for suppression, prevention or treatment.
6. Ease and Speed of Implementation	Quickly achieved regional and national coverage.

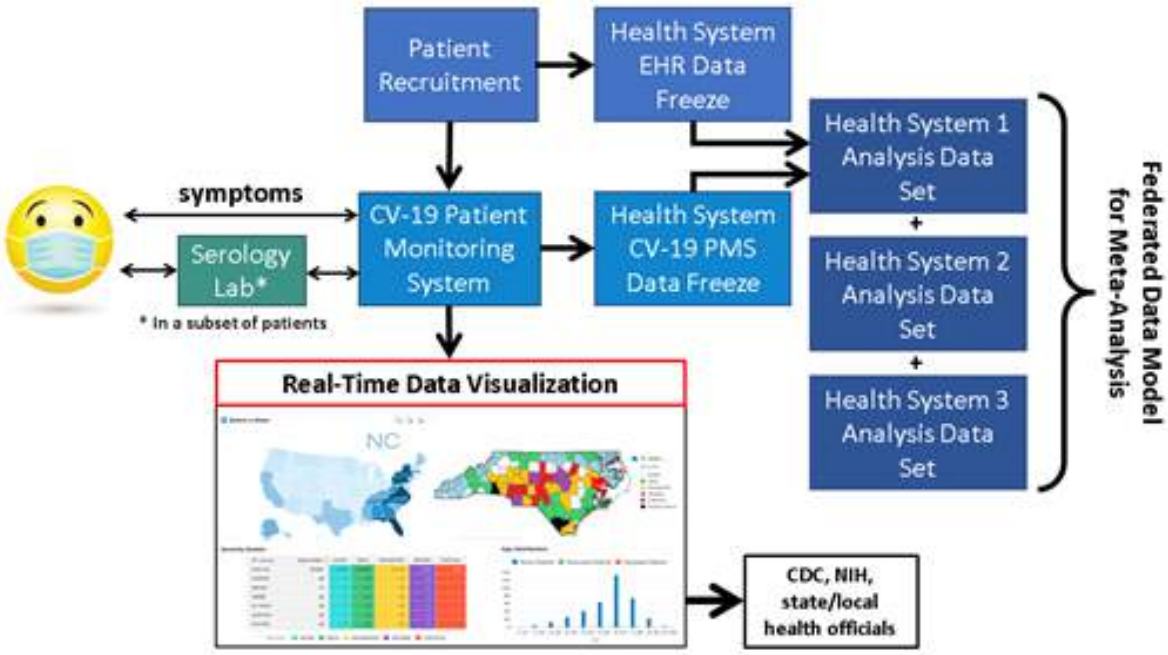

Figure 1. Major design elements of surveillance program.

A Rapidly Scalable Model for Syndromic and Serosurveillance

Figure 1 illustrates the major design elements of this surveillance program. The sampling frame includes all people that received care in our healthcare system. Although not perfectly representative of the population at large, use of collected metadata combined with census tract data will allow statistical adjustments to improve estimates of population prevalence and incidence. Recruitment strategies include use of an electronic patient portal, conventional e-mail, and eventually media channels to solicit invitations, a dedicated patient recruitment website to provide details about the study and obtain informed consent, and a call center to handle project inquires.

Consenting patients receive a personalized and time-limited link to a patient portal that administers a simple baseline questionnaire and solicits daily updates on symptom status, COVID-19 contacts and social distancing behavior. This patient portal, the COVID-19 Patient Monitoring System (PMS) designed by Oracle in close collaboration with federal health officials, clinical investigators at Wake Forest, an integrated clinical research organization (Javara Inc.) and others is able to support millions of patients engaged in daily syndromic surveillance. As a result, there is no practical limit to the number of people in a healthcare system that can be enrolled in this syndromic surveillance platform. The user interface is simple and easy to navigate through email messages or smartphone links. The content is inspired by existing CDC influenza surveillance instruments with additional COVID-19 relevant questions. An important feature of the COVID-19 PMS is a real-time analytics back-end that can produce a variety of visual and tabular reports concerning symptoms and contact reporting and time-trends as a function of socio-demographic factors, zip codes, and other factors. These de-identified summary data are available at any time for use by federal, state and local health officials. Healthcare system-specific data is also available for those contributing patients to the portal.

SAR-CoV-2 Antibody Testing

In-home serosurveillance is accomplished using one of two strategies. In the first, consenting patients are mailed an at-home SARS-CoV-2 IgM & IgG lateral flow test kit (Scanwell Health) and are prompted to download an Android or iOS mobile application that provides step-by-step instructions (short videos, looping GIFs, pictures, and text) on how to complete the test using a drop of blood from a simple finger prick. The application also captures an image of the test cassette and uses computer vision technology to interpret the result (invalid if missing control line, IgM positive/negative, and IgG positive/negative). The results are provided back to the participant using FDA approved language for patients and are automatically returned to the COVID-19 PMS portal. In the second strategy, patients are mailed a simple micro-sampling device to collect a total of 40 microliters of blood using two volumetric absorptive swab tips (Neoteryx) which are returned in the mail for in-lab processing. In–lab high-throughput lateral flow or a similar screening assay is performed with more detailed and quantitative follow-up on positive samples. These strategies are designed to be compatible with social distancing recommendations while in place, and are flexible enough to pivot to alternate in-home or lab-based assays as new information about optimal and sustainable techniques emerge.

Electronic Medical Records

An essential element of this program is the ability to link patient identifiers obtained in the consent process to electronic medical records (EMR). Relevant extracts from the EMR will be combined with syndromic and serosurveillance data to allow detailed analysis of the relationships between prevalent and incident infection and a host of clinical factors such as co-morbid conditions, concomitant medications, incident admissions for potential sequelae of infections, to name a few examples. This design features also provides an opportunity for cooperative meta-analyses using a federated data model to address of important research questions across multiple healthcare systems.

Speed of Implementation

Importantly, the program is designed to facilitate rapid implementation. This is required to assist in the immediate response to the current outbreak, but also to establish a baseline to compare against as public health measures, climatologic conditions and other factors change over the next several months. To support this goal we are making available to any requesting healthcare system a complete portfolio of documents to facilitate local adoption of this program including all IRB and other regulatory documents, all patient recruitment website materials and REDCap programs used to monitor demographics of respondents and to produce data for the COVID-19 PMS portal, standard legal agreements between Oracle and participating institutions that address use of the Patient Monitoring System, and call center scripts to help respond to patient questions about the study mechanics, test kits and results. Dissemination of the design of other existing surveillance programs and coordination among them would also be highly desirable.

Surveillance Costs

No discussion of a surveillance program is complete without a discussion of costs. The current model is designed to support longitudinal syndromic and serosurveillance in an average sized healthcare system for <$20 per syndromic surveillance participant with daily follow-up and <$400 per serosurveillance participant for six serology tests over a one-year period. While these costs are modest on a per participant basis, when applied in a population in sufficient numbers to support reasonable estimates of prevalence and incidence they still translate into significant costs for individual healthcare systems to bear at a time when most normal clinical operations are shut down or are diverted to acute COVID-19 case management. With this commentary, we are urging federal officials and private sector funders to accelerate the mobilization of financial support for this and similar surveillance programs that are desperately needed to help attenuate the transmission and prevent the recurrence of this clinically and economically devastating epidemic.

Implications and Conclusion

As previously noted, the surveillance program described here offers substantial opportunities to expand our knowledge of SARS-CoV-2 epidemiology among the symptomatic but also among the potentially large population of SARS-CoV-2 asymptomatic individuals. Among the many possible uses of this data will be the development of statistical models for identifying and evaluating putative control strategies including population targets of a yet to be developed COVID-19 vaccine. In the long term, this surveillance program will also be critical to the population-level monitoring of seroconversion over time to measure intervention effectiveness such as lock downs and again, vaccines. As the study progresses, data will also be generated in this population to identify clinical sequelae that further strengthen our understanding of the clinical and health system impacts of the pandemic.

Finally, success during this rapidly moving pandemic will require that scientists, clinicians, health system leaders, and funding agencies come together with a level of commitment, effective collaboration, and velocity that has not previously been observed in U.S. and global healthcare.

Disclosures

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Perspectives

Uncertainty and Opportunity

Yenya Hu, M.D., Ph.D.[1]

Spring in academics is a season full of celebrations of accomplishment and expressions of gratitude. Every year, I watch the MD students complete different stages of their training from finishing their first year to getting hooded as these students of-ficially become physicians. I burst at the seams with pride and I have always looked forward to this time of the year. Then, the spring of 2020 happened. Coronavirus disease 2019 (COVID-19) has caused worldwide disruptions in every aspect of life. Medical education is no exception. All of a sudden, distance learning and virtual meetings have replaced face-to-face or bedside teaching. The scheduled board licensing examinations have been canceled or postponed indefinitely. Meticulously organized rotation schedules made just a few weeks ago have been tossed. The celebrations of graduation and hooding ceremonies transitioned completely to virtual events.

Our students have demonstrated extraordinary resilience and grace in this unprecedented time. Some of the first-year students decided to stay in Winston-Salem after the curriculum switched to distance learning, "just in case the hospital needed us." Other students formed a COVID-19 literature search group while others lined up for hospital volunteer positions. Discussion boards were organized to highlight health disparities and racial discriminations currently present locally and nationally. However, with the disruption of the curriculum and rotations, uncertainty levels among the students and faculty inevitably rose.

By definition, uncertainty is a state where things are not known beyond doubt, not clearly identified or defined, or where people do not have specific knowledge. It is woven into both life and medicine. A low level of tolerance for uncertainty is associated with negative outcomes in healthcare, such as burnout.[1] With the high burnout rate among medical students, developing a tolerance for uncertainty is essential.[2]

Uncertainty is interweaved in medical decision making, diagnostic reasoning, and the art of logically and systematically determining the cause of illnesses. However, a search of the goals and objectives of preclinical curriculum mapping revealed that the management of prolonged uncertainty is not a readily visible element. The format of multiple-choice questions (MCQ) is predominant in the assessment in the preclinical M.D. curriculum and the United States Medical Licensing Examination's (USMLE's) Step 1 and Step 2. The hidden curriculum of this type of assessment promotes an unfortunate message to students that there is a "best answer" in all situations and that certainty is expected. Often in medicine, as in life, the only certainty is uncertainty. Tolerance and acceptance of uncertainty are valuable.[3] Currently, teaching the management of uncertainty generally occurs in clinical settings by showing students how to deal with competing for medical evidence through instruction

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and modeling.[4] However, in the preclinical curriculum, the content of deliberately teaching uncertainty is sparse. It can be counterproductive to enforce certainty via the "best answer" in MCQ testing during the first 18-24 months and then expect students to manage or even embrace uncertainty as they enter the clinical curriculum.

Intentionally, integrating training in the management of uncertainty into the preclinical and clinical curriculum is desirable.[3,5,6] It has been integrated into medical decision making and medical humanities before.[7,8] How do we accomplish this goal in the context of a modern, dynamic M.D. curriculum? It is our conviction that the introduction of concepts involving medical uncertainty should occur at the beginning of medical school, in addition to the focus on MCQ performance in the preclinical phase. This should begin in the earliest basic science courses, particularly during Anatomy instruction. What better approach is there than employing anatomic variations to introduce the concept of uncertainty with their students' assigned cadavers, their "first patient?" Stephens and colleagues have found that medical students experience uncertainty within the context of anatomy education.[9]

Similarly, integrating "teaching uncertainty" in the goals and objectives of course mapping into the current preclinical curriculum will make our efforts more intentional and deliberate. Simulation as an instructional method, often deployed in clinical skills and medical decision making instruction, presents an excellent opportunity to engage learners in active learning experiences to promote the management of uncertainty. How will we know that we are successful? One possible method is to incorporate a competency-based assessment with clear and concise developmentally appropriate rubrics into the existing curriculum that is tracked by both the students and their coaches.

With the decision of USMLE Step 1 transitioning away from the 3-digit score reporting, an opportunity to change the culture of certainty in the preclinical phase is on the horizon. Imagine a redesigned preclinical assessment that focuses on the process of learning and less on the final achievement. The assessment would motivate learning and drive curricular design and implementation.[10] Most importantly, the students would have essential training as future physicians in self-assessment, identification of areas needing attention, and outcome-driven improvement.

The uncertainty we are experiencing as a consequence of the COVID-19 pandemic can be stressful, but this lived experience presents an abundance of opportunities to strengthen our preclinical M.D. curriculum. Let us embrace uncertainty and renew our commitment to excellence.

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Perspectives

Out of My Hands: A Third-Year Medical Student's Surgery Clerkship during COVID-19

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I woke up at 4 am on Monday, March 9th, 2020, excited to go into the hospital for my first day of clinical clerkships ever. I was to be on the Transplant Surgery service for the month.

On Monday, March 23rd, 2020, I admittedly woke up at 9 am, not having any obligations until my conference-call after lunch. The coronovirus disease 2019 (COVID-19) pandemic had made itself known in the United States and my classmates and I had been pulled out of our rotations.

It is now Monday, April 27th, 2020, and it's my last week of my "non-clinical" clinical surgery rotation. The COVID-19 pandemic has truly been a lesson in adaptability and practicing gratitude.

As the task force that is at the frontlines of fighting this pandemic, the healthcare community has been at the forefront of having to adapt to all the uncertainty surrounding this situation. This certainly has led hospitals to scramble to adjust to this burden — one of them being the need to temporarily suspend clinical duties for medical students.

I had the privilege of being a medical student during COVID-19, but of all things to learn online, I can assure you that surgery is not the easiest thing to grasp remotely. There was much uncertainty among my classmates on how to achieve a non-clinical clinical rotation. And this same uncertainty was applicable throughout all realms of medicine and healthcare, from medical students unsure how to proceed with their education, patients with conditions that needed to be assessed by their doctors in the office, and transplant surgeons wanting to ensure the safety of their patients during COVID-19.

While I didn't get to have my full three-week experience on the Transplant service, my COVID-19 quarantine afforded me more time for a deeper dive into the realm of transplant surgeries; through my own research, phoning into conference calls of the Transplant Surgery team of my hospital, and the mentorship of my transplant surgeon attending.

I saw parallels in the intricacies that transplant surgeons dealt with regarding patient care during this pandemic, and the compromises that medical students had to make with our educations. Swimming in student loans and our ever-expanding knowledge base of medicine, third-year is our time to figure out what we want to do with the rest of our lives, through which specialty we want to serve our patients in. But I think out of all of this, COVID-19 may be teaching us medical students one of the most

important lessons of being a doctor, a bit early: to accept what comes at you gracefully and adapt.

The surgery faculty at my institution had done just that with our clerkship, providing us with live lectures every day. Normally we are not able to cover all the specialties while we are rotating through, so this has been a wonderful opportunity to hear from physicians in most of the surgical specialties, outside of just transplant, otolaryngology, burns, and trauma that I was going to originally experience. I was also able to discuss pancreas transplantation with my transplant surgeon attending, something I would not have received the opportunity to do otherwise. Upon speaking with my attending, I also learned that our institution was one of the few sites in the area accepting organs for transplantation during this time. Usually, only twenty-five kidney-pancreas transplants occur in a year at our institution, but in the span of a week they did five. I did feel a pang of regret, not being able to experience those surgeries and putting my knot-tying practice to use, or getting to know the patients and witnessing their freedom from dialysis and insulin in their follow-up visits to our transplant clinic.

On March 23rd, I woke up with a multitude of questions about how I would experience surgery without physically scrubbing into cases in the Operating Room. Today on April 27th, those qualms are gone. I feel prepared, now more than ever, to re-start my in-person, albeit shortened, clinical surgery rotation when we can get back in the hospital. I was able to explore and understand surgery in a way that I would not have been able to before, thanks to COVID-19.

My unanticipated surprise from this quarantine was remembering myself outside of medical school. I spent extra time with my family that I had not anticipated. I picked up a pencil to retry my hand at sketching. I played my favorite Bach piece on the piano. I learned how to make café-quality lattes with my sister. This time was a chance to remember ourselves outside of medicine, particularly in the wake of the tireless healthcare workers who could not get to do so, being overwhelmed on the frontlines, braving COVID-19 — thank you for your resilience.

This pandemic was a unique medical school education that perhaps no other class of medical students may experience, at least not anytime soon, hopefully. I've learned to never take things for granted. I do not think I will ever complain of having to wake up at 4 am to have the privilege to round on patients. I've understood that flexibility will be your friend and your commitment to it will bring about innovations. You will be able to turn surgery into an online curriculum, as my professors did. I've seen that in finding the silver lining, it will contribute to that flexibility and innovation. A group of students at my institution and I created a student-led literature review on COVID-19, in the hopes to create a space in which students and clinicians could easily access the most current manuscripts being published. And as important as flexibility and innovation are to have as a physician, so is resilience. During this pandemic, that resilience is paramount as we continue to practice proper social distancing, wash our hands, and wait for this to pass. While I am thankful for the time and experiences that I have been afforded during this quarantine, I am looking forward to feeling that March 9th, 2020 excitement again, when it's time for me to re-start my "clinical" clinical education on June 1st!

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Perspectives

Wake Forest School of Medicine Student Volunteer Engagement During the COVID-19 Pandemic

Abigail Peoples, B.A.[1]

As healthcare systems across the country were stressed by the Coronavirus disease 2019 (COVID-19) pandemic, students stepped up to serve in whatever capacity they could. Through Google Documents, social media, and a GroupMe chat that had over 600 members, students across the county collaborated on projects and shared information about their volunteer efforts.

In Winston-Salem, NC, social distancing and stay at home orders were able to prevent COVID-19 cases from exceeding local hospitals' capacities. Still, the COVID- 19 pandemic caused significant changes to daily life and students at Wake Forest were able to contribute to our local COVID-19 response. When medical students were removed from their clinical learning environment in early March in order to conserve personal protective equipment at Wake Forest Baptist Medical Center, our student body began looking for ways to volunteer their time and skills. Students created projects from scratch and joined existing efforts. These projects and reflections from their student leaders are highlighted below.

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Literature Review Team

Reflection from Student Leaders: Leigh Anne Kline '22 and Shaleen Thakur '22

"The COVID-19 Student Literature Review website began as an endeavor for medical students to assist our Wake Forest community in easily accessing the latest literature on the rapidly evolving severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic. We created a website through which our team of medical students created bite-sized summaries of the latest research as it was published, covering all aspects of the research regarding the pandemic from diagnostics to ethical dilemmas. We were able to widely disseminate the website link across the institution, reaching students, clinicians, and faculty. Overall, the website received a positive response from medical professionals and served as a creative academic opportunity for medical students." The work of the Literature Review team is available on their website: https://sites.google.com/view/wfsom-covid-19/home

Emergency Medical Services (EMS) Reserve Team

Student Leaders: Joseph Humphries '22, Sydney Schieffer '22, and James Jordan '23

In preparation for a possible shortage of EMS workers during the COVID-19 pandemic, medical students volunteered to serve on a reserve team should local EMS need additional personnel. Third and fourth year students along with students with prior EMS experience were eligible to volunteer. The reserve team was not activated.

However, the student leaders coordinated with the School of Medicine and Dr. Tripp Winslow to ensure all student volunteers were appropriately registered for potential volunteer EMS service.

Public Access Screening

Student Leader: Abby Peoples '22

Wake Forest Baptist Medical Center implemented Public Access Screening in early March due to evidence of COVID-19 community spread in Forsyth County. All patients and visitors entering the medical center were required to have their temperature checked and answer screening questions before entering. Some of the designated entrances to the medical center required 24/7 coverage. Many medical students volunteered to staff these screening points. Students played a crucial role in the startup of this new screening system.

Care Packages for Frontline Workers

Reflection from Student Leader: Madison Simas '23

"My Wake Forest Baptist Health (WFBH) family has been nothing but welcoming to me, and I wanted to show them how much I appreciate them while they dealt with the pandemic firsthand. With school online and stay-at-home orders in place, I used social media to reach out for help. Thanks to our Winston-Salem community's support, especially Mr. Bowman Gray and Solarte skincare, I gathered donations for the departments most impacted by the pandemic. When my classmate, Lauren Strickland, and I delivered the care packages, the reception we received made all the work put into this project well worth it."

House Calls

Reflection from Student Leaders: Chloe Ferris '21 and Nikki Mehran '22

"Food security and social isolation are among many challenges that older adults face. These challenges are exacerbated during the COVID-19 pandemic. The WFBH House Call Program provides home health care services to patients in the Winston-Salem community. We recruited 42 health care professional student-volunteers to call 68 pa-tients to assess for safety, social determinants of health, and telehealth readiness during the pandemic. Telephone calls were documented and information was routed to patients' providers who were able to follow-up with patients and provide referrals to patients with food insecurities for a food prescription program."

Senior Services Support

Reflection from Student Leaders: Nikki Mehran '22 and Chloe Ferris '21

"Nikki had the great idea of reaching out to Winston-Salem Senior Services to see if they were in need of volunteers for any of their programs such as Meals on Wheels and Williams Adult Day Care Center. Meals on Wheels had enough volunteers but were interested in recruiting students as back up for meal deliveries. Meals on Wheels was also interested in a food drive. We organized a contactless food drive in which students could drop off food at a Meals on Wheels location without congregating with other students or volunteers."

Novel Mask Design and Testing

Student Leaders: Paula Grisales '22, Jennifer Hadley '22, and Abby Peoples '22

Dr. John Sanders and Dr. Katherine Schaffer sent Abby Peoples '22 an article about making masks out of cotton t-shirts. This team of students used this article as a starting point to design and sew masks made of cotton t-shirts and home air filters. The mask design passed N95 fit testing and was submitted for filtration testing. Paula Grisales '22 reflected on her experience: "When this opportunity became available, it felt like the entire world and our community was getting ready to fall into total chaos. At the same time, I was at home feeling useless and seemingly waiting for things to crumble. I was excited to feel like I was making a contribution to the hospital and my local community, but mostly, I felt like even if it didn't work, I had at least tried instead of waiting for others to hand me the answers. Throughout the process, it was challenging when some of the prototypes failed but it was triply rewarding and exciting when we found designs that worked. Overall, it was intellectually stimulating to work through ideas with colleagues and even though the project did not end in what I expected, it taught me to value the process and take every opportunity that is given."

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I would like to thank the Office of Patient Experience for allowing me to temporarily join their team; I learned so much! Additional thanks to Dr. Marcia Wofford and Dr. Randall Clinch for supporting student engagement in volunteer projects while clinical activities for students were suspended. And of course, thank you to all the student volunteers for their efforts and the reflections they submitted for this article.

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Perspectives

Leading in Society: Learning to Lead on the Sidelines Amidst COVID-19

Isheeta Madeka, B.A.[1] and Roy E. Strowd III, M.D., M.Ed., M.S.[2]

Introduction

The COVID-19 pandemic has rapidly evolved to affect almost every aspect of life. As of July 12, 2020, the United States had more than 3.2 million confirmed cases and 130,000 deaths.[1] Other countries are returning to pre-quarantine normalcy, but cases in the United States continue to rise. In addition to the pandemic, the Black community has once again been the target of racialized violence. COVID-19 and systemic racism are both public health crises facing society. This article details the perspectives of a current fourth-year medical student and an attending physician as they balance the nuances of fulfilling their roles as healthcare providers and respond to the call to lead in society.

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A Medical Student's Perspective

From the start, the pandemic had a profound impact on my personal life. My brother, mother, and father tested positive for COVID-19 in California. Thankfully, my family was able to recover at home. It was anxiety provoking. I struggled to make sense of the constantly evolving situation on my own, and interpreting this for my family felt impossible. As a future healthcare provider, I grappled with the helplessness of being unable to lay my hands on those I love at a time when they needed me the most. I resorted to telephone counseling with dyspnea checks every 6 hours and daily updates to distill information, provide context, and lead from a distance by example.

In March, I was a newly minted fourth-year medical student eager for sub-internships. Then our curriculum quickly shifted to virtual rotations. The months we had spent stressing about our fourth-year schedules suddenly felt futile. Like so much of medicine, we were reminded of the many levers that are outside of our control. As of July, away rotations have been cancelled, USMLE examinations rescheduled, and ERAS application deadlines extended with virtual interviews in place. We continue to live in a period of uncertainty. My classmates and I have embraced flexibility, adaptability, and patience- qualities we have built into our repertoire from the beginning of medical school and now have been thrust into practice. I am both comforted and concerned knowing many medical students are facing similar challenges. Despite our physical and social distancing, we are all in this together.

In many respects, the most difficult challenge has not been any of these concerns: the illnesses of my family, the inability to comfort them, the uncertainty of my current training, or the fear of an unfocused future. The greatest challenge has been far less tangible. Every day I learn about the unimaginable sacrifices that healthcare providers are making on the frontlines of this crisis. I ask with my classmates,

"How can I help?" and "Where can I go?" At our white coat ceremony three years ago, we recited the Hippocratic Oath and were inducted into the profession to serve as leaders. Over the past year, I have further expanded my understanding of interprofessional leadership through the Wake Forest Interprofessional Leaders in Medicine program. I have spent my medical school career witnessing leadership at the bedside and watching humanism through the doctor-patient relationship. Now over the past few months, I have found a unique opportunity to learn an unanticipated new version of leadership — leading in society. I have learned how to be a leader on the sidelines. Whether it be sharing accurate, evidence-based research with friends and family, dispelling myths about mask-wearing on social media, or practicing proper social distancing etiquette, I have set a tone of patience (when possible) and calm resolve (when able) amidst the chaos, uncertainty, and mixed messaging.

Recently, the Black community has once again been the target of brutal, racialized violence. Calls to address systemic racism have reverberated throughout society, including in healthcare. As a future physician, I feel strongly about using the privilege that has been afforded to me to help dismantle structural racism. I attended a local protest with my classmates whilst following proper mask-wearing and social distancing etiquette; I joined a nationwide physician-led book club that aims to unlearn and relearn the nuances of racism in the United States; I am having important, uncomfortable conversations with family and friends to contribute to shifting the conversation and re-focusing our collective discussion on the importance of addressing deeply rooted structural factors that limit workforce diversity, hinder equitable promotion, and maintain antiquated disparities. As part of the next generation of physician leaders, I have recognized that even from the sidelines all healthcare trainees are very much in play. I have learned that adhering to the Hippocratic Oath extends beyond the walls of the hospitals and into every aspect of my life. To me, this means leading as a student-doctor and fulfilling my duty to stand beside frontline healthcare workers. We are all visible and together in this same fight and we have an important responsibility to society. As I step into the next phase of training, I will be ready to fight on the frontlines and lead on the sideline.

A Physician's Perspective

In medicine, we teach many approaches to leadership and place an emphasis on the physician's role in society. The COVID-19 pandemic has provided a unique opportunity to teach how to lead in society often from the sidelines. In many ways, the pandemic has drawn attention to the action inside the bases — do we have sufficient personal protective equipment in our hospitals, ventilators in our intensive care units, and availability of viral testing? We focus on the hot spots and help develop guidelines for policies inside the health system. However, to lead in society, our roles must extend beyond the walls of the hospital. We must lead in wearing masks in grocery stores, social distancing at restaurants, and avoiding large gatherings on beaches. We need leaders who put on their white coat when they leave the hospital as much as when they arrive; who speak up to racism at a sporting event or show up with a mask at a local gathering. Our accountability to society could not be more important amidst the current challenges with the COVID-19 pandemic, systemic racism, pervasive inequities, and a lack of healthcare diversity. As educators, we need to seize this opportunity to educate on leading from the sidelines. We need to lean into our societal responsibility. As our students, colleagues, friends, and family suffer from shock, sickness, judgement, and fear, we must assume our role as leaders from wherever we stand.

Disclosures

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Perspectives

Healthcare Workers' Unique Role in Addressing Anti-Asian American Sentiment During COVID-19

Michelle F. Qiu, B.S.[1]

The coronavirus disease 2019 (Covid-19) pandemic has incited fear and xenophobia towards Chinese Americans. There has been an uptick of anti-Chinese American sentiment, with increased negative rhetoric in the media, physical attacks, and verbal abuse. Due to the difficulty of visually determining someone's nationality, other East Asian groups have been included in the abuse as well. The continued usage of a racialized epithet of the virus ("Chinese virus") in the news and by lawmakers perpetuates and normalizes xenophobia. Healthcare workers are in a unique position to be an ally to Asian Americans, due to their many interactions with patients and credibility within the community.

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The United States has a history of racism towards Chinese people. The most obvious example of this is the Chinese Exclusion Act of 1882, the only race-based exclusion policy the United States has ever passed. It prohibited Chinese people from immigrating to the United States and prohibited citizenship to individuals already here. The Chinese Exclusion Act of 1882 was lifted in 1943 and immigration from China and other Asian countries increased. Now, Asian Americans are the fastest-growing and best-educated racial group in the United States.[1] While strides have been made towards acceptance, the Covid-19 pandemic exponentially increased xenophobia towards Asian Americans, causing financial, physical, and emotional damage.

Much of the anti-Asian American rhetoric conflates Asian Americans with the disease, because the first major outbreak of Covid-19 was in Wuhan, China. In January of 2020, when the virus broke out in China and before it spread to the United States, there was already decreased patronage to Asian American stores, with some reporting an 80% decline in customers.[2] Physical and verbal attacks against Asian Americans increased as well. On February 2, 2020, an Asian woman was called a "diseased b----" and was hit over the head on the New York City subway. On March 14, 2020, three members of an Asian American family, including a 2-year-old and 6-year-old, were stabbed in Texas because the assailant believed they were infecting local residents with the virus. The abuse is so great that the Asian American Pacific Islander (AAPI) Civil Rights Organizations established a website for people to report harassment, shunning, and physical assaults. Since the site's launch on March 19, 2020, they have received over 1,900 reports. The verbal harassment and threats are mirrored online. Twitter has seen a 900% increase in hate speech towards Chinese people due to coronavirus.[3] Even the FBI has warned local law enforcement to prepare for increased hate crimes towards Asian Americans.[4]

Past pandemics have shown us the negative repercussions of naming diseases by geography, which is why the World Health Organization's (WHO) policy for naming diseases is to only use scientific names, not names tied to locations or people.[5] After the Middle East Respiratory Syndrome outbreak in 2012, the WHO cited long-lasting stigmatization of the area and its residents from residual fear. In direct opposition to the WHO's policies, news outlets and lawmakers like President Donald Trump and Secretary of State Mike Pompeo have continually called the virus the "China virus." Conflating the origin of the virus with all Chinese people and their descendants is to dehumanize an entire group of people into harbingers of disease. Even the University California, Berkley, whose student population is 28.6% Asian, assured students that it was okay to feel fear when interacting with "those who may be from Asia," highlighting the normalization of xenophobia.[6] Not addressing these issues for what they are, which is racism, is to slide into complacency.

The potential repercussions include long-lasting stigmatization towards Asian Americans. Even after the pandemic is over, the negative association with Asian Americans could lead to continued verbal and physical attacks as well as economic repercussions from decreased patronage of Asian American owned businesses. Unless allies stand with the Asian American community, the racism will continue.

Healthcare workers have two unique avenues of changing public perception. One avenue is through the personal connection we foster with patients from one-on-one encounters. These intimate interactions can be used to model non-xenophobic behavior and to question patients' negative views. Healthcare workers should only use scientific language to describe the virus and discourage the use of a geographical name. If a patient begins to blame Asian Americans for the virus, healthcare workers can use open-ended, patient-centered communication to ask about the patient's beliefs. Repeating what the patient said back to him/her is a simple way to elucidate his/her thoughts. If the patient's bias is due to false information, such that all Asians have the virus, healthcare workers can educate patient's on how the virus is spread.

Another avenue of change is through the media. The public is looking to healthcare workers now more than ever for advice. As established medical professionals, healthcare workers have credibility and trust from the public, which can be used to speak out against discrimination and myths about the virus. Healthcare workers can use social media platforms, write op-eds for newspapers, or partake in media interviews, as some physicians have already done. For example, Dr. Dagny Zhu, an Instagram-famous ophthalmologist, created a video with other Asian physicians explaining how Asians are not carrying the virus and how in fact, Asian healthcare workers are helping battle it. Her video has been featured on Cable News Network (CNN), Good Morning America, and more.

A fundamental part of healthcare is to be an advocate. Advocacy includes standing up for our colleagues who are discriminated against. There are reports of patients refusing to be treated by Asian doctors and nurse practitioners, who make up 18% and 10% of their fields, respectively.[7] Without Asian healthcare workers taking care of patients due to patients' refusals, the healthcare system will become even more strained. Harassment towards Asian healthcare workers has increased as well. On her way home from work, an anesthesiologist, Dr. Lucy Li, was followed by a man who shouted, "Why are you Chinese people killing us?"[8] Bystanders, especially those who are not Asian American, have significant power to diffuse the situation. Depending on the level of comfort the bystander has, they could redirect the assailant's attention by engaging with him/her, record the encounter, or simply go stand next to the person being attacked. When Dr. Gem Manalo, an anesthesiologist, was verbally harassed on the Boston subway, another passenger assured her they would protect her if the assailant came closer. The bystander's acknowledgement that they saw what was happening and would step in helped her feel much safer.[8]

Treating Asian Americans like they are the disease itself will have long-lasting negative repercussions. Healthcare workers can use their daily interactions with patients and media platforms to take an active stance against discrimination and xenophobia. Covid-19 affects all humans, regardless of their ethnicity or geography. Now is the time for people to unite against a common enemy, to sow seeds of community and care, with healthcare workers leading the charge.

Disclosures

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Perspectives

The COVID-19 Outbreak in Forsyth County Highlights Health Inequities and Calls for Change

Abigail Wehner, B.A.[1] and Tiffany M. Shin, M.D.[2]

Each morning when we check Forsyth County's updated coronovirus disease 2019 (COVID-19) case count, our hearts drop as the impact daily grows more staggeringly imbalanced. The weight of the pandemic here is falling heavily on the shoulders of our minority community, a fact that seems both predictable and preventable. The stage was set for this disparity by pre-existing inequities within our system. In this piece, we provide a closer look at how the COVID-19 pandemic has exacerbated health inequities for Spanish-speaking patients locally, and we propose strategic changes within medical education to address these issues.

There is no doubt that the Hispanic population nationwide has faced more significant financial and health impacts of COVID-19 than their white counterparts, including testing positive for coronavirus at disproportionately higher rates.[1,2] As of this article's writing, patients identifying as Hispanic account for 63% of positive COVID-19 cases in Forsyth county, even though Hispanics represent only 13% of the population.[3,4] Early data from New York City demonstrated that Hispanic people were dying from the virus at twice the rate of white people[5,] and similar trends have occurred throughout the country.

Health information about COVID-19 for Forsyth County from the health department and local hospitals is difficult to find in Spanish, even though the information in English exists. County and city coronavirus websites are without readily accessible translations. Lack of information and care in Spanish impacts patient safety and health outcomes[6] particularly as 41% of Hispanics in the U.S. have limited English proficiency.[7]

Disparities for Spanish speakers likewise are evident in access to healthcare. Nationally, marginalized communities are less likely to have adequate testing centers.[8] Winston-Salem was weeks behind the local coronavirus outbreak in establishing testing locations in the predominantly Black and Hispanic neighborhoods east of Highway 52.[9] Additionally, the pandemic necessitated the rapid transition to telehealth, but Spanish-speaking patients have encountered many barriers, including difficulties using our hospital's online patient portal that is available only in English and unable to host multiple users to perform a video visit with an interpreter.

These disparities call for careful reflection and change, and we must examine our approach to medical education — seeing it as essential to promoting health equity. At our institution, critical work is occurring to address health inequities for Spanish-speaking patients. Faculty are participating in the voluntary development of Spanish-language COVID-19 materials to improve patients' access to information. Students

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and faculty are working to raise community awareness of the disparities in Forsyth County. The medical school's MAESTRO (Medical Applied Education in Spanish through Training, Resources, and Overlearning) program is actively training linguistically competent, culturally humble providers who can provide patient-centered care in Spanish.

We need to prioritize building a healthcare workforce that better supports the Spanish-speaking community by recruiting diverse medical students, resident physicians, attending physicians, nurses, social workers, and other healthcare professionals, including those who identify as Hispanic and who are fluent in Spanish. Additionally, we must train more providers to provide language concordant care to our country's growing Spanish-speaking population. Currently, many U.S. medical schools report medical Spanish educational efforts, ranging from formal courses to ancillary programs to student-led efforts.[10] While these efforts play a crucial role in preparing students to care for Spanish-speaking patients, they often are limited in the number of students they can support and the depth of education they can provide. Building the infrastructure to support a longitudinal, comprehensive medical Spanish curriculum requires significant work and commitment.[11] Ideally, medical Spanish education should be closely integrated with the core curriculum with the opportunity to receive formal credit. English medical language proficiency is never presumed, so students' ability to communicate medical information to patients is assessed through clinical skills exams throughout medical school. Likewise, medical Spanish language proficiency should not be presumed even though a student may be a native or advanced Spanish speaker. Spanish-speaking students still need formal training and formal assessment of patient communication skills in Spanish.

While our focus here is on the Spanish-speaking population, these strategies apply to other languages and cultures. Communication skills training is a core component of medical education, and this training must encompass the ability to communicate well with patients of all backgrounds and languages, including those with limited English proficiency. Students must grasp the importance of language concordant health information and care so that as future healthcare leaders, they can better understand and prioritize their patients' needs. Our diverse community and the world beyond, need medical providers who can meet them where they are. One potential solution is developing and implementing a curriculum for all learners that confers skills for effective in-person and virtual interpreter use. Additionally, incorporating cultural humility education gives students a framework for lifelong self-reflection to continually address their beliefs and biases and how they impact the care they provide.[12]

As it stands, our healthcare system is struggling. It seems insufficiently equipped to provide care for all members of our community and, without change, will only self-propagate these disparities. The current pandemic did not break the system; rather, it shed light on its inequities. These problems did not arise overnight, and solutions will take significant commitment and time. As medical professionals, we should not settle for the status quo, but rather we should acknowledge these inequalities as our responsibility to overcome for the sake of our patients, their families, and their communities. May we do our utmost to uphold our duty to serve our patients well.

Disclosures

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Perspectives

American Policy and Political Polarization during the COVID-19 Pandemic

Lauren N. West-Livingston, PhD., M.S.L.[1]

In recent years, conversations surrounding health policy in the United States have centered around rising costs, limited access, gaps in coverage, and disparities in care. The global pandemic caused by the coronavirus SARS-CoV-2 has exposed the glaring deficiencies in healthcare infrastructure in the United States, and has expedited the need to reassess local, state, and federal responses to massive health crises.

While the first cases of the novel pneumogenic virus in Wuhan were reported by the World Health Organization (WHO) in December 2019, the first case in the United States was confirmed by the Centers for Disease Control (CDC) on January 21, 2020.[1] The precipitous spread of the virus outpaced expectations and containment efforts in numerous countries, leading to an expeditious rise in global cases and subsequent deaths. As of July 2020, the United States recorded staggering statistics, indicating over four million cases and over 140,000 deaths.[2] During a time of collective global suffering and reflection, many have interrogated how a high-income country* and self-described global superpower became a universal cautionary tale for public health policy.[2]

Statistics and Policy Data

In order to examine how the United States response to the COVID-19 pandemic compares to other nations, it is vital to assess the extent to which the virus has relatively impacted each populace. While in July 2020, the United States was the world leader in total cases and deaths resulting from SARS-CoV-2 infections, a per capita analysis of the data reveals the position in the global community. In July 2020, the United States ranked eighth worldwide in total cases with 1,324 cases per 100,000 people, and tenth in the world in COVID-19 related deaths, with 46 deaths per 100,000 people (Figure 1A&B).[3] Notably, the top 10 countries in each of the aforementioned categories primarily consists of other high income countries, with upper middle income countries ranking below the United States in total cases, and only one upper middle income country ranking higher than the United States in deaths per capita.[2,3] Furthermore, when examining the policy initiatives taken by the United States to combat the spread of the novel coronavirus, efforts can be contextualized alongside 16 "peer" countries with comparable economic profiles and healthcare systems. These countries include Australia, Austria, Canada, Denmark, Finland, France, Germany, Italy, Japan, Norway, Portugal, Spain, Sweden, Switzerland, the Netherlands, and the United Kingdom4. Among these peer nations, the United States ranked first in total cases per 100,000 people, and fifth in deaths per 100,000 people (Figure 1C&D).[3,4] Relatively speaking, the United States has done a poor job of containing the outbreak of COVID-19 cases in comparison with other countries comparable in economic standing.

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FOOTNOTE:
*Based on country income designations made by the World Bank, as an effort to avoid describing countries with the less popular and potentially xenophobic designations of "developed," "developing," or "underdeveloped."

A

Country	Total Cases	Per 100,000
Qatar	109,957	3,940
Bahrain	39,482	2,516
San Marino	699	2,069
Chile	347,923	1,858
Oman	77,904	1,613
Kuwait	65,149	1,575
Panama	61,442	1,471
United States	4,332,369	1,324
Armenia†	37,629	1,275
Peru†	389,717	1,218

B

Country	Total Deaths	Per 100,000
San Marino	42	124
Belgium	9,822	86
United Kingdom	45,878	69
Andorra	52	68
Spain	28,436	61
Italy	35,123	58
Peru†	18,418	58
Sweden	5,702	56
Chile	9,187	49
United States	149,071	46

C

Country	Total Cases	Per 100,000
United States	4,332,369	1,324
Sweden	79,494	781
Spain	280,610	601
Portugal	50,410	490
United Kingdom	300,692	452
Italy	246,488	408
Switzerland	34,609	406
The Netherlands	53,647	311
Canada	114,597	309
France	183,079	273
Germany	206,242	249
Denmark	13,811	238
Austria	20,677	234
Norway	9,142	172
Finland	7,404	134
Australia	15,304	61
Japan	31,657	25

D

Country	Total Deaths	Per 100,000
United Kingdom	45,878	69
Spain	28,436	61
Italy	35,123	58
Sweden	5,702	56
United States	149,071	46
France	30,209	45
The Netherlands	6,164	36
Canada	8,901	24
Switzerland	1,703	20
Portugal	1,722	17
Germany	9,122	11
Denmark	613	11
Austria	713	8
Finland	329	6
Norway	255	5
Japan	1,005	<1
Australia	167	<1

†Only countries not designated "high income" by the World Bank. Armenia and Peru are classified as "upper middle income" countries[2].

Figure 1. Statistics on total cases and deaths per 100,000 people in (A) 10 countries with highest number of COVID-19 cases per 100,000 people, (B) 10 countries with highest number of COVID-19 related deaths per 100,000 people, (C) Number of COVID-19 cases per 100,000 people for the United States and peer countries, and (D) Number of COVID-19 cases per 100,000 people for the United States and peer countries.[3,4]

To compare policy initiatives in the United States and aforementioned 16 peer countries, the stringency level of national response can be examined with data from the Oxford University COVID-19 Government Response Tracker.[5] This instrument allows comparison between national responses in policies focused on containment, economic measures, and health-related measures. Through the coding of national responses in areas such as school and workplace closings, income support, public health campaigns, and many other policy measures, multiple countries were assigned stringency levels of policy implementation (Figure 2). Based on these criteria, the United States ranked third in most stringent levels among the previously mentioned peer countries in terms of policy implementation as of July 1, 2020.[5]

This comparison implies that the United States has failed to contain the spread of the SARS-CoV-2 virus, but is able to mitigate deaths resulting from infection to some degree, relative to our peer nations. Furthermore, the United States is among the countries with the most strict policy initiatives. This compels interrogation into which aspects of healthcare in the United States are creating the conditions that have resulted in widespread infection and mass casualty during the COVID-19 pandemic.

Country	Policy Stringency Level	Policies Pertaining to Closures and Containment								Policies Pertaining to Economic Measures		Policies Pertaining to Health Measures		
		C1	C2	C3	C4	C5	C6	C7	C8	E1	E2	H1	H2	H3
Australia	72.96													
United Kingdom	71.30													
United States	69.98													
Canada	69.98													
Germany	69.98													
Portugal	67.13													
Denmark	62.96													
Italy	55.56													
France	48.15													
Austria	43.52													
The Netherlands	39.81													
Sweden	38.89													
Switzerland	35.19													
Norway	34.26													
Spain	33.80													
Finland	29.63													
Japan	24.07													

Policies Pertaining to Closures and Containment

Variable	Definition	Coding
C1	School closing	0 - no measures 1 - recommended closing 2 - require closing at specified levels 3 - require closing at all levels
C2	Workplace closing (work-from-home)	0 - no measures 1 - recommended closing 2 - require for some sectors 3 - required for all but essential
C3	Cancel public events	0 - no measures 1 - recommended cancellation 2 - required cancellation
C4	Restrictions on gatherings	0 - no restrictions 1 - restrictions on >1000 people 2 - restrictions on 101-1000 people 3 - restrictions on 11-100 people 4 - restrictions on <10 people
C5	Close public transport	0 - no measures 1 - recommended closing or reduced service 2 - required closing
C6	Stay-at-home requirement	0 - no measures 1 - recommended 2 - required with exceptions for essential activities 3 - required with minimal exceptions
C7	Restrictions on internal movement	0 - no measures 1 - recommendd not to travel between regions/cities 2 - internal movement restrictions in place
C8	Restrictions on international travel	0 - no measures 1 - screening 2 - quarantine arrivals from high-risk areas 3 - ban arrivals from some areas 4 - ban all regions or border closure

Policies Pertaining to Economic Measures

Variable	Definition	Coding
E1	Income support	0 - no income support 1 - government replacing <50% of lost salary 2 - government replacing >50% of lost salary
E2	Debt/contract relief (stopping loan repayments, banning evictions, etc.)	0 - none 1 - specific relief 2 - broad relief
E3	Fiscal measures (economic stimulus policies)	Monetary value (USD) of fiscal stimuli, including tax cuts not reflected in E4, H4, and H5
E4	Providing support to other countries	Monetary value announced

Policies Pertaining to Health Measures

Variable	Definition	Coding
H1	Public information campaigns	0 - none 1 - public officials urging caution 2 - coordinated campaign
H2	Testing policy	0 - none 1 - symptomatic with specific criteria 2 - anyone with symptoms 3 - open public testing
H3	Contact tracing	0 - none 1 - limited tracing 2 - comprehensive tracing
H4	Emergency investment	Record monetary value of short-term spending on health initiatives
H5	Investment in vaccines	Record monetary value

Figure 2. Comparison of stringency level of policy for the United States and 16 peer nations determined by the Oxford University COVID-19 Government Response Tracker.[4,5]

Unique Aspects of Health Policy in the United States

The approach to healthcare in the United States is fascinating in that there are various contributing factors to how health policy is implemented and how care is rendered. Fundamentally, the United States Constitution asserts that any powers not explicitly delegated to the federal government are reserved to state governments.[6] While healthcare is not delegated directly to the federal government, the Department of Health and Human Services is a federal, cabinet-appointed agency that oversees a great deal of health policy and legislation.[6,7] Furthermore, state and local health departments both support federal regulations, and create policy based on regional needs.[6,7] This dynamic has created variability between the approach of each state in initial efforts to contain and manage the spread of COVID-19. States that allowed business to reopen earlier than others, including Alabama, Colorado, Georgia, Mississippi, Tennessee, and Texas, saw an increase in deaths and total cases by 3 weeks of reopening, despite a decline in number of daily tests.[8] Adherence to federalism in public health policy is one of the major barriers in the ability to unify efforts in mitigating the spread of infections.

Another factor of the United States healthcare system to consider is the advanced technology available for patient care, associated with high costs. The United States is revered for cutting-edge medical treatments and approaches, however these modalities are associated with greater financial burden to patients.[6] The option for progressive treatments may explain why despite having the highest number of COVID-19 cases per capita, the United States did not lead peer countries in deaths per capita resulting from infection. However, the United States is also infamous for health disparities, barriers to access, and lack of insurance coverage for a large portion of the population.[6] These factors, along with difference in state approaches to stringency of policy, likely explain why people who are Black, Indigenous, persons of color, of lower socioeconomic status, or uninsured have fared worse throughout the pandemic concerning morbidity and mortality.[9]

Turbulent Waves: Outlook on the Course of the Pandemic Moving Forward

There has been ample discussion surrounding the "waves" of the pandemic, with many nations seeing a dissipation of the first "wave" of cases while actively preparing for the second during the typical fall flu season.[10] Meanwhile, the United States is still grappling with devastating losses from the first "wave," as many states are in the process of re-opening businesses and schools. Furthermore, protests have erupted in favor of re-opening the economy and lambasting the call for wearing masks in public, replete with mask-burning demonstrations.[11] Opponents to social-distancing and public mask mandates cite conspiracy theories and infringement on personal freedoms as reasons to ignore the global crisis, with these opinions further stoked by the President's vocal skepticism of federal and global agencies like the CDC and WHO.[11] This has led to the pandemic becoming a polarizing political issue, as opposed to a public health crisis. This may be another major cause for why the United States was among the highest cases per capita in the world, as well as why policies were among the most stringent. Perhaps an early laxity in containment, coupled with vocal opposition to current measures, necessitated implementation of stricter policies to address the astronomical rise in cases and spur public cooperation. Thus, the stringency of policies in the United States may have been more of a requirement to address uncontrolled spread, as opposed to rigorous prevention of spread.

As the COVID-19 pandemic continues, American people and policy makers will need to unite behind efforts toward containment. This will require an unprecedented shift from the quintessentially American mindset of individualism, to that of efforts toward collectivism for public betterment. Failure to recognize the potential of an unmitigated outbreak during flu season could lead to cataclysmic levels of infection, morbidity, and mortality, especially with a novel virus with an emerging disease process and unknown long-term effects. While policy and legislation are the tools the United States will need to navigate the upcoming challenges with COVID-19, solidarity and empathy should be our guiding principles.

Disclosures

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Perspectives

A Celebration Overwrought with Hesitation and Isolation

Sarah Jane Sabanis, M.Ed.[1]

Giving Birth During a Pandemic: One Patient's Perspective

"Be sure to make a birth plan."

"Don't make a birth plan, labor and delivery never goes as planned."

Expecting and new parents are bombarded with information and suggestions from everyone they meet. Most families welcoming a new family member at this point in time did not conceive during the pandemic. In 2018, an average of 325.9 babies were born each day in North Carolina.[1] Assuming this trend, this means numerous families have given birth during the state of emergency enacted on March 10, 2020 by Governor Roy Cooper.[2] My due date was April 8, 2020.

Prepartum

When I was eight weeks pregnant in September 2019, I interviewed a doula with the assumption that they would be able to support my husband and I during the birth of our first child. In mid-March 2020, hospitals across the country started implementing strict visitor guidelines. Some systems enacted a policy to allow no one to be present, while others limited a laboring mother to only one support person. I feared that my birthing hospital would ban all support people and I would be alone.

My doula was texting me daily, asking for updates on what our birthing hospital had implemented. When the news broke that I could only have a single support person, I was filled with anxiety and dread. I had to pick between my husband and the doula and ultimately went with my husband. I could not deprive him of being there for the birth of our first child. My doula was kind and understanding, but I felt like I no longer had control over my birth. The plan was already falling apart before labor even began.

On March 16th, I was deemed to be in a high risk population for coronovirus disease 2019 (COVID-19) and was sent home to work. I spent my days watching the news unfold on the pandemic taking a hold all across the globe. Everyone appeared to be working from home, even the newscasters. People were protesting being forced to wear masks. It felt like the world had turned upside down as the number of cases in the United States rose.

Antepartum

My contractions started on April 2nd at 4am. Due to the pandemic, I did not feel comfortable having my doula come over to my home to help me labor. My doula

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was not only a doula, but also a social worker in Guilford County. Because of her occupation in light of the pandemic, I did not want to have her come to my home. This led my husband and I to repeatedly go to the hospital during labor, increasing the risk of contracting the virus. I was admitted on the fourth time I had gone to the hospital in 48 hours from the initial onset of contractions at 6am on April 4th.

As an anxious first time mother, it was hard to only be able to receive comfort from the healthcare workers' eyes. Bedside manner is a critical part of medical training, since most people are not their best selves in the hospital setting and look to those providing care for comfort. I would not be able to pick the healthcare workers who saw me at my most vulnerable out of a line-up. Every worker kept a mask on during the entirety of my care. I received updates about the labor progression and tried to decipher their emotions just by what I could see in their eyes.

I was lucky compared to other women who gave birth just weeks after me. My husband and I did not have to wear masks throughout our stay. We did not have to be tested for COVID-19 upon admission to labor and delivery. My husband was able to come and go as he pleased to get food from outside the hospital and to take care of our dogs at home.

One potential solution to put laboring mothers at ease is upon entry to a patient's room, having the healthcare worker remove their mask, introduce themselves, and place the mask back on from over six feet away, meeting social distancing protocols. This would only be applicable to mothers who tested negative for COVID-19.

Another solution would be to have healthcare workers show their identification badge. However, some workers have not updated their badge since they have been hired, thus not showing an accurate representation. A fun solution to the anonymity of the medical worker could be just fun polaroids tacked up in the room.

Postpartum

The fear of contracting COVID-19 meant that I was not able to get aftercare support from family members. My mother had planned to stay for a week to allow me to sleep when my son was two weeks old, but since she works with the public at the department of social services, she was unable to come. We did not allow anyone to enter our house until our son was 4 weeks old, and even then, they had to be freshly showered in clean clothes with no stops between their house and ours. Everyone had to scrub their hands upon entry to the house. If they had not been practicing social distancing or self-isolating, they were not allowed to come

Figure 1. Maternal grandparents meeting their first grandchild through "corona-vision."

When my son was one week old and we were settled into the house, my husband set up a "corona-vision" window to allow our family and friends to meet our new family member. This was inspired by the Macy's windows he saw growing up in New York City. Since we could not allow people into our home, we introduced our son to everyone through the window, maintaining the social distancing rules and trying to share our joy with our loved ones. While it was not how we imagined introducing our son to our community, it did serve as a little beacon of light during a dark time.

over. Even to this day, I am worried about taking my son to family members' homes due to the potential exposure risk. When my son was one week old and we were settled into the house, my husband set up a "corona-vision" window to allow our family and friends to meet our new family member. This was inspired by the Macy's windows he saw growing up in New York City. Since we could not allow people into our home, we introduced our son to everyone through the window, maintaining the social distancing rules and trying to share our joy with our loved ones. While it was not how we imagined introducing our son to our community, it did serve as a little beacon of light during a dark time.

Figure 2. Son looking out through "corona-vision."

Conclusion

Having a baby is anxiety-inducing, with or without a global pandemic. Expecting and new parents have been forced to navigate a challenging time with more obstacles added. Support during each partum period has been uprooted, forcing patients and their families to adapt to a changing climate while balancing the introduction of a new family member. No one knows what it is like to give birth during a global pandemic other than the people who have done it. Even the most well-intentioned and well-researched birth plan can be thrown out the window in an instant as hospital policy changes. Because of the fluid situation, it is important to consider alternative ways to offer support and emotional connection to help families through a momentous occasion.

Disclosures

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Perspectives

Cultivating Resiliency in Turbulent Times

Paige Bentley, Ph.D.[1]

Background

Shock. Sadness. Anxiety. Grief. Just some of the feelings that arose in March 2020 when the world began to feel surreal. They are also feelings that arose in me years earlier when I received a diagnosis that would change my life.

Prior to Memorial Day in 2007, I never really thought about resilience. Then, in an instant, my life changed. When my body starting acting "weird," I told myself: "It's probably nothing. I've just been stressed." Then came the call. "I hate to do this over the phone," the doctor said. "The test came back positive for multiple sclerosis." In that one moment, my sense of identity and the direction of my life changed forever.

I felt flattened and lost. Moments of mourning the loss of who I used to be and the person I wanted to be collided with anxious fretting about what I was sure was a bleak future. As it turns out, my body was not the enemy. My mind was. As a counselor, I am fortunate to have many psychological tools at my disposal. Even so, healing from the emotional impact of this experience required a commitment to putting those tools into action.

This experience comes back to me as I contemplate recent events in the world, including the global COVID-19 pandemic as well as civil unrest related to issues of social justice and systemic racism. The emotional toll these worldwide crises have and will continue to take on our collective psyche is daunting. Not only are we navigating how to manage our internal experiences of distress, but we are also taking in significant external stressors on a daily basis.

For those in healthcare, factors such as the increasing number of confirmed cases, an intense workload, uncertainty about personal protection, lack of known treatments, fears about being at higher risk, concerns about access to healthcare for all, and moral dilemmas related to care decision-making may add to the risk of mental health issues.[1] For students and faculty in the health professions, the challenges extend to disruption and uncertainty in training and/or research, questions about how to be of service when opportunities may be limited, and concerns about peers and family from whom they may be distanced.[2] Furthermore, we are witnessing death, cruelty, and injustice on a daily basis, which begs us all to look closely inside and ask challenging questions about who we are, who we want to become, and how we intend to get there.

Times of pervasive upheaval and change such as these are understandably wrought with emotion. The human brain is hardwired to ensure we are safe and avoid or be

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cautious in situations that feel uncomfortable or threatening.[3] Furthermore, letting go of the known and moving into the bewildering liminal space of in-between time can leave us feeling disoriented and untethered.[4] Despite this reality, we do not have to languish. We have the potential to flourish.[5]

Resiliency is defined as the ability to recover and even thrive from adversity.[5,6] It is not a hard-wired trait or a limited resource. It is a capacity that is cultivated when we face challenges in life. Researchers and theorists in the fields of positive psychology, mindfulness, and somatic trauma healing among many others have contributed much to the growing body of literature on resilience. [5,7-9] They are finding that we have within us the capacity for self-regulation that allows us to not only survive adversity, but even thrive. We seem designed to adapt. They are also finding that there is much we can do to strengthen our resilience in the face of difficult experiences.[6]

After my diagnosis, I leaned on practices I learned in my training to help process the grief and find a way to live meaningfully with my new reality. The work was not always easy, but it was fruitful. I offer below three practices from the fields of mindfulness and positive psychology that were particularly helpful for me as I worked to integrate the reality of MS into my life. These include self-compassion, shifting our mindset, and taking meaningful action.

Self-Compassion

The pioneering work of Kristin Neff teaches that the practice of self-compassion can help us acknowledge both our internal and external reality — a necessary step for shifting out of a fear reaction and into a growth response.[10] Moreover, compassion practices, in general, have been shown to activate the joy-reward circuitry of the brain in contrast with empathy, which activates the pain circuitry.[11] Self-compassion involves three steps. The first step is mindfully turning toward our experience and acknowledging what is here with curiosity and kindness. The second step is remembering that we are human and that others are suffering in similar ways. The final step is sending ourselves an aspirational wish to be freed from our suffering (e.g., "May you be peaceful").[10] Many people shy away from offering themselves self-compassion under the false belief that it is self-indulgent or will keep them from achieving their goals. The opposite is actually true. Only when we can acknowledge what is really here can we begin to take action toward change. For example, only when a person can say, "I feel horrible because I realize I have implicit bias," can they begin to do the work of change.

In these challenging times, I am practicing self-compassion more than ever. Life's difficult feelings seem to come faster and more intensely of late. I have found that when I get stuck in the thinking patterns that reflect a non-accepting attitude of reality such as: "I do not like this. This feeling needs to go away," the emotion and accompanying body sensations typically get worse. When I notice myself going down this path, I pause and then practice naming what I am feeling. "Overwhelm is here." "Anxiety is arising." "Sadness." I remind myself that I am human, so, of course, I am feeling this way. Then I send myself a compassionate wish: "May you trust in your ability to adapt." "May you find a sense of ground even in the midst of uncertainty." "May you begin to feel a sense of ease inside your body." "May you find peace."

Shifting Our Mindset

Having a framework for how to think in times of upheaval can help us identify thoughts that are helpful and thoughts that are unhelpful.[5] In a groundbreaking study in the late 1970s, Suzanne Kobasa found evidence that challenged the belief that stressful events must be debilitating and suggested that three specific personality qualities related to mindset (i.e., mental behavior) help to mitigate the impact of stressful events.[12] These include commitment, control, and challenge, which she described as the 3Cs of stress hardiness.[12] Commitment refers to a tendency to identify aspects of the current experience that are meaningful and purposeful and persist toward a long-term goal despite obstacles. Control refers to recognizing where one can be influential and avoiding trying to change the things we can't. Finally, the challenge quality reflects an awareness that change is a part of life and a bias toward seeing change as an opportunity for stimulation and growth rather than a threat. In these times, there is much that we can't control, but we can control how we think and where we focus our attention. We can ask ourselves: Where will I put my limited energy? What larger value am I committed to serving? Am I willing to seize this as an opportunity for growth?

Meaningful Action

A critical step in bouncing back from adversity is turning toward meaningful action in the world.[6] Having the sense that one's existence is meaningful or purposeful is considered a critical component of life satisfaction and well-being.[13-15] Action tends to have a strong effect on mood, and reminds us that we are not helpless.[6] Furthermore, action begets motivation, which can be low during times of distress. If I wait around to feel like doing something, I likely will never do it. If I push myself over that "activation energy hump" and get started, the likelihood is that I will be more motivated to do it again. This action does not have to be dramatic. It could be as simple as saying thank you to the mail carrier or helping someone with groceries. In this time of quarantine and social distancing, even small actions such as these that build interpersonal connection are helpful for cultivating our sense that we can make it through difficult times.

It is important during these times of upheaval and change to pay attention to how we are thinking and what we are doing, and, as best we can, choose thoughts and behaviors that support our overall peace of mind and well-being. My own experience reminds me that even in the midst of uncertain times, we can remain mentally healthy and cultivate our capacity for resiliency. It requires a willingness to be open to the disorienting and sometimes painful feelings inherent in the process of change. As we turn toward our experience rather than away from it, we may find our difficult feelings to be pivotal internal guides, deepening our connection to what is most important in our lives.

For me, as I worked through the transition from my old identity to being someone who permanently lives with this uninvited guest of MS, I ultimately arrived at a mental place where I could acknowledge the reality before me. Even though I did not like it, it was here. I focused on what I could control rather than what I could not — most of the time. I began to look on my diagnosis as a gift rather than a threat. I began to take action in the world that aligned with my values, and I used my diagnosis as a daily reminder that moments are all we have. In the words of Albert Camus I learned, "In the midst of winter, I at last discovered there was within me an invincible summer."[16]

Disclosures

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Original Science

Racial and Ethnic Differences in Clinical Characteristics and Outcomes from COVID-19

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Abstract

Background: Studies on coronavirus disease 2019 (Covid-19) suggest that racial disparities, along with age, gender, obesity, and specific comorbidities like cardiovascular disease and diabetes, strongly influence outcomes. Racial differences may be associated with comorbidities or social factors. In this analysis, we studied outcomes by race with an emphasis on an outbreak occurring in a work setting that affected several ethnic groups.

Methods: In a retrospective, observational cohort study, we examined patients in the Wake Forest Health Network who tested positive for SARS-CoV-2 via qualitative polymerase-chain-reaction assay between March 17 and June 2, 2020. We stratified patients based on self-reported race and ethnicity. The primary end points of this study were hospitalization and in-hospital death.

Results: A total of 783 patients who tested positive are included in the analysis. Of these patients, 52% were males, 39% Hispanic, 33% White non-Hispanic, 17% Black non-Hispanic, 11% Asian, and 1% Other. Of the 783 patients, 225 (29%) required hospitalization, and 93 (41%) of hospitalized patients required care in the intensive care unit (ICU). Of hospitalized patients, 35% were White non-Hispanic, 35% Hispanic, 23% Black non-Hispanic, and 6% Asian. Of patients in the ICU, 37% were Hispanic, 34% were White, 23% were Black, and 6% were Asian. There were 140 workers who tested positive related to a work setting in a city served by the Wake Forest Health Network. In a multivariate analysis, increasing age, Hispanic race, asthma, and coronary artery disease were independently associated with increased odds for hospital admission. In a multivariate model, increasing age, Hispanic race, Asian race, and transfer from an outside hospital to Wake Forest Baptist Medical Center were independently associated with in-hospital mortality.

Conclusions: In a single-center cohort of Covid-19-positive patients, Hispanic and Asian race were independently associated with in-hospital mortality after adjusting for differences in age, race/ethnicity, gender, BMI, diabetes, asthma, and coronary artery disease.

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Introduction

Coronavirus disease 2019 (Covid-19), the disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first reported in Wuhan, China in December 2019 and quickly spread throughout the world. In February 2020, the first case in the United States was documented in Washington state.[1] As of June 29, 2020, the United States has had approximately 2.5 million confirmed cases and 125,000 deaths.[2] The clinical presentation of Covid-19 varies along a spectrum from mild to severe, with a higher proportion of severe cases affecting older adults with chronic comorbidities such as cardiovascular disease, diabetes, and lung disease.[3] In addition to identifying these diseases as important risk factors for developing severe illness, studies also suggest that increasing age and male gender increases the risk for poor outcomes.[3–5]

Recent studies have explored the impact of race and ethnicity on outcomes of Covid-19 and reveal that Black non-Hispanic (hereafter referred to as Black) patients are disproportionally affected compared to White non-Hispanic (hereafter referred to as White) patients.[6] However, there is limited data on outcomes for Hispanic patients. In North Carolina, Hispanic individuals represent 9.6% of the North Carolina population yet comprise 46% of affected Covid-19 cases.[7,8] While differences in comorbidities could be a factor explaining differences in outcomes between races and ethnicities, social factors are likely also important.[6,9] Working conditions of essential workers that require close proximity to others in congregate work settings may explain, in part, socioeconomic disparities.[10] Factory outbreaks occurred throughout the United States during the months of April and May, 2020, including an outbreak at a work setting in a city served by the Wake Forest Health Network.

At the time of this investigation, North Carolina had 63,484 confirmed cases and 1,325 deaths.[8] Forsyth County reported 2,978 confirmed cases with 34 deaths. The objective of this report is to describe the clinical characteristics of patients treated in the Wake Forest Health Network and examine the outcomes among each race and ethnicity.

Methods

Study Design and Data Collection

This retrospective, observational study included patients from the Wake Forest Health Network who tested positive for SARS-CoV-2 by qualitative polymerase-chain-reaction assay between March 17 and June 2, 2020. In-hospital deaths were assessed through July 4, 2020. Forsyth Country and neighboring counties are primarily served by two healthcare systems. The Wake Forest Health Network includes patients treated by Wake Forest Baptist Medical Center, High Point Medical Center, Lexington Medical Center, and Wilkes Medical Center; these centers are included in this analysis. The Other healthcare system is a larger health network that also cares for patients in Forsyth County and surrounding counties; patients treated by the Other system were unable to be obtained and are therefore not included in this analysis. The institutional review board at Wake Forest Baptist Medical Center approved this study (IRB00064578).

Clinical data were obtained manually from the electronic medical record and entered into the Research Electronic Data Capture software (REDCap, Vanderbilt University). Information collected included demographics, medical comorbidities, symptoms at presentation, medical complications, and laboratory values upon first medical contact (whether it was in the outpatient setting, in the Emergency Department, or on the floor of the hospital). Comorbidities were ascertained directly from chart review of the admission. ZIP codes were used to assess the location of confirmed cases and were visualized with Google Cloud Platform's Geocoding API and Maps Static API.[11] Patient ZIP code coordinates were mapped with a jitter function to maintain confidentiality.

Statistical Analysis

Descriptive statistics were used to summarize the data according to race and ethnicity (Asian, Hispanic, Black, White, and Other). Asian includes individuals of Middle Eastern origin. The race classified as Other contains four patients, two of whom self-identified as American Indian or Alaska Native, one Native Hawaiian or Pacific Islander, and one who did not identify with any race or ethnicity. Continuous measures are represented as medians and

interquartile ranges after evaluating for normality by the Shapiro-Wilk test. No imputation was made for missing data. The percent of each of the variables missing is described in Supplemental Table 1.

Risk factors for hospitalization were determined by applying multivariate logistic regression. Risk factors associated with in-hospital death were evaluated with Cox proportional hazards models. Model covariates were selected through a combination of clinical relevance (race, ethnicity, obesity) and stepwise variable selection models (transfer to Wake Forest Baptist Medical Center from an outside hospital, tobacco use within the last 30 days, and history of cancer) that minimized the Akaike information criterion (AIC). Additional covariates included in the models were those previously shown to be associated with the outcome, including age, gender, diabetes, asthma, and coronary artery disease. Odds ratios (OR) and hazard ratios (HR) along with 95% confidence intervals and p-values were generated, and both the odds and hazard ratios for Asian and Black race, and Hispanic ethnicity, are relative to White race. P-values were calculated using the Fisher's exact test for proportions and the Kruskal-Wallis test for medians. A p-value of < 0.05 was considered to be statistically significant. Analysis was performed using R v4.0.0 software in RStudio.

Results

Characteristics of Covid-19-Positive Patients

A total of 783 patients who tested positive for SARS-CoV-2 are included in this analysis. Among these patients, the median age was 48 years (IQR, 33-61) and 72% of all patients were less than 60 years old (Table 1). 52% of patients were male. Most of the patients in our cohort were Hispanic (39%) or White (non-Hispanic) (33%), while 17% were Black (non-Hispanic), 11% were Asian, and 1% were Other.

While all groups reported similar levels of known exposure to a source of transmission (approximately 40-50% of patients from each race had a known exposure), the median age among each racial and ethnic group showed marked variation. Asian and Hispanic patients were younger, with a median age of 40 years (IQR, 28-56) for Asian patients, 41 years (IQR, 27-50) for Hispanic patients, 55 years (IQR, 38-66) for Black patients, and 58 years (IQR, 45-67) for White patients (Figure 1A). In accordance with the younger mean age observed, Asian and Hispanic patients had a statistically significant lower prevalence of hypertension, coronary artery disease, and chronic obstructive pulmonary disease. Asian patients had the lowest prevalence of obesity ($p < 0.001$), defined as a body-mass-index of 30 or greater, whereas Black patients had the highest prevalence of cerebrovascular disease ($p = 0.007$).

The frequency of symptoms at the initial visit that prompted Covid-19 testing were similar among each group (fever, dyspnea, cough, myalgia), although Black patients were less likely to present with sore throat ($p < 0.001$) and Asian patients were less likely to experience dyspnea ($p = 0.002$) or diarrhea ($p = 0.009$). A lower percentage of Black patients (34%) initially presented to an outpatient setting vs. the emergency room compared to White (51%), Hispanic (51%), and Asian (49%) patients ($p = 0.008$). Patients from all groups sought initial medical attention after a similar duration of symptoms (4-5 days). A total of 17 patients were transferred to Wake Forest Baptist Medical Center from regions across North Carolina (Supplemental Table 2). Of these patients, 13 (76%) were from rural counties (as defined by the North Carolina Rural Center[12]).

Outcomes of Hospitalized Patients

A total of 225 (29%) of the patients who tested positive for Covid-19 were hospitalized. Of those patients who tested positive in the emergency department (ED), 61% of White patients, 58% of Black patients, 54% of Hispanic patients, and 33% of Asian patients were subsequently admitted to the hospital. Of those patients who tested positive in the primary care setting, 2% of White patients, 2% of Hispanic patients, 2% of Black patients, and 0% of Asian patients were sent to the ED for hospital admission. Of all patients admitted to the hospital, White patients and Hispanic patients comprised the majority (35% and 35% of all hospitalized Covid-19 patients, respectively). Black patients constituted 23% of hospitalized patients and Asian patients 6%. All racial groups had elevated markers of inflammation on initial presentation to the ED, including elevated LDH, C-reactive protein, and D-dimer levels (Table 2).

The most common acute medical complications that developed among hospitalized patients were acute kidney injury and acute respiratory distress syndrome (Table 3). Among the

225 patients admitted for hospitalization, 93 (41%) required care in the ICU. The proportion of hospitalized Hispanic, White, Black, and Asian patients admitted to the ICU were similar (40-43%). Of patients admitted to the ICU, a majority of Hispanic patients (56%) were younger than 60 years old. In contrast, a minority of Black (29%), White (19%), and Asian (0%) patients in the ICU were less than 60 years.

To compare the severity-of-illness among Covid-19 patients requiring care in the ICU, SOFA scores for individual patients were obtained upon entry into the ICU and we subsequently calculated the mean SOFA score for each group. Black patients, on average, had a higher severity-of-illness as measured by a median SOFA score of 4, whereas the median SOFA score was 2 for Hispanic patients, 2 for White patients, and

Table 1. Baseline Characteristics of Covid-19 Patients

Characteristic	Overall	Asian	Hispanic	Black non–Hispanic	White non–Hispanic	Other	*p*
N	783	84	307	130	258	4	
Age (years) – median [IQR]	48 [33, 61]	40 [28, 56]	41 [27, 50]	55 [38, 66]	58 [45, 67]	50 [46, 54]	<0.001
Gender (male) – no. (%)	411 (52)	59 (70)	165 (54)	64 (49)	120 (47)	3 (75)	0.002
Tobacco use in last 30 days – no. (%)	56 (7)	7 (9)	14 (5)	13 (10)	22 (9)	0 (0)	0.177
Nursing home – no. (%)	31 (4)	1 (1)	1 (0)	7 (5)	22 (9)	0 (0)	<0.001
Coexisting conditions							
Hypertension – no. (%)	267 (44)	15 (31)	69 (30)	64 (60)	118 (55)	1 (50)	<0.001
Coronary artery disease – no. (%)	30 (5)	3 (6)	4 (2)	4 (4)	19 (9)	0 (0)	0.01
Hypercholesterolemia – no. (%)	97 (21)	10 (24)	20 (12)	24 (28)	43 (27)	0 (0)	0.002
Congestive heart failure – no. (%)	18 (3)	1 (2)	2 (1)	4 (4)	11 (5)	0 (0)	0.078
Cerebrovascular disease – no. (%)	30 (5)	0 (0)	5 (2)	11 (10)	14 (6)	0 (0)	0.007
Obesity (BMI ≥ 30) – no. (%)	282 (36)	4 (5)	111 (36)	62 (48)	104 (40)	1 (25)	<0.001
Asthma – no. (%)	64 (11)	4 (8)	18 (8)	19 (18)	23 (11)	0 (0)	0.092
COPD – no. (%)	33 (5)	0 (0)	0 (0)	6 (6)	27 (12)	0 (0)	<0.001
Diabetes – no. (%)	154 (26)	11 (22)	59 (26)	35 (33)	49 (23)	0 (0)	0.311
CKD ≥ Stage 3 – no. (%)	43 (7)	4 (8)	8 (3)	11 (10)	20 (9)	0 (0)	0.054
Cancer – no. (%)	15 (2)	2 (4)	1 (0)	5 (5)	7 (3)	0 (0)	0.041
HIV – no. (%)	8 (1)	0 (0)	3 (1)	5 (5)	0 (0)	0 (0)	0.016
Symptoms							
Cough – no. (%)	512 (65)	50 (60)	200 (65)	84 (65)	174 (67)	4 (100)	0.479
Fever – no. (%)	478 (61)	54 (64)	189 (62)	74 (57)	158 (61)	3 (75)	0.815
Dyspnea – no. (%)	253 (32)	13 (15)	101 (33)	52 (40)	85 (33)	2 (50)	0.002
Myalgia – no. (%)	266 (34)	32 (38)	99 (32)	42 (32)	89 (34)	4 (100)	0.077
Headache – no. (%)	224 (29)	20 (24)	95 (31)	33 (25)	73 (28)	3 (75)	0.173
Fatigue – no. (%)	181 (23)	17 (20)	62 (20)	30 (23)	71 (28)	1 (25)	0.286
Sore throat – no. (%)	135 (17)	19 (23)	62 (20)	7 (5)	44 (17)	3 (75)	<0.001
Diarrhea – no. (%)	94 (12)	3 (4)	30 (10)	21 (16)	40 (16)	0 (0)	0.009
Location of presentation (%)							0.008
Primary care	377 (48)	41 (49)	158 (51)	44 (34)	132 (51)	2 (50)	
Emergency department	387 (49)	42 (51)	138 (45)	85 (65)	120 (47)	2 (50)	
Other or unknown service area	18 (2)	0 (0)	11 (4)	1 (1)	6 (2)	0 (0)	

Table 2. Laboratory Findings of Covid-19 Patients

Characteristic	Overall	Asian	Hispanic	Black non-Hispanic	White non-Hispanic	Other	*p*
N	387	42	138	85	120	2	
Vitals signs on admission							
Systolic blood pressure (median [IQR])	127 [114, 143]	132 [121, 138]	118 [110, 135]	131 [114, 150]	132 [118, 145]	144 [144, 144]	0.004
Heart rate (median [IQR])	90 [78, 102]	90 [82, 99]	92 [81, 102]	90 [80, 99]	85 [77, 103]	76 [76, 76]	0.569
Oxygen saturation (median [IQR])	95 [94, 98]	96 [94, 98]	95 [94, 98]	95 [94, 97]	95 [93, 97]	96 [96, 96]	0.85
Supplemental oxygen (median [IQR])	2 [1, 4]	0 [0, 0]	2 [2, 6]	3 [2, 5]	2 [2, 3]	N/A	0.04
Serum laboratory measures on admission							
Sodium (median [IQR])	135 [133, 138]	132 [128, 136]	136 [132, 137]	136 [134, 139]	136 [133, 139]	132 [132, 132]	0.01
Potassium (median [IQR])	4 [4, 4]	4 [3, 4]	4 [4, 4]	4 [4, 4]	4 [4, 4]	3 [3, 3]	0.187
Bicarbonate (median [IQR])	24 [22, 26]	24 [22, 26]	23 [21, 25]	26 [23, 27]	24 [22, 26]	N/A	0.104
BUN (median [IQR])	17 [11, 28]	15 [9, 22]	12 [8, 19]	18 [11, 32]	19 [14, 33]	14 [14, 14]	<0.001
Creatinine (median [IQR])	1 [1, 1]	1 [1, 1]	1 [1, 1]	1 [1, 2]	1 [1, 1]	N/A	<0.001
AST (median [IQR])	38 [27, 55]	49 [28, 56]	38 [30, 55]	35 [28, 69]	37 [24, 53]	65 [65, 65]	0.655
ALT (median [IQR])	29 [22, 42]	27 [24, 52]	35 [25, 47]	27 [19, 39]	28 [18, 39]	45 [45, 45]	0.129
LDH (median [IQR])	296 [235, 426]	303 [272, 341]	340 [268, 477]	318 [215, 422]	236 [208, 322]	N/A	0.016
Hemoglobin (median [IQR])	13 [12, 15]	15 [13, 16]	14 [12, 15]	13 [12, 14]	14 [12, 15]	18 [18, 18]	0.026
White-cell count (median [IQR])	6 [5, 9]	6 [4, 8]	8 [5, 11]	6 [5, 9]	6 [4, 7]	8 [8, 8]	0.001
Platelet count (median [IQR])	183 [138, 233]	134 [112, 209]	201 [160, 254]	178 [138, 226]	168 [129, 211]	184 [184, 184]	0.031
C-reactive protein (median [IQR])	104 [56, 151]	72 [53, 81]	122 [75, 203]	92 [40, 138]	84 [61, 149]	N/A	0.314
ESR (median [IQR])	50 [38, 66]	7 [7, 7]	45 [44, 49]	56 [39, 69]	63 [56, 68]	N/A	0.057
Procalcitonin (median [IQR])	1 [0, 2]	0 [0, 0]	1 [0, 1]	2 [1, 2]	2 [1, 6]	N/A	0.112
PT (median [IQR])	12 [11, 13]	12 [12, 12]	11 [11, 13]	13 [11, 15]	11 [11, 15]	N/A	0.636
PTT (median [IQR])	30 [27, 33]	33 [33, 33]	29 [27, 32]	30 [29, 36]	30 [26, 33]	N/A	0.704
D-dimer (median [IQR])	970 [600, 1910]	850 [410, 1710]	740 [445, 1815]	1505 [738, 2225]	820 [710, 2010]	N/A	0.232

Table 3. Treatments and Outcomes for Hospitalized Covid-19 Patients

Characteristic	Overall	Asian	Hispanic	Black non-Hispanic	White non-Hispanic	Other	*p*
N	225	14	79	51	80	1	
Admitted to ICU – no. (%)	93 (41)	6 (43)	34 (43)	21 (41)	32 (40)	0 (0)	0.992
Required mechanical ventilation – no. (%)	45 (20)	5 (36)	18 (23)	11 (22)	11 (14)	0 (0)	0.269
Treatments							
Remdesivir – no. (%)	41 (18)	2 (14)	18 (23)	8 (16)	13 (16)	0 (0)	0.734
Tocilizumab – no. (%)	6 (3)	0 (0)	3 (4)	1 (2)	2 (2)	0 (0)	0.923
Hydroxychloroquine – no. (%)	45 (20)	3 (21)	12 (15)	9 (18)	21 (26)	0 (0)	0.466
Convalescent plasma – no. (%)	24 (11)	4 (29)	11 (14)	4 (8)	5 (6)	0 (0)	0.101
Azithromycin – no. (%)	89 (40)	7 (50)	32 (41)	19 (37)	30 (38)	1 (100)	0.701
Corticosteroids – no. (%)	18 (8)	2 (14)	9 (11)	1 (2)	6 (8)	0 (0)	0.206
Complications							
AKI – no. (%)	24 (11)	2 (14)	6 (8)	7 (14)	9 (11)	0 (0)	0.652
ARDS – no. (%)	14 (6)	1 (7)	6 (8)	3 (6)	4 (5)	0 (0)	0.891
Coagulation disorder – no. (%)	6 (3)	0 (0)	4 (5)	0 (0)	2 (2)	0 (0)	0.433
Bacterial pneumonia – no. (%)	7 (3)	0 (0)	3 (4)	2 (4)	2 (2)	0 (0)	0.876

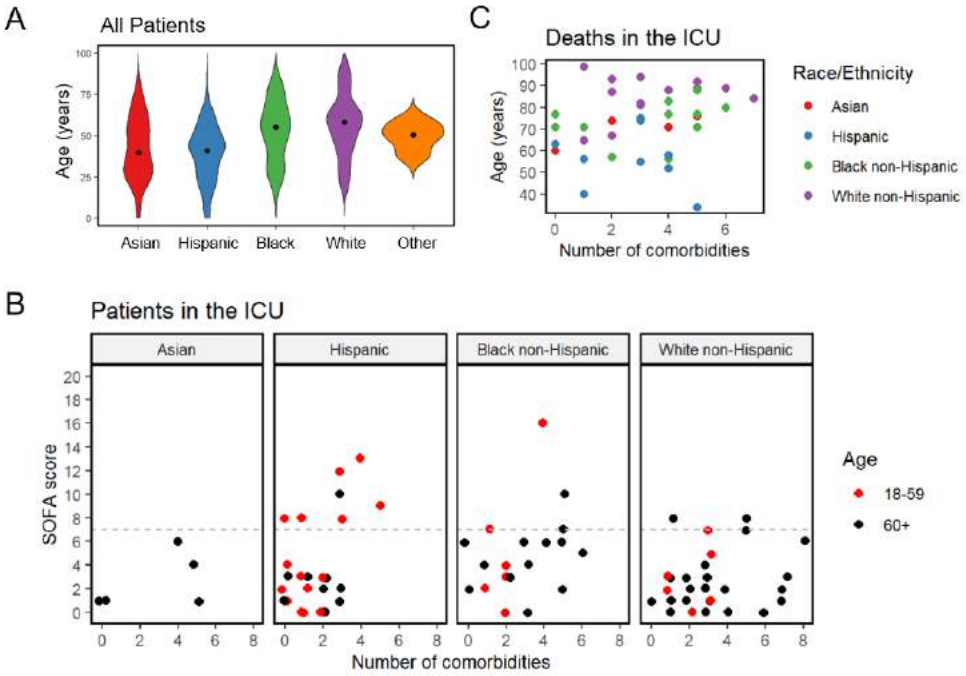

Figure 1. Covid-19 differences among racial and ethnic groups.

(A) A distribution of ages is shown for all Covid-19-positive patients for each racial and ethnic group as a violin plot, which displays the probability density for each age. Median age for each race and ethnic group is shown within each violin plot. (B) All patients in the ICU are stratified by race and ethnicity and plotted according to their severity-of-illness (as measured by SOFA) and respective total number of comorbidities. A threshold SOFA score of 7 is shown. (C) Patients who died in the hospital are plotted according to their age and respective total number of comorbidities.

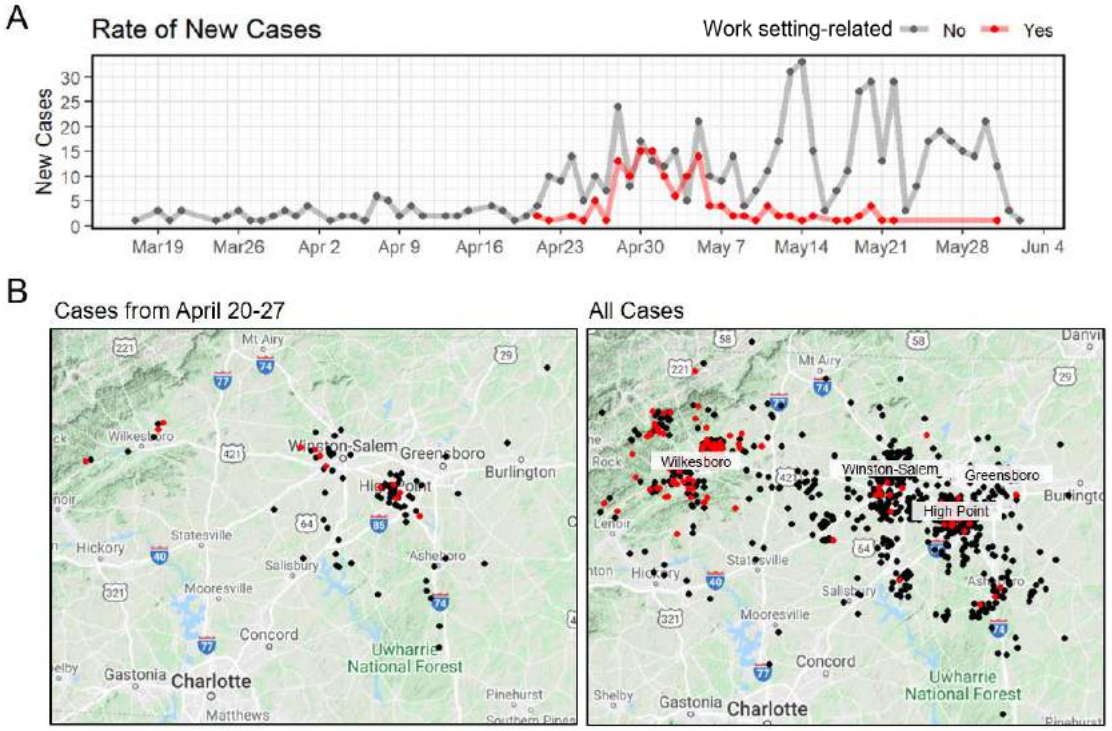

Figure 2. Work setting outbreak and its relationship with community spreading.

(A) Rate of new cases for work-related and non-work-related patients is shown over time. (B) Spatial information of affected patients is shown using each patient's ZIP code. The initial incidence of work-related cases is shown (left) and for all affected cases (right). This data represent cases treated within the Wake Forest Health Network and is not a comprehensive description of the outbreak.

1 for Asian patients. Examining the SOFA scores and the number of comorbidities for each patient did not reveal a strong relationship (Spearman rank correlation, $\rho = 0.22$), suggesting that a higher severity-of-illness is not adequately explained by having more comorbidities (Figure 1B). Six middle-aged Hispanic patients (age group, 18-59 years) developed severe disease as exemplified by a SOFA score of 7 or higher, whereas patients of the same age group from other races mostly had SOFA scores less than 7.

Across all racial groups, the time from hospital admission to initiation of mechanical ventilation was similar (2-3 days). Of Asian patients in the ICU, 83% required invasive mechanical ventilation. This is in contrast to the proportion of Hispanic (53%), Black (53%), and White (34%) patients in the ICU who required mechanical ventilation. 40% of all patients received azithromycin, 20% received hydroxychloroquine, 18% received remdesivir, 11% received convalescent plasma, 8% received corticosteroids, and 3% received tocilizumab. Of the 38 patients that died in the hospital, 34% were White, 29% were Black, 24% were Hispanic, and 13% were Asian. Of the 9 Hispanic patients that died, 6 (67%) were under 60 years. In contrast, of the 13 White patients that died, none were under 60 years (Figure 1C). The overall unadjusted case-fatality rate among admitted, hospitalized Covid-19-positive patients was highest for Asian patients (36%) compared to Black (24%), White (20%), and Hispanic patients (15%).

Outbreak at a Work Setting

During late April and early May, a Covid-19 outbreak occurred at a work setting located in a city served by the Wake Forest Health Network. Of note, this investigation includes only those patients treated in the Wake Forest Health Network and is not a comprehensive description of the outbreak. From April 20-27, there were a few initial work-related cases (Figure 2A) which preceded the larger work-related outbreak. The incidence of all cases treated in the Wake Forest Health Network, both related to the work setting and unrelated, is shown in Figure 2B.

Of the 783 patients in our analysis, 140 (18%) were workers or family members of workers at the work setting. Of these 140 patients, 121 were employees and 19 were family members, with 49 (35%) White, 38 (27%) Hispanic, 24 (17%) Black, and 29 (21%) Asian. Most of these patients presented directly to the ED, and 12 (24%) White patients, 9 (24%) Hispanic patients, 1 (4%) Black patient, and 1 (3%) Asian patient were admitted for hospitalization. A total of 23 work-related patients were admitted for hospitalization and 11 of these patients required care in the ICU. Of the 9 Hispanic patients admitted, 6 (67%) were admitted to the ICU. Of the 12 White patients admitted, 5 (42%) were admitted to the ICU. Four of the 6 work-related Hispanic patients in the ICU (67%) required mechanical ventilation, while only 1 of 5 work-related White patients (20%) required mechanical ventilation. Four work-related Hispanic patients died during hospitalization, and all other work-related patients were eventually discharged from the hospital. Of the 4 work-related patients that died, 3 were employees and 1 was a family member of an employee.

Risk Factors for Hospitalization

In a multivariate analysis, Hispanic ethnicity (odds ratio, 2.09; 95% confidence interval [CI], 1.10 to 4.03), increasing age (odds ratio, 1.05; 95% CI, 1.03 to 1.07), asthma (odds ratio, 2.42; 95% CI, 1.11-5.27), and coronary artery disease (odds ratio, 3.08; 95% CI, 1.23-8.16) were independently associated with an increased odds of hospital admission (Table 4). The association between an increasing BMI with increased hospital admissions trended toward statistical significance. Female gender (odds ratio, 0.44; 95% CI, 0.26-0.73) and recent tobacco use (odds ratio, 0.26; 95% CI, 0.06-0.81), defined as tobacco use within 30 days prior to testing positive for Covid-19, were independently associated with a lower odds of hospital admission. In our cohort, Asian, Black, and Other race were not associated with increased odds for hospital admission.

Table 4. Multivariate Odds Ratios for Hospitalization

Variable	Odds Ratio (95% CI)
Asian	0.59 (0.19-1.69)
Hispanic	2.09 (1.10-4.03)
Black non-Hispanic	1.76 (0.91-3.42)
Other	3.00 (0.11-80.11)
White non-Hispanic	1
Age	1.05 (1.03-1.07)
Female gender	0.44 (0.26-0.73)
BMI	1.02 (0.99-1.05)
Diabetes mellitus	1.62 (0.94-2.78)
Asthma	2.42 (1.11-5.27)
Coronary artery disease	3.08 (1.23-8.16)
Tobacco use (within last 30 days)	0.26 (0.06-0.81)

Risk factors Associated with In-Hospital Death

In a multivariate time-to-event analyses, increasing age (hazard ratio, 1.10; 95% CI, 1.05-1.15), Hispanic ethnicity (hazard ratio, 7.04; 95% CI, 1.89-26.30), Asian race (hazard ratio, 6.96; 95% CI, 1.42-34.22), and transfer to Wake Forest Baptist Medical Center from an outside hospital (hazard ratio, 3.73; 95% CI, 1.14-12.24), were independently associated with in-hospital mortality (Table 5). Notably, Black race was not associated with in-hospital death relative to White race. Of the 10 Hispanic patients that died (4 were related to the work setting, 6 were unrelated), 8 (80%) required translators and 3 (30%) did not have a documented primary care physician (PCP). Of the 11 Black patients that died, 1 (9%) did not have a documented PCP. Of the 13 White and 5 Asian patients that died, all of them had documented PCPs. No other patients that died required translators.

Table 5. Multivariate Hazard Ratios for In-Hospital Death

Variable	Hazard Ratio (95% CI)
Asian	6.96 (1.42-34.22)
Hispanic	7.04 (1.89-26.30)
Black non-Hispanic	2.85 (0.95-8.51)
White non-Hispanic	1
Age	1.10 (1.05-1.15)
Female gender	1.18 (0.51-2.71)
BMI	1.01 (0.96-1.08)
Diabetes mellitus	0.93 (0.44-1.97)
Asthma	1.04 (0.19-5.58)
Coronary artery disease	0.77 (0.21-2.77)
Cancer	2.08 (0.62-7.01)
Transfer to WFBMC	3.73 (1.14-12.24)

Discussion

This study aimed to identify the characteristics and clinical outcomes among different racial and ethnic patients with Covid-19 in western North Carolina. The majority of patients in our study were under 60 years old and were White or Hispanic. Males and females were equally affected, although males were more likely to be hospitalized. Unlike other reports, Black patients did not comprise the majority of cases.[6,13] On average, Hispanic and Asian patients were 20 years younger than Black and White patients. This observation was not explained by a higher prevalence of comorbidities such as cardiovascular disease, pulmonary disease, obesity, or diabetes. Of all patients in our analysis, less than one-third of patients were admitted to the hospital. Among hospitalized patients, slightly more than one-third were admitted to the ICU. The case-fatality rate among all patients with confirmed Covid-19 in our study was 5% which is higher than the 2.6% case-fatality rate across the United States although the duration of time used for the United States case-fatality rate is as of October 28, 2020 and is likely decreased compared to the start of the pandemic as evidence for effective treatments has emerged (see discussion below regarding the RECOVERY trial)[14] in addition to the more widespread use of testing in asymptomatic patients (i.e. pre-procedural).

In our study, we observed differences in the presenting symptoms and complications among the different racial groups. The most common presenting symptoms of Covid-19 were similar to previously described findings with the addition that Asian patients were less likely to report dyspnea or diarrhea and Black patients were less likely to report sore throat.[15–17] While all patients presented for medical care after 4-5 days of symptoms on average, Black patients were less likely to present to the outpatient setting for testing and were more likely to initially present to the ED. Hispanic and Asian patients were more likely to develop a higher severity-of-illness at younger ages and had a higher increased risk of death. Interestingly, transfer to Wake Forest Baptist Medical Center from an outside hospital was found to be an independent risk factor for mortality. While a majority of transferred patients lived in rural counties, the need for transfer is likely an indicator of severity of disease rather than an indicator of a geographical risk factor (i.e. increased number of comorbidities, poorly controlled comorbidities, regional outbreaks with a particularly virulent strain) given that the multivariate model controls for comorbidities including cardiovascular disease, diabetes mellitus, and obesity. Furthermore, a majority of transferred patients had unique and geographically diverse ZIP codes which suggests against localized outbreaks of a more virulent strain as a possible cause of the increased risk for poor outcomes. Consistent with findings from other studies[6,18,19] Covid-19 had a disproportionate impact on Black, Hispanic, and Asian patients. While similar proportions of patients from each racial group were admitted to the ICU, Black patients in the ICU had the highest severity-of-illness score on average (as

measured by SOFA). However, Black race was not found to be an independent risk factor for hospitalization nor death, suggesting that other factors such as the interplay between socioeconomics and comorbidities likely contributed to a higher severity-of-illness. Furthermore, a disproportionate number of younger Hispanic patients had higher SOFA scores compared to other young patients from other racial groups. This trend is also seen among the patients who died: a majority of the Hispanic patients who died were less than 60 years of age, whereas only a minority (or none) of Asian, Black, and White patients were in this same age group. Similar to our findings, data from the Centers for Disease Control suggests that Hispanic patients between the ages of[40-59] are infected at a rate that is five times higher than that of White patients of the same age group[20,21], and that more than 25% of Hispanic patients who died were less than 60 compared to only 6% of White patients of the same age group.

The reasons why non-White patients, especially Hispanic and Asian patients, are at an increased risk of death is likely multifactorial. One possible explanation is that these patients may have a stronger host immune response against the viral pathogen with subsequent generation of a cytokine storm and acute respiratory distress syndrome. For example, Puerto Ricans are disproportionately affected with asthma in part because of the presence of specific asthma-susceptibility loci.[22,23] It is possible that other genetic loci contribute to the aggressive host immune response to SARS-CoV-2, such as the ABO locus genotype.[24]

The cohort studied in this investigation were treated prior to the preliminary findings of the RECOVERY trial, which demonstrated that dexamethasone administered to patients with an oxygen requirement decreased mortality.[25] These findings have since influenced our institution's treatment protocol. Similarly, the use of remdesivir was low in this cohort, though similar across all groups.

Additional explanations that might account for the disparate proportion of minority groups affected by Covid-19 include socioeconomic factors such as employment status, residential segregation, economic inequality, and healthcare access. The observation that Black patients were less likely to present to a primary care provider may suggest unequal access to care as outpatients, which in turn may be associated with the chronic development of other comorbidities that were shown to be risk factors for hospitalization. This may also be, in part, explained by distrust in the healthcare system due to a history of receiving unequal care.[26] Of the patients that died, not all Hispanic and Black patients had a documented primary care physician, and it is possible that these patients had unknown medical comorbidities that likely contributed to hospitalization and death but were unable to be accounted for in our multivariate model. In contrast, White and Asian patients that died all had established primary care physicians.

Many of the patients were also essential workers (food service, healthcare, transportation) who were unable to work from home and whose jobs required close contact with others. Early on in the outbreak, the use of masks was not considered as vital in reducing the spread of the virus and it is possible that the lack of mask use in congregate work settings contributed to the spread of cases. However, even among affected employees from the work setting outbreak, which included patients from all racial groups, Hispanic patients had the highest morbidity. This disparity, even among essential workers of different races and ethnicities, suggests that there are yet still other factors that exist and place minorities at increased risk. Another possible reason for the increased risk of death for Hispanic and Asian patients may be related to long-term exposures to air pollution. A nationwide study, controlled for race and ethnicity, showed that long-term exposures to small increases of fine particulate matter significantly increases the Covid-19 death rate.[27] However, while examining the ZIP codes with the highest number of Covid-19 cases did not reveal a disproportionate number of any particular race or ethnicity, the top five affected ZIP codes notably represented communities where the percentage of low-income residents exceeds the North Carolina benchmark of 35.6%.[28] Altogether, these findings suggest that the outcomes for Covid-19 may be explained by a complex combination of genetic influences, underlying comorbidities, and socioeconomic factors like living conditions and environmental pollutions, access to healthcare, or linguistic challenges in implementing preventative or treatment strategies.[22,27,29–33] While a majority of the young Hispanic patients who died required translators, we are unable to draw conclusions as to the significance of this observation.

Interestingly, recent tobacco use was independently associated with a decreased odds of hospital admission. In light of this, published studies regarding the effects of tobacco and Covid-19 are conflicting. A recent meta-analysis examined 13 studies from China and found a significantly reduced prevalence of smokers who were hospitalized with Covid-19 compared to the overall smoking prevalence in China.[34] Another study in Israel found that observed current smokers were significantly less prevalent among Covid-19-positive patients compared to expected numbers.[35] The decreased rates of hospitalized smokers might be related to nicotine's potent anti-inflammatory properties, as it has been shown to suppress pro-inflammatory cytokine expression in vitro[36] and reduce lung inflammatory infiltrates, edema, and cytokine production in animal models of ARDS.[37] Despite these findings, other studies link tobacco use with increased odds of severe Covid-19.[38,39] Further studies are needed to elucidate these contradictory findings, and the FDA still endorses the benefits of smoking cessation during the Covid-19 pandemic.[40]

This study has several limitations. First, the sample size in our analysis is relatively small, which limits the statistical conclusions that may be drawn. We also do not have data regarding which patients may have died at home rather than in-hospital. Second, this study contains patients from one healthcare system in western North Carolina and thus may have limited generalizability to other settings. As this study does not contain data from the Other healthcare system, we do not know the true underlying hospitalization rate among each race and ethnicity within our community, and it is possible that patients of a particular race and ethnicity preferentially sought medical care at one hospital system over another. In addition, we are reporting on the first group of cases seen in the Wake Forest Health Network and did not perform a population-based study. We also only have a small percentage of patients with immunosuppressive diseases and so are unable to assess the impact of Covid-19 on this potentially vulnerable group of patients. Lastly, obtaining data from the electronic medical record in a retrospective study precluded obtaining important information such as whether patients were wearing masks at the time of exposure, the duration of direct exposure to a contact known to have Covid-19, and on the social determinants of health — many of these key components, for which their presence or absence can help elucidate the differential outcomes observed among patients with Covid-19, were not documented in the electronic medical record for the majority of patients. Moving forward, widespread effort should be made to capture this information and continuously engage in conversations with patients about the social determinants of health to help better understand the barriers each individual may face. Furthermore, the data in the electronic health record is dependent on the accuracy of the primary care team in updating complete patient information. Despite these limitations, our study contributes epidemiological data to our particular region of the country and includes a large proportion of Hispanic patients not seen in many other published studies.

Disclosures

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Supplemental Table 1. Percent of each of the variables missing

Variable	Percentage missing in data
Age	0%
Gender	0%
Tobacco use in last 30 days	1%
Nursing home	1%
Hypertension	2%
Coronary artery disease	2%
Hypercholesterolemia	20%
Congestive heart failure	1%
Cerebrovascular disease	2%
BMI	18%
Asthma	2%
COPD	2%
Diabetes	2%
CKD >= Stage 3	2%
Cancer	2%
HIV	2%
Cough	0%
Fever	0%
Dyspnea	0%
Myalgia	0%
Headache	0%
Fatigue	0%
Sore throat	0%
Diarrhea	0%
Location of test	2%
Systolic blood pressure	0%
Heart rate	1%
Oxygen saturation	1%
Supplemental oxygen	1%

Supplemental Table 2. Counties in North Carolina of patients who were transferred to WFBMC from an outside hospital.

Zip code	Major County	Classification (https://www.ncruralcenter.org/about-us/)	Number of patients
27017	Surry	Rural	1
27020	Wilkes, Yadkin	Rural	1
27021	Stokes	Rural	1
27028	Davie	Rural	2
27041	Surry	Rural	1
27265	Guilford	Urban	1
27295	Davidson	Suburban	1
27299	Davidson	Suburban	1
27360	Davidson	Suburban	2
28344	Sampson	Rural	1
28360	Caldwell	Rural	1
28645	Caldwell	Rural	1
28651	Wilkes	Rural	2
28654	Wilkes	Rural	1
28655	Burke	Rural	4
28659	Wilkes	Rural	1
28697	Wilkes	Rural	

Reviews

Systematic Literature Review of COVID-19: Quality and Source of Primary Clinical Data

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Abstract

As of April 10, 2020, there were 1.7 million confirmed cases of COVID-19 worldwide and 496,500 cases in the US, with an ongoing surge in the number of reported cases and deaths. It is important to know the strengths and weaknesses, quality, and source of clinical data that was available at an important time point in the surge to help physicians caring for patients with COVID-19. We performed a systematic literature review of all clinical studies published in Pubmed® regarding COVID-19. We included all articles identified from a search of the keywords "COVID-19" or "COVID 19" from January 1, 2020 to April 10, 2020. We identified the type of study, number of patients studied, country of origin, whether multivariate regression was used, and other characteristics. Of 3337 articles, only 490 (15%) were clinical studies that analyzed primary patient clinical information. Of the 490, there were 310 (63%) retrospective cohort studies, 136 (28%) case reports, 16 (3%) prospective cohort studies, 24 (5%) cross-sectional studies, and 4 prospective clinical trials (1%). Of the 490 studies, 74% were from China, 15 (3%) from the US, and 111 (23%) from other countries. Chinese patients accounted for 31,050 (79%) of the 39,477 individuals studied. Excluding a letter to the editor that included 3,615 patients, there were only 81 patients from the US included in publications at a time when there were 496,500 affected individuals in the US. While papers were accepted for publication rapidly (mean time from submission to acceptance 9.4 ± 9.6 days), they were primarily descriptive, statistical analysis was limited, and publications did not address the critical clinical questions facing clinicians and public health officials at a critical time during the pandemic. As cases of COVID-19 reached 1.7 million worldwide and 496,500 in the US, almost all clinical studies were published by Chinese authors studying individuals in China affected with COVID-19. Studies were in general small and accepted quickly, with limited statistical analysis. With rapidly emerging infectious outbreaks and pandemics, the US and other countries must be better prepared to quickly publish clinically important studies that will improve insights and improve patient care.

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Introduction

As of April 10, 2020, there were 1.7 million reported cases of COVID-19 worldwide and almost 500,000 cases in the US.[1] Despite the very large number of cases, there were unanswered clinical questions that were critical to the care of COVID-19 patients. For example, a validated multi-center risk score for hospitalization and mortality was critically needed to help triage patients and identify healthcare workers at high

risk for infection who might need to avoid direct patient contact. Other critical questions at this time included whether individuals who have received hydroxychloroquine and azithromycin had a better outcome than other affected individuals[2-8], and whether individuals who were receiving angiotensin converting enzyme inhibitors or angiotensin receptor blockers had better or worse outcomes.[9-11]

Unlike early observational epidemiologists like John Snow and Florence Nightingale, investigators today have access to computer capabilities that facilitate communication, data collection and analysis through databases that can be easily built in programs like REDCap (Research Electronic Data Capture)[12], and the ability to perform more advanced statistical analysis such as multivariate regression with statistical programs such as SAS (Cary, NC) and free software such as The R Project for Statistical Computing.[13] However, clinical researchers must also comply with investigational review boards and data sharing agreements between academic centers and nations. Clinical researchers may also have additional clinical responsibilities.

The purpose of this investigation was to determine how the knowledge base regarding COVID-19 was formed at a critical time point during the pandemic and to determine how clinical researchers around the world responded to the COVID-19 pandemic and potential obstacles. This study is a historical document but also provides insight into the course of action for the months ahead as the pandemic continues to evolve. In order to analyze currently published knowledge on COVID-19, we reviewed manuscripts that were published in PubMed with the keyword "COVID_19" as of April 10, 2020.

Methods

All studies that included the keyword "COVID-19" or "COVID 19" were identified using PubMed®. We chose PubMed® because it is used almost exclusively by clinicians for clinical information and because we were not studying basic science articles that may be represented in other databases. PubMed® includes the following search terms when "COVID-19" is searched: COVD-2019, severe acute respiratory syndrome coronavirus 2, 2019-nCoV, SARS-CoV-2, 2019nCoV, Wuhan combined with coronavirus, or the term coronavirus occurring after December 2019. To be included in this study, each reference required a title, English abstract, and, if an abstract was not available, a link to an article in English that provided the information collected (see below). An initial review was manually performed by reviewing each abstract to limit the database to articles that provided primary clinical information regarding COVID-19. Primary clinical information was defined as research that included information collected directly about individuals with COVID-19. We excluded all studies that were basic science articles (defined as articles studying COVID-19 in the laboratory and not including patient specimens, reviews, editorials, or clinical guidelines on the COVID-19 pandemic. We also excluded studies that did not directly concern COVID-19 (e.g. population attitudes about COVID-19). Clinical studies were classified as follows: Case reports included all studies of a single individual affected with COVID-19. Retrospective cohort studies included studies with at least two patients that collected information from at least two time points. Prospective cohort studies were non-interventional studies that collected pre-defined data prospectively. Cross-sectional studies were observational descriptions of patients at a single point in time. Epidemiologic studies were defined as studies that provided limited or no patient-specific information and focused almost exclusively on temporal or geographic trends related to COVID-19 spread. We defined studies as meta-analyses or systematic reviews if they were described as such in the title or abstract, and this was verified on review of the publication. The country of origin was identified as the country in which all or most of the cases occurred. As a marker of the analytical complexity of studies, we analyzed how many clinical studies performed multivariate regression. We determined how many studies were focused on radiologic, obstetric/gynecologic, and pediatric findings. Radiologic studies were tabulated because of the large number of such studies noted upon initial review. Obstetric/gynecologic and pediatric studies were tabulated because of interest in the specific subgroups. For each clinical article, we obtained the date of publication from PubMed® and attempted to obtain the dates of submission and acceptance by manuscript review; these dates were not always available. Due to the vast majority of studies originating from China and concerns regarding multiple publications on the same patient population, a closer examination of the origin of these

studies was performed. We determined from which hospitals the patient population originated and reviewed the number of authors who contributed more than one manuscript. There were two studies that used the same database of 1590 individuals from China.[14,15] Both studies were included in our analysis, but we included the number of patients in our calculations only one time. Each abstract was reviewed by one of the co-investigators, with review of the entire article as needed for the collection of data. Studies analyzing primary data on clinical subjects were reviewed by two faculty members. We used data from the Coronavirus COVID-19 Global Cases by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University website 1 to calculate the number of affected individuals in affected regions by date.

Data was entered in Microsoft Excel, with subsequent analysis performed by SAS statistical software (Cary, NC), using standard analytic techniques for discrete and continuous variables.

Results

There were 3380 studies reviewed, of which 43 were excluded for insufficient data. There were 3,337 articles reviewed and published online from January 1, 2020 to April 10, 2020 for which there was adequate data available (including title, abstract (or link to text), and date of publication). There were 2563 (77%) articles that were classified as basic science articles, editorials, narratives, clinical recommendations, ethical reports, and other opinion pieces that were not included in further review. There were 144 (4%) epidemiologic articles, 16 meta-analyses (<1%) and 7 systematic reviews (<1%). Fig 1 shows the publication of articles over time. The publication of narratives, editorials, reviews, and similar articles increased rapidly, while there has been only a slow increase in clinical articles.

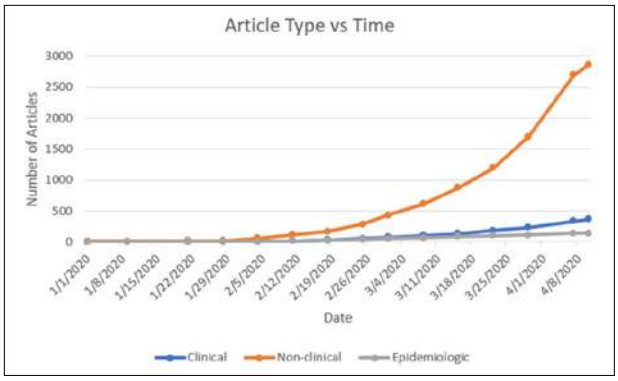

Figure 1. Cumulative publication of articles by type and date. Non-clinical articles have increased much more rapidly than clinical articles.

There were 490 articles that analyzed primary patient data, accounting for only 15% of all articles. Of the 490 clinical studies, there were 310 (63%) retrospective cohort studies, 136(28%) case reports, 16 (3%) prospective cohort studies, 24 (5%) cross-sectional studies, and 4 prospective clinical trials (1%). Of these 490 studies, 74% were from China, 15 (3%) from the US, and 111 (23%) from other countries). Fig 1 shows the increase over time in the number of articles published and the publication type. Fig 2 shows the increase over time of different clinical articles.

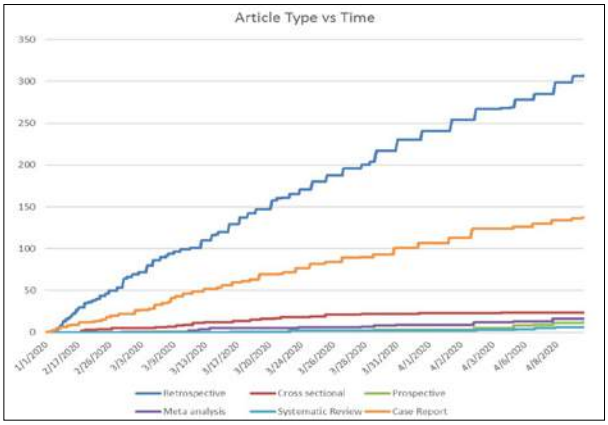

Figure 2. Cumulative publication of articles by type and date of clinical articles.

Study quality: The level of evidence of clinical studies is shown in Table 1. The majority of the studies provided a low level of evidence. There were four prospective clinical trials. One was a well-executed and designed trial of 199 patients that found lopinavir-ritonavir was not beneficial in severe COVID-19.[16] Another study from France was an open-label non-randomized clinical trial showing 20 patients treated with hydroxychloroquine and azithromycin had a significantly faster decrease in viral load.[17] There was another study of chloroquine, in which 22 patients were randomized into two groups with 10 treated with chloroquine 500 mg orally twice per days for 10 days and 12 patients receiving lopinavir/ironavir.[18] The percentage of patients whose COVID-19 viral load became negative in

the chloroquine group was slightly higher at day 7, 10, and 14. There was also a prospective non-randomized trial of ACE2-mesenchymal stem cells in patients with COVID-19 pneumonia. (7 treated and 3 controls).

Table 1. Level of quality for clinical studies

Type	Description	N (percent)	China (5)	US (%)	Other (%)
1	Properly powered randomized trial	1 (0%)	1(100%)		
	Systematic review	7(1%)	4(57%)		3(43%)
	Meta-analysis	16(3%)	14(88%)	1(6%)	1(6%)
2	Prospective cohort study	16 (3%)	12(75%)		4(25%)
	Prospective nonrandomized trials	3(0%)	2(67%)		1(33%)
3	Retrospective cohort studies	310 (60%)	263(85%)	5(2%)	42(13%)
4	Cross-sectional studies	24 (5%)	19(79%)		5(21%)
5	Case Reports	136 (27%)	67(49%)	10(7%)	59(44%)

Table 2 shows the number of patients included in clinical studies. There were only 13 studies that included more than 500 patients, and 10/13 (77%) were from China. Twenty-nine percent of studies were case reports and 72% included less than 50 individuals. The total number of patients included in studies from China were 31,050, in the US 3,696, and from other countries 4,731. There was one US study that was a letter to the editor that included 3,615 patients and looked at weight and age associated with hospital admission and ICU admission only.[19] Excluding this study, there were only 81 patients in the US studies, a time when nearly 500,000 individuals were affected with COVID-19. There were 454 clinical studies from which the number of centers could be determined (see table 2). Eighty-three percent of Chinese, 100% of US, and 94% of studies from other countries were single center studies. China had 17 studies including more than 5 centers.

Table 2. Number of patients in Clinical studies

Class	Number	China	US	Other
Case reports	138	69(50%)	10(7%)	59(43%)
2 to <50	207	165(78%)	4(2%)	38(18%)
2 to <100	62	58(94%)	0	4(6%)
100 to <500	62	56(90%)	0	6(10%)
500 to <1,000	5	4(80%)	0	1(25%)
>= 1,000	8	6(75%)	1(13%)	1(13%)

The time from submission to study acceptance was very short in most cases. From 227 publications with data available, 31 (14%) were accepted on the day of submission and 80 (35%) were accepted within three days of submission. Statistical analysis was cursory in most instances with only 36% of studies with > 100 patients having multivariate analysis. 11% of studies were pediatric, 19% radiologic, and 5% obstetric. 75% of studies included information about diagnosis and 49% about prognosis.

Of the 490 studies, 364 (74%) were from China (Table 3). Chinese patients accounted for 80% of the patients who were studied, though this analysis was limited by the possibility of Chinese patients being included in multiple reports and possible inaccurate reporting of the number of affected individuals in China.[20] As of April 10, 2020, there were 82,900 reported affected individuals in China, suggesting inclusion of a significant number of patients in these observational studies. As of April 10, 2020, there were 496,500 patients infected in the US, with only 84 individuals reported in full articles. As of April 10, 2020, with Italy having 147,600 patients, there were 22 studies including 2,024 patients, including one study with 1,591 patients.[21] Fig 3 shows the rate of publication of clinical studies for the US, Italy, and China, together with the number of reported cases from each country. Chinese studies were published earlier and included more centers and individuals. As of April 10, 2020, Italy had reached 500 cases of COVID-19 43 days ago. At this time point, China had 112 publications with 7,542 patients vs. 22 publications and 2,024 patients from Italy. As of April 10,202, the US had reached 500 patients 32 days previously and had 14 full publications of 81 patients vs. 50 publications with 1,610 patients from China at a similar time point.

Table 3. Number of centers in clinical studies

Number of centers	Number	China	US	Other
1	392	283(72%)	13(3%)	96(25%)
2	23	21(91%)	0	2(9%)
3	14	13(93%)	0	1(7%)
4	6	5(83%)	0	1(17%)
5 or greater	19	17(89%)	0	2(2%)

We then specifically examined the clinical studies from China that collected and analyzed primary data. Of 357 with clinical data available, 171 (48%) were from Wuhan province and included 20,208 patients. There were 186 studies from other provinces and included 10,581 individuals. The three most common centers from which patients were studied included Tongji Hospital of Tongji Medical College, (46 (9%)), Union Hospital of Tongji Medical College (29 (6%)) and Wuhan Children's hospital (13 (3%)). In general, studies appeared to come from many different authors, multiple medical centers, and many different geographic areas of China.

Studies of clinical relevance: There were several larger studies that identified the relative importance of risk factors associated with increased severity of COVID-19 infection. Liu et al. studied 78 individuals admitted to three regional hospitals and identified factors associated with progression to severe disease.[22] Multivariate logistic analysis indicated that age (odds ratio [OR], 8.54; 95% confidence interval [CI]: 1.63-44.86; P=0.011), history of smoking (OR, 14.3; 95% CI: 1.58-25.0; P=0.018), maximum body temperature at admission (OR, 9.0; 95% CI: 1.04-78.147, P=0.046), respiratory failure (OR, 8.7, 95% CI: 1.9-40; P=0.016), serum albumin (OR, 7.35, 95% CI: 1.1-50; P=0.003), and serum C-reactive protein (OR, 10.5; 95% CI: 1.2-34.7, P=0.028) as risk factors for disease progression. In a large study, Guan et al. studied 1590 patients with COVID-19 and evaluated the risk of severe adverse outcomes, with a composite endpoint of admission to intensive care unit, invasive ventilation, or death, which occurred in 131 patients (8.3%).[15] After adjusting for age and smoking status, the following risk factors were of highest significance in a multivariate model: COPD (hazards ratio (HR) 2.7, 95% confidence interval (95%CI) 1.4-5.0], diabetes (HR 1.59, 95%CI 1.03-2.45), hypertension (HR 1.58, 95%CI 1.07-2.32) and malignancy (HR 3.50, 95%CI 1.60-7.64). The HR was 1.79 (95%CI 1.16-2.77) among patients with at least one comorbidity and 2.59 (95%CI 1.61-4.17) among patients with two or more comorbidities. Grasselli et al. reported on 1,591 patients admitted to intensive care units in Italy.[21] These authors found that the majority of patients admitted to the intensive care unit were older men, and a large proportion required mechanical ventilation with positive end-expiratory pressure, with an ICU mortality of 26%. This study did not develop a risk score and was descriptive in nature. In a letter to the editor, a US study of 3,615 reported that patients with a body mass index between 30 and 34 were 2.0 times (95%CI 1.6-2.6, P<0.001) more likely to be admitted and 1.8 (1.2-2.7, P=0.006) times more likely to be admitted to acute and critical care units. The authors did not control for diabetes, hypertension, or other comorbidities.[19]

There had been a number of editorials regarding the potential effects of angiotensin converting enzyme inhibitors or angiotensin receptor blockers[9,23-32], but little data had been obtained in this regard. In a study by Peng et al. 33 of 112 patients admitted to Union Hospital, with a group of 16 critical patients, the use of angiotensin converting enzyme inhibitors or angiotensin receptor blockers was not associated with an increased incidence of poor outcomes. Meng et al. studied 417 patients with COVID-19 admitted to the hospital, including 17 patients treated with angiotensin converting enzyme inhibitors or angiotensin receptor blockers and 25 who did not receive these medications.[34] During hospitalization 12 (48%) of the patients not receiving angiotensin converting enzyme inhibitors or angiotensin receptor blockers developed severe disease vs. 4 patients (24%) in the patients receiving these medications.

Similarly, there had been numerous editorials regarding the benefits and risks of hydroxychloroquine[10,17,18,35-40], but there have only been two very small studies (see above).

Discussion

This investigation summarizes the state of medical knowledge regarding COVID-19 as of April 10, 2020. At this time point, there were 496,500 cases in the US, with 34 days since 500 cases reported (3/7/2020). Clinical knowledge from the US was based on 15 US manuscripts describing 3,696 patients. Excluding a letter to the editor regarding 3,615 patients[19], clinical judgement was based on 14 articles describing 81 of the 496,500 individuals affected with COVID-19. As of April 10, 2020, there were 147,600 cases in Italy, with 43 days since 500 cases reported. There were 22 studies of 2,024 patients at this time. The majority of clinical information regarding COVID-19 stemmed from China, where 89,200 patients were reported with COVID-19 and 80 days had elapsed since 500 cases were reported (1/21/2020). Chinese publications accounted for 74% of the publications, with 80%

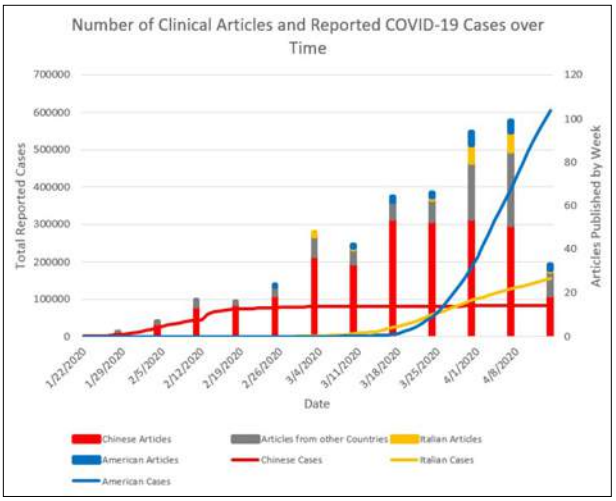

Figure 3. Number of reported COVID-19 affected individuals by country (China, US, and Italy) and number of articles published by country.

of the patients studied. While the duration of the COVID-19 pandemic was significantly longer than in other countries, publications lagged at similar time points for the US and Italy compared to China.

Chinese investigators, primarily from Wuhan province and Tongji Medical College, published a large number of manuscripts, which provided the basis for clinical understanding of the COVID-19 pandemic. A number of the studies from China were multi-center, and authorship did not appear to be concentrated at one hospital. There was a focus on radiologic studies, accounting for 21% of clinical studies. Studies were published quickly, with many studies accepted within several days of submission. However, study quality appeared to have been limited by the haste of editors to publish. The lack of more advanced statistical analysis (such as multivariate regression) was a hindrance to our understanding of risks associated with COVID-19 infection.

Data to answer critical questions remained unavailable (see Table 4). Given the large number of affected patients, a multivariate risk score to predict patients at increased risk of admission and death could have been developed but was not. Such risk scores would have been useful in the admissions process, in determining which healthcare personnel should not interact with patients, and in assisting in determining individuals who were at very low risk and might be able to return to work. A single-center study in the US identified a body mass index greater than 30 as a significant risk factor for severity of disease.[19] A study from China with multivariate regression identified smoking status, age, COPD, diabetes, hypertension, and malignancy as important risk factors, as well as an increased number of comorbidities.[15] Validation of these results, together with inclusion of race as a covariate, is critical to our understanding of individuals at risk from COVID-19.

Table 4. Critical unanswered questions regarding coronavirus as of April 10, 2020:

1)	Is there a risk score that would help identify individuals at increased risk of hospitalization for COVID-19 infection?
2)	Is there a risk score that would help identify individuals at increased risk of death from COVID-19 infection?
3)	Is hypertension an independent risk factor for death?
4)	Is African American race an independent risk factor for death?
5)	Are gender and obesity independent risk factors for death?
6)	Is the use of angiotensin converting enzyme inhibitors or angiotensin receptor blockers beneficial or disadvantageous to survival after COVID-19 infection.?
7)	Is consumption of commonly used medications such as non-steroidal anti-inflammatory agents a risk factor for death?
8)	Does administration of hydroxychlorquine and azithromycin improve outcomes?
9)	Why is mortality so high in Italy?
10)	Are healthcare workers at increased risk of death from coronavirus?
11)	Is there a clinical score that could predict futility of outcome?
12)	Is influenza vaccination beneficial to survival?

Early studies of hydroxychloroquine that included less than 50 patients were used to guide treatment for over 500,000 patients. Retrospective case control studies of the more than 100,000 individuals who have received this medication would have been helpful to detect adverse effects and identify potential benefit. An observational study of 1,446 patients from a single medical center was published on May 7, 2020, showing no association of hydroxychloroquine use with intubation or death (hazard ratio 1.04, 95% confidence interval 0.82-1.32).[41] This analysis was published after there were already 1.25 million patients diagnosed with COVID-19, many of whom had received hydroxychloroquine as a therapy. Similarly, the identification of hypertension as a risk factor needed further explanation. The binding of COVID-19 to ACE2 receptors in the lungs pointed to possible effects of angiotensin converting enzyme inhibitors and angiotensin receptor blockers on patient survival. Similarly, case control studies could have been performed to resolve this issue.

While there were a large number of editorials and reviews reflecting interest in COVID-19 in the US and other countries,

primary data analysis was limited. Whereas, historically, early epidemiologic studies focused on infectious diseases, more recently, epidemiologic studies have focused on large aggregations of data and chronic diseases. Such data collection is not time-sensitive, and protection of individual privacy, especially with genetic information, has been a priority. This environment has fostered the slow, methodical collection of data that has multiple safeguards for participants, academic centers, and countries involved in research. Patients are protected by institutional review board approval at each site, and the Health Insurance Portability and Accountability Act (HIPAA) further complicates data sharing. Academic centers are protected by data sharing agreements between sites, and countries are protected by a variety of laws overseeing research. Unfortunately, this structure is likely providing critical obstacles in the study of a rapidly emerging epidemic. The very limited number of multicenter studies pointed to an environment that did not foster rapid collaboration. In addition, the ability to obtain funding quickly to perform these studies is extremely limited.

Living in an environment which allows the rapid movement of individuals worldwide results in the possibility of rapid spread of new viruses. To counter these pandemics, real-time epidemiology is required, with the development and utilization of tools that are similar to those used in industry to obtain real-time collection of data and data analysis. The tools for such data collection are available, but administrative obstacles must be overcome for their use.

Real-time collection of basic data including demographics, comorbid conditions, medications, and outcomes should be performed at individual centers. This real-time collection of data at individual centers will help each center in their response to viral outbreaks and collectively could provide answers to critical questions in a rapid manner. However, even the real-time collection of data is not as important as well performed clinical trials, which require time to design and execute.

A primary weakness of this article was the lack of inclusion of more databases. We chose PubMed® because it is the most common database used by academic clinicians and includes publications from all major clinical journals. Other weaknesses include the possible inaccurate reporting of the number of cases in China, which would make their publication record appear more favorable. We could not ascertain how many patients were included in more than one study from China, though we showed that there was data available from many academic centers. We also included only articles in PubMed® and did not explore other methods of publication. In addition, the rapid publication of articles may result in changes in trends of publication and analysis.

While we have looked at general trends in COVID-19 publication, it is important to also point out the importance of primarily literature from China in providing us information about subsets of our patient population. For example, studies in children[42], pregnant women[43], hemodialysis patients[44], and cancer patients[45] provide important guidance even though they are descriptive and have small patient numbers.

In summary, clinically reported data on COVID-19 as of April 10, 2020 were limited and primarily from China. While articles were accepted quickly, data analysis was poor, and the vast majority of publications did not address the critical issues facing patients, clinicians, and public health officials at this time. Clinical researchers and leaders of medical centers and governments must identify obstacles to collection and dissemination of data and overcome them quickly. We must overcome administrative obstacles and develop a real-time approach to the collection of data and its analysis to help prevent morbidity and mortality during pandemics.

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Reviews

Cutaneous Manifestations of COVID-19

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Abstract

In light of the ongoing COVID-19 pandemic, large volumes of research have surfaced in an effort to better characterize the disease. In addition to systemic symptoms, various organ-specific findings have emerged. In this article, we systematically reviewed the cutaneous manifestations of COVID-19. Data for the review were identified by a PubMed search using the terms "COVID skin," "COVID cutaneous," "COVID vasculopathy," "COVID acral," "coronavirus skin," "coronavirus cutaneous," "coronavirus vasculopathy," and "coronavirus acral." A total of 105 articles and a pooled sum of 1,903 patients were included. The most commonly reported skin findings were chilblain-like acral lesions, maculopapular eruptions, urticarial eruptions, and vesicular eruptions. Knowledge of these findings and their potential diagnostic and prognostic importance may help providers better recognize and understand the disease.

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Introduction

Since its initial detection in December 2019, COVID-19 has caused a pandemic declared a public health emergency by the World Health Organization.[1] Physicians and researchers have reported their findings, however limited, in an attempt to alert others and contribute to our evolving understanding of this disease. Several characteristic clinical features have emerged, including dry cough, fever, fatigue, loss of appetite, diarrhea, and isolated anosmia.[2,3] Cutaneous manifestations are increasingly being reported and may provide useful diagnostic and prognostic information.[4] Herein, we perform a systematic review of the literature pertaining to the cutaneous manifestations of COVID-19.

Methods

Articles were identified by a PubMed search using the terms "COVID skin," "COVID cutaneous," "COVID vasculopathy," "COVID acral," "coronavirus skin," "coronavirus cutaneous," "coronavirus vasculopathy," and "coronavirus acral." Articles published between January 1, 2020 and June 25, 2020 were included. Articles were initially screened by title for relevance; subsequently full-text articles were screened. Screening and review were performed in accordance with the Preferred Reporting Items for Systematic and Meta-Analysis (PRISMA).

Results

A total of 210 full-text articles were assessed for eligibility; 105 were included in the review (Figure 1). Of these 87 were case reports or small case series with 20 or fewer patients (Table 1) and 18 were larger case series or observational studies with more than 20 patients (Table 2). Published studies included twenty different countries; 76% (n=80) of studies were conducted in Europe and 30% (n=32) were conducted in Italy specifically. Of the 14 observational studies, 6 provided estimates of prevalence and/or incidence of skin manifestations in COVID-19 patients; estimates ranged from 1.5% to 36%.[5-10] A total of 1,903 patients were described and of these 641 (34%) were laboratory-confirmed to be COVID-19 positive. Some patients had multiple different skin manifestations. The most commonly reported skin finding was chilblain-like acral lesions (52%, n=998), followed by maculopapular eruption (17%, n=331), urticarial eruption (8%, n=150), vesicular eruption (7%, n=142), "other" lesions (7%, n=142), non-chilblain-like acral lesions (4%, n=71), erythema multiforme-like lesions (3%, n=56), and vascular lesions including livedo reticularis or racemosa, retiform purpura, and acute digit/limb ischemia or necrosis (3%, n=54). Other lesions included cutaneous small vessel vasculitis, pityriasis rosea, Kawasaki-like eruptions, bullous or Steven-Johnson Syndrome-like eruptions, and purpuric eruptions.

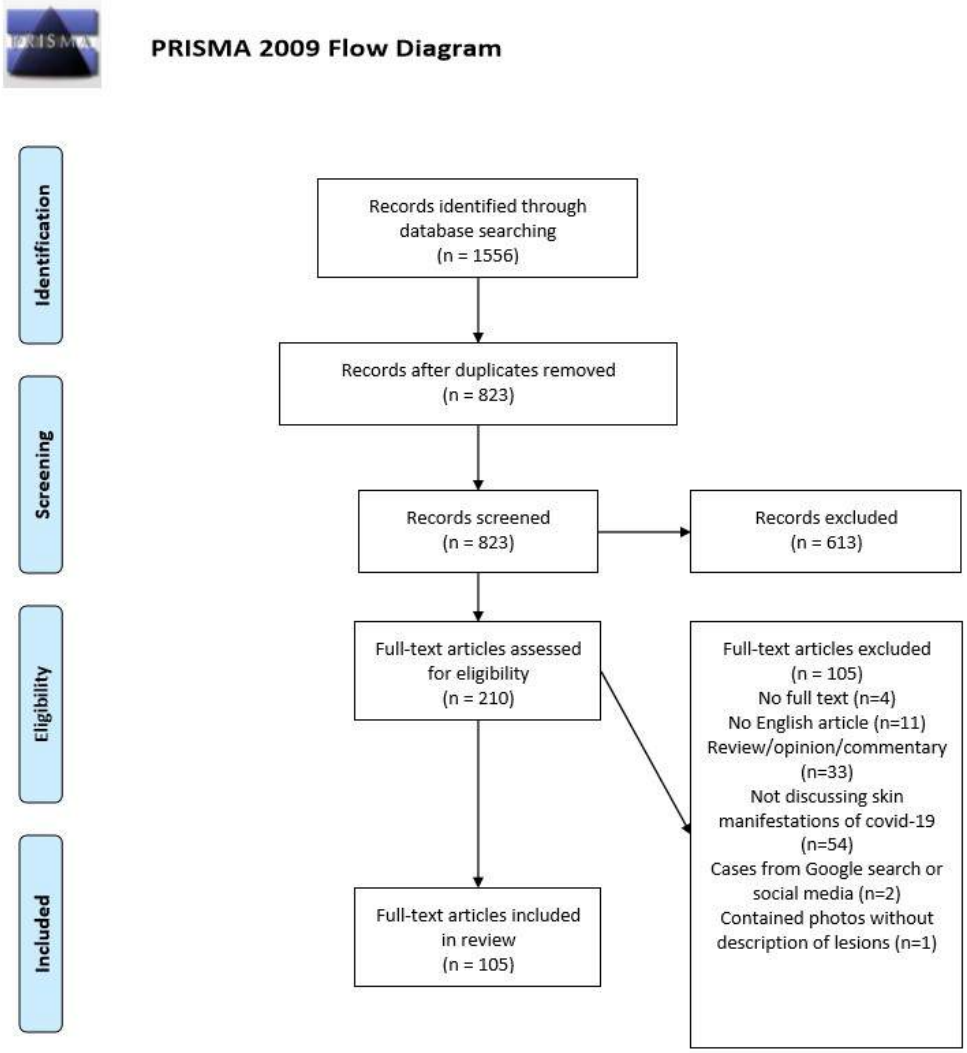

Figure 1.

Table 1. COVID-19 case reports and small case series (n<=20) with cutaneous manifestations

Citation	Country of Origin	Number of subjects	Number of subjects with positive COVID-19 test	Skin manifestations	Comments
Magro et al.[17]	US	3	3	Retiform purpura on buttocks (n=1), dusky purpuric patches on palms and soles (n=1), livedo racemosa on trunk and limbs (n=1)	
Guarneri et al.[18]	Italy	3	3	Acral chilblain-like lesions (n=3)	Age 14-18yo, all had complete resolution
Sachdeva et al.[19]	Italy	3	3	Pruritic maculopapular rash on trunk resembling Grover's disease (n=1), diffuse maculopapular rash on trunk followed by macular hemorrhagic rash on legs (n=1), pruritic papulovesicular rash on submammary folds, trunk, hips (n=1)	
de Medeiros et al.[20]	Brazil	1	1	Painful edematous erythematous plaques on limbs involving into bruises after first exposure, pruritic urticarial lesions on shoulders/inguinal region and pruritic erythematous lesions on palms after a second exposure	Patient developed skin manifestations on two separate occasions after exposures—resolved with betamethasone in both cases
Estébanez et al.[21]	Spain	1	1	Confluent erythematous-yellowish papules on bilateral heels that evolved into hardened pruritic erythematous plaques	
Henry et al.[22]	France	1	1	Urticarial eruption. Disseminated pruritic erythematous plaques with face and acral involvement	
Sakaida et al.[23]	Japan	1	1	Pruritic erythematous indurated limb lesions after treatment with antibiotics 2 days prior à admission for COVID symptoms/pneumonia and worsening lesions à new maculopapular eruption on face and trunk with petechiae	Authors suggest COVID-19 may have predisposed patient to drug hypersensitivity; state that path showed deep lymphocytic infiltrate uncharacteristic of drug eruptions
Olisova et al.[24]	Russia	1	1	3-4 mm purpuric eruptions and erythematous macula on eyelids, forehead, and temporal region along with swollen tongue and pronounced lingual papillae	Developed after cessation of fever. Resolved in 3 days without treatment
Mahé et al.[25]	France	3	3	2-3 mm vesicles with excoriation on trunk (n=3) with upper limb/face involvement (n=1)	Evidence of direct viral effect on histology
Mahé et al.[26]	France	1	1	Erythematous rash on bilateral antecubital fossa that spread to trunk and axillary folds, similar to Symmetrical Drug-Related Intertriginous and Flexural Exanthema (SDRIFE)	Resolved after 5 days
Tosti et al.[27]	Italy	4	0 (not tested)	Erythematous plaques or papules on heels (n=3) and/or toes (n=2)	Patients mildly symptomatic or asymptomatic
Jimenez-Cauhe et al.[28]	Spain	4	4	All had erythema multiforme-like eruption. Erythematous papules on upper trunk that became erythemato-violaceous patches with dusky center and central pseudo-vesicle (n=4). Typical target lesions that coalesced on back and spread to face and limbs sparing palms/soles (n=2), palatal petechiae and macules (n=3)	All patients were hospitalized. One developed skin findings during hospitalization, the others developed lesions after discharge

Table 1. *Continued*

Llamas-Velasco et al.[29]	Spain	1	1	Livedoid purpuric lesions: purple ischemic digits on bilateral hands, livedoid purplish retiform patches in non-ischemic fingers and on volar and dorsal hands and feet	Lesions during hospitalization for bilateral pneumonia and DKA, patient had heterozygous Factor 5 Leiden mutation
Ahouach et al.[30]	France	1	1	Diffuse fixed erythematous blanching maculopapular lesions on limbs and trunk with burning sensation in palms	Rash occurred 2 days prior to fever onset
Bursal et al.[31]	Turkey	3	3	Erythema similar to rash of roseola (n=1), pruritic maculopapular rash (n=2). All spread from face to extremities to trunk	All were hospitalized pediatric patients; roseola-like rash was in 8-month old
Colonna et al.[32]	Italy	4	0 (tested negative)	Erythematous macules and/or edema on feet (n=4) and hand (n=1)	Age 5-11. All patients had symptoms of or exposure to COVID-19
Gianotti et al.[33]	Italy	5	0	Diffuse maculopapular eruption on trunk clinically suggestive of Grover's disease (n=2), purpuric maculopapular vesicular rash (n=1), papular erythematous exanthema on trunk (n=1), macular livedoid hemorrhagic eruption (n=1)	COVID-positivity is implied but no mention of test results
Gianotti et al.[34]	Italy	3	3	Widespread erythematous macules (n=3) on arms and trunk (n=2) with lower limb involvement (n=1), with pruritus and papules (n=1)	All rashes resolved within 10 days
Gianotti et al.[117]	Italy	5	0 (not tested)	Diffuse macular papular rash on trunk and upper limbs (n=2), pernio-like lesions and vesicular lesions resembling erythema multiforme (n=1), polymorphic findings including reticulated pigmented dermatitis, psoriasiform lesions on elbows/buttocks, papules and plaques on upper limbs, erythematous macular lesions on lower limbs (n=1), erythrodermic psoriasis (n=1)	All patients had comorbidities (cancer, HIV, polycystic kidney disease, guttate psoriasis) and presented for a dermatosis. All were suspected COVID-19 due to systemic symptoms but unable to be tested
Skroza et al.[35]	Italy	1	1	Urticarial vasculitis secondary to adverse drug reaction: multiple raised erythematous wheals, some with central purple hyperpigmentation on head, trunk, and upper arms with intense pruritus	Eruption happened 18 days after hospitalization for COVID-19 pneumonia, at which time patient tested negative
Falkenhain-López et al.[36]	Spain	1	1	2 necrotic ulcers with raised, sharply demarcated borders in the inferior medial aspect of the right labia minora and a single oral aphthous ulcer	At initial presentation bacterial culture of ulcer and HSV PCR were negative
Cordoro et al.[37]	US	6	0 (all tested negative)	Red to violaceous macules and dusky purpuric plaques on mid/distal toes (n=6), more critically ill had edema with superficial bullae and hemorrhagic crust, some had livedo reticularis on forearms, hands, or feet (n=3)	All were previously healthy adolescents. 1-2 week delay between skin lesion onset and upper respiratory infection symptoms
Torrelo et al.[38]	Spain	4	1	Chilblains on feet (n=4) and hands (n=2) with pruritus (n=3) and erythema multiforme-like target or targetoid lesions (n=4). Mild pain in n=1.	Ages 11-17. All had complete resolution within 1-3 weeks

Table 1. *Continued*

Gaspari et al.[39]	Italy	18	18	Exanthematous rash (n=9), acral vasculitis eruptions (n=6), urticarial eruptions (n=2), varicelliform eruption (n=1)	Rash present at symptom onset (n=2), after in other 16 patients
Cepeda-Valdez et al.[40]	Mexico	2	2	Urticarial eruption: disseminated annular rash with irregular wheals on shoulders, elbows, knees, and buttocks	Treated with antihistamines and moisturizers and disappeared within 48 hours of onset
Genovese et al.[41]	Italy	1	1	Varicella-like rash: 40 erythematous papules and few vesicles scattered bilaterally symmetrically on trunk, limbs, face, genitals with sparing of mucous membranes	8yo female; rash presented in the setting of a 6-day history of mild cough
Elsaie et al.[42]	Egypt	2	2	Painful vesicular rash (n=2) in right groin (n=1) or chest/neck (n=1) consistent with herpes zoster	
Elsaie et al.[43]	Egypt	1	1	Papules and vesicles on erythematous base in dermatomal distribution on back and chest consistent with herpes zoster	
El Hachem et al.[44]	Italy	19	7 (positive serology). All had negative PCR	Chilblain-like lesions: toe swelling and erythema (n=19)	All were adolescents with flu-like symptoms 1-2 months prior to skin findings
Rodríguez-Jiménez et al.[45]	Spain	1	0	Acute urticaria: confluent wheals on anterior and posterior trunk	In the setting of hospital admission for fever/cough
Klimach et al.[46]	UK	1	1	Erythematous papular eruption in axillae with cervical lymphadenopathy, tender erythematous papules on plantar surface of feet, erythematous macules and petechiae on lower extremities, followed by annular lesions on lower extremities 2 days later	Adolescent patient. Developed 24 hours after systemic symptoms. Resolved within 14 days
Mahieu et al.[47]	France	10	0 (all tested negative)	Acral chillblain-like lesions (n=10) with bullous evolution (n=5)	Median age 27. All had negative PCR, negative antibodies, and biopsy negative for SARS-CoV-2 if performed.
Kanitakis et al.[48]	France	17	2	Chilblain-like lesions on toes, feet, and/or fingers (n=17)	Histopathology showed features similar to idiopathic and autoimmune chilblains. Eosinophils in dermal infiltrate may be a new finding
Annunziata et al.[49]	Italy	4	3	Maculopapular rash on trunk (n=1), erythematous rash on abdomen with vesicles and crusts (n=1), pruritic erythematous papules and vesicles on trunk (n=1), pruritic urticarial lesions on legs (n=1)	
Negrini et al.[50]	Italy	1	1	Non-pruritic vesiculo-bullous lesions on neck and dorsal hands, path consistent with bullous hemorrhagic vasculitis (leukocytoclastic vasculitis)	Patient was hospitalized, had comorbidities, and succumbed to disease

Table 1. *Continued*

Najafzadeh et al.[51]	UK	1	0 (tested negative, confirmed based on CT)	Urticaria: 1.5-8 cm generalized pruritic hives	
de Perosanz et al.[52]	Spain	2	1	Urticarial vasculitis (n=2). Painful erythematous patches on trunk and hips that left residual purpura when fading (n=1), Pruritic erythematous and edematous plaques with purpuric center on buttocks (n=1)	1 patient was hospitalized for bilateral pneumonia and succumbed to disease
Bosch-Amate et al.[53]	Spain	1	1	15 cm painful retiform purpuric violaceous patches with hemorrhagic blisters and crusting on bilateral legs	
Conforti et al.[54]	Italy	1	1	Transient livedo reticularis: asymptomatic livedoid patches on back, abdomen, and face along with livedoid maculae that blanched involving bilateral periorbital skin, nose, and frontal region conferring a mask-like appearance	Skin lesions developed 2 weeks into hospitalization for pneumonia. Lesions resolved after starting heparin
Rossi et al.[55]	Italy	1	1	Maculopapular eruption on trunk, upper limbs, face without palmoplantar involvement	Rash resolved after discharge
Locatelli et al.[56]	Italy	1	1	Chilblain-like lesions: multiple asymptomatic edematous erythematous partially eroded macules and plaques on dorsal fingers and one toe	Clinico-pathologic findings consistent with idiopathic chillblains. Lesions lasted >20 days
van Damme et al.[57]	Belgium	2	2	Acute extensive urticaria (n=2)	Presenting symptom along with fever
Calvão et al.[58]	Portugal	1	1	Acro-ischemic lesions: petechial lesions on fingers and toes that progressed to hemorrhagic bullae and necrotic plaques on hands and feet	Patient succumbed to disease
Rodríguez-Villa et al.[59]	Spain	1	1 (positive serology but negative PCR)	Acral chilblain-like lesions	Patient was adolescent caregiver of COVID+ individual
Rosell-Díaz et al.[60]	Spain	12	12	Pruritic papular exanthem with cephalocaudal spread and islands of sparing (n=12), some developed violaceous areas and/or target-like lesions (n=7), some developed fever and facial edema (n=3)	All patients were hospitalized. Histology in 2 patients was compatible with drug reaction
Gargiulo et al.[61]	Italy	1	1	Erythema multiforme-like eruption: erythematous edematous patches on trunk and limbs (coalescing on trunk), isolated target lesions on thighs	Patient succumbed to disease
Colmenero et al.[62]	Spain	1	0 (tested negative)	Acral chilblain-like lesions	*Remaining 6 cases in article are included in a large case series by Andina et al.
Kalner et al.[63]	US	2	2	Periorbital erythema (n=2): dusky red nonpruritic nonblanching periorbital dyschromia with no other symptoms	Was sole presenting sign. Both patients completely recovered
Tehranchinia et al.[64]	Iran	1	0 (not tested)	Generalized red-purple papules and plaques (lichenoid eruption) on lower extremities	Histology showed vacuolar interface dermatitis

Table 1. *Continued*

Putra et al.[65]	Indonesia	1	1	Multiple discrete lenticular erythematous papules up to 3mm on extremities along with pins and needles sensation that became darker/more apparent	Skin lesions eventually exfoliated on day 14
Del Guidice et al.[66]	France	1	1	Acute bilateral necrosis of legs and feet	Patient succumbed to disease. Had coagulopathy/ DIC but no antiphospholipid antibodies
Ho et al.[67]	Singapore	2	2	Papulopustular exanthema (n=1): erythematous blanchable non-follicular papules on trunk, thighs, and intertriginous areas with nonfollicular pinpoint pustules in intertriginous areas. Purpuric plaques on abdomen and back (n=1)	Both patients' skin lesions resolved with topical steroids
Papamichalis et al.[68]	Greece	1	1	Signs of ischemia in upper and lower extremities with hypoperfused regions	Cutaneous and subcutaneous ischemia resolved after administration of rt-PA, enoxaparin, and tocilizumab. Patient was found to have AML and died from bacteremia
Lagziel et al.[69]	US	1	1	Bullous interface dermatitis initially thought to be SJS/TEN	Initial COVID tests were negative. Biopsy suggested bullous drug reaction with EM-like reaction pattern versus SJS/TEN
Castelnovo et al.[70]	Italy	2	0	Widespread urticaria involving thigh and peri-malleolar area (n=1), vasculitic purpura of legs followed by fleeting erythematous rash (n=2)	
Avellana et al.[71]	Spain	1	1	Generalized pruritic morbilliform rash with cephalocaudal progress respecting palmoplantar areas and mucosa, developed transient scaly reaction 4 days later	
Recalcati et al.[72]	Italy	14	0 (5 were tested)	Erythemato-violaceous papules and macules with possible bullous evolution or digital swelling on feet (n=8), hands (n=4), or both (n=2). Erythematous papular targetoid lesions on hands/elbows (n=2)	All patients were children/young adults. All had complete resolution
Ehsani et al.[73]	Iran	1	0 (not tested but had CT findings)	Pityriasis rosea: scaly erythematous annular plaque on left forearm, then developed into generalized papules and plaques on trunk and upper extremities resembling drooping pine-tree branches	Patient was an otherwise healthy 27-year-old male
Mayor-Ibarguren et al.[74]	Spain	1	1	Cutaneous small vessel vasculitis: palpable purple papules and blisters on lower legs, feet, toes	Histopathology consistent with leukocytoclastic vasculitis
Krajewski et al.[75]	Poland	2	2	Cutaneous hyperesthesia (n=2) with fine scaly pink pruritic rash on the mammary area that spread to neck and abdomen (n=1)	Patient with rash had experienced similar rash and hyperesthesia with previous viral infections

Table 1. *Continued*

Paolino et al.[76]	Italy	1	0 (not tested)	Erythematous maculopapular rash with craniocaudal spread on face, neck, and trunk and urticaria-like lesions on lower limbs	
Morey-Olivé et al.[77]	Spain	2	2	Confluent erythematous maculopapular rash that spread from trunk to extremities with palmar involvement (n=1). Urticaria-like eruption with cephalocaudal spread (n=1)	Pediatric patients. Skin lesions completely resolved.
Dominguez-Santas et al.[78]	Spain	1	1	Cutaneous small vessel vasculitis: purpuric macules and papules on bilateral legs with Koebner phenomenon on right knee, no livedo reticularis or retiform purpura	
Bitar et al.[79]	US	6	6	Exfoliative shock syndrome: erythematous to dusky plaques with superficial exfoliation on trunk (n=2), rash and mucositis or SJS-like eruption: dusky vesicles and bullae with denudation on back (n=1), non-uremic calciphylaxis with thrombotic vasculopathy: painful retiform purpura on bilateral legs (n=1), maculopapular rash (n=2)	1 patient with exfoliative shock syndrome expired
Kamali Aghdam et al.[80]	Iran	1	1	Cutaneous mottling	15-day-old neonate with sepsis. Fully recovered.
Herrero-Moyano et al.[81]	Spain	8	8	Maculopapular exanthema (n=8) on trunk in all cases and on flexures, face in some cases	Late-onset and during hospitalization
Papa et al.[82]	Italy	1	1	Chilblain-like lesions on feet with dyschromic ulcerative lesions of nails	11 year old female, symptoms completely resolved
Larrondo et al.[83]	Chile	1	1	Acute symmetric purpura on buttocks, thighs, and axillae with papules coalescing into plaques	Completely resolved
García-Gil et al.[84]	Spain	1	0 (negative test)	Pruritic purpuric eruption and vesicles on bilateral heels	Pediatric patient otherwise asymptomatic. Histology showed purpura-erythema multiforme consistent with thrombotic vasculopathy
Karaca et al.[85]	Turkey	1	1	Erythematous purpuric rash in left inguinal region	
Reymundo et al.[86]	Spain	7	7	Maculopapular eruption involving trunk (n=7) and proximal limbs (n=6)	
Jones et al.[87]	US	1	1	Met diagnostic criteria for Kawasaki disease: initially had erythematous rash that became a blanching polymorphous maculopapular rash along with prominent tongue papilla, swelling of hands and lower extremities	6-month-old female
Zulfiqar et al.[88]	France	1	1	Petechiae	Diagnosed with Immune thrombocytopenic purpura

Table 1. *Continued*

Zhou et al.[89]	China	1	1	Venous thrombosis and arteriosclerosis obliterans of lower extremity	Patient had severe course with multiple comorbidities and succumbed to disease
Schultz et al.[90]	US	2	2	Fingertip ischemia (n=2). Mottled dusky distal phalanges and nail beds (n=1), ischemic changes with pulp necrosis and dusky fingertips (n=1)	One patient succumbed to disease (70 yo female with no medical history who developed ARDS and septic shock), other patient recovered
Diaz-Guimaraens et al.[91]	Spain	1	1	Erythematous maculopapular rash with petechiae affecting buttocks, anterior thighs, and popliteal fossae	Patient fully recovered
Sernicola et al.[92]	Italy	NR	NR	Erythematous to violaceous lesions on palmar and dorsal fingers with targetoid shape, erythema multiforme-like	Young patients
Perini et al.[93]		2	2	Acute limb ischemia (n=2)	Patients were 53 and 37 years old with no atherosclerosis and otherwise healthy
Davoodi et al.[94]	Iran	1	1	Redness, warmth, and swelling of leg due to deep vein thrombosis	
Ciccarese et al.[95]	Italy	1	1	Erythematous maculopapular rash with petechiae on lower extremities and erosion, ulceration and crusting on inner lips with palatal petechiae	Adolescent patient with complete resolution
Fernandez-Nieto et al.[96]	Spain	1	1	Urticarial rash	Resolved completely
Lorenzo-Villalba et al.[97]	France	3	3	Facial and trunk erythema (n=1), petechial purpura on lower limbs (n=2), oral mucosal hemorrhagic bubbles (n=1)	All patients recovered
Noakes et al.[98]	UK	2	0 (not tested)	Chilblain-like lesions: pruritic tender erythematous plaque on toes (n=2), with dry erythematous facial rash (n=1)	Young adults
Ramondetta et al.[99]	Italy	7	0 (not tested)	Chilblain-like lesions (n=7)	Ages 4-60
Tammaro et al.[6]	Italy, Spain	3	3	Vesicular lesions (n=3): vesicles surrounded by erythematous halos with mild pruritus (n=2), numerous isolated vesicles on back (n=1)	Authors suggest lesions may have been caused by herpesvirus based on appearance. Prevalence estimate in one of the hospitals: 1.5%
Verdoni et al.[114]	Italy	8	8	Kawasaki-like symptoms (n=8)	Study compared incidence of Kawasaki symptoms in groups of children before and during COVID-19 pandemic; there was a 30-fold increase
Hedou et al.[9]	France	5	5	Erythematous rash (n=2), urticarial (n=2), oral HSV-1 reactivation (n=1)	5/103 COVID confirmed patients had cutaneous manifestations

Table 2. COVID-19 larger case series and observational studies (n>20) with cutaneous manifestations

Citation	Country of Origin	Study Type	Number of patients	Number of patients with confirmed COVID-19	Skin manifestations	Comments
Marzano et al.[100]	Italy	Multi-center case series	22	22	"Varicella-like" exanthem: scattered or diffuse papulovesicular lesions on trunk and extremities	
Colonna et al.[101]	Italy	Single-center case series	30	0 (6 were tested; all negative)	Chilblain-like lesions on feet, ankles, and/or hands (n=30)	All pediatric, median age 11
Andina et al.[102]	Spain	Single-center retrospective	22	1 (19 were tested)	Chilblain-like lesions on feet, some with swelling or superficial blistering	All pediatric patients between ages 6-17
Freeman et al.[103]	Multi-country registry	International registry-based case series	318	23	Chilblain-like lesions of feet, hands, or both (n=318)	
Fernandez-Nieto et al.[104]	Spain	Nationwide retrospective	132	2 (11 were tested)	Chilblain-like lesions on fingers/toes (n=95), erythema muliforme-like lesions (n=37)	
Mastrolonardo et al.[105]	Italy	Single center case series	38	0	Acral chilblain-like lesions (n=29), acral ecchymoses (n=21)—overlap because some had both	Patients were otherwise asymptomatic children
Rubio-Muniz et al.[106]	Spain	Single center case series	34	17	Maculopapular exanthema (n=10), pseudo-chilblain/ livedo lesions (n=10), targetoid lesions (n=5), palpable purpura (n=4), acute urticarial/urticarial exanthem (n=4), prurigo (n=1)	
Bouaziz et al.[107]	France	Nation-wide retrospective	54	14	Chilblain-like lesions (n=42), exanthema (n=4), vesicular rash (n=2), urticarial rash (n=1), porcelain-like violaceous macules (n=1), livedo (n=1), purpura (n=2), eruptive cherry angioma (n=1)	40 patients with chilblain-like lesions were unconfirmed; 6/40 were tested
Recalcati et al.[5]	Italy	Single hospital prospective	18	18	Erythematous rash (n=14), widespread urticarial (n=3), vesicles (n=1)	Was the first study to attempt prevalence of skin findings in COVID-19: 20.4%
Fernandez-Nieto et al.[108]	Spain	Single hospital prospective	24	24	All had vesicular lesions (n=24). Diffuse pattern: widespread papules, vesicles, pustules in different stages or localized pattern: monomorphic lesions located centrally in same stage	

Table 2. *Continued*

Docampo-Simón et al.[109]	Spain	Regional prospective	58	1 (39 were tested)	All lesions were acral. Chilblain-like lesions (n=42), purpuric lesions (n=3), vesiculobullous lesions (n=3), eczematous lesions (n=3), paronychia (n=1), ulcer (n=1), desquamation (n=1)	All patients who presented with acral findings in a 3-week period were tested for COVID-19
Garcia-Lara et al.[110]	Spain	Single hospital retrospective	27	0 (9-11 were tested)	Acral purpuric lesions on hands/feet: chilblain-like (n=25), erythema multiforme-like (n=2)	All were pediatric patients
Saenz Aguirre et al.[111]	Spain	Single hospital retrospective	74	1 (11 had testing)	Acral lesions on hands/feet: chilblain-like (n=57), purpuric macules (n=30)—overlap as some patients had both	Median age 14.5
Galván Casas et al.[112]	Spain	Nationwide prospective consensus using survey data	375	234	Chilblain-like lesions (n=71), vesicular lesions (n=34), urticarial eruption (n=73), maculopapular lesions (n=176), livedo/necrosis (n=21)	
de Masson et al.[113]	France	Nationwide retrospective	277	25 (34 were tested)	Urticarial lesions (n=26), vesicular lesions (n=41), acral lesions (n=142), maculopapular rash (n=25), petechial rash (n=7), livedo reticularis (n=4), other (n=41)	
Guarneri et al.[7]	Italy	Single hospital prospective	34	18	Widespread urticarial (n=2), panniculitis (n=3), erythematous rash (n=2), chilblain-like lesions (n=23), acrocyanosis and leg thrombosis (n=2), reactivation of oral herpes simplex (n=2)	Report prevalence of skin manifestations: 10.4%
De Giorgi et al.[8]	China, Italy	Binational multicenter prospective	53	53	Erythematous rash (n=37), diffuse urticarial (n=14), vesicular rash (n=2)	Prevalence of cutaneous manifestations: 7.8%
Askin et al.[10]	Turkey	Single hospital prospective	52	34	Maculopapular rash (n=11), pityriasis rosea-like rash (n=1), urticarial (n=7), petechial purpuric rash (n=4), necrosis (n=4), enanthema/apthous stomatitis (n=3), vesicular rash (n=3), chilblain-like lesions (n=1), erythematous scaly rash on hands (n=17)	Rashes on hands thought to be due to hand-washing. 36.1% of hospitalized COVID-19 patients had skin findings; 24% occurred during COVID-19 infection

Discussion & Conclusions

Galván Casas et al. categorized the cutaneous manifestations of COVID-19 into 5 patterns with varying prognostic significance: acral chilblain-like lesions (least severe, younger patients), vesicular eruptions, urticarial eruptions, maculopapular eruptions, and livedo or necrosis (most severe, older patients).[11] Suchonwanit et al. delineate two broad categories — exanthematous eruptions and vasculopathy — and suggest that the former are a non-specific inflammatory response to the virus itself that does not correlate with disease severity; while the latter, which range from benign chilblain-like lesions to acute ischemia, may be useful indicators of severity.[12,13]

Our literature review of published cases parallels this finding. Chilblain-like lesions present as non-blanching purpuric or deeply erythematous macules or patches on acral skin. In our review those with chilblain-like lesions tended to be younger, mildly symptomatic or asymptomatic, and generally experienced full recovery. Other vascular findings include livedo reticularis, livedo racemosa, retiform purpura, and acute limb ischemia. Livedo reticularis manifests as net-like or mottled skin discoloration and suggests physiologic reversible disturbance of blood flow to the skin. Livedo racemosa indicates partially occluded cutaneous vessels and retiform purpura indicates completely occluded cutaneous vessels; both are pathologic signs of cutaneous thrombosis.[14] Those with severe vasculopathic findings in our review had a more severe disease course. Of the 9 reported patients who succumbed to their disease, 5 had ischemic skin findings. However, not all patients with ischemia were older or had comorbidities; 2 confirmed COVID-19 patients with acute limb ischemia were relatively young (37 and 53 years old) and otherwise healthy with no atherosclerosis.[15] Finally, while we found no reports of exanthematous eruptions correlating with disease severity, some did suggest their prognostic utility. Van Damme et al. reported two patients with acute extensive urticaria that, along with fever, were the presenting sign of COVID-19,[16] and Joob et al. reported a patient who presented with petechial skin rash presumed to be Dengue fever, which led to delayed COVID-19 diagnosis.[17]

These skin findings, along with dermoscopic and histopathological correlates, may provide clues regarding COVID-19 pathophysiology. Navarro et al. analyzed dermoscopic findings in COVID-19-related chilblains. They described a background area in all cases; this was generally red, purple, or brown in color. These colors indicate vascular dilation, red blood cell extravasation, and hemosiderin deposition, respectively.[18] Gianotti et al. examined histopathology from PCR-positive COVID-19 patients and found dermal edema, dilated capillaries, red blood cell extravasation, and increased eosinophils in exanthematous and papular lesions. In a patient with a livedoid exanthematous eruption admitted to the ICU for severe systemic symptoms, Langerhans cells were found in the epidermis and microthrombi with nuclear and eosinophilic material were found in the dermis. The authors suggest that these findings provide valuable insight into the mechanism of the disease. They theorize that the SARS-CoV-2 virus travels through the vascular system, possibly inducing vessel damage along the way, and that it alerts the immune system by activating Langerhans cells. While circulating immune complexes may induce short-lived urticarial reactions, progression of the disease and immune complex-mediated induction of cytokine activation may be responsible for downstream thrombotic complications.[19]

Knowledge of cutaneous manifestations and their potential significance may heighten provider awareness and recognition of this disease. Furthermore, these findings and their histopathologic correlates may help elucidate the mechanism by which SARS-CoV-2 causes disease. The American Academy of Dermatology has established a COVID-19 patient registry where providers can enter patient details of COVID-19 patients with cutaneous eruptions. This registry currently has several thousand entries from across multiple countries (https://www.aad.org/member/practice/coronavirus/registry).

Limitations

Our review was limited to published English language studies, which report on a very small fraction of the total number of COVID-19 cases worldwide. Only 34% of patients had confirmed COVID-19 positivity. Limited testing, possible false negative tests, and potential low antibody response in minimally symptomatic individuals may impact which cases are published. Most patients hospitalized for COVID-19 were exposed to multiple different medications simultaneously and therefore drug eruptions or other causes for skin findings could not be excluded. As COVID-19 cases continue to rise, additional studies may better describe cutaneous manifestations in a larger patient population.

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Reviews

SARS-CoV-2: A Review of the Virus's Biology

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Abstract

The coronavirus disease 2019 (COVID-19) pandemic has affected over 185 countries and infected over 6.3 million people as of June 2, 2020. Without a reliable and effective treatment or vaccine, the number of infected patients continues to rise daily. The causative agent for COVID-19 is severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a highly infectious virus that is transmitted through respiratory droplets. In this review, we provide an overview of the history, biology, life cycle, potential therapeutic targets, and lab techniques used to study this virus. We compare SARS-CoV-2 to other viruses within the betacoronavirus genus, including bat coronaviruses and SARS-CoV, and discuss the life cycle and structural variations that explain the differences in infectivity among the viruses. We also discuss the potential life cycle stages that can be targeted with therapeutic interventions and the types of experiments that are done to confirm their effectiveness. This is a unique time in science and medicine as the entire world is collaborating to share new information on this novel coronavirus. By having a firm understanding and foundation on the molecular biology of the virus, we will be one step closer to discovering a cure.

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Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of coronavirus disease 2019 (COVID-19), is a 29.9 kilobase (kb), enveloped, positive-sense ribonucleic acid (RNA) virus from the betacoronavirus genera and is responsible for the current pandemic affecting many countries across the world.[1] Originating in Wuhan, China with the first case reported in December 2019, there are now over 6,300,000 confirmed cases of SARS-CoV-2 and over 375,000 related deaths in the world as of June 2, 2020 (Figure 1). In comparison to SARS-CoV and the Middle East Respiratory Syndrome Coronavirus (MERS-CoV), two other viruses in the betacoronavirus genera responsible for outbreaks in 2002 and 2015, respectively, SARS-CoV-2 has been shown to be more infectious.[2] Although SARS-CoV and SARS-CoV-2 are genetically similar and thought to have similar origins in the horseshoe bat, the 2002-2003 SARS-CoV outbreak was limited to five countries with 88% of the 8,096 confirmed cases occurring in China and Hong Kong.[2,3] SARS-CoV-2 has currently infected approximately 780 times more people than SARS-CoV with over a quarter of the infections reported in the United States. With many papers already published on SARS-CoV, this virus has been used as an incredibly helpful tool to understand which modifications in SARS-CoV-2 allowed it to become so infectious and dangerous, such as its altered spike glycoprotein and the novel furin-like cleavage site.[2,4]

SARS-CoV-2 is highly infectious due to its structural and non-structural proteins responsible for host cell entry and its transmissibility via respiratory droplets. Thus, in order to effectively develop therapeutics and vaccines against SARS-CoV-2 infection, it is critical to have a firm understanding of the virus's structure and life cycle. With new information regarding the virus being discovered and published every day, it is necessary for readers to regularly update themselves with the latest findings on this topic. In this review, we discuss the most up-to-date findings involving various topics related to SARS-CoV-2's biology, including its structural and non-structural proteins, its life cycle, viral proteins that are currently being identified as potential therapeutic targets, and different laboratory techniques used to study SARS-CoV-2 in vitro and in vivo. The race to control the SARS-CoV-2 pandemic has been marked a global priority, and by having a strong foundation and understanding of the virus's biology, we will be a step closer to achieving this goal.

History of SARS-CoV and SARS-CoV-2

Overview of the coronavirus family

Coronaviruses are a large family of positive-sense, non-segmented, enveloped RNA viruses, all with a characteristic crown-shaped appearance due to unique spike surface proteins.[5] Based on phylogeny, the family is further divided into four genera: alpha-, beta-, gamma-, and deltacoronaviruses. Although these viruses are classified into four separate groups, they all have many similar features, especially when involving their conserved genomic organization.[6,7] The genome of the coronaviruses is organized starting with the leader sequence at the 5' end, then the untranslated region (UTR), replicase, spike (S) protein, envelope (E) protein, membrane (M) protein, nucleocapsid (N) protein, UTR, and 3' poly (A) tail with genes encoding accessory proteins interspersed throughout the structural genes.[6,8] They also have a unique ability to frequently undergo homologous and nonhomologous recombination, allowing the coronavirus family to evolve over time and obtain novel gain-of-function mutations.[9-12] Coronaviruses are also known to host shift, which may explain why most human-infecting coronaviruses have animal reservoirs. For example, two members of the betacoronavirus genus, human coronavirus OC43 (HCoV-OC43) and bovine coronavirus (BCoV), show over 90% homology both at the nucleotide and protein levels for all of its open reading frames, including their structural and non-structural proteins.[13] Using molecular clock analysis, it was hypothesized that HCoV-OC43 originated from BCoV in the 1890s through host shifting.[13] HCoV-229E is another human coronavirus hypothesized to arise from a bat alphacoronavirus about 200 years ago.[14] With strong evidence of coronaviruses having the capability of viral evolution and cross-species transmission, it is not surprising for these viruses to originate from zoonotic hosts and then eventually mutate to infect humans. This explains why both SARS-CoV and likely SARS-CoV-2 emerged from bat carriers, and are responsible for the two pandemics in the 21st century.

Origins of SARS-CoV and SARS-CoV-2

The 2002-2003 SARS-CoV pandemic was the first pandemic of the 21st century and although it did not infect or spread to as many countries as SARS-CoV-2 (Figure 1), both viruses caused unprecedented global panic. Originating from Foshan City, Guangdong Province, China with the first case reported in November 2002, SARS-CoV infected people via respiratory droplets, direct contact with infected individuals, and possibly fomites.[15] By February 2003, the virus spread to Hong Kong, China, which is approximately 83 miles from Foshan City.[16] When comparing the genome sequences of SARS-CoV in infected patients to animals from a live animal market in Shenzhen, China, it was hypothesized that the virus originated from raccoon dogs, viverrids, mustelids, and canids.[16,17] Strangely, by the end of the pandemic, there was a 29 nucleotide deletion in the S gene of SARS-CoV in infected patients when compared to the viral sequences of zoonotic hosts.[18] Bats, on the other hand, have been identified as natural reservoirs for other viruses. When sequencing swab samples for SARS-CoV in the horseshoe bat in the Rhinolophus genus, it was found that these viral sequences also lacked the 29 nucleotides in the S gene, suggesting that these bats may have served as reservoirs for SARS-CoV.[19]

Almost two decades later, in December 2019, the first case of SARS-CoV-2 was reported in Wuhan, China at a Huanan Seafood wholesale market.[20] SARS-CoV-2 most likely has a zoonotic origin but it is not completely clear if the first patient was infected by environmental factors or directly from an

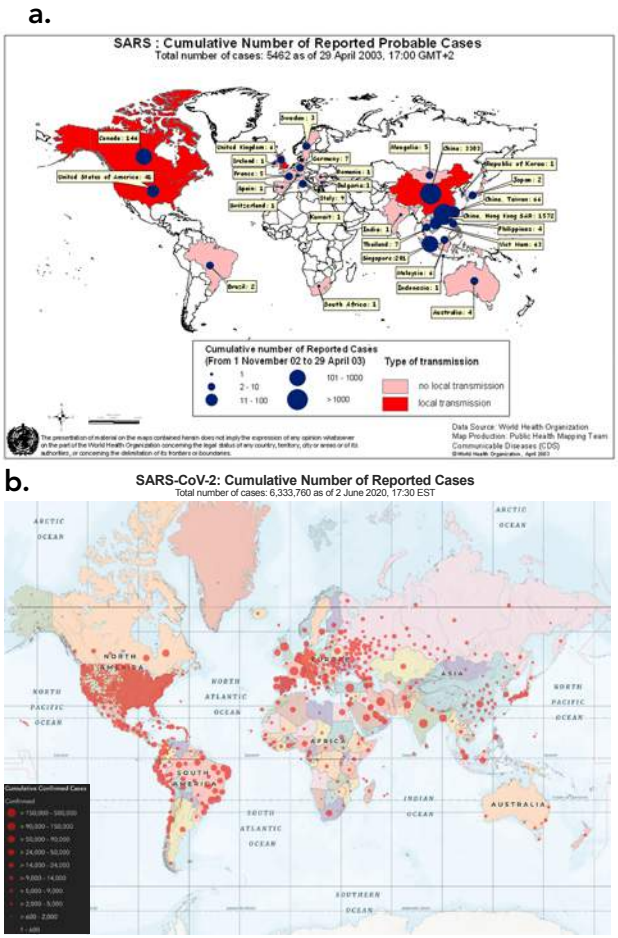

Figure 1. Prevalence of SARS-CoV and SARS-CoV-2 during their outbreaks. Web screenshots of the world prevalence of (a) SARS-CoV during the SARS-CoV pandemic in 2003 (https://www.who.int/csr/sars/SARS2003_4_8.jpg) and of (b) SARS-CoV-2 as of May 17, 2020 (https://coronavirus.jhu.edu/map.html). On the SARS-CoV-2 prevalence map, the number of reported SARS-CoV-2 cases and related deaths are indicated for each country and state by clicking on the red circles.

animal. Through genomic sequencing and phylogenetic analysis of various surface samples at the market, those samples showed significant similarity to the virus's genome of the first infected patient.[2] SARS-CoV-2 is stable and viable on surfaces, such as plastic, stainless steel, cardboard, and copper, for up to 72 hours so it is possible for it to be transmitted that way.[21] However, without direct animal and environmental sampling during the start of the outbreak, it is impossible to confirm the virus's origins. Similar to SARS-CoV, SARS-CoV-2 may also have bats as reservoirs since the Rhinolophus affinis bat RaTG13 and RmYN02 coronaviruses are the most closely related viruses to SARS-CoV-2 with their genomes almost 96% identical (Figure 2a).[22] However, it is unlikely for these bats to be the original hosts of the virus because the bat coronaviruses lack the variations in the S protein found in SARS-CoV-2. This variation may explain why bat S proteins have reduced binding affinity to the angiotensin-converting enzyme 2 (ACE2) receptor in humans, which supports the claim that bats are reservoirs for SARS-CoV-2, but not the original host. Some Malayan pangolin coronaviruses, on the other hand, do not display as strong of a genetic similarity to SARS-CoV-2, but their S protein has a much higher affinity to the human ACE2 receptor, similar to the S protein of SARS-CoV-2. It is also interesting to note that human coronavirus NL63, a common, circulating coronavirus in the alphacoronavirus genera, has significant protein homology to bat coronavirus RaTG13 (Figure 2b). The origins of SARS-CoV-2 are unclear and difficult to validate but there are a few theories.[23]

Currently, there are no animal coronaviruses that have both polybasic cleavage sites and the S protein found in SARS-CoV-2, making it difficult to confirm the direct ancestor to the virus. With a wide diversity of coronaviruses that have not been sequenced yet, it is possible for the virus to have mutated in zoonotic hosts living in high population densities, allowing the virus to gain novel insertions and deletions in its genome before the virus infected humans. The second theory is that a less virulent strain of SARS-CoV-2 infected humans and over time with a myriad of unrecognized human-to-human transmissions, the virus could have evolved to what it is today. The last theory suggests the possibility of a lab accidentally creating SARS-CoV-2 with extensive cell culture and animal passaging. This theory is least likely to be true because SARS-CoV-2 requires O-linked glycosylation for it to become mature, which typically requires enzymatic modifications from the human immune system, or cell culture and animal models expressing human ACE2 receptors for S protein affinity maturation. None of these studies have been previously reported before the pandemic.[23] Even if a lab did perform these experiments, the development of SARS-CoV-2 is extremely unlikely to occur.

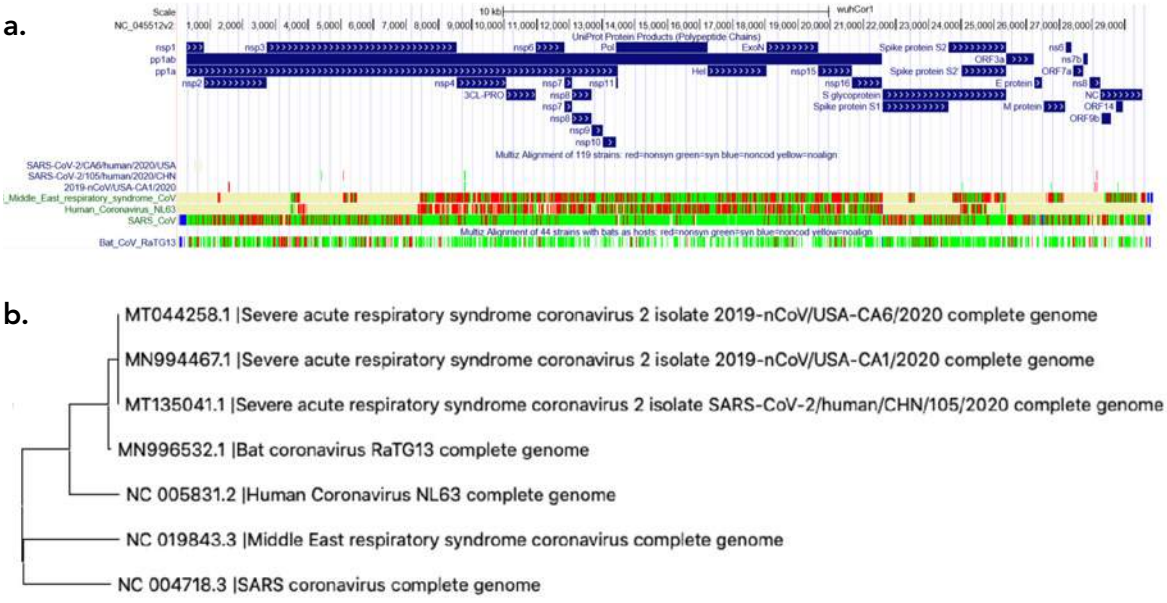

Figure 2. Genomic alignment of SARS-CoV-2 to other related coronaviruses. (a) A genomic alignment of various strains in the beta coronavirus family, including SARS-CoV, MERS-CoV, SARS-CoV-2 and RaTG13, is shown. The wuhCor1 assembly was used as the reference genome. Areas that are yellow indicate regions that cannot be aligned to the reference, areas that are green indicate silent polymorphisms, areas that are red indicate missense polymorphisms, and areas that are blue indicate regions outside the coding region. Areas that do not have any of these colors indicate homology to the reference strain. The University of California Santa Cruz Genome Browser (https://genome.ucsc.edu/) was used to create these alignments.[102] (b) Phylogenetic tree of the beta coronaviruses and NL63 (alpha coronavirus) from aligned amino acid sequences. Phylogenetic tree reconstruction using MEGA X (version 10.1.8 build 10200331) to create a Neighbor-Joining tree using p-distance.

Genomic and structural differences between SARS-CoV and SARS-CoV-2

SARS-CoV and SARS-CoV-2 arise from the same betacoronavirus genus and are both genetically similar with a homology of approximately 79% (Figure 2a).[4] The genome for SARS-CoV-2 consists of various open reading frames (ORFs) encoding for replicase machinery and structural proteins, similar to SARS-CoV.[24] ORF1a and ORF1b have been noted to encode for the 16 non-structural proteins (nsps) identified within the first two-thirds of the genome.[24] The nsps make up many important proteins crucial for the virus's life cycle, including the RNA-dependent RNA polymerase (RdRP) and papain-like proteases, which aid in viral survival within host cells.[24]

Although both viruses are relatively similar genetically and structurally, their infection rates vary significantly. Over the span of the entire SARS-CoV pandemic, which was only about 8 months, there were 8,096 confirmed cases while in only 7 months, there are currently over 6.3 million confirmed cases of SARS-CoV-2 in the world (Figure 1). The most notable difference between the two viruses that may explain the difference in infectivity is the presence of a longer S protein in SARS-CoV-2, which contains a novel furin-like cleavage site.[2,4] This modification most likely allows the SARS-CoV-2 S protein to bind to human ACE2 receptor with greater affinity than the SARS-CoV S protein.

Structural and non-structural proteins of SARS-CoV-2

RNA polymerase

SARS-CoV-2 RdRP (106 kilodaltons or kDa), also known as nsp12, is a large multimeric protein that is paramount for the virus's life cycle. The RdRP interacts with other co-factors, specifically nsp7 and nsp8, which have primase activity and are necessary for catalytic activity.[25] SARS-CoV-2 RdRP is also homologous to most other RdRPs in the coronavirus family, sharing approximately 96% protein homology with SARS-CoV RdRP.[26] Studies exploring molecular docking at

the catalytic region of the RdRP can be crucial in discovering new therapies targeting this area.[6,25,27,28]

The architectural domain of RdRP consists of the palm, the finger and the thumb, similar to most RdRPs. The highest conserved residues are located within the inner architecture of the polymerase, which is in direct contact with the nascent viral RNA. Through RdRP crystallography, nsp7 and nsp8 have been identified as co-factors to nsp12, stabilizing and forming the large multimeric structure, nsp12-nsp7-nsp8.[25,28] SARS-CoV-2 has also been identified to have a homologue to nsp14, which has exonuclease activity in SARS-CoV. Although SARS-CoV-2 RdRP also has 3'-5'exonuclease activity, it is still prone to errors, allowing viral evolution.[26,29]

Spike protein

S protein (180 kDa) is a structural, transmembrane protein crucial for viral attachment and entry into host cells (Figure 3). S protein consists of homotrimers that span and extend from the viral surface, providing its unique crown-like, spike shape. It is currently subjected to extensive study as it is a major target for antiviral therapies. Although SARS-CoV-2 is homologous to SARS-CoV, one of the most important distinctions that differentiate their infectivity mechanisms is the S protein: the SARS-CoV-2 S protein shares only approximately 76% amino acid homology with the S protein of SARS-CoV.[30]

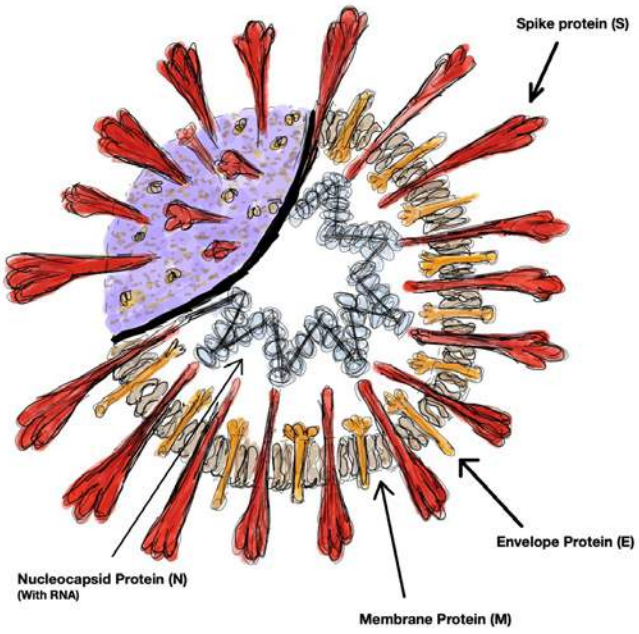

Figure 3. Illustration of SARS-CoV-2. Schematic drawing of SARS-CoV-2 with its four structural proteins: S, E, M, and N proteins. All the structural proteins are assembled in the ER-Golgi intermediate compartment before the viral progeny are released out of the cell.

Based on the function and the location of a unique cleavage site, the SARS-CoV-2 S protein can be divided into two proteins: S1 and S2. The S1 protein contains a highly conserved receptor-binding domain (RBD) located in the C-terminal domain (CTD), critical for facilitating attachment to human ACE2 receptor.[23] In order for S1 to attach to the human ACE2 receptor, there is significant hinge-like movement at the RBD, altering the protein conformation and allowing accessibility to the ACE2 receptor.[31] After binding to the human ACE2 receptor, the S protein homotrimers are destabilized, allowing for shedding of the S1 subunit. These structural changes allow for a stable S2 conformation, facilitating further viral fusion.[30,31] Furthermore, it is postulated that there are two distinct spontaneous conformations for the SARS-CoV-2 S protein homotrimers: open and closed. The open S trimer conformation allows access to the receptor-binding motifs, which are buried in the case of closed trimer conformation. Thus, the S open trimers have been identified in highly pathogenic strains, while those that remain in the closed conformation are associated with milder symptoms.[30]

Additionally, at the S1 and S2 junction, there is a proline residue inserted in front of the cleavage site.[23] The proline can potentially stabilize the cleavage site and is predicted to insert a turn which can be subjected to further O-linked glycosylation. Although the role of additional O-glycans is ambiguous at this site, they have previously been identified in creating domains that help shield important viral proteins, helping evade immune surveillance.[23,32]

The S1 CTD is highly variable and helps facilitate the interaction between S protein and human ACE2 receptor. The N-terminal domain (NTD), on the other hand, does not have any affinity towards human ACE2 receptors.[33,34] SARS-CoV-2 CTD interacts with human ACE2 receptor through strong polar interactions, specifically with hydrogen bonds and salt bridges, extensively in the ACE2 binding domain.[34] Six key amino acids have been identified in the CTD that are involved in binding to human ACE2 receptors.[23] Out

of the six amino acids, five differ between SARS-CoV and SARS-CoV-2. Structural and biochemical analysis speculate that these differences might be one of the factors leading to enhanced affinity noted between SARS-CoV-2 and human ACE2.[23,33]

Furthermore, there are 24 residues identified on the human ACE2 receptor which are involved in interacting with the SARS-CoV-2 CTD. Out of the 24 residues, 15 are in more direct contact through van der Waals interactions at the SARS-CoV-2 CTD and human ACE2 interface.[34] This interaction at the atomic level leads to stronger binding affinity when compared to the SARS-CoV RBD and human ACE2 interface. This was further confirmed by Wang et al. using surface plasmon resonance (SPR), which calculated the equilibrium dissociation constants for SARS-CoV RBD, SARS-CoV-2 S1, and SARS-CoV-2 CTD as 408.7 ± 11.1 nM, 94.6 ± 6.5 nM, and 133.3 ± 5.6 nM, respectively.[34] This demonstrated approximately 4 times greater affinity between SARS-CoV-2 CTD and human ACE2, despite sharing 73.9% homology with SARS-CoV RBD.

Another novel aspect of SARS-CoV-2 is the insertion of four amino acids between the S1 and S2 sites, also known as the furin-like cleavage site.[23,30,35] The furin-like cleavage site is crucial in enhancing infectivity and increasing viral tropism. The site is cleaved by furin, a serine protease encoded by the FURIN gene that is ubiquitously expressed in various mammalian tissues. Furin is an important regulator for various cellular processes, but it is also utilized by pathogens and tumors. Furin has been noted to be highly expressed in lung tissues, thus allowing exploitation by respiratory tract pathogens.[35] This might explain the further expanded tropism and infectivity of SARS-CoV-2. It is important to note that although furin-like cleavage sites are not identified in SARS-CoV, they are not unique to SARS-CoV-2. They have also been identified in various viruses such as MERS-CoV, HIV, and influenza.[30,35] Following cleavage of the furin-like cleavage site, host proteases further cleave at the S2 site, allowing for significant conformational changes of S2. These conformational changes activate fusion peptides, initiating the endocytic process (Figure 4).[33,35] The S protein has a critical role in viral pathogenesis and is one of the prime targets for vaccine development and novel therapies.

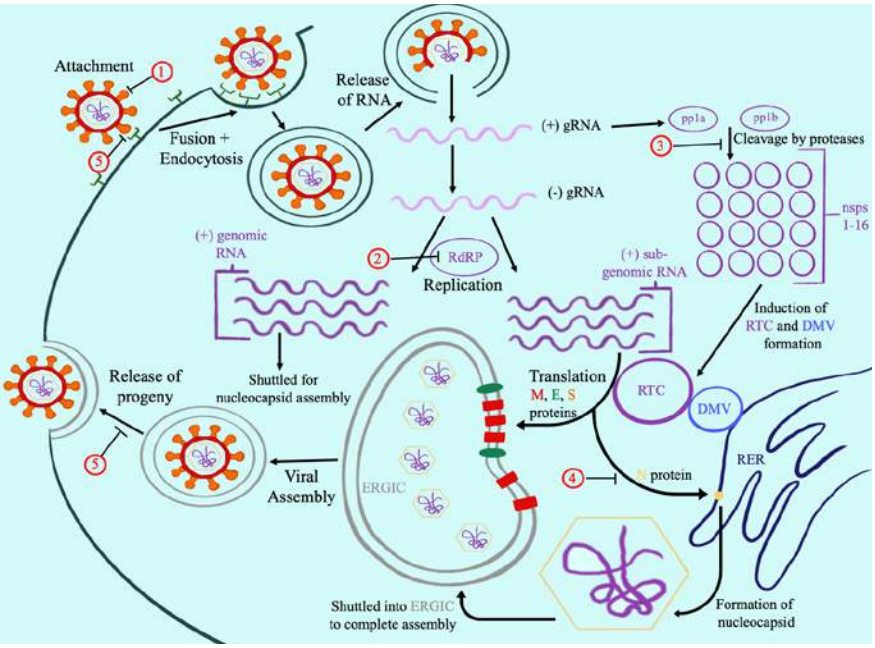

Figure 4. Life cycle of SARS-CoV-2 with antiviral targets. SARS-CoV-2 attaches to the host cell through interactions between the S protein of the virus and an ACE2 receptor on the host cell membrane. Following conformational changes of the S protein, the virus fuses to the host membrane and enters the cell through endocytosis. Once viral genomic RNA is released into the cytoplasm, its replicase gene expresses the pp1a and pp1b proteins. These proteins are cleaved by proteases to produce 16 nonstructural proteins, each of which serve a crucial role in the replication and assembly of viral progeny. In addition, the viral (+) genomic RNA is transcribed into (-) gRNA. Replication of the (-) gRNA produces (+) gRNA, which is used as the genomic RNA for the viral progeny, and (+) sub-genomic RNA, which is translated into the M, E, S, and N structural proteins. The N protein is used to form the nucleocapsid, while the M, E, and S proteins interact to induce envelope formation and completion of the viral assembly. Lastly, the viral progeny is released from the host cell. Several antiviral targets are currently being studied as treatments for the virus due to their inhibition of various stages of the virus's life cycle. These therapeutic treatments include (1) chloroquine and hydroxychloroquine, (2) remdesivir, (3) lopinavir and ritonavir, (4) ivermectin, and (5) arbidol.

Envelope protein

E protein (8-12 kDa) is the smallest structural protein abundantly expressed in SARS-CoV-2 (Figure 3). It is an integral membrane protein involved in many stages of the virus's life cycle, including viral assembly, intracellular trafficking processes, budding, and pathogenesis.[6,36] The E protein is ubiquitously expressed during viral replication with a majority of the protein being localized to sites responsible for cellular trafficking, such as the endoplasmic reticulum (ER) and the Golgi apparatus. Using mass spectrometry and an adapter protein 3 pull-down assay, the E protein was identified to be involved in intracellular trafficking and maintenance of secretory pathways.[36] Furthermore, E protein is also known to interact with PDZ domains located on intracellular signaling and transport proteins. Recent molecular evidence has also identified that the E protein has excess ion channels, which have cation selective activity, mediating membrane permeabilization.[6,36] Blocking these ion channels could be a potential target for future drug design and therapy.

Due to the E protein's multifunctionality and ambiguity in the virus's life cycle and the promising results found through focusing on other promising therapeutic targets, there has been less focus on this protein. Nevertheless, it plays a major role in the virus's life cycle, maturation, and virion release. When the E protein is knocked out, some studies demonstrated significantly lower viral titers most likely due to a decrease in the production of fit viral progeny.[6,36] E protein is also known to have various interactions with M protein in order to formulate the viral envelope.

Since the SARS-CoV and SARS-CoV-2 E proteins share approximately 95% protein sequence, most of our understanding comes from the SARS-CoV E protein.[26,36] The BCL-2 homology 3 (BH3)-like motif is one of the highly conserved sequences in the E protein. The BH3-like motif, which is located on the C-terminal, has been noted to interact with host BcL-xL, a mitochondrial transmembrane protein involved with anti-apoptosis in host cells which consequently aids viral survival.[36] It is possible that SARS-CoV E protein could play a role in protecting the host cell from apoptosis in the early stages of the infection while help induce host cell apoptosis in the later stages of infection. Although the entirety of this mechanism is unclear, it serves to demonstrate one of the unique ways in which the E protein is able to modulate host cellular processes.

Membrane protein

M protein (25-30 kDa) is the most ubiquitously found structural protein in SARS-CoV-2, comprising of a triple spanning transmembrane region which helps with structural integrity (Figure 3).[6] The protein is highly conserved amongst the members of the coronavirus family, especially in SARS-CoV. It is important in viral assembly, as well as mediating other key interactions, including association with the N protein and maintenance of viral shape. Many studies have also shown that the M protein significantly interacts with all other SARS-CoV structural proteins.[37] It has been postulated that the interaction between the M and E proteins facilitate the budding process, regulating the viral life cycle.[6,37,38]

M protein contains an important N-terminus, located external to the virus, as well as a C-terminus that extends within the viral particle. The N-terminal region is highly glycosylated, aiding in attachment and fusion facilitation through interaction with S protein.[6] Further research needs to be conducted to identify these interactions in SARS-CoV-2, although they may share similar functions since the protein homology between the M proteins in SARS-CoV and SARS-CoV-2 is approximately 90%.[26] The interaction with S protein could facilitate attachment and propagate virulence, making it another potential antiviral target.

An interesting aspect noted in the SARS-CoV M protein is its role in various host signaling pathways, allowing it to evade immune surveillance and to mediate cellular apoptosis. For example, the C-terminus in the SARS-CoV M protein was involved in mediating NF-κB and PKB/Akt signaling pathways.[39,40] Through various interactions in these signaling pathways, downstream products can be altered, affecting gene expression. This can help enhance viral pathogenicity and viral evasion from the host's immune system.[39,40] It is likely that the SARS-CoV-2 M protein could also modulate a similar mechanism, enhancing viral pathogenicity, although further research is needed for this to be confirmed.

Nucleocapsid protein

N protein (~50 kDa) is the last structural protein to be encoded in the viral genome, located near the 3'-UTR (Figures 2,3).[41] It has an important association with viral RNA and is crucial in forming the ribonucleoprotein core, which is where the viral genome is contained.[26,42] The N protein also has extensive functions involving viral envelope assembly, viral RNA synthesis, and viral pathogenesis that enhances the virus's infectivity and replication within the host cell.[42] N protein is further expressed during infection and can potentially play a role in facilitating the reproductive cycle of the host cell.

The N protein is rich in positively charged domains and has high affinity for non-specific nucleic acids. The large number of coils and several highly disordered regions within the N protein transiently allow it to bind to various targets as it simultaneously maintains its structural conformation.[42] This function is crucial because it allows the virus to bind to a wide array of targets without losing its structural integrity.

Structurally, the N proteins of both SARS-CoV and SARS-CoV-2 are highly conserved with a protein homology of approximately 90.52%.[42] They also have three distinct, conserved regions: the flexible linker chain which allows the protein to maintain structural conformation during binding; the NTD region which interacts with viral RNA through electrostatic interactions; and the CTD region which is responsible for dimerization that is crucial to the overall protein structure.[42] The highly disordered nature of the N protein, specifically within the linker region, potentiates its ability to bind to non-specific nucleic acid targets with high affinity while retaining its structural integrity. Since the N protein plays such an important role in infectivity and pathogenesis, it is no surprise that various host antibodies against it have been identified in recovering patients. IgA, IgG, and IgM antibodies against N protein have been recovered from patients' serum samples.[41,42] Thus, the N protein presents as a potential target for vaccines and diagnostic tests since it is a natural target by our immune system. Specifically, the NTD region of the N protein has been a therapeutic target due to its involvement with the RNA binding domain.

Life cycle of SARS-CoV-2

Entry and fusion

The life cycle of SARS-CoV-2 is fortunately very similar to the life cycle of SARS-CoV (Figure 4), which allowed researchers to spend more time focusing on structural proteins and potential therapeutic targets rather than elucidating the virus's life cycle. The initial phase of the viral life cycle is characterized by attachment and entrance into the host cell. Cleavage of the S protein at the S1/S2 site allows for the RBD at the C-terminus of the S1 protein to bind with the host cell receptor in a "standing-up" conformation.[30] Studies have shown that SARS-CoV-2 primarily binds to the peptidase domain on human ACE2 receptors.[43] The ACE2 receptor is characterized as a transmembrane protein found on cell surfaces of several organs, including the lungs, heart, kidneys, testes, and intestines. The protein serves a role in the conversion of angiotensin II to angiotensin 1-7, which are components of the renin-angiotensin system that significantly influences cardiovascular disease.[44] Following receptor binding, the protease, TMPRRS2, cleaves the S2 site, revealing the protein's fusion peptide and inducing conformational changes.[30] This rearrangement allows for viral incorporation into the host cell membrane, resulting in fusion and entry of the viral genome into the cytoplasm.[45]

Replication and translation

Once the genome is inserted into the host cell, translation of the replicase gene within the viral genome RNA (gRNA) is initiated.[46] Two ORFs encoded by the replicase gene, rep1a and rep1b, are expressed to produce the polyproteins, pp1a and pp1b.[47] Due to the overlapping of ORF1a and ORF1b, ribosomal frameshifting occurs in order for the expression of both polyproteins to occur. This frameshifting is a result of a slippery sequence (5'UUUAAAC-3') and a downstream pseudoknot structure. The slippery sequence causes the viral polymerase to halt, the reading frame to shift by one nucleotide (-1), and the polymerase to continue expressing pp1a and pp1b.[6]

Viral progeny assembly

Cleavage of pp1a and pp1b by a papain-like cysteine protease (PLpro), main protease (Mpro) and 3C-like cysteine proteinase

(3CLpro) produces 16 nsps.[48] Each nsp plays a crucial role in viral pathogenesis (Table 1).[47] Following the synthesis of nsp3, 4, and 6, these proteins activate the formation of double-membrane vesicles (DMVs), which are extensions of the RER that are hypothesized to hide viral gRNA from host cell antiviral mechanisms.[49] Several nsps then aggregate into a replicase-transcriptase complex that binds to the DMV, an interaction necessary for the replication and transcription of the sub-genomic RNAs. Following the assembly of these proteins, negative-sense genomic and sub-genomic RNA strands are synthesized. The negative-sense genomic RNA strands are used as templates in the synthesis of positive-sense genomic RNA, and the sub-genomic RNA strands are translated into structural and accessory proteins, including the transmembrane structure proteins (S, M, and E). Once synthesized, the S, M, and E proteins are cotranslationally inserted the host cell's ER membrane and are transported to the ER-Golgi intermediate compartment (ERGIC). It is

Table 1. Function of each nonstructural protein

Nonstructural protein (nsp)	Function	Source
Nsp1	Promotes host mRNA degradation through interaction with the host 40S ribosomal subunit	https://www.sciencedirect.com/science/article/pii/S2211383520302999
Nsp2	Binds to prohibitin proteins 1 and 2	https://link.springer.com/content/pdf/10.1007/s10930-020-09901-4.pdf
Nsp3	Induces DMVs and CMs through host cell membrane rearrangement	https://www.sciencedirect.com/science/article/pii/S0042682217302386
Nsp4		https://www.sciencedirect.com/science/article/pii/S0042682217302386
Nsp5	Forms the Main protease (Mpro)	https://jvi.asm.org/content/87/23/12611.short
Nsp6	Induces cellular autophagy	https://www.sciencedirect.com/science/article/pii/S0163445320301869?casa_token=huHegEKsFRQAAAAA:u9MHMFaIllxOtYEU8cSRYRtq1_GgZadG-SiZLPp4KXn_4GHruA578ShF0vHattx30yId7HzZ
Nsp7	Complexes with nsp8 to serve as a sliding clamp for RdRP	https://portlandpress.com/biochemj/article/477/5/1009/222182/Processing-of-the-SARS-CoV-pp1a-ab-nsp7-10-region
Nsp8	Complexes with nsp7 to serve as a sliding clamp for RdRP	https://portlandpress.com/biochemj/article/477/5/1009/22218
Nsp9	Binds to ssRNA and provides stability during replication and transcription	https://link.springer.com/article/10.1007/s10969-007-9024-5
Nsp10	Regulates viral replicase	https://www.jbc.org/content/289/37/25783.short
Nsp11	Unknown	
Nsp12	Forms RdRp	https://www.sciencedirect.com/science/article/pii/S0166354220302072?casa_token=dVYKe3TojMMAAAAA:SUnkN5YLN3ZwGuz6ASSZ06loHOjwDdSv3DdXL4gGLeapfIlRx0w5M0p-xzjlDlZxYRPya1Te
Nsp13	Forms helicase	https://www.sciencedirect.com/science/article/pii/S0166354213003379?casa_token=BReYpHJV_VUAAAAA:ayWPsqrHYpdXBRaAlFkmmvDPZp2tM4QhtJWkwFWH_6-543BQYCHIy0syHITW8Kn61eYR1uzd
Nsp 14	Forms 5'RNA-cap methyltransferase and exonuclease	https://www.sciencedirect.com/science/article/pii/S0166354213003379?casa_token=BReYpHJV_VUAAAAA:ayWPsqrHYpdXBRaAlFkmmvDPZp2tM4QhtJWkwFWH_6-543BQYCHIy0syHITW8Kn61eYR1uzd https://www.sciencedirect.com/science/article/pii/S0166354220302072?casa_token=dVYKe3TojMMAAAAA:SUnkN5YLN3ZwGuz6ASSZ06loHOjwDdSv3DdXL4gGLeapfIlRx0w5M0p-xzjlDlZxYRPya1Te
Nsp15	Forms endoribonuclease	https://onlinelibrary.wiley.com/doi/full/10.1002/pro.3873
Nsp16	Forms 5' RNA-cap methyltransferase	https://www.sciencedirect.com/science/article/pii/S0166354213003379?casa_token=BReYpHJV_VUAAAAA:ayWPsqrHYpdXBRaAlFkmmvDPZp2tM4QhtJWkwFWH_6-543BQYCHIy0syHITW8Kn61eYR1uzd

important to note that the N protein does not follow this pathway, but rather is synthesized in the cytoplasm and encapsulates the viral gRNA progeny in the RER to form their helical nucleocapsids.[50] Thorough research has found the M protein to be a crucial regulator of viral assembly in the ERGIC, specifically through its primary role in developing the viral envelope.[51]

Virus-like particles (VLPs) are then assembled through co-expression of the M, E, and N proteins. While the M protein was found to be the most abundant of the structural proteins and to play a key role in the viral assembly, studies have found that the S, N, and E proteins play key roles in stabilizing and improving the efficiency of virogenesis.[52] Specifically, the S protein utilizes the M protein to embed itself in the viral progeny and N protein interacts with M protein to stabilize nucleocapsid formation.[53] Although the role of the E protein in this phase is not fully understood, studies have demonstrated that the absence of E protein results in a significant decrease in VLP production and virion release.[54] Furthermore, studies have found the E protein to play a role in the morphogenesis of the virus by initiating curvature of the envelope, giving the virus its spherical shape.[53] Following the production of VLPs, the C-termini of the M and N proteins interact with each other to complete formation of the viral progeny.

Following the outbreak of SARS-CoV-2, many have called into question whether or not this virus exhibits a latency period, a phase characterized by a halt in the viral life cycle while in the host cell. In order for a virus's life cycle to involve latency, it must demonstrate that, in addition to exhibiting a halt in replication and expression while in the host cell, the virus must be eventually able to resume proliferation.[55] The coronavirus family is currently not believed to have a latency period as no studies have found substantial evidence; however, it is still a possibility. Recent studies discovered gRNA of mouse hepatitis virus, a betacoronavirus, in isolated neuronal and glial cells, signifying that this virus has the ability to remain dormant in a human cell and could influence the development of multiple sclerosis in individuals.[56,57] Further research is necessary to explore the potential of a latency period in the coronavirus family.

Antiviral targets of SARS-CoV-2

In the absence of a standardized treatment, great focus has been placed on repurposing FDA-approved and investigative drugs to combat SARS-CoV-2. In this section, we will discuss the activity of drugs that are being studied, with S protein, RdRP, and Mpro emerging as some of the main targets for intervention.

Chloroquine (CQ), an anti-malarial and anti-inflammatory drug, has been shown to increase endosomal pH, preventing viral-endosome fusion of SARS-CoV. It was also shown that terminal glycosylation of the ACE2 receptor is decreased in the presence of CQ, inhibiting SARS-CoV S protein attachment to ACE2 and therefore viral entry.[58] Because SARS-CoV-2 also utilizes the ACE2 receptor for cell entry, CQ is a promising therapeutic.[30,59] In vitro, CQ was able to inhibit SARS-CoV-2 with a half-maximal effective concentration (EC50) of 1.13 μM with a selectivity index (SI) > 88.50 according to Wang et al., while an EC50 of 5.47 μM was reported by Yao et al.[60,61] Using the reported maximum plasma concentration (Cmax) of 1500 mg CQ administered orally in humans over 3 days, Arshad et al. derived a lung Cmax/EC90 of 1.261 for CQ, noting varying EC50s in the literature limited interpretation of their results.[62,63] Considered with CQ's in vitro efficacy against several viruses, the potential to exceed the EC90 in the lungs suggest the possible utility of CQ against SARS-CoV-2; however, in vivo success has been limited.[64]

Hydroxychloroquine (HCQ), a CQ analog with fewer off-target effects, has been used extensively in SARS-CoV-2 prophylaxis and treatment, but differences in their mechanisms are not well understood.[65] Yao et al. reported an EC50 of 0.72 micromoles (μM) for HCQ, but did not report 50% cytotoxic concentration (CC50) data.[61] In an observational study of 96,052 patients with 14,088 in treatment groups, treatment with CQ or HCQ with or without macrolides was not associated with a clinical benefit but was associated with increased mortality.[66] However, due to concerns about the data, this study was retracted.[66] Another study of 1438 patients found no significant difference in mortality, but a significant increase in cardiac arrest for patients receiving hydroxychloroquine and azithromycin (adjusted OR, 2.13 [95% CI, 1.12-4.05]), but not hydroxychloroquine alone

(adjusted OR, 1.91 [95% CI, 0.96-3.81]). The lack of proven efficacy and toxicity concerns has led the FDA to revoke the emergency use authorization (EAU) for CQ and HCQ.[67] Thus, the case of CQ and HCQ provides an apt example of promising in vitro data that should be interpreted with caution.

Viral entry and fusion are also thought to be targeted by the anti-influenza drug, Umifenovir (trade name Arbidol). In vitro testing showed that Arbidol effectively inhibited SARS-CoV-2 (EC50 = 4.11 μM, CC50 = 31.79 μM, SI = 7.73) by decreasing virion release from endolysosomes and lowering binding efficiency by approximately 67%.[68] Molecular modeling and structural analysis showed that S protein trimerization, which is necessary for the protein's function, is inhibited by Arbidol.[69] Taken together, this is consistent with the finding that Arbidol inhibits viral fusion at both entry and post-entry steps.[68]

Another strategy employed against SARS-CoV-2 is the use of nucleotide analogs to inhibit RdRP. Remdesivir is an adenosine analog that was under investigation for use against the Ebola virus.[70,71] Specifically, remdesivir acts on nsp12 and is resistant to nsp14 exonuclease, which mediates tolerance in comparison to other nucleoside analogues such as ribavirin and 5-fluorouracil.[71,72] An in vitro study determined that remdesivir incorporates at position i as remdesivir triphosphate (RDV-TP) and causes termination at position i+3 for SARS-CoV-2 with a 0.26 selectivity value, which is the ratio of nucleotide triphosphate incorporated into the nascent RNA strand to nucleotide analog incorporated. Other inhibitors of RdRP, including ribavirin, sofosbuvir, and favipiravir, all incorporate with much lower selectivity at >>1000, 570, and 1056 respectively.[70] Remdesivir demonstrated high potency and SI in vitro (EC50 = 0.77 μM, CC50 > 100 μM, SI >129.87), while ribavirin (EC50 = 109.50 μM, CC50 > 400 μM, SI > 3.65) and favipiravir (EC50 = 61.88 μM, CC50 > 400 μM, SI > 6.46) were significantly less potent.60 In a randomized, double-blind, placebo-controlled study, patients with symptoms for 10 days or less receiving 200 mg on day 1, followed by 100 mg daily infusions of remdesivir for up to 10 days had a faster time to clinical improvement than patients receiving a placebo, though results were not statistically significant (hazard ratio 1.52 [95% CI 0.95–2.43]). The study was terminated before the predetermined sample size could be recruited, and as such, the study was underpowered.[73] In another randomized, double-blind, placebo-controlled study using the same dosing guidelines with 1063 patients, significantly lower median recovery time, mortality, and adverse events were reported for patients receiving remdesivir.[74]

As an essential component of the SARS-CoV-2 life cycle, Mpro has also been investigated as a potential antiviral target. The Mpro of SARS-CoV-2 is inhibited by the HIV-1 protease inhibitors, lopinavir and ritonavir, according to multiple in silica studies.[75-77] Previously, lopinavir was shown to have antiviral activity against SARS-CoV in vitro[78]; however, there are currently no in vitro studies of lopinavir/ritonavir against SARS-CoV-2. In a randomized, controlled, open-label trial of 199 patients with severe SARS-CoV-2 infection, lopinavir and ritonavir were not shown to decrease time to clinical improvement, mortality, or viral load.[79] A rationale for this failure is provided by Catteano et al., who state that measured in vivo concentrations of lopinavir, which range from 10 to 40 μM in COVID-19 patients, were too low to achieve the likely toxic 90% inhibitory concentration (IC90) of 4680 μM and 393 μM lopinavir in plasma and epithelial lining fluid, respectively.[80] This clearly shows the importance of considering in vivo pharmacokinetics when suggesting the repurposing of an FDA-approved drug.

An anti-parasitic with antiviral activity, ivermectin, has also shown potential against SARS-CoV-2 in vitro. Ivermectin has been shown to impede viral replication of RNA viruses that use nuclear importin IMPα/β1 for nucleocytoplasmic shuttling of viral proteins, including the SARS-CoV nucleocapsid protein.[81,82] Thus, Caly et al. hypothesized that SARS-CoV-2 may be inhibited by the destabilization of IMPα/β1 by ivermectin, although this mechanism is still not completely clear. Their in vitro study revealed a 93% reduction in viral RNA in the supernatant at 24 h and a ~5000 fold decrease in viral RNA at 48 h in samples treated with 5 μM ivermectin with an IC_{50} of approximately 2 μM.[83] However, this is another example illustrating the importance of considering pharmacokinetics data, as calculations by Schmith et al. suggest that lung concentrations of ivermectin would only reach 1/20th of the IC_{50} of 2 μM using the approved dose of ivermectin.[84]

Lab Techniques

With limited clinical data and with only several FDA-approved drugs with inhibitory activity against SARS-CoV-2 currently available, it is incredibly important to discover new therapies as efficiently as possible. To achieve this goal, reliable and safe cell culture and animal models are needed to discover new compounds, their mechanisms of action, and their effectiveness as potential prophylactic and therapeutic agents. In this section, we will discuss a few popular in vitro and in vivo techniques used in many labs across the world to study SARS-CoV-2.

In vitro cell culture models

The general scheme to test different chemical agents against any infectious agent with a cell-culture model is as follows: seed cells onto a well plate, add the compound of interest at various concentrations before (if prophylactic) or after (if therapeutic) the infectious agent is added to the media, and then analyze how well the compound inhibits infection via reporter proteins, RT-PCR, ELISA, confocal microscopy, or other types of quantitative and qualitative analyses to extrapolate the IC_{50}. In parallel, a cytotoxicity assay is usually performed to assess the cytotoxicity of the compound and extrapolate the 50% cytotoxic concentration (CC_{50}). Depending on the infectious agent, the cell line is selected based on whether it has the correct receptors, enabling it to be susceptible to infection.

Many groups have been testing different drugs by either infecting cells with different isolated strains of SARS-CoV-2 or through a vector with the S protein. When using viral strains, VeroE6 cells (African green monkey kidney epithelial cell line), Huh7 cells (hepatocellular carcinoma cell line) and Calu-3 cells (human lung cancer cell line) have widely been used due to their ACE2 receptor expressivity.[85-88] Various modifications to these cell lines to co-express other genes, including hSLAM and TMPRSS2, have been used to enhance isolation of SARS-CoV-2 in vitro. VeroE6/TMPRSS2, for example, showed elevated viral RNA levels after infection by over 100 times in comparison to VeroE6 cells.[85] For consistency, most groups seem to infect their cells with the isolated viral strain at an MOI of 0.01 for 2 hours before or after adding the compound of interest, and to analyze infectivity by measuring viral RNA via RT-PCR.

A safer approach to studying SARS-CoV-2 is to infect cells with S protein or a pseudovirus rather than the infectious viral strain. One common method is using 293T cells transfected with a vector encoding the S gene and green fluorescent protein (effector cells) and using ACE2/293T cells as target cells. By co-culturing these cells together, the S gene in the effector cells will be able to bind to the ACE2 receptor in the target cells, and "infection" can then be quantitatively measured via fluorescence. This model can then test various inhibitors, such as Xia et. al.'s compounds EK1C4 lipoprotein and EK1-scrambled peptides, to assess potency.[89] Others have adopted this model and used ACE2/293T target cells against SARS-CoV-2 pseudovirus containing the S glycoprotein and a luciferase reporter or fluorescence protein for quantitative analysis.[90,91]

Lastly, the physiologically based pharmacokinetic (PBPK) model and simulation is a relatively new tool that predicts pharmacokinetic behavior in humans and dose regimens based on preclinical data. It can also incorporate age, ethnicity, disease status, and drug-drug interactions into the model's calculations. Although there are limitations, this model advances the field of drug discovery and development by providing better guidance on drug safety and effectiveness in humans without spending months on pharmacokinetic studies.[92] This tool has recently been gaining more popularity in the SARS-CoV-2 field and has been used to predict the loading and maintenance doses of various drugs, including HCQ and CQ.[86,93] As mentioned previously, although ivermectin showed impressive in vitro data against SARS-CoV-2[87], one group used the PBPK model to show that ivermectin would not be effective in humans.[94]

In vivo animal models

Unlike other viruses, such as hepatitis C, that require chimeric humanized immunodeficient mice for infection, SARS-CoV-2 can infect many wild type animals without any significant genetic alterations, such as mice, golden Syrian hamsters, ferrets, and monkeys. Nevertheless, there are limitations since they do not exhibit the same symptoms as infected humans. Mice, for example, express their own ACE2 receptors, and human and mouse ACE2 receptors are structured differently, leading to different interactions with SARS-CoV-2. Transgenic mice expressing human

ACE2 receptors[95] were then the next best phenotypic model since they present with similar human-like SARS-CoV-2 symptoms. This model, however, still co-expresses its own mouse ACE2 receptor, and currently, there are no mouse models that solely express humanized ACE2 receptors.[96]

Another animal model gaining popularity are golden Syrian hamsters. The pathogenicity and transmission of SARS-CoV-2 are relatively similar between humans and hamsters because the virus can be transmitted to neighboring hamsters, likely through respiratory droplets, and the hamsters recover from the virus after neutralizing antibodies are detected 2 weeks after infection.[97] Viral infection in ferrets also presents with similar findings to human infection, such as airborne transmission and detection of viral antigens in various organs, including the lungs and intestines.[98] Cynomolgus and rhesus monkeys and cats have also made their way into labs due to their susceptibility to the virus.[99-101]

These animal models can be used depending on the type of study that is being performed. If a group is studying the pharmacokinetics or the prophylactic and therapeutic effects of different chemical agents, then transgenic mice, hamsters or ferrets are suitable models. They are less expensive and easier to work with than monkeys, they exhibit similar symptoms to humans, and litters can be expanded easily. When evaluating vaccine safety or immune protection, monkeys could be used due to their close genetic relationship to humans; however, they are very expensive and are quite resilient to viral infection which may make them more difficult to study viral symptoms. Once an animal model is established, there are numerous tools available for data interpretation and analysis. A few popular techniques are enzyme-linked immunosorbent assay (ELISA), which can be used to detect neutralizing antibodies in serum samples, reverse transcription polymerase chain reaction (RT-PCR), which can be used to detect viral loads after infection, and confocal microscopy, which can be used to qualitatively visualize co-localization of SARS-CoV-2 with ACE2 receptors.[89,96-101]

Conclusion

While much is known about the structure, life cycle, and pathogenesis of SARS-CoV-2, the steadily rising number of daily COVID-19 cases demonstrates the urgent need to find effective therapeutics and vaccines. Rapid drug and vaccine development depend on an understanding of the key viral structures as they relate to the life cycle and pathogenesis of the virus, as well as the current therapies used and research methods employed to study the virus.

The S, E, M, and N proteins are all potential antiviral targets, as they play a role in the viral life cycle, but no antiviral has been proven effective against these targets in vivo. Future research directed towards development of inhibitors of the S protein RBD, cation-selective ion channels of the E protein, and the NTD of N protein may provide effective antiviral treatments. Other targets include TMPRSS2, Mpro, and 3CLpro. In the development of such therapies, and during repurposing of other drugs, consideration of in vivo pharmacokinetics data is essential to avoid unnecessarily costly and time-intensive efforts. With chloroquine and hydroxychloroquine showing increased toxicity and no clinical benefit and lopinavir/ritonavir showing no clinical benefit, the RdRP inhibitor remdesivir is the only therapy shown effective in a randomized, double-blind, placebo-controlled phase III clinical trial. This highlights the need for novel antiviral development, in addition to further research regarding prophylactic utilization of these treatments.

Taken altogether, our review provides an overview of the current SARS-CoV-2 structural, life cycle, antiviral, and lab technique literature to serve as the basis for future research.

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Reviews

A Review of COVID-19 Epidemiology, Immune Response, and Clinical Presentation

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Abstract

The COVID-19 pandemic is one of the greatest public health and infectious disease events of the past century. Understanding how this disease develops within individuals and spreads between individuals is key in order to combat the pandemic. In this literature review, we discuss the epidemiology of SARS-CoV-2 and the modes of transmission as it is currently understood. We will also examine the immunological response to the SARS-CoV-2 virus within the human body with focus on its unique aspects as targets for therapy. Finally, we will briefly review the basic clinical presentation of the COVID-19 disease from a system-by-system perspective.

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Epidemiology

In December 2019, several clusters of patients with pneumonia of unknown etiology were reported in Wuhan, China. The local health authorities linked these cases to a popular seafood and animal market while the World Health Organization (WHO) confirmed that the cause was a novel strain of coronavirus not previously seen in humans. The clinical disease was eventually dubbed coronavirus disease-2019 (COVID-19) and the virus itself was termed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1]

Vector

Early analyses of SARS-CoV-2 showed that the virus was likely of zoonotic origin similar to other pathogenic beta coronaviruses like severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS). Specifically, SARS-CoV-2 shares large nucleotide sequences and an overwhelming portion of its whole genome with bat coronaviruses.[2] Malayan pangolins are the suspected intermediate host between bats and humans. Virus isolated from the lung tissue of the dead pangolins was found to share over 90% of its genome with both SARS-CoV-2 as well as a strain of bat coronavirus called BatCoV RaTG13.[3] While epidemiologically useful, this early association with zoonosis has since been criticized because much of the early research on SARS-CoV-2 focused on animal-to-human transmission.[4] However, it is the human-to-human transmission of the virus that allowed COVID-19 to become a global pandemic with confirmed infections in over 5 million people worldwide as of May 24, 2020.[5]

Host

As COVID-19 spread in China and around the world, it became apparent that the virus causes a wide spectrum of disease in human hosts. Risk factors for critical illness

and death include male sex, age >65, and smoking history. In addition, comorbidities such as hypertension, diabetes, cardiovascular disease, and respiratory disease all increase the risk for more severe infection and increase mortality.[6] Data from China shows that most COVID-19 cases have occurred in the 35-69 age group with fewer than 3% of overall cases occurring in children ages 0-18 years of age.[7] Further studies demonstrate that pediatric patients (<18 years) suffer from a milder course of illness than adults, with older adults more likely to suffer from severe disease.[8] Interestingly, however, clinicians have found that children who experience even just mild COVID-19 infection are at risk for developing a Kawasaki-like syndrome.[9] Several case series regarding this syndrome in children have been published naming it Multisystem Inflammatory Syndrome in Children (MIS-C), and the CDC has now published guidelines establishing it as a reportable syndrome.[10-12] MIS-C as defined by the CDC is seen in Table 1. More research on MIS-C is needed to determine optimal treatment.

Table 1. Multisystem Inflammatory Syndrome in Children (MIS-C) Case Definition

An individual <21 years-old who presents with fever, laboratory evidence of inflammation, and evidence of clinically severe illness requiring hospitalization with multisystem involvement; AND
No alternative plausible diagnoses; AND
Positive for current or recent SARS-CoV-2 infection by RT-PCR, serology or antigen test (or exposure to a suspected or confirmed COVID-19 case within 4 weeks prior to onset of symptoms)

Fever is defined as greater than 38° C for at least 24 hours or a report of subjective fever for at least 24 hours. Inflammatory markers include elevated C-reactive protein, erythrocyte sedimentation rate, fibrinogen, procalcitonin, d-dimer, ferritin, lactic acid dehydrogenase, IL-6, elevated neutrophils, decreased lymphocytes, or low albumin.[12]

Like SARS, researchers have determined that SARS-CoV-2 uses angiotensin-converting enzyme 2 (ACE2) to gain entry into cells. Thus, scientists are hypothesizing that variations in this gene could affect COVID-19 morbidity and mortality.[13] The X-linked nature of this gene may also play a role in morbidity and mortality discrepancies between males and females.[14] However, more data is needed to determine the degree to which variations in this gene affect clinical disease and prognosis as well as if existing pharmaceuticals could be of therapeutic use.

Environment

In addition to host characteristics, environmental factors also play a key role in the propagation of infectious disease. Weather factors such as humidity and temperature are suspected of contributing to the COVID-19 pandemic and are a big topic of current discussion in the scientific community. Coronaviruses are known to inactivate at higher temperatures and higher degrees of relative humidity with temperature having the biggest influence in virus survivability.[15] One team of researchers found that temperatures between 13-24o C and humidity of 50-80% were conducive to SARS-CoV-2 survival, while lower temperatures hindered the virus.[16] This is in contrast to another study which demonstrated that most of the outbreaks globally have occurred in the northern hemisphere in an isothermal zone between 5 and 11°C.[17] Obviously, more research on optimal SARS-CoV-2 ex vivo conditions is needed. Though these two studies present differing temperature ranges, neither study supports viral survival in warmer climates. This may support the assumption that there will likely be seasonality of COVID-19 with incidence increasing in the winter months, which is why a second wave of infections may occur later this year. The temperature patterns may also explain why certain regions of the world have experienced lower disease burden from COVID-19 thus far in the global pandemic. However, as many of these warms regions also tend to be more resource poor, further research is needed to verify this.

Another foundational Chinese study showed a correlation between mean daily temperature and the number of new cases 14 days later.[18] If temperature and humidity patterns can be established, epidemiologists may be able to predict regions where potential new viral outbreaks may occur or where incidence of disease may suddenly rise. The hope is that such models would ultimately be able to help decrease the morbidity and mortality associated with COVID-19.

Transmission

The first zoonotic to human transmission of COVID-19 is believed to have originated in a seafood market in Wuhan, China. Although this market sold many live animals, including raccoon dogs, snakes, birds, civet cats, studies suggest that bats likely had a major role in harboring and transmitting the disease.[19-21]

After the original zoonotic infections in Wuhan, the virus was found have human to human transmission. SARS-CoV-2 is mainly spread through respiratory droplets and close contact, but fomite and airborne transmissions have also been shown.[22-25]

There are variations in recommendations for preventing droplet and airborne transmission amongst healthcare organizations. The WHO recommends droplet precautions for standard patient care and airborne precautions for procedures with high risk of producing aerosolized particles.[25] Such procedures include endotracheal intubation, turning patients prone, manual ventilation, cardiopulmonary resuscitation, tracheostomy, administering nebulizer treatments, and disconnecting ventilators.[25]

The Centers for Disease Control and European Center for Disease Prevention recommend wearing N95 or higher respirators or masks during all patient care for confirmed and suspected COVID-19 cases, and only using surgical masks and other face coverings when there are PPE shortages.[26,27]

One meta-analysis found that surgical masks offer comparable protection from flu-like viruses to N95 masks when performing standard patient care.[27] However, this analysis drew upon data from various viruses, and was not based solely on COVID-19.

COVID-19 particles have been found on fomites including infected patient hospital room air vents, toilets, sinks, and door handles.[28] A study showed that SARS-COV-2 is viable on several surfaces including plastic and stainless steel for up to 72 hours.[29] Given these findings, fomite transmission has been deemed possible, and sufficient cleaning of hospital rooms and other potentially infected surfaces could reduce virus transmission.[30]

Pets and other domestic animals have also been considered as a possible means of COVID-19 transmission.[31] Early in the pandemic course, there were reports that household animals had potentially tested positive for SARS-COV-2.[31-33] Later studies have found that ferrets and cats are susceptible to COVID-19 infection, but other animals such as dogs, ducks, pigs, and chickens are less likely to be infected.[31-34]

Receptor Targets

For both SARS-CoV and SARS-CoV-2, the ACE2 receptor is the binding target in humans.[35] As seen in Figure 1, ACE2 (angiotensin-converting enzyme 2) is a part of the renin-angiotensin system and has physiologic roles in breaking down angiotensin II (Ang II), a vasoconstrictor and pro-inflammatory agent, into Ang(1-7), which is a vasodilator.[36,37] Dysregulation of this balance between Ang II and Ang(1-7) has been shown to have a role in the development of diseases such as heart failure, hypertension, and diabetic complications.[35]

ACE2 is expressed on a variety of organs including the heart, liver, intestines, brain, and notably the lungs. ACE2 expression in the lungs is an important factor in the development of COVID-19 infections as the virus is primarily spread via respiratory droplets entering the respiratory tract. ACE2 in the lungs are mostly expressed by the type 2 pneumocytes.[13] Other factors can influence a relative increase or decrease in ACE2 expression. Some studies on female rats have shown an upregulation of ACE2 expression with increased estrogen levels during pregnancy.[36] Other animal studies also showed lower ACE2 expression in neonates than in adults.[36] Smoking also seem to play a role in ACE2 expression. One study looking at data from China showed a correlation between

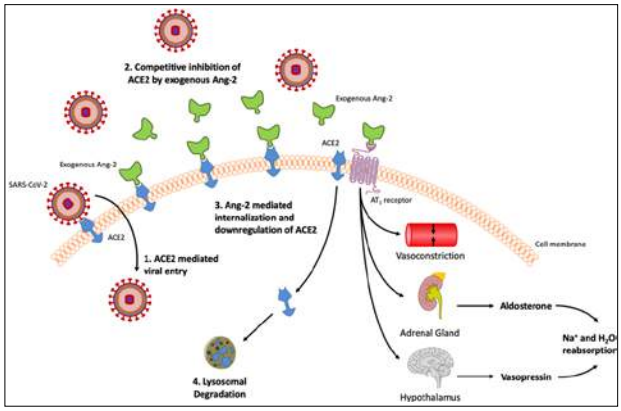

Figure 1. ACE2-mediated SARS-CoV-2 Entry and Effect of Ang II. SARS-CoV-2 gains entry into a host cell by binding to the endogenous ACE2 cellular receptor. This receptor can also be bound by Ang-2 (or Ang II) which is a part of the renin-angiotensin system. Ang-2, when bound to the AT1 receptor, causes vasoconstriction and aldosterone secretion. Ang-2 is metabolized into Ang(1-7) by ACE2. When Ang-2 is present in excess, the ACE2 expression is downregulated. Reprinted under open license from Critical Care, 24(136); Busse, Chow, McCurdy & Khanna, "COVID-19 and the RAAS – a potential role for angiotensin II?" Copyright 2020.

smoking and prevalence of COVID-19, and it also showed histologic evidence of upregulation of ACE2 in smokers compared to nonsmokers.[38]

In regards to lung injuries, ACE2 seems to be a protective factor with its role in increasing Ang(1-7) and decreasing Ang II. In knockout studies in mice, an acute respiratory distress syndrome (ARDS)-like injury developed in those without ACE2.[36] SARS-CoV-2 infection also downregulates the expression of ACE2 in the lungs, which would seem to contribute to the development of lung injury in COVID-19 patients. Similar effects are seen on cardiac myocytes and the development of cardiac injury in COVID-19 infections.[35]

From a therapeutic aspect, the ACE2 receptor does offer some possibilities. One possibility is ACE2 activators which include diminazene aceturate, xanthenone and resorcinolnaphthalein, which all possibly increase the protective effect of ACE2.[36] Another idea is also to use existing ACE inhibitors to upregulate the expression on ACE2 in the body.[36] Continued research into the outcomes and feasibility of these treatments is still needed.

Immune Response

Innate Immune Response

The human response to infectious agents can be divided into innate and adaptive immune responses. The innate response is nonspecific to the pathogen, but it is also the first of the two to occur. Physical barriers, such as the skin, are the first line of defense before pathogen entry. Once the virus enters the host, there are several pattern recognition receptors (PRRs) that are present in or on host cells that recognize generic pathogen-associated molecular patterns (PAMPs).[39] Toll-like receptors are PRRs that recognize lipids, proteins, and nucleic acids on pathogens. RIG-I-like receptors are PRRs that recognize nucleic acids particularly from RNA viruses.[39] Activation of these PRRs causes down-stream activation of intracellular pathways (Figure 2) that release inflammatory cytokines, such as type 1 interferons, IL-1, and IL-6,[40,41] as well as activate antigen-presenting cells (APCs) to breakdown the virus and present it to other immune cells.[39]

The APCs in the human body include dendritic cells, B cells, and macrophages. The most important APCs in regards to COVID-19 infection are the dendritic cells. Dendritic cells start immature with a high affinity for phagocytosis of infected cells. When the PRRs are activated, the dendritic cell matures and increases major histocompatibility complex II (MHC II) expression to present antigen which subsequently activates the adaptive immune system.

This PRR activation and release of cytokines are important for destroying the virus, but prolonged activation can lead to "cytokine storm," which is one of the key causes of organ damage in patients. Also, the systemic effects of cytokines, such as tumor necrosis factor (TNF), can lead to septic shock.[40] The balance in the immune response seems to shift to be more over-reactive in those who are over 60 or with co-morbidities, which could explain why this population is more prone to severe COVID-19 symptoms.[40]

Since the innate immune response is the first defense against pathogens, several hypothesized therapies involve altering the innate response to act sooner or differently. One option is activation of toll-like receptor 5 (TLR5), which is normally only activated in response to bacteria.[42] By activating TLR5 with flagellin, production of IL-22, INF-beta, and type 1 INF were increased.[42] Another possible treatment is with monolizumab, a NKG2A receptor antibody, that increases the activity of natural killer (NK) cells and cytotoxic T cells.[43] The activity of these cells is important for clearing the virally infected cells in the body. Treatment with JAK kinase inhibitors, which inhibit intracellular signal transduction, has been shown to increase levels of interleukins (IL) and interferon response and is an area of active clinical research.[44]

Adaptive Immune Response

The second phase of the human immune response to COVID-19 infection is the adaptive immune response. Once the antigen-presenting cell (APC) has encountered and processed the antigen, it then activates naïve T cells in the body. They can either be CD8+ T cells, which help with viral clearance, or CD4+ T cells, which help stimulate B cell maturity.[39] These cells continue to produce cytokines which promote the inflammatory and antiviral response. One cytokine, IL-6, has a role in promoting Th17 cells which have been shown to contribute to an eosinophilic immune response. There is possibility in inducing the Th17 response to enhance immune responses to a COVID-19 vaccine.[45] For

longer-term immunity, the memory T cells develop after T cell activation, but more research is needed to assess efficacy in preventing COVID-19 reinfection.[46]

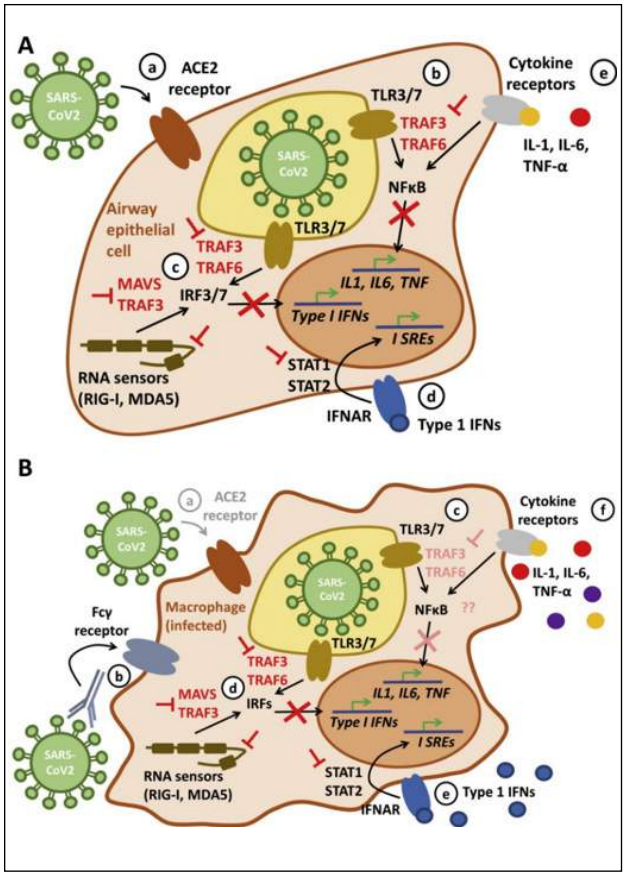

Figure 2. Immune response to SARS-CoV-2 infection. A) SARS-CoV-2 infects respiratory epithelial cell via ACE2 (a). TLF3/7 (b) and RIG-I and MDA5 (c) are activated by RNA viruses in endosomes and the cytosol, respectively. Pathways inhibited by SARS-CoV-2 include the STAT1/2 pathway which limits the type 1 interferon response (d). Systemic cytokines bind to cell to activate viral response mechanisms (e) B) Tissue monocytes and macrophage response, with down regulated ACE2 expression (a). Ineffective antibodies bound to SARS-CoV-2 are brought into the cell, infecting it (b). Intracellular receptors activated by RNA virus (c,d). Inhibited type 1 interferon pathway (e) and unopposed cytokine response (f) contribute to pro-inflammatory state. Reprinted from Clinical Immunology 215, Felsenstein et al., "COVID-19: Immunology and treatment options," Copyright 2020, with permission from Elsevier (License 4853130020958).

As the activated T cells continue to proliferate and clear infected cells, the B cells have also been activated by these T cells and begin antibody production. This response is one of the slowest of the human immune system, but it conveys the longest protection against reinfection. It has been seen that at about 4-8 days after symptom onset, antibody levels are detectable in the serum.[40] By three weeks after symptom-onset, enough mature antibodies should be present so that it begins to be neutralized.[40] The most likely target of these IgG antibodies is the spike (S) protein present on the SARS-CoV-2 capsule based on research with the MERS-CoV virus.[39] Early development of antibodies during the disease course is shown to increase the severity of lung injuries from the disease, but more studies are necessary with recovered patients to determine the efficacy of monoclonal antibody therapies.[40] Convalescent plasma therapy is another opportunity for treatment with donated plasma from recovered COVID-19 patients given to those with severe COVID-19 infections. While this therapy has shown some benefits in case series, randomized trials need to be performed to establish dosing and treatment indications.[47]

Clinical Presentation

Coronaviruses typically cause non-specific common cold signs and symptoms, such as fever, cough, myalgia, and malaise. Generally, infected individuals are asymptomatic after infection. An American group performed an epidemiological analysis of 181 cases and found that the median incubation period, from exposure to symptom onset, was approximately 4 to 5 days, and 97.5% of symptomatic patients had symptoms within 11.5 days after infection (CI, 8.2 to 15.6 days).[48] Extending the cohort to the 99th percentile results in almost all cases developing symptoms within 14 days after exposure.[48]

The clinical spectrum of COVID-19 (Table 2) varies from asymptomatic forms to clinical conditions characterized by respiratory failure that necessitates mechanical ventilation, to multi-organ and systemic manifestations such as sepsis and septic shock. Fever (85-90%), cough (65-70%), fatigue (35-40%), sputum production (30-35%), and shortness of breath (15-20%) are among the most reported clinical manifestations, although the full spectrum remains to be determined.[49] Some patients have gastrointestinal symptoms, including anorexia, nausea, and diarrhea.[50,51] Anosmia and ageusia have also been reported.[52,53] Thus, the extreme ranges of COVID-19 severity make it difficult to diagnose, predict, and manage.

Table 2. Common Clinical Presentation of COVID-19

Symptoms	Signs
Fever	Lymphopenia
Cough	Elevated LDH
Fatigue	Elevated PT
Nausea/Vomiting	Elevated D-dimer
Diarrhea	Transaminitis
Shortness of Breath	Elevated troponin
Hypoxia	Abnormal chest imaging

The following reviews some of the literature on the effects of COVID-19 on the respiratory, cardiovascular, neurological, gastroenterological, and renal systems:

Respiratory

The 3 primary symptoms of COVID-19 are fever, cough, and shortness of breath. In some series of hospitalized patients, shortness of breath developed a median of 5 to 8 days after initial symptom onset[51,54]; its occurrence is suggestive of worsening disease. While the majority of patients present with a lower respiratory tract infection, 17-29% of patients are reported to develop ARDS.[55]

A retrospective cohort study of 201 patients with confirmed COVID-19 pneumonia admitted to Wuhan Jinyintan Hospital in China investigated clinical characteristics associated with the development of ARDS and progression from ARDS to death. Older age, neutrophilia, and organ and coagulation dysfunction were found to be risk factors.[56] Additionally, patients with ARDS who received methylprednisolone, a corticosteroid, were more likely to survive (46.0% mortality) compared to those who did not (61.8% mortality).[56] Therefore, treatment with methylprednisolone may be beneficial for patients who develop ARDS and may decrease mortality. Another steroid, dexamethasone, has shown promise at reducing mortality by one-third for ventilated COVID-19 patients in a randomized clinical trial.[57,58]

Cardiovascular

Overall, individuals with underlying cardiovascular disease (CVD) are at increased risk of severe disease. The use of ACE inhibitors and angiotensin II blockers (ARBs) is more frequent among patients with COVID-19 than among controls because of their higher prevalence of CVD. However, an investigation performed by Mancia et al. showed no evidence that ACE inhibitors or ARBs affected the risk of COVID-19.[59]

Individuals with underlying CVD are more vulnerable to worse outcomes due to the virus. For example, severe SARS-CoV-2 appears to affect the myocardium, consequently causing myocarditis.[60] Sporadic autopsy cases suggest infiltration of myocardium by interstitial mononuclear inflammatory cells.[60] Cases of severe myocarditis with reduced systolic function have been also reported after COVID-19.[61,62] Additionally, cardiac biomarker studies suggest a high prevalence of cardiac injury in hospitalized patients.[60,63]

In a case series of 187 patients with COVID-19 performed by Guo et al., 27.8% of patients had myocardial injury, which resulted in cardiac dysfunction and arrhythmias.[63] Overall, 66 (35.3%) had underlying CVD including hypertension, coronary heart disease, and cardiomyopathy, and 52 (27.8%) exhibited myocardial injury as indicated by elevated troponin T (TnT) levels.[63] Patients with high TnT levels showed a higher incidence of complications, including ARDS, malignant arrhythmias, acute renal injury, and acute coagulopathy.[63] The prognosis of patients with underlying CVD without myocardial injury, however, was relatively favorable. Therefore, myocardial injury is likely associated with infection-related myocarditis and/or ischemia and is an important prognostic factor in COVID-19.

COVID-19 associated coagulopathy is also a complication found in severe cases of COVID-19. Laboratory changes include elevated fibrinogen and d-dimer, while PT, aPTT and platelet counts remain relatively unchanged in early disease.[64] This can lead to microvascular thrombosis or venous thromboembolism, both of which have a negative impact on mortality. Also, COVID-19 patients are at risk of developing endothelialitis which can could contribute to microvascular thrombosis.[65] At this time, current guidelines for treating and preventing venous thromboembolisms or pulmonary embolisms with anticoagulation should be followed for COVID-19 patients.[64]

Neurological

Some patients with COVID-19 have been exhibiting atypical neurological symptoms as initial symptoms (such as headaches, cerebral hemorrhage, and cerebral infarction) instead of fever, cough, and shortness of breath.[66] In a recent study of 214 patients with COVID-19, 78 (36.4%) patients had neurological manifestations, such as headache, dizziness, acute cerebrovascular diseases, and

impaired consciousness.[67] Of these 214 patients, 40 (18.7%) patients required intensive care unit (ICU) interventions for severe neurological involvement.[67]

Interestingly, patients with COVID-19 often suffer from coagulopathy and prolonged prothrombin time, both of which are also contributing factors to secondary cerebral hemorrhage. In contrast, no cases of secondary cerebral infarctions have been reported in patients with COVID-19. However, an increase in D-dimers may be seen with COVID-19,[66,68] which could indicate a coagulopathy that could lead to thrombotic vascular events. These laboratory findings suggest that COVID-19 has the potential to induce cerebral venous and/or arterial infarctions.

Gastrointestinal

Clinicians should recognize that digestive symptoms, such as diarrhea, can also be among the presenting features of COVID-19. One study of 204 patients with COVID-19 found that, although most patients presented to the hospital with fever or respiratory symptoms, 103 patients (50.5%) reported a digestive symptom, including lack of appetite (81 [78.6%] cases), diarrhea (35 [34%] cases), vomiting (4 [3.9%] cases), and abdominal pain (2 [1.9%] cases).[50] Also, as the severity of the disease increased, digestive symptoms became more pronounced. Of note, patients with digestive symptoms had a longer time from symptom onset to admission, evidence of prolonged coagulation, and higher liver enzyme levels.[50]

There is also evidence of clinical pancreatitis as a clinical presentation of COVID-19.[69] In this study, among the 52 patients with COVID-19 pneumonia, the incidence was 33% for heart injury (abnormal LDH or creatine kinase), 29% for liver injury (any abnormality in aspartate amino transferase (AST), alanine transaminase (ALT), gamma-glutamyl transferase (GGT) or alkaline phosphatase (ALP)), 17% for pancreatic injury, 8% for renal injury (abnormal creatinine), and 2% for diarrhea.[69] Without a larger sample size, it is unclear if pancreatitis is a presenting symptom or associated manifestation of the disease.

Although larger sample studies need to be performed, both studies suggest that the index of suspicion for COVID-19 infection should be higher in the at-risk population presenting with digestive symptoms.

Renal

COVID-19 may also be able to target and infect the kidneys.[70] Acute kidney injury (AKI) has been reported as one of the complications that occur during the progression of COVID-19 in both patients comorbid with kidney disease and those who are not.[71] One retrospective analysis showed that 6% of patients with SARS had an AKI and the incidence of an AKI in these patients was similar to that found in patients with SARS.[71]

Furthermore, Huang et al. reported on 41 patients with COVID-19, among whom 10% had elevated creatinine (>133 μmol/L) on admission and 7% had AKI.[54] Laboratory tests showed that the levels of blood urea and creatinine increased progressively in the development of COVID-19.[54] The data suggest that patients with severe cases of COVID-19 showed signs of kidney damage, even in patients without underlying kidney problems before infection.

Outlook

Despite all the knowledge acquired about the SARS-CoV-2 virus, there is still much more to research as therapies and vaccines need to be developed. Continuing studies on existing antivirals and their usefulness in treating COVID-19 are of great importance given that production of these drugs will be much easier to scale up and distribute to areas around the world. For example, remdesivir, an antiviral which was initially developed for the Ebola virus, has been shown to decrease hospital stay for those with COVID-19.[72,73] Also, more studies with anti-inflammatory drugs are being conducted, which could offer another line of therapy with existing medications. As previously discussed, the steroid dexamethasone has been shown to decreased mortality with patients requiring oxygen support.[58] Finally, as of this writing, several promising vaccines are in different stages of development, such as one which uses viral mRNA to mount an immune response.[74] The outlook on future research on COVID-19 seems abundant given the institutional and governmental support for research from around the world.

Conclusion

The coronavirus pandemic has had a tremendous impact across the health care system and will continue to do so for years to come. Clinicians from all specialties should attempt

to have a basic understanding of the pathophysiology and epidemiology of COVID-19, as it impacts every patient population in some aspect. Continued research is also needed to clarify the epidemiology and clinical presentation of COVID-19 and to guide therapeutic developments in the future.

Disclosures

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Reviews

COVID-19: Local and Global Epidemiology

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Abstract

Since the first reported case of coronavirus disease 2019 (COVID-19) in December 2019, the disease has progressed rapidly into a global pandemic, affecting 28,637,952 patients and leading to 917,417 deaths as of September 13, 2020. The current estimates suggest a mortality rate of around 3.4%. Countries have implemented various strategies to combat the prevalence of the disease. Although no unified pharmacological intervention has been approved, the World Health Organization (WHO) has recommended preventive measures, including handwashing, mask-wearing, and social distancing. Countries that have created innovative testing and surveillance strategies, such as South Korea, have also observed drastic improvements in COVID-19 case numbers. North Carolina has implemented several similar preventive measures to protect the general public, especially vulnerable populations. Of concern, however, is the disproportionately higher prevalence among different ethnic groups.

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Introduction

Since the first reported case of a pneumonia of unknown etiology in Wuhan City, China in December 2019, coronavirus disease 2019 (COVID-19) has progressed rapidly into a global pandemic.[1] COVID-19 is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1] Its clinical manifestations vary from mild, non-specific symptoms, such as cough, fever, and fatigue, to severe pneumonia with organ dysfunction.[2] Modes of transmission of COVID-19 are through droplet transmission, fecal-oral route, conjunctiva, and fomites.[2] The virion is stabilized and has higher survival rates at lower temperatures.[2,3] Additionally, current data suggests that the half-life of SARS-CoV-2 in aerosols (1.5 h), copper (1 h), cardboard (3.4 h), stainless steel (5.6 h), and plastic (6.8h) are different.[2,3] SARS-CoV-2 virions can be shed throughout the clinical course, meaning that patients with COVID-19 can spread the infection prior to symptom presentation, during the symptomatic course, and during the clinical recovery period.[2] In this paper, the symptomatology, global and local (NC) epidemiology, as well as current treatment and prevention strategies will be discussed.

Background

There have been two additional coronavirus epidemics in the past twenty years: severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV).[4,5] SARS-CoV provoked a large-scale epidemic beginning in China in 2002, involving 37 countries with approximately 8,000 cases and

800 deaths.[4] In contrast, the MERS-CoV was first identified in Saudi Arabia in 2012 with approximately 2,500 cases, 850 deaths, and continued sporadic cases.[4] Both SARS-CoV and MERS-CoV share many symptoms of SARS-CoV-2, such as fever, cough, and dyspnea.[5] However, gastrointestinal symptoms are more common in SARS-CoV and MERS-CoV, and MERS-CoV has a higher incidence of renal failure.[5] Table 1 demonstrates further epidemiological comparisons of SARS-CoV, MERS-CoV, and COVID-19. As seen in Table 1, COVID-19 has the highest incubation period (4-7 days) and the least number of days (48 days) to infect the first 1000 people.[6,7] Current estimates calculate the mortality rate of COVID-19 to be around 3.4% although the accuracy of this number is limited by bias toward symptomatic and sick patients as well as variability in testing accuracy.[7,8] Additionally, individuals may be receiving either a diagnostic test or an antibody test. The two types of diagnostic tests, molecular (detects the virus's genetic material) and antigen, identify active infections, whereas antibody tests identify past exposures.[9]

Symptomatology

The most common symptoms of COVID-19 have been fever (77.4-98.6%), cough (59.4-81.8%), fatigue (38.1-69.6%), dyspnea (3.2-55%), and myalgia (11.1-34.8%).[5] Among patients who developed severe disease, the median time to develop dyspnea ranged from 5-8 days, the median time to acute respiratory distress syndrome (ARDS) ranged from 8-12 days, and the median time to intensive care unit (ICU) admission ranged from 10-12 days.[10] Olfactory and gustatory dysfunctions have also been a typical symptom of SARS-CoV-2 infection.[11] A cross-sectional study in northern Italy reported a total loss of olfaction and taste in 64% and 60% of cases, respectively.[11] SARS-CoV-2 is more likely to infect people with chronic comorbidities, such as cardiovascular disease, cerebrovascular diseases, and diabetes. The highest proportion of severe cases occurs in adults over 60 years of age, and in those with the above underlying conditions.[12]

Global Epidemiology

The first reported case of COVID-19 to the World Health Organization (WHO) was in December 2019 in Wuhan, China, and the outbreak was declared an International Public Health Emergency a month later.[13] Since the first reported case, COVID-19 has become a global pandemic, affecting 28,637,952 patients in more than 180 countries/regions and leading to 917,417 deaths as of September 13, 2020, of which around 22.3% (6,386,832) of the total confirmed cases and 20.9% (191,809) of total deaths were from the United States.[14,15] Figure 1 shows the number of confirmed COVID-19 global cases and deaths as reported by the WHO between February 20 – July 20, 2020.

Table 1. An epidemiological comparison of SARS-COV, MERS-COV, and COVID-19

	SARS-COV	MERS-COV	SARS-COV2
Outbreak date	Nov. 2002	April 2012	Dec. 2019
Incubation period	2-7 days	5-6 days	7-14 days
Days to infect first 1000 people	130	903	48
R0*	1.5-4.0	0.5-1.0	1.5-30.0
Total cases	8096	2519	28,637,952
Total deaths	744	866	917,417
Mortality rate	9.2%	34.4%	3.4%

*R0= The basic reproductive number or the expected number of cases generated from one case.

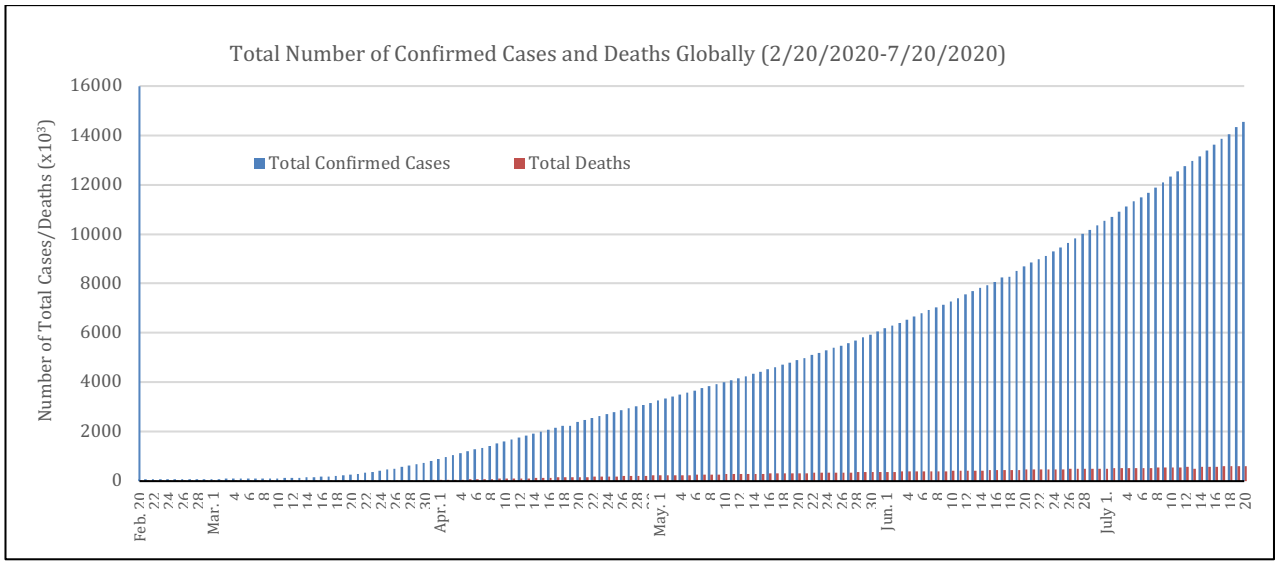

Figure 1. The number of confirmed cases and deaths worldwide as reported by WHO situation reports between February 20 to July 20, 2020.

Case-Fatality Rates and Disease Prognosis in Different Populations

The spectrum of symptomatic infection ranges from mild to critical, with most infections not being severe and less than 15% of patients seeking hospital services.[16] The current estimates suggest a mortality rate of around 3.4%.[17] However, the challenges in estimating the mortality rate of COVID-19 include: 1) lack of available tests, especially in the earlier phases of the pandemic, 2) the varying reliability of available tests, 3) asymptomatic cases or mild cases that do not undergo testing - some analyses suggest up to 18% of infected individuals may be asymptomatic[18], 4) underestimation of the number of COVID-19 deaths as some patients are not hospitalized and/or not tested before or after death, 5) delays in data reporting, and 6) assigning an accurate cause of death as there is a lack of standardized definitions for possible COVID-19 related mortality.[19,20]

Mortality rates are higher among those who are hospitalized. In a study of 2,634 patients hospitalized for COVID-19 in the New York City area, 14% were treated in the ICU and 12% received invasive mechanical ventilation, with an 88% mortality rate among those receiving mechanical ventilation.[21] However, the analysis was limited to patients who had either been discharged or had died during the admission, representing fewer than half of the total patients admitted for COVID-19.[21]

In addition to variations in mortality rates among hospitalized vs. non-hospitalized COVID-19 patients, the fatality rates may also vary by location. For example, the case-fatality rate ranged from 5.8% in Wuhan to 0.7% in the rest of China, with the adjusted case-fatality rate in mainland China being 1.4%.[16] This variation in the fatality rate within China may be explained by a shortage of health resources in Wuhan during the early phase of the epidemic.[16] The geographical variations in mortality may also be due to the population's demographics, such as underlying comorbidities or age distribution. In Italy, where the median age of patients with COVID-19 infection is 64 years, the estimated case-fatality rate was 7.2% in mid-March.[22] In contrast, the estimated case-fatality rate in mid-March in South Korea was 0.9%, where the median age of COVID-19 patients was in the 40s.[22]

Although individuals at any age can become infected with COVID-19, middle age and older individuals are most commonly affected, with the median age ranging from 49 to 56 years.[23] In a report from the Chinese Center for Disease Control and Prevention, case-fatality rates were 8% in those aged 70-79 years and 15% among those 80 years or older, while the case-fatality rate among the entire cohort was 2.3%.[23]

In addition to older age and the aforementioned underlying chronic conditions, male sex has also been associated with a worse prognosis in COVID-19 patients.[24] While the rates of infection among men and women are about equal, men are more likely to progress to severe disease and death. A proposed hypothesis for this sex difference was a higher rate of smoking among men, which can increase the production of surface protein angiotensin-converting enzyme 2 (ACE2) used by SARS-CoV-2 to infect cells.[25] However, current analyses suggest that this hypothesis is unlikely, with only 1.4-12.5% of patients being current smokers, and the current literature does not support smoking as a predisposing factor.[25] Additionally, men may have higher rates of underlying comorbid conditions, such as obesity, diabetes, and cardiovascular disease.

In the United States, analyses of COVID-19 deaths from some states show that there are more deaths in African Americans than in White Americans, likely due to socioeconomic and environmental rather than biological explanations.[24] Underserved communities may be more likely to have medical comorbidities, live in close quarters, and are more likely to be "essential workers," with jobs in the service industries, including grocery workers, custodial staff, retail workers, public transit employees, and health care workers. However, data on the risk factors and potential underlying causes of COVID-19 complications for ethnic/racial minorities are limited and not yet available globally.[24]

Although the pediatric population is susceptible to COVID-19, the disease in children has a mild course, with fever and cough being the most common symptoms and ICU admissions and deaths being extremely rare.[26] In a review study, out of the 31 infected pregnant mothers with COVID-19, no COVID-19 infection was detected in their neonates or placentas. However, two mothers died from COVID-19-related respiratory complications after delivery. Thus, based on the current, limited data, there is no evidence for vertical transmission of COVID-19, but mothers may be at increased risk of respiratory complications. A separate study also showed similar results with no vertical transmission of COVID-19 and negative SARS-CoV-2 reverse transcriptase polymerase chain reaction (RT-PCR) tests in breast milk or amniotic fluid.[27]

COVID-19 in Different Regions

Table 2 demonstrates epidemiological data for the 10 countries most affected by COVID-19 worldwide based on the highest number of deaths proportional to the number of confirmed cases as of June 30, 2020.[28] The mortality rate of COVID-19 (deaths per 100,000 people) and case-fatality rate (number of deaths proportional to the number of cases) vary across regions and countries as seen in Table 2. These numbers could be affected by variations in reporting and testing availability in different countries/regions. As of June 30, 2020, the case-fatality rate worldwide ranges from 13.9% in the UK to 0.1% in Qatar and Singapore.[28]

Table 2. Epidemiological data of the 10 most-affected countries by COVID-19, based on case-fatality% as of 6/30//2020.[27,28,29]

Country	First case	Confirmed cases	Deaths	Case-fatality %	Deaths/ 100k population
UK	1/30/2020	321,640	43,598	13.9%	56.6
Mexico	2/27/2020	220,657	27,121	12.3%	21.5
Ecuador	2/29/2020	55,255	4,502	8.1%	26.4
Indonesia	3/1/2020	55,092	2,805	5.1%	1.0
US	1/22/2020	2,590,552	126,140	4.9%	38.5
Iran	2/18/2020	225,205	10,670	4.7%	13.0
Egypt	2/13/2020	66,754	2,872	4.3%	2.9
Brazil	2/25/2020	135,106	9,888	4.3%	5.09
Iraq	2/23/2020	47,151	1,839	3.9%	4.8
Columbia	3/5/2020	91,995	3,256	3.5%	6.6

As of June 2020, countries in Africa have been less affected than other regions such as China, the United States, and European countries. Based on reports on June 24, 2020, there were 239,548 confirmed cases on the African continent, compared to 2,581,602 cases in Europe and 2,295,272 in the United States.[13] Despite the slow arrival of COVID-19 in Africa, the 1.2 billion people living in the region are at risk.[30] Some of the obstacles in African countries include people living together in close quarters and without access to clean running water as well as a shortage of ICU beds.[30] However, heads of state, ministries of health, hospitals, clinics, and community health organizations are taking immediate action, such as containment efforts, travel restrictions, and banning social gatherings.[30]

Local Epidemiology–North Carolina, United States

The first confirmed case of COVID-19 in North Carolina (NC) was on March 3, 2020, in Wake County.[31] The individual had recently traveled out of state to Washington where he/she was exposed to a long-term care facility with a COVID-19 outbreak.[31] The WHO declared the virus a Public Health Emergency of International Concern on January 30, 2020, and on March 13, 2020, the U.S. president formally proclaimed a national emergency with a total of 1,645 confirmed cases from 47 states.[32,51]

By February 11, 2020, Governor Cooper of NC set up the Novel Coronavirus Task Force, an organized body to provide state surveillance of COVID-19 and work alongside the Centers for Disease Control and Prevention (CDC), to monitor and prepare for the virus in NC.[33]

In March 2020, Governor Cooper signed Executive Order No. 116, declaring a State of Emergency in NC with 763 COVID-19 cases across the 60 counties.[34,35] Governor Cooper then issued Executive Order No. 121, outlining a stay-at-home order to prevent community transmission.[36] Many other states were implementing similar stay-at-home guidelines, allowing residents to leave their homes for essential activities (e.g. grocery stores), encouraging social distancing of at least six feet, limiting the size of social gatherings, and closing nonessential businesses like restaurants and gyms.

As of June 6, 2020, the state of North Carolina has 34,625 confirmed cases with a total of 973 deaths, with over 400,000 tests completed.[37] The top 3 counties with the highest number of cases in NC are: Mecklenburg (5358 cases and 106 deaths), Wake (2155 cases and 40 deaths), and Durham (2025 cases and 48 deaths).[35] The top 3 counties with the highest number of deaths include Mecklenburg, Guilford (1573 cases and 78 deaths), and Durham.[35] Based on reports from the North Carolina Department of Health and Human Services on June 6, 2020, which includes 34,625 cases in NC, the distribution of race of confirmed cases is: White 54% (13,540), Black/African American 28% (7,001), Other 14% (3,551), Asian 3% (667), American Indian/Alaskan Native <1% (228), and Native Hawaiian or Pacific Islander <1% (95).[35] Based on ethnicity, Hispanics comprised 41% (14,196), and non-Hispanics comprised 59% (20,429) of COVID-19 cases.[35] Data on race is missing in 9,543 cases (27.6% of total cases), and data on ethnicity is missing in 10,616 cases (30.7% of total cases).[35]

Among the COVID-19 patients in NC, 49% (16,851) were male and 51% (17,300) were female. Data on gender is missing in 474 cases (1.4% of total cases). In contrast, males comprised 53% of death cases (523) while 47% of death cases were female (465).[35]

The distribution of cases by age group was as follows: 7% in the 0-17 age group (n=2548), 10% in the 18-24 age group (n=3606), 45% in the 25-49 age group (n=15469), 21% in the 50-64 age group (n=7435), 8% in the 65-74 age group (n=2623), and 8% in the 75+ age group (n=2932).[35] The absolute number of deaths was largest in the 75+ (624 deaths, 63% of total deaths), 65-74 (198 deaths, 20%), and 50-64 (124 deaths, 13%) age groups.[35]

Nursing homes, with their populations of elderly patients with comorbid conditions, have been associated with 3,567 cases and 523 deaths.[38] Nursing homes account for almost 54% of death cases. Other congregate living settings, such as residential care facilities, have been associated with 914 cases and 75 deaths, and correctional facilities with 1,354 cases and 22 deaths.[38] There have been outbreaks (2 or more laboratory-confirmed cases) in 98 nursing homes, 51 residential care facilities, and 20 correctional facilities in NC.[38]

By May 8, 2020, North Carolina started implementing a multi-phased plan for the gradual return of the general public to normal activities while attempting to reduce the spread of COVID-19. In the two weeks prior, the average number of new daily cases was 414 (ranged from 155 to 639 cases) and positive test percentage averaged 8.6% (ranged from 6.5% to 12.9%) with the number of daily tests averaging 5,186 (range of 2,134 to 9,339 tests).[35] The average number of daily deaths was 382 (range of 269 and 507).[35] Phase 1 continued to encourage people to only leave home for essential needs like food and medications.[35] Retail businesses (both essential and non-essential) were allowed to open at 50% capacity, and outdoor gatherings were limited to 10 people with the encouragement to practice social distancing and wear masks. Childcare centers were open for working parents, though teleworking was still encouraged.[35] Restaurants were restricted to take-out and delivery, and bars and nightclubs, salons, theaters, and gyms remained closed. Long-term care visitation was still not allowed.[35]

Phase 2 was implemented on May 22, 2020, which lifted the stay-at-home order to a safer-at-home recommendation.[35] Restaurants reopened with limitations on occupancy and requirements for disinfection and social distancing of at least 6 feet between individuals. Pools reopened to 50% capacity, and social gatherings were limited to 10 (indoors) and 25 (outdoors) people.[35] Cloth face-coverings continued to be strongly recommended but not required, especially when leaving the household and in public.[35] In the two weeks between Phase 1 and 2, the average number of new cases was 433 (range: 281 to 854), with an average of 8,645 (range: 2,741-12,313) tests daily and an average of 620 (range: 527-716) deaths daily.[35]

During Phase 2 there had been an upward trend of new cases reported, peaking in mid-July (2,486 new cases).[35] This is not dissimilar to the national average of new cases, which also peaked in July.[35] Phase 2.5 was implemented on September 4, 2020. Mass gathering capacity increased to 25 people indoors and 50 people outdoors.[35] Outdoor activities expanded to include the opening of playgrounds, museums, and aquariums at 50% capacity and fitness facilities at 30% capacity with a requirement to wear a face-covering at all times except during strenuous activity.[35] Bars, night clubs, and other entertainment facilities remained closed.[35] As of September, according to the CDC, North Carolina still has one of the highest new daily case counts (number 6 after Texas, California, Florida, Wisconsin, and Illinois) and total case counts (number 9 after California, Texas, Florida, Georgia, Illinois, New York City, Arizona, New York) compared to other states/territories.[56] As of September 26, 2020, the number of total cases in North Carolina was 204,331.56 North Carolina is the 18th highest state/territory in terms of total deaths, with 3,409.[56]

Socioeconomic Implications in North Carolina

African Americans make up 22.2% of the NC population and 30% of confirmed COVID-19 deaths.[35] Hispanics or Latinos comprise 9.6% of the state population and account for 39% of the cases.[38] In North Carolina, Executive Order No. 143 oversees equitable distribution of relief funds, universal community access to COVID-19 testing and related health care, measures to increase awareness of relief services and resources, efforts to protect feeding operations at food banks and schools, and support for testing of food-processing plant workers and migrant farmworkers.[40]

North Carolina is not unique to these statistics. In cities across the United States, mortality rates are higher in African Americans (73 per 100,000) and Latinos (36 per 100,000) versus White counterparts (22 per 100,000).[41] The cause of this is multifactorial. Racial/ethnic minorities often have underlying comorbidities (e.g. diabetes, cardiovascular disease, lung disease, or COPD).[41,42] These comorbidities increase the risk for severe complications with COVID-19.[41,42] Furthermore, racial/ethnic minorities disproportionately live in crowded settings or neighborhoods, increasing the risk for transmission.[41,42] They are also more likely to be employed as "essential workers" (e.g. service industry jobs like grocery workers, custodial staff, retail workers, and public transit employees), and continue employment during the pandemic in order to maintain an income despite the increased risk.[41,42]

Non-Pharmaceutical Interventions

One of the major preventive measures endorsed by the WHO is frequent handwashing and mask-wearing. Homemade cloth masks can be superior to no masks in helping prevent the transmission of the virus.[43] A study assessing the impact of mask-use (even homemade cloth masks) by the general public showed that, singularly, the broad adoption of face masks reduced transmission and decreased hospitalizations and deaths.[43]

Physical distancing measures such as isolation, quarantine, and community containment have also been implemented.[43,45] China was one of the first countries to implement physical distancing measures in response to the growing infection rates. By January 30, 2020, China issued the largest quarantine in history, which included several interventions.[46] For travelers, national borders were closed in entries to cities where community containment was implemented.[46] Incoming travelers were required to quarantine in designated facilities with medical observation for 14 days in their home country.[46]

Testing and Surveillance

Besides wearing masks and social distancing, testing is another method for preventing the spread of the virus. There are two types of COVID-19 tests available: viral (molecular) tests and antibody (serological) tests.[47] RT-PCR is used to test for current infection, detecting the presence of an antigen or genetic material of the contagion. The PCR method has been used as a "gold standard" for detecting an infection and entails collecting and multiplying a segment of genetic material. One of the greatest challenges is that a negative viral test may not necessarily rule out a current infection, especially in cases with low viral loads at the time of sampling. In a study of 205 patients with confirmed COVID-19, 398 pharyngeal swabs were taken with 126 (32%) positive results, and 8 nasal swabs were taken with 5 positive results (63%).[48] Ultimately, initial tests may be inefficient to rule out COVID-19, and reliance on history and chest imaging may be necessary. False-negative results can have public health implications as the individuals may be sources of infection spread.

Although RT-PCR is the most utilized test, serology tests have a major role in public health and health surveillance. Serology tests detect previous or cleared infections as they detect antibodies or immunoglobulins (Ig).[47] There are three main types of serology tests: rapid diagnostic test (RDT), enzyme-linked immunosorbent assay (ELISA), and neutralization assay. Each test has different characteristics, including the time to get results (RDT takes minutes, ELISA takes hours, and neutralization assay takes days), the level of expertise needed to administer the test (RDT takes the least), and the range of sensitivities. Currently, serology tests have a range of 87-93% sensitivity and 95-100% specificity.[47] Serologic tests provide useful public health information and surveillance regarding the immune status of the community (herd immunity), which can then help inform and guide policies that target population behavioral changes for the prevention and spread of COVID-19.

Testing Challenges and Strategies

Due to the increasing number of suspected cases of COVID-19, there have been global shortages in the molecular reagents used for testing, resulting in an inability to meet the demand for testing of suspected cases. There are other logistical limitations to diagnostic tests, including manufacturing and mass distribution. In most countries, especially in the early phase of the pandemic, testing was reserved for those with acute symptoms, vulnerable patients or those at risk for developing severe complications, as well as health care workers to prevent nosocomial transmission. The WHO recognizes the shortage of tests, disproportionate access, and difficulty in receiving timely test results.[49]

South Korea is considered an example of a country that has successfully decreased case burden due to an aggressive testing program, in addition to other factors.[50] Despite the global challenges to testing accessibility, South Korea has been able to quickly establish and mobilize testing resources, including RT-PCR testing kits.[50] By mid-March, South Korea had performed 5,200 tests per million inhabitants.[48] In February, case counts had been in the hundreds in South Korea, but by March, active cases had gone to as low as 74.[50] In comparison, the United States had performed 74 tests per million inhabitants when the number of confirmed

cases by Mid-March was 4,226 and increasing by at least 500 new cases each day.[46,52] South Korea had also created drive-through screening centers for efficiency and safety, with patients being able to communicate through their phone while driving through booths.[51] Once registered, body temperature measurement and questionnaire are performed, and if there is high suspicion for infection, a respiratory sample is retrieved, and the test is designated to a hospital. The entire service takes about 10 minutes.[51]

Germany was also one of the first countries to initiate a large scale program for serological testing. One of the intentions was to produce "immunity certificates" that would allow those who had antibodies to SARS-CoV-2 to return to work and resume normal activities.[47] As this information can give an idea of herd immunity, it could help inform when schools could reopen and mass gatherings can be planned.

Discussion/Conclusion

The COVID-19 pandemic is a global threat, affecting more than 180 countries. Although Extensive measures to reduce person-to-person transmission of COVID-19 have been implemented around the world, the virus continues to spread.[13] In the United States, most states have started to lift stay-at-home orders, reopen businesses, and relax social distancing measures, resulting in increased cases of COVID-19 in states such as Florida, Nevada, and South Carolina.[54] Current forecasts predict total COVID-19 deaths to be between 130,000 and 150,000 by July 18th.[55] On May 18, 2020, more than 300,000 deaths were reported to the WHO, but as stated by WHO director-general, Dr. Tedros Adhanom Ghebreyesus, "The numbers do not even begin to tell the story of this pandemic. Each loss of life leaves a scar for families, communities, and nations."[17]

Disclosures

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Reviews

Personal Protective Equipment (PPE) during the SARS-CoV-2 Pandemic: A Literature Review

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Introduction

Personal protective equipment (PPE) is defined by the Occupational Safety and Health Administration (OSHA) as equipment used to "minimize exposure to a variety of hazards".[1] PPE comprises an array of equipment, including gloves, masks, goggles, gowns, respirators, and full body suits.[2] The types of PPE utilized for the transmission prevention of a pathogen is based on a pathogen's mode of transmission, size, infectious dose, survival susceptibility, host susceptibility as well as the environmental setting of exposure.[3] Classically, goggles are used to prevent droplets from infecting ocular tissue in respiratory viruses such as Influenza, Rhinovirus, and H1N1.[1,4] Surgical masks and gowns protect the wearer from contact with splashes, droplets or sprays from the mouth, nose or non-intact skin.[5] Respirators and full body suits are utilized when the infectious threat is transmissible through airborne particles.[6]

Prior guidelines for respiratory viruses of similar characteristics to SARS-CoV-2 dictated the recommendations for transmission prevention of this novel virus. SARS-CoV-2 was estimated to measure approximately 120nm [7], allowing for transmission via droplets, direct contact[8] as well as through aerosols.[9] Therefore, guidelines implemented by the Center for Disease Control (CDC) recommended the use of N95 respirators, gowns, gloves, and eye protection in the healthcare setting.[10]

At the time that this manuscript was written, preliminary data was also supportive of public facemask use, with the intention of transmission prevention from infected individuals as well as the prevention of infection of healthy individuals.[12] However, it was recommended that the public utilize surgical masks and surgical mask alternatives rather than N95 respirators. N95 respirators are better suited for the transmission prevention of aerosols through their high filtration capabilities, and therefore, should be reserved for healthcare workers where the risk of infection through aerosolization is higher than in the community[3] due to aerosol generating procedures[13], such as positive pressure ventilation, bronchoscopy, and intubation.[14]

CDC recommendations for public facemask use, alongside increased demands of N95 respirators and surgical masks in the healthcare setting, instigated national PPE shortages that were propagated by underfunded Infection Prevention and Control (IPC) programs as well as breaks in global and local supply chains.[11] Two areas of study emerged in efforts to combat these shortages. The first area of study explored the protection of healthcare workers, which led to the design of medical grade mask alternatives as well as CDC endorsed decontamination procedures in efforts to prolong PPE lifespan.[15] The second area of study entailed investigation of

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facemask alternatives and their decontamination within the community setting, in efforts to preserve medical grade masks for healthcare workers.

The United States faced a significant public health crisis with the onset of the SARS-CoV-2 pandemic. The response involved research into protecting healthcare workers and our communities. These avenues of research remain critical to the continued fight against SARS-CoV-2, as the course of the pandemic and existing PPE supply remain undetermined.

Contributing Factors to the National PPE Shortage

In January 2020, as a consequence of the SARS-CoV-2 pandemic, the PPE demand increased by 400%, rising up to 585% by February 2020.[16] The dramatic demand for PPE was aggravated by local, national, and global circumstances, including historically unprioritized IPC programs and insufficient stockpiles.

During infectious disease threats, hospitals rely on the guidance of IPC programs. These programs are designed to provide education, reinforce infection prevention standards, establish a process to identify, isolate, and inform potential cases as well as ensure hospitals are meeting CDC and World Health Organization (WHO) guidelines.[17] However, these programs have been historically faced with understaffing and competing priorities due to the expense associated with biopreparedness. IPC programs dedicate a majority of their time to reporting requirements and Medicare reimbursements rather than preparing for infectious disease threats, which are considered unlikely to occur.[17] Due to the low level of priority assigned to infectious disease threat preparation and maintenance, many hospitals were found to have an inadequate supply of PPE relative to the surge in demand during the SARS-CoV-2 pandemic. With such a dramatic increase in demand, even adequately staffed IPC programs endured challenges with maintaining appropriate PPE stockpiles.[16]

During non-emergent periods, the government does not intervene with the regular operations and supply chain of healthcare equipment. However, when local or national supplies of PPE are at risk of depletion, the federal government can call upon the CDC's Strategic National Stockpile (SNS) to deploy supplies. The SNS is the largest national storage of medical supplies that is available for deployment during a public health crisis. However, even as the largest repository of medical supplies, the SNS stockpile is not large enough to combat the high demand for PPE during this pandemic, as evidenced by the 2009 H1N1 pandemic. The demand for N95 respirators and face masks during the H1N1 pandemic exceeded the supply of the SNS stockpile, consequently causing a two to three year backlog.[18]

The global supply chain has also contributed to the PPE shortages observed in the United States. Since the late 1990s, a majority of mask manufacturers moved their production overseas, resulting in 90% of the masks used by the United States being manufactured outside of the country.[19] China, in particular, produces approximately half of the world's face masks, but exportation ceased as SARS-CoV-2 spread through the country.[20] This phenomenon was mirrored in other overseas facilities as the virus spread throughout the world, with countries focusing on PPE production for their own use. As a result, exportation to the United States significantly decreased.[21]

The delicate balance of supply and demand, through which the United States market operates, was further disturbed by limited capabilities to surge production rapidly in country due to nationalization and export restrictions.[18] Upscaling production to meet demand was met with additional challenges due to SARS-CoV-2 transmission, requiring production personnel to comply with health guidelines such as social distancing and limiting the number of personnel within work zones. This in turn decreased production rate and increased time required to produce PPE.[22]

Public Facemask Use

There have been discrepancies between the CDC and WHO regarding the recommendations for public facemask use. The WHO stated that medical masks are not recommended for community use, citing preservation of PPE for healthcare workers as well as lack of data showing a mask's utility in protecting healthy individuals from infection.[23] They endorsed hand hygiene and social distancing (at least six feet) as sufficient transmission prevention in the community[24], warning that public facemask use provided a false sense of security.

While the WHO's guidelines have remained unchanged with the progression of pandemic, the CDC has altered their stance on public facemask use since December 2019. At this time, the CDC recommends the voluntary public use of cloth masks or homemade face when social distancing cannot be enforced.[12] Similar to the WHO, the CDC recommends reserving surgical masks and N95 respirators for healthcare workers, while also highlighting the importance of appropriate hand hygiene and social distancing in the community.[12,24]

The discord between guidelines adopted by these two agencies caused significant confusion amongst the public. The confusion was further propelled by lack of data identifying the purpose for public facemask use. Two predominant theories regarding the protection offered by facemasks were viral transmission prevention from an infected symptomatic or asymptomatic individual and infection prevention in a healthy individual. Consequently, these two theories were heavily studied in efforts to determine public facemask use efficacy.[25]

Current data demonstrates that facemask use prevents transmission from an infected individual. Trials show that aerosols and particles are captured via adhesion to the internal surface of both improvised and medical grade masks, as demonstrated through the detection of cytokines on the mask's interior surface hours after aerosolization. This data suggests that mask use significantly reduces viral load transmission from an infected individual, although further quantification is necessary.[26] This is a valuable consideration for infected individuals, with emphasis on asymptomatic individuals whom may not be adhering to isolation guidelines.[27] Proof of transmission prevention was further demonstrated in a Chinese case study, where a symptomatic, unmasked individual transmitted the virus to five of thirty-nine bus passengers. In contrast, transmission was prevented after he utilized a facemask as none of the passengers of the second bus of his journey became infected.[28] Even though the study does not mention whether other passengers were wearing masks, it does highlight the importance of wearing a mask by infected individuals to prevent transmission to healthy people. Nonetheless, further research is needed to determine if the masking of infected individuals is enough to prevent transmission when healthy bystanders are unmasked.

There is limited data demonstrating efficacy of facemask use in infection prevention of healthy individuals while exposed to SARS-CoV-2 infected people in the community. However, a study showing the utilization of masks by healthy occupants against influenza-like illnesses in households with an infected occupant, demonstrated that the adherent use of a mask alone significantly decreased the risk of infection of the healthy occupant.[29] Furthermore, three randomized controlled trials (RCT) in households or university residence halls suggested the reduction of transmission of influenza-like illnesses to healthy masked individuals.[30–32] Although diligent hand hygiene was a confounding factor in this particular study, another study supported a statistically significant ($p<0.5$) protective effect of facemasks.[32] Additionally, a 76% rate of facemask use alongside other precautions during the SARS outbreak in Hong Kong demonstrated decreased infection rates.[33] Lastly, although not based on RCT data, a model study designed to test the hypothetical efficacy of population-wide mask use to prevent influenza-like illness transmission, determined that their use would delay a pandemic.[34] While these studies are not SARS-CoV-2 specific, they highlights mask efficacy in infection prevention of similar pathogens.

While preliminary data supports public face mask use, several limitations are common across most of the aforementioned studies. These limitations include results gathered through self-reporting or case studies, multiple confounding factors, and limited sample size. Most importantly, however, alternative viruses to SARS-CoV-2 were studied; the applicability of this data to SARS-CoV-2 is still under question and warrants further research.

Alternatives, Decontamination, and Reuse in the Community Setting

Prior studies demonstrated that facemask use by infected and/or healthy individuals within the community may prevent SARS-CoV-2 transmission. Relative to cloth masks, surgical masks have a two-fold reduction in particle penetration[45], however due to medical mask shortages, trials are simultaneously exploring alternatives to the surgical mask as well as decontamination protocols of both surgical and homemade masks.

Research emphasis, thus far, is on cloth masks due to the material's widespread availability, simple construction, ability to recruit the general public for production, and reuse capabilities. Trials on cloth masks deduced that type of cloth, number of layers, and material quality played a role in filtration efficacy.[61] Several trials confirmed quilter's cotton61 and polyester cotton[46] as fabrics of choice. Number of layers also played a role in filtration efficacy with flannel (Wake Forest Regenerative Medicine) or kitchen paper towels[47] being utilized in conjunction with the cotton materials to add an additional layer of filtration. Alternative trials determined that four layers of silk exhibited greatest filtration efficiency, followed by one layer of chiffon, and one layer of flannel, respectively. This trial also confirmed that cotton exhibited increased filtration capability at higher thread count.[48]

An important consideration in mask design is mask fit; it was demonstrated that filtration efficiency decreased by 60% when a mask exhibited improper fit. Although studies may deduce high mask efficacy during trials, these masks may not reflect this data in practice due to discrepancies in fit and air leakage.[48,49] While variation in data exists, a mathematical model of face mask use by a population during an influenza pandemic demonstrated that even if masks are 20% effective, then 25% mask use by the population would reduce infectivity by 30%.[49] While some mask designs are superior to others, current data suggests that even simple designs may reduce infection risk when combined with other preventative measures.

In addition to research on surgical mask alternatives, efforts are also underway in establishing decontamination protocols, with an emphasis on easily accessible equipment. Multiple studies on steam sterilization have thus far demonstrated the ability to inactivate viral and bacterial particles by steaming samples over boiling water, with reported time lengths varying from 5 to 10 minutes between studies.[50] Comparison of masks that underwent the steam sterilization relative to unused masks did not demonstrate a difference in permeability nor were structural differences observed upon visual inspection.[51] This suggests mask integrity was maintained. Rice cooker-steamers are another accessible and efficient kitchen tool that can reduce viable organisms on cloth masks by 5log10 in a single 13-15 minute cycle; no observable qualitative changes to the mask were evident, albeit quantitative examination of integrity has not been conducted.[52] Dry sterilization at 121°C for 15 minutes[51] or utilizing a heating oven set to 70°C for 30 minutes[53] exhibited microbe inactivating capabilities. Utility of easily accessible equipment in one's home offers the community ways to reuse surgical masks, N95 respirators as well as cloth masks to preserve as much PPE as possible for healthcare workers. However, several limitations to these processes must first be addressed. It is yet to be established precisely how many cycles of dry or steam sterilization a mask can undergo before its integrity is compromised. Additionally, these decontamination studies utilized alternative bacterial and viral microbes for testing, lacked quantitative analysis of mask integrity, and did not consider the effects on mask integrity with human wear.

While several methods of decontamination have thus far exhibited the ability to maintain mask integrity, some methods have failed to gain recommendation by compromising mask integrity. Bleach particles lingered on masks[54], microwave ovens melted parts of the mask, while alcohol and chlorine removed charges within the fibers and potentially retained harmful gases.[55]

Alternatives, Decontamination, and Reuse in the Healthcare Setting

Protection of healthcare workers became paramount with the rising number of cases in the face of inadequate hospital PPE stockpiles. Research efforts focused on addressing these concerns through comparison of surgical mask efficacy relative to N95 respirators, designing alternatives comparable to N95 respirators, as well as medical grade mask decontamination protocols.

It has been determined that surgical masks are not a replacement for N95 respirators. For N95 respirators, the National Institute for Occupational Safety and Health (NIOSH) requires a 95% filtration efficacy of particles 100-300 nm in order for masks to be considered adequate for sale. In contrast, surgical masks are not required to meet these standards.[35] Therefore, these masks are not designed to assume the function of N95 respirators in the healthcare setting. While surgical masks are less effective in

transmission prevention relative to N95 respirators, their use has demonstrated protective potential by decreasing infection in healthy individuals as well as preventing transmission from infected individuals. Recent trials demonstrated that surgical mask use significantly reduced seasonal coronavirus emission in both respiratory droplet and aerosols.[36]

Researchers are also conducting trials on repurposed medical and non-medical equipment. Surgical tray wrapping, which is made of Haylard H300 two-ply spun polypropylene is impenetrable to water, bacteria, and particles, blocking 99% of particulate matter; this level of impermeability is comparable to that of the N95 respirator.[60] Anesthesia masks are another piece of common hospital equipment that has been repurposed by Wake Forest Baptist Health's Anesthesiology Department. The masks were modified through the addition of a viral/bacterial filter produced by Medline Industries as well as two rubber tourniquet straps. The filter has a 99.99-99.9999% filtration efficacy and the modified mask has successfully passed fit testing for all trial participants.[62] This trial demonstrates another solution for the modification of an easily accessible piece of hospital equipment.

Lastly, 3D printing has been explored due to the ability to streamline production and offer quality consistency across products. 3D printed prototypes have not only demonstrated the advantage of personalization of fit based on individual face scans, but also ease of access to the software, which can be downloaded worldwide. However, limited data is available pertaining to mask efficacy, leakage properties, and mask reusability.[37] Other alternatives have utilized 3D printed components, such as Duke University's modification of the Stryker Flyte helmet via attachment of a 3D printed manifold. The system demonstrated appropriate filtration efficacy.[38]

Since N95 respirator alternatives are still under investigation, the need for simple, efficient, and accessible methods of decontamination without compromising mask integrity was also necessary. Current CDC guidelines acknowledge the risks associated with the extended use of N95 respirators, but have created provisions for circumstances requiring extended N95 use.[39] Several processes have been identified as effective methods of decontamination, including vaporized hydrogen peroxide (VPH), ethylene oxide, UVGI, oven heating as well as dry and steam sterilization.[40]

Duke University utilized its Bioquell Clarus C system to decontaminate masks by vaporizing 35% hydrogen peroxide, which provides the benefit of maintaining mask fit and filtering capacity for approximately 50 cycles. This protocol conservatively offers the ability to rewear a mask 30 times[41], which can dramatically reduce supply stress; however, system inaccessibility presents an obstacle to widespread use of this method. Similarly, the Barnes Jewish Hospital in collaboration with Washington University School of Medicine and BJC Healthcare, outlined its process for N95 respirator collection and VPH decontamination utilizing the Bioquell Z-2 system. The decontamination and aeration process was cited to last 4.5 hours with a turnaround return time of N95 respirators to healthcare providers of 24 hours. At this time, a single cycle can decontaminate 1500 N95 respirators.[42]

Ultraviolet C light (UVC) is another method of decontamination under investigation. It has been previously established that a UVC minimum dose of 1J/cm2 is required for inactivation of other viral particles.[43] It is yet to be determined whether this dosing is adequate for SARS-COV-2 inactivation. There are several methods of UVC application under investigation, including UVC boxes as well as low pressure mercury room decontamination devices, which may vary in viral reduction efficacy.[44] There are many decontamination procedures under investigation, although at this time, the FDA has only approved the use of VPH systems through Emergency Use Authorization (EUA), under section 564 of the Federal Food, Drug, and Cosmetic Act (FD&C Act).[40]

Mask Adverse Effects

The required use of PPE amongst healthcare workers and other essential employees has manifested in common adverse effects within the healthcare community. A survey administered to frontline healthcare workers reported that N95 masks and goggles were worn for 5.9 hours a day. Symptoms most commonly included headaches 56 and a myriad of dermatologic symptoms.[57–59] Overall headache prevalence increased in addition to the exacerbation of pre-existing headaches amongst providers.[56] Additionally, various

dermatological manifestations have been documented as a result of prolonged PPE use. Erythema, papules, dryness, and scaling were reported at the nasal bridge, cheeks, and hands.[57] Irritant contact dermatitis was observed with N95 mask respirators, gloves, and gowns; acne was mainly observed on the cheeks and nose.[58] Furthermore, pressure sores on the bridge of the nose were an additional dermatologic finding. Alleviating efforts such as hydrocolloid dressings only seemed to aggravate sores because the intense stickiness of the dressing caused more damage during removal.[59] The adverse reactions associated with prolonged PPE use highlight the importance of manufacturing better fitting PPE. Physical discomfort and adverse symptomatology could have an effect on the physical, professional, and mental wellbeing of the provider.

Future Outlook/Direction/Conclusion

The swift, widespread, and multi-disciplinary response to medical grade mask shortages impressively demonstrated the capability to design and execute decontamination protocols as well as develop alternatives to these PPE. Trials demonstrated that N95 respirators and medical grade masks can effectively be decontaminated for extended use; medical equipment can also be repurposed to mimic N95 respirators. Studies exhibited that cloth masks can adequately prevent viral transmission prevention, but that cloth type, design, and fit play a role in the mask's efficacy. Common limitations across studies have included the use of alternative viral and bacterial particles to SARS-CoV-2, limited quantitative analyses of mask efficacy and degradation, as well as limited evaluation of designs and protocols in an uncontrolled laboratory setting. Further research is needed to address these limitations as well as refine protocols and designs.

The SARS-CoV-2 pandemic highlighted the inadequacies of programs developed to undertake infectious disease threats. Programs, like IPCs, were unable to appropriately supply hospitals across the country with the correct skills and equipment. In retrospect, we have learned that contributing factors were lack of funding and inappropriate allocation of work duties. Programs designed to establish and maintain adequate infectious disease protocols and supply chains should be revised and maintained going forward.

Additionally, the PPE supply chain requires further attention. If the manufacturing of the equipment or their parts cannot be returned to the country, then guidelines need to be created to facilitate the transition of production companies from varying industries to manufacture PPE during times of crisis. These guidelines should be federally established to ensure reproducibility and standardization across the country. Similarly, public education should be consistent, especially in the early phases of a crisis to ensure appropriate information is disseminated to the masses. Addressing the challenges that the United States faced in the wake of the SARS-COV-2 pandemic will hopefully ensure adequate supplies in the continued fight against this pandemic as well as future ones. While the goal remains to produce and maintain enough medical grade PPE, the efforts addressed in this literature review should not be overlooked due to the looming uncertainties regarding the SARS-CoV-2 trajectory.

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Reviews

Curbing COVID: A Review of the Therapeutic Treatments for SARS-CoV-2

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Abstract

The COVID-19 pandemic has drastically changed the world around us, with the SARS-CoV-2 virus having affected millions of individuals worldwide. Many therapeutics have been proposed and evaluated as potentially effective against COVID-19. This review summarizes the treatments which were utilized as of June 2020, and evaluates the evidence of available therapies early in the pandemic. The therapeutics being pursued for the treatment of SARS-CoV-2 at the time were categorized into six main groups: antivirals, immunomodulators, corticosteroids, antimalarials, convalescent plasma, and mesenchymal stem cells (MSC). Antivirals were the most studied, with remdesivir having been shown to reduce recovery time. Immunomodulators such as monoclonal antibodies target pro-inflammatory pathways such as cytokine receptors and JAK-STAT, and dexamethasone, the widely-used corticosteroid, demonstrated reduction in mortality. Hydroxychloroquine is an antimalarial that received substantial attention in the media; however, evidence substantiating its efficacy was limited by biases including study design. Convalescent plasma therapy was shown to improve clinical outcomes, but the evidence at the time was largely limited by sample size and lack of controls. MSC therapies also had very limited data available but many clinical trials were actively recruiting. As more clinical trials emerge, more and stronger evidence is available to evaluate the use of these therapies in the treatment of COVID-19.

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Introduction

The origins of the coronavirus disease 2019 (COVID-19) pandemic dates back to December 2019, when a novel coronavirus was isolated from the lower respiratory tract of patients with pneumonia in Wuhan, China; these patients were found to be epidemiologically linked to the Huanan Seafood Market.[1] High-throughput, unbiased sequencing was used to isolate the previously unknown beta-coronavirus which was initially named 2019-nCoV.[1] The 2019-nCoV virus belongs to the same genus as acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome (MERS)-CoV and was subsequently named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). On January 20, 2020, the CDC confirmed the first positive test for SARS-CoV-2 in the United States from the nasopharyngeal and oropharyngeal swabs of a 35-year-old male in Snohomish County, Washington; this individual had returned from Wuhan with a cough and subjective fever.[2] The SARS-CoV-2 virus causes COVID-19 which has continued to spread rapidly worldwide leading to the present COVID-19 pandemic.

The dramatic spread of SARS-CoV-2 is largely due to human-to-human transmission. The virus primarily affects the respiratory system which leads to the widely recognized symptoms of fever, cough, sore throat and dyspnea. Risk factors for COVID-19 mortality include older age and comorbidities including hypertension (HTN), diabetes, and coronary heart disease.[3,4] While the exact pathogenesis remains unknown, there is evidence to suggest that the virus enters the host cell by binding its S protein to the host's angiotensin converting enzyme 2 (ACE2) receptors which are expressed in the lung, heart kidney, bowel and bladder.[5,6] Severe COVID-19 is a multi-organ disease resulting from the host's immune response to the virus and an uncontrolled inflammatory response and cytokine storm.[7] Many suggested therapeutic strategies against COVID-19 involve immunopathologic targeting.

As of June 30, 2020, a total of 10,434,835 cases have been reported in 188 countries with 509,779 deaths including 2,629,372 total confirmed cases and 127,322 reported deaths in the United States.[8] At the time we did our literature review, there were no U.S. Food and Drug Administration (FDA) approved treatments or preventative vaccines for COVID-19. With the rising case numbers and unprecedented global toll of the pandemic, there was and continues to be an urgent need to identify effective therapeutics for COVID-19.

Of note, this literature review summarizes the landscape of therapeutics and vaccines that were being studied to treat COVID-19 as of June 2020. Recently, as of December 2020 the FDA had approved the use of Remdesivir to treat COVID-19. The Adaptive COVID-19 Treatment Trial (ACTT-1) showed the Remdesivir group had shorter time to recovery (10 days) than placebo (15 days) in hospitalized adult patients with evidence of lower respiratory tract infection.[9] However, the interim WHO Solidarity Trial results have not shown a mortality benefit.[10] The FDA had also granted authorization for the use of two monoclonal antibody treatments (bamlanivimab; and a combination of casirivimab and imdevimab) to help prevent progression to severe COVID-19 for at-risk patients.[11] As of January 2021, two vaccines have been authorized for emergency use by the FDA for administration in the US: the m-RNA Pfizer-BioNTech vaccine and the m-RNA Moderna vaccine.[12] Many more vaccines are in development and several others have been approved for use worldwide. The juxtaposition of these updated therapeutics to our data from June 2020 is a testament to the complexity of this virus and the effort to efficiently and efficaciously combat the pandemic.

Methods

A literature review was performed using online databases including PubMed, MEDLINE, and LitCovid (https://www.ncbi.nlm.nih.gov/research/coronavirus/). Searches included publications between January 2020 and June 2020. Key terms included: "SARS-CoV-2," "coronavirus," "COVID-19," in combination with "treatment," "therapeutics," "pharmacology," "immunomodulators," "antivirals," "convalescent plasma," "anti-malaria," "stem-cell," "mesenchymal." Following preliminary screening, manuscripts were then assessed for relevance using manuscript titles and abstracts. Therapeutic treatment options of most clinical evidence and research were included in this manuscript. Active clinical trials for SARS-CoV-2 were identified using the search term "SARS-Cov-2" and specific drugs of interest using ClinicalTrials.gov (Table 1). Adverse effects, contraindications, and cautionary warnings for drugs of interest were obtained using Epocrates and UpToDate.com. Adverse effects unveiled from the active trials mentioned in Table 1, as well as the manuscripts referenced within the text of the manuscript were not included. The most common adverse effects mentioned in Epocrates, as well as UpToDate were included in the table. The COVID-19 Vaccine Tracker (Regulatory Affairs Professionals Society, RAPS.org) was used to identify vaccine candidates in various phases of investigation. While we do not provide the exact number of initial papers reviewed and removed during screening, we believe that we included the most relevant papers available by June 2020 in our final selection.

Potential Therapeutics

Antivirals

Antiviral agents are drugs which inhibit a particular stage of viral replication[13] and are currently the most studied therapeutic treatment for COVID-19, especially due to their efficacy in the treatment of SARS and MERS.[14] The SARS-CoV-2 virus is a part of the coronavirus family, a family of enveloped viruses with a positive-sense, single-stranded

Table 1. Therapeutic Treatment Options for SARS-CoV-2

Drug Category	Drug	MOA	Clinical Trials Identifier	Adverse Effects	Contraindications
Antivirals					
	Remdesivir	Nucleoside Analog	Active Trials include: NCT04292899; NCT04292730. 8 trials currently recruiting or enrolling patients by invitation.	• Hyperglycemia • Increased LFTs • Acute renal failure • Decreased eGFR • Increased serum creatinine • Fever • Infusion related reaction	• Hypersensitivity to remdesivir
	Lopinavir-Ritonavir	Protease Inhibitor	Active Trials include: NCT04386876; NCT04328285. 19 trials currently recruiting or enrolling patients by invitation.	• Skin rash • Hypercholesterolemia • Increased serum triglycerides • Increased gamma-glutamyl transferase • Dysgeusia, Nausea/vomiting/ diarrhea • Abdominal pain • Increased LFTs • URI	• Hypersensitivity to lopinavir or ritonavir • Co-administration with drugs that are dependent on CYP3A for clearance
	Ribavirin	Guanosine Analogue	Completed trial: NCT04276688. No Active trials. No trials currently recruiting or enrolling patients by invitation. 2 trials not yet recruiting.	• Alopecia, dermatitis, pruritus, diaphoresis, skin rash, xeroderma -Growth retardation, weight loss • Hyperuricemia, Nausea/ vomiting/diarrhea, dyspepsia, • GI disease • Abdominal pain • Xerostomia • Anemia (hemolytic) • Lymphopenia, neutropenia • Hyperbilirubinemia • Viral infection • Erythema/inflammation at injection site • Anxiety, chills, depression, dizziness, fatigue, headache, insomnia, irritability, lack of concentration, nervousness, rigors • Arthralgia, musculoskeletal pain, myalgia • Cough, dyspnea, pharyngitis, sinusitis, • URI • Fever	• Hypersensitivity to ribavirin -Pregnancy • Males whose female partners are pregnant • Hemoglobinopathies • Concomitant use with didanosine • combination therapy with α interferons • CrCl <50 mL/minute (for Ribasphere capsules and Rebetol capsules/solution)
	Favipiravir	viral RNA polymerase inhibitor	Completed Trials: NCT04349241; NCT04376814. Active Trials include: NCT04336904; NCT04434248. 9 trials currently recruiting or enrolling patients by invitation.	• Decreased appetite • Nausea/vomiting/diarrhea • Hyperuricemia • Neutropenia • Hepatic injury • Increased LFTs	• Inhibits CYP2C8: drug interactions with Influenza Virus Vaccine, Pyrazinamide, and Repaglinide • Pregnancy • Breast-feeding

Table 1. *Continued*

Immunomodulators					
	Tocilizumab	IL-6 Receptor Antibody	Active Trials include: NCT04320615; NCT04315480; NCT04331808; NCT04347031; NCT04346693. 40 Trials currently recruiting or enrolling patients by invitation.	• Transient, dose dependent neutropenia • Alterations in serum lipid profiles, • Increased LFTs • Injection site reactions • GI perforation	• Hypersensitivity to tocilizumab • Caution in patients with a history of opportunistic infections, including tuberculosis • ANC < 2000 • Plt <100,000 • Hepatic disease
	Sarilumab	IL-6 Receptor Antibody	Active Trial: NCT04324073. 12 Trials currently recruiting or enrolling patients by invitation.	• Increased LFTs • Hypertriglyceridemia • Neutralizing antibody development • Injection site reaction • GI perforation	• Hypersensitivity to sarilumab • Caution in patients with a history of opportunistic infections, including tuberculosis
	Ruxolitinib	JAK1 and -2 Inhibitor	No Active Trials. 7 Trials currently recruiting or enrolling patients by invitation.	• Anemia • Thrombocytopenia • Neutropenia • Elevated LFTs • Ecchymosis	• Hypersensitivity to ruxolitinib • CrCl < 15 • Hepatic impairment • Plt < 50,000 • Breastfeeding
	Baricitinib	JAK1 and -2 Inhibitor	Completed Trial: NCT04358614. No Active Trials. 10 Trials currently recruiting or enrolling patients by invitation.	• Neutropenia • Lymphopenia • Anemia • Thrombosis • Elevated LFTs • Increased Lipids • URI infections • Nausea • HSV infection	• Hypersensitivity to Baricitinib • eGFR <30 • Hgb <8 • Lymphocytes < 500 • ANC<1000
Corticosteroids					
	Dexamethasone	Anti-inflammatory and immunosuppressive	Completed Trial: NCT04445506. Active Trials: NCT04327401; NCT04425863. 9 Trials currently recruiting or enrolling patients by invitation.	• Cushingoid appearance • Weight gain/abdominal distension • Appetite changes • Anaphylaxis • Adrenal insufficiency • Steroid psychosis and myopathy • Cardiovascular arrhythmia or failure • Depression, emotional ability • Acne vulgaris, alopecia, ecchymosis	• Hypersensitivity • Breastfeeding • Systemic fungal infection • Cerebral malaria • Ocular HSV infection

Table 1. *Continued*

Anti-Malarials					
	Hydroxychloroquine	Weak base and Immunomodulator	Completed Trials: NCT04261517; NCT04434144; NCT04423991; NCT04376814; NCT04389320; NCT04343768; NCT04308668. Active Trials include: NCT04429867; NCT04333225; NCT04345653; NCT04353271; NCT04372017; NCT04358068; NCT04303507; NCT04328285; NCT04332991; NCT04345159; NCT04452617; NCT04322123; NCT04347031; NCT04328467; NCT04316377. 112 trials currently recruiting or enrolling patients by invitation.	• Retinopathy • Cardiomyopathy, ECG changes (prolonged QTc interval), torsades de pointes, ventricular arrhythmia • Skin rash, SJS, TEN, urticaria • Hypoglycemia, Nausea/ Vomiting/Diarrhea • Abdominal pain • Agranulocytosis, • Anemia, aplastic anemia, bone marrow failure • Leukopenia, thrombocytopenia • Abnormal LFTs • Acute hepatic failure • Angioedema • Drug reaction with eosinophilia • Ataxia, dizziness • Emotional lability, fatigue, headache, irritability, psychosis, • Seizure • Asthenia • Corneal changes, decreased visual acuity, macular degeneration, maculopathy, scotoma, visual field defect • Deafness, tinnitus • Bronchospasm • Renal insufficiency	• Hypersensitivity to hydroxychloroquine
	Chloroquine	weak base and immunomodulator	Active Trial: NCT04303507. 16 trials currently recruiting or enrolling patients by invitation.	• AV block, bundle branch block, cardiac arrhythmia, cardiac failure, cardiomyopathy, ECG changes, torsades de pointes, ventricular arrhythmia • Alopecia, blue-gray skin pigmentation, SJS, TEN, urticaria • Hypoglycemia • Abdominal pain • Nausea/vomiting/diarrhea, Agranulocytosis • Anemia (aplastic, hemolytic) • Neutropenia, pancytopenia, thrombocytopenia • Hepatitis, increased LFTs • Anaphylaxis, angioedema • Drug reaction with eosinophilia -agitation, anxiety, confusion, headache, hallucination, insomnia, psychosis, seizure • Myopathy, asthenia • Accommodation disturbances, blurred vision, corneal opacity, macular degeneration, maculopathy, night blindness, retinal pigment changes, retinopathy, transient scotomata, visual field defect • Hearing loss, tinnitus	• Hypersensitivity to chloroquine • Presence of retinal or visual field changes of any etiology

Table 1. *Continued*

Convalescent plasma / Immunoglobulin Therapy					
	Convalescent plasma	Passive immunity via neutralizing antibody titers	Completed Trials: NCT04407208; NCT04346446; NCT04441424; NCT04401085; NCT04442958 Active Trials: NCT04323800; NCT04333251; NCT04377672; NCT04373460; NCT04373460; NCT04355767; NCT04344535; NCT04359810; NCT04358406; NCT04364737; NCT04362176; NCT04361253; NCT04421404; NCT04385199; NCT04390503; NCT04397757; NCT04408040; NCT04418518; NCT04377568; NCT04438057; NCT04442191	• Nausea • Skin erythema • Fever • Anaphylaxis • Transfusion-related lung injury • Transfusion-transmitted infections (e.g. HBV)	• IgA deficiency • Hypersensitivity to plasma transfusions
Mesenchymal stem-cell infusion					
	Mesenchymal stem-cell infusion	Immunomodulation	Active Trials: NCT04315987; NCT04302519; NCT04288102; NCT04273646; NCT04252118; NCT04299152; NCT04269525; NCT04276987; NCT04313322 6 trials currently recruiting or enrolling patients by invitation.	-Fever -Graft-versus-host disease	-High CMV load -High hypercoagulability

Note: ANC = absolute neutrophil count; AV = atrioventricular; CrCl = Creatinine Clearance; CMV= cytomegalovirus; ECG = echocardiogram; eGFR = Estimated Glomerular Filtration Rate; GI = Gastrointestinal; Hgb = Hemoglobin; HBV= Hepatitis B Virus; HSV = Herpes Simplex Virus; LFT = liver function tests; Plt = Platelets; SJS = Stevens-Johnson Syndrome; TEN = Toxic Epidermal Necrolysis ; URI = upper respiratory infection

ribonucleic acid (RNA) genome.[15] The most noticeable characteristic of coronaviruses is the spike (S) protein. Once the receptor is attached, S proteins are involved in viral replication by facilitating the fusion of the virus with the host cell membrane.[16] During attachment and penetration, the virus is able to inject its viral RNA genomic material into the host cell resulting in the replication and assembly of the viral genomic material and the ultimate release of newly-formed virions into the host organism.[17] The antivirals discussed in this review as potential treatments for COVID-19— remdesivir, lopinavir-ritonavir, ribavirin, and favipiravir—act upon the different stages SARS-CoV-2 viral replication. Remdesivir has shown the most promise of the antiviral treatments, and of treatments overall. While remdesivir is not yet FDA approved, the FDA has accelerated its development to potentially treat COVID-19.[18]

Remdesivir

Remdesivir,a nucleoside analog that inhibits viral RNA polymerases has been shown to be effective in vitro activity against SARS-CoV-2 by inhibiting the virus' ability to proliferate.[19] Remdesivir exhibits in vitro activity against RNA virus families such as Coronaviridae, Flaviviridae, Filoviridae, Paramyxoviridae, and Pneumoviridae.[20] It was initially developed as a potential treatment for Ebola and has been shown to be effective against coronaviruses such as SARS and MERS in vitro.[21] There are two active trials and eight trials which are currently recruiting or enrolling patients by invitation (Table 1). A Phase 2 double-blind, placebo-controlled clinical trial conducted by the U.S. National Institute of Allergy and Infectious Diseases, concluded that the use of remdesivir was superior to the placebo group in shortening the recovery time in adults hospitalized due to COVID-19.[22] Remdesivir has received an Emergency Use Authorization for the treatment of COVID-19 given it ability to reduce the recovery time of patients with severe COVID-19 disease. It is now undergoing Phase 3 clinical trials through the Gilead "SIMPLE" Trials –two studies conducted to assess the safety and efficacy of two dosing durations of remdesivir, a 5- and 10-day course in patients with severe COVID-19 disease.[23] Three-hundred and ninety-seven patients underwent treatment, 200 for five days and 197 for ten days, with no placebo-controlled participants. Investigators concluded that there was no significant difference in outcomes for those undergoing the 5-day versus 10-day course of remdesivir.[24] The limitations of this trial to be considered are the lack of a placebo group and the open-label nature of the study, and thus the inability to interpret the non-inferiority of the 5-day versus 10-day course of remdesivir. The second SIMPLE trial was aimed at evaluating the safety and efficacy of 5-day and 10-day dosing in patients with moderate COVID-19 disease, and the preliminary results showed that a 5-day course has better outcomes for moderate presentation.[25] Gilead continues to be in discussion with organizations for future clinical trials.[26] Remdesivir has already been recommended to be authorized as the first medicine for treatment against COVID-19 by the European Union, which is awaiting the final report submissions from Gilead in December, 2020.[27]

Lopinavir-Ritonavir

Lopinavir-Ritonavir is a fixed-dose combination of two protease inhibitors which has been previously studied as an adjunctive medication in the treatment of SARS.[28] In vitro, inhibition of SARS-CoV-2 viral replication has been shown to be effective; thus protease inhibitors, which inhibit viral replication are plausibly effective.[29] Protease inhibitors are a class of antiviral drugs that inhibit the proteases required for the cleavage of viral polyprotein precursors—a critical step in viral assembly. Lopinavir-Ritonavir (Kaletra) is currently used to treat HIV infections and has been shown to be clinically effective.[30] Though clinical trials are ongoing, there is still no clinical evidence to suggest that lopinavir-ritonavir is effective in treating COVID-19.[31] For lopinavir-ritonavir, there are two active trials and 19 trials which are currently recruiting or enrolling patients by invitation (Table 1). A randomized, controlled, open-labeled Phase 2 clinical trial conducted in Wuhan, China concluded that there were no benefits identified in using lopinavir-ritonavir in patients with severe COVID-19 disease, with no accelerated clinical improvements, reduced mortality, or diminished detection of viral load from throat RNA swabs noted compared to patients receiving symptomatic supportive care (i.e. supplemental oxygen, noninvasive and invasive ventilation, antibiotic agents, vasopressor support, renal-replacement therapy, and extracorporeal membrane oxygenation).[28] The limitations of this study include the lack of blinding, smaller sample size, and potential confounding variables such as concomitant use of glucocorticoids. In another Phase 2 clinical trial, the UK

RECOVERY trial, similar findings were found showing no significant differences in rates of survival, decreased rates of progression to mechanical ventilation or decreased length of hospital stay in patients receiving.[32] Thus far, there has been no significant benefit shown utilizing lopinavir–ritonavir as a treatment for COVID-19.

Ribavirin

Ribavirin is a guanosine analogue which helps to inhibit viral replication and has broad spectrum activity against RNA and DNA viruses.[33] Ribavirin has been approved for use in treating RSV, Lassa fever virus, influenza A and B, and hepatitis C[34], as well as SARS and MERS patients.[35] In vitro, ribavirin has been shown to have activity against SARS-CoV-2, with immunomodulatory ability in suppressing pro-inflammatory cytokines.[36] For ribavirin, there is one completed trial, no current active trials or trials which are recruiting or enrolling, and two trials which are not yet recruiting (Table 1). In Hong Kong, a multicenter, prospective, open-label, randomized, Phase 2 trial was conducted to determine the safety and efficacy of an antiviral triple therapy regimen (interferon beta-1b, lopinavir-ritonavir, and ribavirin) in treating COVID-19.[37] Researchers concluded that early antiviral triple therapy was superior and safer in treating mild to moderate COVID-19 than lopinavir-ritonavir alone. Triple therapy reduced the duration of viral shedding and hospital stay, and alleviated symptoms including fever, cough, sputum, malaise, and diarrhea. Notable limitations included the lack of blinding, small sample size, and the concurrent use of therapies, which could confound which of the therapies was actually effective and may point to the lack of efficacy in lopinavir-ritonavir treating COVID-19. Another conclusion that could be drawn from this study is that ribavirin and interferon therapy should be investigated further without the concurrent use of lopinavir-ritonavir. More clinical trials are needed in order determine ribavirin's use as an efficacious treatment for COVID-19 and to determine drug interactions with ribavirin, given its current use and experimentation in multi-drug regimens to treat COVID-19.[35]

Favipiravir

Favipiravir is a viral RNA-dependent RNA polymerase inhibitor, which has been approved for use against influenza infections in some Asian countries and has also been used in the treatment of Ebola, SARS, and MERS.[38] In vitro studies have shown that favipiravir is able to produce an adaptive immune response via expansion of cytotoxic T-cells leading to viral clearance, which could be beneficial in treating COVID-19.[39] Furthermore, with SARS-CoV being an RNA virus, the mechanism of action of favipiravir could be useful in the treatment of COVID-19. For favipiravir, there are two completed trials, two active trials, and nine trials currently recruiting or enrolling patients (Table 1). In China, an open-label, randomized, before-after controlled study was conducted to examine the effects of favipiravir versus lopinavir-ritonavir.[40] Findings demonstrated that the favipiravir group showed better therapeutic responses to COVID-19 in regard to disease progression and viral clearance, along with less adverse events than those administered lopinavir-ritonavir. Limitations of this study were small sample size, lack of blinding, and lack of placebo group. Though there is some promise of favipiravir as a future treatment for COVID-19, more clinical trials need to be conducted to determine its efficacy, particularly with more participants, double-blinding, and placebo-control.

Immunomodulators

The pathogenesis of SARS-CoV-2 begins with an early viral response phase that is then followed by a pulmonary and hyperinflammatory phase.[41] It is during these two latter stages where SARS-CoV-2 becomes a lethal disease, leading to significant respiratory dysfunction in the lower respiratory tract in the form of pneumonia and acute respiratory distress syndrome (ARDS).[42] Elevated serum levels of cytokines (interleukin [IL]-1, IL6-10, Interferon-γ [INF-γ], and tumor necrosis factor-α [TNFα]) and chemokines (CXCL10 and CCL2) have been found in patients diagnosed with SARS-CoV-2. Specifically, pro-inflammatory cytokines such as IL-6 mediate inflammatory cascades through Nuclear Factor (NF)-κB and Janus kinase-Signal Transducer and Activator of Transcription (JAK-STAT) signaling pathways leading to further amplification and activation of the so called "cytokine storm," that is characteristic of ARDS. These events then lead to immune dysregulation, lymphocyte proliferation and differentiation, and oxidative stress.43 Given this knowledge and an understanding of the immunopathogenic features of ARDS, potential therapeutic targets aimed towards dampening the pro-inflammatory phase (cytokine

receptor inhibitors including tocilizumab and sarilumab) and downstream signaling pathways (JAK-STAT inhibitors including ruxolitinib and baricitinib) in patients with SARS-CoV-2 are of utmost interest.

Monoclonal Antibodies: Tocilizumab and Sarilumab

Tocilizumab is a humanized, monoclonal antibody that functions as an IL-6 receptor antagonist, binding to both soluble and membrane-bound IL-6 receptors.[44] Traditionally, tocilizumab has been used for the treatment of various auto-immune disorders such as rheumatoid arthritis (RA) and giant cell arteritis[44,45], as well as the life-threatening cytokine release induced by chimeric antigen receptor T cell therapy.[46,47]

Initial studies using tocilizumab in patients with SARS-CoV-2 have shown promise.[48,49,50,51,52] One of the first studies to examine the efficacy of tocilizumab in patients with SARS-CoV-2 in China demonstrated that in patients receiving a single dose of tocilizumab 91% of patients showed clinical improvement as evidenced by a decreased physiologic need for additional oxygen therapy and significant decreases in the percentage of circulating lymphocytes, C-reactive protein (CRP) and computed tomography (CT) study abnormalities.[53] This initial study was limited by its relatively small sample size and lack of comparative sample prompting additional researchers to examine the potential benefits of tocilizumab in the setting of SARS-CoV-2. In a single-center study conducted in Italy, patients diagnosed with SARS-CoV-2 related pneumonia, not needing mechanical ventilation were either treated standard therapy at the time (hydroxychloroquine, lopinavir and ritonavir) or were later treated with one dose of tocilizumab (400 mg, 324 mg, or 800 mg, depending on the patient) within four days of admission once the drug became available. Findings from the study suggest that tocilizumab treatment showed significantly higher survival rates compared to patients receiving standard therapy.[48] In a larger clinical study of hospitalized patients with SARS-CoV-2, a hospital treatment algorithm including a one-time tocilizumab treatment provided additional evidence to suggest improved oxygenation and dampening of inflammatory biomarkers (IL-6 and CRP), and improved survival, particularly in black and Hispanic patients.[51] Surprisingly, elevated D-dimer levels following tocilizumab therapy, was unanticipated and concerning given the existing increase in thromboembolic events in patients with SARS-CoV-2. Additional studies are needed to further elucidate these findings to confirm whether tocilizumab may predispose patients to hypercoagulable events, as well as to delineate the optimal dose for SARS-CoV-2. Further insight into which patients may benefit most from tocilizumab is critical to optimize treatment efficacy as higher levels of serum IL-6 post treatment may distinguish non-survivors from survivors.[54] There are currently five active, randomized clinical trials (RCTs) underway, with 40 additional trials recruiting and enrolling patients (Table 1).

Sarilumab is a fully human IL-6 receptor antagonist, that is mechanistically equivalent to tocilizumab and is similarly FDA approved to treat moderate-to-severe RA.[55] In one case report, investigators in Italy reconstituted sarilumab for intravenous (IV) administration in eight patients diagnosed with SARS-CoV-2, in addition to the standard daily therapy cocktail that included hydroxychloroquine, azithromycin, darunavir, cobicistat and enoxaparin. Two separate sarilumab 200mg dosages were administered at 48 and 96 hours of hospitalization. Results found a 30% reduction in oxygen requirement from initial baseline hospitalization measurements, as well as progressive reduction in inflammatory markers of serum amyloid A and CRP. These findings provide preliminary evidence for the early intervention and adjunctive therapy of sarilumab patients with SARS-CoV-2 as all but one patient was discharged within 14 days of hospitalization.[56] Current evidence is limited by a lack of randomization and potential selection bias; however, there is an active Phase 2/3 trial examining the therapeutic effect and tolerance of sarilumab treatment in patients with moderate-severe pneumonia associated with SARS-CoV-257 (Table 1), as well as 12 additional RCTs recruiting participants.

JAK/STAT Inhibitors

Ruxolitinib is a potent and selective JAK 1 and 2 inhibitor with selectivity against tyrosine kinase 2 and JAK3, respectively. Ruxolitinib is currently approved in patients with primary myelofibrosis and polycythemia vera and has also been shown to be effective in conditions where cytokine release is a hallmark feature for pathogenesis such as in graft-versus-

host disease and hemophagocytic lymphohistiocytosis.[58-59] Repurposing ruxolitinib and other small-molecule JAK inhibitors is of particular interest given the similarities in cytokine profiles between SARS-CoV-2 and the cytokine-driven inflammatory conditions mentioned above.[60] Cao et al. demonstrated a numerically faster clinical improvement in patients receiving ruxolitinib plus standard of care treatment compared to controls just receiving standard of care treatment, median 5 days vs 8 days respectively.[61] Significant chest CT improvements and a faster recovery from lymphocytopenia, compared to controls was also observed. Rosée and colleagues provided a case series of 14 patients with pro-inflammatory syndromes who were treated with ruxolitinib with 12 out of 14 patients achieving a significant reduction in the newly developed COVID hyperinflammatory score index and sustained clinical improvements in 11 out of 14 patients.[62] In another case report of a 55-year-old patient with SARS-CoV-2 and significant comorbidities including primary myelofibrosis (an indication for ruxolitinib treatment), HTN, obesity and chronic kidney disease, continuation of the patient's original ruxolitinib to prevent a fatal cytokine storm and ARDS was decided.[63] The patient's condition remained stable, without progression to mechanical ventilation or vasopressors, and was discharged after two weeks of hospitalization. Although this report's findings do not prove that ruxolitinib alone was the sole agent of this patient's recovery, the report does provide evidence for the continuation of ruxolitinib therapy, especially in the setting of patients with existing hyperinflammatory conditions such myelofibrosis in preventing the "cytokine storm" in SARS-CoV-2 pneumonia. Another case report demonstrated a therapeutic response to ruxolitinib therapy in a patient with severe SARS-CoV-2 refractory to anti-IL6 therapy.[64] In a report featuring two patients in different stages of SARS-CoV-2 infection, ruxolitinib treatment resulted in a soft tissue infection in one patient and cutaneous purpura reaction and reduction in hematocrit levels in both patients.[65] Additional large scale studies are needed to further clarify the safety profile of ruxolitinib administration in the setting of SARS-CoV-2 (e.g. determine which patients are most at risk for developing hyperinflammatory syndromes) and to better characterize the precise role of the JAK-STAT pathway in the pathophysiology of SARS-CoV-2 immune dysregulation. There are currently seven clinical trials underway (Table 1). Though these results are encouraging, more robust, randomized trials are required to determine the efficacy of JAK/STAT inhibitor therapy.

Similar to ruxolitinib, baricitinib is a small-molecule, reversible JAK-inhibitor currently approved for moderate-severe RA.[66] Stebbing et al., observed in a cohort of patients with bilateral SARS-CoV-2 pneumonia that baricitinib treatment was associated with both clinical and radiologic recovery, as well as a rapid decline in viral load and inflammatory markers.[67] In an earlier review, baricitinib therapy in the setting of SARS-CoV-2 was met with some caution given the risk of lymphocytopenia with therapy in patients with whose absolute counts were already reduced.[68] A case series that treated hospitalized patients with moderate SARS-CoV-2 pneumonia with baricitinib,in addition to the standard ritonavir-lopinavir therapy, demonstrated improved respiratory function parameters were improved from baseline, compared to the controls receiving lopinavir/ritonavir and hydroxychloroquine.[69] Additional studies using larger cohorts are needed to better understand whether baricitinib therapy may be generalized to a larger population of patients infected with SARS-CoV-2 to limit the cytokine-release syndrome seen in this disease.

Corticosteroids

Dexamethasone, the widely used synthetic corticosteroid has recently gained significant attention following groundbreaking evidence from a UK government funded trial showing that the therapy may reduce SARS-CoV-2 related deaths.[70] Dexamethasone is a long-acting, corticosteroid that functions to dampen the inflammatory response by suppression of neutrophil migration, decreased production of inflammatory mediators and suppression of the hosts normal immune response.[71] Given how inexpensive and widely used dexamethasone is in the medical arena, as well as its broad-spectrum immunosuppressive effects, researchers have found that it may be useful for the short-term in patients who are present with severe SARS-CoV-2 infection.[72] What is less known, is how longer treatment of dexamethasone may impact clinical outcomes given it limits both the production and down-stream pathways of inflammatory cytokines, and the protective function of T and B cells. These effects may potentially lead to a dysregulated host response and

subsequent increased viral load. Investigators in the UK who were a part of the RECOVERY trial demonstrated that mortality was reduced in patients receiving dexamethasone by one-third in ventilated patients and one-fifth in patients receiving oxygen therapy only.[73] Given the speed at which this press release came out, many are speculative of the findings and are accepting the results at arm's length; however, these promising findings point to yet another potential therapeutic that may have been sitting right in front of us this whole time.

Antimalarials

Chloroquine and hydroxychloroquine are originally used as malarial prophylaxis and treatment, with hydroxychloroquine utilized in certain rheumatologic conditions such as systemic lupus erythematosus (SLE) and RA.[74] Chloroquine is a quinoline derivative which has activity against the erythrocytic stage of malaria infection, by inhibiting heme polymerase activity which results in the accumulation of cytotoxic free heme.[75] Hydroxychloroquine, an aminoquinoline, is a derivative of chloroquine, and though it has been shown to be much less effective against malaria than chloroquine[76], it is less toxic in its cardiac-related adverse effects.[77] In the case of the rationale behind their use as antiviral therapies, they have shown, in vitro and in vivo, to demonstrate the direct inhibition of viral entry and spread.[78] Chloroquine and hydroxychloroquine both act as weak bases which elevate the pH of acidic intracellular organelles (such as endosomes and lysosomes), and thus help to disrupt viral membranes, along with additionally inhibiting viral entry.[77] Thus far, the use of antimalarials for COVID-19 has been preclinical, and clinical trials have yet to bolster their use against the SARS-CoV-2 virus. For hydroxychloroquine use for the treatment of COVID-19, there have been 7 completed clinical trials, there are 15 active trials, and 112 trials currently recruiting/enrolling patients (Table 1). For chloroquine use for the treatment of COVID-19, there is one active trial and 16 trials currently recruiting/enrolling patients (Table 1).

There has been much discussion surrounding the use of hydroxychloroquine and chloroquine in the realm of prophylaxis and treatment of COVID-19, with hydroxychloroquine being the first choice as it is less toxic, as previously mentioned. Those in the academic community have critiqued the current quality of the evidence to support the use of these therapies in the treatment of COVID-19, particularly calling into question the efficacy of the studies and the heterogeneity of the outcomes measured between different studies.[77] One randomized, control trial done in Shanghai, with 30 participants concluded that while the prognosis of a moderately-ill COVID-19 patients is "good" in hydroxychloroquine-treated patients, a larger study needs to be done to fully investigate the use of hydroxychloroquine in treating COVID-19.[79] Though some preclinical data provides a rationale for the use of these antimalarial drugs for COVID-19, recently completed clinical trials for the use of hydroxychloroquine have begun to indicate that its use is not effective for the prevention and treatment of COVID-19. In one such trial, hydroxychloroquine was tested as a post-exposure prophylaxis within four days of a known COVID-19 exposure, and it was concluded that it was not effective in the prevention of the development of a COVID-like illness or a confirmed infection.[80] In the randomized and double-blinded CloroCovid-19 Phase 2 trial, which assessed the adverse risk profile of chloroquine, they identified some benefit of chloroquine's early use in COVID-19 cases due to its anti-inflammatory properties[81], but cited the need for more research in this regard. Of note, this study also pointed to the high risk profile associated with increased dosing of chloroquine. As for limitations, this study only had a sample size of 81 participants and did not have a placebo group. More robust evidence through clinical trials, must come out regarding the use of chloroquine and hydroxychloroquine in preventing and treating COVID-19, particularly given the hope that initially surrounded these therapies in the media.[82] Their irrational use outside of healthcare settings, particularly given their side effect profile if not managed correctly, has led to adverse events for the irresponsible user.[83]

Convalescent Plasma Therapy

Convalescent plasma therapy (CPT) is a form of passive immunization in which plasma containing antibodies are collected from recovered individuals and transfused into patients with active disease. The approach of using plasma from recovered individuals as a therapeutic approach to treating novel pathogens including Ebola and SARS has been around for over a century, with the most convincing data arising from the treatment of Argentine hemorrhagic

fever.[84] With the urgent need for prophylactic and therapeutic interventions against COVID-19, many studies on CPT for the SARS-CoV-2 have emerged.

The goal of CPT for treating SARS-CoV-2 is to collect plasma from individuals who have recovered from COVID-19 and are without symptoms for at least 2 weeks and transfuse the antibody-containing plasma into patients with severe COVID-19 in order to neutralize the virus in the affected individuals.[85,86] This form of passive immunization provides patients receiving the donor plasma with neutralizing antibodies that target the virus for destruction as well as other anti-inflammatory cytokines and defensins that may provide patients with additional immunologic benefits to the severe inflammatory response to SARS-COV-2.[87] Key studies examining the potential of CPT for COIVD-19 are discussed below.

The results of a pilot study in Wuhan, China suggest that CPT is clinically beneficial[88], and demonstrated patients had a reduction of symptoms including fever, cough and shortness of breath within three days and had reduction pulmonary lesions on CT imaging. In addition to CPT, patients were receiving antiviral medications and steroids.

Preliminary findings of a case series (n=5) in Shenzhen, China suggest that convalescent plasma containing neutralizing antibodies might be beneficial in treatment of severely ill patients with COVID-19.[89] Patients with COVID-19 and ARDS, on mechanical ventilation, methylprednisolone and antivirals (interferon and lopinavir/ritonavir) received administration of CPT. Clinical outcomes of each of the five patients showed improvement with ARDS resolving in four patients,12 days after transfusion, and three patients were weaned from mechanical ventilation within 2 weeks of CPT.

The results of the first randomized clinical trial of CPT, an open-label, multicenter RCT in Wuhan, China (n=103) suggest that CPT when added to standard treatment does not lead to statistically significant clinical improvement in patients with severe COVID-19.[90] The results showed no significant differences in outcomes between patients who received the CPT combined with standard treatment and patients who received standard therapy alone in mortality and time-to-discharge outcomes. While there were no statistically significant differences, there was an observed overall mortality difference of 24% versus 15.7% for patients who received standard vs standard and CPT.[90,91]

In South Korea, two patients with severe COVID-19 received convalescent plasma in addition to lopinavir/ritonavir and hydroxychloroquine and showed marked clinical improvement (oxygenation and reduction in viral loads) and radiologic improvement on chest x-rays. after CPT administration.[92]

In a case series of patients with severe COVID-19, patients had no adverse events from therapy (as measured by safety measurements shortly following CPT administration) and 76% had improvement in clinical status.[93] Nine patients (36%) improved from baseline by seven days post-CPT, while 13 (52%) had no change, and 3 deteriorated. By post-transfusion day 14, 19 patients (76%) showed improvement in at least one of World Health Organization's ordinal scale for clinical improvement.

A controlled case series of patients with COVID-19 (n=20) treated with CPT showed that 7- and 14-day case fatality rate was higher in the control compared to the CPT group.[94] The study found also found that respiratory status of patients treated with CPT was similar to that of controls and a similar proportion of patients in the control and CPT groups were discharged.

There are several key limitations to the current COVID-19 CPT studies. First, most studies are case series, and many are uncontrolled. There is a lack of large, multicenter RCTs; the first RCT for CPT by Li et al.[90] had limited sample size (n=103), and the study was terminated early due to decreased recruitment.[86] Second, most studies are small and drastically limited by sample size (n=2 to n=103), and thus significantly underpowered. Third, in all studies, patients who received CPT were also receiving additional therapies including antivirals, steroids, and hydroxychloroquine despite the uncertainty of the efficacy of these therapies; therefore, it is possible that the additional agents could have contributed to recovery or synergistically contributed to outcomes. Fourth, timing and dosage of CPT were not standardized and could have affected the outcomes in each of the studies. Lastly, most studies had additional biases such as variations in patient ages, comorbidities, and poor reporting within studies.

Overall, CPT studies in patients with COVID-19 showed positive preliminary clinical outcomes and suggest improved mortality; however, the evidence is limited by high risk of bias and thus low certainty of the evidence presented in the limited studies available. More large-multicenter RCTs with significantly larger study populations and more rigorous study design are needed to draw a definitive conclusion on the efficacy of CPT for COVID-19. CPT was shown to have a good safety profile with most studies reporting limited or no adverse effects from CPT. In addition to larger RCTs, additional studies are needed to determine the optimal timing, dosage and titer levels for CPT to be most effective in COVID-19. There are currently at least 20 active trials examining CPT (Table 1). If subsequent studies demonstrate the efficacy of CPT for patients with COVID-19, then the next steps would include investigating the efficacy of hyperimmune globulin, or concentrated antibodies.

Mesenchymal Stem Cell Infusion

Mesenchymal stem cells (MSCs) have been proposed as a possible therapeutic treatment for COVID-19 due to their immunomodulatory properties, high proliferation and low invasive nature. Sources of MSCs include adipose tissues, bone marrow, umbilical cords, abdominal fat, and Wharton's jelly.[95,96] The goal of MSC therapy is to prevent the cytokine storm seen in severe COVID-19 and promote endogenous repair in the host.[97] MSCs mitigate inflammation by releasing paracrine factors as well as preserve and restore alveolar epithelium; the stem cells have been shown to regenerate type II alveolar epithelial cells directly via their differentiation.[98]

In a pilot clinical trial conducted in Beijing, China, ten patients with COVID-19 were enrolled to study the effects and safety of MSC therapy.[99] Clinical improvement within two days was seen in the patients who received MSC transplantation along with decreases in CRP and TNF-α and no adverse reactions were reported within 14 days after infusion.

In a retrospective study of 15 patients receiving MSC transfusion, all patients showed clinical improvement, and 64% of patients had radiologic improvement demonstrated by chest CT scans.[100] Three patients had adverse events involving liver dysfunction, heart failure, and allergic rash. There were no statistically significant changes in inflammatory markers after treatment compared to before treatment.

In a prospective, non-blinded, non-randomized trial at a single hospital center, 24 patients with COVID-19 received bone marrow-derived MSC exosome infusion.[101] Patients were initiated on hydroxychloroquine and azithromycin prior to receiving infusion. Results from this study showed reversal of hypoxia and significant reductions in CRP, ferritin and D-dimer after single-dose infusion treatment.

Overall there is limited evidence to support the use of MSC therapy for treating patients with COVID-19. Studies are small, lack randomization and blinding, thus there is a need for large, randomized clinical trials to establish the efficacy of MSC for COVID-19. On April 5, 2020, the FDA approved MSC therapy for use in severe COVID-19 under the expanded access compassionate use pathway. There are currently 21 clinical trials investigating MSC therapies for COVID-19 (Table 1).

Vaccines

The most effective and long-term solution to SARS-CoV-2 would be the development of a targeted vaccine against COVID-19. An effective and safe vaccine, through the traditional vaccine development pathway, can take up to ten years to manufacture. In the case of the COVID-19 pandemic, an expedited process has been enacted through and coordinated by the Coalition for Epidemic Preparedness Innovations, with the hopes of a SARS-CoV-2 vaccine being available in early 2021.[102] As of June 30, 2020, there are 14 potential SARS-CoV-2 vaccines in clinical trials per ClinicalTrials.gov.[103]

Limitations

This literature review has several limitations worth mentioning. In a rush to find a cure for SARS-CoV-2, the speed and sheer volume of which research and clinical trials are being conducted, makes this area of science constantly evolving. Our current review includes clinical research and trials up until June 2020, after which significant, and new clinical recommendations and research findings have undoubtedly changed. Second, the current review predominantly covers the treatment of SARS-CoV-2 in adult populations. Thus, treatment recommendations specific to pediatric populations were not included in this review. Third, heterogeneity in patient demographics and drug dosage and duration are limitations in reviewing the adverse effects for potential

COVID-19 therapeutics. Additionally, there is a large number of large-scale, RCTs underway (Table 1). The current review includes many findings from case reports and/or case series, so it will be critical to compare the current evidence and recommendations from this review to more robust data once it becomes available. For example, the cytokine storm hypothesis has recently been challenged since this manuscript has been researched.

Conclusion

This review examines the different therapeutic treatments being utilized and studied as of June 2020 for treating patients with COVID-19, and it highlights the different mechanisms of actions of each class of therapy while evaluating the current evidence. As more clinical trials continue to emerge, more insight is being gained into potential effective therapies. In the beginning of 2021 there are now more FDA approved treatments and two vaccines now available under emergency use authorization in the US. Although astounding progress has been made with international contributions in record time, continued efforts to develop therapeutics and preventative therapies are needed in the hopes of curbing the pandemic.

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Reviews

The Diagnostic Challenges and Developments of COVID-19

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Abstract

The 2019 coronavirus disease (COVID-19) pandemic has infected over 26.6 million people as of February 3, 2021 and has spread to every country with unprecedented speed and efficacy. COVID-19 has presented a diagnostic challenge to the medical community due to its highly variable presentation between individuals and population cohorts. Diagnostic methods and capabilities play a large role in identifying affected individuals, mitigating viral spread, and influencing public policies throughout the course of the pandemic. In this review, we provide an overview of the varying clinical manifestations, diagnostic nuances for specific populations, testing indications, and a review of current and future diagnostic testing methods.

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Introduction

The severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) pandemic was first documented in December 2019 in Wuhan, China. Subsequently, SARS-CoV-2 has spread to every country with unprecedented speed and devastating consequences. The 2019 coronavirus disease, also known as COVID-19, caused by SARS-CoV-2 has presented a diagnostic puzzle to medical communities, since documented presentations have varied greatly between individuals and distinct patient populations. Disease manifestations range from asymptomatic to acute hypoxic respiratory failure refractory to medical therapy. SARS-CoV-2 spreads rapidly through populations, and patients with severe disease have overwhelmed intensive care units across the globe. In an effort to slow the transmission of SARS-CoV-2, many nations including the United States have implemented policies restricting public interaction and travel. Accurate diagnostic information is essential to informing the implementation and gradual repeal of these strategies and restrictions. Diagnosing COVID-19 solely based on signs and symptoms is ineffective due to high symptom variability. This demand for sophisticated and reliable diagnostic testing has catalyzed the creation and invention of novel testing modalities with varying utility and accuracy. In this article, we review the available diagnostic tests for COVID-19, from detailed symptomatic history-taking to facility-directed antibody testing, and associated limitations in the setting of such a widespread and rapidly developing pandemic.

Clinical Diagnosis

The extreme range of COVID-19 manifestations continues to challenge diagnosticians and public health officials alike. According to CDC guidance, symptoms supportive of a COVID-19 diagnosis are cough and shortness of breath or two of the following:

fever, chills, shaking, myalgia, headache, sore throat, or new onset anosmia and/or ageusia.[1] The most common symptoms of mild to moderate COVID-19 are fever, fatigue, and dry cough.[2] Additionally, up to one-third of individuals infected with SARS-CoV-2 may experience anosmia, dysgeusia or both and do not correlate with clinical course.[3,4] However, the fact that anosmia and dysgeusia are normally common in the population, especially following upper respiratory tract infection, further complicates this diagnostic factor for mild disease.[5]

In its most severe form, COVID-19 is complicated by acute respiratory distress syndrome (ARDS), acute cardiac injury, acute kidney injury (AKI), septic shock, thrombotic stroke, and limb ischemia. In one of the first descriptive studies of COVID-19 patients, 29% developed ARDS, 12% experienced acute cardiac injury, 7% had AKI, 10% had secondary infection and 7% developed septic shock.[6] Severe presentation of COVID-19 typically involves dyspnea, respiratory distress and hypoxemia requiring ventilatory support. Individuals at increased risk of severe presentation are those older than 65 years and those with concurrent obesity, hypertension, chronic obstructive pulmonary disease (COPD), and diabetes.

Although not included in the CDC case definition, gastrointestinal symptoms have been reported in up to a quarter of patients and include abdominal pain, nausea, vomiting, diarrhea, and loss of appetite.[7] Gastrointestinal symptoms have often been reported to be the first manifestation of COVID-19, especially in the immunocompromised, and should increase suspicion of infection.[8,9] Such atypical presentations may dissuade patients from seeking out testing and may preclude their qualification for testing due to limited supplies.

Asymptomatic individuals, thought to make up close to half of the population infected with SARS-CoV-2[10], pose a major challenge to diagnosing COVID-19 based on clinical presentation alone. In a survey of Icelandic populations, symptomatic presentation alone was not found to be a reliable predictor of COVID-19, as 43% of those who tested positive were asymptomatic and 29% of those who tested negative had symptoms consistent with mild COVID-19.[11] The prevalence of asymptomatic and pre-symptomatic COVID-19 infection has been shown to be even higher in limited studies of populations that have undergone universal screening; at a homeless shelter, 87.8% who tested positive were asymptomatic[12] and of pregnant women who tested positive at a single hospital 87.9% were asymptomatic.[13]

The rate of symptom development in pre-symptomatic individuals is unknown because most large cohort studies do not follow participants over time. However, multiple investigations have concluded that transmission by pre-symptomatic individuals is significant, thus self-isolation after travel or suspected contact with SARS-CoV-2 is an important factor in limiting disease spread.[14,15] Asymptomatic and pre-symptomatic phases of infection make diagnosis based on exposure history a challenge, mostly due to unknown exposures to asymptomatic and pre-symptomatic patients.

Population-Specific Signs and Symptoms

In addition to variability in disease manifestation among the general population, ranging from asymptomatic to ARDS, COVID-19 presentation also has been shown to vary greatly between distinct population groups. In pediatric patients, symptoms are similar but milder than in adult patients, notably presenting with a lower prevalence of fever and cough.[16] Coinfection with other respiratory viruses is also more common in pediatric patients. Upon imaging, in addition to ground glass opacities as seen in adult patients, pediatrics patients also can present with a characteristic "consolidation with surrounding halo sign.[17] Pediatric patients also uniquely present with elevated procalcitonin levels (80%), unlike adult patients.[18]

Although other previous epidemics of viral infections have typically resulted in poor obstetrical outcomes, COVID-19 infection has not been shown to increase risk of maternal death.[19] There are small number of case series that have found evidence of vertical transmission of SARS-CoV-2 in third trimester infection by testing the fetal side of the placenta and testing neonates after birth.[20] However, no vertical transmission to date has resulted in serious disease progression for neonates.

In elderly patients, the most common symptoms following disease onset are fever (87.5%), cough (77.1%), and dyspnea (74.0%), which is not dissimilar from the general adult population.[21] However, atypical presentation of COVID-19 is

more common in older adults. Symptoms may be nonspecific such as falls, delirium, and functional decline.[22] Symptoms of chronic conditions can mask acute illness and alter the ability to perceive or report symptoms.[23] This may result in signs such as fever being diminished or absent in elderly populations.[24] In this population, emerging recommendations are emphasizing the consideration of COVID-19 in older adults with any significant change from baseline with a low threshold for testing.[25,26]

There is limited data on the prevalence and outcomes of COVID-19 in immunosuppressed patients and most data consists of case reports. Due to risk of severe disease manifestations in immunosuppressed patients, providers should have a low threshold to test for SARS-CoV-2 regardless of symptomology or risk of exposure to SARS-CoV-2 in immunocompromised patients.[27]

Indications for Testing

Multiple groups including the Infectious Disease Society of America, the Department of Defense, and the CDC have released evidence-based indications for testing. These guidelines have evolved and will continue to evolve during the pandemic. As our understanding of SARS-CoV-2 and COVID-19 increases, the diagnostic technologies available to hospitals and health agencies have also rapidly adapted. These diagnostic testing guidelines will change in order to remain up to date with current best practices.

The Infectious Diseases Society of America (IDSA) strongly recommends testing of individuals in the community with one or more symptoms even if suspicion of COVID-19 is low. Similarly, the CDC recommends that people with symptoms specific for COVID-19 are eligible for testing, and those with mild infection should self-isolate at home.[28] SARS-CoV-2 nucleic acid amplification testing of asymptomatic individuals in the hospital and community is recommended for people with a high likelihood of exposure or in an area of high COVID-19 prevalence. Testing is not recommended for asymptomatic individuals with low likelihood of exposure. It is also recommended that people entering places of close contact will require testing, including skilled nursing facilities (SNFs), group homes, and detention centers. It is also recommended that individuals with a high likelihood of exposure who might spread disease, including doctors performing aerosolizing procedures, should be tested.[29] Similarly, the IDSA advises that asymptomatic patients undergoing surgery procedures also be tested for SARS-CoV-2 infection unless PPE is available, in which case testing is unnecessary. However, testing is unnecessary for patients undergoing aerosol generating procedures, such as intubation or bronchoscopy, when appropriate PPE is available.

Diagnosis by Laboratory Tests

Nucleic acid amplification testing (NAAT): NAAT is currently the most utilized form of COVID-19 detection in the United States. It detects and amplifies specific viral antigens and therefore requires the virus to be present for a positive result. The most widely used PCR test in the United States was created by the CDC. It contains two primer-probe sets that target viral nucleocapsid genes N1 and N2. The kit also contains a probe for the human RNase P gene to act as a positive control indicating successful extraction.[30] Based on reports, this test is both highly sensitive and specific with little cross reactivity for other pathogenic viruses, although it is challenging to reliably assess accuracy in the absence of a control.

Reverse transcription polymerase chain reaction (RT-PCR) testing can be used to quantify viral levels. Multiple studies have endeavored to correlate viral load with symptoms or disease severity, but the results of these studies have been variable. While some studies found a direct correlation between disease severity and viral load[31], other publications have drawn the conclusion that viral load is most predictive of timing and disease progression.[32] This variability is also likely due to the overall variable disease prevalence in given populations, in turn altering the negative and positive predictive values of the tests even before they are used for patient diagnostics.

Preferred testing platforms, methods and modalities are likely to change and for this reason we have not included a table of FDA approved COVID-19 tests. A full list of FDA approved tests can be found on the FDA website as well as a list of COVID-19 tests for which the FDA has withdrawn authorization.[33]

Method of Collection

Nasopharyngeal or mid-turbinate nasal swabs are preferred over oropharyngeal or saliva swabs based on analysis of accuracy and safety. Mid-turbinate swab had the highest specificity (100%) and sensitivity (100%) of the collection modalities.[34] The data used to inform this recommendation showed that nasopharyngeal sampling had the highest specificity (99.3% (95% CI: 0.986-1.001) and sensitivity (96.4% (95% CI: 0.925-1.005)) when results were pooled across studies. Oral sampling showed the lowest specificity (56.2% (95% CI: 0.351-0.772). When collecting a specimen, it is important to use a flocked swab with an aluminum or plastic shaft as cotton swabs or wood shafts may contain substances that inhibit PCR testing.[35]

Collection of a lower respiratory sample is only indicated if an upper respiratory sample is negative but suspicion for severe SARS-CoV-2 infection is high. This is based on evidence that lower respiratory tract samples are more specific than upper respiratory tract samples but are more difficult to obtain and increase risk of aerosolization and droplet production.[36] SARS-CoV-2 is also found in feces of infected patients. Urine and stool detection methods are under investigation and may be indicated in certain future circumstances, such as testing wastewater for presence of SARS-CoV-2 in specific populations.[37] The virus appears to be shed for longer in the nasopharynx and feces than other sites, and some evidence suggests that viable virus may be shed for up to one month after symptom onset. This may have implications for both testing and isolation, as patients with previous infection concerning for continued transmission could be tested at these alternate sites.[38]

Antibody Tests

The current role for antibody testing is controversial. The most recent guidelines released by the IDSA recommends utilizing the serum IgG antibody test for symptomatic patients when there is high clinical suspicion for COVID-19 and two or more negative nasopharyngeal PCR tests. It is recommended that the IgG test not be used within 2 weeks of symptom onset due to decreased accuracy; pooled data on IgG testing demonstrated 23% sensitivity before 2 weeks compared to 68% sensitivity and 99% specificity at 2 weeks.[39] This is unsurprising given the time required to produce IgG in response to a novel infection. IgM testing may detect immune response to SARS-CoV-2 infection earlier than IgG, however, there are currently no guidelines on the use of IgM testing. The IDSA supports the use of IgG or total antibody testing to detect evidence of past infection, however, detecting past infection is of little clinical use given the uncertain risk of reinfection and longevity of anti-SARS-CoV-2 antibody response. Detection of past infection via antibody testing is most useful in the research and epidemiology setting. Some investigators and laboratories suggest using antibody testing for past infection, but the durability of antibody response remains in question. The CDC is still collecting data to assess the parameters of antibody tests.[40] The FDA has released data on the accuracy of the 12 antibody tests approved under emergency use authorization (EUA) status.[41] These 12 assays were also independently tested, and findings have been published as a pre-print.[42]

It has yet to be determined whether the presence of anti-SARS-CoV-2 antibody is protective against reinfection, or whether protective effects are sustained over a significant time period. Currently, antibody tests are being used for research and to test for recent previous infection. The optimal time frame for widespread antibody testing is at least three to four weeks after symptom onset and at least two weeks after symptom resolution.[43] Antibody testing could be used to identify previous exposures to create contact tracing maps and determine points of transmission. This information could be used to determine locations and activities where transmission is highest, allowing for more focused public policy on quarantine and testing.

Point of care tests (POCT)

The FDA has approved multiple low complexity, rapid point of care tests for clinical use, including Abbott ID NOW (Abbott Laboratories), BioFire FilmArray (bioMerieux), cobas Liat (Roche Diagnostics) and GeneXpert (Cepheid).[29] POC serologic testing technologies include single-use, lateral flow tests where the presence of antibody is demonstrated on a color changing paper strip. Although simple to use and theoretically promising for widespread testing capacity, meta-analyses of POCT performance have shown these commercially available testing kits to be scarcely reliable in real-world settings, reporting a pooled sensitivity of only 64.8%.[44] Although the further development of POCT has the potential to improve current COVID-19 testing capacity,

the World Health Organization (WHO) has stated that the use of these tests should be limited only to research settings, and that their use in clinical decision-making should be avoided.[45] Indeed, after the FDA granted emergency use authorization for the Abbott ID NOW POCT platform, there were 15 adverse event reports about the device that suggest users are receiving inaccurate negative results.[46]

Other Forms of Testing

The development of inexpensive, rapid, and reliable tests for SARS-CoV-2 will have an increasingly important role in informing ongoing pandemic control measures, including contact tracing, quarantine, and social isolation. Alternative forms of testing are under development with hopes of outcompeting PCR for efficiency and cost. Mammoth Biosciences and Sherlock Biosciences, both co-founded by the inventors of CRISPR, are developing novel tests based on this technology.[47] This test takes 30 minutes, had a positive predictive value of 95%, and a negative predictive value of 91.7% in a sample of 36 COVID-19 patients and 42 patients with other viral respiratory infections.[48]

Other creative modes of testing are being developed to compensate for the lag in available biologic tests. A recently developed app (AI4COVID-19) uses automated intelligence to distinguish the sound of a cough associated with SARS-CoV-2 infection from other causes of cough. The creators posit that the AI engine can distinguish COVID-19 patient cough from other coughs with 90% accuracy. The authors also note that this is not meant to be a definitive diagnostic test but could be used to influence pretest probability and direct testing.[49]

Radiographic Testing

Radiologic imaging, such as chest x-ray and computed tomography (CT) of the chest, is not recommended for diagnosing COVID-19.[50] Imaging is currently only recommended when complications arise from infection. Multiple trials have revealed imaging patterns in patients with COVID-19.[51] These patterns can be used to increase pretest probability in persons-under-investigation and may be used to evaluate disease severity in patients with confirmed COVID-19 infection. CT of the chest is more sensitive than chest x-ray; a retrospective review of 51 COVID-19 patients that compared RT-PCR testing vs CT found that CT imaging was 98% sensitive for COVID-19 compared to 69% for chest x-ray.[52,53] Despite this, chest CT is still not recommended in the initial diagnosis of SARS-CoV-2 infection.

The most common finding on chest CT is ground glass opacities with or without consolidation in a peripheral and basilar-predominant distribution (56.4%).[54] Patients with more severe forms of the disease experience an evolution of imaging findings, with more extensive opacification of the lung parenchyma. Interlobular septal thickening, air bronchograms, and pleural effusions are also more prevalent.[47] Artificial intelligence is being employed for the detection of COVID-19 pneumonia via chest x-ray. Qure.ai has repurposed its x-ray interpretation program, qXR, to identify COVID-19 via chest radiograph with a self-reported sensitivity of 91.2% and specificity of 77.5%.[55] As SARS-CoV-2 continues to spread through the general population, software like qXR may enable physicians to identify COVID-19 patients presenting with non-specific signs of pneumonia with even greater sensitivity and specificity.

Outlook

Indications for testing are likely to change as evidence on the transmission and common symptoms of COVID-19 are gathered. It is reasonable to expect that larger trials will be conducted to assess test accuracy, especially those most used such as PCR. These trials will likely yield insight into the flaws of current tests and will prompt the creation of more accurate tests, for example RNA primers with higher reliability in those PCR tests. This may have significant implications for past studies; based on inaccuracy, margins for error would be increased. It is likely that the false negative rate is higher than estimated, especially in the community where there are many factors that increase the likelihood for a negative test such as dry mucous membranes, low viral loads and poor collection technique.

It is important to interpret test results in the appropriate context; there is no ideal control against which COVID-19 tests can be validated, therefore absolute accuracy cannot be assessed. Many questions remain regarding the pathogenesis of COVID-19 and there appears to be great variability between individuals, manifested in the broad range of symptoms that people may experience. Together, these points of uncertainty

make it essential that test results be interpreted critically in clinical and research settings. For example, there have been many reports of COVID-19 like illness with negative repeat testing and reports of asymptomatic individuals who test positive for SARS-CoV-2.[11] Initially these reports are paradoxical but could be explained by differences in testing. Both reports used PCR testing, however, different primers were used, and collection methods were slightly different. Variations in collection site and test threshold could result in 'false' negatives in the case of negative a test in a symptomatic individual or low threshold positive result in asymptomatic individuals. Even individual variations such as amount of mucous secretion during testing could influence results.

In addition to the development of reliable PCR primers, creation of reliable antibody tests may prove to be important in the monitoring of long-term immunity and tracking of vaccine efficacy over time. The duration and efficacy of acquired immunity will determine the susceptibility of individuals, the need for vaccination and the reliability of immunity in multiple populations. Variation in acquired immunity between individuals could also provide information about the pathogenesis if SARS-CoV-2; for example, it is possible that those with a mild, short course do not develop lasting immunity while those with a severe or prolonged course do. This information could be used to direct vaccination and determine the reliability and longevity of immunity.

There have been many issues with the creation of POC testing. Many of these tests are expedited through approval because of demand and have been subsequently recalled. COVID-19 test results must be judged critically; if there is a high suspicion for SARS-CoV-2 infection but tests are negative, appropriate precautions should be taken whether this be time away from the hospital for healthcare workers or close follow-up for patients with recent exposure or symptoms.

Conclusions

Diagnostics based solely on symptomatic presentation are unreliable for COVID-19 due to high variability in disease presentation among individuals and between population cohorts. However, symptoms such as fever, dry cough, shortness of breath, headache, or myalgia should increase pretest probability for COVID-19. If testing is not available, individuals with symptoms should self-isolate at home. History of exposure or impending transfer to a facility particularly susceptible to COVID-19 should prompt testing even in the absence of symptoms. Once it is deemed necessary for a patient to be tested and evaluated for COVID-19, a number of diagnostic modalities may be utilized with varying degrees of efficacy. The most reliable method of testing is currently RT-PCR, and most hospitals or outpatient facilities are now capable of testing for SARS-CoV-2 infection using such methods. Reliable antibody tests have been created, although the utility of these tests remains to be determined because the duration of post-infection antibody response and immunity status are unknown at this time. Although chest imaging is not indicated for diagnostic testing, chest x-ray or CT can be useful in the evaluation of disease severity. The most common findings on chest CT in those admitted for COVID-19 were bilateral infiltrates and pleural effusion. Suspicion for severe COVID-19 should be high in those who present with respiratory distress or hypoxemia and those over 65 years old or with multiple comorbidities including obesity, COPD, hypertension, cardiovascular disease and diabetes. Unfortunately, respiratory distress is a common reason for admission and has many etiologies, and comorbidities are ubiquitous in the population. Nevertheless, appropriate precautions should be taken to prevent viral transmission from persons-under-investigation and those who test positive for SARS-CoV-2 and possess risk factors for severe disease should be monitored as an inpatient or outpatient. National measures to prevent SARS-CoV-2 transmission have effectively decreased the rate of transmission and consequently prolonged the course of the COVID-19 pandemic; this statement is based on the concept of herd immunity which posits that a disease will cease to spread after a specific proportion of the population has been exposed and gain immunity. The time that it will take to reach that proportion will be increased if transmission is delayed. As a result, continued testing and surveillance is required, especially as communities transition back to pre-pandemic function and social distancing laxity. The role of targeted testing, identification, and treatment of those at the highest risk of severe disease will be essential to mitigating injury and mortality caused by COVID-19.

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