# MEDICC Review

July–October 2022
Vol 24, No 3–4

## On COVID-19





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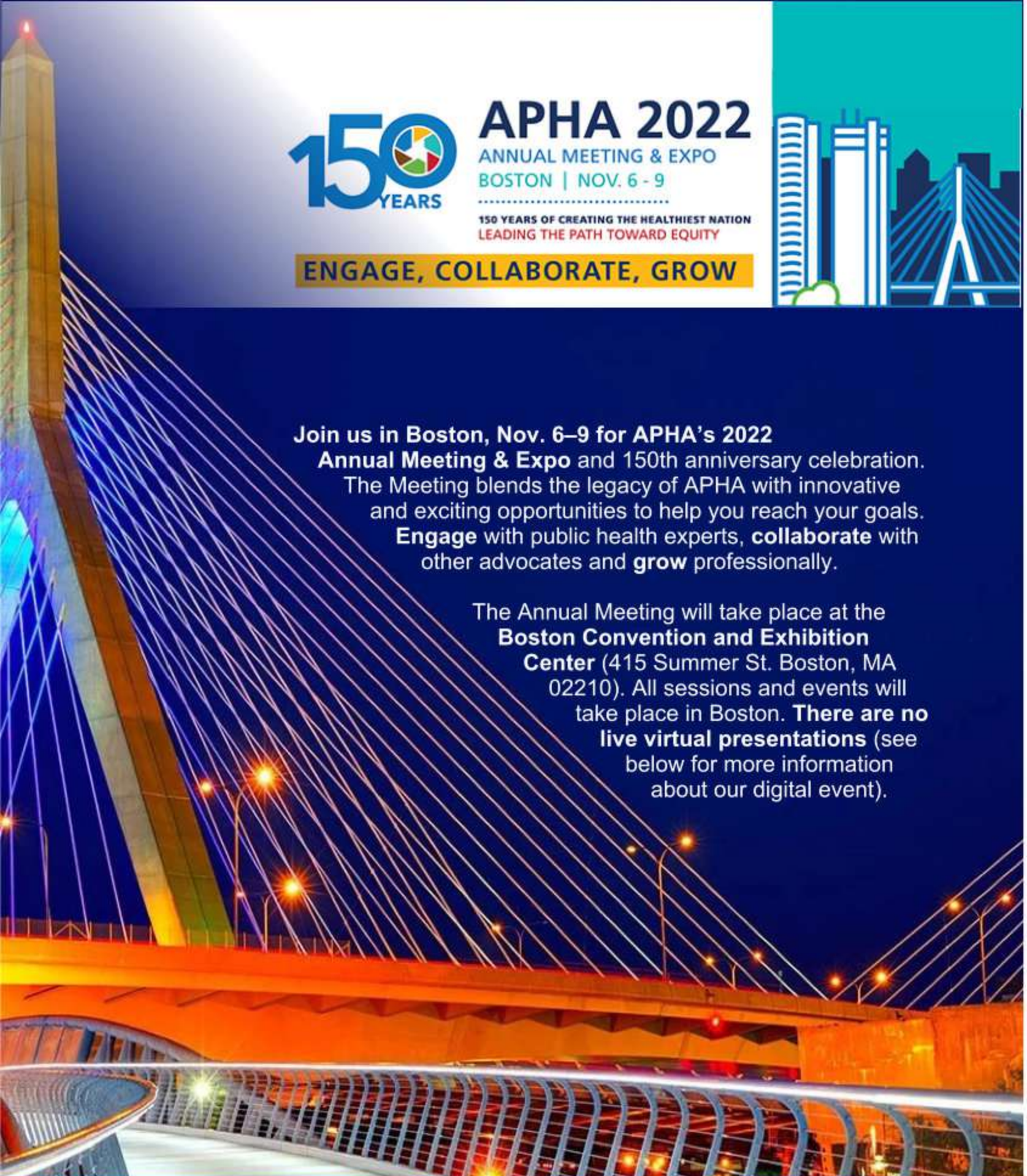

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# MEDICC Review

July–October 2022, Vol 24, No 3–4






MEDICC Medical Education Cooperation with Cuba

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MEDICC Review

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MEDICC Review

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# Global COVID-19 Scorecard: Science, 1—Science Diplomacy and Equity, 0

Wherever you may be reading this: thank science. As you dress your children for school, commute, commune, worship or workout: thank science. As you plan a wedding, year-end celebrations, a trip, surgery, or dental cleaning: thank science. Our very survival is thanks to collaborative research and science that delivered safe, effective COVID-19 vaccines in record time.

So why is our collective pandemic response categorized as a "massive global failure"[1] that "puts the whole world at risk?"[2] The current COVID-19 scenario—despite vaccines—is a dangerous panoply of failed systems, inequities and inadequate social protections:

- **Millions dead:** Over 7 million reported COVID-19-related fatalities and an estimated 17.5 million excess deaths;[1]
- **Millions orphaned:** An estimated 10.5 million children orphaned due to COVID-19-related deaths;[3] in the United States, Black and Hispanic children lose a parent or caregiver at twice the rate of White children;[4]
- **Millions with long COVID:** An estimated one in eight people develop long COVID symptoms;[5]
- **Hundreds of COVID-19 variants:** Some 300 omicron sublineages now circulate,[6] alongside rising incidence of preventable diseases including cholera, malaria, and influenza, plus outbreaks of zoonotic diseases like monkey pox;
- **Millions pushed into extreme poverty:** An estimated 263 million more people pushed into extreme poverty due to COVID-19, rising global inequality, and inflation affecting prices of food and other basic necessities;[7]
- **Millions without vaccines:** While 75% of people in high-income countries are fully vaccinated, in low-income countries, the rate drops to a devastating 19%, with just 23% receiving even a single dose.[8]

These statistics reveal the backstory that led us to this syndemic crossroads: protectionist policies exposed gross inequities to life-saving commodities across and within countries; unprepared, inequitable and fractured health systems buckled under pandemic surges; the politicization of science paralyzed safe and effective health interventions, contributing to the infodemic and the preventable death toll; and the neocolonial approach to the Global South multiplied the effects of systemic violence heaped upon lesser-developed nations by the Global North, eroding the foundation for effective collaboration in the process. In short: a failure of equity-driven science diplomacy.

Most importantly, the disassociation of human health from planetary health, coupled with the denial of health care as a human right, continues to imperil everyone regardless of race, place or station.

The deadline for redacting this narrative is upon us—governments, policymakers, industry leaders and funders must harness the political will to prioritize an agenda of multilateral cooperation focusing on global health security, knowledge transfer, and full access to primary health care (PHC). Equity, transparency, clear public health messaging and an all-society, holistic approach—what the *Lancet* Commission terms "prosociality"[9]—must underpin actions designed to enhance human and planetary heath. Without exception.

Claims that we've turned the corner on COVID-19—President Biden declared "the pandemic is over" and WHO stated the end of the pandemic is "in sight"—when more than one million people have died of the virus between January 2022 and this writing, are another failure of science diplomacy.[10,11] They erode trust in health authorities,[12] threaten current and future health interventions, fuel the North-South disconnect and further disenfranchise the most vulnerable, millions of whom still wait for life-saving vaccines.

**The disassociation of human health from planetary health, coupled with the denial of health care as a human right, continues to imperil everyone regardless of race, place or station.**

Science makes clear the road map forward, but it still depends on political will. To build real global health security while addressing long-term COVID-19 ramifications—including clinical sequelae and adverse effects on mental health—requires rapidly scaled-up funding to support resilient, responsive health systems and action towards universal access. Needed are in-country capacity building; knowledge transfer around surveillance, contact tracing and follow-up during disease outbreaks; and shared examples of preventive, rather than reactive, health strategies.

Progress is too slow, but there are some indications that the pandemic's darkness may lead to a new dawn: WHO, *The Lancet* and others argue for a new global health paradigm founded on, among other things, accessible, community-based primary care.[1,9,13] Even some in the United States are awakening to this imperative: the *Paul Farmer Memorial Resolution*, introduced in the US House of Representatives, is designed to curb preventable deaths by strengthening health systems; aligning funding with "local plans and priorities, not the development industry;" increasing US global health spending; and forging a more just and democratic global economy, including the power for poorer countries to decide for themselves how to allocate health funding from the World Bank and IMF.[14] Actions proposed in this resolution, known as 'North Star,' offer pragmatic guidance for coordinated initiatives to improve human and planetary health.

Sweeping corrections to international financial mechanisms beyond health are also imperative for confronting future global emergencies. The pandemic has left economies in near collapse and "stopped sustainable development in its tracks."[15] Urgently required are aggressive investments in health systems imbued with an equity and gender focus, sustained support for comprehensive social protection programs, and private-public cooperation pegged to the interests of whole populations, not just individuals.

The litany of global threats now upon us—emerging, re-emerging and treatment-resistant diseases, the climate catastrophe and

zoonotic spillover, not to mention worsening and entrenched inequities—argue for more science-based collaboration and more, much more, North-South funding. Barriers to collaboration including sanctions and economic protectionism are not only unjust and prejudicial to everyone's health: they are fossils from a bygone era, a bygone world.

In contrast, a new global health paradigm calls for multilateral science solidarity emphasizing in-country development and production to empower lower- and middle-income countries (LMICs). This would liberate LMICs from exploitative international policies that prioritize the interests of foreign governments and industry over their own needs and criteria. Promoting LMIC self-reliance also requires technology transfer, regulatory expertise including harmonizing standards across regions, and overhauled intellectual property and patent statutes that promote, rather than obstruct, health and well-being.

**A new global health paradigm calls for multilateral science solidarity emphasizing in-country development and production to empower LMICs.**

Failure to promote such LMIC scientific independence carries dire consequences, warns former PAHO director Dr Carissa Etienne: "our health and economies are dependent on the production, availability and equitable access to pharmaceutical products, vaccines, medical supplies and diagnostics…and Latin America and the Caribbean has been found wanting."[16] Dr Etienne points out that there are exceptions; Cuba is one of them, as a delegation of international experts discovered on a recent visit to better understand the country's COVID-19 vaccine development and vaccination strategy.

Following three days' discussions and site visits with Cuban researchers, developers and regulators responsible for producing several safe, efficacious vaccines (including one with potential as a universal booster), the delegation issued its report recommending above all that "multilateral and bilateral mechanisms for health promotion and pandemic prevention should actively engage Cuban scientists in dialogue, academic exchange and joint research." During their visit, the group of US, Caribbean and African scientists interacted with investigators from Cuba's decades-old biotech industry that produces novel biologics and 8 of the 11 vaccines included in the country's childhood immunization program. They also heard from Cuban public health experts on the national vaccination strategy that relied on the strengths of the island's primary healthcare facilities and professionals to vaccinate 90% of the population by mid-2022. This rated included 97.5% of children over the age of 2, making Cuba the only country to achieve such high vaccination rates in children this young so early in the pandemic. Such high vaccination compliance and the potential for pediatric immunization to blunt infection rates in the general population were of particular interest to the delegation, which recorded its findings and recommendations in the full Technical Report and its Executive Summary we publish in this issue.

A community-based universal model, like the one Cuba introduced almost 40 years ago, has proven advantageous in disease detection and control, strengthening public trust in and compliance with health measures, and improving overall population health. This issue's interview with Dr Ileana Morales, Director of Science & Technological Innovation in Cuba's Ministry of Public Health, explores how the country harnessed science and the strengths of its health system to confront the pandemic even in perilous economic times.

Training health workers from the community, for the community, has shown to be especially effective in LMICs, particularly when coupled with strategies recognizing health care as a human right.[9,17] The late Dr Paul Farmer, our second interview in this issue, was dedicated to such a 'pro-poor,' rights-based strategy. From rural Haiti to Rwanda and even the United States, the physicians and health workers he trained, the communities he supported and the patients he served are his legacy. Codifying this legacy is up to us.

In sobering and sad news, ***MEDICC Review*** offers its condolences to family, friends and colleagues of Dr F. Douglas Scutchfield, who died in May. A physician and champion of preventive and community-based medicine, 'Scutch' was founding director of San Diego State University's Graduate School of Public Health and founding dean of the University of Kentucky School of Public Health. In addition to many awards and scholarly publications, he was founding co-editor of the *Journal of Appalachian Health* and served on the Editorial Board of ***MEDICC Review*** since its inception as a peer-reviewed journal. He will be sorely missed by us all.

**The Editors**

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## In Memoriam
## Carmen Landau MD
## (1977-2022)


*Dr Carmen Landau (fourth from right, in back) celebrates graduation day at the Latin American School of Medicine, Havana, 2007.*

As this issue of ***MEDICC Review*** was finalized for publication, we received tragic news about the sudden death of family physician Dr Carmen Landau, among the first US graduates from Havana's Latin American School of Medicine (ELAM), class of 2007. Following graduation, Dr Landau completed her residency at the University of New Mexico's Department of Family and Community Medicine and became a staff physician at Southwestern Women's Options in Albuquerque, New Mexico. Her community of local and migrant women relied on Dr Landau not only to provide quality care, but also to defend their right to safe abortions, without stigma.

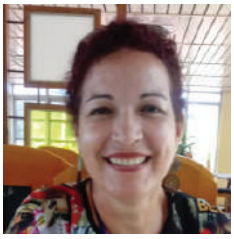
In a prescient 2015 article for our journal (**Under the Cover of Night: Abortion Across Borders**), she decried laws that obligate women to follow often dangerous routes to fulfill that right: "Restrictive abortion laws in many US states and countries force women into these 'ranks of the desperate,' endanger their lives and violate their rights. Why must they pay such a price?"

Dr Landau's solidarity with and commitment to the most vulnerable went beyond her service to low-income and disadvantaged women in New Mexico: she died in Puerto Rico on October 16, 2022 while helping victims of Hurricane Fiona.

Dr Landau was a force for change who embodied the right to compassionate, science-based health care upon which ELAM was founded. The editors of ***MEDICC Review*** offer our deepest condolences to Dr Landau's family, friends, colleagues and community of patients. She is survived by her husband and two children.

https://doi.org/10.37757/MR2022.V24.N3-4.12

**Alina Artiles-Santana MD**
Family physician and assistant professor, Prenatal Diagnostic Ultrasound Department, Chiqui Gómez Lubián Teaching Polyclinic, Santa Clara, Cuba. With more than three decades in public health services, Dr Artiles has taught residents in family medicine in courses on geriatrics, maternal and child health, and ultrasound diagnostics in primary health care.



**Anabel Fernández-Abreu MS PhD**
Chemical engineer with a master's degree in bacteriology and a doctorate in health sciences. Dr Fernández is associate researcher and associate professor at the National Reference Laboratory for Acute Diarrheal Diseases, Pedro Kourí Tropical Medicine Institute (IPK), Havana, Cuba. Her research focuses on surveillance of pathogens causing diarrheal diseases.

**Jorge Amado Grau-Abalo PhD**
Clinical psychologist with a doctorate in the psychological sciences. Dr Grau is a full professor and senior researcher at the Medical University of Havana, Cuba. He also chairs the National Psychology Group at the Cuban Ministry of Public Health. Dr Grau is a founder and vice-president of the Latin American Psychology Association (ALAPSA). His work has contributed to guidelines for providing mental health support during the COVID-19 pandemic.

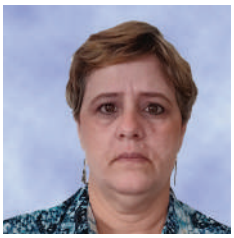
**Julio César Hernández-Perera MD PhD**
Internist with a doctorate in medical sciences. Dr Hernández is a senior researcher at the Medical-Surgical Research Center (CIMEQ) in Havana, Cuba, and is a full professor at the Medical University of Havana. He is a member of the hepatology and hepatic transplant group at CIMEQ.

**Olga Esther Infante-Pedreira MS**
Psychologist with a master's degree in health psychology. Ms Infante is an associate professor and associate researcher at the Medical University of Havana, Cuba, and heads the Psychology Department at the Manuel Fajardo Faculty of Medicine. She is also Secretary of the National Psychology Group at the Cuban Ministry of Public Health and President of the Latin American Psychology Association (ALAPSA).

**Yuliannis Lugones-Ladrón de Guevara MS**
Pharmacist with a master's degree in pharmacology. Ms Lugones is an assistant researcher at the National Center for Animal and Plant Health (CENSA) in San José de las Lajas, Cuba. She is currently pursuing a doctoral degree in cellular and molecular biology at the Universidad de La Frontera in Chile.

**Calixto Machado-Curbelo MD PhD DSc FAAN**
Neurologist and neurophysiologist with a doctorate in medical sciences and an advanced doctorate in sciences. Dr Machado is a senior professor and researcher at the Neurology and Neurosurgery Institute in Havana, Cuba, where he chairs the Clinical Neurophysiology Department and heads the Neurophysiological Laboratory for Cerebrovascular Diseases and Intensive Care Unit. He is president of the Cuban Society of Clinical Neurophysiology, chair of the Network on Defining Death of the International Bioethics Association and chair of the Cuban Commission for Brain Death Diagnosis.

**Minerva Montero-Díaz MS PhD**
Mathematician with master's and doctoral degrees in mathematics and statistics. Dr Montero is a senior researcher at the Mathematical Cybernetics and Physics Institute, Havana, Cuba, where her investigations have focused primarily on data analysis and statistical modeling. In the area of health, she has worked with UNICEF-Cuba on designing anthropometric tables for nutritional evaluation of pregnant women. In 2021, she received the Cuban Academy of Sciences Annual Award in the category of biomedical sciences.



**Lissette Pérez-Santos MS PhD**
Microbiologist with a master's degree in virology and a doctorate in health sciences. Dr Pérez is associate professor and senior researcher at the Virology Department, Pedro Kourí Tropical Medicine Institute (IPK), Havana, Cuba. Her research has focused on antiretroviral therapies and drug resistance, as well as herpes viruses as opportunistic infections in AIDS and transplanted patients, among others.

**Felipe Neri Piñol-Jiménez MD MS PhD DSc**
Gastroenterologist with a master's degree in infectious diseases, a doctorate in medical sciences and an advanced doctorate in sciences. Dr Piñol is a full professor and senior researcher at the National Center of Minimally Invasive Surgery (CNCMA) in Havana, Cuba. His research focuses primarily on digestive conditions and biliary acids.

# Peer Reviewers

All original scientific articles appearing in ***MEDICC Review*** are subject to double-blind international peer review. ***MEDICC Review*** is indebted to the following colleagues for their collaboration as peer reviewers in 2022:

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## COVID-19, VIRUSES AND DEMENTIA

**To the Editors:**
COVID-19 pandemic continues testing the stamina of both societies and health care services. Until now, we have focused on the acute effects of SARS-CoV-2 but, as more people convalesce from the infection, our attention is drawn to its more long-term effects. With increasing frequency, we examine outpatients with cognitive dysfunction after COVID-19 infection.

SARS-CoV-2–induced increased inflammatory response, brain vasculature disorders and respiratory difficulties/hypoxia have a negative impact on the cognitive functions of patients and may possibly accelerate cognitive decline, even after many years.[1]

Findings from previous epidemics have shown that respiratory coronaviruses may invade the brain and cerebrospinal fluid, causing various neurologic symptoms, such as cognitive dysfunction, and especially affecting anatomic areas and structures, such as the temporal lobe and hippocampus. Possible hippocampal damage raises the question of whether this could be held responsible for the acceleration of neurodegenerative processes in Alzheimer's dementia or the beginning of disease in previously asymptomatic people.[2]

Many studies have shown that viral infections affect cognitive functions, through direct or indirect mechanisms, contributing especially to manifestations of minor cognitive impairment and dementia. Both the high prevalence and the overlap of these conditions underlie the need to understand better the role viruses, such as SARS-CoV-2, may play in the pathogenesis of dementia.[1]

There is increasing interest concerning the role of infectious agents in the pathogenesis of dementia, but current evidence is restricted because high-quality population studies are lacking. Systemic infections may trigger brain responses through microglial activation and release of pro-inflammatory cytokines. Furthermore, antigenic activation may contribute to age-related remodeling of the immune system and accelerate neurodegeneration in disorders, such as dementia.[2]

The current pandemic could serve as an opportunity to study the effects of viral infections on cognitive functions and dementia. Therapeutic approaches toward this way could prove important, taking into consideration global aging and high prevalence of dementia.

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# Putting Science to Work: Cuba's COVID-19 Pandemic Experience

## Ileana Morales Suárez MD MS

### Director, Science & Technological Innovation, Ministry of Public Health, Cuba

**Gail A. Reed MS**

It was just before New Year's Eve, 2019 when an emerging virus in China caught the attention of Dr Ileana Morales, director of Science and Technological Innovation in Cuba's Ministry of Public Health. She had already participated in implementing Cuban protocols to prevent Ebola and address diseases such as Zika and dengue. But this was different, and COVID-19 would prove the biggest challenge in her decades-long career in public health leadership. As she put it: a "trial by fire." Over two years later, in March 2022, she spoke with ***MEDICC Review*** providing a firsthand account of the Cuban strategies, results and lessons learned in the nation's colossal efforts to stem the tide of a pandemic that still threatens the world.


***MEDICC Review:*** **After that glimpse of the virus detected in China, what were the first steps you and the Ministry took?**

**Ileana Morales:** From that start, we began gathering all the information available internationally, and then as a Ministry and a government, we got to work planning. We knew we needed a coordinated national plan involving everybody, from the community level to the various ministries, which would be used across the country. So, every ministry was asked to revise their protocols for addressing epidemics, including first and foremost, the Ministry of Public Health (MINSAP). By January 30, 2020, the first version of the *National Plan to Address COVID-19* was approved at the highest levels. [The first cases of COVID-19 were diagnosed in Cuba on March 11, 2020.--Eds.][1]

***MEDICC Review:*** **And that plan began to change your life, and that of many others…**

**Ileana Morales:** It certainly did. At that point, the Plan emphasized prevention, surveillance—these were at the heart of our Ministry's efforts. But my life really changed on February 12, when the National Science Group was created to confront the coming epidemic… that was perhaps the first big step, and the Group was co-chaired by myself and Dr Rolando Pérez, who heads research at BioCubaFarma, our biotech conglomerate. But the group had a multisector design from the start, and we began bringing in experts from every field…public health, biotech, social sciences, animal health, you name it. There were about 15 of us at that point, and we began meeting right away, and we've met daily ever since, to this day.

***MEDICC Review:*** **What have been the Science Group's main responsibilities over the course of the pandemic?**

**Ileana Morales:** We had three main tasks, which later evolved of course. At the start, it was to keep abreast of the available information on the disease throughout the world, monitor how it was behaving, what was new. From there, we created a COVID-19 Observatory online where we posted everything available globally. Second, we were tasked with finding out what was available in Cuba itself to address such a virus: epidemiological research, biotech products that might be repurposed, or others that were in development. Third, we delved into health system studies that could help us better organize our response. The whole idea was—and is— to put science to work. Science became the engine for our whole pandemic response.

***MEDICC Review:*** **The Ministry of Public Health has had a fundamental role in the process, and in the creation of the National Protocols as well, correct?**

**Ileana Morales:** Of course. The first national treatment protocol, step-by-step guidance, was created on February 16th. At that point, it was basically a list of what might be used to treat the disease, and a few days later we included the steps to be taken for epidemiological monitoring. All the proposals were put on the table for the Science Group to consider, and on February 26th, the Innovation Committee was formed, also chaired by Dr Pérez and myself. Included in the Innovation Committee were other key actors from research and manufacturing centers, as well as the directors of the national regulatory authority and of the clinical trials coordinating center. These last two became doubly important when we began developing our own vaccines.

Thus, we had the Science Group making recommendations, and the Innovation Committee choosing what aspects, products or strategies to introduce, in coordination with government. Together, final decisions were made. The Innovation Committee also approved all versions of the national protocols applied across the country, which were revised periodically based on our own experi-

ence in Cuba and those of other countries. That is, these were not set in stone, but periodically revised, informed by the results as we continued to study COVID-19.

We began to include other important elements regarding not only prevention but also diagnosis and of course refining the treatments needed according to severity of symptoms, comorbidities, etc. The idea was to get this information to our health professionals as fast as possible, so they would have the latest and best guidance, whether at the community level or a tertiary care facility. The idea is that there isn't a single medical decision taken that isn't supported by a study, a trial or an approved therapy. Today's protocol has over 200 pages!

**The idea is that there isn't a single medical decision taken that isn't supported by a study, a trial or an approved therapy.**

Each new protocol version was presented to the National Temporary Technical Group, chaired by President Miguel Díaz-Canel, for final authorization. And by April 2020, both the Science Group and the Innovation Committee were meeting weekly with the President. The aim was to generate evidence-based decisions throughout the pandemic.

***MEDICC Review:*** **Several Cuban biotech products have been introduced into the treatment protocol over the last two years. How was this done?**

**Ileana Morales:** I remember early in 2020, one of our biotech experts brought to the table the idea of using Cuban interferon to treat some patients, and so right there, we decided to launch a clinical study to determine its potential, a study approved by the clinical trials coordinating center and the regulatory authority. And then there were other products, already in use, but which had to be evaluated specifically for treating COVID-19. This was true, for example with some that had anti-inflammatory potential to curb the cytokine storm. Each one had to be tested, studied and the results assessed amidst the rising tide of the epidemic. There was no time to lose, but we also couldn't afford to make superficial decisions. People's lives were at risk. People were counting on us.

***MEDICC Review:*** **Nevertheless, the country was under other pressures, especially economic ones, with the US embargo still in effect, the collapse of tourism, and the needs of the health system itself. With the decision to re-open airports in November 2020, the number of COVID-19 cases began to surge. In this context, how were the epidemiological priorities considered?**

**Ileana Morales:** Virtually every decision passed through the lens of science. At that point in particular, we were looking at Cuba's rating on the Oxford COVID-19 Government Response Tracker (Oxford index), how we were doing compared to other countries internationally.[2] We had a rating of nearly 100, the highest on the scale in terms of stringency of measures. So, in November and December 2020, we were seeing good epidemic control, and with that in mind, the Science Group experts began to analyze how to open the country. Our epidemiologists and mathematicians recommended opening because of the low incidence of cases we had managed to achieve at that point. But what happened was that in January 2021, the delta variant entered into play, which we hadn't foreseen. Delta was responsible for the great case surge we experienced from June through September in various provinces, such as Havana, Matanzas and Ciego de Ávila. Those months were very hard, but also full of lessons, especially for the clinical protocols and the medicines to be used, who were the most vulnerable patients, how to work with children and with the elderly, and so on.

***MEDICC Review*: Amidst all this, the government decided to develop Cuban vaccines. How did this come about? How did it interface with your own work?**

**Ileana Morales:** In May 2020, President Díaz-Canel met with scientists at the Finlay Vaccine Institute, and they all concluded that developing Cuban vaccine candidates offered the best hope of being able to vaccinate our whole population. And so, the biotech centers, including Finlay and the Genetic Engineering and Biotechnology Center, began their research, based on their decades of experience developing and producing vaccines.

Thus began the challenge of pre-clinical research and later the clinical trials, all of which had to be presented first to the Science Group and thereafter to our national regulatory authority for approval—the design, certification of trial sites, data collection, everything. And finally, the Science Group had to address vaccination rollout: if and when we had efficacious vaccines authorized for emergency use, how were we going to distribute them? Who would get them first? How would we prepare health personnel, and certify vaccination centers in primary health care? Keep records and provide the necessary follow-up for adverse reactions?

***MEDICC Review*: How did the vaccine clinical trials and other studies evolve towards mass vaccination?**

**Ileana Morales:** From mid-August 2020 to March 2022, 20 vaccine clinical trials have been conducted. Plus another 30 related to other products. And after preliminary results from phase 2 clinical trials for the SOBERANA and Abdala vaccines, we initiated an intervention study, involving mainly health workers as well as biotech scientists, who received either of the two vaccine regimens (see graphic). We mounted a study protocol, with external research ethics committees and evaluation through site visits and monitoring of results and adverse events.

This was followed by the public health intervention, involving an even larger number of people in territories where the surge was peaking. Again, this was a complicated process, since the vaccines were still in clinical trials, although we had excellent safety and efficacy results in hand from phase 2 trials. But this required a ministerial resolution, based on our Public Health Law, which gives the minister specific extraordinary authority in health emergencies. The Science Group provided a risk-benefit analysis to guide the decision, and thus it became the most complex public health intervention we have ever undertaken in Cuba. We began vaccinating adults in four Havana municipalities, the ones experiencing the greatest incidence, and later extended the process to other municipalities experiencing surges. And if we hadn't done that, who knows how high the peak of cases would have reached!? Havana was able to resist the delta variant better because of the public health intervention.

**Havana was able to resist the delta variant better because of the public health intervention.**

***MEDICC Review:* And parallel to that, the phase 3 trials of both the Abdala and the SOBERANA vaccine regimens were continuing, correct?**

**Ileana Morales:** Yes, and in July 2021, Abdala received emergency use authorization from our regulatory agency, and in August the SOBERANAs also received authorization, in both cases for adults 19 years of age and older. Thus, in July, the national vaccination rollout went forward. In summary: some 100,000 people participated in the clinical trials, about 160,000 in the intervention study, 2.5 million in the health intervention and 8 million in mass vaccination. Every step, all the procedures, regulatory processes, good clinical practice guides, logistics, data collection and follow-up, were previously submitted to the Innovation Committee. Our regulatory agency, the Center for State Control of Medicines and Medical Devices (CECMED), made thorough reviews of all these aspects, inspected clinical trial sites and vaccine manufacturing facilities, and assessed the vaccine dossiers. During the process, CECMED authorities met often with vaccine producers and the clinical trials' principal investigators to gain clarity and determine any changes in study protocols. Finally, after considering the results of each successive study and clinical trial, CECMED made the final decisions on emergency use authorization.[3]

***MEDICC Review:* An extraordinary process. What has it meant for you, for your work schedule?**

**Ileana Morales**: It was very, very tough. An 18-hour day on average. And the hardest part was the responsibility you carried with you every minute. We would start early in the morning and wouldn't leave the ministry until 1:00 or 2:00 in the next morning, seven days a week. It has been like a war, where everyone was needed on the frontlines. And it's been very emotional. Dr Francisco Durán, national head of epidemiology, and I would finish our daily video chat with the provinces at about midnight, and then he would drop me home. We left with a lump in our throats, often with heavy hearts. Feeling the weight of the responsibility, desperately wanting to do more. I sometimes felt like I was carrying a whole building on my back. But all of us felt that way, I'm sure.

At the same time, we had confidence because we had so many talented people on our side, so much organization brought to bear for the whole country--the schools, teachers, even tourism workers pitched in. Not to mention the hospital and primary care professionals, the students. The whole country was involved. How could we not have confidence? But it was still hard.

***MEDICC Review*: What about omicron now?**

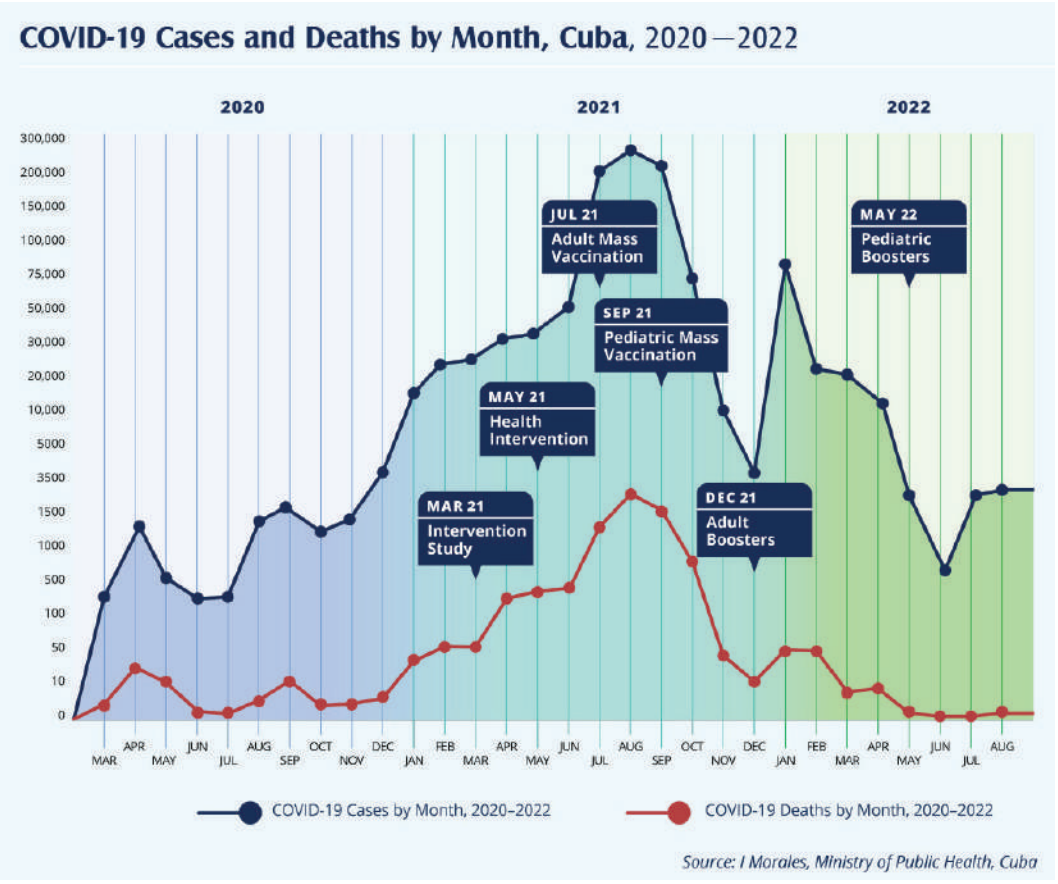
**Ileana Morales:** Thus far, we haven't seen a big rise in cases. For me, the explanation is that Cuba vaccinated virtually the whole population very quickly, and then boosted. For some time, Cuba has been among the first, if not the very first country, in terms of doses applied per 100 population. That includes the three-dose original schedule, plus boosters. Our vaccination rates are over 90%.

***MEDICC Review:* And children vaccinated?**

**Ileana Morales:** The vaccination of youngsters from 2 to 18 years was crucial. That happened after emergency use authorization and was critically important. Children are carriers and can impact transmission in the general population, which is especially dangerous for elderly people at home. Since vaccinating, we haven't had one child die from COVID-19, not one pregnant woman, either. For some time, we haven't had children with severe disease. The only critical cases we are seeing now are in immunocompromised adults, people in terminal stages of other diseases, and elderly with four or five comorbidities.

***MEDICC Review:* What has Cuba done differently than some other countries?**

**Ileana Morales:** Our strategy has been different from some others in at least four ways. First, it has been a single strategy,



applied throughout the country. It is flexible, of course, but essentially the same wherever you are, the clinical guidelines the same, too. Second, it's a protocol that begins in the community and ends in the community. Why? Because it's like a staircase…beginning with prevention and diagnosis in the community, and finally with convalescent patients followed up in their communities. In both cases, the neighborhood family doctors and nurses are key since they are at the heart of community care.

Third, it is a protocol that covers all aspects of pandemic response: from preventive measures to therapies and rehabilitation, including mental health. It's all there, and that is also why we have taken care to update it periodically with the latest experiences and information. We developed a stratification model to accompany it. Which leads me to the fourth difference: the protocol depends on getting ahead of the curve. That is, if we applied a medication at one time when patients had become critical, later we decided to apply it *before* that stage, to prevent such severe complications. Thus, this was a kind of staircase: the aim was to prevent illness, and if a person became ill, then to prevent serious disease, and if they became serious, then to prevent death, and finally, for convalescents to recover as quickly as possible.

***MEDICC Review:*** **Lessons learned?**

**Ileana Morales:** Many. First, that evidence-based decision-making provides the best, surest guide to action. I think that's a lesson learned by the entire society, the entire government, too. Second, that health is too important to leave entirely to the Ministry of Public Health. So, it made sense for everyone to be at the table who had something to contribute: mathematicians, physicists, sociologists, demographers. Multi- and transdisciplinary discussions were essential, and nobody was asking permission to talk with anybody else. If you needed to talk with the university rector, you simply called her, or with a government official at any level. And for some of the most renowned scientists, you had to see them going with me to the provinces, to the communities, to the primary health care level. The borders were simply erased, red tape cut. And that made for more streamlined, coordinated action… something we need going forward. We also learned that we could introduce earlier some measures or medicines with the required studies when the situation and the evidence warrant.

We ratified the importance of the links between biotechnology and public health, between the universities, the various fields of study and population health. The aim is for our cumulative knowledge to be at the service of people's health, to be able to quickly introduce the results that science can offer. And that is the challenge.

Finally, we once again learned how important international cooperation is, scientific and public health collaboration, sharing of experiences. This is vital. We have approved about one thousand research studies about COVID-19, 300 of these national studies that could help inform the global scientific community. And we're certain that such potential exists elsewhere as well. All these lessons we learned during an experience that was traumatic. We learned them during our 'test of fire.'

**We have approved about one thousand research studies about COVID-19, 300 of these national studies that could help inform the global scientific community.**

***MEDICC Review:*** **And that trauma extended to your own family.**

**Ileana Morales:** Yes, my grandson, the light of my life, contracted COVID-19 early in the pandemic. Then my daughter and my son-in-law. He was the most serious case in the family, but received several of the Cuban biotech medications, and thankfully recovered. Then I tested positive, too. But I have to say, all this was not extraordinary. In one way or another, all of us have someone close to us who contracted the disease.

***MEDICC Review:*** **What can you say about the role of women in the sciences in this colossal effort? Cuba has a record of being one of the countries with the greatest percentage of women in the sciences, and health sciences in particular.**

**Ileana Morales:** Yes, that's been the norm for some time. What is interesting now is the role of women leading in the sciences. Because you could see many women as researchers, health workers, teachers…but it's quite another thing to see women leading. For example, women are leading 70% of the primary research projects approved by the Innovation Committee. What's more, when I look over the report we made to parliament in 2021—*Scientific Advances to Confront COVID*—you see women in the majority, leading the efforts and the results in virtually every aspect. And that includes vaccine development as well as the vaccination rollout.

***MEDICC Review:*** **Anything else, going forward?**

**Ileana Morales:** I'm reminded that our meetings used to start with a standard Cuban saying: "gentleman, what more can we do?". But of course, this just isn't right. It has to be *at least* "ladies and gentlemen"! The day I write a book about all this, that just may be the title. Science empowers, and it has empowered women.

**Science empowers, and it has empowered women.**

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# Health Care is a Right, Not a Commodity: The Legacy of Dr Paul Farmer MD PhD

**Introduction and excerpts by Conner Gorry MA**
**From an interview by Connie Field for the film *¡Salud!***


Still from the film *¡Salud!*

Deaths averted, doctors trained, clinics staffed, communities reached, minds changed, lives touched: the metrics for measuring the impact of the late Dr Paul Farmer (1959-2022) are impressive in themselves. As a physician, medical anthropologist, professor, author and public health activist, Dr Farmer's commitment to advancing health equity is felt from Boston to Peru, Rwanda to Haiti—contexts where he worked on the ground and shoulder-to-shoulder with some of the world's most vulnerable populations.

While living in and serving these communities, training local health professionals and learning from their experience, Farmer cemented a philosophy and approach based on equity, humanism and access to quality care that take into account social determinants and structural violence.

These were radical ideas in 1987 when Farmer co-founded Partners in Health (PIH), an international NGO dedicated to guaranteeing health care as a human right by providing services and training, catalyzing policy innovation and improving health systems through a community-based clinical model. From its first programs and initiatives in rural Haiti, PIH now offers care and training in 12 countries including Lesotho, Liberia, Malawi, Kazakhstan, Mexico and the Navajo Nation (USA).

This goal to shift the global health paradigm towards quality care for all—especially in the world's most impoverished contexts—forged Farmer's reputation as a 'partner to the poor.' As Chair of the Department of Global Health and Social Medicine at Harvard Medical School and chief of the Division of Global Health Equity at Brigham and Women's Hospital, he inspired students and colleagues alike to value all lives equally and provide a level of care that reflects that commitment. In addition to his multiple roles and awards, Paul Farmer is author or co-author of a dozen books (see Sidebar) and was editor-in-chief of the peer-reviewed journal *Health and Human Rights*.

***MEDICC Review*** shares the vision of health for all, our editorial focus dedicated to moving the needle towards a more just, equitable and healthy world underpinned by health care as a human right. In honor of Dr Farmer's contribution to the health, education and empowerment of patients, practitioners and communities where he worked, we excerpt this exclusive interview conducted in 2006 for the MEDICC-produced documentary exploring the Cuban health system, ***¡Salud!.*** This previously unpublished conversation between Dr Farmer and ***¡Salud!*** Director Connie Field of Clarity Films dives deep into his philosophy, dedication to his patients, the importance of community-based primary care, strengthening health systems globally and lessons he learned working with populations and professionals, including Cuban doctors, in vulnerable contexts.

**Partners in Health started in Haiti, one of the most disadvantaged countries in the world. After several decades of in-country experience, what have you learned and how has your work evolved?**

Haiti, which you know suffers from the worst poverty in this half of the world, has literally hundreds, if not thousands, of non-governmental organizations and church groups. Usually, NGOs go into poor parts of Latin America and Africa and set up shop all alone—they're silos. And they're really doing almost nothing to support public education and public health. We'd been there ten years working, hard work, yeoman's work, trying to deliver basic health services to poor people in central Haiti before we asked ourselves: 'what have we done to beef up the public sector?' A decade is a long time to ask that question. We concluded that we hadn't done enough; from that point forward, we decided all of our expansion would be solely in the public sector. So, we began expanding rather rapidly in the public sector from a base in the middle of a squatter settlement. And now we're trying to bring other NGOs on board.

The public sector is the primary guarantor of health and education to the poor. This flies in the face of the current fashion in Latin America and the Caribbean, which is privatize, privatize, privatize. And it's not an accident for example that in Haiti, the least literate country in this hemisphere, 85% of all education is private. It's in the hands of NGO's or churches, not in the public sector. And you see the same thing in health care…you can't ignore the public sector if you're interested in health.

It's easy in a place like Haiti for groups like ours to say 'we're doing great. We've built an operating room, we've put in a blood bank'… but you know you can always do better. Before I came to Haiti, I used to think, 'pre-natal care is how you stop maternal mortality.' Curbing maternal mortality doesn't only have to do with prenatal care. It also has to do with whether or not there's access to cesareans, to surgery, and often, a blood transfusion. But above all, it's a problem of trained personnel. The standard of care, the best that we can do, is to have a physician trained in doing cesarean sections and nurses who are trained in giving anesthesia for example. And we quickly learned training community health workers to provide follow-up care can improve that standard of care. With the systems we put in place in rural Haiti and rural Rwanda, we train community health workers to give good follow-up care.

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**Community-based primary care, supported by specialists and integrated into the national public health system, is the backbone of the Cuban approach at home and abroad. Can you speak about your work alongside Cuban doctors?**

After working for over 20 years in central Haiti, one of the most significant problems we've had is recruiting medical specialists. There are almost none in Haiti serving the rural poor. We knew we were going to have a hard time recruiting and retaining specialists and we tried the obvious routes—recruiting Haitian specialists—but there wasn't a lot of interest in living in rural Haiti where they might not have electricity or hot water and their families don't feel comfortable. So, we started campaigning early on for help from Cuban physicians, specifically requesting a senior surgeon and a pediatrician, a request approved by both the Haitian Ministry of Health and the Cuban medical aid program. The express purpose of the Cuban program is to help strengthen the public sector, which is exactly what was needed.

There were delays, but the first two specialists finally arrived and spent two years working with us. The Cuban surgeon had been practicing for 30 years and could do any kind of emergency surgery, general surgery; he was very broadly experienced. The pediatrician had 27 years of work experience. One of the things that I admire was their tremendous work and professional ethic. They were great teachers and it was wonderful, almost magical, how humane they both were. The pediatrician for instance…you would think after 27 years, someone would be hardened and used to the tragedy around pediatrics. But she was so emotional in the best way, so open and kind to families. And I realized that it wasn't just about personality. It was about their training. They had been trained to be humane and ethical physicians.

We have some very committed Haitian physicians and nurses, but the presence of these two very mature physicians, with a lot of public health experience, served to raise the level of care across the entire hospital. Having experienced doctors impart their way of delivering care to others—to trainees, to those who are younger or less experienced—there's no substitute for that in medicine. Cuban health professionals have been cycling through Haiti for two-year stints since then.

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**As a professor at one of the United States' most respected teaching hospitals, and with a long history working with Cuban doctors, can you talk a bit more about what differentiates their medical training?**

The Cuban medical system is exceptional in that physicians are required to know public health. Even clinicians—a urologist, for instance or an ophthalmologist—are expected to know something about public health. They are also trained to know the communities they serve. In Cuba, your neighbors are also your

**The Cuban medical system is exceptional in that physicians are required to know public health.**

patients and physicians do home visits, for instance. These are largely gone from American medicine and are something we've tried to reintroduce in our training and service programs at Harvard; the physicians who train with us, whether they work in Boston or Rwanda or Haiti or Peru, they all do home visits. And of course, they're all being trained in public health. But I think that's an exception for medicine in the United States. In Cuba, it's the rule.

In Cuba, prevention and health promotion are also an important part of the medical school curriculum from the first year and throughout training. These concepts are highly regarded and even surgeons, for instance, are expected to know about health promotion and preventive medicine. Contrast this to training in the United States where prevention, maintenance and promotion just aren't a focus. These fields of medicine are not highly regarded among students, faculty and trainees, who are more focused on pathophysiology and responding to illness, not preventing it.

Let me add that the Cuban medical system, unlike some other countries, hasn't pushed primary care forward to the detriment of sub-specialty care. I wouldn't want to live in a place where there's no ophthalmologist or urologist and everybody is a primary healthcare practitioner. But Cuba has managed to provide specialist care, promote research on a very limited budget and at the same time strengthen primary health care. And in Cuba, health care is considered a right, not a commodity; a commodity is something you buy and a right is something you have—whether you can buy it or not. It's yours because you're human.

In order to respond to complex health problems among the poor, you need a public health system. And you need physicians trained in public health. That's the strong point of the Cuban training program, it's visionary and is helping us find our way back to public health and primary health care.

**Promoting health care as a right underpins your work in the United States and around the world. Why is it slow to take root?**

If you look at schools of public health, for example, in the United States, they're teaching about 'health care reform' or 'cost recovery' or 'sustainability.' None of these strategies are about health care as a right. And indeed, if you go to a US school of public health and talk about the right to health care, you are smirked at. Sure, the response is, 'oh, yes, we all agree' but obviously, some people don't agree that health care should be a right. Or it would be—certainly in a place where it could be, like the United States.

**If you strengthen the notion of health care as a right and put in place strong measures to protect that right, then you would be ready for whatever disaster came upon you.**

If we could push that forward as the number-one priority, other things would follow. For instance, if you strengthen the notion of health care as a right and put in place strong measures to protect that right, then you would be ready for whatever disaster came upon you. And it's not about the money: it's not that there's not enough money to do this work, to assure health care globally—there's plenty of money, even if we didn't have support from other affluent countries, the United States could pay the bill itself.

**If it's not about the money, why is over half the world's population still without access to primary care?**

It's complex, but brain drain is one of the reasons: for much of Africa and especially sub-Saharan Africa, professionals leave on the classic trajectory: from poorer African nations to South Africa (the continent's wealthiest country), and from there to England, over to Canada and the United States. The standard interpretation is that health professionals are seeking better wages. And they are. But that's not the whole explanation by any stretch of the imagination. If you study a hospital in Africa and talk to the physicians and nurses, they don't only talk about salaries and providing for their children. They're also going to say: 'we have lots of sick people but we don't have any diagnostic tools or medicines. We can't do our jobs.' Physician burnout is another reason.

In the United States, I wouldn't call it burnout, but rather a disenchantment or dissatisfaction with the medical system. 'Too much bureaucracy, too many lawsuits, insurance costs too much' is what a lot of physicians I meet around the country are saying. They are worn out. But I think some of it also has to do with knowing that more than 40 million Americans are without health insurance.[1] I work at a fantastic hospital in Boston—I don't think I've ever seen a better hospital anywhere in the world, with such an astounding level of care—but if you go down to the emergency room, you'll see patients coming in with very unremarkable problems. And you know that those patients should have received care from primary care physicians, not in the emergency room. I've seen it time and time again: health problems that should have been managed by a primary care physician before it became a problem and landed people in the ER; you really can't deliver good primary health care in the emergency room. And that's just discouraging to US physicians. A big chunk of our population is not well-served under the current system.

**It seems like providing access to quality care to all—especially in underserved areas, whether in US inner cities or the most impoverished nations—is an intractable problem. What solutions do you see?**

I think there are three things we can do to wipe out 60% of the health problems in poor areas. First, is the personnel issue. We should be training people living in poverty to become health professionals. Second, we need to assure these professionals have access to the 'tools of the trade:' medicines, diagnostics and other basics to deliver primary care (to say nothing of a tertiary facility like a hospital). Third, linking these first two to the notion of health care as a right.

This approach is what I call a 'pro-poor strategy:' we have this problem, we have the tools to solve this problem and we have to make these tools available to everyone as rights, not commodities to sell. In short, we have to train people living in poverty to be protagonists in this narrative and involve them in the work to make these services their right. It's hard, but rewarding, and I think this is the way forward.

**Cuba has bet big on training thousands of doctors from these poor communities, including from the United States, at the Latin American School of Medicine (ELAM) in Havana.[2] Do you think it will make a difference?**

Inviting poor kids from all over the world—the ones I know personally from Haiti who are attending ELAM are poor kids—to give them a full medical education is miraculous to me. The idea is these young people have grown up in or around poverty, they speak the language and are sympathetic to the destitute sick and understand the context and challenges. Training them to be good, humane and equitable physicians and return to poor communities to practice is part of the strategy. The question is, will it work?

The issue remains: once they become physicians, is health care a right or a commodity where they're practicing? If it's the latter, they're going to face the same problems that everybody else does. They'll just have better training. Until there are fair systems for financing health care, the full potential of these students is not going to be realized. Real support for this school in Cuba, or similar ones elsewhere, means creating jobs that allow the graduates of these schools to work equitably for poor people. Otherwise, the full promise of ELAM won't be met.

> **We have to train people living in poverty to be protagonists in this narrative and involve them in the work to make these services their right.**

This fits into the pro-poor strategy and transforming poor people into protagonists that I mentioned before. Whether it's a student from a squatter settlement in Haiti or highland Bolivia attending ELAM, we have to remove barriers, creating a job for him or her once they graduate so they can deliver health care to the poor in their home country. Otherwise, the risk is they will go home and end up serving in the capital just like any other doctor.

**Can you summarize what you believe has been Cuba's contribution to global health?**

The most important contribution Cuba has made to global health is the power of its own example. The idea that you can introduce the notion of a right to health care, establish a comprehensive public health system and wipe out the diseases of poverty—that's a stirring example, especially for other poor countries. Their mandate for equity, South-South technological transfer and vaccines are other areas of Cuban influence globally.

Another important contribution are the Cuban physicians and health professionals serving all over the world. I'm not sure what we would have done in rural Haiti without the Cuban physicians over the past few years; there were probably more Cuban doctors in rural Haiti than Haitian doctors and that's true in other places, too. I've run across Cuban doctors in nooks and crannies in Haiti and Africa where they're the only providers. Although it's hard work and takes adapting, it strikes me that these doctors are game for anything.

Cuba's holistic approach to medicine was also ahead of the curve. Cuban doctors are trained to understand a patient's illness and design effective interventions by looking at the whole picture—how social conditions and a person's habits determine their experience of chronic disease, for example. This is complemented by problem- and patient-centered learning emphasizing detailed patient exams and clinical histories to provide context for laboratory results.

What I've seen with the Cuban doctors with whom I've worked is a very high level of commitment to the profession and their patients. Their exchanges with patients are extraordinarily warm and that creates respect for and adherence to doctors' recommendations. It's touching. There are shortcomings to Cuban medicine—crises over the past two decades have hurt the health system—but there's this inherent justice in that system that inculcates certain ethical values among physicians, and those values are highly regarded in Cuban society and beyond. To me, this is largely attributable to Cuba's guarantees of universal access and health as a right.

## Notes and References

1. This interview was conducted before the Affordable Care Act was enacted in 2010. Currently, some 26 million people in the United States are uninsured.
2. Since its establishment in 1999, some 30,000 students from 103 countries, including 200 from the United States, have graduated from Cuba's Latin American School of Medicine.

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# Epidemiological Characterization of Patients in the First Eight Weeks Following Detection of SARS-CoV-2 B.1.1.529 (omicron) Variant in Cuba

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## ABSTRACT

**INTRODUCTION** In November 2021, omicron—a new SARS-CoV-2 variant—was identified in South Africa and almost immediately, WHO declared it a 'variant of concern'. In view of its rapid worldwide spread and its imminent introduction in Cuba, genomic surveillance was strengthened.

**OBJECTIVE** Describe cases during the first eight epidemiological weeks (epiweeks) of SARS-CoV-2 infection attributable to omicron variant in Cuba by clinical and epidemiological variables.

**METHODS** From epiweek 48, 2021 to epiweek 4, 2022, 288 nasopharyngeal swabs were processed for sequencing of a 1836 bp fragment of the S gene. Variants were identified according to GISAID database and confirmed by phylogenetic analysis. Variants' association with clinical and epidemiological outcomes was assessed.

**RESULTS** The first cases of omicron variant were imported, mostly from African countries and the United States. During the period studied, omicron was detected in 83.0% (239/288) of cases processed, while the delta variant was found in 17.0% (49/288). Most persons infected with omicron were symptomatic (63.2%; 151/239) and fully vaccinated (65.3%; 156/239); severe cases and deaths occurred mainly among patients aged ≥65 years (92.9%; 13/14), and 12 of these deaths occurred in fully vaccinated persons (92.3%; 12/13). Omicron spread rapidly throughout the country (from 10% of cases in epiweek 48, 2021, to 100% by epiweek 4, 2022), displacing the formerly predominant delta variant.

**CONCLUSIONS** Omicron's rapid expansion in Cuba was associated with increased incidence but not with a higher case fatality rate. The relatively milder disease in those infected with this variant could be influenced by the high vaccination coverage, along with the natural immunity acquired as a consequence of previous virus infection.

**KEYWORDS** Pandemics, epidemiology, epidemiological monitoring, COVID-19 testing, COVID-19, SARS-CoV-2, COVID-19 vaccines, Cuba

## INTRODUCTION

Several SARS-CoV-2 variants have circulated worldwide. On May 31, 2021, WHO introduced labels for SARS-CoV-2 variants of concern (VOC) and variants of interest (VOI) to be used with the scientific nomenclature. WHO updates the existing and emergent variants according to their detection frequency, transmissibility, severity or immune response.[1]

On November 24, 2021, South Africa reported detection of a new SARS-CoV-2 variant, designated as B.1.1.529. Two days later, WHO recognized this variant as a new VOC, named omicron.[2] Omicron has numerous mutations that could increase transmissibility, confer resistance to therapeutics, or partially escape from infection or vaccine-induced immunity.[3–5] Preliminary evidence suggested that infection with the omicron VOC might have a milder clinical presentation compared to delta. However, further analysis showed that clinical evolution was more influenced by previously achieved immunity levels, rather than the intrinsic characteristics of the SARS-CoV-2 variant.[6,7] More recently, different omicron subvariants have been described, such as BA.1, BA.1.1, BA.2, BA.3, BA.4 and BA.5, according to WHO.[8]

**IMPORTANCE** This is the first description of SARS-CoV-2 infection by the omicron variant in Cuba, including the variant's association with epidemiological and clinical variables during the first omicron surge, December 2021–January 2022.

In Cuba, genetic surveillance of SARS-CoV-2 was started at the beginning of the Cuban epidemic, in March 2020. Circulation of 18 variants was detected in 2021 including: the Wuhan virus (Clade L), D164G (Clade G); the VOCs alpha, beta, gamma and delta; one VOI (lambda); and two previous VOIs (A.2.5.1 and Zeta/P.2). However, beta (34.8%), delta (24.9%) and D614G (19%) variants were the most frequently detected in 2021. Co-circulation of different predominant variants was observed in all epidemic waves; however, delta increased from 1.4% in May 2021 to 27.1% by June, 70.1% in July and 94.1% in August. By September, 100% of the studied samples were classified as delta, displacing the other variants circulating in the country; this suggested that delta had genetic advantages over other previously circulating variants.[9–11]

Immediately after detection of omicron in South Africa, Cuba's Ministry of Public Health (MINSAP) intensified genetic surveillance of SARS-CoV-2.[12,13] This study aims to summarize the characteristics of the first SARS-CoV-2 cases in Cuba attributable

to the omicron variant, detected during epidemiological weeks (epiweeks) 48, 2021–4, 2022.

## METHODS

**Study design** This is a cross-sectional study, with viral genome analysis of nasopharyngeal swabs obtained from patients confirmed with infection by SARS-CoV-2 from epiweek 48, 2021 to epiweek 4, 2022. Samples included travelers from various countries and all Cuban provinces. Samples were collected at different points in the period studied, from patients exhibiting differences in clinical severity, from within areas with a significant increase of cases, and from diverse age groups (Table 1). Samples were sent to the Reference Laboratory for Influenza and Respiratory Viruses, in the Virology Department of the Pedro Kourí Tropical Medicine Institute (IPK), in Havana, Cuba, where genomic analysis was performed. As not all samples fulfilled the required quality attributes for amplification or sequencing, the number of samples with valid sequences varied by week.

**Sequencing and variant designation** Total RNA was extracted using the QIAcube Automated DNA/RNA Purification System and the QIAamp Viral RNA mini Kit (QIAGEN, Germany), following manufacturer's instructions. cDNA synthesis and amplification of a 1836 bp fragment of the S gene (positions 21,976 to 23,812) was performed using the commercial kit One Step RT-PCR (QIAGEN, Germany), following manufacturer's instructions. Primers for RT-PCR were those described in Protocols for SARS-CoV-2 Sequencing by the US Centers for Disease Control (CDC, Atlanta, USA).[14] The Dye Terminator Cycle Sequencing (DTCS) Quick Start commercial kit (Beckman Coulter, USA) was used for the sequencing reaction, with four primers spanning the entire amplified fragment of the S gene (positions 21,976 to 23,812).

Sequencing products were purified also according to the DTCS Quick Star Master Mix kit (Beckman Coulter, USA) and analyzed in a Beckman Coulter automatic sequencer model CEQTM8800 using the raw data analysis procedure for PCR products. Obtained sequences were assembled and edited using the Sequencher program (Sequence Analysis Software, Version 4.10.1, Gene Codes Corporation, USA). The complete Wuhan-Hu-1 sequence (NC_045512.2) was used as the reference nucleotide sequence. Mutations were identified using the CoVsurver interpretation algorithm: Mutation Analysis of hCoV-19, from the GISAID database (https://www.gisaid.org/epiflu-applications/covsurver-mutations-app). Cuban variants were named according to the mutational profile described in the GISAID database,[15] and confirmed by phylogenetic analysis, using the bioinformatics tool NGPhylogeny (https://ngphylogeny.fr).

**Statistical analysis** Descriptive statistics and graphics were obtained using Microsoft Office Excel 2010.

**Ethics** IPK's Ethics Committee approved the study protocol. Epidemiological and clinical information was obtained from the case registry of MINSAP's surveillance system fully protecting patient identities. WHO guidelines for clinical management of COVID-19[16] were used as criteria for clinical classification of asymptomatic and symptomatic (mild and severe) patients.

## RESULTS

During the study period, 288 nasopharyngeal swab specimens from persons with confirmed SARS-CoV-2 infection were sequenced. Table 1 describes the epidemiological and clinical characteristics of these first omicron cases detected in Cuba, during epiweeks 48, 2021–4, 2022.

**Table 1: Demographic and clinical characteristics of persons studied, by SARS-CoV-2 variant: Cuba, epiweeks 48, 2021–4, 2022**

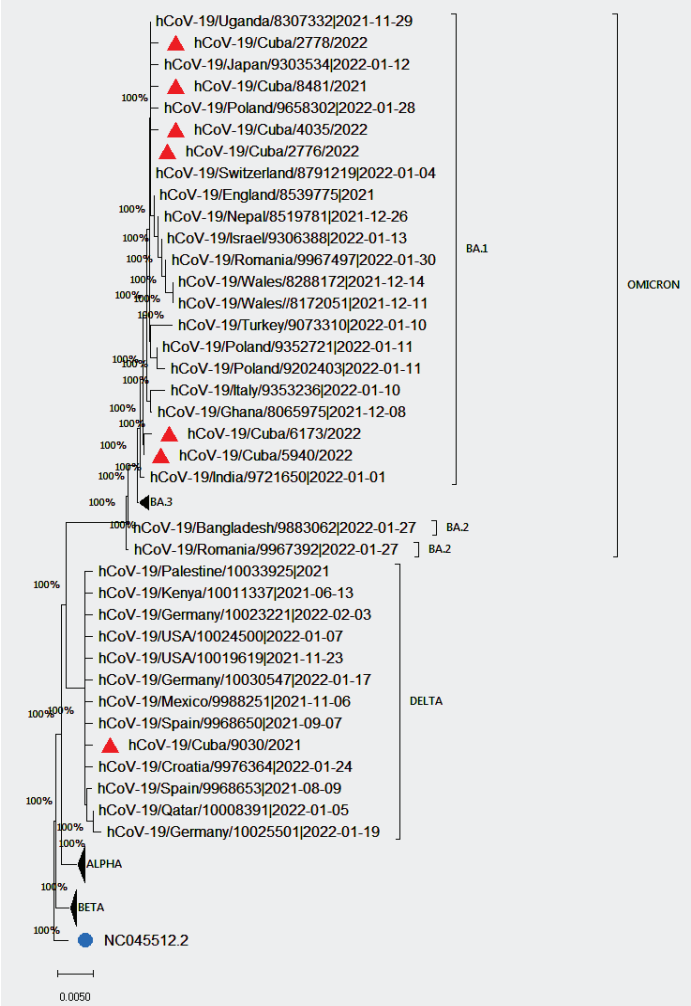
| Characteristics | Delta | Omicron | Total |
|---|---|---|---|
| Persons studied | 49 (100%) | 239 (100%) | 288 (100%) |
| **Dates of SARS-CoV-2 diagnosis (epiweek)** | | | |
| Nov 28–Dec 04, 2021 (48) | 10 (20.4) | 1 (0.4) | 11 (3.8) |
| Dec 05–11, 2021 (49) | 6 (12.2) | 0 (0.0) | 6 (2.1) |
| Dec 12–18, 2021 (50) | 3 (6.1) | 7 (2.9) | 10 (3.5) |
| Dec 19–25, 2021 (51) | 15 (30.6) | 58 (24.3) | 73 (25.3) |
| Dec 26–Jan 1, 2022 (52) | 11 (22.4) | 44 (18.4) | 55 (19.1) |
| Jan 2–8, 2022 (1) | 0 (0.0) | 36 (15.1) | 36 (12.5) |
| Jan 9–15, 2022 (2) | 4 (8.2) | 41 (17.2) | 45 (16.6) |
| Jan 16–22, 2022 (3) | 0 (0.0) | 19 (7.9) | 19 (6.6) |
| Jan 23–29, 2022 (4) | 0 (0.0) | 33 (13.8) | 33 (11.5) |
| **Age group (years)** | | | |
| ≤18 | 0 (0.0) | 22 (9.2) | 22 (7.6) |
| 19–39 | 8 (16.3) | 77 (32.2) | 85 (29.5) |
| 40–64 | 11 (22.4) | 75 (31.4) | 86 (29.9) |
| 65–80 | 4 (8.2) | 32 (13.4) | 36 (12.5) |
| >80 | 2 (4.1) | 18 (7.5) | 20 (6.9) |
| Unknown | 24 (49.0) | 15 (6.3) | 39 (13.5) |
| **Sex** | | | |
| Male | 29 (59.2) | 113 (47.3) | 139 (48.3) |
| Female | 20 (40.8) | 126 (52.7) | 149 (51.7) |
| **Recent International Travel** | | | |
| Yes | 12 (24.5) | 54 (22.6) | 66 (22.9) |
| No | 37 (75.5) | 185 (77.4) | 222 (77.1) |
| **COVID-19 vaccination status** | | | |
| Unvaccinated | | 6 (2.5) | 6 (2.1) |
| Partially vaccinated | 0 (0.0) | 14 (5.8) | 14 (4.8) |
| Fully Vaccinated* | 26 (53.1) | 156 (65.3) | 182 (63.2) |
| Vaccinated plus booster dose | 0 (0.0) | 32 (13.4) | 32 (11.1) |
| Unknown | 23 (46.9) | 31 (13.0) | 54 (18.8) |
| **Symptom profile** | | | |
| Symptomatic | 27 (55.1) | 151 (63.2) | 178 (61.8) |
| Asymptomatic | 1 (2.0) | 78 (32.6) | 79 (27.4) |
| Unknown | 21 (42.9) | 10 (4.2) | 31 (10.8) |
| **Outcomes** | | | |
| Severe case | 0 (0.0) | 19 (7.9) | 19 (6.6) |
| Death | 0 (0.0) | 5 (2.1) | 5 (1.7) |

*Vaccinated with the complete schedule according to different vaccine requirements (two or three doses)

On December 1, 2021 (epiweek 48), the first case of COVID-19 attributable to omicron variant was identified in a Cuban traveler from Mozambique. This variant expanded rapidly, replacing delta, which had been predominant since July 2021.[13] By January 29, 2022, 239 omicron samples had been reported through genomic surveillance, all classified as omicron BA.1 subvariant (Figure 1). The rapid extension of the omicron variant was accompanied by an increase in case numbers (Figure 2A). However, case fatality rates (CFR) did not increase significantly (Table 1, Figure 2B).

By the first epiweek of 2022, all sequenced samples were omicron, with the exception of four from the Isle of Youth Special

**Figure 1: Phylogenetic tree with SARS-CoV-2 partial nucleotide S gene sequences of omicron and delta variants**



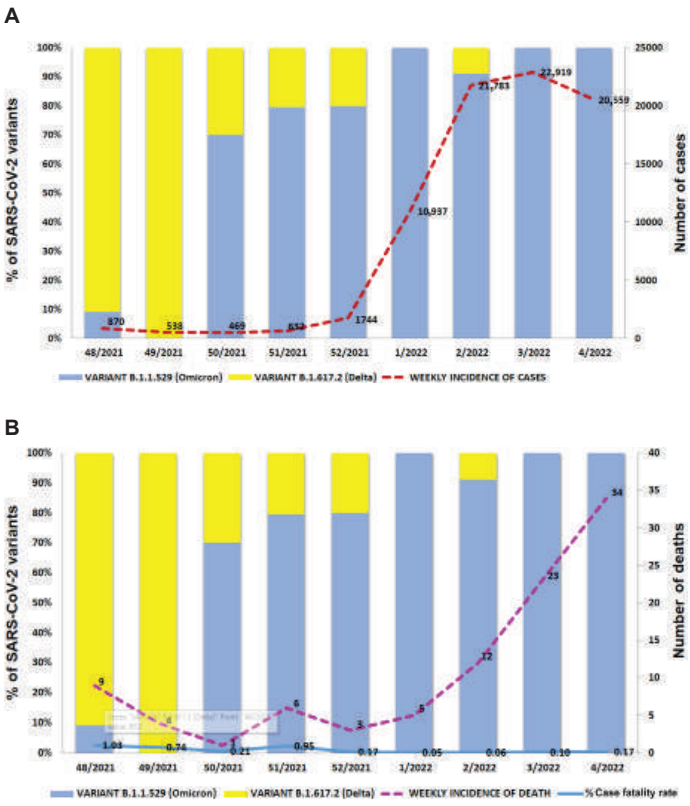

Analysis included 50 reference nucleotide sequences of the S gene, and 7 Cuban sequences from the present study (1836 bp). Duplicate sequences were removed using the elimdupes tool (https://www.hiv.lanl.gov/content/sequence/elimdupesv2/elimdupes.html). Sequences were aligned using the Mega version 11.0.11 and the bioinformatics tool NGPhylogeny (https://ngphylogeny.fr/). The evolutionary history was inferred using the NJ method (https://ngphylogeny.fr). The consensus tree, inferred from 10,000 replicates, was used to represent the evolutionary history of the analyzed sequences. Our study samples are labeled with a red triangle. Sequences of alpha and beta variants are labeled with a black triangle. A blue circle shows the Wuhan-Hu-1 sequence, that was used as the outgroup.

Municipality, where the delta variant was still circulating. As of epiweek 3, 2022, all Cuban provinces had identified at least one omicron autochthonous case (Figures 2 and 3).

Most of the persons studied were aged 19–64 years (59.4%; 171/288), with no differences for those infected with delta and omicron variants; in children (≤18 years, 7.6%; 22/288), only the omicron variant was detected (Table 1).

Sixty six recently arrived international travelers were positive for SARS-CoV-2 infection and classified as imported cases (Table 1). Fifty-four of them (81.8%; 54/66) carried omicron variant. Countries most represented in the introduction of this variant were the United States (15), Angola (11), South Africa and Eswatini (6 each), and Canada (5) (Figure 4).

**Figure 2: SARS-CoV-2 delta and omicron variants in Cuba, per number of cases and deaths, by epidemiological weeks**




Omicron imported cases were predominant in epiweeks 48–52, 2021 (51/54); autochthonous cases increased rapidly starting in epiweek 51, 2021 (Figure 5).

One hundred and eighty two (63.2%; 182/288) persons infected had completed the full 3-dose COVID-19 vaccination schedule, mainly with the Cuban vaccine Abdala, and 32 (11.1%; 32/288) had received additional booster doses; 17 of 22 children had been fully vaccinated, the remaining 5 were aged <2 years and therefore not eligible for vaccination, since the Cuban regulatory agency had only provided emergency use authorization for the Cuban vaccines beginning at 2 years old.[17] Vaccination status could not be determined in 54 individuals (18.7%; 54/288), most of whom were imported cases (Table 1).

Overall, 178 (61.8%) patients presented with symptoms and 79 (27.4%) were asymptomatic. In those infected with the omicron variant, the percentage of symptomatic cases was slightly higher (63.2%; 151/239). Information on symptoms was lacking in a high proportion of those infected with the delta variant (46.9%; 23/49) (Table 1); 24 patients infected with the omicron variant developed severe disease and five of them died (Table 1). As described in Table 2, most severe or fatal cases (13/14, 92.9%) with documented age, were aged ≥65 years, and 12 of them had completed the vaccination schedule (Table 2).

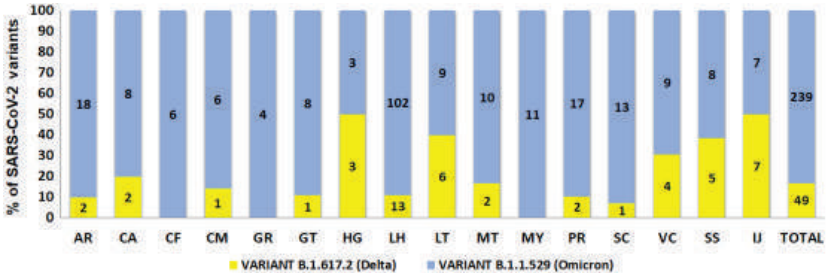
## DISCUSSION

By epiweek 10, 2022, 446,987,500 COVID-19 cases and 6,022,374 deaths had been reported worldwide since the beginning of the

pandemic; Cuba had reported 1,072,560 cases and 8500 deaths.[18]

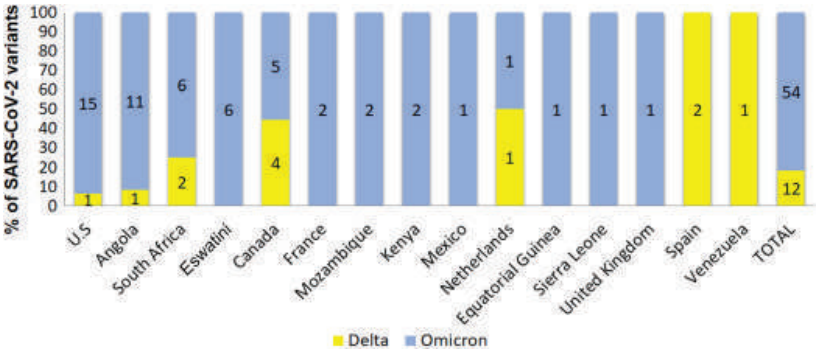
The first case of COVID-19 attributed to the omicron variant was detected in Cuba during epiweek 48, 2021, only a few days after WHO declared this variant a VOC.[2,19] Initially, this variant was detected in travelers, arriving mainly from African countries (29 cases) or the USA (15 cases) where this variant had already been reported.[20,21] All Cuban isolates were classified as BA.1, as had been reported for most of the sequences worldwide.[8]

**Figure 3: SARS-CoV-2 delta and omicron variants in Cuba by province, epidemiological weeks 48, 2021–4, 2022***



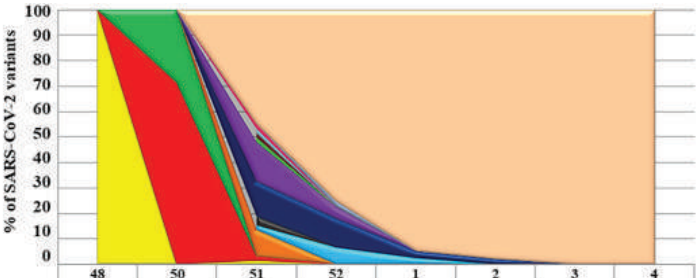

*Figures inside bars indicate number of cases of each SARS-CoV-2 variant
AR: Artemisa; CA: Ciego de Ávila; CF: Cienfuegos; CM: Camagüey; GR: Granma; GT: Guantánamo; HG: Holguín; LH: Havana; LT: Las Tunas; MT: Matanzas; MY: Mayabeque; PR: Pinar del Río; SC: Santiago de Cuba; VC: Villa Clara; SS: Sancti Spíritus; IJ: Isle of Youth Special Municipality

**Figure 4: SARS-CoV-2 delta and omicron variants introduced in Cuba, by percentage and country of origin, November, 28 2021–January 29, 2022***




*Figures inside bars mean number of cases of each SARS-CoV-2 variant

**Figure 5: Distribution of omicron cases in Cuba by country of origin, stratified by epidemiological week of detection (epiweeks 48, 2021-4, 2022)**




| | 48 | 50 | 51 | 52 | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|---|---|---|
| Cuba | | | 26 | 33 | 34 | 40 | 19 | 33 |
| Equatorial Guinea | | | | 1 | | | | |
| Sierra Leone | | | 1 | | | | | |
| United Kingdom | | | 1 | | | | | |
| Netherlands | | | 1 | | | | | |
| Mexico | | | 1 | | | | | |
| Angola | | | 9 | 2 | | | | |
| United States of America | | | 8 | 5 | 1 | 1 | | |
| France | | | 2 | | | | | |
| Canada | | | 1 | 3 | 1 | | | |
| Eswatini | | | 6 | | | | | |
| Kenya | | 2 | | | | | | |
| South Africa | | 5 | 1 | | | | | |
| Mozambique | 1 | | 1 | | | | | |

As in other countries, omicron emerged in Cuba when delta was the predominant circulating variant and rapidly displaced it, due to its higher transmission rate, infectivity and evasion of vaccine-induced immunity.[22,23] At the time, Cuba was in a favorable epidemiological context with low numbers of new cases (7-day moving average of daily new cases: 67, in epiweek 50, 2021) and extremely low number of deaths (7-day moving average of daily deaths: 0 on epiweek 50, 2021), principally due to high levels of immunity achieved—attributable to high vaccination coverage—including pediatric immunization (2–18 years), and to the natural immunity acquired as a consequence of previous infection during the delta wave. When omicron was detected in Cuba (epiweek 48, 2021) 10,192,588 (91.1%) persons in Cuba had received at least one dose of the Cuban vaccines Abdala or Soberana and 83% of them had completed the 3-dose vaccination schedule. Only 3.9% had received a fourth (booster) dose.[24]

Emergence of the omicron variant in Cuba was accompanied by an increase in case numbers, but not higher CFR. By March 7, 2022, number of new cases had dropped to <600 cases per day (7-day moving average of daily new cases: 520).

Omicron's first-wave peak in Cuba took place in epiweeks 2–4, 2022. The Cuban wave was less intense compared to other countries, including those with high vaccination coverage. As of January 29, 2022, Cuba reported 268.08 new COVID-19 cases per million population (pmp), while the UK, USA and Germany reported 1307.72, 1589.32 and 1740.56 cases pmp, respectively.[25] European countries with higher vaccination rates were better prepared for the autumn 2021 wave, despite the emergence of the more transmissible SARS-CoV-2 delta and omicron variants.[6]

A relatively high number of omicron-infected individuals were asymptomatic, as reported in other countries,[4,23,26] which has been attributed to a preferential infection of the upper respiratory tract.[27] Cuba's high immunization coverage also may have facilitated the mild disease occurrence observed during the first omicron wave.[24] In fact, it has been argued that a milder clinical evolution in omicron cases may be associated with acquired immunity due to virus circulation or vaccination, rather than to intrinsic characteristics of this SARS-CoV-2 variant.[7]

Nevertheless, severe disease and even fatal outcomes have been reported globally from the omicron variant, mainly in older adults and those with

**Table 2: Demographic and clinical characteristics of persons infected by omicron, by COVID-19 vaccination status: Cuba, epiweeks 48, 2021–4, 2022[a]**

| Characteristics | Fully vaccinated[b]/ Vaccinated plus booster | | | | | Unvaccinated/ Incomplete vaccination | | | | | Unknown vaccination status | TOTAL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Age (years) | <18 | 18–39 | 40–65 | >65 | Total | <18 | 18–39 | 40–65 | >65 | Total | | |
| Asymptomatic (%) | 2 (11.8) | 14 (21.9) | 23 (38.3) | 6 (14.3) | 45 (24.6) | 0 (0.0) | 5 (62.5) | 2 (50.0) | 0 (0.0) | 7 (38.9) | 15 (68.2) | 67 (30.0) |
| Mild symptoms (%) | 15 (88.2) | 50 (78.1) | 36 (60.0) | 24 (57.1) | 125 (68.3) | 5[c] (100.0) | 3 (37.5) | 2 (50.0) | 0 (0.0) | 10 (55.6) | 2 (9.1) | 137 (61.4) |
| Severe cases (%) | 0 (0.0) | 0 (0.0) | 1 (1.7) | 9 (21.4) | 10 (5.5) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1[c] (100.0) | 1 (5.6) | 4 (18.2) | 15 (6.7) |
| Deaths (%) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 3 (7.1) | 3 (1.6) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (4.5) | 4 (1.8) |
| TOTAL (%) | 17 - | 64 - | 60 - | 42 - | 183 (82.1) | 5 - | 8 - | 4 - | 1 - | 18 (8.1) | 22 (9.8) | 223 (100.0) |

[a]Differences observed between the information in Table 1 and the information analyzed in this table are due to missing data on patient age and/or clinical status.
[b]Vaccinated with the complete schedule according to the different vaccine requirements (two or three doses)
[c]Unvaccinated

various comorbidities.[28] Coinciding with previous reports, most severe cases and deaths observed in our study occurred in elders, probably associated to a less robust vaccine immune response, and a shorter durability of omicron-neutralizing antibodies due to immunosenescence.[29]

This study has some limitations. The number of samples studied was determined by the laboratory sequencing capacity. A number of samples positive for SARS-CoV-2 proportional to the number of cases in the country was selected each week; nevertheless, not all resulted in the required quality either during amplification or sequencing; in consequence, the number of samples sequenced was not homogeneous each week.

Although whole-genome sequencing was not performed, the sequences obtained covering the S gene from positions 21,976 to 23,812 permitted identification of SARS-CoV-2 variants circulating in Cuba.

## CONCLUSION

Beginning in late 2021 and into early 2022, the omicron BA.1 subvariant of SARS-CoV-2 rapidly replaced delta the variant in Cuba. Although the case numbers also increased, case fatality rates did not increase proportionally. The relatively milder form of the disease in individuals infected with this variant could be influenced by the high vaccination coverage achieved, including childhood immunization beginning at two years of age, along with the natural immunity acquired as a consequence of previous infection during the earlier delta wave.

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# Temporal–Spatial Distribution of *Vibrio cholerae* in Cuba: July 1997–December 2019

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## ABSTRACT

**INTRODUCTION** *Vibrio cholerae* is a microorganism that causes acute diarrheal diseases and cholera, one of the leading causes of global morbidity and mortality, especially in children under five years old. It is present in many regions and has been isolated from diverse sources such as water, soil and food. Surveillance of this microorganism in Cuba from 1985 through June 1997 showed circulation of non-epidemic non-O1/non-O139 serogroups, but surveillance continued to identify distribution of *V. cholerae* serotypes and serogroups in the different geographic regions of the country during the following years, due to the risk of introducing cholera-causing serogroups that provoked cholera epidemics in other countries of the region.

**OBJECTIVE** Describe the temporal–spatial distribution of serogroups and serotypes of *V. cholerae* in Cuba.

**METHODS** A cross-sectional study was conducted that included isolates from passive surveillance of *V. cholerae* in 16 hygiene and epidemiology centers throughout Cuba from July 1997 through December 2019, submitted to the National Reference Laboratory for Acute Diarrheal Diseases of the Pedro Kourí Tropical Medicine Institute in Havana, Cuba. The timeline was subdivided into three five-year periods and one eight-year period. The centers submitting isolates were grouped into three geographical regions: western, central and eastern Cuba. A total of 1060 *V. cholerae* isolates were studied, from the 1438 samples sent from 15 Provincial Hygiene, Epidemiology and Microbiology Centers and the Municipal Hygiene, Epidemiology and Microbiology Center of the Isle of Youth Special Municipality. Genus, species and serotype of all specimens were studied and reviewed in the context of the outbreaks of acute diarrheal diseases reported in the country.

**RESULTS** All 1060 isolates were confirmed as *V. cholerae*. In the distribution by time period and region, the highest percentage occurred in the 2012–2019 period, and the eastern region contributed the most isolates in all periods. Approximately 63.9% (677/1060) were from outbreaks, and in the 2012–2019 period, the most epidemic-causing isolates came from the western region. Approximately 52.8% (560/1060) were identified as non-O1/non-O139 *V. cholerae*, and 47.2% (500/1060) as O1 *V. cholerae*; of these, 96.4% (482/500) corresponded to Ogawa serotype and 3.6% (18/500) to Inaba. Circulation of non-O1/non-O139 *V. cholerae* occurred throughout the entire period. The O1 serogroup began to circulate in 2012 and continued through 2016; however, since 2017, it has not been identified again. In the western region, there were smaller percentages of isolates of non-O1/non-O139 *V. cholerae* in all periods, except 2012–2019. In that period, *V. cholerae* O1 was identified to a lesser degree in the central region.

**CONCLUSIONS** *Vibrio cholerae* circulated in all three Cuban regions during the years studied, with a higher percentage of isolates of the non-O1/non-O139 serogroup, which caused outbreaks or sporadic cases of diarrhea in the eastern region, with the exception of the 2012–2019 period, when epidemic outbreaks of the O1 serogroup (which causes cholera) occurred in all three regions, with higher percentages in the western region.

**KEYWORDS** *Vibrio cholerae; Vibrio cholerae* O1; *Vibrio cholerae* non-O1; *Vibrio cholerae* O139; dysentery; cholera; epidemiological monitoring; infectious diarrheal disease; disease transmission, infectious; gastrointestinal diseases; Cuba

## INTRODUCTION

Acute diarrheal diseases—including cholera—are one of the main causes of global morbidity and mortality, especially in children under five years old.[1] Causal factors include various bacteria; among these, *Vibrio cholerae* is one of the most important.[2,3]

Morphologically, the microorganism has a curved structure with a polar flagellum.[4] Based on the O antigen of the capsular lipopolysaccharide, *V. cholerae* is classified in more than 200 biochemically identical serogroups, including O1 and O139, the two epidemic serogroups that cause cholera.[5] Non-O1/non-O139 serogroups are not considered epidemic, although they can cause outbreaks or sporadic cases of diarrhea with clinical symptoms and signs different from cholera.[3]

**IMPORTANCE** Results of microbiological surveillance for *V. cholerae* in Cuba provide useful and relevant information for the national health system and the Foodborne Diseases Program to channel the human resources and materials needed to control such acute diarrheal diseases.

*V. cholerae* is present in several regions of the world and can be isolated from diverse sources such as water, soil and food. Humans are an accidental and transitory host, yet they are the ones who disseminate it in the environment.[6,7] Intestinal infections are the most clinically relevant, particularly cholera,[8] but extra-intestinal infections can occur as well, such as fasciitis, bacteremia, meningitis, otitis and those that can develop in surgical wounds.[9]

Since 1991, in the region of the Americas, various cholera outbreaks have been reported[10] as well as sporadic cases of gastroenteritis

or bacteremia caused by non-O1/non-O139 *V. cholerae*.[8,11,12] In 2010, acute diarrheal diseases caused by *V. cholerae* spread rapidly in Haiti,[13] a new scenario that could lead to reemergence and dissemination of cholera in countries such as the Dominican Republic, Cuba and Mexico, where positive cases were reported.[14,15] Considering circulation of this pathogen in the region, the National Reference Laboratory for Acute Diarrheal Diseases of the Pedro Kourí Tropical Medicine Institute (IPK) established in 1995 a surveillance system to identify outbreaks and sporadic cases of diarrheal illnesses that could be caused by *V. cholerae*.[16] This surveillance documented evidence of the circulation of non-O1/non-O139 strains from 1985 through June 1997, but surveillance of sporadic outbreaks of diarrheal diseases and epidemiological events possibly attributable to *V. cholerae* has continued, in order to provide relevant information to health authorities. The purpose of this study was to describe the temporal–spatial distribution of *V. cholerae* serogroups and serotypes in Cuba from July 1997 through December 2019.

## METHODS

**Design and samples** A cross-sectional study was conducted from July 1997 through December 2019. It was subdivided into four periods: P1: 1997–2001, P2: 2002–2006, P3: 2007–2011 and P4: 2012–2019 (three five-year periods and one eight-year period, the latter in order to include the most recent results).

Of the 1483 *V. cholerae* isolates from passive surveillance in the culture collection of the IPK's National Reference Laboratory for Acute Diarrheal Diseases, 1060 viable isolates (microorganisms with the possibility of multiplying) were studied; 423 resulted nonviable or were contaminated. The isolates were originally collected in 15 of Cuba's Provincial Hygiene, Epidemiology and Microbiology Centers (CPHEM) and the Municipal Hygiene, Epidemiology and Microbiology Center (CMHEM) of the Isle of Youth Special Municipality.

The centers submitting isolates were grouped into three regions: western, central and eastern Cuba. The western region included CPHEMs of Pinar del Río, Artemisa, Mayabeque, Havana, and Matanzas provinces, as well as the CMHEM of the Isle of Youth Special Municipality; the central region included CPHEMs of Cienfuegos, Ciego de Ávila, Villa Clara, Sancti Spíritus and Camagüey provinces; and the eastern region included CPHEMs of Las Tunas, Holguín, Granma, Santiago de Cuba and Guantánamo provinces.

Isolates were classified according to genus, species, serogroup and serotype, and those related to outbreaks were identified. Cases were considered to be an outbreak when the unit submitting the *V. cholerae* isolates reported that they belonged to a single event in which two or more patients had been in contact with one another. The moment of onset of symptoms, the place where the episodes occurred, and the individuals' characteristics were all recorded.[17]

**Procedures** Isolates were preserved in Pasteur's conservation medium for Enterobacteriaceae; they were inoculated in brain-heart infusion broth and anaerobically incubated for 18–24 hours at 37 °C. After incubation, a loopful of the broth culture was seeded by colony depletion plating on plates with selective medium for *Vibrio* thiosulfate-citrate-bile salts-sucrose (TCBS) agar (Biolife, Italy), MacConkey agar (Biolife, Italy) and blood agar (5% sheep blood) and incubated under recommended conditions.[18]

After incubating for 24 hours, three or more colonies were selected, based on their characteristics in the corresponding media: i) convex, with regular borders and sucrose fermenters in TCBS agar; ii) translucent, convex, with regular borders in MacConkey agar; and iii) hemolytic or not, convex, with regular borders in blood agar.

All colonies were inoculated by puncture and streaked in the primary differentiated media (Kligler's iron agar with double sugar fermentation and lysine iron agar). Both were aerobically incubated from 18–24 hours at 37 °C.[18]

Once incubation was complete, cultures that exhibited the following characteristics were selected: a) they did not oxidize or ferment lactose, they did oxidize and ferment glucose, they did not produce gas or hydrogen sulfide in Kligler's iron agar iron with double sugar fermentation; and b) they decarboxylated L-lysine in iron and lysine agar.[18]

In all isolates, presence of cytochrome oxidase enzyme was confirmed, in accordance with the Kovacs method. Those that were oxidase positive were submitted to a complementary physiological study for confirmation in the *Vibrio* genus and *V. cholerae* species.[18]

To identify the genus, the Möeller method was utilized for biochemical tests measuring use of amino acids and carbohydrates.[18] To identify the species, isolates were submitted to tolerance tests for sodium chloride (NaCl) and use of sucrose. Serological agglutination tests were performed on slides using *V. cholerae* O1 and *V. cholerae* O139 polyvalent antisera to identify these serogroups.[18]

**Analysis** Descriptive statistics were used, such as frequency and percentages, to analyze and present results.

## RESULTS

The 1060 isolates were confirmed as *V. cholerae* as they were Gram-negative bacilli, facultative anaerobic, positive oxidase, with positive reaction to lysine and ornithine decarboxylase tests and negative for arginine dihydrolase; they grew in tryptone soy broth with 6% NaCl concentration and used sucrose and mannitol, not inositol.

The highest percentage of *V. cholerae* isolates occurred in the 2012–2019 period and in the eastern region in all periods (Table 1). Approximately 63.9% (677/1060) came from outbreaks. Figure 1 shows the proportion calculated for each period, according to geographic region. In the 1997–2001 period, the lowest percentage of isolates coming from outbreaks was reported in the western region (5.0%; 34/677), but in the 2012–2019 period, that region accounted for the highest proportion of isolates from outbreaks (95.0%; 643/677).

Of total isolates, 52.8% (560/1060) were identified as *V. cholerae* non-O1/non-O139 and 47.2% (500/1060) as *V. cholerae* O1, of which 96.4% (482/500) were Ogawa serotype and 3.6% (18/500) Inaba. None of the isolates were identified as *V. cholerae* O139.

Figure 2 shows the temporal distribution of *V. cholerae* serogroups over the 23 years studied. As observed, *V. cholerae* non-

O1/non-O139 were in continuous circulation all those years. The O1 serogroup began circulating in 2012; the number of O1 isolates increased in 2013 and 2014, decreased in 2015, and since 2017, the O1 serogroup has not been reported at all.

**Table 1: Distribution of confirmed isolates of *Vibrio cholerae* by geographic region. Cuba, July 1997–December 2019**

| | Western | Central | Eastern | Total |
|---|---|---|---|---|
| 1997–2001 | 20 | 105 | 100 | 225 |
| 2002–2006 | 14 | 80 | 103 | 197 |
| 2007–2011 | 8 | 14 | 36 | 58 |
| 2012–2019 | 187 | 157 | 236 | 580 |
| Total | 229 | 356 | 475 | 1060 |

**Figure 1: Proportion of isolates of *Vibrio cholerae* (n = 677) from outbreaks of diarrheal diseases by geographic region. Cuba, July 1997–December 2019**

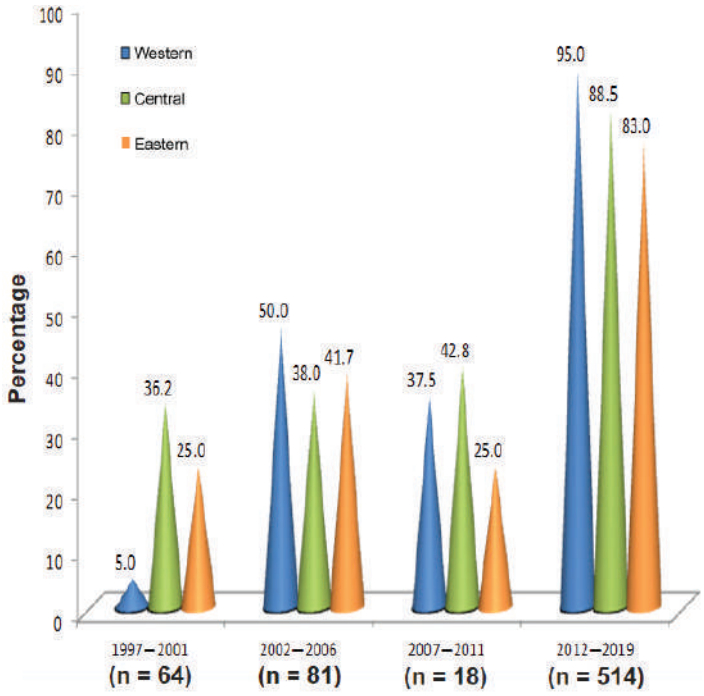


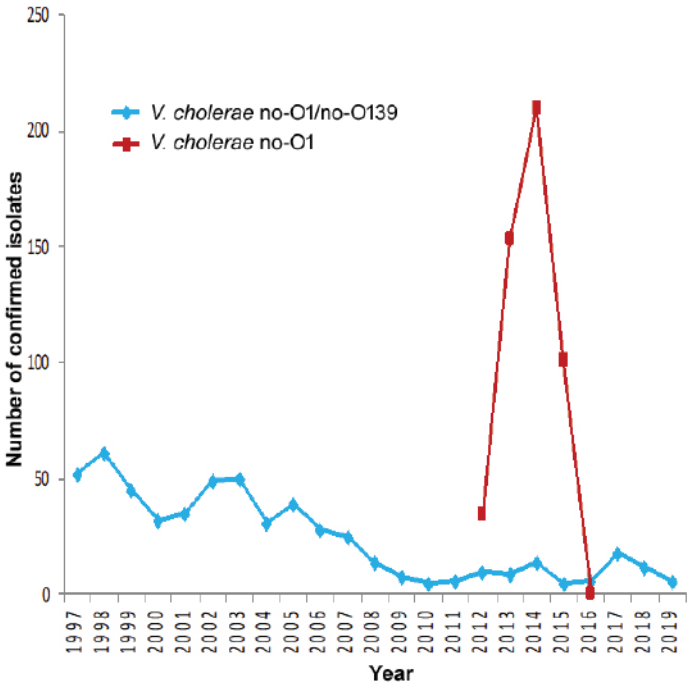

Figure 3 presents the distribution (in percentages) of serogroup strains according to geographic region. The western region presented the lowest percentage of non-O1/non-O139 *V. cholerae* isolates in all periods except 2012–2019. In that same period, *V. cholerae* O1 was identified in the central region, but exhibiting the lowest percentages.

## DISCUSSION

Worldwide, *Vibrio* spp. are widely distributed in aquatic environments.[18] It has been suggested that as a result of climate change, increases in ocean temperatures may be responsible for outbreaks of *Vibrio* infections in countries such as Israel, Denmark, Spain, Chile and the United States.[19,20]

According to data published by the Global Burden of Diarrheal Diseases Collaborators (GBDDC), acute diarrheal diseases have persisted in Latin America as a significant public health problem.

**Figure 2: Distribution over time of *Vibrio cholerae* O1 serogroup and *Vibrio cholerae* non-O1/non-O139 serogroup (n = 1060) responsible for diarrheal diseases. Cuba, July 1997–December 2019**




[21] Levels during the last three decades have been relatively stable and mortality in several countries has dropped, which Herrera-Benavente attributes to the success of WHO-sponsored control programs (under auspices of PAHO).[1]

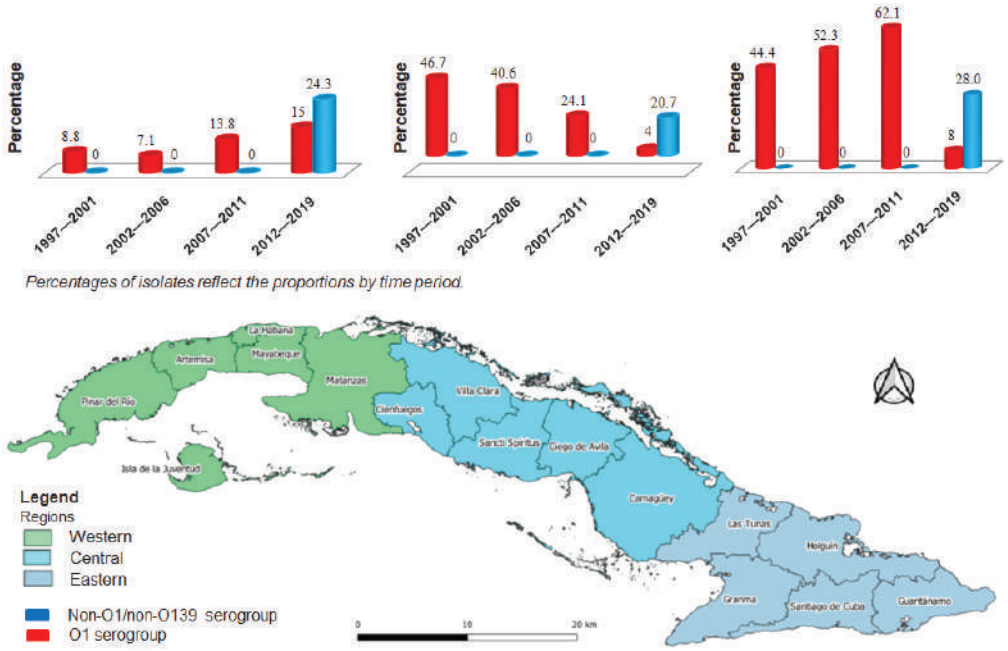
In a study prior to implementation of Cuba's surveillance system, Bravo[16] found that in the three Cuban regions, non-O1/non-O139 *V. cholerae* circulated from 1985 through 1997 in patients with acute diarrheal diseases, most commonly in the eastern region. The author suggested that an ecological niche in that region might favor persistence of diarrheas caused by this agent.

In June 2012, *V. cholerae* O1 was isolated in Cuba, when the Ministry of Public Health reported a cholera outbreak in Granma province.[22] Although no events caused by this strain had been observed in previous years, it is not unusual for such events to occur. The cholera-causing agent cannot be eliminated from the environment since it is a native species and is associated with marine habitats such as algae and crustaceans. In addition, changes in climate patterns favor the growth of *V. cholerae* in aquatic ecosystems and contribute to the occurrence of outbreaks and epidemics.[23,24]

In Cuba, more outbreaks and cases of diarrhea occur in the summer months, in part due to high temperatures and summer rains.[25] However, no studies have been conducted on Cuban climate variability and its effect on the temporal–spatial behavior of *V. cholerae*.

The epidemic O1 and O139 *V. cholerae* serogroups produce cholera, particularly in developing countries due to faulty infrastructure affecting basic sanitation and drinking water, as well as poor access to quality medical services,[2,26,27] while non-O1/non-O139 strains are often isolated in the environment and are associated with sporadic cases of gastroenteritis and extraintes-

**Figure 3: Distribution of *Vibrio cholerae* serogroup O1 and *Vibrio cholerae* non-O1/non-O139 responsible for diarrheal diseases (n = 1060) according to geographic region. Cuba, July 1997–December 2019**




tinal infections.[28]

From 2012 to 2016, there were various outbreaks in Cuba associated with the circulation of *V. cholerae* O1.[22,29] In the present study, an increase in isolates was observed from 2012 to 2019 from outbreaks in the country's western region, which could be related to the joint circulation of the epidemic serotypes that can cause outbreaks of greater magnitude.[27]

In a 2012 study in Cuba by Romero-Placeres to investigate whether shortcomings in the quality of drinking water and sanitation influenced the burden of acute diarrheal diseases, it was found that more municipalities in the west faced high risk of a diarrheal outbreak due to both these problems. In addition, Havana is located in western Cuba, and is the province with the largest population and most dealings (through business, tourism, travel) with the rest of the island and also with other countries.[30] Another important factor is that 96.8% of households in the capital receive water via aqueduct; the supply service is inconsistent and in some places has low pressure, which along with the current poor condition of distribution pipes, can lead to water contamination.[31]

According to the 2012 census by Cuba's National Statistics Bureau,[31] despite the fact that the country enjoys extensive coverage of water and sanitation services, it still faces deficiencies from deteriorating infrastructure due to aging distribution systems, with high risk of microbiological contamination, a conclusion coinciding with observations by Romero-Placeres.[30]

The 2012 and 2013 cholera outbreaks in Havana were associated with food markets, where several asymptomatic carriers worked and manipulated foods.[25] The O1 serogroup contains three serotypes; the two most common—Ogawa and Inaba—are capable of producing cholera outbreaks. The difference between the two lies in methylation of the capsular lipopolysaccharide by the enzyme methyltransferase, codified by the *webT* gene. When an isolate presents a methylated lipopolysaccharide, it belongs to the Ogawa serotype, and when it does not, it is Inaba.[32]

In endemic areas, the change from one serotype to another is not a random process, rather, it depends on several factors.[33] Genetic studies suggest that the mobile elements present in the genome of *V. cholerae,* such as those related to virulence (CTXØ), antibiotic resistance (SXT ICE) and phages and their resulting clonal expansion, can contribute to a preponderance of a particular serotype during a specific time period. Another important factor in the change of serotype is pressure generated by the immune system, because when a specific serotype circulates in a population over a long time, the host will present immunity to this serotype. The effect of environmental factors on the viability of these two serotypes is not well known.[34]

In stool samples of patients with diarrhea obtained from 2006 through 2016 in sentinel centers in Nepal, *V. cholerae* O1 was isolated, mostly the Ogawa serotype,[35] and in 2015 and 2016 this strain was also isolated in stool samples of patients with acute diarrheal disease and identified in samples of water used for drinking and domestic chores in Ghana, coexisting with non-O1/non-O139 serogroup.[36]

During cholera outbreaks in Cuba, circulating together with *V. cholerae* O1 were the non-epidemic serogroups, as reported in Thailand, Iraq and Japan.[37] The coexistence of toxigenic and nontoxigenic serogroups represents a global health emergency, due to the horizontal transfer of genes that contribute virulence and antimicrobial resistance among serogroups of the same species, which can lead to the appearance of more virulent strains.[38]

The results of the present study on circulation of serogroups and serotypes coincided with the above-cited works and with cocirculation of *V. cholerae* O1 and non-O1/non-O139 from 2012 to 2016, underscoring the importance of epidemiological surveillance of this bacterium in Cuba.

In January 1991, a cholera epidemic broke out on the coast of Peru, attributed to the O1 serogroup, which quickly spread to almost all Latin American countries, although Cuba was not affected.[25] In October 2010, cholera was introduced into Haiti, and from then through 2018, persistent circulation and outbreaks of cholera were reported, associated with poor sanitary conditions and lack of access to safe drinking water in that country.[13] Subsequent outbreaks in Cuba were related to the Haitian epidemic, according to Cuban

health officials, who cited the ongoing interaction between the two countries.[39]

During the period studied, *V. cholerae* O139 was not reported in Cuba. This serogroup, first identified in 1992 in Bangladesh, has caused outbreaks elsewhere in the past, but in recent years it has been reported only in sporadic cases, always in the Asian region.[27]

The principal limitation of this study is that unidentified outbreaks may have occurred in the country's rural zones and cases not included because the infected individuals did not seek treatment in a medical center.

## CONCLUSIONS

Our temporal–spatial heterogeneity study confirmed that *V. cholerae* circulated for 23 years in all three regions of Cuba. Additionally, non-O1/non-O139 serogroups that caused outbreaks or sporadic cases of diarrhea were isolated in all time periods and regions, most often in the eastern region, with the exception of the last period, when epidemic outbreaks of the cholera-causing O1 strain occurred in all three regions, most commonly in the western region. This study shows that gastrointestinal infections from *V. cholerae* constitute a health problem in Cuba that needs to be addressed and suggests that the results should be used to design focused strategies, in accordance with the microorganism's temporal-spatial distribution pattern.

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# Validation of a New Diagnostic Index to Determine Metabolic Obesity Phenotypes in Normal-Weight Women in Early Pregnancy

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## ABSTRACT

**INTRODUCTION** It would be useful to have diagnostic indices for obesity phenotypes in pregnant women based on morphological traits and the specific distribution of abdominal adipose tissue. This type of practical resource would allow for the classification of obesity phenotypes in normal-weight women in early pregnancy and would contribute to primary healthcare followup of pregnant women.

**OBJECTIVE** Validate a new diagnostic index for the metabolically unhealthy obese, normal-weight phenotype, as a determinant for cardiometabolic risk in normal-weight pregnant Cuban women in the first trimester of pregnancy.

**METHODS** A cross-sectional study of 526 pregnant women at a gestational age of 12 to 14 weeks seen at the ultrasound service of the Chiqui Gómez Lubián Teaching Polyclinic, Santa Clara municipality, Villa Clara province, Cuba, was conducted from January 2016 through July 2020. Subcutaneous, preperitoneal and visceral abdominal fats, as well as anthropometric and blood chemistry variables, were measured. The women were divided into three groups based on metabolic phenotypes, taking into account body mass index in the normal weight range, visceral adiposity index values and the lipid accumulation product starting at the 75th percentile. The new index, called the abdominal adipose deposit index, was obtained by multiplying the subcutaneous fat thickness by visceral fat thickness, both measured by ultrasound. A cut-off point was established that facilitated discernment of an unhealthy phenotype: normal weight but metabolically obese, a cardiometabolic risk factor.

**RESULTS** Receiver operating characteristic (ROC) analysis of the abdominal adipose deposit index to distinguish the metabolically unhealthy obese, normal-weight phenotype in normal-weight pregnant women showed an area under the curve of 0.707 (95% CI: 0.62–0.79, p <0.001), greater than that of the body fat index (0.630; 95% CI: 0.54–0.72), the fat accumulation index (0.637; 95% CI: 0.55–0.73) and other established ultrasound indices of abdominal adiposity, with a prevalence of 6.3%.

**CONCLUSIONS** The abdominal adipose deposit index is better than other traditional indicators at detecting the risk of metabolic obesity in early pregnancy in normal-weight women, facilitating early intervention in clinical practice to prevent or delay progression of cardiometabolic disease in these women.

**KEYWORDS** Abdominal adipose tissue, abdominal fat, pregnant woman, phenotype, metabolic syndrome, diagnostic ultrasound, Cuba

## INTRODUCTION

Consensus is lacking on clinical management of obesity during pregnancy as there is not consistent evidence justifying one or another change in this regard. The prominence of adipose tissue in the genesis of metabolic and cardiovascular complications related to obesity[1] has increasingly drawn interest to the differentiation between specific adipose tissue depots due to their association with obesity phenotypes that contribute to development of type 2 diabetes and cardiovascular disease.[2]

The body's response to central adiposity is complex. Under conditions of obesity, visceral adipose tissue becomes the first store of triglycerides when faced with the inability of subcutaneous adipose tissue to store such excess energy ('overflow hypothesis'), which causes lipotoxicity and insulin resistance (IR). According to some researchers, subcutaneous adipose tissue is related to and influences IR, at least as much or more than perivisceral adiposity. Subcutaneous adipose tissue possesses a considerably higher adipose mass, which influences insulin sensitivity due to the volume of free fatty acids that it sends into general circulation, considered an insulin resistance phenomenon via a non-portal mechanism.[3,4]

**IMPORTANCE** Based on ultrasound measurement of abdominal fat, an index was obtained to diagnose an unhealthy (metabolically obese, normal-weight) phenotype in early pregnancy, facilitating identification and prevention of cardiometabolic risk in pregnant women.

The essential mechanism for development of metabolically abnormal phenotypes is disruption in the ability of subcutaneous tissue adipocytes to proliferate and differentiate, which leads to a limitation of adipocyte hyperplasia and to adipocyte hypertrophy, with an increase in the flow of fatty acids to the visceral adipose tissue and consequent accumulation of fat in other ectopic tissues. This represents an emerging clinical problem that in the near future may materialize as higher rates of 'healthy obese' and 'thin sick' individuals, which require coordination of diagnostic criteria on metabolic phenotypes with other studies that examine the relevance of the various definitions and findings.[5]

During pregnancy, when waist circumference is changing significantly, different criteria have been used to classify metabolic syndrome (MetS). Its high prevalence, the metabolic risks caused by the physiological changes of pregnancy and postpartum, the lack of indicators to classify the syndrome, as well as inconsistencies

regarding its prevalence and associated factors in women with no prior diseases, support the need for research on the subject.[6]

The US National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) criteria,[7] which include abdominal obesity as an independent risk factor for cardiometabolic disease,[8] have been used to predict MetS in pregnant women due to the simplicity of their application.

Few studies have researched central adipose tissue deposits and glucose homeostasis in pregnancy.[9] An obese woman has a metabolic increase of IR in early pregnancy, with results such as glucose intolerance and fetal overgrowth, that clinically manifest in late pregnancy. While a single mechanism has not been identified to explain the adverse perinatal results associated with maternal obesity, nonetheless maternal pregestational insulin resistance, inflammation and oxidative stress could contribute to early placental insufficiency.[10]

Ultrasonography has often been used as a necessary substitute for waist circumference measurements during pregnancy.[11] These measurements, together with the usual first trimester ultrasound examination, may provide additional information more useful than body mass index (BMI) regarding association with cardiometabolic risk factors and IR.[12]

Evidence shows that ultrasonographic indices of abdominal adiposity, such as abdominal fat index (AFI),[13] fat accumulation index (FAI),[14] body fat index (BFI)[15] and others, combined with use of anthropometric measurements and laboratory studies such as visceral adiposity index (VAI),[16] are better cardiometabolic risk indicators than BMI. However, despite the existence of cutoff points that appear optimal for diagnosing obesity and MetS, studies in several countries and ethnic groups have arrived at different conclusions.[17] No single definition exists for the metabolically obese phenotype in a normal-weight pregnant woman. This makes it difficult to compare results[18] or to apply them to the Cuban population, least of all to pregnant Cuban women.

There are no studies on abdominal adiposity using ultrasound (US) in Cuba, despite its wide availability in our healthcare institutions, and thus its potential to diagnose obesity has not yet been reached, particularly in early pregnancy.

Considering that some cardiometabolic risk factors can be identified using obesity phenotypes,[19] creating a new abdominal adipose deposit index (AADI) based on direct measurement of subcutaneous and visceral fat using US and its validation in the first trimester of pregnancy, would provide an instrument for early differentiation of such risks in this population group,[6,20,21] and would support a new clinical approach for managing obesity and its implications during pregnancy.

The objective of this study was to validate a new diagnostic index to determine a metabolically-unhealthy and obese phenotype in normal-weight pregnant women in Cuba, as an indicator of cardiometabolic risk in early pregnancy.

## METHODS

**Design and population** A cross-sectional study was conducted with 2357 women of all nutritional statuses in the first trimester of pregnancy from various community polyclinic catchment areas in Santa Clara municipality, Villa Clara province, Cuba, seen at the Prenatal Ultrasound Service of the Chiqui Gómez Lubián Teaching Polyclinic, from January 2016 through June 2020. The study population comprised 526 pregnant women after applying the following criteria:

*Inclusion criteria* Classified as normal weight according to BMI, aged 20–35 years, pregnant with a single fetus and at a gestational age of 12–14 weeks (confirmed by US).

*Exclusion criteria* Presence of metabolic disease including diabetes mellitus in any of its stages, dyslipidemia, psychiatric disorders, uterine fibroids and regular consumption of prescription drugs.

*Exit criteria* Termination or loss of pregnancy and incomplete or unreliable data.

**Study variables**

*Ultrasound variables and their determination* Thickness in millimeters (mm) of subcutaneous abdominal fat (SAF), preperitoneal fat (PPF) and visceral fat (VF) were measured by US. SAF was measured in the upper half of the anterior abdominal wall above the umbilicus, for which a longitudinal scan was done perpendicular to the skin, at the lowest point between the wall and the *linea alba* at the level of the xiphoid process. PPF was measured at the maximum point, behind the anterior abdominal wall and at the level of the xiphoid process, between the *linea alba* and the peritoneum that covers the liver surface, placing the transducer perpendicular to the skin. SAF thickness was obtained by placing the electronic cursors located in the skin-fat interface (excluding the skin) to the *linea alba*; to measure PPF thickness, cursors were placed in the fat-muscle interface, between the *linea alba* and the liver surface, keeping them almost parallel to the skin, according to the Suzuki technique.[13] To measure VF, cursors were placed at the internal edge of the rectus abdominis muscle, without including it, and at the anterior edge of the spine, at the level of the fourth lumbar vertebra (L4–L5), placing the transducer between one and two centimeters above the umbilicus, according to Armellini.[22] No standardized cutoff points or reference values have been reported for these variables in pregnancy.

*Abdominal adipose deposit index (AADI)* The new index proposed in this study was obtained by multiplying the thickness of the subcutaneous fatty tissue by the thickness of visceral fatty tissue, both measured by ultrasound: AADI = SAF × VF. The AADI result is expressed in $mm^2$ and includes both fatty depots, which are anatomically and functionally related in their metabolic effects. The creation of this index is supported by theories on the accumulation of abdominal adipose tissue that suggest that the maximum expansion capacity of SAF leads to accumulation of VF, which has been associated with various cardiometabolic risk factors. When the superficial subcutaneous compartment reaches its full capacity for fat storage, the secondary depots, such as the deep subcutaneous and visceral depots, assume the main role in the accumulation of the excess triglycerides, manifested in an increase of the values of this index. As will be indicated later, the cutoff point for the AADI was established at 350 $mm^2$. Values above this threshold identified the metabolically obese, normal-weight phenotype in pregnant women.

*Fat accumulation index (FAI)* Calculated as the sum of SAF and PPF.[14]

*Anterior abdominal wall adiposity index (simplified to abdominal fat index, AFI)* Calculated as the quotient between PPF and SAF.[14]

*Body fat index (BFI)* Calculated by the equation: BFI = (PPF × SAF)/Height.[15]

*Anthropometric variables* Weight (kg) and height (m) were measured for the BMI calculation when the pregnancy was reported; if a pregnant woman had a value 18.8–25.6 kg/m$^2$ she was classified as normal weight, according to the Anthropometric Pregnancy Tables (one of which is Cuban).[23,24] Waist circumference (WC) was considered a risk indicator at >88 cm.[25]

*Blood chemistry risk variables* Blood glucose (BG) >4.4 mmol/L,[26] triglycerides (TG) >1.7 mmol/L and high-density lipoprotein cholesterol (HDLc) <1.3 mmol/L.[25]

*Combined variables (combination of blood chemistry and/or anthropometric variables)* The lipid accumulation product (LAP) and the visceral adiposity index (VAI) were used,[18] for which risk variables were established starting at the 75th percentile of the study population.

All study variables were continuous quantitative variables.

**Procedures** A high-resolution Sonoacer5 scanner (Samsung Medison Co., Ltd, South Korea) was used for ultrasound measurement of abdominal fat. Measurements were taken by the same specialist—a professional in imaging technique with training in fetal echocardiography and over 15 years' experience in prenatal US diagnosis. The transducer was placed perpendicular to the skin, after exhalation, with the woman in supine decubitus position, arms at her sides. The defined area from the xifoid process to two centimeters above the umbilicus on the xifo-umbilical line was covered in conductive gel.

To validate AADI, the criteria proposed by the NCEP-ATP III[7] were used as the reference standard test,[27] modifying the cutoff point for blood glucose, with >4.4 mmol/L considered a risk value for pregnant women, according to the Second Cuban Consensus on Diabetes and Pregnancy.[26]

Panel III was selected as reference criteria for AADI validation since there are no risk criteria or standardized cutoff points associated with abdominal obesity and MetS in pregnant women in Cuba. Thus, the rest of the variables were evaluated according to Panel III criteria, also used in the Cuban guide for diagnosis, evaluation and treatment of hypertension.[25] Such criteria, in addition to their simplicity and applicability in the clinical context, have been used successfully by various authors to diagnose MetS in early stages of pregnancy.[6,20,21]

To evaluate AADI's discriminant ability to detect the metabolically obese, normal-weight phenotype (MONW), as well as MetS, we turned to the Receiver Operating Characteristic (ROC) curve. AADI was used as a contrast variable (test variable), and qualitative variables were selected from the group as explanatory variables, which were assigned numeric values to identify phenotypes or the respective presence or absence of MetS.

The study population was stratified by taking into account BMI values in the normal weight range,[23] and VAI and LAP values starting at the 75th percentile, according to the criteria of Du,[18] including the metabolically healthy obese, normal-weight phenotype (MHONW) as a transition phenotype,[3] resulting in the following groups of metabolic phenotypes:

- Metabolically healthy, normal weight (MHNW): 18.8 ≤BMI ≤ 25.6 kg/m$^2$ and VAI <2.37,
- Metabolically healthy obese, normal weight (MHONW): 18.8 ≤BMI ≤25.6 kg/m$^2$ and VAI ≥2.37,
- Metabolically unhealthy obese, normal weight (MUONW): When criteria for the MHONW phenotype are met and they also have LAP values ≥55.1.

A cutoff point was calculated seeking a balance between sensitivity and specificity, in keeping with the aim of the diagnostic test to identify the highest number of pregnant women with the MUONW phenotype or MetS, as appropriate.

To carry out the validation process, the study population of 526 pregnant women was stratified into two groups, according to presence or not of MetS, based on the modified NCEP-ATP III criteria for the blood glucose cutoff point. A pregnant woman was considered to have MetS if she met three or more of the following conditions: waist circumference >88 cm; fasting glucose >4.4 mmol/L; HDL cholesterol <1.30 mmol/L; triglycerides >1.7 mmol/L and systolic blood pressure >130 mmHg and/or diastolic blood pressure >85 mmHg.[7]

**Statistical analysis** Information was processed with the IBM SPSS Statistics version 22.0 for Windows and Statgraphics Centurion XV professional software packages.

Analysis of variance (ANOVA) was conducted, and when the null hypothesis that the means are equal was rejected, comparisons were made a posteriori (post hoc) to which Fisher's least significant difference (LSD) method was applied to identify groups that differed among them.

Summary measures were used to describe quantitative variables, measures of central tendency, dispersion and location (mean and standard deviation, SD) for data with symmetrical distribution, and median and interquartile range (IQR) for non-symmetrical distributions. For qualitative variables, the frequency distributions expressed in absolute and relative values (number and percentage) were calculated.

For all hypothesis testing performed, p value = 0.05 was considered the threshold for statistical significance. Results were presented in tables and figures.

**Ethics** Written informed consent was provided by all participants, and the anonymity of their personal and clinical data protected. The research was approved by the Chiqui Gómez Lubián Teaching Polyclinic's Ethics Committee.

## RESULTS

Table 1 shows AADI in normal-weight pregnant women who were the subject of the study, based on their metabolic phenotypes. Prevalence of MUONW phenotype was 6.3%.

Through ANOVA analysis, significant differences in AADI were verified between phenotype groups, and an F-distribution value of 10.16 was obtained with an associated p value ≤0.001; likewise, the post hoc multiple range tests by Fisher's LSD method generated statistically significant differences among all phenotype groups.

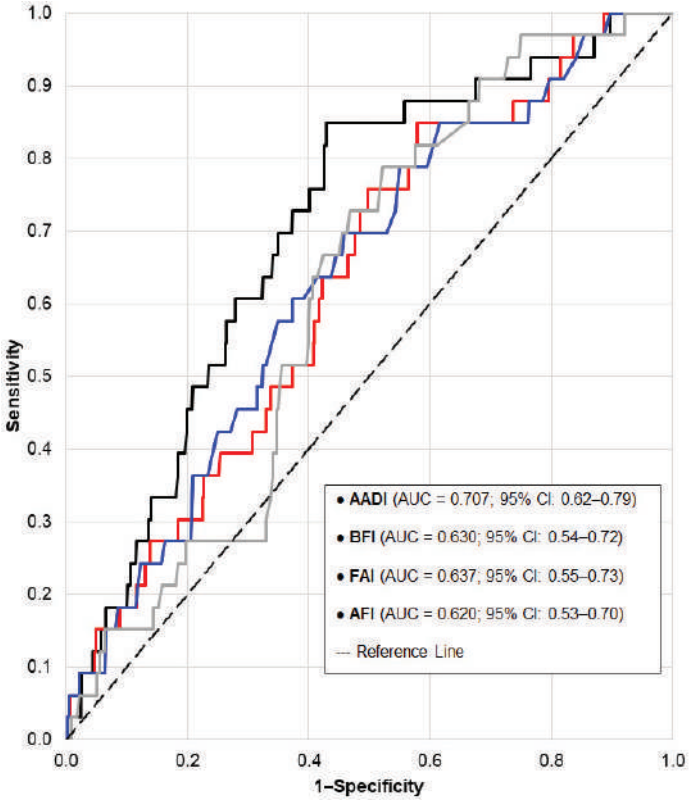
**Table 1: Descriptive statistics of the abdominal adipose deposit index (AADI) in each metabolic phenotype**

| | MHNW (n = 393) 74.7% | MHONW (n = 100) 19.0% | MUONW (n = 33) 6.3% |
|---|---|---|---|
| Mean ($mm^2$) | 389.71[a] | 435.64[b] | 531.52[c] |
| Standard deviation | 191.23 | 172.08 | 180.06 |
| Minimum value | 73.10 | 96.60 | 184.30 |
| Maximum value | 1196.00 | 957.60 | 949.96 |

[a,b,c]Different superscripts identify groups that differ from each other, according to Fisher's LSD test (F-distribution = 10.16 and p ≤0.001)

MHNW: Metabolically healthy, normal-weight phenotype; MHONW: Metabolically healthy obese, normal-weight phenotype; MUONW: Metabolically unhealthy obese, normal-weight phenotype

**Figure 1: ROC curves of ultrasound indicators to distinguish the metabolically unhealthy obese, normal-weight (MUONW) phenotype in normal-weight pregnant women studied**




AADI: Abdominal adipose deposit index; AFI: Abdominal fat index; AUC: Area under the curve; BFI: Body fat index; FAI: Fat accumulation index; 95% CI: 95% confidence interval

**Discriminant capacity of AADI** Figure 1 presents the ROC curve which showed the greatest area under the curve for AADI (0.707; 95% CI: 0.62–0.79, p <0.001).

The cutoff point selected to detect the MUONW phenotype corresponded to an AADI value of 350 $mm^2$, located in the graph of Figure 1 at the coordinates Sensitivity = 0.879 (87.9% correctly diagnosed pregnant women) and 1–Specificity = 0.558 (55.8% false positives).

Table 2 shows the prevalence of MetS, as well as the descriptive statistics of the US variables in study participants.

When the Panel III criteria modified to identify MetS in pregnant women were applied as the gold standard, it was confirmed that 62 had MetS (11.8%), in whom a highly significant AADI increase was observed as an expression of increased abdominal adiposity (Table 2).

In the ROC curve (Figure 2) used to evaluate the discriminant ability of the AADI to detect MetS, the area under the curve was 0.705 (95% CI: 0.57–0.78), with a standard error of 0.064 and p = 0.003.

AADI's cutoff point was calculated at 350 $mm^2$, for presence of MetS, identified at coordinates 0.457 and 0.781 on the x- and y-axes, respectively, corresponding to 78.1% of participants with correct diagnosis of MetS and 45.7% false positives.

**Diagnostic test** The contingency table (Table 3) shows the cross-classification between modified MetS based on NCEP-ATP III criteria and AADI with its cutoff point.[28]

## DISCUSSION

Prevalence of the MUONW phenotype in normal-weight pregnant women was 6.3%, coinciding with the highest AADI values among all women studied. These results show that some pregnant women, despite classification as normal weight, exhibit increased abdominal adiposity along with an underlying metabolic abnormality.[2] This phenomenon has been noted by other authors such as Ahmadi, who detected MetS in 5.3% of non-obese women in the first trimester of pregnancy based on US evaluation of visceral fat thickness.[20] Pinto Lima reported a 3.0% prevalence of MetS in early pregnancy according to Panel III criteria, also using anthropometric measures, blood pressure, metabolic profile, and US measurements of SAF and VF thickness.[6]

The different capacity of the SAF to store triglycerides and the uncontrolled and growing flow of free fatty acids to other tissues with ectopic fat deposit and lipotoxicity are evident in the MUONW phenotype. This demonstrates that such a fat layer is not an inert depot, but rather can contribute to the pathogenesis of IR with greater circulation of cytokines and free fatty acids, elevating risk of complications during pregnancy.[29]

Research on subcutaneous adipose deposits and IR in non-pregnant women emphasizes the superficial and deep compartments of the subcutaneous fat layer and describes two histologically unique tissues separated by a discrete fascial plane. The fact that the thickness of the deep subcutaneous tissue layer varies with obesity and has been shown to be a better predictor of IR than usual indicators would explain that in

phenotypes classified as normal weight by their BMI, but which are actually obese due to their abdominal adipose tissue distribution, the layer of deep subcutaneous adipose tissue reveals a differentiation in metabolic behavior. This explains the characterization of the relative contribution of SAF and VF in dysmetabolism in pregnant women.[9] In the MUONW phenotype, the layer of subcutaneous adipose tissue is greater (while we were unable to discriminate between its superficial and deep layers, taking them into account together instead).

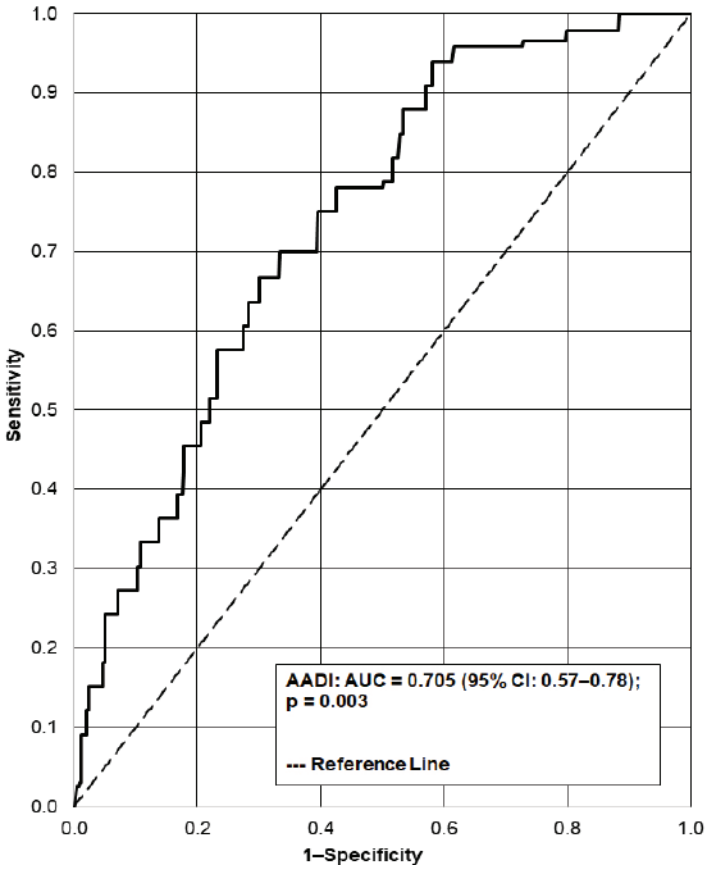
**Table 2: Prevalence of modified metabolic syndrome and descriptive statistics of the ultrasound variables in normal weight pregnant women**

| Variable | Modified metabolic syndrome | |
|---|---|---|
| | Absence (n = 464) 88.2% | Presence (n = 62) 11.8% |
| | Means (SD)[a] and Medians (Q1–Q3)[b] | |
| SAF | 11.28 (3.69) | 13.39 (3.61) |
| PPF | 10.60 (8.43–12.7) | 10.00 (8.73–12.88) |
| VF | 34.43 (9.35) | 35.25 (9.62) |
| AFI | 0.908 (0.72–1.14) | 0.793 (0.72–0.92) |
| FAI | 21.70 (8.03) | 23.97 (5.47) |
| BFI | 0.766 (0.38) | 0.91 (0.40) |
| AADI | 398.31 (188.7) | 474.88 (190.12) |

[a]Standard Deviation (SD); [b]Interquartile Range (IR)

AADI: abdominal adipose deposit index; AFI: Abdominal fat index; BFI: Body fat index; FAI: Fat accumulated index; PPF: Preperitoneal fat; SAF: Subcutaneous abdominal fat; VF: Visceral fat

**Figure 2: ROC curve for the abdominal adipose deposit index (AADI) to identify metabolic syndrome (MetS) in normal-weight pregnant women studied**




AADI: Abdominal adipose deposit index; AUC: Area under the curve; 95% CI: 95% confidence interval

Increased subcutaneous adipose tissue would cause the visceral fat to increase its triglyceride accumulation function, causing a greater metabolic dysregulation, detectable by applying the proposed AADI.

AADI values in the different groups studied, characterized by notable differences between the phenotypes MHNW and MHONW, and between MHONW and MUONW, confirm the common characteristics that identify metabolically unhealthy obesity with MetS in the determination of the MUONW phenotype. This confirms what is posited in other studies that reveal an association of subcutaneous and visceral adipose tissue—not just general adiposity—with various cardiometabolic risk factors.[30]

These results confirm the benefit of applying AADI in early pregnancy for normal-weight women, even when clinical manifestations of MetS have not been identified, which would allow for timely guidance regarding healthy behaviors.

The sensitivity demonstrated by the AADI diagnostic test with a high number of pregnant women correctly diagnosed with MetS, as well as the discriminant ability of this index for the MUONW phenotype in normal-weight women in early pregnancy, allows for diagnosis of the hidden risk of cardiometabolic disease based on ultrasound evaluation of SAF and VF. This demonstrates the robustness of the new index, which can be used in primary health care to reliably diagnose metabolic disorders at the start of pregnancy, with a practical value from the clinical perspective.

The principal limitation of this study is that the distinctive features of abdominal adiposity distribution were not studied, nor was its relationship with cardiometabolic risk factors in pregnant women according to their reproductive status.

The ultrasound measurement of SAF and VF and its later expression in the AADI together with the routine exam for normal-weight women in the first trimester of pregnancy is simple, low cost and feasible, given the availability of US machines in primary health care in Cuba, as part of the nationwide Maternal-Child Care Program.

Unlike Panel III, here the direct measurement of abdominal fat is performed, which allows for reliable diagnosis of unhealthy obesity phenotypes like metabolically obese, normal-weight phenotype with increased risk of cardiovascular disease and MetS. In this way, complications may be avoided during pregnancy among women apparently at low obstetric risk.

## CONCLUSIONS

AADI is better than other traditional indicators at detecting the risk of metabolic obesity in early pregnancy in normal-weight women, facilitating early intervention in clinical practice to prevent or delay progression of cardiometabolic disease in these women.

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# Adjusting Iron Deficiency for Inflammation in Cuban Children Aged Under Five Years: New Approaches Using Quadratic and Quantile Regression

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## ABSTRACT

**INTRODUCTION** Ferritin is the best biomarker for assessing iron deficiency, but ferritin concentrations increase with inflammation. Several adjustment methods have been proposed to account for inflammation's effect on iron biomarker interpretation. The most recent and highly recommended method uses linear regression models, but more research is needed on other models that may better define iron status in children, particularly when distributions are heterogenous and in contexts where the effect of inflammation on ferritin is not linear.

**OBJECTIVES** Assess the utility and relevance of quadratic regression models and quantile quadratic regression models in adjusting ferritin concentration in the presence of inflammation.

**METHODS** We used data from children aged under five years, taken from Cuba's national anemia and iron deficiency survey, which was carried out from 2015–2018 by the National Hygiene, Epidemiology and Microbiology Institute. We included data from 1375 children aged 6 to 59 months and collected ferritin concentrations and two biomarkers for inflammation: C-reactive protein and α-1 acid glycoprotein. Quadratic regression and quantile regression models were used to adjust for changes in ferritin concentration in the presence of inflammation.

**RESULTS** Unadjusted iron deficiency prevalence was 23% (316/1375). Inflammation-adjusted ferritin values increased iron-deficiency prevalence by 2.6–4.5 percentage points when quadratic regression correction model was used, and by 2.8–6.2 when quantile regression was used. The increase when using the quantile regression correction model was more pronounced and statistically significant when both inflammation biomarkers were considered, but adjusted prevalence was similar between the two correction methods when only one biomarker was analyzed.

**CONCLUSIONS** The use of quadratic regression and quantile quadratic regression models is a complementary strategy in adjusting ferritin for inflammation, and is preferable to standard regression analysis when the linear model's basic assumptions are not met, or when it can be assumed that ferritin–inflammation relationships within a subpopulation may deviate from average trends.

**KEYWORDS** Alpha-1-acid glycoprotein, C-reactive protein, anemia, iron deficiency, ferritin, acute phase protein, Cuba

## INTRODUCTION

Interpreting iron indicators in the presence of inflammation is a topic of particular interest to public health.[1] Serum ferritin concentration is recognized by WHO as the best indicator of populations' iron deficiency,[2] but inflammation can affect ferritin concentrations, as it is an acute phase protein (APP).[3] For this reason, WHO suggests accompanying ferritin measurements with measurements of other APPs to confirm the presence of inflammation.[1,2] Among the most widely-used inflammation biomarkers in clinical practice and nutrition research are C-reactive protein (CRP) and Alpha-1-acid glycoprotein (AGP).[4]

Several approaches have been proposed which use APPs to adjust for inflammation's effects on ferritin levels and other biomarkers,[5–8] but there is still no consensus as to a preferred method.[4] In addition to taking the advantages and limitations of each method into account, the choice of method must be weighed against the burden of infection in the country or region where it is applied.[9] Most studies have been conducted in low- to middle-income countries with moderate to high infection burdens.[10–15]

**IMPORTANCE** This study demonstrates the usefulness of two new approaches for correcting ferritin concentrations in the presence of inflammation, which would improve methods for evaluating iron deficiency in Cuban children aged under five years , and thus provide more reliable data on iron deficiency prevalence in the country.

Cuba is a developing country considered to have a population with a low level of inflammation. The most recent study on iron deficiency in children aged under five years supports the hypothesis that ferritin concentrations change in magnitude according to the state of inflammatory processes in the first five years of life.[16]

Iron status in Cuban children aged under five years is modified in the presence of inflammation.[16] In Pita's study,[16] the ferritin concentrations of 1375 children were adjusted for inflammation (measured by CRP and AGP), using four of the most well-known approaches in recent literature: a) higher ferritin cut-off point (>30 g/L); b) excluding subjects with inflammation (CRP >5 mg/L or AGP >1 g/L); c) CRP- or AGP-based arithmetic correction factors; and d) regression correction using the method proposed by the BRINDA group (Biomarkers Reflecting Inflammation

and Nutritional Determinants of Anemia).[17] The significant disparity between unadjusted ferritin values and those adjusted by some of the above approaches underlines the importance of correcting for inflammation and the need to develop adequate tools to examine the validity of the methods used for correction. Regression correction (RC) is recommended,[7] but the need for continued investigation into other methods of adjusting ferritin concoentrations is emphasized.[18]

The RC approach is based on subtracting inflammation's effects from observed ferritin concentrations. This approach is less subject to bias and allows for continuous correction of ferritin even for lower reference values for inflammation than those traditionally used.[19] However, it is based on the assumption of a linear effect of inflammation on iron deficiency indicators; in practice, relationships between iron status biomarkers and APPs (CRP and AGP) are not linear.[4] Additionally, different types of relationships could exist for subpopulations that deviate from average trends for heterogenous distributions. It may therefore be necessary to use more flexible regression models, like quadratic regression models[20] and quantile regression models.[21–24]

Quantile regression (QR) is considered a natural extension of the standard regression model and allows for separate regression models to be used for different parts of the dependent variable's distribution. QR's additional flexibility may broaden the description of inflammation's effect on ferritin's conditional distribution. An additional advantage to QR is that it does not depend on normality assumptions or transformations.

Some exogenous factors—such as observations below detection limits—can alter parameters of the dependent variable's conditional distribution. It is common practice to fill in undetected or censored data with a value equal to or less than the detection limit. However, when there are a considerable number of such replacements, estimates of mean effects and standard errors of least-squares regression models will not be reliable, risking erroneous conclusions. QR is more robust against these types of outliers. Therefore, QR models could be used to not only detect heterogenous CRP and AGP effects at different quantiles of ferritin values, but also to obtain more precise estimates compared to mean regression when normality assumptions are breached, or when there are outliers and long tails.[24]

Taking into account the need for more robust adjustment models that offer more precise measurements, we set out to estimate possible non-linear relationships and the usefulness and relevance of quadratic regression and quadratic regression by quantile models to explain ferritin concentration's relationship with CRP and AGP inflammation biomarkers in Cuban children aged under five years.

## METHODS

**Population, study area and variables** We used data pertaining to population, study area and variables obtained from Cuba's national anemia and iron deficiency survey, a cross-sectional study carried out by the National Hygiene, Epidemiology and Microbiology Institute (INHEM), from February through April each year from 2015–2018 in four randomly-selected regions of the country. The sample included 1375 presumably healthy children, with no diagnosis of chronic disease, aged 6 to 59 months, and complete serum ferritin, CRP and AGP records. A detailed description of sample selection can be found in the aforementioned article by Pita.[16]

**Case definition** Iron deficiency was defined as ferritin concentration <12 µg/L, the cut-off point recommended by WHO.[2] Acute inflammation was defined as CRP ≥5 mg/L and chronic inflammation as AGP >1 g/L.[2]

**Statistical analysis** Graphs and simple statistics were used to study the distribution of the three biochemical variables. All showed some kind of positive skew and were transformed to their natural logarithms to avoid disproportionate ranges. Once data was transformed, histograms and normal probability plots were constructed to visually judge normality.

To explore the relationship between ferritin and inflammatory biomarkers, children in the sample were divided into 10 subgroups, determined by intervals of equal length, according to ln(CRP) or ln(AGP) values. Box-and-whisker plots were constructed to observe ferritin concentration patterns in the different ranges of each inflammation biomarker.

APP's effect on ferritin was evaluated using models on a continuous scale. We used linear regression, quadratic regression ($R_c$) and quantile quadratic regression ($QR_c$). Models were adjusted considering each biomarker's effect (CRP and AGP) both separately and jointly. We constructed scatterplots to show bivariate associations.

To interpret iron stores in the presence of inflammation, ferritin concentrations were adjusted using two approaches: quadratic regression correction ($R_cC$) and quantile quadratic regression correction ($QR_cC$).

These analyses were conducted using the statistical software R, version 3.5.3 (Free Software Foundation, USA).[25] We used the The lm function of the Stats statistical package was used to fit models with the least squares method. Model fit by quantiles was performed using the rq function of the quantreg package.[26]

**Estimation of quantile regression's effects** While ordinary least squares regression (OLS) offers only information on the conditional mean, QR allows us to estimate conditional quantiles of a response variable's distribution $Y$ based on a set $\boldsymbol{X}$ of *p* predictor variables.

Analogous to linear regression, where $E(Y|\mathbf{X}) = \boldsymbol{X\beta}$, the QR model for a conditional quantile $\theta$ can be formulated as:

$$Quant_{\theta}(Y|\mathbf{X}) = \boldsymbol{X\beta_{\theta}},$$

where $0 < \theta < 1$ and $Quant_{\theta}(.|.)$ denote the conditional quantile function for the $\theta$–$\theta$th quantile. $Y_{[n]}$ is the response vector, $\boldsymbol{X}_{[n\times p]}$ is the explanatory variable matrix and $\boldsymbol{\beta}_{\boldsymbol{\theta}_{[p]}}(\theta)$ is the vector for unknown parameters for the generic conditional quantile $\theta$.

Unlike OLS regression, in which a single regression line is fitted, QR has multiple lines, and therefore, as many coefficient vectors $\boldsymbol{\beta}_{\theta}$ as quantiles are considered.

Parameter estimates in linear QR models are interpreted in the same way as any other linear model. Therefore—as in the OLS model—in the case of a multivariate QR with *p* explanatory variables, the QR model coefficient $\beta_{\theta j}$ can be interpreted as the rate of change of the *Θth* quantile of the dependent variable distribution by unit change in the jth regressor:

$$\beta_{\theta j} = \frac{\partial Quant_{\theta}(Y|\boldsymbol{X})}{\partial \boldsymbol{x}_j}$$

A median regression ($\theta$ = 0.5) of ferritin concentrations on inflammation biomarkers specifies changes in median ferritin concentration as a function of predictors. Indicators' effects on median ferritin concentration can be compared with their effects on other ferritin quantiles. As we increase from 0 to 1, we can determine the full distribution of $\boldsymbol{Y}$, conditional on $\boldsymbol{X}$.

**Quantile regression correction** Suppose that we have data $\{(Y_i, \boldsymbol{X}_i),\ i = 1,2,\ldots,n\}$, and that the parameter of interest is the conditional quantile $\theta$ of $Y_i$, given by $\boldsymbol{X}_i$. Pairs $(Y_i, \boldsymbol{X}_i)$ are assumed to be observations of randomly-selected individuals from a population.

While the effects of CRP and AGP are uniquely calculated in linear regression, in quantile regression they vary depending on the desired quantile. It is possible to identify for each individual the QR model that can best predict the response variable to provide a unique vector of coefficients.[23]

Consider the QR model for a given conditional quantile *θ:*

$$Quant_{\theta}(\hat{\boldsymbol{y}}|\mathbf{X}) = \boldsymbol{X}\hat{\boldsymbol{\beta}}_{\theta},$$

The generic element of matrix $\hat{\boldsymbol{Y}}_{[n\times k]}$ is the dependent variable's estimate corresponding to the *i*th individual according to the *θ*th quantile.

The best estimate for each individual is the one that minimizes the difference between observed and estimated values for each of the *k* models:[23]

$$\theta_i: argmin_{\theta=1,\ldots,k}|y_i - \hat{y}_i(\theta)| \quad (1)$$

Once $\theta_i$ is identified for each individual *i,* ferritin concentrations are adjusted by subtracting the estimated effects of inflammation on the corresponding quantile assigned to each individual.

Take, as an example, the measurements of ln(ferritin) and ln(CRP) of the random sample of Cuban children aged under five years. The quantile quadratic model used to evaluate the effect of each inflammation biomarker on different parts of ferritin's conditional distribution is expressed as follows:

$$ln(ferritin)_{\theta} = \beta_{0\theta} + \beta_{1\theta}\ln(CRP) + \beta_{2\theta}ln^2(CRP) \quad (2)$$

where $\beta_{0\theta}$ is the intercept and $\beta_{1\theta}$ and $\beta_{2\theta}$ are the regression coefficients of the *θ*th quantile.

For all QR models formulated in this research, five conditional quantiles (θ = 0.10, 0.25, 0.50, 0.75, 0.90) were used for synthesis purposes.

The graphs of each quadratic quantile function are parabolas in the form of ∪, so the vertex is their lowest point. In this study, a threshold for inflammation was defined as the point at which the quadratic quantile function was minimized. This occurs when:

$$ln(CRP)_{\theta} = -(\hat{\beta}_{1\theta}/2\hat{\beta}_{2\theta})$$

Where $\hat{\beta}_{1\theta}$ and $\hat{\beta}_{2\theta}$ are the estimates of the regression coefficients of each quantile function in (2). Once identified $\theta_i$ for each individual according to the criteria in (1), ferritin concentrations were adjusted by performing the following transformation:

$$ln(ferritin^*)_{\theta_i} = ln\ (ferritin)_{\theta_i} - \hat{\beta}_{1\theta_i}\left[ln(CRP) - ln(CRP_{ref})_{\theta_i}\right] - \hat{\beta}_{2\theta_i}\left[ln^2(CRP) - ln^2(CRP_{ref})_{\theta_i}\right]$$

To avoid overfitting, the correction was applied according to the following expression:

$$ln(ferritin)_{adj} = \begin{cases} ln(ferritin^*)_{\theta i}, & if \quad ln(CRP)_i > ln(CRP_{ref})_{\theta_i} \\ ln(ferritin), & if \quad ln(CRP)_i \leq ln(CRP_{ref})_{\theta_i} \end{cases}$$

The *adj* subscript refers to the ferritin concentrations' fitted values. The *ref* subscript refers to inflammation reference values, under the assumption that they mark the cutoff points of inflammation biomarkers, from which ferritin concentrations increase.

Results on the values of inflammation-adjusted ferritin concentrations were expressed in the original measurement scale. Iron deficiency was determined by applying a ferritin cut-off of <12µg/L[2] to inflammation-corrected ferritin concentrations.

**Ethics** This research was approved by INHEM's research ethics committee, and that of the Cybernetics, Mathematics and Physics Institute. Information was kept confidential, without revealing the childrens' identity.

## RESULTS

**Participant characteristics** After an exploratory analysis, 10 children with extreme values were excluded from the study: 3 with CRP values >76 mg/L, and 7 with AGP values ≤0.012 g/L. The sample was thus comprised of 1365 children. Sample distribution by age and sex was as follows: 36.3% (496/1365) <2 years; 63.7% (869/1365) ≥2 years; 50.4% (688/1365) boys; 49.6% (677/1365) girls.

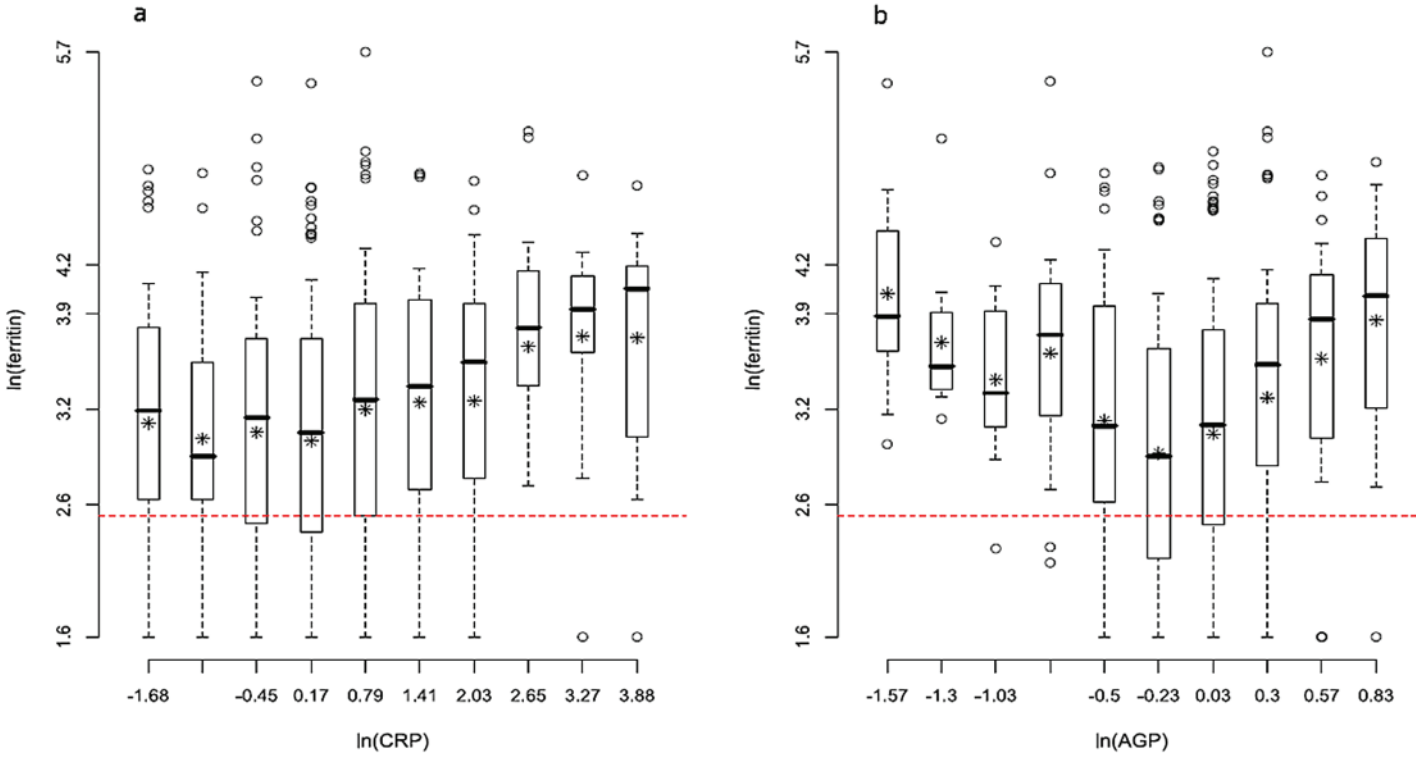
Iron deficiency prevalence (ferritin <12 µg/L) was 23.2% (317/1365). The prevalence of acute inflammation (CRP ≥5 mg/L) was 11% (150/1365) and that of chronic inflammation (AGP >1 g/L) was 30.8% (420/1365).

Ferritin concentrations changed when considered together with CRP and AGP values that were above or below the cutoff points established to define inflammation. Median ferritin concentration in children CRP-measured inflammation was 46.3 µg/L (Q1 = 20.8, Q3 = 64.1) and that in children without inflammation was 24.0 µg/L (Q1 = 12.2, Q3 = 47.5). Median ferritin concentration of AGP-measured inflammation was 34.5 µg/L (Q1 = 17.1, Q3 = 53.65) and that of children without inflammation was 23.0 µg/L (Q1 = 11.7, Q3 = 44.3).

**Relationship between inflammation and iron deficiency** Figure 1 shows the means and the 0.10, 0.25, 0.50, 0.75 and 0.90 quantiles of ln(ferritin) in each subgroup, of the variables ln(CRP) and ln(AGP), respectively.

The distribution of ln(ferritin) conditioned to ln(CRP) is similar in the first subgroups, but as the magnitude of inflammation increases, the distribution of ln(ferritin) shifts toward higher values. Moreover,

**Figure 1: ln(ferritin) concentration distributions in each subgroup according to: (a) ln(CRP) and (b) ln(AGP)**




AGP: Alpha-1-acid glycoprotein; CRP: C-Reactive protein
The mean of ln(ferritin) in each subgroup is represented by asterisks, while the median is represented by the line that cuts the box delimited by the first and third quartiles. The extremes represent the symmetrical quartiles 0.10 and 0.90. The dashed horizontal red line represents the cut-off point for iron deficiency (ferritin <12 µg/L). The values indicated on the vertical axis correspond to the values of the quantiles 0.10, 0.25, 0.50, 0.75 and 0.90 of the variable ln(ferritin) for the entire sample. The values on the horizontal axis correspond to the upper limits of the intervals defined by the subgroups.

this variability does not seem to be constant (Figure 1a). Figure 1b shows a non-linear pattern in which ferritin concentrations are low when inflammation is moderate, and are high, for both high and low ln(AGP) values.

**Effects of CRP and AGP on ferritin estimation** In univariate linear regression models, the effect of ln(CRP) on ln(ferritin) was significant (0.105, p <0.000), suggesting that on average, when CRP values increase, ferritin concentrations also increase, but mean ln(ferritin) concentrations did not change significantly (0.066; p = 0.298) with increasing ln(AGP). When both inflammation biomarkers were included in a model, both effects were significant. The estimated effect of ln(CRP) was positive (0.128, p = 0.021), but the effect of ln(AGP) was negative (–0.162, p = 0.025).

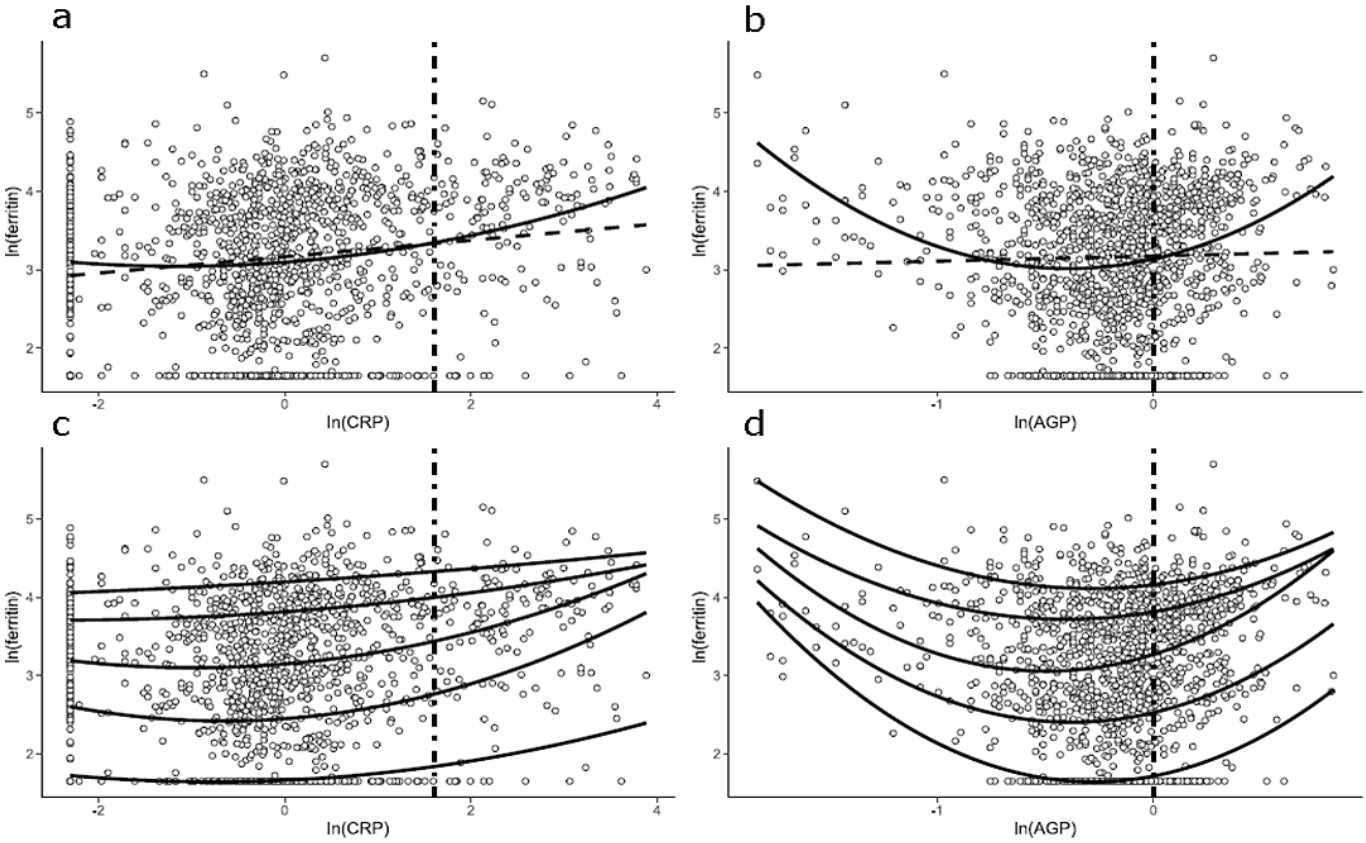
Graphs were used to find inconsistencies as per assumptions of models' normality, linearity and homoscedasticity. Using information from Figure 1 as a guide, we evaluated quadratic functions in order to achieve a better fit. For illustrative and comparative purposes, Figure 2 shows two graphs that represent the linear and quadratic regression functions for each of the univariate models.

The minimum value of the nonlinear function is reached when ln(CRP) is –1.182, so the CRP value from which inflammation begins to positively influence ferritin values is 0.31 mg/L (Figure 2a), but it is nearly impossible to visually discriminate ferritin values below the vertex of the quadratic function. Inside the range of CRP values defined by the segment where linear and quadratic functions intersect, the fit of the two functions is similar, and ferritin concentrations increase more rapidly as inflammation increases.

The quadratic model (Figure 2b) shows a U-shaped relationship between ln(ferritin) and ln(AGP), with the vertex at ln(AGP) of –0.397. Thus, inflammation begins to positively influence ferritin from AGP = 0.67 g/L. On average, ferritin concentrations tend to rise in individuals with high AGP values.

Table 1 shows the estimates of the quadratic regression models ($R_c$) for the conditional means of the combined and individual

**Figure 2: Linear and quadratic relationships between: (a) ln(CRP) and ln(ferritin); (b) ln(AGP) and ln(ferritin). Quadratic relationship by the following quantiles: (θ = 0.1; 0.25; 0.50; 0.75; 0.9) between (c) ln(CRP) and ln(ferritin); (d) ln(AGP) and ln(ferritin)**




AGP: Alpha-1-acid glycoprotein; CRP: C-Reactive protein
Gray dots represent the concentrations of the iron deficiency biomarker (ferritin). In graphs (a) and (b) the dashed line is the linear regression line and the solid line represents the quadratic function fitted to the data. In figures (c) and (d) the curves represent the quadratic functions, by quantile. The vertical dashed line indicates the WHO-recommended cut-off point defining inflammation (5 mg/L for CRP and 1 g/L for AGP).

inflammation biomarkers: $R_c$-(CRP), $R_c$-(AGP) and $R_c$-(CRP, AGP). Estimated coefficients were significant ($p < 0.05$). Adjusted $R^2$ values were small, but higher than those obtained using linear models. Due to the high heterogeneity of the data, the quadratic models can only explain a small portion of ln(ferritin)'s variation around the mean. Associations explaining ferritin's relationship to CRP values may be different in other parts of the conditional ferritin distribution.

**Quantile regression feasibility and adequacy** Quantile regression shows variation between ln(ferritin)'s quantile distribution. In different parts of the distribution, proposed models show an inflammation–iron deficiency relationship, which, as expected, was not linear and was identified in regression to the mean. Table 1 shows estimated coefficients for the 0.10, 0.25, 0.50, 0.75 and 0.90 quantiles of three models: $QR_c$, $QR_c$-(CRP), $QR_c$-(AGP) and $QR_c$-(CRP, AGP).

Quadratic regression models $R_c$-(CRP) and $QR_c$-(AGP) showed a significant parabolic correlation ($p < 0.05$) between ferritin concentrations and inflammation. In the case of the $QR_c$-(AGP) model, estimated coefficients in all quantiles differ significantly from zero. In the $QR_c$-(CRP), the coefficients associated with the linear ln(CRP) at the 0.10 quantile and the quadratic $ln^2$(AGP) at the 0.90 quantile were not significant. As in the case of regression to the mean, the $QR_c$ models confirm that the relationship between ferritin and both inflammation biomarkers is not completely linear.

The graphic versions of the quadratic fits of the estimated conditional quantiles in Table 1 (models 4 and 5) can be seen in Figures 2c and 2d.

Inflammation's effect on ferritin seems to be accentuated as CRP values increase (Figure 2c), as seen in children in the 0.25 and 0.50 percentiles. In the two highest quantiles, ferritin values also increased due to the effect of inflammation; but the increase is discrete and almost linear.

Figure 2d shows a positive increase of ferritin from each quantile function's vertex. As AGP values increase, the effect of inflammation decreased toward the tail's upper distribution (0.75 and 0.90 percentiles).

When both inflammation biomarkers were considered in the $R_c$-(CRP, AGP) quadratic regression model, all estimated coefficients showed a statistically significant positive effect, with non-linear ferritin growth (Table 1).

Estimation by quantiles in the $QR_c$-(CRP, AGP) model suggests a more complex situation (Table 1), since the influence of explanatory variables on ferritin varied from one quantile to another, and some were significant in only some quantiles. The ln(CRP)'s effect was only statistically significant in the subpopulation of children who had the highest iron reserves. The $ln^2$(AGP) variable is the

**Table 1: Parameter estimates (and standard errors) of quadratic OLS and quantile regression models for ln(ferritin)**

| | Mean | Quantile | | | | |
|---|---|---|---|---|---|---|
| | | 0.10 | 0.25 | 0.50 | 0.75 | 0.90 |
| **Models** | **R-(CRP²)** | **QR-(CRP²)** | | | | |
| Intercept | 3.097* (0.029) | 1.664* (0.014) | 2.448* (0.059) | 3.147* (0.043) | 3.814* (0.031) | 4.196* (0.036) |
| ln(CRP) | 0.092* (0.018) | 0.059 (0.033) | 0.088* (0.031) | 0.099* (0.023) | 0.086* (0.018) | 0.073* (0.021) |
| ln(CRP)² | 0.039* (0.009) | 0.031 (0.016) | 0.067* (0.017) | 0.051* (0.010) | 0.017* (0.007) | 0.006 (0.011) |
| Adjusted $R^2$ and pseudo $R^2_\theta$ | 0.036 | 0.001 | 0.021 | 0.034 | 0.020 | 0.013 |
| | **R-(AGP²)** | **QR-(AGP²)** | | | | |
| Intercept | 3.137* (0.026) | 1.701* (0.027) | 2.524* (0.053) | 3.269* (0.041) | 3.815* (0.026) | 4.159* (0.032) |
| ln(AGP) | 0.617* (0.087) | 0.418* (0.133) | 0.650* (0.166) | 0.862* (0.129) | 0.478* (0.064) | 0.328* (0.100) |
| ln(AGP)² | 0.779* (0.087) | 0.751* (0.143) | 0.859* (0.124) | 0.884* (0.127) | 0.588* (0.096) | 0.574* (0.110) |
| Adjusted $R^2$ and pseudo $R^2_\theta$ | 0.053 | 0.027 | 0.032 | 0.034 | 0.024 | 0.020 |
| | **R-(CRP²,AGP²)** | **QR-(CRP², AGP²)** | | | | |
| Intercept | 3.079* (0.031) | 1.704* (0.020) | 2.428* (0.062) | 3.147* (0.048) | 3.792* (0.031) | 4.126* (0.038) |
| ln (CRP) | 0.074* (0.030) | 0.042 (0.031) | 0.058 (0.038) | 0.091* (0.028) | 0.069* (0.021) | 0.076* (0.028) |
| ln (AGP) | 0.330* (0.111) | 0.410* (0.091) | 0.417* (0.198) | 0.304* (0.135) | 0.237* (0.082) | 0.066 (0.135) |
| ln (CRP)² | 0.023* (0.009) | 0.018 (0.012) | 0.053 (0.018)* | 0.033* (0.010) | 0.007 (0.008) | 0.004 (0.012) |
| ln(AGP)² | 0.595* (0.096) | 0.745* (0.094) | 0.677* (0.109) | 0.450* (0.126) | 0.438* (0.062) | 0.414* (0.133) |
| Adjusted $R^2$ and pseudo $R^2_\theta$ | 0.064 | 0.028 | 0.041 | 0.045 | 0.030 | 0.027 |

AGP: Alpha-1-acid glycoprotein; CRP: C-Reactive protein; OLS: Ordinary least squares regression; QR: Quantile regression

Standard errors appear in parentheses. The * indicates significant coefficients (p <0.05) adjusted $R^2$ and pseudo $R^2_\theta$ represent the capacity of the independent variables to explain the variation of ferritin in the OLS and QR regression models, respectively.

most important in explaining ferritin's variation throughout the conditional distribution.

**Statistical significance of OLS and QR estimate difference** Within the 0.25 to 0.90 quantiles of the $QR_c$-(CRP) model, estimates of ln(CRP)'s effects are within the estimation interval of the OLS regression (Figure 3a), which indicates that in this part of ferritin's conditional distribution, the linear relationships identified by $QR_c$ are the same as those suggested by classical regression. However, the effect of $ln^2$(CRP) at the 0.25 quantile is significantly higher than the OLS estimate, suggesting that in this part of the distribution, CRP's quadratic effect is higher than the OLS estimate.

Figure 3b shows that the estimates of the effect of ln(AGP) in the QRc-(AGP) model is significantly higher at the 0.50 quantile of the distribution when compared to the mean estimate, indicating that around the median of the conditional distribution of ferritin, the linear effect of AGP could be greater than that estimated by regression to the mean.

The estimates of the linear effects of the two inflammation biomarkers on ln(ferritin) at almost all quantiles in the $QR_c$-(CRP, AGP) model are within or very close to the OLS estimate's confidence interval limits (Figure 3c), while the effect of $ln^2$(CRP) at the 0.25th percentile is significantly larger than the estimate for the mean, confirming that CRP's quadratic effect is larger than that estimated by classical regression in this part of the distribution.

**Statistical significance of differences between estimated coefficients at conditional quantiles** The analyses of greatest interest focused on the 0.10, 0.25, and 0.50 quantiles, where adjusting for the effect of inflammation on ferritin concentrations was most likely to produce a change in iron deficiency prevalence, since they represent the subpopulation of children with ferritin concentrations around the WHO-recommended cut-off point defining iron deficiency.

The effects of CRP (both linear and quadratic) are similar across quantiles in the $QR_c$-(CRP) model. The coefficient equality test[27] shows that there are no statistically significant differences between estimated values in the 0.10, 0.25 and 0.50 quantiles. In the $QR_c$-(AGP) model, statistically significant differences were found (F = 5.0548, p <0.05) in AGP's linear effect between the 0.10 and 0.50 quantiles.

The tests for the equality of the estimated coefficients in the QRc-(CRP, AGP) model indicate that there are significant differences in the quadratic effect of CRP between the 0.10 and 0.25 quantiles (F = 4.1486, p <0.05) and in the effect quadratic of the AGP between quantiles 0.10 and 0.50 (F = 4.5198, p <0.05).

**Impact of adjustments on estimated iron deficiency prevalence** Table 2 summarizes the median ferritin estimates and adjusted and non adjusted iron deficiency prevalences for the two correction approaches. Adjusting ferritin concentrations using internal reference values for inflammation produced a mean increase in iron deficiency prevalences of 2.6, 2.7 and 4.5 percentage points according to the $R_cC$-(CRP), $R_cC$-(AGP) and $R_cC$-(CRP, AGP) methods. Iron deficiency prevalence calculated using the $QR_cC$-(CRP), $QR_cC$-(AGP), and $QR_cC$-(CRP, AGP) methods led to a mean increase of 2.8, 2.8, and 6.3 percentage points (Table 2).

The differences in the estimated prevalence of iron deficiency before and after adjustment for RcC-(CRP), QRcC-(CRP), RcC-(AGP) and QRcC-(AGP) do not reach statistical significance,

(see graph in Table 2), but they could be important from an epidemiological point of view, since the upper limit of the intervals reaches differences in prevalence >6% with respect to the unadjusted model. However, their confidence intervals overlap, so it cannot be ruled out (with an error probability of less than 5%), that the small prevalence differences obtained from these models are due to chance.

The differences in the prevalence of iron deficiency estimated before and after adjustment by RcC-(CRP, AGP) and QRcC-(CRP, AGP) models are statistically significant ($p < 0.05$). Estimated prevalence is higher when QRcC-(CRP, AGP) is used. In this case, the confidence interval's upper limit for the differences in prevalence is greater than 9%.

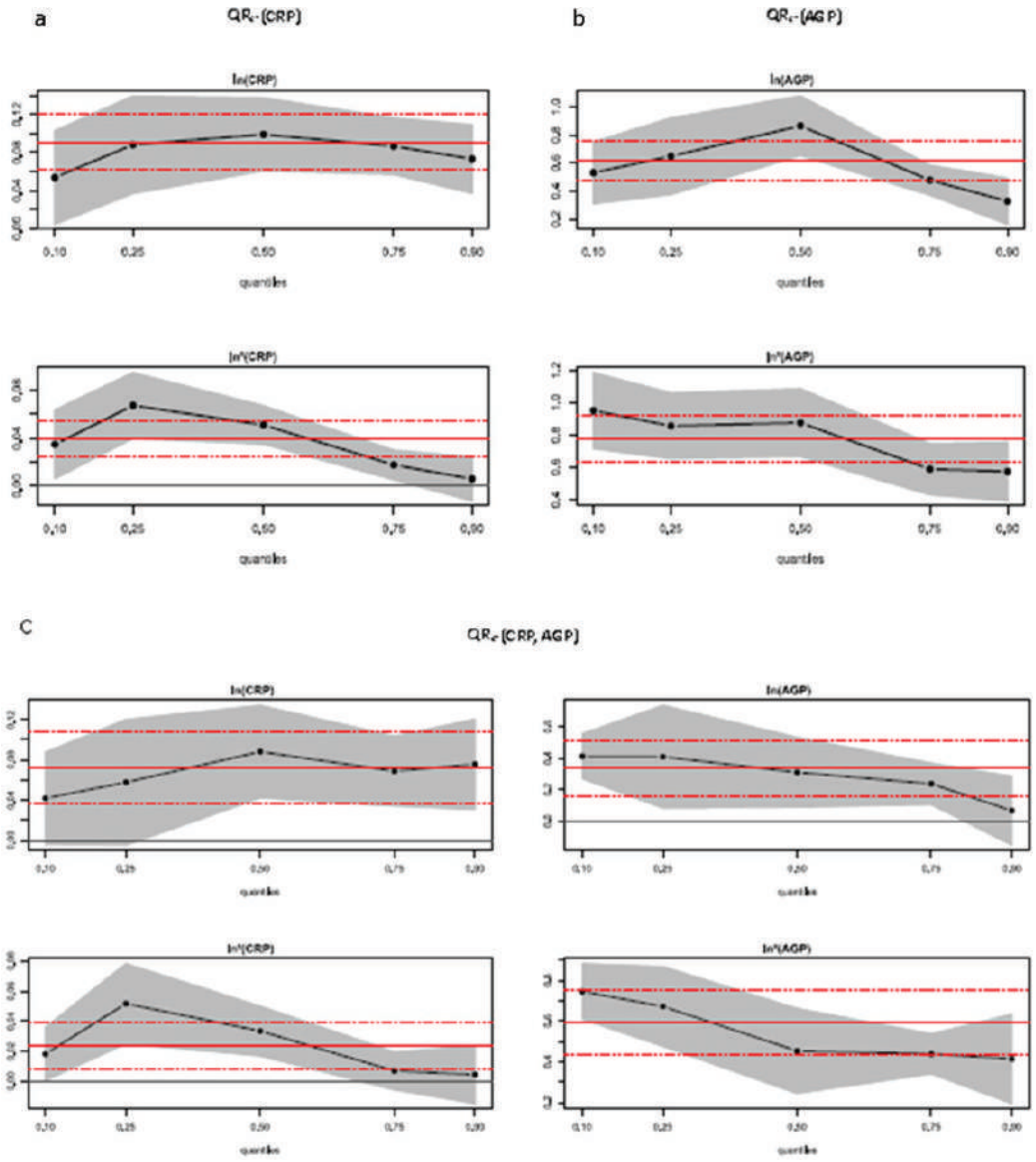
## DISCUSSION

Coefficients estimated by linear regression for the sample of 1365 children were slightly different than those obtained by Pita,[16] due to the exclusion of the 10 individuals with outliers in AGP and CRP values.

The small values of the $R^2$ and pseudo $R^2$ fit measures[28] and the significant p-values of coefficients in the quadratic regression and quantile quadratic regression models indicate that although the data show high variability, there is a non-linear trend to the response which offers relevant information on the relationship between ferritin and biomarkers for inflammation. Note that the goal of regression correction methods is not to predict ferritin concentrations, but to remove inflammation's effect on ferritin concentrations. In quantile regression, the best estimate for each individual is the one that minimizes the absolute difference between observed values and estimated values for each of the quantile models.

Quantile regression was able to detect that inflammation may have different effects on different parts of iron status' conditional distribution. In agreement with other studies,[6,29] we found that high concentrations of CRP and AGP are associated with high ferritin concentrations, but it would be risky to generalize the effect of inflammation on these values. These results suggest that inflammation exerts less influence on ferritin concentrations in children with the highest iron stores.

**Figure 3: Regression coefficients estimated by OLS and QR**




AGP: Alpha-1-acid glycoprotein; CRP: C-Reactive protein; OLS: Ordinary least squares regression; QR: Quantile regression

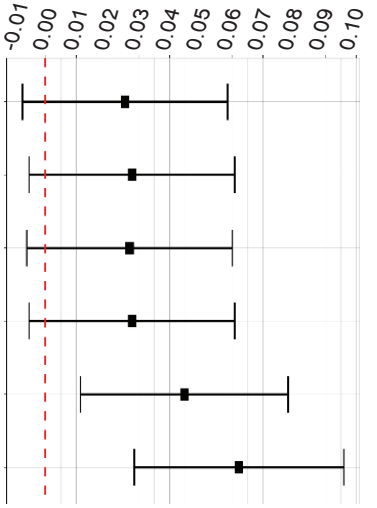
The x-axis represents the different conditional quantiles. In each panel, the horizontal red lines represent the point estimate (solid line) and the 95% confidence interval (dashed lines) of the OLS regression coefficients. Black dots interconnected by solid lines represent point estimates of the QR coefficients. The gray area shows the 95% confidence intervals.

Reference values for serum ferritin concentrations fall within the range of 15–300 µg/L, and are lower in children.[9] Apparently-healthy children were selected in the current study, so it is expected that in the subpopulation with high ferritin concentrations resulting from high iron stores, physiological homeostasis would be expected to control inflammation's effect on regulating deviations in iron status indicator ranges, thus maintaining a state of balance in the body.

The use of quadratic regression models to estimate inflammation's influence on ferritin allows us to recognize that the mean effect of inflammation on ferritin concentrations in this sample is manifested from AGP and CRP values below the limits WHO designates as clinically relevant (CRP ≥5 and AGP >1).[2]

**Table 2: Medians and interquantile ranges (IQR) of unadjusted and inflammation-adjusted ferritin; estimated prevalence of iron deficiency (% ID), unadjusted and adjusted for inflammation. Prevalence differences (proportions and 95% confidence intervals) of ID before and after adjustment by each correction method**

| Models | Ferritin | | Iron Deficiency | | 95% CI and proportion differences |
|---|---|---|---|---|---|
| | Median | IQR | % ID | n | |
| Unadjusted | 25.30 | 13.00–48.7 | 23.22 | 317 | |
| Adjusted | | | | | |
| $R_cC$-(CRP) | 23.04 | 11.69–41.58 | 25.79 | 352 | |
| $QR_cC$-(CRP) | 22.70 | 11.51–40.76 | 26.01 | 355 | |
| $R_cC$-(AGP) | 23.28 | 11.59–41.21 | 25.93 | 354 | |
| $QR_cC$-(AGP) | 21.90 | 11.57–42.58 | 26.01 | 355 | |
| $R_cC$-(CRP, AGP) | 21.68 | 10.94–38.46 | 27.69 | 378 | |
| $QR_cC$-(CRP, AGP) | 21.11 | 10.31–40.50 | 29.45 | 402 | |




AGP: Alpha-1-acid glycoprotein; CI: Confidence interval; CRP: C-Reactive protein; QRcC: Quantile quadratic regression correction; RcC: Quadratic regression correction
N = 1365

Interest in correcting ferritin focused on the 0.25 quantile, which represents the subpopulation of children with ferritin concentrations close to the cut-off point recommended by WHO to signal iron deficiency (ferritin <12 µg/L). According to the $QR_cC$-(CRP, AGP) method, 80.8% of children with potentially overestimated ferritin values—who, after applying the inflammation correction changed their status from adequate iron stores to iron deficiency—were part of the 0.25 quantile subpopulation.

Inflammation's effect on the population of children with the highest (0.75 and 0.90 quantiles) and lowest (0.10 quantile) iron stores also provoked an overestimation of ferritin values. However, while an individual correction for inflammation would result in a decrease in estimated ferritin concentration values, these changes would not modify the children's iron status classification, or, consequently, iron deficiency prevalence.

Using QR as a method of estimating the effects of inflammation on ferritin reduces the bias that undetected ferritin values can introduce. In this investigation, the censored data comprise part of the 10% of observations located below the regression line of the 0.10 quantile, so when substituting the unknown value for the minimum detected value, there is no significant change in estimates of the effects of inflammation biomarkers on ferritin at the distribution's lower end.

Inflammation's effects may be more important in some subpopulations than in others, but if the effects estimated in the conditional quantiles are considered equivalent to the effect estimated using only the conditional expectation, the $R_cC$ approach may be preferred over the $QR_cC$ method, due to its simplicity; especially in population studies. But before making decisions based on these results, the $QR_c$ estimate's confidence intervals should be checked to see whether they include values with important epidemiological implications.

Another aspect to consider is the choice of inflammation cut-off points, because iron deficiency estimates depend not only on figures of iron reserves in the population, but also on the presence of inflammation in the population. To avoid overfitting, corrections of ferritin concentrations are restricted to individuals whose inflammatory biomarkers exceed reference values. The BRINDA group[7] recommends using the upper limit of the first decile of each biomarker as a reference value. Based on this criteria, Pita[16] obtained a CRP reference value of 0.10 mg/L and an AGP reference value of 0.54 g/L.

In the current study, the non-linear trend between inflammation and ferritin concentrations obtained with the quadratic regression models $R_c$-(CRP) and $R_c$-(AGP) showed that in a population with low inflammation levels (such as those in the Cuban population),[16] the threshold from which we can assume that inflammation begins to exert influence on ferritin concentrations may be greater than that determined by the upper limit of the first decile (CRP = 0.31 mg/L and AGP = 0.67 g/L).

The $QR_cC$ approach reflects the underlying relationship between ferritin and inflammation better than the RC approach, but differences in the estimated effects along the conditional distribution of ferritin with respect to mean effects did not produce important differences in iron deficiency prevalences adjusted by both methods.

Inflammation's effect on ferritin may be greater in some subpopulations and therefore the adjusted concentrations in these subpopulations will decrease more compared to adjusted concentrations in other subpopulations. However, these differences will only be important to prevalence if they occur in the subpopulation of children whose ferritin values are around the 0.25 quantile, which is the quantile closest to the cut-off point recommended by WHO to define iron deficiency (ferritin <12 µg/L).[2]

In this investigation, the effects of inflammation that were statistically significant at the 0.25 quantile only occurred for CRP's quadratic effect in the $QR_c$-(CRP) and $QR_c$-(CRP, AGP) models.

Compared with unadjusted prevalence, the $R_cC$ and $QR_cC$ approaches led to similar iron prevalence estimates when ferritin concentrations were adjusted for CRP or AGP.

The highest estimates were obtained when ferritin concentrations were adjusted for both biomarkers, particularly when the $QR_cC$ approach was used. Developing tools to examine correction method validity is both merited and necessary.

One limitation of this study is that the children selected for the study came from a two-stage cluster sample,[16] so

the results may not reflect the diversity of the entire Cuban population. Furthermore, its cross-sectional nature precludes analysis of any seasonal influence of inflammation on ferritin concentrations.

## CONCLUSIONS

The combined use of quadratic regression and quantile regression is a useful analytical resource to explain the peculiarities of how ferritin levels change in the presence of inflammation. Each function's vertex can be a guide suggesting the threshold from which inflammation begins to influence ferritin concentrations. The quantile regression correction allows estimating higher prevalences of iron deficiency if CRP and AGP values are included at the same time. Correction methods using quadratic regression and quantile quadratic regression models confirm that inflammation can lead to underestimating iron deficiency prevalence in Cuban children aged under five years.

The proposed approach can be used to complement standard correction analysis. Comparisons using different correction methods can reduce discrepancies between statistical estimates, while helping interpret results in both biochemical and epidemiological terms.

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# Effect of Cuban Porcine Pulmonary Surfactant (Surfacen) and rCmPI-II Protease Inhibitor on Neutrophil Elastase Activity

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## ABSTRACT

**INTRODUCTION** In inflammatory respiratory diseases, the imbalance between proteases and endogenous protease inhibitors leads to an exacerbated activity of human neutrophil elastase (a protease that destroys the extracellular matrix and stimulates proinflammatory cytokine release). Elastase is considered a target in the search for therapeutic treatments for inflammatory respiratory diseases. Pulmonary surfactant is a promising product for this purpose, because in addition to its biophysical function, it has anti-inflammatory properties.

**OBJECTIVE** Evaluate effect of the Cuban porcine pulmonary surfactant (Surfacen), the rCmPI-II elastase inhibitor, and the Surfacen/rCmPI-II combination on activated neutrophil elastase activity in vitro, and determine if Surfacen's interface property changes in the presence of the inhibitor.

**METHODS** The anti-elastase effect of Surfacen, rCmPI-II and the Surfacen/rCmPI-II combination was evaluated in an in vitro model of activated neutrophils, previously purified from the blood of healthy subjects. The cells were stimulated with LPS/fMLP and were incubated with different concentrations of Surfacen, rCmPI-II and the Surfacen/rCmPI-II combination. Elastase activity was measured. The interface property was determined on a Langmuir surface balance.

**RESULTS** Surfacen at 10 mg/mL inhibited 71% of stimulated neutrophil elastase activity. rCmPI-II at 0.1 µM reduced 20% of elastase activity; at 200 µM—the maximum concentration evaluated—inhibition was 68%. Both products had a dose-dependent effect. The Surfacen/inhibitor combination (0.5 mg/mL/80 µM) did not affect the surfactant interface property or the inhibitory activity of rCmPI-II against human neutrophil elastase.

**CONCLUSIONS** Surfacen and the rCmPI-II inhibitor have an anti-elastase effect on an activated neutrophil model. rCmPI-II does not affect Surfacen's interface property and, therefore, both can be evaluated for combined use in treating inflammatory lung diseases.

**KEYWORDS** Pulmonary surfactants, elastase inhibitor, drug carriers, neutrophils, Cuba

## INTRODUCTION

Endogenous pulmonary surfactant is a complex mixture of lipids and proteins. It is synthesized and secreted into the alveolar space by type II pneumocytes. Its main function is to reduce surface tension at the air–liquid interface in the alveolus, preventing alveolar collapse and reducing respiratory effort. Pulmonary surfactant is essential and its absence, deficiency or inactivation is associated with several pulmonary diseases, such as neonatal respiratory distress syndrome (NRDS, or hyaline membrane disease), acute respiratory distress syndrome (ARDS), meconium aspiration syndrome, asthma and chronic obstructive pulmonary disease.[1] In addition to improving pulmonary function and oxygenation, surfactant is a key modulator of innate and acquired pulmonary immunity.[2,3]

Natural exogenous surfactant preparations are quite safe and effective standard therapy in newborns with NRDS.[4] The preparations used in neonatology services come from bovine or porcine surfactant extracts. Surfactants for clinical use are obtained from bronchoalveolar lavage or from lung tissue. Hydrophobic lipid and protein fractions, which contain the surfactant's main tensoactive components, are extracted using organic solvents, usually chloroform/methanol. Five surfactant preparations have been marketed by large pharmaceutical companies, all registered for use in NRDS: Curosurf (Chiesi Farmaceutici, Italy); Survanta (AbbVie, USA); BLES (BLES, Canada); Infasurf (ONY Biotech, USA), and Alveofact (Boehringer-Ingelheim, Germany).[5] Cuba also markets a registered surfactant preparation called Surfacen.[6] WHO includes these kinds of products on its Essential Medicines List.

**IMPORTANCE** Surfacen, rCmPI-II, and the Surfacen/rCmPI-II combination are shown to inhibit elastase in vitro in a stimulated neutrophil model, which supports research targeting its therapeutic application in inflammatory respiratory diseases.

Several respiratory diseases characterized by a strong inflammatory response involve an imbalance between proteolytic enzymes and their inhibitors. Neutrophils (and especially neutrophil elastase) are the main inflammatory mediators involved in acute alveolar injury and interstitial edema related to an increase in vascular permeability in ARDS.[7] In other chronic inflammatory lung conditions (cystic fibrosis, chronic obstructive pulmonary disease and bronchiectasis), abundant neutrophils are present in lung tissue. Excessive release of neutrophil elastase accelerates lung tissue damage, which causes a decrease in pulmonary function.[8]

Uncontrolled neutrophil elastase proteolytic activity is regulated by various endogenous protein inhibitors; however, this enzyme can evade this regulation using several pathways.[9] Recognizing neutrophil elastase as a therapeutic target in inflammatory lung diseases has led to discovery of new elastase inhibitors,[10–12] now undergoing clinical studies.[13,14]

Recently, two surfactants in clinical use, Alveofact and Curosurf, were shown to inhibit formation of neutrophil extracellular traps (NETs) by decreasing elastase, myeloperoxidase and cell-free DNA (cfDNA) levels.[15]

Surfacen is a natural porcine surfactant, approved for use in Cuba in 1995 by the country's regulatory agency, the Center for State Control of Medicines, Equipment and Medical Devices (CECMED) (health registration No. 0800).[6] It was introduced into medical practice in all neonatal intensive care units to treat hyaline membrane disease or NRDS in preterm infants.[16] Later, in 2011, Surfacen was approved for ARDS treatment in adults,[17] and in 2014, for ARDS in pediatric patients.[18] Surfacen is a safe product; no adverse reactions have been reported in any of the clinical trials conducted, and it has a safety profile similar to the other surfactants marketed internationally.[19] In the Cuban protocol for clinical management of patients with COVID-19 published in 2020, Surfacen is one of the medicines recommended for use.[20]

In addition to its biophysical function, Surfacen has an in vitro anti-inflammatory effect, decreasing tumor necrosis factor-alpha (TNF alpha) and interleukin 6 (IL-6),[21] and has shown an anti-inflammatory and anti-allergic immunomodulatory effect in an animal model of allergen-induced asthma.[22]

Pulmonary surfactant has been evaluated as a drug carrier or vehicle since the 1990s;[23] it has been used as a budesonide carrier in premature newborns at risk of developing bronchopulmonary dysplasia and is currently undergoing clinical trials.[24,25] Those studies provide the first clinical proof of concept for a surfactant-drug combination and justify research on its possible use in combination therapies with other drugs.

Given the importance of neutrophil elastase in respiratory diseases, the combination of Surfacen with inhibitors of this enzyme could lead to new therapeutic options for these diseases. CmPI-II, a Kazal-type serine protease inhibitor, is isolated from the *Cenchritis muricatus* marine snail. CmPI-II is a strong inhibitor of serine proteases, especially human neutrophil elastase.[26,27] The recombinant variant of this inhibitor (rCmPI-II) has functional and molecular characteristics similar to the natural inhibitor. Its ability to inhibit human neutrophil elastase has suggested important biomedical applications, including in inflammatory diseases.[28]

Very few in vitro and in vivo studies have been conducted on the combination of surfactants and protease inhibitors. Belai[29] demonstrated that use of Survanta pulmonary surfactant with alpha-1 antitrypsin had a positive effect on oxygenation and surfactant metabolism in surfactant-deficient rats. Cochrane[30] demonstrated that the combination of elafin and a surfactant in an animal model of lung damage decreased the lung damage.

The objective of this study was to evaluate the effect of Surfacen pulmonary surfactant, the rCmPI-II elastase inhibitor, and the Surfacen/rCmP-I-II combination on elastase activity in an in vitro model of activated neutrophils, as well as the surfactant's interface property when combined with the inhibitor.

## METHODS

### Evaluated products

*Surfacen* Natural surfactant obtained from porcine pulmonary lavage. The final product is supplied as a sterile white lyophilized powder in a 6R vial containing 50 mg total phospholipids. Composition: phospholipids (95%), hydrophobic proteins (SP-B and SP-C, 1.5%) and other lipids (3.5%).[6,31] Surfacen is produced in the National Center for Animal and Plant Health (CENSA) in collaboration with the National Biopreparations Center (BIOCEN), both in Cuba. Each vial is reconstituted in 2 mL of water for injection, obtaining a phospholipid concentration of 25 mg/mL.

*rCmPI-II* Elastase inhibitor obtained at the University of Havana Biology Faculty's Protein Studies Center (CEP) via recombinant pathway in *Pichia pastoris* yeast. Its characteristics are similar to those of the CmPI-II natural protein from the *Cenchritis muricatus* marine snail. The inhibitor concentration was determined by measuring absorbance at 280 nm using the coefficient of extinction ξ1%, 280 nm = 16.1, previously determined for the natural inhibitor. The concentration was 2 mg/mL. Molecular mass of rCmPI-II is 5485 Da.[32]

**Human neutrophil purification from human blood** Neutrophils were obtained from 10 mL of venous blood from healthy volunteers using Ficoll density gradient cell separation (Ficoll-Histopaque-10771, density: 1.077 g/mL, Sigma-Aldrich, USA).[33] First, blood was collected in EDTA tubes (Vacuette 4 mL K3EDTA, Greiner Bio-One, Germany) and centrifuged at 1500 rpm (DL6M centrifuge, Kaida, China) for 15 minutes at 20 ºC. Plasma was collected and centrifuged at 2500 rpm for 5 minutes at 20 ºC, to obtain a platelet-poor supernatant, which was remixed with the rest of the blood. After gravity sedimentation gradient was performed using Ficoll-Histopaque, plasma was collected and put through a second centrifugation gradient at 2500 rpm for 30 minutes at 20 ºC with Ficoll-Histopaque. To eliminate the remaining Ficoll, the precipitate obtained was resuspended in 5 mL of phosphate-buffered saline and centrifuged at 2500 rpm for 5 minutes at 20 ºC. Finally, the precipitate was collected and resuspended in 1 mL of pH 7.2 phosphate-buffered saline (PBS) solution supplemented with 0.1% bovine serum albumin (BSA, Sigma-Aldrich, USA). Purity of purified neutrophils (>95%) was determined in the MacsQuant 10 flow cytometer (Miltenyi, Germany) by fluorescence-activated cell sorting (FACS) using anti-CD45-FITC and anti-CD3-PE antibodies (Miltenyi, Germany).

**Neutrophil activation assay** Purified neutrophils were adjusted to a concentration of $1\times10^7$ cells per mL in PBS solution supplemented with 0.1% BSA (Sigma-Aldrich, USA). Then, 100 mL of the neutrophil suspension was activated for 10 minutes through incubation with 10 mL of cytochalasin B from *Drechslera* (Sigma-Aldrich, USA) at a final assay concentration of 5 µg/mL, and then stimulated for 5 minutes through the addition of 10 mL lipopolysaccharides (LPSs) from *Escherichia coli* O55:B5 (Sigma-Aldrich, USA) at a final assay concentration of 1 µg/mL. Simultaneously, 10 mL of the N-formyl-methionyl leucyl-phenylalanine (fMLP, Sigma Aldrich, USA) stimulant was added at a final assay concentration of 50 nM, along with the compounds to be evaluated (surfactant and inhibitor) and incubated for 30 minutes at 37 °C while mixing in a thermo shaker (TS 100, Fisher, USA). Surfacen was used at the final assay concentrations of 0.25 mg/mL to 10 mg/mL, and rCmPI-II was used at final concentrations of 0.1 µM to 200 µM. In the Surfacen/rCmPI-II com-

bination assays, 0.5 mg/mL of Surfacen and 80 µM of inhibitor were used. The final assay volume was adjusted to 200 mL with PBS. Samples corresponding to the different designs were centrifuged at 2500 rpm for 10 minutes at 22 °C, and the supernatant was used to determine elastase activity.

In parallel, a positive control was performed with activated/stimulated neutrophils, and a negative control was performed with untreated neutrophils. To evaluate the effect of Surfacen and rCmPI-II, these were treated with neutrophils that were not activated/stimulated. The maximum elastase percentage present in neutrophils was determined by lysing activated/stimulated cells with 0.1% hexadecyltrimethylammonium bromide (HTAB) detergent (Sigma-Aldrich, USA) for 15 minutes at 4 °C.

**Elastase enzyme activity** Elastase enzyme activity was evaluated in 90 µL of the supernatant by measuring at 405 nm hydrolysis of methoxysuccinyl-Ala-Ala-Pro-Val-p-nitroaniline substrate; 10 µL, 2.8 mM dissolved in dimethyl sulfoxide (DMSO, Calbiochem, Merck, Germany), in Tris HCl 0.02 mol/L, NaCl 0.5 mol/L pH 8.0 (100 µL), for 30 minutes, in a plate reader (ChemWell Manager, EIA mode, USA). Each reaction (200 µL) was done in triplicate in a 96-well plate. The initial velocity of the reaction was calculated using the slope of the curve (DDO/Dt) in the linear area. Enzyme activity was expressed in U/mL.

**Determining kinetics of spreading** Equilibrium surface pressure was determined on a Langmuir surface balance. Surfacen (25 mg/mL), rCmPI-II (10 and 80 µM), and the Surfacen/rCmPI-II combination were applied with a 10 µL syringe (Hamilton, USA) to the air-liquid interface in the shape of a T, from a Langmuir balance made with teflon (Nima Technology, UK). The subphase volume is 15 mL (Tris buffer 5 mM, NaCl 150 mM, pH 7.0). Samples were applied at one end of the container, while a pressure sensor with surface plate (typically a small piece of filter paper) monitored changes in surface pressure of the active surface material, as a function of time. The kinetics of spreading (p vs. time) was obtained at 37 ± 1 °C for 300 seconds.

**Statistical analysis** This was performed using GraphPad software version 5.0 (GraphPad Software, USA). The mean and standard deviation (SD) were used as descriptive statistics, and the groups were compared using analysis of variance and a posteriori comparisons using Tukey's test. The p value of 0.05 was used as the statistical significance threshold.

## RESULTS

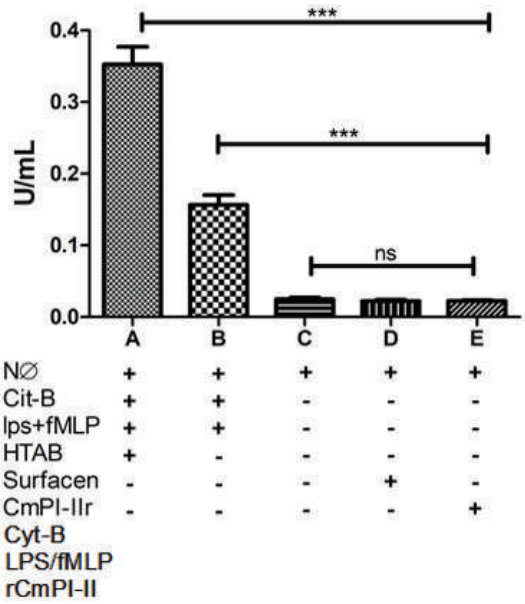
Elastase enzyme activity present in the positive control after neutrophil lysis with the HTAB detergent was 0.35 ± 0.05 U/mL (Figure 1, bar A). This value corresponds to total elastase enzyme activity in the neutrophil and was considered the maximum enzymatic activity.

The amount of elastase released in response to activation with cytochalasin B and to LPS and fMLP stimuli (positive control, Figure 1, bar B) corresponds to about 45% of the maximum enzyme activity in neutrophils, determined by cell lysis. In the unstimulated, untreated neutrophils (negative control, Figure 1, bar C), spontaneous release of the enzyme was detected, representing about 10% of the positive control value (Figure 1, bar B). In the inactivated, unstimulated neutrophils treated with surfactant and elastase inhibitor (Figure 1, bars D and E), the enzyme activity value was similar to the negative control, which suggests that these compounds do not cause neutrophil activation.

**Effect of Surfacen, rCmPI-II, and the Surfacen/rCmPI-II combination on activated neutrophil elastase activity** At concentrations of 0.25, 0.5 and 4 mg/mL, Surfacen inhibited 45% to 57% of elastase activity. At 10 mg/mL, inhibition was 78% (statistically significant value compared with other studied concentrations) (Figure 2-A).

In activated and stimulated neutrophils, incubated with rCmPI-II, elastase activity was inhibited at a magnitude dependent on the inhibitor concentration (Figure 2-B). rCmPI-II at 0.1 µM reduced 20% of elastase activity; at the maximum concentration evaluated (200 µM), inhibition was 68%. rCmPI-II is a competitive inhibitor of this enzyme and, therefore, increasing its concentration increases its inhibition. This characteristic has been described for the inhibitor in an enzyme assay with a chromogenic substrate. To

**Figure 1: Neutrophil elastase enzyme activity**

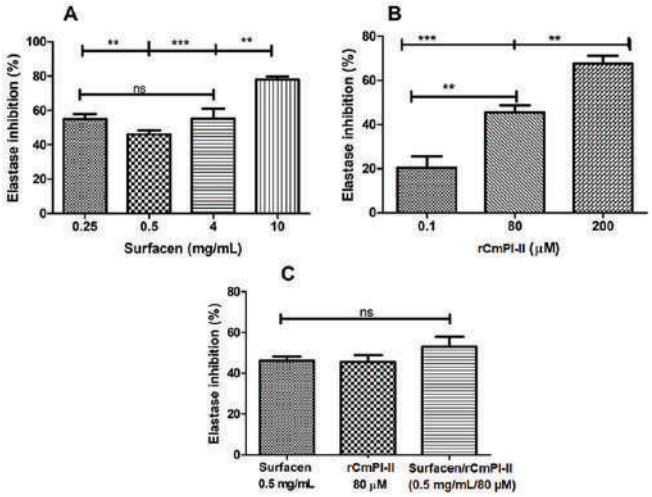



The bars represent the means of four independent experiments in U/mL. In the analysis of variance with Tukey's test, the significant differences are identified with asterisks:
***: p <0.001 (A vs. B, C, D, and E); ***: p <0.001 (B vs. C, D, and E); ns: nonsignificant (C vs. D, and E).

A: neutrophils activated and stimulated with cytochalasin B and LPS+fMLP and lysed with HTAB (maximum percentage of elastase present in neutrophils)
B: neutrophils activated and stimulated with cytochalasin B and LPS+fMLP (positive control)
C: untreated neutrophils (negative control)
D: unactivated, unstimulated neutrophils, treated with Surfacen
E: unactivated, unstimulated neutrophils, treated with rCmPI-II

Cyt-B: cytochalasin B; fMLP: formylmethionyl leucyl-phenylalanine; HTAB: hexadecyltrimethylammonium bromide; LPS: lipopolysaccharide; NØ: neutrophils; rCmPI-II: *Cenchritis muricatus* (Gastropoda, Mollusca) isolated elastase inhibitor obtained via recombinant pathway (Protein Studies Center, Faculty of Biology, University of Havana); Surfacen: natural porcine pulmonary surfactant (CENSA, Cuba).

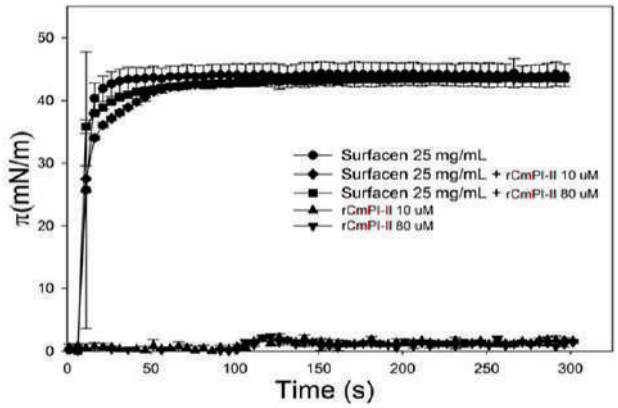
**Figure 2: Effect of Surfacen (A), rCmPI-II (B) and the Surfacen/rCmPI-II combination (C) on activated and stimulated neutrophil elastase activity**




The bars represent the mean ± standard deviation of four independent experiments. In the analysis of variance with Tukey's test, the significant differences are identified with asterisks: **: p <0.01, ***: p <0.001, ns: nonsignificant.

rCmPI-II: *Cenchritis muricatus* (Gastropoda, Mollusca) isolated elastase inhibitor obtained via recombinant pathway (Protein Studies Center, Faculty of Biology, University of Havana); Surfacen: natural porcine pulmonary surfactant (CENSA, Cuba).

**Figure 3: Spreading assays of Surfacen and Surfacen/rCmPI-II combination in a Langmuir surface balance**




Vertical axis: interface pressure in millinewton per meter. Horizontal axis: time in seconds.

rCmPI-II: *Cenchritis muricatus* (Gastropoda, Mollusca) isolated elastase inhibitor obtained via recombinant (Protein Studies Center, Faculty of Biology, University of Havana); Surfacen: natural porcine pulmonary surfactant (CENSA, Cuba).

study the effect of the product combination, the concentration that resulted in about 50% inhibition in the assays of each product was selected: for rCmPI-II, 80 µM, and for Surfacen, 0.5 mg/mL. The Surfacen/rCmPI-II combination did not affect the elastase inhibitor activity observed for both independently (Figure 2-C).

**Effect of rCmPI-II on Surfacen interface properties** Figure 3 compares the kinetics of spreading of Surfacen and the Surfacen/rCmPI-II combination, measured using changes in surface pressure (p) over time. Surfacen adsorbs very efficiently at the air-liquid interface, with an equilibrium surface pressure around 42 mN/m in less than 1 minute. The addition of rCmPI-II to Surfacen did not modify the kinetics of spreading. At the two concentrations evaluated, rCmPI-II did not modify the surface pressure.

## DISCUSSION

Human pulmonary surfactant improves pulmonary function and oxygenation, and modulates innate and acquired immunity, regulating the lung's inflammatory processes. Various surfactant preparations decrease respiratory burst cytokine release and nitric oxide production in inflammatory cells (neutrophils, monocytes and macrophages).[34,35] Among them, Surfacen, the Cuban porcine surfactant, decreases TNFa and IL-6 production in human monocytes responding to *Staphylococcus aureus*.[21]

There are few in vitro model studies on the modulatory effect of surfactants in clinical use on neutrophil activation. In 1996, Tegtmeyer evaluated the modulatory effect of surfactants in clinical use on the function of neutrophils responding to different damage-inducing agents.[36] The surfactants tested did not produce neutrophil activation. However, when the neutrophils stimulated with interleukin-8 (IL-8), neutrophil-activating peptide-2 (NAP-2), or fMLP were incubated with different surfactant combinations, elastase release depended on the type of surfactant. Exosurf and Alveofact had little effect on the elastase release induced by the three mediators, while Curosurf and Survanta inhibited cell response in a dose-dependent manner. Schulz demonstrated that Curosurf and Alveofact had an inhibitory effect on in vitro NET formation in neutrophils stimulated with phorbol-12-myristate-13-acetate (PMA). In both preparations, the effect was dose- and time-dependent; the effect was stronger with Alveofact. When the neutrophils were incubated with these preparations before PMA activation, the inhibitory effect was higher, which suggests a preventive effect. The authors suggest that these preparations reduce neutrophil activation and highlight pulmonary surfactant as a possible candidate for attenuating inflammation.[15]

This study showed that Surfacen inhibits the activity of released elastase, present in activated/stimulated human neutrophils in vitro. Some surfactants show different effectiveness against this enzyme in in vitro models of activated neutrophils, likely due to differences in its composition.[15,36] The inhibitory activity of Surfacen could be related to the release process of this enzyme by neutrophils once activated/stimulated. In this model, Surfacen's elastase inhibition mechanism is unknown; however, for other surfactants (Survanta and Infasurf), the enzyme release mechanism is known to be modulated by their action on neutrophils, and it is mediated by depolarization and release of intracellular $Ca^{++}$ through activation of the G protein pathway.[37] Recently, pulmonary surfactant preparations in clinical use have been shown to decrease expression of P2Y6, a G-coupled extracellular receptor that participates in NET formation by interacting with calcium pathways.[15]

rCmPI-II is an inhibitor of serine proteases, including human neutrophil elastase, that reduces elastase activity in an in vitro activated/stimulated neutrophil assay in a dose-dependent manner. This inhibitory activity is not affected by the presence of Surfacen. Several preclinical trials have investigated the therapeutic potential of neutrophil elastase inhibitors Sivelestat, Sirtinol, DX-890 and

BAY 85-8501 for treating lung diseases. The synthetic inhibitor Sivelestat is one of the most studied. In a murine model of acute lung injury involving the lung tissue repair process, the inhibitor decreased neutrophil accumulation in bronchoalveolar lavage and collagen deposition in lung parenchyma, and improved lung compliance.[38] In a clinical trial in patients with ARDS, Sivelestat increased ventilator-free days and 180-day survival.[39]

The mechanism of action of the synthetic inhibitor Sirtinol in an in vitro activated neutrophil model was to inhibit elastase enzyme activity, since its effect was not mediated by protein kinase A, or by calcium and kinase pathways regulated outside the cell; therefore, it did not directly affect neutrophil function. In an animal model of LPS-induced acute lung injury, Sirtinol reduced neutrophil recruitment and lung edema.[40]

DX-890, a peptide enzyme inhibitor, inhibits activity of elastase released by fMLP-stimulated neutrophils obtained from healthy individuals and patients with cystic fibrosis, inhibits neutrophil transmigration through epithelial cells, and decreases IL-8 secretion in nasal epithelial cells and sputum of patients with cystic fibrosis.[41] BAY 85-8501, another synthetic enzyme inhibitor, had a favorable safety and tolerance profile when administered over 28 days to patients with bronchiectasis not associated with cystic fibrosis; however, additional studies of prolonged treatment are needed to evaluate its clinical efficacy.[14]

rCmPI-II's ability to reduce human neutrophil elastase activity, not only in experiments with a commercial enzyme,[26] but also in an in vitro model of activated/stimulated neutrophils obtained in this study, suggests that rCmPI-II might be used in inflammatory processes in which neutrophils are involved.

The combination of the surfactant Survanta and the alpha-1 antitrypsin elastase inhibitor in an in vivo model showed that the addition of alpha-1 antitrypsin improved oxygenation and surfactant metabolism in surfactant-deficient rats.[29] Here, Surfacen is shown to be absorbed very efficiently into the air–liquid interface with an equilibrium surface pressure of about 42 mN/m in less than 1 minute. The results of this study show that rCmPI-II does not affect the Surfacen surface properties.

Pulmonary surfactant may be combined with medications; to do so, both the surfactant and the drug transported by the surfactant must preserve their therapeutic functions. This study shows that the Surfacen/rCmPI-II combination did not modify the properties of either one in vitro. Inhibition of neutrophil elastase did not increase with respect to the values obtained with each compound separately, which could be related to different mechanisms of action of these compounds. Understanding these mechanisms on activated/stimulated neutrophils would open the door to using the Surfacen/rCmPI-II combination to treat inflammatory lung diseases.

Despite these advances, transferring the results with elastase inhibitors from the preclinical phase to the clinical phase is a challenge. Preclinical and clinical research continues, alongside development of new, more powerful and selective inhibitors.[42] Only two of these medications are used in clinical practice to date: alpha-1 antitrypsin (Prolastin), approved by the US Food and Drug Administration in 1987,[43,44] and Sivelestat (ONO-5046), approved for clinical use to treat ARDS and acute lung injury associated with systemic inflammatory response syndrome in Japan and South Korea.[13]

Respiratory failure is one of the causes of death in patients with COVID-19.[45,46] Clinical trials of pulmonary surfactants in COVID-19 are in process.[47,48] Surfacen is indicated in the Cuban COVID-19 Protocols for treatment of these patients.[6,49] The potential benefit of neutrophil elastase inhibitors in patients with severe COVID-19 is being investigated because these inhibitors could mitigate elastase damage on the lung connective tissue and limit the virus spreading capabilities by preventing S protein proteolytic activation.[50]

A limitation of this study is that it does not provide information about the mechanism of action of each of the compounds.

## CONCLUSION

The anti-elastase effect of the two Cuban products, Surfacen and the rCmPI-II inhibitor, is demonstrated in an activated neutrophil model. rCmPI-II does not affect Surfacen's interface property and, therefore, both can be evaluated for combined use in treating inflammatory lung diseases.

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# High Levels of Serum Bile Acids in COVID-19 Patients on Hospital Admission

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## ABSTRACT

**INTRODUCTION** Bile acids are signaling molecules with immune, metabolic and intestinal microbiota control actions. In high serum concentrations they increase inflammatory response from the liver-gut axis, until causing multiorgan failure and death; therefore, they may be associated with COVID-19's clinical progression, as a consequence of tissue and metabolic damage caused by SARS-CoV-2. While this topic is of considerable clinical interest, to our knowledge, it has not been studied in Cuba.

**OBJECTIVE** Study and preliminarily characterize patients admitted with a diagnosis of COVID-19 and high levels of serum bile acids.

**METHODS** A preliminary exploratory study was carried out with descriptive statistical techniques in 28 COVID-19 patients (17 women, 11 men; aged 19–92 years) who exhibited high levels of serum bile acids (≥10.1 µmol/L) on admission to the Dr. Luis Díaz Soto Central Military Hospital in Havana, Cuba, from September through November 2021.

**RESULTS** On admission patients presented hypocholesterolemia (13/28; 46.4%), hyperglycemia (12/28; 43.0%) and hyper gamma-glutamyl transpeptidase (23/28; 84.2%). Median blood glucose (5.8 mmol/L) and cholesterol (4.1 mmol/L) were within normal ranges (3.2–6.2 mmol/L and 3.9–5.2 mmol/L, respectively). Severe or critical stage was the most frequent (13/28) and median serum bile acids (31.6 µmol/L) and gamma-glutamyl transferase (108.6 U/L) averaged well above their respective normal ranges (serum bile acids: 0–10 µmol/L; GGT: 9–36 U/L). Arterial hypertension was the most frequent comorbidity (19/28; 67.9%).

**CONCLUSIONS** Severe or critical stage predominated, with serum bile acids and gamma-glutamyl transferase blood levels above normal ranges. The study suggests that serum bile acid is toxic at levels ≥10.1 µmol/L, and at such levels is involved in the inflammatory process and in progression to severe and critical clinical stages of the disease. In turn, this indicates the importance of monitoring bile acid homeostasis in hospitalized COVID-19 patients and including control of its toxicity in treatment protocols.

**KEYWORDS** COVID-19, SARS-CoV-2, bile acids and salts, gamma-glutamyl transferase, pregnant women, postpartum, Cuba

## INTRODUCTION

Toxic action of bile acids has been reported since 1933 and their biological activities were described in the late 1990s. Bile acids are signaling molecules and, when interacting with nuclear receptors and cellular transporters, express a sequence of actions linked to physical-chemical properties, conjugation with glycine or taurine, primary or secondary synthesis, and hepatic and intestinal transport, among others.[1,2] These properties explain their pleiotropic activities, such as intestinal absorption of fats and fat-soluble vitamins, and control of energy, lipid, glycemic, cholesterol and immune-system metabolism. This would also explain their influence on the specific activity of organs such as the heart, lungs, kidneys and nervous system.

**IMPORTANCE** This is the first report in Cuba of COVID-19 cases with high levels of serum bile acids on hospital admission. Its findings suggest disease progression could be linked to the toxic effect of bile acids, such as worsening of the clinical stage and high levels of gamma-glutamyl transferase. The study establishes several premises for further research related to serum bile acids in management and follow-up of COVID-19 patients.

[3,4] These concepts offer a comprehensive view of their biological activities within the framework of novel approaches in human physiology.[5]

In vivo and in vitro studies show that high levels of bile acids maintained on plasma membranes and cellular DNA generate an inflammatory sequence that increases oxidative stress, protein denaturation and misfolding, decreased calcium and iron ion levels, and formation of secondary structures in DNA, as well as dysfunction of mitochondria and other organelles, responsible for genesis and persistence or amplification of the inflammatory and proinflammatory response.[5,6]

Reference levels considered normal vary according to the different assays and units of measurement used, and may be standardized internationally, regionally, and even by institutions and reagent manufacturers. Thus, the normal range used by the International System of Units (SI) is 10–19 µmol/L,[7] Chinese researchers use 0–12 µmol/L,[8] while the German company DiaSys Diagnostic Systems (GmbH) uses a normal range of 0–10 µmol/L.[9]

Despite reports of digestive and extradigestive toxic effects of serum bile acids and use of new therapeutic interventions, such as ursodeoxycholic acid in various conditions and in pregnancy,[10] there has

been virtually no published research on serum levels in COVID-19, the results of which could explain clinical and tissue changes in patients, conditioned by homeostatic decontrol of bile acids.

This is the first report in Cuba of cases with COVID-19 and high serum levels of bile acids (≥10.1 µmol/L) and it aims to describe characteristics of patients on admission, as a first step to considering eventual changes in clinical teaching, care and research regarding this potential association.

## METHODS

A case study was conducted for reasons of feasibility, its flexible design enabling a preliminary approach to the problem, hypothesis generation and formulation of bases for future studies.

The Dr. Luis Díaz Soto Central Military Hospital is a general hospital that also serves the adjacent civilian population and has a gynecology-obstetrics service. In the pandemic, this Havana facility also received all pregnant COVID-19 confirmed cases in the city.[11] During September–November 2021, 91 PCR-confirmed COVID-19 patients were admitted. On admission, all patients underwent serum bile acid assay, found to be high in 28 patients who comprised the case series studied; all were aged >18 years, and the group included six pregnant and two postpartum women.

Patients were interviewed; physical exams, imaging studies and blood tests were carried out.

**Study variables** Evaluated were: clinical stage (mild: patient with mild symptoms, such as upper respiratory symptoms, no pneumonia; moderate: patient with mild pneumonia without respiratory failure or inflammatory response; severe: patient presenting pneumonia with acute respiratory failure, and pneumonia with inflammation and hypercoagulability; critical: patient with intubation and assisted ventilation, shock and multiorgan failure),[11] clinical and imaging assessment, blood tests (blood glucose, cholesterol, gamma-glutamyl transferase or GGT, and serum bile acids) and comorbidities.

Bile acid testing at admission was done using a stand-alone clinical chemistry analyzer (Cobas c 311, Roche Diagnostics International Ltd, Switzerland) that fulfilled parameters for calibration and calculation stability with standards in µmol/L established by DiaSys Diagnostic Systems GmbH (Germany), which consider reference values of 0–10 µmol/L as normal and ≥10.1 µmol/L as high.[9] For blood glucose, cholesterol and GGT, methods standardized in Cuba's clinical laboratories and approved by the country's Ministry of Public Health were used.[12]

Results were presented in frequency tables.

**Ethics** This study followed the principles of the Declaration of Helsinki.[13] Patients or legal guardians (in cases of critical and disabled patients) provided written informed consent and patient anonymity was maintained.

## RESULTS

Table 1 shows the results of the case series. Of the total admitted cases, 30.8% (28/91) had high serum bile acids at admission. Ages ranged from 19 to 92 years, and 60.7% of cases (17/28) were women. The series had a mean serum bile acid of 23.9 µmol/L, with a median of 28.7 µmol/L. Values were higher the more severe the clinical picture (Table 2). GGT figures were the furthest from the normal range, with a median of 76.5 U/L, higher in patients at the severe or critical stages (108.0 U/L). All cases had a clinical and imaging diagnosis of pneumonia or bronchopneumonia on admission. There was a remarkable abundance of comorbidities.

**Table 1: Case series of patients with COVID-19 and high serum bile acid levels on admission to the Dr. Luis Díaz Soto Central Military Hospital, Havana, 2021**

| Patient No. | Age (years) | Sex | Clinical Stage | BG* mmol/L | CL* mmol/L | GGT* U/L | SBA* µmol/L | Comorbidities |
|---|---|---|---|---|---|---|---|---|
| 1 | 19 | F | mild | 3.7 | 3.4 | 11 | 11.3 | HTN |
| 2 | 28 | M | severe | 4.8 | 4.5 | 99 | 38.9 | HTN |
| 3 | 30 | M | severe | 6.5 | 4.1 | 78 | 20.4 | HTN |
| 4 | 48 | M | severe | 13.1 | 4.6 | 42 | 30.7 | Healthy |
| 5 | 51 | M | mild | 6.9 | 3.8 | 46 | 12.5 | HTN, OB, LD |
| 6 | 51 | M | mild | 6.9 | 3.8 | 46 | 12.5 | HTN, OB, LD |
| 7 | 57 | M | moderate | 6.8 | 5.5 | 108 | 33.9 | HTN, CVD |
| 8 | 72 | F | mild | 5.5 | 5.3 | 75 | 28.1 | HTN, BA |
| 9 | 74 | M | mild | 5.3 | 3.0 | 129 | 29.4 | HTN, DM2, CVD |
| 10 | 74 | F | severe | 5.3 | 3.1 | 53 | 29.0 | HTN, CVD, OB, LD |
| 11 | 76 | F | mild | 6.3 | 5.2 | 163 | 31.5 | Healthy |
| 12 | 76 | F | mild | 3.6 | 2.8 | 101 | 34.0 | HTN, DM2, OB, LD |
| 13 | 76 | M | severe | 14.2 | 5.1 | 93 | 12.8 | HTN, BA |
| 14 | 79 | M | moderate | 5.6 | 2.3 | 186 | 31.6 | HTN, DM2 |
| 15 | 80 | F | moderate | 5.8 | 3.1 | 30 | 31.2 | HTN |
| 16 | 82 | F | critical | 5.7 | 4.3 | 63 | 30.4 | HTN, GBR |
| 17 | 85 | F | severe | 5.6 | 5.5 | 82 | 11.8 | HTN |
| 18 | 86 | F | mild | 6. 2 | 3.1 | 61 | 29.4 | HTN, DM2, CVD |
| 19 | 92 | M | mild | 5.9 | 3.8 | 130 | 32.6 | HTN, DM2, CVD |
| **Deceased** | | | | | | | | |
| 20 | 72 | M | critical | 7.5 | 2.8 | 320 | 10.3 | HTN, OB, GBR |
| **Post Partum** | | | | | | | | |
| 21 | 29 | F | severe | 4.3 | 5.0 | 174 | 28.4 | OB |
| 22 | 32 | F | mild | 4.4 | 4.5 | 120 | 45.6 | GBR |
| **Pregnant** | | | | | | | | |
| 23 | 24 | F | severe | 7.8 | 5.0 | 18 | 10.1 | AN |
| 24 | 26 | F | critical | 20.0 | 1.9 | 99 | 11.5 | AN, GD |
| 25 | 27 | F | severe | 6.4 | 4.6 | 43 | 11.0 | IUGR, HTN-Pr |
| 26 | 33 | F | severe | 7.1 | 4.1 | 24 | 10.5 | AN, HTN |
| 27 | 33 | F | mild | 4.8 | 6.0 | 14 | 34.4 | AN, IUGR |
| 28 | 34 | F | mild | 4.6 | 3.5 | 45 | 17.3 | AN, GD |

AN: anemia; BA: bronchial asthma; BG: blood glucose; CL: cholesterol; CVD: cardiovascular disease; DM2: diabetes mellitus 2; F: female; GBR: gallbladder removed; GD: gestational diabetes; GGT: gamma-glutamyl transferase; HTN: hypertension; HTN-Pr: hypertension-preeclampsia; IUGR: intrauterine growth retardation; LD: liver disease; M: male; PC: prostate cancer; OB: obesity; SBA: serum bile acids
*Normal ranges: BG: 3.2–6.2 mmol/L; CL: 3.9–5.2 mmol/L; GGT: 9–36 U/L; SBA: 0–10 µmol/L

**Table 2: Medians for blood chemistry variables, serum bile acids and gamma-glutamyl transferase, by clinical stage**

| Clinical stage (n) | BG* mmol/L | CL* mmol/L | GGT* U/L | SBA* µmol/L |
|---|---|---|---|---|
| Severe or critical (13) | 5.8 | 3.1 | 108.0 | 31.6 |
| Moderate (3) | 5.3 | 3.8 | 68.0 | 29.4 |
| Mild (12) | 6.5 | 4.5 | 78.0 | 12.8 |
| All (28) | 5.8 | 4.1 | 76.5 | 28.7 |

BG: blood glucose; CL: cholesterol; GGT: gamma-glutamyl transferase; SBA: serum bile acids
*Normal ranges: BG: 3.2–6.2 mmol/L; CL: 3.9–5.2 mmol/L; GGT: 9–36 U/L; SBA: 0–10 µmol/L

Hypertension was the most frequent, present in 67.9% (19/28) of patients; 6 were diabetic and 6 were obese (21.4%; 6/28). Five (17.9%; 5/28) had 4 or more comorbidities and only 2 (7.1%; 2/28) previously had no comorbidities.

## DISCUSSION

High rates of mortality, morbidity and sequelae from COVID-19 are related to immune and metabolic disorders that are the pathophysiological basis of the disease.[14] These disorders involve the release of multiple proinflammatory mediators that trigger an inflammatory response in various organs and tissues. These tissues contain angiotensin-converting enzyme 2 (ACE2) receptors, used by SARS-CoV-2 for cell entry. Once inside the cell, the viral particles cause damage that results in immune intolerance and metabolic dysregulation in general, especially of cholesterol. It should be noted that 50% of derivatives of the end product of hepatic cholesterol catabolism are bile acids.[15]

When SARS-CoV-2 infects susceptible cells in various organs, especially those of the cholangiolar epithelium (cholangiocytes) of the bile canaliculi and bile ducts, it triggers an inflammatory response that results in functional damage and lysis, which impacts bile acid secretion and transport.[16]

The epithelial cells of the terminal ileum have the second highest distribution of ACE2 receptors in the body. Upon infection, the absorption, intestinal microbiota and intracellular transport of bile acids are dysregulated.[17]

In severe and critical COVID-19 patients, clinical manifestations of intestinal failure have been observed, presenting as uncontrolled metabolic homeostasis and proinflammatory mediators released by immune system cells. There is also uncontrolled intestinal motility, mucus secretion and breakdown of the intestinal barrier. This inflammatory process can lead to alterations in intestinal permeability, bacterial translocation, local or systemic sepsis and in extreme cases multiorgan failure and death. Bile acids may influence or be one of the factors that trigger the process.[18]

However, there is a notable absence of reports in the medical literature of patients with COVID-19 whose clinical manifestations (cardiac, renal, pulmonary, hematological, neurological and intestinal) are associated with homeostatic imbalance of bile acids or evidence of the toxic damage they cause.[2,4]

Furthermore, bile acids are neither part of the optional or required blood chemistry in care of COVID-19 patients on admission nor throughout their disease, whether severe or critical. These tests are not included in the followup of patients in intensive care, or in any of the existing national[11] and international protocols,[19] despite all the accumulated evidence of their effect on other diseases that progress to severity and of their importance in the management of pregnancy with cholestasis or fatty liver.

This study has limitations inherent to exploratory designs based on case series, whose purpose is to describe potential clinically relevant findings while not aspiring to signal causal relationships or other types of associations. Nevertheless, these preliminary results suggest the need to design and execute analytical studies that will enable a deeper understanding of the probable role of bile acids in the clinical course of patients with COVID-19.

## CONCLUSIONS

The patients admitted with COVID-19 and high serum levels of bile acids levels in this case series were primarily women in the severe or critical stage, with GGT above normal for established ranges.

Coupled with the scarce evidence from recent literature, the study suggests at least two considerations: (1) that serum bile acid levels ≥10.1 µmol/L are toxic and are involved in the inflammatory process and in disease progression to unfavorable clinical stages, and (2) that it is important to monitor bile acid homeostasis in hospitalized COVID-19 patients, and to include control of its toxicity in treatment protocols.

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# Polyserositis as a Post–Covid-19 Complication

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## ABSTRACT

**INTRODUCTION** Polyserositis is described as inflammation with effusion of more than one serous membrane. There is very little published literature linking it to COVID-19 as a late complication.

**OBJECTIVE** Present and describe a case of post–COVID-19 polyserositis.

**METHODS** Data were collected from the medical record of a female patient admitted for fainting spells and marked weakness. The patient underwent a clinical evaluation, additional hematology, imaging and histopathology tests, and a surgical procedure.

**RESULTS** We present the case of a 57-year-old female patient admitted to hospital for fainting spells and marked weakness, four months after COVID-19 infection. She also had a history of obesity, asthma, type 2 diabetes mellitus and a cholecystectomy in December 1992 for gallstones. Clinical assessment revealed pericardial effusion and bilateral pleural effusion, in addition to a tumor-like lesion outside the pericardium, proximal to the right ventricular wall. A surgical procedure and findings from additional tests led to diagnoses of thymic remnants and polyserositis.

**CONCLUSIONS** This is a case of polyserositis in a post–COVID-19 patient. After other causes of polyserositis were ruled out, and since there is a likely physiological and pathogenic mechanism operating between the two diseases, the polyserositis was determined to be a late complication of COVID-19. To date, it is the second case reported in the world and the first reported in Cuba.

**KEYWORDS** COVID-19, SARS-CoV-2, colchicine, pericardial effusion, pleural effusion, pericarditis, thoracoscopy, Cuba

## INTRODUCTION

COVID-19 has presented extraordinary global challenges in virtually all aspects of public and private life, prompting continuing debate and research.[1] On the medical front, complications of this viral infection have included reports of isolated inflammation of the pericardium, pleura and peritoneum.[2–4]

The medical community has also focused on the persistent symptoms identified after resolution of acute COVID-19. These symptoms have been called by different names: post-COVID conditions, long COVID, and post-acute COVID syndrome. Most of these descriptions involve onset of symptoms one to three months after the patient recovers from acute infection. [5,6]

After reviewing acute and late COVID-19 conditions, we found very few reports of polyserositis in adults not attributable to another comorbidity.

The objective of this report was to present and describe a case of post–COVID-19 polyserositis—the first in Cuba and the second in the world considered as a post–COVID-19 complication.[7]

## METHODS

The patient's medical record included data collected from the patient interview, the physical exam, additional blood and imaging test results and surgeries, as well as the histopathology test of pleural and pericardial fluid samples and tissue bordering the pericardium. All research was conducted by technicians and professionals in laboratories and hospital departments, following good clinical and laboratory practices.

We searched the Scielo, MedCarib, PubMed, LiLacs, and Google Scholar databases for previous reports of polyserositis as a post–COVID-19 complication.

We also conducted an interdisciplinary review of the case.

**Ethics** The patient voluntarily provided her written informed consent to be studied for her health condition, as part of her healthcare regimen. We took special care to respect the patient's privacy and anonymity and ensured the integrity and accuracy of the data disclosed.

**IMPORTANCE** This case of polyserositis as a post–COVID-19 complication is the second case reported in the world and the first in Cuba.

## RESULTS

This is the case of a 57-year-old female patient with obesity (body mass index 34.4 kg/m$^2$), a history of bronchial asthma and a 10-year history of type 2 diabetes mellitus regularly controlled with oral hypoglycemic agents. The patient underwent a cholecystectomy in December 1992 for gallstones.

In July 2021, the patient completed her SARS-CoV-2 vaccination series with three doses of Abdala, a Cuban COVID-19 vaccine. On September 15, 2021, she received a diagnosis of COVID-19; she had a mild case, and recovered quickly.

She was admitted to the Medical-Surgical Research Center (CIMEQ), in Havana, Cuba, on January 25, 2022, for fainting spells and sweating that started approximately two weeks before

admission, with progressive worsening in intensity and frequency. The spells were accompanied by noticeable weakness.

In the interview, the patient reported dyspnea on moderate physical exertion and when attempting supine position.

Physical examination revealed decreased vesicular breath sounds in both lung bases, rhythmic and somewhat muffled heart sounds, heart rate at 80 beats per minute, blood pressure (in the right arm) at 110/70 mmHg and soft and rounded abdomen. The rest of the physical examination was normal.

Additional tests showed abnormally high blood glucose (>20 mmol/L), requiring a change in diabetes treatment (discontinuation of oral hypoglycemic agents in favor of insulin), which brought the symptoms under control. Laboratory tests also revealed high levels of high-sensitivity troponin T, aspartate aminotransferase, alanine aminotransferase and gamma-glutamyl transpeptidase. The results of the remaining blood tests were unremarkable (Table 1).

The electrocardiogram was normal.

Other findings were as follows:

**Abdominal ultrasound** The liver had increased echogenicity due to steatosis; both kidneys were normal; the pancreas was normal; the uterus was small and involuted with thin endometrial lining; and a small amount of fluid was found at the bottom of the pouch of Douglas.

**Computed tomography (CT)** The noncontrast study showed pericardial thickening on the outer border of the right atrium, pericardial effusion and bilateral pleural effusion, more evident on the right side. There was no mediastinal lymphadenopathy. The liver showed uniform density. A small amount of fluid was observed in the bottom of the pouch of Douglas. The spleen measured 128 mm. Kidneys were normal in appearance, without stones. The pancreas was normal. There was no retroperitoneal lymphadenopathy. There were incipient degenerative changes in the lumbosacral spine.

**Transthoracic echocardiogram (TTE)** Valve apparatuses were normal. The right atrium and right ventricle were collapsed due to effusion. The echocardiogram identified a tumor, proximal to the right atrium, which seemed to be attached to the pericardium, but could not be further pinpointed with this study.

**Contrasted heart CT** The arterial phase showed a hyperdense lesion measuring 1.7 x 4.3 cm, oval in shape, enhanced with a contrast agent, located in close contact with the outer border of the right atrium (Figure 1). This lesion coincides with the pericardial thickening reported in the noncontrast study.

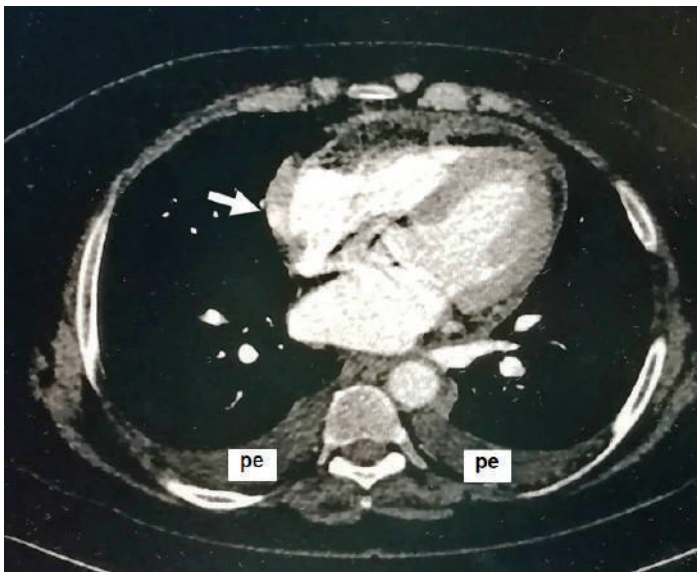
**Thoracoscopy** Considering the previous findings and especially the tumor-like lesion in the pericardial region, a thoracoscopy was performed to pinpoint whether there were pleural abnormalities and to urgently study the pleural

**Table 1: Results of additional laboratory tests performed at hospital admission**

| Test | Result | Unit of measurement | Normal reference value/range |
|---|---|---|---|
| Hemoglobin[a] | 142 | g/L | (120–150) |
| Hematocrit[a] | 38.2 | % | (37–47) |
| MCV[a] | 91.7 | fL | (80–92) |
| MCH[a] | 34* | pg | (27–32) |
| MCHC[a] | 371* | g/L | (320–360) |
| Leukocytes[a] | 11.05* | $10^9$/L | (2–8) |
| Lymphocytes[a] | 2.3 | $10^9$/L | (1–5) |
| Monocytes[a] | 4.1* | $10^9$/L | (0.1–1) |
| Neutrophils[a] | 4.6 | $10^9$/L | (2–8) |
| Eosinophils[a] | 0 | $10^9$/L | (0–0.4) |
| Basophils[a] | 0 | $10^9$/L | (0–0.2) |
| Platelets[a] | 138 | $10^9$/L | (150–400) |
| AST[b] | 130* | U/L | (≤46) |
| ALT[b] | 124* | U/L | (≤40) |
| Alkaline phosphatase[b] | 75 | U/L | (≤117) |
| GGT[b] | 213* | U/L | (≤60) |
| Cholesterol[b] | 2.30 | mmol/L | (3.9–5.2) |
| Triglycerides[b] | 1.38 | mmol/L | (0.5–1.6) |
| HDL-C[b] | 0.08 | mmol/L | (≥1) |
| VLDL[b] | 0.63 | mmol/L | (≤1.16) |
| LDL-C[b] | 1.59 | mmol/L | (≤3.4) |
| β-lipoprotein[b] | 6.33 | mg/dL | (<30) |
| Total bilirubin[b] | 8 | µmol/L | (5.1–20.5) |
| Direct bilirubin[b] | 2 | µmol/L | (0–5.1) |
| Total proteins[b] | 60 | g/L | (40–80) |
| Albumin[b] | 37 | g/L | (38–54) |
| Blood glucose[b] | 13.4* | mmol/L | (3.9–5.8) |
| Creatinine[b] | 60 | µmol/L | (61.9–115) |
| Total CK[b] | 49 | U/L | (30–170) |
| CK-MB[b] | 11 | U/L | (<25) |
| hs-Troponin T[c] | 45.7* | ng/L | (12.7–24.9) |
| $T_3$[c] | 1.4 | nmol/L | (1.1–3) |
| $T_4$[c] | 95.05 | nmol/L | (64–155) |
| TSH[c] | 4.22 | mUI/L | (0.5–5) |
| Procalcitonin[c] | 0.2 | ng/mL | (<0.5) |
| CEA[c] | 3.5 | ng/mL | (3.8–5) |
| CA 125[c] | 58.9* | UI/mL | (<35) |
| CA 15-3[c] | 17.7 | UI/mL | (<30) |
| CA 19-9[c] | 12.4 | UI/mL | (<39) |
| CA 72-4[c] | <0.2 | UI/mL | (<6.9) |

*Values above maximum normal value
[a]Test conducted with an automatic hematology analyzer (Mindray BC5800, China)
[b]Test conducted with the Cobas C311 blood chemistry analyzer (Roche Diagnostics, Germany)
[c]Test conducted with the Cobas E411 immunoassay analyzer (Roche Diagnostics, Germany)
ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; CA 15-3: Carbohydrate antigen 15-3; CA 19-9: Carbohydrate antigen 19-9; CA 72-4: Carbohydrate antigen 72-4; CA 125: Carbohydrate antigen 125; CEA: Carcinoembryonic antigen; CK: Creatine kinase; CK-MB: Creatine kinase myocardial band; GGT: Gamma-glutamyl transpeptidase; HDL-C: High-density lipoprotein; hs-Troponin T: High-sensitivity troponin T; LDL-C: Low-density lipoprotein; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; MCV: Mean corpuscular volume; T3: Triiodothyronine; T4: Thyroxine; TSH: Thyroid stimulating hormone; VLDL: Very-low-density lipoprotein

**Figure 1: Axial slice of heart CT scan during arterial phase**




CT: computed tomography; pe: pleural effusion
The arrow points to the contrast-enhanced lesion projected out from the pericardium, with oval appearance, located in close contact with the outer border of the right atrium. The study was conducted with a 128-slice SOMATOM Definition AS scanner (Siemens, Germany).

fluid. Slightly cloudy, light-yellow fluid, with fibrin formation and exudate appearance, was collected and analyzed in the pathology department. Malignant neoplasm was ruled out; only isolated lymphocytes were observed.

During the same surgical procedure, a median sternotomy was performed to study the pericardium and remove the lesion reported in the imaging tests. Moderate pericardial effusion was confirmed. The gross characteristics of the pericardial fluid extracted were similar to those described in the pleural fluid.

A lesion with fatty appearance outside the pericardium at the right pericardiophrenic angle was identified and completely removed. A pericardial window was also performed as a therapeutic measure to prevent recurrence of pericardial effusion and to prevent risk of developing cardiac tamponade.

**Histopathology** A cytopathology test of the pericardial fluid confirmed absence of malignant neoplasm and presence of isolated lymphocytes.

**Anatomic pathology report** Analysis of the specimen removed during surgery yielded a determination of fibroadipose tissue with Hassall's corpuscles, leading to the conclusion that this tissue was related to thymic remnants and that this condition is not associated with development of pericardial effusion in this case.[8]

**Followup** The patient recovered quickly and started treatment with colchicine at low doses (0.5 mg every 12 hours). In the followup conducted one month after the procedure, the patient did not have pleural or pericardial effusion, and she maintained normal cardiac function and diabetes control.

**Final diagnosis** The patient received a diagnosis of polyserositis, possibly as part of a post–COVID-19 complication.

## DISCUSSION

The imaging finding of a pericardial lesion with pericardial effusion, along with manifestations of right ventricular failure, led to several possible diagnoses.

Pericardial conditions are variable in clinical practice and may occur as an isolated process or associated with certain systemic conditions. Different benign or malignant diseases can also be established in the pericardium.[9,10]

Initial identification of a pericardial mass, with the aid of TTE and CT, led to different presumptive diagnoses. First, pericardial metastases had to be considered because they are the most common malignant neoplasms of the pericardium.

The most common metastases are those from malignant lung, breast and kidney tumors.[10] Primary pericardial neoplasms are extremely rare. Mesothelioma is the most frequent, followed by fibrosarcomas and other types of sarcomas, lymphomas, neuroectodermal tumors and other benign lesions such as lymphangiomas, hemoangiomas, teratomas, neurofibromas and lipomas.[10] For these reasons, doctors initially turned to a thoracoscopy to evaluate the nature of the pleural effusions and rule out a malignant pleural effusion.[11]

Identifying fatty-like tissue bordering the pericardium with thymic remnants fully ruled out the primary nature of a pericardial lesion, as well as a primary or metastatic malignant lesion. While it may be an uncommon finding, thymic remnants are present in more than half of adults.[12]

After ruling out thymic remnants and neoplastic, infectious and autoimmune diseases as plausible causes for polyserositis in this case, a diagnosis of polyserositis associated with COVID-19 was proposed, a condition that to date has been reported in only one case in a scientific publication.[7] Nevertheless, the characteristics of the pleural and pericardial fluids (exudate) and the high levels of high-sensitivity troponin T support such a diagnosis. Evidence has also emerged indicating that SARS-CoV-2 can cause direct damage to the heart, either affecting the myocardium (myocarditis) or the pericardium (pericarditis).[13]

Polyserositis is a condition characterized by inflammation and effusion in more than one site, including the pericardium, pleura and the peritoneum. Because of the rarity of polyserositis in medical practice, the diagnosis is based on clinical recognition and subsequent screening of probable causes.[9] It has been suggested that polyserositis onset in these patients occurs as a result of immunological phenomena caused by COVID-19, characterized by a disregulated cytokine and chemokine production.[14]

Experience using colchicine to treat acute pericarditis and prevent recurrences indicates that this drug is safe for patients with COVID-19 and can be used during the early phases of the disease and for complications such as pericarditis and pleuritis.[15,16] The previous report of polyserositis as a post-COVID complication also documented successful treatment with colchicine.[7]

## CONCLUSION

This report described a patient with clinical symptoms consistent with post–COVID-19 polyserositis, which occurred three months after infection by SARS-CoV-2. While it is not possible to establish a definitive causal relationship between polyserositis and history of COVID-19, this link cannot be ignored, especially after ruling out other possible causes of polyserositis and finding a plausible physiological and pathological mechanism between these two diseases. Thus, the studies conducted in this patient led us to conclude that polyserositis may be a late complication of COVID-19. This case is the second reported in the world and the first in Cuba describing such a nosological association.

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# Families in Grief: Need for Psychological Care and Support for Those Who Lost Loved Ones to COVID-19

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## ABSTRACT

The COVID-19 pandemic has caused notable changes in all areas of our lives. Pandemic-coping strategies include attention and care at various levels, for different people and in various scenarios. Death is one of the most feared consequences of COVID-19 for both patients and their families; for the latter, the grief and adaptation processes to loss require that care for grievers be an important part of the public health response to the COVID-19 pandemic.

Grief from losses due to COVID-19 has distinctive features: it is not anticipatory (with virtually no time or progressive stages to facilitate adaptation to loss); closure or goodbyes are not possible (in-person social support decreases due to distancing to minimize risk of infection); it may affect various close relationships (a relevant predictor of complicated grief); it may imply stigmatization by peers, friends and neighbors; it is preceded by a period of absence of fluid and in-person communication between family members and the hospitalized patient; and those who break the news of the death are often professionals in red zones who are stressed and do not always have the skills or the ability to properly communicate bad news.

The death of a family member from COVID-19 generally causes an unexpected crisis in the family, which is already affected by the pandemic and its daily consequences. This has prompted an analysis of COVID-19 loss on family life and how best to mitigate its consequences.

During the COVID-19 pandemic, care and monitoring of the grief of family members and those who were close to the deceased require psychological action within a framework of comprehensive care, which demands preparation of healthcare professionals. Experiences described are taken from some actions developed in Cuba.


**KEYWORDS** Grief, psychology, death, attitude to death, COVID-19, SARS-CoV-2, Cuba

## INTRODUCTION

The pandemic caused by SARS-CoV-2 has led to changes in all areas of our lives, with important consequences that require development of various adaptation strategies, including those that help us grapple with the emotional and psychological repercussions. Perhaps the most terrifying personal aspect of the pandemic—besides our own death—is the loss of family members and other loved ones. By the time of this writing—end of October 2021—244 million people had suffered from the coronavirus worldwide, of whom nearly 5 million had died, and both cases and deaths were on the rise.[1]

On the individual level, COVID-19 has caused substantial changes at one of the most difficult times in any life: saying goodbye to a loved one. The special circumstances surrounding the adaptation processes to these losses may become a risk factor hindering a normal grieving process, and must therefore be examined and prioritized. This is not a simple task; it demands extreme delicacy and competence. It requires training, communications skills and willingness on the part of health professionals to support readjustments to family life. At the same time, it is increasingly becoming a real public health problem. There are no known long-term studies that determine the proportion of normal grief or complicated grief due to COVID-19.

**IMPORTANCE** Grief monitoring and management for people who lost a loved one to COVID-19 is a public health problem that is now of particular importance. This paper describes grief manifestations and the care recommended in the pandemic context. Some Cuban experiences in this field are described. It also comments on the need to prepare health professionals for such urgent patient care, to which psychologists can and should be important contributors.

This paper presents our understanding of grief among people who have lost family members to COVID-19, with recommendations for managing their care, including evaluation, monitoring, support and counseling as part of a comprehensive approach to therapy. Experiences in Cuba serve as point of reference, including initial actions taken to address grief-stricken families and loved ones.

## DEVELOPMENT

**Grief as a loss adaptation process and distinctive features of COVID-19 grief** The process that ensues after a significant loss, aimed at restructuring life around acceptance of that loss, is known as 'grief.'[2,3] During the COVID-19 pandemic, losses may be of a diverse nature; some may be temporary; others permanent. Many of us have experienced the most painful loss: the death of a family member, friend or other close relationship.

In general, loss of a loved one engenders a normal adaptive process with a beginning and an end; it is often long and painful, which involves undoing, little by little, ties with the loved one, while keeping his or her image present in our inner world.[2–4]

There are three main components of grief: 1) feelings of sorrow, suffering and internal pain, without which grief cannot be resolved; 2) longing and nostalgia due to the physical absence

of the deceased person; and 3) expressive behaviors of loss (i.e. mourning, the forms of which have changed throughout history and depend on cultures and personal characteristics).[2−6]

The latest glossaries for classifying mental disorders define grief as disposed to receiving medical attention, as it may cause symptoms similar to depression and other conditions.[7] That is, while there is consensus that grief is not an illness, if it progresses to 'complicated grief,' it becomes a risk factor for illness. Obviously, the line differentiating normal grief from complicated grief requires clear diagnosis criteria that allow for timely decision-making and intervention.[3,8]

Prevalence estimates for complicated grief vary among different populations and can be influenced by culture, age and other factors (involving such situations as death of a close relative, prevalence of certain chronic conditions in the close space a given country or territory, personal capacity to make sense of the loss, attitudes and beliefs concerning death, critical scenario competencies and personal ability to handle emotions, degree of self-care, sentiments of guilt, unresolved mourning from past losses, etc.).[8]

For example, Jacobs estimated complicated grief at 10%−20% of grievers;[9] Bonnano and Kaltman, 15%;[10] and Maercker found a prevalence of just 7.4% in older persons.[11] Thus, according to these and other classic studies, grief follows a normal course in >70% of cases, requiring only monitoring and support. In <30% of cases it becomes complicated grief, when it lasts many years and the griever is incapable of social reintegration, or when the griever acquires pathological characteristics, with extreme nervous strain, excessive identification with the deceased, drug addiction or risk of suicide, all conditions requiring therapeutic management. [3,5,6,8,12]

There are no reliable estimates regarding the relative frequency of normal and complicated processes in grievers due to COVID-19. If each deceased person left an average of five grieving individuals, and of these, 15% had complicated grief, by the end of October 2021, there would be millions of people experiencing complicated grief worldwide, with their numbers increasing over time.

Certain vulnerability factors are linked to the risk of complicated grief: the bereaved's connection with the death (intensity, ambivalence, emotional dependence); emotions and their expressions (anger, blame, inability to express emotions); the illness and its characteristics (speed of progression, delay in diagnosis, knowledge of the family member's prognosis, duration of care); uncontrolled symptoms such as anxiety, chest pain, nausea, rage, emotional lability, dysthymia or other depressive states; personal medical history; unresolved previous grief; financial problems; presence of children and/or disabled individuals in the family; and lack of family support.

There are also protective factors that comprise a resilient or 'hardy' personality that include self-control mechanisms (such as meditation, deep-breathing exercises), ability to handle emotions, perception of stressors as challenges rather than catastrophes, positive attitude towards life and the world, and marked spirituality, among others. Protective factors can also be influenced by interpretations and beliefs (religious beliefs, confidence in recovery, ability to find meaning in what happened, sense of purpose in the care of the patient); and attitudes and skills in managing various situations (ability to plan in the face of difficulties and consider alternatives, ability to communicate and carry out self-care, ability to feel positive emotions and plan enjoyable activities).[3,8] These factors play a large part in the grieving process, its duration and prospects for recovery.

Various studies indicate that any grief passes through stages lasting between one and two years, but there is no consensus in this regard. Most people go through an 'early' stage that lasts 3–6 months and then a 'late' stage that lasts up to 12–18 months. In general, there are four stages: 1) a first phase of bewilderment, numbness and denial, which may last a few hours *(it can't be true…)*; 2) a second, more prolonged phase of yearning and nostalgia, where the person misses the deceased and tries to compensate for the loss *(I miss him/her so much…)*; 3) a third phase, characterized by disorganization and desperation when trying to continue living *(a piece of me is missing…)*, and 4) a last phase of reorganization and recovery, always positive, where the person begins to use various skills to compensate for their loss, starts to feel the will to continue living, embraces help and the company of others, and can reminisce without as much pain *(I am more calm, I should try to rebuild my life…)*.[3,6]

People often misunderstand the meaning of 'grief resolution.' This does not mean disloyal forgetting. Physical presence ends, but not the emotional relationship, which continues to change, continues to 'restructure;' the griever does not 'give up' the deceased, but rather tries to find an appropriate place for the deceased in the griever's inner life, leaving space for new actions and life experiences.[3,6,13]

Table 1 summarizes grief types and their characteristics. [3,6,14,16–18]

**Specificities of COVID-19 loss and grief** COVID-19 pandemic has brought about losses that affect practically all spheres of life: freedom of movement (defined by lockdown restrictions); affectionate connection through physical contact and in-person social relationships (especially among adolescents and youths); work and opportunities; in-person studies; family finances, etc. All of these imply a complex adaptive process in multiple dimensions. This global impact inherent to COVID-19, along with grief specificities, present a challenge for professionals who must assist and monitor the grief process.[14,15] Main specificities of grief from losses due to COVID-19 are summarized in Table 1.

Professionals assisting grievers should understand that family members and even health professionals may experience a great variety of mixed emotions and confusion due to the physical and psychological exhaustion accompanying the situation; even though these are normal reactions, they may be long-lasting. It is common for there to be many questions with no clear answers regarding what has happened. In addition to losing a family member, there are other special circumstances surrounding the loss of a loved one from COVID-19, such as not being able to take care of or accompany the family members or say goodbye during their last moments. This increases the risk of inadequate physical and psychological regulation associated with this immediate situation (palpitations, feeling of precordial chest pressure, or a knot in the throat or stomach, headaches, dry mouth, dizziness, mood changes, impatience, difficulty concentrating, etc.).[14−16]

**Table 1: Grief types and their characteristics**

| Grief type | Characteristics |
|---|---|
| Normal | Presents with suffering, longing and various expressive behaviors of mourning, but gradually decreases over time and as the griever recovers his or her life |
| Anticipatory | Generally, when family members are prepared for the loss of a person who has a long-term illness; grief manifestations precede the loss |
| Delayed, frozen or denial | When the person refrains from expressing his or her emotions or denies them, the person does not confront the reality of their loss and may experience false euphoria or rapid acceptance that may suggest a pathological tendency |
| Complicated (chronic or pathological) | Intense symptoms or evolution towards chronicity; requires therapeutic support |
| **Special*** | |
| Due to unexpected or unforeseen death (sudden death, homicide, accident, suicide) | Generally complicated due to insufficient time to 'process' the loss; may include griever insistence to find people responsible or at fault |
| Deferred | When various losses occur and the last of these causes the griever to break down emotionally |
| Due to natural or man-made disasters | Impacts many people in many ways, and usually suddenly or unexpected |
| Collective | When a well-known or admired public figure passes away |
| At a distance | When the body of the deceased, which would validate the reality of death, never appears |
| In special groups | In the elderly, children, parents who have lost a child, siblings; pregnant women who have miscarried; even loss or death of pets, etc. |
| **Due to COVID-19 losses** | • Does not have characteristics of anticipatory grief, where there has been time and progressive stages for loss adaptation; nor is it grief for an unexpected or unforeseen death<br>• Emotions related to sadness and sorrow merge with others stemming from characteristics of the pandemic and the conditions in which people die; there may be blame, rage and exasperation, helplessness, feelings of frustration and injustice, fear<br>• Closure or saying goodbye is not always possible; due to the risk of infection, many farewell rituals must change; in-person social support is decreased in the early period after death<br>• Multiple losses of various significant others may be frequent (a risk predictor for complicated grief), which combines various types of losses<br>• There may be stigmatization by peers, friends and neighbors due to fear of transmission<br>• Generally, grief is preceded by a period where there is a lack of fluid and in-person communication between family members and the hospitalized patient<br>• News of the death is communicated by professionals who are in the red zone, are stressed and do not always have the full complement of skills or opportunity to properly communicate the news to the significant others of the deceased<br>• It can also affect professionals and service providers, especially those who have spent the patient's last days at their bedside, supporting him or her and trying to save his or her life; it can therefore be associated with work-related stress, burnout syndrome or so-called 'compassion fatigue' |

* There will be as many specific types of grief as there are types of losses, levels of attachment, and age groups, among other factors.[3,6,14]
*Source: References 3,6,14,16–18*

**Recommended guidelines for care of families who have lost members to COVID-19** It is difficult to identify a single protocol for grief care that is equally applicable to everyone. Generally, guidelines or recommendations must be accepted in a flexible and time-sensitive way for each grieving individual or family. We are dealing with a pandemic that we experience as continuous alarm. We should not be overly demanding of ourselves or grievers, as many of the current circumstances are out of our control. The aim is to strengthen grievers by patiently setting them on a continuous path towards acceptance and recovery.[13]

In general, the recommended measures for any grief are applicable in the face of losses due to COVID-19 (Table 2).[5,7−10,13]

Children require special management that is dependent on their concept of death, related to developmental growth (generally children acquire a concept of death similar to adults after 10 years of age); prior experiences with death in the family or outside of it; the family's emotional stability and their relationships with other adults; the opportunity to share emotions and feelings; their relationship with the deceased; the environment surrounding the child at home and elsewhere; and the cause of the loss. In the case of COVID-19, it may be associated with prior feelings of threat due to the child's excessive exposure to information from the media surrounding the pandemic.[13−18]

Over our 30 years of clinical practice, we have been approached multiple times by families seeking guidance on how to manage grief, not only in adults, but also in children. Not knowing how to tell a child about the death of a close family member (parent, grandparent, sibling) is a source of insecurity that sometimes leads to quick 'solutions' that include lying, avoidance, or using mystical explanations that complicate the normal course of grieving for the child later on. In Cuba, since family doctor-and-nurse offices are close by, embedded in every neighborhood, family members often seek orientation from either the family physician or the psychologist assigned to the office, sometimes before the death actually occurs. This guidance facilitates handling the grieving process with children in particular.

In the case of COVID-19 losses and children, a number of suggestions for support adapted from the broader spectrum

**Table 2: Recommended general measures to treat all grief types, applicable to COVID-19 losses**

- Do not prevent the emotional venting of the griever; let him or her express emotions freely
- If possible, provide the griever with details regarding what happened with the deceased
- Do not criticize or impose cultural or familial stereotypes (e.g. 'men don't cry')
- Help the griever consider his or her emotions and thoughts as normal, let the griever know that the suffering that may initially seem unbearable will end, that the magnitude of his or her feelings cannot be measured in a given time. Explain that grief has its stages and that there are factors that can hinder or facilitate this process
- Emphasize that the griever's relationship with the deceased does not disappear over time, but rather changes
- Advise against 'geographical cures' (moving to other places or changing homes), or making important decisions early in the grieving process
- Do not pressure the griever to avoid memories or get rid of the belongings of the deceased
- Do not abuse psychotropic drugs, since they may 'freeze' the grief process
- Advise against too early a return to work, indicating it is usually preferable to spend several days at home with support from family members and friends

**For children**

- Do not lie to them or tell them something you may have to retract later
- Adapt information to their chronological age and emotional maturity
- Do not link death with sleep
- Do not encourage false expectations regarding the return of the deceased
- Do not delegate explanations to other people (it will always be best if parents or persons closest to the child explain)
- Do not associate death with opposition that may lead to blame
- Encourage adults not to hide their own grief, but share it with children
- Give the child the opportunity to talk about the person who died

*Source: References 3,6,13,14*

of grief counseling can be applicable (Table 2).[13−18] Professional advice centers on not separating children from the reality that adults are experiencing, since they notice what is happening and it affects them if the news is not shared with them. While it is painful and difficult, it is usually preferable to tell them what happened as soon as possible, in the proper time and place using simple and sincere words (*Something very sad has happened; Grandpa will no longer be with us, he is no longer alive*), making the explanation as brief as possible (*you know that he has been sick*), and using age-appropriate language.

For the family as a whole, upon learning of the person's death, it is common for grievers to feel that they should do 'something', such as participate in a ritual of mourning. Rituals are symbolic acts that help express feelings in the face of loss, establish a certain order for life events, and allow the social construction of shared meanings. They also open the door to awareness of the grieving process. Some may be longer or shorter in terms of time: it is not the same to write a letter as it is to start a diary or create a permanent altar. What is important is that they help integrate what has happened and the way in which the loss is experienced. These rituals can be in-person or remote.[14−18]

It is generally preferred that in-person farewell rituals are more private events, or that only those with whom the person was closest are present; each person's manner and personal space should be respected. Any variant can be performed, informing the people around them, always mindful of accompanying and caring for the closest loved ones. Depending on the situation, some mourning rituals may be held immediately, or others postponed. It is usually good to prepare something in writing for when it is possible for all the deceased's loved ones to gather and make a small in-person tribute, or to record a video and share it with others via social media. Narrative therapy techniques are useful: writing a letter to the deceased, to a specific emotion (sadness, rage), to God, to life, to other people…it is healthy to write poems and messages with positive content (something that you would say to the person if they were present), to express memories and feelings of appreciation and forgiveness.

In remote farewell rituals, it may be advisable to schedule a meeting after consulting with family members, where everyone shares and expresses what they might like to do in a virtual ceremony. It may also be useful to post on social media, as a tribute, referring to aspects of the person's legacy through words, music or images.

Grief guides with specific orientation for children and adolescents and people with cognitive disabilities, as well as for healthcare professionals, insist that euphemisms not be used (*he is gone; he has left us; she has gone to sleep forever*). It is preferable to speak about death naturally, attuned to the griever's emotional reaction.[14−18] Children, the elderly and people with cognitive disabilities should not be excluded from these rituals, but rather participate in all ways their condition and age allow. Each person needs to feel loved and give love, be cared for and care, feel safe and provide safety, feel validated, strengthened, understood, respected, and accompanied in their life processes; therefore, it is also necessary that each one be able to reciprocate what they receive. This involves building social networks rather than walls.[16] We agree with Bucay; this is not easy, but it is possible—and it takes time.[13]

On the personal front, grievers may find solace in compiling works by authors with whom they identify, starting a diary or a series of drawings to express their feelings. A corner of a room can be set aside for remembrance, displaying a photo of the deceased or an object that symbolizes the relationship with that person, decorating it with flowers and candles, to pray, to express what is felt, and to call up memories.

Any normal grief (or uncomplicated grief) requires monitoring with support, accompaniment, and counseling, but not necessarily therapy.[3,14] However, psychiatric evaluation and treatment should be considered when grief manifests in a severe way, especially with suicidal ideation or suicidality or an increase of addictive substance use, when symptoms are severe and/or persistent and do not resolve with support interventions and counseling, or when there is other evidence of complication risk. In such cases, anxiolytic or antidepressant medications may be necessary for a short time, but these are not always needed, and their dose should be decreased progressively as soon as possible. The goal is not for the psychotropic drugs to 'freeze' the grieving process, which can lead to its chronic progression (a chronic grieving process).[19]

In cases of complicated grief, people often turn to specialized professionals who attempt to direct the process towards a normal evolution. Different models, methods and techniques have been used successfully for this, including problem solving, positive reevaluation, balance in emotion-focused coping, seeking out social and general support, encouraging spiritual values, even third-generation cognitive therapies such as mindfulness and acceptance and commitment therapy (ACT).[20−22] What is important is the timely detection of complicated grief, which can be done with a brief interview or questionnaires that may even be completed by telephone.[5,14]

**Repercussions of COVID-19 loss on family life** Family represents the social group with the greatest significance for most people. The first life lessons and fundamental learning in the process of personality formation usually take place in the family. For that reason, relationships established in the family and events within it can be sources of satisfaction, wellbeing and positive feelings, or alternatively, pain, frustration and despair.

The loss of a family member inexorably gives rise to a family crisis, which may be normative (related to the expected family life cycle) or para-normative (unexpected loss), but it is a high-impact event in family life, regardless. Death of a close relative from COVID-19 is a para-normative and unexpected event that occurs in a family that has already been affected—as have nearly all families—by the crisis generated by the pandemic and its impact on daily life.

Many family-related variables determine the response to the loss of a family member, such as communication, cohesion, family structure, roles, coping resources, lifestyle characteristics and belief systems, among others. Among the risk factors for complicated grief that should be considered when deciding whether professional interventions are necessary are: a) relational (age of the deceased and relationships of dependency, conflict or ambivalence); b) circumstantial (conditions in which the death occurred, uncertain death); c) personal (age of the survivor, poor stress management, comorbidities, previous grief); and d) social (isolation, socioeconomic difficulties, childcare, stigmas).[23]

During the pandemic, these processes are influenced by other conditions that add to the complexities surrounding the loss. Intra-family transmission of the virus (quite common) may cause feelings of guilt that add to those that death commonly produces in family members. Having thoughts such as: *If I had protected myself better or required him or her to protect him or herself better*; *if I hadn't left the house*; *if I had taken him or her to the hospital earlier,* may hinder the normal course of grief. The inability to accompany family members during their hospitalization and to be present in critical moments, including at the time of death, greatly impedes or limits communication precisely when, in other circumstances, farewells, forgiveness, resolution of pending problems and satisfaction of last wishes would take place. This impossibility causes anguish in the family. The uncertainty regarding the way in which the family member died also contributes to these states of anxiety.

A review of research on the impact of grief on families during the pandemic[24] shows the primary need expressed by families is to be nearer to their loved one during the process of dying, and the impossibility of doing so becomes traumatic. Additional traumatic events include the feeling of loneliness during end-of-life care and lack of counseling for the family by healthcare personnel, as well as insufficient information or contradictory information provided by such personnel. These results confirm the urgent need for further preparation of healthcare personnel to more effectively support families facing loss.

If supporting a grieving person at any time is difficult, doing so while adhering to COVID-19 measures presents a bigger challenge. When in-person gatherings are not possible, it is important to find a virtual way to organize a collective farewell with the main grievers. It is important to show understanding; the situation is difficult, and the family may be overwhelmed or even difficult to contact at first try.[14−18] Yet, it is also helpful to stay in touch with them via telephone and social media to offer support (*I just want to know how you are, and I want you to know that I am available if you want to talk. If you need to talk, you can call me, etc.).* Active and empathetic listening is always preferable to trite expressions of sympathy (avoiding phrases such as: *Be strong; cheer up; do it for your kids; don't cry anymore, you are torturing yourself; life goes on.)* The main message is always *I want you to know that I am here, and I am thinking of you*, and self-care should be encouraged and supported.

**Experience in Cuba** Cuba has conditions that favor family accompaniment during the grieving process and therapeutic follow-up as needed. In particular, its potential includes a universal health system that ensures equitable and free access to its citizens, a social orientation that prioritizes psychosocial problems, and availability of psychologists throughout the health system. Psychologists also work at the primary care level with family doctors and nurses, an ideal setting to offer follow-up care, help modify attitudes (in terms of their cognitive, emotional and behavioral components) and assist in charting a course towards a renewed positive sense of life among the grieving.[25–28]

Given the peculiarities of the COVID-19 situation, the first task undertaken by the Ministry of Public Health and the National Psychology Group was to institute further training of professionals in the field. Scientific articles were widely distributed, accompanied by a newly-developed manual on providing support to the population, and precise orientation was provided via videoconferencing to the chairs of provincial chapters of the Cuban Society of Health Psychology and heads of provincial psychology services, to be shared with colleagues in each territory, along with the experiences accumulated.[27,28]

These guidelines were reinforced throughout the pandemic through various academic activities carried out face-to-face or remotely. The theme has been the subject of various manuscripts submitted for publication and in presentations at several international conferences. On a more personal level, psychologists and psychiatrists serving in the red zones also joined in mourning there, which even brought together other health professionals mourning the loss of their own family members.

Grief management was also a recurring theme in social communication throughout the country, considering the delicacy needed to address it, with care not to transmit hopelessness or reinforce fears created by the situation, and at the same time, to legitimize the difficult conditions for handling grief, as well as ways to satisfy the needs for emotional release and farewell rituals. Thus, the theme was the subject of various special television programs and spots.[29,30]

Psychological orientation for addressing grief was in constant demand during the epidemic in Cuba, especially in 2021 at the height of cases and deaths. Hence, the psychology and psychiatry services that remained open offered consults through video and WhatsApp groups. A hotline for psychological support that functioned 24 hours a day throughout the country also responded to calls seeking orientation on the grieving process. Due to the magnitude of the pandemic, and its effect on the entire population, there is obviously much work and follow-up still to be done. Thus, it is a priority, both for care and for continuing research on the subject.

At this stage in the pandemic, another factor influencing grief is the exhaustion of family members, with dwindling resources to address the crisis, and they may simply be at risk of burnout. This can have health implications for all family members, especially the most vulnerable, and may affect the completion of important family functions such as protection and care.

The greater extent of losses during the pandemic—of family members, friends, neighbors, coworkers and other acquaintances—has brought the notion of death closer to home, adding additional pain and fear for the health of the family's remaining members and for oneself. Such specific conditions created by the spread of COVID-19 must be kept in mind when conducting preventive measures, family support or psychotherapeutic care in the ensuing period.

## FINAL CONSIDERATIONS

Grief management demands urgently needed comprehensive care for family members of the deceased. Even though this is a normal adaptive process necessary to restructure life after loss, which passes through several phases with variable duration, in the case of losses due to COVID-19, it takes on a special character: the absence of an acceptable farewell, as well as other specific characteristics, determine the forms grievers may use to adapt to the loss of their family member. Managing the process, according to specific guidelines, is beneficial not only for the griever closest to the deceased, but also for the entire family, for friends, people who were close to the deceased, and even for other health professionals.

It must not be forgotten that grief is a part of life and that, while difficult and painful, it is necessary to continue personal and family development. As Viktor Frankl said: "If you can no longer change a situation that causes you pain, you can always choose the attitude with which to confront the suffering,"[25] or, in the words of Jorge Bucay: "tears are, therefore, welcome…they mean we are on the road."[13]

We believe that care for the general population during and post-pandemic will be incomplete if it does not entail attention to family members and significant others of the deceased. It is important that we are all prepared to participate in this care, particularly health professionals, with a special role for psychologists and psychiatrists.[29]

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# A Shift in SARS-CoV-2 Omicron Variant's Entry Pathway Might Explain Different Clinical Outcomes

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## ABSTRACT

Globally, SARS CoV-2 omicron variant has led to a notable increase of COVID-19 diagnoses, although with less severe clinical manifestations and decreased hospitalizations. The omicron wave swelled faster than previous waves, completely displacing the delta variant within weeks, and creating worldwide concern about final, successful pandemic control. Some authors contend that symptoms associated to omicron differ from 'traditional' symptoms and more closely resemble those of the common cold.

One major COVID-19 symptom frequent with other variants—loss of taste and smell—is rarely present with omicron. This may be of interest, since it has also been suggested that direct SARS-CoV-2 invasion into the brainstem through the olfactory nerves by transsynaptic pathways could provide one explanation for the acute respiratory distress syndrome refractory to treatment. Brainstem infection by SARS-CoV-2 can severely damage the respiratory center, triggering functional deviations that affect involuntary respiration, leading to acute respiratory distress syndrome refractory to treatment, the main cause of death in COVID-19 patients. A shift in the omicron SARS-CoV-2 entry pathway from cell-surface fusion, triggered by TMPRSS2, to cathepsin-dependent fusion within the endosome, may affect transmission, cellular tropism and pathogenesis. Therefore, we can hypothesize that this entrance modification may impact transmission from the olfactory nerve to the brainstem through transsynaptic pathways. A decrement of the virus's direct invasion into the brainstem could diminish respiratory center dysfunction, reducing acute respiratory distress syndrome and the need for mechanical ventilation.

**KEYWORDS** SARS-CoV-2, COVID-19, olfactory nerve, COVID-19 pandemics, respiratory center, smell, anosmia, taste, ageusia, brain stem, cathepsins, endosomes

## INTRODUCTION

Globally, the SARS CoV-2 omicron variant has led to a significant increase of COVID-19 diagnoses with less severe clinical manifestations and decreased hospitalizations. The omicron wave has swelled faster than previous waves, completely displacing delta variant within weeks, and creating new worldwide concern about the ability to achieve final pandemic control.[1]

Omicron is the fifth variant to be named as a variant of concern by WHO and the third (after alpha and delta) to achieve global dominance.[2] Omicron was first documented in the city of Tshwane, Gauteng Province, South Africa, on November 9, 2021, in travelers from Hong Kong who were quarantined.[1] At the time of this writing, it had split into three divergent sublineages (BA.1, BA.2, and BA.3), of which BA.1 spread rapidly around the world.[1,2] Several initial reports suggested a less severe disease, including from researchers in South Africa, where the variant spread quickly.[1] In young people, omicron is 40%–70% less severe than delta variant. Chen studied omicron variant infectivity, vaccine breakthrough, and antibody resistance. The author concluded that omicron variant infects and replicates 70 times faster in the human bronchi than delta variant and the original SARS-CoV-2 virus, which may explain why omicron is more transmissible than previous variants.[3] A recently published study from Hong Kong found that omicron replicates faster in bronchi than all other SARS-COV-2 variants, although less efficiently in the lung parenchyma. This could explain its increased transmissibility, but reduced severity.[4]

**IMPORTANCE** The SARS-CoV-2 omicron variant's cell entry pathway differs from that of earlier variants and could explain the lower prevalence of acute respiratory distress, due to diminished invasion of the brainstem through the olfactory nerve. This change could have implications in patient management, as intractable respiratory distress syndrome is less likely to occur.

Some authors have emphasized that symptoms associated with omicron differ from 'traditional' COVID-19 symptoms, more closely resembling those of the common cold.[5] One major COVID-19 symptom is rare in omicron patients: loss of taste and smell.[6,7] Some research suggests that 48% of people carrying the original mutation of the novel coronavirus lost smell, and 41% had a loss of taste; these numbers decreased to 23% for loss of taste and 12% for loss of smell among omicron-infected patients.[8] Consequently, many people may not realize they have contracted COVID-19, since fewer experience cough, fever, or loss of taste or smell. A virus in the upper respiratory tract is associated with increased transmissibility, but with a less severe disease, exhibiting similarities with flu strains.[9]

The most deadly syndrome in COVID-19 patients is acute respiratory distress syndrome (ARDS), leading to severe respiratory failure and the need for mechanical ventilation in intensive care units (ICUs). Initial reports from Wuhan hospitals in China revealed that 11.1% received high–flow oxygen therapy, 41.7% received noninvasive ventilation, and 47.2% received invasive ventilation. These data suggest that most COVID-19 ICU patients (about 89%) could not breathe on their own.[10,11]

One explanation for ARDS resistance to treatment could be SARS-CoV-2 direct invasion into the brainstem. Such an invasion can severely damage the respiratory center, triggering functional

deviations that affect involuntary respiration, leading to ARDS refractory to treatment, the main cause of death in COVID-19 patients. Evidence accumulated to date has led to a much wider acceptance of the neuroinvasive potential of SARS-CoV-2 for inducing respiratory failure in some patients.[11–13,15,16]

Nevertheless, there have been conflicting hypotheses, based on the fact that brain damage leading to respiratory failure is usually accompanied by other signs of brain dysfunction, not reported in any of the initial Chinese case series.[14]

It is suspected that cranial nerves (CNs), particularly the olfactory nerve, contribute to the neuroinvasiveness of SARS-CoV-2.[17] The virus has been found in the olfactory mucous membrane, signaling involvement of the olfactory neuroepithelium. It can reach the olfactory bulb through anterograde axonal transport and can subsequently gain access to other neuroanatomical areas, such as the respiratory and cardiovascular centers of the medulla oblongata, via endocytosis and exocytosis for transsynaptic transfers. Numerous neurotropic agents, including parasites, bacteria and viruses, can reach the CNs via the olfactory nerve.[8,11] Other possibilities for neuroinvasion of SARS-CoV-2 are through hematogenous spread via either the blood–brain barrier (BBB) or the blood–cerebrospinal fluid barrier (B-CSFB).[18]

Coronaviruses cell entry depends on the viral spike (S) protein binding to cellular receptors and its priming by host cell proteases. SARS-CoV-2 uses the angiotensin-converting enzyme 2 (ACE2) receptor for entry and the plasma membrane serine protease 2 (TMPRSS2) for protein S priming.[19,20] The virus enters the nasal and mouth tissues through the ACE2 receptor and the proteolytic activation of the spike protein by TMPRSS2 in olfactory epithelia;[21] ACE2 is also expressed in brain locations (choroid plexus and olfactory bulb). Cell types expressing ACE2 include excitatory and inhibitory neurons, and some non-neuronal cells (astrocytes, oligodendrocytes and endothelia). Viral colonization of nose and mouth may cause temporary damage to smell and taste nerves; this damage tends to disappear within one to two weeks after disease onset.[22]

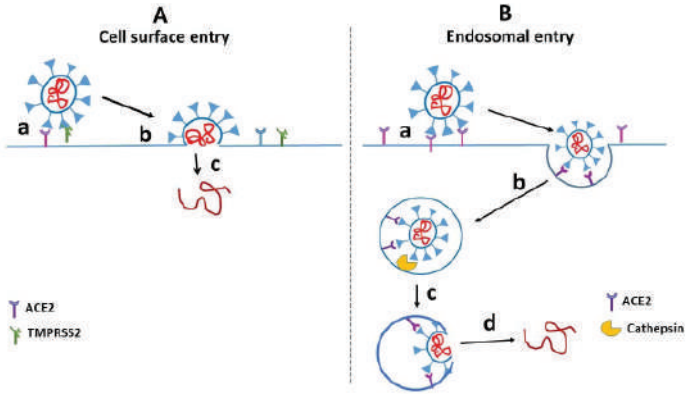
Recently, an alternate mechanism for central nervous system (CNS) entry of SARS-CoV-2 has been proposed, where neurons of the nervus terminalis—also known as cranial nerve '0' at the olfactory epithelium in the nasal cavity—would be the carrier, rather than the olfactory nerve. Nervus terminalis neurons project directly to the hypothalamus in the brain, bypassing the olfactory bulb. Once the hypothalamus is reached, SARS-CoV-2 can cross the blood–brain barrier and reach neural circuits connected to the hypothalamus, including brainstem nuclei involved in respiration. As other cell types in the brain, the nervus terminalis expresses ACE2 but not TMPRSS2. This would favor the omicron variant's preferential pathway for entering the cells, making this nerve a prime target for brain entry.[23]

SARS-CoV-2 spreads to the brainstem in the area of the medullar respiratory control centers and may produce discoordination of the inspiration–expiration sequence, explaining the appearance of other abnormal respiratory patterns: hyperpnea, tachypnea, hyperventilation, hypoventilation and not necessarily dyspnea.[11–13] The omicron variant's spike protein exhibits a series of mutations that affect affinity for the ACE2 receptor. It has been suggested that omicron switched its entry route into human cells, from cell surface fusion to cathepsin-dependent fusion within the endosome.[24] This fundamental shift is likely to influence omicron spread and the types of cells it can hijack. These changes may also affect the pathogenesis and severity of disease, and require further evaluation in population-based studies.[20,24]

The entry of SARS-CoV-2 and related coronaviruses can proceed via two routes[24] (Figure 1):

1. Cell surface fusion following proteolysis by TMPRSS2
2. Fusion in the endosome after endocytosis and activation by the endosomal proteases cathepsin B or L (independent of TMPRSS2).

**Figure 1: The entry of SARS-CoV-2 and related coronaviruses can proceed via two routes**




1A. a) Binding of the S1 subunit of the viral S protein to the ACE2 receptor at the cell membrane surface and proteolytic activation of the spike by the transmembrane protease serine 2 (TMPRSS2); b) conformational change in the S2 subunit and large-scale rearrangements of the S protein, resulting in virus–cell membrane fusion; c) uncoating and release of viral nucleocapsid into host-cell cytoplasm.

1B. a) Binding of the S1 subunit of the viral S protein to the ACE2 receptor at the cell membrane surface; b) endocytosis of the viral particle; c) activation by the endosomal proteases cathepsin B or L, which leads to fusion within the endosome; d) uncoating and release of viral nucleocapsid into host-cell cytoplasm.

The ability of SARS-CoV-2 to achieve cell-surface fusion is dependent on its S1/S2 polybasic cleavage site; this is absent from most closely related sarbecoviruses, which are confined to endosomal fusion.[24] Omicron, like pangolin CoV, has optimal properties for endosomal entry. Therefore, while delta entrance is improved for fusion at the cell surface (Figure 1A), omicron preferentially enters through endosomal fusion (Figure 1B). The previously mentioned modification in omicron's entry mechanism could impact transmission, cellular tropism and pathogenesis, and offers an explanation for reduced cell fusion or syncytia formation by omicron-infected cells. Syncytia have been reported on autopsy in COVID-19 cases, and the efficient cleavage at the furin site underlying syncytia formation has been associated with enhanced disease severity in animal models.[25] This may explain the decreased disease severity, since these properties can substantially change the virus's cellular tropism and disease pathogenesis.[24]

Concerning the neuroinvasive potential of SARS-CoV-2, modification of omicron's biological properties might result in less effective transmission along the olfactory nerve and its projection to the brainstem, reflected clinically in less frequent impairment of smell and taste. We hypothesize that it could preferentially direct trans-

mission from the olfactory nerve to the brainstem through transsynaptic pathways. Hence, decreased viral invasion directly into the brainstem would diminish respiratory center dysfunction, while reducing ARDS complication and the need for mechanical ventilation. Evidence strongly supports the association of neurological involvement with the lethality of SARS-CoV-2 infection, and omicron's severity and lethality are much lower than those of other previous variants.[26]

Other causes may explain why the omicron variant is related to a milder symptomatology and a reduced viral access to the CNS: 1) omicron infection in the lung is significantly less frequent than that of the original SARS-CoV-2, thus leading to lower severity and, subsequently, lower prevalence of respiratory distress; 2) lower pathogenic effect could generate a lower viral load and less local or systemic inflammation; 3) acquired immunity (by previous infection or vaccination) during more than two years of pandemic.

The possible interpretations proposed here should be reviewed cautiously. Understanding SARS-CoV-2's entry mechanisms into the CNS is still challenging, despite intense research on this topic. Two years of investigation are insufficient to arrive at definitive conclusions in any area of biological research. In the case of the omicron variant, this is especially so. More studies are required to establish the exact mechanisms of how the SARS-CoV-2 variants reach the brain stem, and to what extent this phenomenon can contribute to inducing ARDS resistant to treatment. We must keep in mind that the route and molecular mechanisms of neuroinvasion are not the only factors involved in ARDS development in COVID-19 patients. Other processes must be considered, such as previous specific immunity and disregulated innate immune responses leading to neuroinflammation.

## CONCLUSIONS

A shift in the omicron SARS-CoV-2 entry pathway from cell-surface fusion, triggered by TMPRSS2, to cathepsin-dependent fusion within the endosome, may affect transmission, cellular tropism and pathogenesis. Therefore, we can hypothesize that this entrance modification may impact transmission from the olfactory nerve to the brainstem through transsynaptic pathways. Decreased direct viral invasion of the brainstem could diminish respiratory center dysfunction, reducing acute respiratory distress syndrome and the need for mechanical ventilation.

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INSIGHTS FROM

MEDICC

# Cuba's COVID-19 Vaccine Enterprise: Report from a High-Level Fact-Finding Delegation to Cuba

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# Context for the Delegation's Visit to Cuba

In June 2022 an international delegation of scientists from the United States, the Caribbean and Africa traveled to Havana, Cuba for a three-day fact-finding mission to better understand Cuba's COVID-19 vaccine development and vaccination efforts. The delegation was comprised of experts in public health, infectious diseases, biotechnology, and vaccine research and development. The purpose of the fact-finding mission was three-fold: first, to learn how and why a relatively small country of some 11 million people—one facing considerable economic hardships—chose to develop, manufacture and deploy its own vaccines; second, to understand the country's strategy for vaccine rollout and its preliminary results; and third, to explore Cuba's approach to science in the context of public health, with implications for what a safe, effective Cuban vaccine might have for COVID-19 vaccine equity—particularly for the most vulnerable in the Global South.

When we visited in mid-2022, COVID-19 infections were spiking around the world with the appearance of new immune-evasive variants. At that time, there were over 843,000 new confirmed COVID-19 cases and 1874 deaths per day worldwide, with only 60% of the global population fully vaccinated and much lower rates in low-income countries.[1,2] In contrast, Cuba reported fewer than 20 new infections daily and zero deaths,[3] while 90% of the population, including 97.5% of children over the age of 2, were vaccinated with vaccines developed and produced on the island. Our delegation was tasked with examining the science, manufacturing processes, regulatory mechanisms and vaccination strategy. All of those are credited with shifting the pandemic burden in Cuba from a peak of some 10,000 new infections and almost 100 deaths reported daily in August of 2021, just as a nationwide vaccination campaign was launched,[4] to the lower levels reported in June 2022.

The Cuban vaccines used nationwide—Abdala, SOBERANA 02 and SOBERANA Plus—were developed and underwent clinical trials between spring 2020 and spring 2021. Both Abdala and the SOBERANA regimes are protein sub-unit vaccines that generate immunity via the SARS-CoV-2 spike protein. Following analyses of results from pre-clinical studies and phase 1, 2 and interim results from phase 3 trials, **all three received emergency use authorization (EUA) for use in adults from Cuba's national regulatory authority** (NRA), the Center for State Control of Medicines and Medical Devices (CECMED), in the summer of 2021. EUA for use in Cuba's pediatric population was authorized in fall 2021 after examining results from phase 1 and 2 clinical trials in children, and phase 3 results from clinical trials in young adults—known as immunobridging:[5]

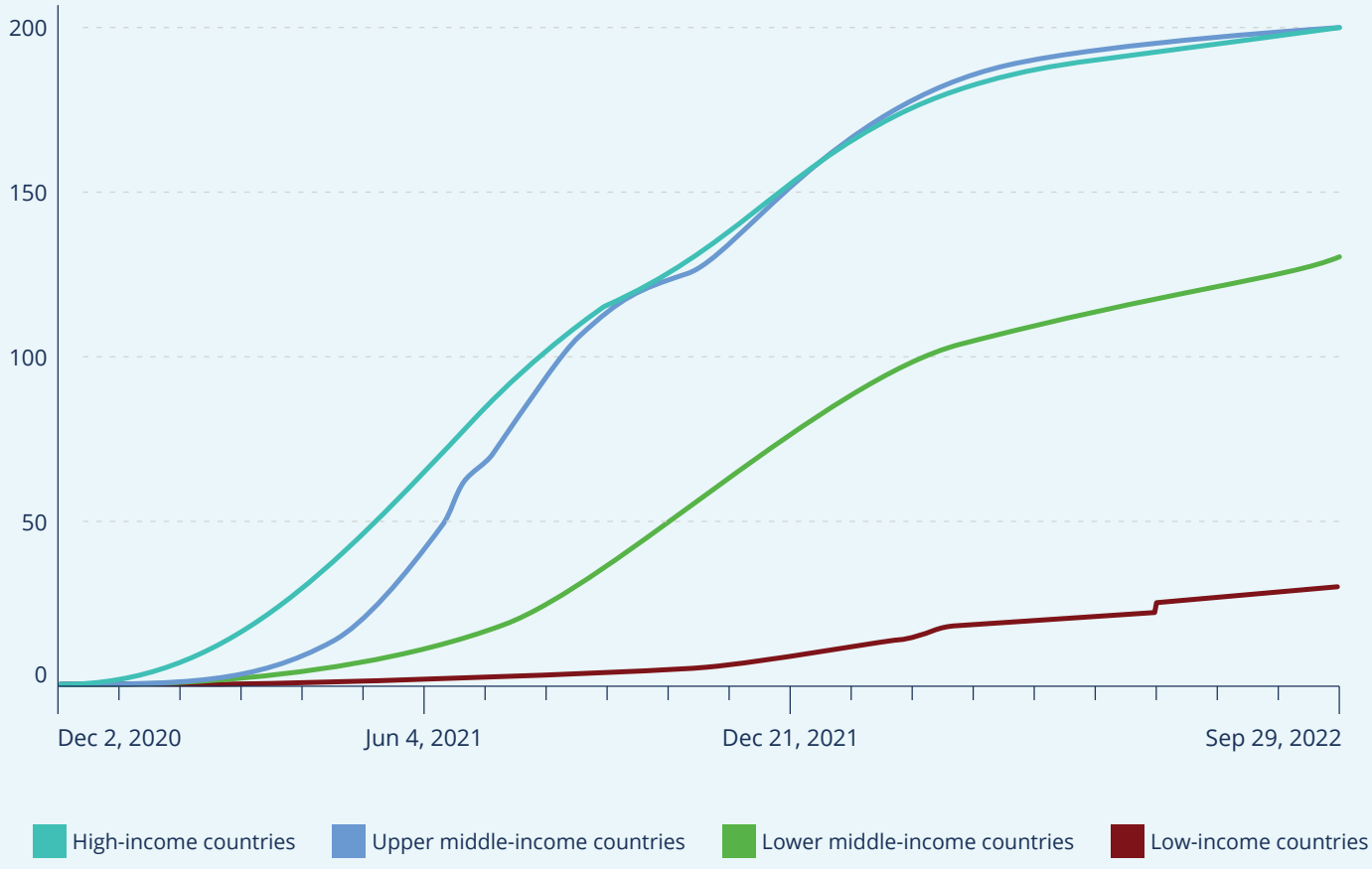
- **July 9, 2021:** Abdala vaccine receives EUA for use in adult population
- **August 20, 2021:** SOBERANA 02 and SOBERANA Plus vaccines receive EUA for use in adult population
- **September 3, 2021:** SOBERANA 02 and SOBERANA Plus receive EUA for use in pediatric population
- **October 27, 2021:** Abdala receives EUA for use in pediatric population

At this writing, Cuba's COVID-19 vaccines have received EUAs from several countries that have also signed commercial contracts, including Mexico, Iran, Viet Nam, St. Vincent & the Grenadines, Belarus and Venezuela. Abdala is currently being considered for Emergency Use Listing (EUL) by the World Health Organization (WHO), and the dossier for the SOBERANAs has been prepared for submission. An exploratory study has also been completed of SOBERANA Plus to assess its reactogenicity and immunogenicity in 30 Italian volunteers previously vaccinated with Pfizer, Moderna, AstraZeneca or Johnson & Johnson vaccines; clinical trials for SOBERANA Plus use as a universal booster are ongoing. At the time of our fact-finding mission, certain data on safety and efficacy of the Cuban COVID-19 vaccines had yet to be published in peer-reviewed journals, a process important to the scientific community and WHO evaluation. (*See Appendix C for updated publications list.*)

The delegation was mindful of predictions that the world is perilously close to the next pandemic, with crossover zoonotic infections—which already account for 75% of emerging infectious diseases—on the rise amidst advancing climate change.[6] Even as the specter of COVID-19 appeared to wane, we were also alarmed by the inequitable vaccine distribution that continues to threaten entire continents, countries and communities.[7]

## **Worldwide COVID-19 Doses Administered per 100 People,** By Income Group

**All doses, including boosters, are counted individually**




*Source: Official data collated by Our World in Data, World Bank*

# The MEDICC Delegation and Its Unique Mission

This fact-finding trip to learn more about the island's COVID-19 vaccines was the first time in five years a scientific delegation with significant US presence had engaged in discussions with scientists in Cuba. African and Caribbean members of the delegation offered developing-country scientific perspectives, and together, delegation members represented a broad range of expertise. *(See Appendix A for the list of delegation members issuing this report; see Appendix B for the list of participating Cuban scientists and their institutional affiliations.)*

The visit was organized by MEDICC (Medical Education Cooperation with Cuba), a US-based non-profit that promotes health-related dialogue and collaboration. Since 1997, MEDICC has facilitated exchanges between Cuban and US health professionals, scholars, policymakers, foundations, students, and leaders of medically underserved communities. Delegation organizing and travel were supported in part by a grant from the Open Society Foundations.

It is important to note that the MEDICC delegation was not functioning as a regulatory, certification or scientific review body. Likewise, it was out of its scope to seek independent verification of the data presented regarding COVID-19 vaccines, vaccination coverage and voluntary vaccination compliance. Nor did the group intend to perform a rigorous evaluation of Cuba's national, multisector pandemic response strategy that incorporated the vaccine initiatives. Rather, we sought to engage in frank, open and direct exchanges with Cuban scientists and regulatory, industry and public health experts—without involvement by high-level government officials—an aim that was fulfilled.

During our three days in Havana, MEDICC delegation members met with experts involved in the science, clinical trials, regulatory processes, production, and public health vaccination campaigns for Cuba's COVID-19 vaccines. Specifically, we met with directors of Cuba's national regulatory authority, CECMED, that regulates all biotech products developed and used in Cuba. We received detailed briefings from scientists at the institutions that led the country's COVID-19 vaccine development (the Finlay Vaccine Institute and the Genetic Engineering and Biotechnology Center, CIGB), as well as from those responsible for conducting clinical trials (the Pedro Kourí Tropical Medicine Institute and Hematology and Immunology Institute). We also visited three vaccine production facilities, including a new one at the Mariel Biotech Industrial Complex west of Havana, which was being equipped to expand production of the Abdala vaccine, as well as other vaccines and biotech products for diseases prioritized by Cuba's national health system. Finally, our delegation met with Cuban health professionals who led the country's national COVID-19 adult and pediatric vaccination campaigns, and visited a local elementary school where children and teachers described their experiences with vaccination efforts.

Delegation discussions covered many aspects of the country's COVID-19 vaccine development and immunization experience. Key areas we examined were:

- Cuba's experience with novel vaccine development and production as part of its broader biotechnology sector, in existence since the 1980s.
- The rationale behind Cuba's decision early in the pandemic to embark on independent COVID-19 vaccine development and production.
- The regulatory process utilized to grant Emergency Use Authorization for Cuba's COVID-19 vaccines.
- The Cuban COVID-19 vaccine technologies and studies undertaken to establish safety and efficacy of their vaccine candidates.
- The strategy that leveraged Cuba's universal public health system to achieve widespread COVID-19 vaccine coverage, including vaccination of children as young as two years old, the world's first country to do so.[8]

# Cuban Vaccine Development and Biotechnology: 1981–2022

As has been documented elsewhere and confirmed by our visit, Cuba's COVID-19 vaccine development capacity is rooted in a decades-long effort by the Cuban government to create a biotech R&D sector, which began in 1981 with the opening of the Biological Research Center (CIB), just five years after the first biotech company opened its doors in the USA; that year, CIB produced Cuba's first biotech product, human leukocyte interferon alfa.[1] Today, Cuba's biotech industry includes 32 research and development institutes and manufacturing entities. They operate under the umbrella of the state-owned conglomerate BioCubaFarma, with a collective mandate to develop pharmaceuticals and products to address health problems in Cuba first. Marketing these products abroad proceeds once domestic needs are met, fueling the centers with new resources, in what scientists term a 'closed-loop approach'.[2]

BioCubaFarma officials reported that their companies have a portfolio of over 900 products, including novel therapies and vaccines, biosimilars, diagnostic tests and reagents, medical technologies, and agriculture and animal health products. They noted that consortium members have joint ventures, licensing, co-development and/or commercial representation agreements in more than 40 countries, including Spain, France, Germany, Brazil, Russia, China, India, the United States, Iran and South Africa.

For example, between 2016 and 2018, joint trials were conducted in the United States between the Roswell Park Comprehensive Cancer Center and Cuba's Molecular Immunology Center (CIM) to test CIMA-vax-EGF, a CIM-developed therapeutic vaccine designed to halt the advance of lung cancer and thus extend survival times for patients. Phase 1 trial results were published in the peer-reviewed journal **Frontiers in Oncology** shortly after our visit.[3] In Iran, a manufacturing plant was opened in May 2022 to begin producing SOBERANA 02 under the name PastoCoVac, after Iran's Pasteur Institute ran its own clinical trials on the vaccine from Cuba's Finlay Vaccine Institute. Technology transfer agreements between Cuba and Brazil began in 2004 with institutions from the two countries pursuing joint biotechnology projects, including the production of recombinant erythropoietin and pegylated interferon. Technology transfer of the Finlay Vaccine Institute's meningitis AC vaccine enabled scale-up in Brazil for export to halt a vast disease outbreak in Africa (at the request of WHO).[1]

Prior to embarking on the COVID-19 vaccine effort, the two institutions involved in creating Abdala and SOBERANA—CIGB and IFV—already had an international reputation for developing safe, effective vaccines, including a recombinant hepatitis B vaccine (approved for use in Cuba since 1992), another against *Haemophilus influenza* type b (Hib; in use in Cuba since 2003), and the world's first effective vaccine against a deadly form of meningococcal meningitis caused by serogroup B meningococcus (MenB; in use in Cuba since 1989). Additionally, BioCubaFarma companies were producing 8 of the 11 vaccines administered through the country's national childhood immunization program.

The basic technology used in the Abdala vaccine is similar to that used for CIGB's hepatitis B vaccine, Heberbiovac. Another CIGB product for treating chronic hepatitis B infections, HeberNasvac, is a therapeutic intranasal hepatitis B vaccine that received regulatory approval in 2015; CIGB scientists used HeberNasvac as a model for developing the Mambisa nasally-administered COVID-19 vaccine candidate. At the time of our visit, Mambisa was being tested as a potential booster for people who had received Abdala—the first to begin clinical trials in humans for a COVID-19 booster administered nasally.

Developed by IFV and CIGB scientists, Cuba's *Haemophilus influenza* type b or Hib (which, despite its name, is a bacterial disease, not a form of flu) vaccine, Quimi-Hib, is a conjugate vaccine; it received WHO approval for international use in 2010.[4,5]

IFV is perhaps best known for its pioneering work in 1989 to develop the world's first effective vaccine against meningitis produced by serogroup B meningococcus, a rare but often deadly disease.[6] The Institute also developed biosimilars' vaccines against typhoid fever and leptospirosis, a bacterial disease that poses a risk of kidney damage and brain inflammation. IFV's SOBERANA vaccine regimen was based in part on the Institute's previous experience with conjugate vaccine technology—both Quimi-Hib and Quimi-Vio, the latter a Cuban vaccine against *Streptococcus pneumoniae* causing pneumococcal disease, are conjugate vaccines.

In addition to highlighting the country's vaccine research and novel products development capacities, BioCubaFarma directors pointed to investments in advanced biotech manufacturing, exemplified by the Mariel Biotech Industrial Complex west of Havana that the delegation visited. Such investments also include a growing capacity to manufacture monoclonal antibodies for use in both vaccines and biological therapies, infrastructure that is often lacking outside wealthy countries. For example, CIM has developed a monoclonal antibody called Itolizumab used to fight damaging inflammatory responses in diseases like rheumatoid arthritis. During the pandemic, Cuban regulators noted that they granted EUA to prescribe Itolizumab for treating the dangerous and potentially fatal inflammatory immune reaction occurring in COVID-19 infections, dubbed the 'cytokine storm'. (*See Appendix C for publications.*) Several members of the MEDICC delegation visited a manufacturing facility operated by the Center that, during the pandemic, had pivoted from producing monoclonal antibodies to producing viral proteins in mammalian cell cultures for the SOBERANA vaccines.

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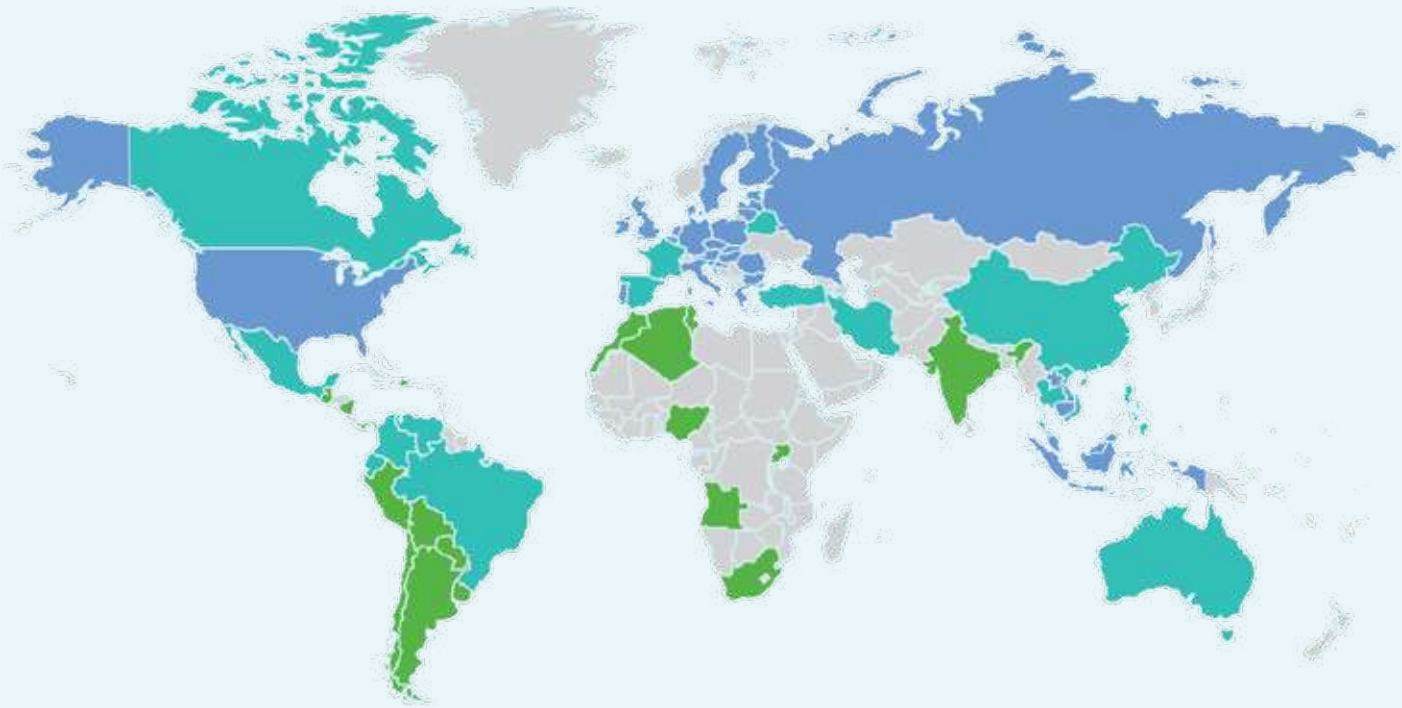
## Cuban Biotech in the World, 2021



| Exports | | Business Modalities | | | Exports & Business Modalities | |
|---|---|---|---|---|---|---|
| Angola | Paraguay | Armenia | Greece | Myanmar | Australia | Spain |
| Algeria | Peru | Austria | Hungary | Netherlands | Belarus | Thailand |
| Argentina | St. Vincent & the Grenadines | Belgium | Indonesia | Poland | Brazil | Turkey |
| Bolivia | South Africa | Brunei | Ireland | Portugal | Canada | Venezuela |
| Chile | Tunisia | Bulgaria | Italy | Republic of Korea | China | Viet Nam |
| Dominican Republic | Uganda | Cambodia | Kazakhstan | Romania | Colombia | |
| Guatemala | Uruguay | Croatia | Kyrgyzstan | Russia | Ecuador | |
| India | | Cyprus | Laos | Slovakia | France | |
| Jamaica | | Czechia | Latvia | Slovenia | Iran | |
| Morocco | | Denmark | Lithuania | Sweden | Mexico | |
| Nicaragua | | Estonia | Luxembourg | United Kingdom | Philippines | |
| Nigeria | | Finland | Malaysia | USA | Serbia | |
| Panama | | Germany | Malta | | Singapore | |

*Source: BioCubaFarma, Havana, 2022*

# Pivoting for the Pandemic: Why, How and Which Vaccines?

An important area of inquiry for the MEDICC delegation was why, given Cuba's limited resources, officials made the potentially risky decision to invest in an independent COVID-19 vaccine development program. This approach contrasted with decisions by many low- and middle-income countries that sought vaccines via the COVID-19 Vaccines Global Access program called COVAX. This is an international effort co-led by the Coalition for Epidemic Preparedness—along with Gavi, the Vaccine Alliance and WHO—that has helped some low- and middle-income countries acquire affordable vaccines, although not supplying the quantities or guaranteeing the accessibility originally hoped for.[9]

## An Early Decision to Develop COVID-19 Vaccines Domestically

Cuban experts meeting with the MEDICC delegation cited three key reasons the country decided in March 2020 to place its bets on a domestic COVID-19 vaccine program.

- **Cuban scientists and health officials were confident in their abilities to domestically produce safe, effective vaccines and rapidly deploy them across the Cuban population.** Cuban scientists from BioCubaFarma (the umbrella, state-owned conglomerate responsible for biotech and pharma, see Sidebar) said they were relatively confident that their decades of experience in vaccines provided a strong foundation for developing effective ones for COVID-19. In addition, they believed Cuba's track record of supplying safe, effective childhood vaccines had earned public trust in domestically-produced vaccines—and this trust would facilitate public compliance with COVID-19 vaccination campaigns.
- **They foresaw a long wait for Cuba to be able to access other vaccines, particularly given that it was not one of the poorest countries singled out for free vaccines by COVAX.** When Cuban scientists began their COVID-19 program, the global scramble for vaccines had just started, later including full-blown hoarding by some wealthy countries. Even in a best-case scenario, Cuban health officials told us, they believed that it would take a long time for the world's major vaccine developers to meet global demand, and certainly to do so equitably. They also noted that requisites for COVAX eligibility precluded Cuba from receiving sufficient vaccines within a practical timeframe, even if it paid millions to purchase them.[10]
- **Producing vaccines domestically was the only cost-effective and sure way to achieve high levels of coverage.** Cuban health officials said they were concerned that the combined impact of US sanctions and the loss of tourist revenue during the pandemic would make it difficult to purchase enough vaccines (at prices yet unclear) on the international market or through COVAX to cover their whole population. In addition to vaccinating adults, Cuban health officials also believed early on that their vaccination efforts would have to extend to millions of children in order to stem the pandemic.

## Why Cuba Developed Various Vaccine Candidates and Decided to Use Two Vaccines

Cuban researchers at Finlay and CIGB began working simultaneously on various vaccine candidates, realizing that not all would make the final cut of success in human trials. Finally, as we learned, they moved forward with two different COVID-19 vaccine regimens utilizing two different technologies that require separate manufacturing processes: the SOBERANAs and Abdala. These vaccines also require different immunization schedules in terms of the number of days between doses. According to our discussions with Cuban scientists and public health officials over the course of our visit, several factors seem to have driven the decision to use both vaccines.

One factor was the pace of development. The Abdala vaccine advanced faster than the SOBERANA vaccines, in part because it relied on simpler manufacturing technologies. Secondly, production capacities by individual manufacturers likely also influenced the decision since existing facilities could not guarantee a sufficient volume of one vaccine at a single production plant. Ultimately, Abdala became a more practical option to halt the rapid surge of the delta variant in 2021, due to the shorter time between doses, enabling full vaccination faster. When it came time to vaccinate children, however, the SOBERANA vaccines appeared as a first choice, given that they were based on conjugate vaccine technologies that have been used for decades in Cuba for childhood immunizations.

## Regulatory Oversight: Cuba's National Regulatory Authority

Central to Cuba's COVID-19 vaccine development program was regulatory oversight of biotech products by its national regulatory authority (NRA), the Center for State Control of Medicines and Medical Devices (CECMED).

Established in 1989, we learned that the Center provides regulatory reviews and approvals of all medicines, biopharmaceuticals, vaccines and medical devices produced in Cuba or imported for use in the country's health system. A presentation by the CECMED director noted that the Pan-American Health Organization (PAHO) certified the agency as a Collaborating Center for Regulation of Health Technologies and a Regional Reference National Regulatory Authority. The director also explained that CECMED has retained PAHO/WHO certification as a Level 4 National Regulatory Authority of Reference since 2011 (requiring PAHO/WHO evaluation and certification of: regulatory system; registration and marketing; vigilance; market surveillance and control; licensing establishments; regulatory inspection; laboratory testing; clinical trials oversight; and lot release).[11]

As was the case for NRAs worldwide that were overseeing other COVID-19 vaccine development efforts, CECMED regulators were in uncharted waters due to the need to rapidly expedite complex regulatory reviews—with far less personnel and fewer resources than their US and European counterparts. Worldwide and in Cuba, regulators were challenged to accelerate reviews without sacrificing scientific rigor.

For example, in times of global health emergencies, vaccine developers may employ adaptive trial designs with overlapping phases (seamless trials) for generating initial data on vaccine safety and immune response. Immediate continuation from one phase to another requires having sufficient data from the initial phase to justify moving to the next, and only if the trial design elements overlap.[12]

At the time of our visit, 13 temporary modifications to regulatory processes or procedures had been made during the pandemic, including permissions for seamless trials. Regulatory officials also reported they were able to accelerate reviews of the Abdala and SOBERANA vaccines because both relied on technology platforms utilized in previously approved Cuban-developed vaccines and were manufactured in facilities already certified by CECMED.

CECMED regulators said coordination among the national regulatory authority, Cuba's biotech companies and its national health system helped expedite regulatory reviews while attending to evidence of safety and efficacy. They noted that this coordinated, streamlined approach was buttressed by an Innovation Committee—comprised of regulatory officials, researchers, physicians in various clinical specialties, epidemiologists, virologists and directors of relevant national programs like maternal-child health—established by the Ministry of Public Health. The Committee met regularly to discuss all aspects of the country's efforts to prevent and treat COVID-19 infections. CECMED's director explained that such coordination enabled regulatory questions to be answered more quickly and discussed more thoroughly, and for changes to be enacted faster when required in clinical trials organization.

Cuban regulatory officials said they hoped to emerge from the pandemic with a well-established process for conducting regulatory reviews in the context of health emergencies. CECMED's director also said the next step for her agency was to become a WHO Listed Authority, the highest WHO level reserved for the world's most trusted NRAs, whose opinions are relied upon internationally.

# Cuba's COVID-19 Vaccines: Science, Clinical Trials and Emergency Use Authorization

During the three-day fact-finding trip, one group met with scientists at meetings hosted by CIGB, where the Abdala vaccine was developed; a second group was hosted at IFV for similar briefings on the SOBERANAs vaccines developed there.

Scientists at both institutions explained that before proceeding with clinical testing, all trials for COVID-19 vaccine candidates were reviewed and approved by CECMED and clinical sites inspected and certified for adherence to international good clinical practices (GCPs). The trials were conducted by external institutions (i.e., not by those producing the vaccines); their progress and how they were conducted were monitored by external ethics research committees; and adherence to GCPs were analyzed by Cuba's National Clinical Trials Coordinating Center. Researchers noted that, in addition to specific inclusion/exclusion criteria, trial participation required participants' written informed consent.

Trials involving pediatric groups 2–18 years old required written informed consent from the child's parents or legal guardians; volunteers 12 years and older also had to provide written consent. (A complete list of clinical trial sites, study purpose, design, inclusion/exclusion criteria, formulations, technical and ethical considerations, and funding for each trial is available at the Cuban Public Registry of Clinical Trials, a WHO Primary Registry.)

In addition to the three vaccine formulations later authorized for use, our delegation received brief information about three innovative Cuban biopharmaceuticals—Nasalferon, Itolizumab and Jusvinza—that were repurposed to treat COVID-19 and received CECMED emergency use authorization for expanded-access use. We were told that another COVID-19 vaccine candidate, Mambisa, was still in clinical trials, and scientists emphasized its potential as a nasal-spray vaccine.

## The Abdala Vaccine

Developed by CIGB, Abdala is a protein sub-unit vaccine that relies on vaccine technology similar to that used by the Center for its hepatitis B vaccine, Heberbiovac, included in Cuba's childhood vaccination program since 1992 and WHO-approved for use internationally since 2001. The goal with Abdala, according to developers, was to generate immunity with the receptor binding domain (RBD) portion of the SARS-CoV-2 spike protein. The virus employs the spike protein to penetrate cells; Abdala generates antibodies that interfere with this process. Many COVID-19 vaccines in use globally—including those available in the United States and Europe—rely on some aspect of the spike protein to generate immunity. Like the hepatitis B vaccine, Abdala relies on a recombinant viral protein produced in *Pichia pastoris* yeast and then formulated with an aluminum hydroxide-based adjuvant. (An adjuvant is a substance intended to help a vaccine generate a strong immune response.) Abdala is administered intramuscularly in three doses 14 days apart.

Below, we summarize the information received about Abdala's clinical trials and their results:

### Clinical Trials in Adults

From December 2020 through February 2021, Abdala underwent randomized, double-blind, placebo-controlled phase 1/2 trials to test the vaccine candidate's safety and immunogenicity. The combined (or seamless) trial design was authorized by CECMED and conducted at the Saturnino Lora Provincial Clinical–Surgical Teaching Hospital in Santiago de Cuba in the eastern part of the country. Phase 1 studied 132 healthy participants 19-54 years old; Phase 2 studied 660 healthy participants 19-80 years old.[13]

After CECMED approval, phase 3 multicenter, randomized, double-blind and placebo-controlled clinical trials began in March 2021 with 48,290 healthy volunteers 19-80 years old. The trials were designed to test Abdala's safety, immunogenicity and efficacy and conducted through early June 2021 at 18 certified clinical trial sites in the eastern provinces of Santiago de Cuba, Granma and Guantánamo. Results from this phase 3 trial, announced on June 21, 2021, showed vaccine efficacy at 92.28% against symptomatic infection, 100% against severe disease and 100% against death.

Post-vaccination surveillance data revealed about 16,000 adverse events, divided almost equally between recipients of the placebo and actual vaccine. Scientists involved with the trial said the adverse events were mainly mild issues, such as pain at the injection site; there were no serious adverse events with a clear link to the vaccine. Phase 3 Abdala clinical trials occurred before emergence of the omicron variant, and results were available as a preprint at the time of the MEDICC delegation

visit. (See Appendix C: A phase 3, randomized, double-blind, placebo-controlled clinical trial for adult evaluation of the efficacy and safety of a SARS-CoV-2 recombinant spike RBD protein vaccine, in medRxiv.)

### Clinical Trials in Children and Adolescents

A combined phase 1/2 trial ('Ismaelillo Clinical Trial') was conducted in 592 healthy pediatric volunteers 3-18 years old at 11 certified clinical trial sites in Camagüey province in central Cuba from July through October, 2021. This was a multi-center, randomized, double-blind trial. In late 2021, a similar, second phase 2 trial ('Meñique Clinical Trial') was conducted in 703 healthy pediatric volunteers 3-18 years old. The objective of these trials was to assess safety and immunogenicity, as well as to compare these results to adult cohorts receiving the same vaccine regimens.

Principal investigators reported 99% of trial participants 3-18 years old experienced high seroconversion levels of RBD antibodies following the full, three-dose Abdala vaccination regimen. They also presented adverse event data to our delegation: 32% of participants in the Ismaelillo trial experienced at least one adverse event (causally related or not) after the three-dose regimen; total number of adverse events was 413. The most common were pain and redness at injection site, headache, sleepiness and fever, with lower frequencies of adverse events in the 3–11-year-old age group. No vaccine-related serious events were observed. CIGB scientists opted not to conduct phase 3 pediatric trials due to ethical considerations around using a pediatric placebo control group, and instead used phase 1, 2 and immunobridging results for requesting EUA (see below, as the same strategy was applied for the SOBERANAs).

## The SOBERANA Vaccines

The SOBERANA vaccine relies on a three-dose regimen, combining two different vaccines to generate protection (known as a heterologous regimen). The first two doses are with the SOBERANA 02 vaccine, the third with SOBERANA Plus. They are administered via intramuscular injection 28 days apart. The viral proteins used in the SOBERANA vaccines are produced in mammalian cells. The technology required to manufacture vaccine proteins in mammalian cells makes production of the SOBERANAs somewhat longer and more expensive.

Finlay scientists noted that the SOBERANA 02 vaccine is based on conjugate vaccine technologies developed in the 1980s that are now utilized in vaccines globally. For this vaccine, Finlay researchers linked (conjugated) the RBD portion of the SARS-CoV-2 spike protein with a protein (tetanus toxoid) taken from the tetanus bacterium (*Clostridium tetani*). Scientists have found that, with conjugate vaccines in general, linking a protein isolated from a disease pathogen to one from an unrelated virus or bacteria is a safe and effective way to elicit a strongly protective immune response, involving helper T cells.

Our delegation learned that scientists decided to consider a third dose because results from early clinical testing indicated two doses of SOBERANA 02 were not producing sufficiently high titers of neutralizing antibodies. What's more, early results indicated that use of SOBERANA Plus as a third dose produced results superior to a third dose with SOBERANA 02 in terms of neutralizing antibodies.

SOBERANA Plus is not a conjugate vaccine. Instead, it relies on vaccine technology like that used for Abdala. However, Finlay scientists noted that SOBERANA Plus utilizes a slightly different version of the RBD protein (called a dimeric protein) that they viewed as well-suited for boosting immunity provided by vaccination or prior infection. This protein was then formulated with an aluminum hydroxide-based adjuvant to amplify its immune-generating potential.

> Data presented by principal investigators showed 92% efficacy of the SOBERANA regimen for preventing symptomatic disease and 100% effective for preventing severe disease and death.

### Clinical Trials in Adults

Two phase 3 trials of the SOBERANA vaccine regimen were conducted in adult populations. Both were performed in the spring of 2021: one in Cuba, (before detection of the omicron variant on the island in December 2021) involving

44,031 healthy volunteers 19–80 years old and another in Iran in partnership with the Pasteur Institute there, involving nearly 24,000 participants in a similar age range.

For the Cuban trial, researchers described random assignment of 44,031 participants into three groups: one received two doses of SOBERANA 02; the second received the full regimen—two doses of SOBERANA 02 followed by a single dose of SOBERANA Plus; the third group received a placebo. All doses were administered 28 days apart. This trial was conducted during circulation of beta and delta variants in Cuba.

Data presented by principal investigators showed 92% efficacy of the SOBERANA regimen for preventing symptomatic disease and 100% effective for preventing severe disease and death. Reported efficacy of the two-dose regimen was significantly lower than the three-dose combination: 69.7% against symptomatic COVID-19 and 74.9% against severe disease. (The small number of deaths—2 in vaccinated and 3 in the placebo group—precluded a point estimate of vaccine efficacy for preventing this outcome.)

The clinical trials demonstrated safety of the SOBERANA vaccines and used WHO definitions for adverse events. Reported adverse events included some level of pain or swelling at the injection site, headache or fatigue—all mainly after the first dose. These events were more common in the vaccinated group compared to placebo group. There were isolated reports of systemic adverse events including dengue infection, hypertension and erythema multiforme, but no reports of severe or potentially life-threatening events involving any of the volunteers.

Peer-reviewed safety and immunogenicity results from open-label parallel phase 1 and 2a trials for SOBERANA 02 in homologous or heterologous regimens were published in the journal ***Vaccine*** shortly after our fact-finding mission.[14] Included in this manuscript are in vitro results indicating a strong T-cell response induced by SOBERANA 02 which could be instrumental in protecting against immune-evading variants. The journal ***Med*** later published safety and immunogenicity results for a phase 2b placebo-control trial, reaffirming selection of the heterologous regimen.[15] Phase 3 results presented to the delegation had been submitted for publication but, at the time of our meetings, had not yet appeared in a peer-reviewed journal.

The phase 3 trials in Iran were randomized, double-blind and placebo-controlled and involved nearly 24,000 healthy participants 18–80 years old. This was a multi-center trial conducted in eight cities throughout the country; the first cohort (n = 17,972) received a two-dose regimen of SOBERANA 02, 28 days apart, while the second cohort (n = 5,987) received an additional dose of SOBERANA Plus on day 56. This trial was conducted during exclusive circulation of the delta variant.

The Iranian trial reported 51% efficacy against symptomatic disease in trial participants who received the two-dose SOBERANA 02 regimen and 65% efficacy in the group that received the heterologous regimen (SOBERANA 02 and SOBERANA Plus). The two-dose regimen showed 80% efficacy against severe disease, which jumped to 96.5% with the three-dose regimen. There was one COVID-related death observed in the placebo group.

## Clinical Trials in Children and Adolescents

An open-label combined phase 1/2 trial was conducted at the Juan Manuel Márquez Pediatric Teaching Hospital in Havana in the summer of 2021 involving 350 children and adolescents 3–18 years old. The purpose of the trial was to assess safety, reactogenicity and immunogenicity using the heterologous SOBERANA regimen in healthy children and compare these results to phase 3 trial results in healthy young adults, in whom the SOBERANA vaccines had already demonstrated significant protection.

Results of this pediatric trial, available as a preprint, revealed the regimen was safe and generated a strong immune response, demonstrating non-inferiority to that obtained in young adults.

Cuban researchers opted not to conduct phase 3 pediatric trials due to ethical considerations around using a pediatric placebo control group, and instead used phase 1/2 pediatric results and those from phase 3 in young adults to request emergency use authorization. This process, known as immunobridging, has also been recommended by the US Food and Drug Administration when evaluating COVID-19 vaccines for children.[16]

## Clinical Trials in COVID-19 Convalescents

SOBERANA Plus underwent phase 1 and 2 clinical trials in Havana during the spring of 2021 for use in convalescent adults 19-80 years old, its results published.[17]

A second open-label phase 1/2 trial with 518 COVID-19 convalescent volunteers 2–18 years old was conducted at the Juan Manuel Márquez Pediatric Teaching Hospital in Havana and the Paquito González Cueto University Pediatric Hospital in Cienfuegos. These participants received a single dose of SOBERANA Plus with the objective of measuring safety, reactogenicity and immunogenicity in children 2–18 years old previously infected with symptomatic or asymptomatic COVID-19. These results, also published, were compared to those of convalescent adults 19–29 years old and to healthy (non-convalescent) children 3–18 years old.[18]

# Public Health Strategy for Vaccinating Cuba's Population Against COVID-19

Cuba's Ministry of Public Health formulated a strategy aimed at achieving rapid vaccination for the vast majority of the Cuban population. This relied on domestically produced vaccines that could be stored at household refrigerator temperatures and thus distributed across the island for use in the universal health system's primary health care centers. In a presentation to the MEDICC delegation, ministry analysts described a multi-phase rollout of the Abdala and SOBERANA vaccines, including an intervention study and public health intervention, accompanied by a large cohort study in Havana; mass vaccination of adults, and children 2-18 years old; and booster regimens for adults and pediatric populations.

Cuban public health experts credited past investments in nationwide primary healthcare infrastructure—including neighborhood family doctor-and-nurse offices acting as primary care providers and nearly 500 community clinics—with facilitating rapid immunization of the general population in the context of a universal health system. They also noted that Cubans of different skin colors experience similar rates of various co-morbidities, such as hypertension, and that basic COVID-19 assessments by both phenotype and skin color did not reveal differences in rates of disease incidence or adverse events post-vaccination. We did not receive such information concerning socio-economic status.

The mass vaccination campaign utilized 120,000 health workers, who administered vaccines through 11,000 vaccination centers. For the pediatric population, vaccinations were administered either at these centers (for younger children) or at schools certified as vaccination centers. Officials reported that, at the peak of the campaign, Cuban health professionals were administering 300,000 COVID-19 vaccine doses per day.

Cuban protocols called for each person vaccinated to be checked by a health professional, typically, the individual's primary care provider, before they were immunized and evaluated again one hour after, as well as stocking vaccination centers with medications to address adverse events. Vaccines were taken to people who were unable to leave their homes.

## Intervention Study

In March 2021 based on safety results demonstrated in phase 2 trials,[13,14] CECMED approved intervention studies to further evaluate the Abdala and SOBERANA vaccines in high-risk groups for symptomatic COVID-19 infection, severe disease and death and to record adverse events. The cohort was comprised of 164,000 essential workers in the country's health facilities and biotech industry. Most of that group, about 140,000, were frontline health workers. The remainder were involved in product development in BioCubaFarma research and manufacturing enterprises.

The Abdala study included 120,000 volunteers from Havana, Santiago de Cuba, Granma and Guantánamo provinces, who received 3 doses of the vaccine beginning in late March 2021. The remainder of the cohort—in Havana—received two doses of SOBERANA 02, followed by SOBERANA Plus.

## Public Health Intervention

Utilizing an approach commonly used in clinical vaccine trials, Cuban scientists undertook an interim analysis (with a data cutoff date 14 days after the third dose) of the Abdala phase 3 trial that provided preliminary evidence of the vaccine's safety and efficacy. In May 2021, these results were presented to the Ministry of Public Health. In consultation with the Innovation Committee, the Ministry decided to move forward with a public health intervention to vaccinate a large number of people with Abdala (and later, SOBERANA) in areas where the caseload was increasing most rapidly.[19] This decision, based on a risk-benefit analysis made in consultation with the Innovation Committee, was taken within the legal framework of Public Health Law #41, which permits such actions in health emergencies.[20] The emergency at hand was a sharp rise of delta variant cases and deaths in certain territories.[21] Ultimately some 3 million Cubans were vaccinated in various municipalities of Havana, Matanzas, Ciego de Ávila and Santiago de Cuba provinces—regions that were experiencing the sharpest surge of infections.

At the time, neither Abdala nor the SOBERANA had received emergency use authorization, and public health officials we spoke with noted that they had been criticized in the international media for this public health intervention. However, they maintained that the subsequent drop in cases and deaths vindicated their decision.

On July 9, 2021, CECMED granted EUA for Abdala for the country's adult population based on interim phase 3 trial results, data from the intervention study mentioned above (unpublished) and 'real world effectiveness' evidence from the public health intervention. This permitted deployment of the vaccine in the mass vaccination campaign for Cuba's adult population.

## Mass Vaccination: Adult and Pediatric Populations

Vaccination of Cuba's adult population began in July 2021 with the three-dose Abdala regimen. Analysis of previous results and surveillance for adverse effects continued in post-marketing studies. Researchers presented data from a study conducted in Havana of 1.35 million Cubans who received the Abdala vaccine from July 9 through August 31, 2021. That analysis focused on protection against severe disease and death; effectiveness for both outcomes in those fully vaccinated was over 98%. When the delegation visited, the study was available as a preprint, later published.[22]

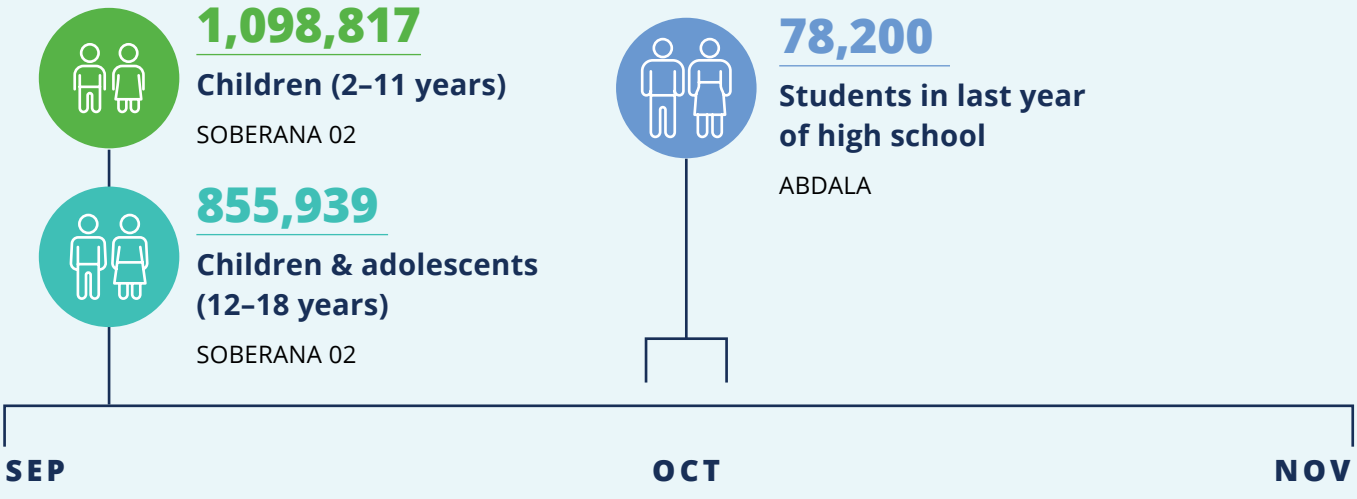
On August 20, 2021, SOBERANA 02 and SOBERANA Plus also received EUA from CECMED for use in Cuba's adult population based on interim phase 3 results.

On September 3, 2021, the SOBERANA vaccine regimen received EUA from CECMED for pediatric immunizations for children aged two and over. In reviewing EUA for the SOBERANA regimen in children and adolescents, CECMED considered the phase 1/2 safety and immunogenicity results in pediatric volunteers and efficacy data from phase 3 trials with young adults using the same heterologous schedule. Additionally, based on phase 1/2 results from the pediatric trials in Havana and Camagüey and comparisons to young adult trial results mentioned above, CECMED granted Abdala EUA for use in pediatric populations on October 27, 2021.

The pediatric vaccination campaign was rolled out as follows: some 78,000 students in their last year of high school received the Abdala vaccine beginning in October 2021; almost 856,000 children and adolescents 12-18 years old and just over 1 million children 2-11 years old, received the SOBERANA regimen between September and November. Health officials presenting to the delegation reported that from September through November 2021, approximately 2 million Cuban children and adolescents 2-18 years old were vaccinated with Abdala and the SOBERANA vaccines.

Our delegation learned that **by June 2022 when we visited, 97.5% of the pediatric population was fully vaccinated** (some 200,000 infants under 2 years old were ineligible for vaccination). Of vaccinated children, 0.01% reported adverse events, none life-threatening.

### Cuba: COVID-19 Vaccination in Pediatric Ages, 2021




*Source: I Morales, Ministry of Public Health, Havana*

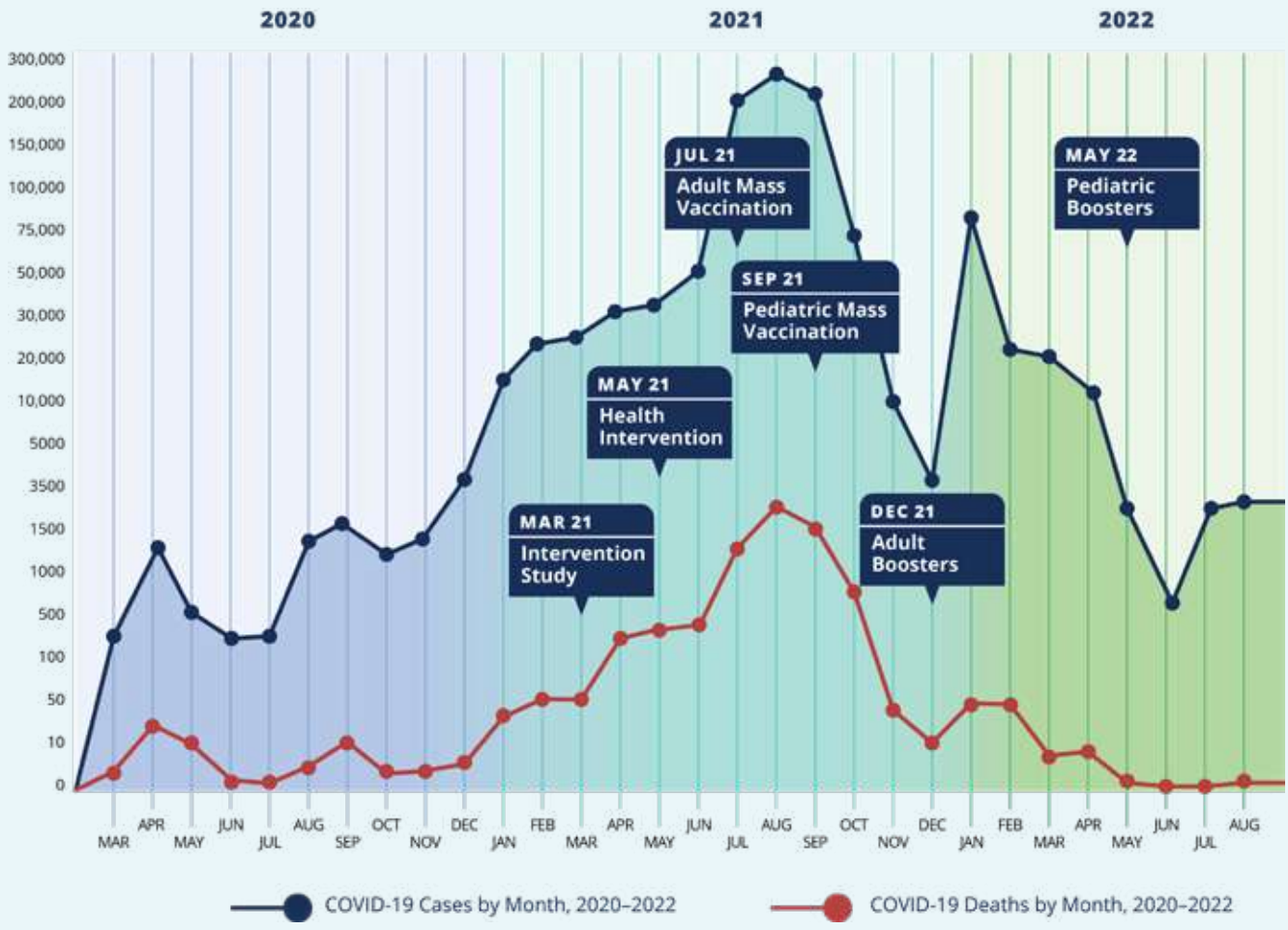
## Booster Strategy and Rollout

In our meetings, Cuban public health officials explained that the booster strategy was a phased approach like that used in the earlier rollout: first, frontline health workers and scientists, followed by high-risk groups and territories, and finally, adults 19 years and older and the pediatric population 12-18 years old. Use of the Abdala and SOBERANA vaccines as boosters was included in the designs of CECMED-approved clinical trials.

Cuban health officials recommend SOBERANA Plus as a booster for COVID-19 convalescent adults 19 years and older, COVID-19 convalescent children and adolescents, and as a universal booster (see below) for those vaccinated with any other COVID-19 vaccine. Both Abdala and SOBERANA Plus are recommended as boosters three months after completing the initial immunization series, while SOBERANA Plus is recommended for COVID-19 convalescents two months after infection.

Cuba began applying boosters in the adult population in December 2021; pediatric boosters in children and adolescents aged 12–18 years, in May 2022. A few months after our fact-finding trip, it was reported that Cuba started applying boosters in children aged 2–11 years in Cienfuegos province, later extended to the rest of the country.[23]

### COVID-19 Cases and Deaths by Month, Cuba, 2020—2022




*Source: I Morales, Ministry of Public Health, Cuba*

# Delving Deeper: Delegation Discussions with Cuban Vaccine Scientists

The comprehensive presentations from IFV and CIGB scientists, principal investigators, Cuban regulators and public health specialists sparked extensive discussions with our delegation. Key issues covered included: potential vaccine effectiveness against omicron variants; immunity duration and booster strategy; the possibility for SOBERANA Plus to serve as a universal booster; the role of primary healthcare systems in health emergencies; reporting adverse events; vaccination compliance; and funding for vaccine R&D in Cuba.

## Preliminary Evidence of Effectiveness Against Omicron Variant

At the time of the delegation's visit, surveillance data indicated that Cuba had yet to be challenged by the omicron BA.4 and BA.5 subvariants causing a significant spike in cases elsewhere in the world. However, the original omicron strain already had moved through the Americas, and delegation members asked for insights into potential effectiveness of the Cuban COVID-19 vaccines against it.

IFV researchers responded with preliminary data from an unpublished study in children and adolescents 2–18 years old, comparing pre-immunization infection rates during the delta wave in the summer of 2021 with those post-immunization during the first omicron wave in early 2022. These data showed that infection rates fell from 40 per 1000 during delta (pre-vaccination) to 8.6 per 1000 during omicron (post-vaccination). Meanwhile, infection rates in unvaccinated children under 2 fell only modestly, from 56 to 39 per 1000. Finlay scientists concluded that the sharp reduction in the vaccinated group could provide preliminary evidence of the vaccine's potential to protect against the omicron variant.

Finlay scientists indicated that they focused on pediatric infection rates because they are interested in whether high vaccination coverage in this group could help blunt the nationwide impact from variants like omicron that better evade vaccine-acquired immunity. They noted that Portugal experienced high omicron breakthrough infection rates despite high vaccination rates in adults and pointed out that Portugal did not have similar vaccination rates in children and adolescents. An important issue is whether children and adolescents play such a large role in disease transmission across populations that high vaccine coverage in this group could reduce the overall impact of immune-evading variants. For example, there was discussion about whether vaccination of older children contributed to the modest drop in COVID-19 infections in children under 2 years old (delta vs. omicron) who had not been vaccinated.

## Immunity Duration and Booster Strategy

As mentioned, at the time of the delegation's visit, boosters were already being administered to Cubans 12 years of age and older who had received the full schedule of either SOBERANA or Abdala vaccines. Delegation members were presented findings (unpublished) indicating that the level of neutralizing antibodies generated by both vaccines had dropped six months post-vaccination. Discussion centered on whether this loss in quantity also indicated the same degree of loss in quality of antibody protection—in other words, whether there still was enough to provide at least a modest level of protection. In this regard, Cuban scientists provided intriguing data on B- and T-cell immunological memory response that warrant further study.

Health officials noted that at the time of our visit, some 67% of the Cuban population had received at least one booster. They indicated that either Abdala or SOBERANA Plus can be used as a booster, regardless of the vaccine originally applied—including mRNA or others.

CIGB scientists reported unpublished data from a study in 1083 adults indicating that a fourth dose of Abdala administered six months after primary vaccination restored protection to earlier levels. They also shared data from the pediatric Ismaelillo trial showing that neutralizing antibodies dropped after 7.5 months in the 3–11-year-old age group and after 8 months in the 12–18-year-old age group. Cuban health officials now recommend a second booster for adults aged 50 years and over.

## Investigating SOBERANA Plus as a Universal COVID-19 Booster

IFV researchers presented data regarding their ongoing efforts to evaluate SOBERANA Plus as a universal booster for elevating immune response in two different populations: people who were previously infected, but not vaccinated; and people who were vaccinated with one of the many COVID-19 vaccines currently administered around the world.

They presented peer-reviewed results from a phase 2 trial published in June 2022 in the journal ***Lancet Respiratory Medicine*** that found previously infected (but not yet vaccinated) people who received a single dose of SOBERANA Plus experienced a 31-fold increase in neutralizing antibodies against the alpha, beta and delta variants of concern.[24] The study was conducted before the emergence of omicron.

The Finlay team noted they were evaluating data from a small clinical trial in 30 subjects from Turin, Italy who received SOBERANA Plus after being vaccinated with either the Pfizer, Moderna, AstraZeneca or Johnson & Johnson COVID-19 vaccines. Participants in the SOBERANA Plus Turin trial received one dose of SOBERANA Plus at least three months after completing the full schedule of other vaccines. No serious adverse events were reported. Finlay scientists also referenced ongoing studies involving 100,000 volunteers utilizing SOBERANA Plus as a booster for people who have received China's Sinopharm vaccine, the results of which they plan to submit for peer-reviewed publication.

## Adverse Events: Recording and Reporting

A vaccine surveillance and pharmacovigilance expert from Havana's Pedro Kourí Tropical Medical Institute (which led clinical trials of SOBERANA 02 and SOBERANA Plus in Cuba's adult population) explained that adverse events are documented via systematic surveillance of adverse events following immunization (AEFI), a mechanism used in Cuba's national immunization program since 1999.[25] With the COVID-19 vaccines, active surveillance was conducted the first hour after vaccination, followed by 15 days of passive surveillance. The latter depended on manual recording of adverse events by health professionals in the primary healthcare system.

Data presented for the Abdala vaccine, for example, showed 5,828 adverse events reported after application of 26,991,674 doses in adults. Of these, 5,769 were mild events; 20 were moderate; and 39 serious—9 related to vaccination and 30 unrelated to vaccination. Additionally, there were 20 adverse events of special interest: 8 facial paralysis events (moderate and recovered) and 12 seizures (moderate).

Similar data was presented for adverse events in Cuba's pediatric population that received the Abdala vaccine from October 2021 through April 2022. In this period, 348,704 doses were applied and 22 AEFI reported; the most common were headache, hypertension, low-grade fever and vomiting. In pregnant women who received Abdala, there were 7 mild and 2 serious AEFI after application of 145,147 doses from July 2021 through April 2022. The two serious events were high blood pressure (not vaccine related) in two women who were then admitted to hospital and stabilized.

Of the nearly 1.9 million children and adolescents who received the SOBERANA regimen, 223 reported an adverse event, or 0.01%, a rate of 10.2 X $10^5$ applied doses. As of this writing, no cases of myocarditis, pericarditis or multisystem inflammatory syndrome have been reported for those who received Abdala or the SOBERANAs. Scientists at the Finlay Vaccine Institute noted that following phase 3 clinical trials for both vaccine lines, youngsters received active follow-up for one year; following mass pediatric vaccination, children and adolescents are followed up by their neighborhood family physicians for at least one year.

> Health officials noted that at the time of our visit, some 67% of the Cuban population had received at least one booster.

## Vaccination Compliance

Asked about vaccine hesitancy or anti-vaccination sentiments, health officials stated that the decision to receive COVID-19 vaccination (or not) was voluntary and left to individuals. They credited several factors with high rates of compliance, including: long-standing public confidence in childhood vaccines developed by Cuban scientists, administered through the National Immunization Program that reports over 98% vaccination rates,[26,27] as well as consistent messaging and extensive public information offered by scientists on vaccine safety profiles and efficacy, presented on national prime-time television, in social media and elsewhere, and the relationship of family doctors and nurses to residents in the neighborhoods where they live and serve.

They said that eligible people who chose not to get vaccinated sometimes cited an acute illness or concern about severe co-morbidities.

## Funding for Cuba's Vaccine Effort

Funding for a costly undertaking like vaccine development, production, clinical trials and deployment amidst a global pandemic—especially for a tourism-dependent country also under stringent US sanctions—was another line of inquiry of interest to the MEDICC delegation. Although we did not fully discuss this issue, subsequent perusal of public records indicates that financing for development of both vaccine regimens was provided by Cuba itself, including reallocated funds from the BioCubaFarma conglomerate. For the Abdala clinical trials, funds were provided by CIGB; for the public health interventions using the Abdala vaccine, by CIGB and Cuba's Ministry of Public Health (MINSAP); and for the SOBERANA clinical trials and vaccination campaigns, by IFV and the Cuban Fund for Science and Innovation of the Ministry of Science, Technology and the Environment.

Later, in January 2022, the Central American Bank for Economic Integration provided a €46.7-million credit for the *Project to Strengthen the Cuban Biopharmaceutical Industry to Confront COVID-19 in Cuba and the Region*, to be implemented via the United Nations Development Programme (UNDP). Objectives of the grant include: modernizing manufacturing technology, strengthening production infrastructure, scaling up COVID-19 vaccine innovation, and production and acquisition of personal protective equipment. This followed an initial grant to Cuba in 2020 from the same bank for €935,600 to purchase of RT-PCR tests.[28] Vaccine finances have been supplemented by various donations, such as those from MediCuba-Europe, MediCuba-Suiza and the Swiss Agency for Development and Cooperation.[29]

Funding information, plus clinical trials registration and authorization dates, ethics committees, clinical sites, selection criteria, study design and outcomes for all biopharmaceuticals and medical devices can be accessed at the Cuban Public Registry of Clinical Trials.

# Delegation Findings

**The MEDICC delegation highlighted the following key observations based on our site visits and information from Cuban scientists and public health experts in Havana in June 2022, as well as documents in the public record:**

1. In a difficult socio-economic context, Cuban scientists developed and tested two lines of COVID-19 vaccines, each exhibiting over 95% efficacy rates against severe disease and death, with good safety profiles. This result alone merits attention by the global scientific community to control and prevent pandemics, and to build more equitable in-country capacities for doing so.
2. The two lines of Cuban vaccines (the SOBERANAs and Abdala) rely on classic protein sub-unit technologies that the country's biotech institutions have used previously with success. These vaccines require refrigeration but not deep freeze—an important factor for health systems to carry out mass vaccination in resource-constrained settings.
3. The Cuban vaccines were used to fully vaccinate some 90% of the Cuban population by the time of the delegation's visit, as compared to 60% global rates of vaccination, 54% in lower middle-income countries and 13% in low-income countries.
4. Cuba's vaccination rate in children and adolescents 2–18 years old (97.5%) is the world's highest and may be a factor in controlling infections caused by immune-evasive variants, a line of research worth pursuing.
5. As elsewhere, continued monitoring of Cuban COVID-19 vaccines and their effectiveness against new variants is warranted, as well as duration of immune response. In this context, innovations including SOBERANA Plus as a potential universal booster are intriguing for their possible contribution to pandemic control.
6. Previous biotechnology research, development, production and regulatory experience allowed Cuba's BioCubaFarma centers to quickly pivot towards creating COVID-19 vaccines and to repurpose existing therapies for treating severe infections.
7. Cuba has a robust, well-documented history of international biotech collaboration and technology transfer for health—particularly with other countries of the Global South—which continued through the pandemic.
8. A new high-tech manufacturing plant at the Mariel Biotech Industrial Complex promises to boost Cuba's vaccine manufacturing capacities to at least 15 million doses monthly, a minimum that incorporates production of Abdala at Mariel and the SOBERANAs at their current plants. This would enable Cuban exports to contribute to global COVID-19 vaccine shortfalls, especially if priced affordably for low- and middle-income countries.
9. Lag in publishing peer-reviewed phase 3 results has been disadvantageous to Cuba's COVID-19 vaccine program, likely delaying availability of these vaccines internationally. WHO emergency use listing is vital to such global registration and potential use of the Cuban vaccines.
10. Our delegation and our Cuban colleagues benefited from open, transparent scientific engagement, a prerequisite for the bilateral and multilateral collaboration urgently needed to effectively address global health emergencies.

# Recommendations from the Fact-Finding Delegation

1. **Multilateral and bilateral mechanisms for health promotion and pandemic prevention should actively engage Cuban scientists in dialogue, academic exchange and joint research. This is our main, overarching recommendation.** Current threats to health, alarming global inequities and the specter of recurring pandemics mandate science-based international collaboration and policymaking to protect our populations and our planet. Vital to these efforts are scientists in developing countries such as those in Cuba, who have important contributions to make based on decades of experience and success in disease eradication, and emergency preparedness and response. We learned that dozens of countries and institutions have signed bilateral agreements with Cuban health and biotech sectors, in the USA as well. These should serve as frameworks for research and joint programs to improve population health and equity—in the Americas and beyond. Decades of WHO/PAHO and UNICEF partnerships with Cuba should be leveraged, and newer multilateral mechanisms such as the Coalition for Epidemic Preparedness Innovations (CEPI) and summits on global health security should enlist Cuban expertise and experience to draw lessons from:

- **Key results and prospects for Cuba's COVID-19 vaccines and biotech pharmaceuticals.** Several features of Cuba's protein-based vaccines deserve attention: duration of their immunogenicity, use in pregnant and nursing women, ability to address new variants, and SOBERANA Plus's effectiveness as a universal booster for other internationally available vaccines. In addition, it is important to determine if the types of vaccines developed in Cuba provide any advantages for people with comorbidities and for children; or, due to less stringent storage requirements and affordable pricing, for low- and middle-income countries.
- **Cuba's vaccination strategy and national vaccine rollout, considering its experience rapidly vaccinating 90% of its population, including 97.5% of children and adolescents two years and older.** Two aspects merit immediate attention: Cuba is the only country to have vaccinated such a high percentage of children at such an early age so early in the pandemic, an experience that promises abundant, valuable information on whether pediatric vaccination can help blunt transmission rates in the general population. Second, the high COVID-19 vaccination rates also speak to how Cuba met the health equity challenge that has plagued so many other nations, where vaccine access and take-up rates have fallen short or been uneven by population subgroups. This in turn relates to vaccine compliance and its relationship to Cuba's universal health system, primary healthcare coverage, public health workforce, public messaging and population health literacy levels—all globally pertinent as even childhood vaccination rates plummet, influenced by vaccine hesitancy and refusal. Several recent studies also have considered the role of primary health care for public education, contact tracing, accessible treatment and vaccine administration. The Cuban experience may be relevant in this regard as well, with its 11,000 family doctor-and-nurse offices and nearly 500 community clinics involved in all these aspects, including vaccination.
- **Health and biotech capacity-building in Cuba, a critical component for other low- and middle-income countries aiming to develop domestic capabilities, build more equitable health systems and contribute to more equitable pharmaceutical access worldwide.** Cuba's pre-existing and integrated vaccine development, manufacturing, regulatory and immunization capabilities enabled rapid vaccine rollout—rare among countries of its size or economy. As we learned, Cuba entered the pandemic with key components of a national biopharmaceutical–public health pandemic response strategy that facilitated a pivot to address COVID-19. The country had cultivated a brain trust of vaccine scientists and a workforce pipeline; built state-of-the-art manufacturing facilities; established a national biomedical regulatory authority that interacts with PAHO/WHO and retains high-level certifications from both; and established a universal health system relying on strong primary care. How did the country accomplish this on a shoestring budget? Does the Cuban experience hold clues to success for other developing countries?
- The scientists we met explained that Cuba's vaccine manufacturing strategy is designed to satisfy domestic need first and consider export thereafter. In this context, Cuba may provide an example of the advantages of local vaccine and biotech manufacturing,

particularly in the face of global shortfalls, hoarding and inequitable access. Additionally, opportunities exist for Cuba to work with the Africa Union/Africa CDC as they pursue establishment of vaccine manufacturing on the continent (see the Partnerships for Vaccine Manufacturing report and Now is the Moment to Launch an African Vaccine Industry).

- As the world searches for 'pancorona' vaccines to cover a broader range of viruses, it is essential to recall that in most of the world, not only are the current vaccines in short and unequal supply, but few countries actually have the capabilities to produce their own. Cuba's vaccine development experience stands in sharp contrast.
- **The experience of Cuba's regulatory authority, contributing to fast-tracking harmonization of regulatory processes in the Americas and globally.** During evaluations to consider emergency use authorization for the country's COVID-19 vaccines, Cuba's regulatory authority (a PAHO/WHO-certified Level 4 Regulatory Authority of Reference since 2011) faced challenges common to its counterparts everywhere, particularly how to balance the urgency of product approvals with rigorous evidence review. But it had the additional challenge of assessing products developed by publicly funded/operated institutions subject to clinical trials organized by government-run public health institutions. Regulator independence has long been a subject of concern with research conducted by large, financially flush conglomerates, but the path to authorization through integrated, publicly operated research and regulatory institutions has been less studied. Cuba's experience provides an opportunity to do just that, advancing criteria for the harmonization process and its ethical underpinnings.

2. **External trade and financial barriers that hamper development, production, use or cost recovery for Cuba's biotech and pharmaceutical products should be removed.** Moreover, we advocate collaboration with Cuba to boost production capacities for its COVID-19 vaccines and other proven biologics, and to advance research and public health initiatives. In this context, we applaud assistance from WHO/PAHO, UNICEF, the European Union and others, and urge the new Financial Intermediary Fund for Pandemic Prevention, Preparedness and Response, hosted by the World Bank, to include Cuban vaccine developers in considerations by implementing entities strengthening pandemic preparedness and response.

   The Mariel Biotech Industrial Complex—poised to scale up production of at least one COVID-19 vaccine plus other therapeutics—should be supported, as it may also help address global vaccine shortfalls and inequities when coupled with Cuba's record of collaboration with other developing countries. Havana's Finlay Vaccine Institute has received good marks on the Fair Pharma Scorecard, and the Cuban health ministry has gone on record stating that the country's vaccines will be priced affordably or even provided free for poorer nations. Thus, we commend Cuba's application to WHO for Abdala emergency use listing and encourage SOBERANA developers to follow suit as soon as possible; if Cuban vaccines ultimately receive such approval, it will open the door to their registration by other countries, especially low- and middle-income nations that often rely on PAHO/WHO-certified findings. In such a context, we encourage Cuban biotech companies to become regional vaccine suppliers.

   Our delegation learned that Cuba has established working partnerships with institutions in Africa, the Latin American-Caribbean region and Asia—examples of South-South training, technology transfer and joint venture initiatives that also merit recognition and support.

3. **International partners should invest in modernizing Cuba's health-sector data systems, to better position the country for the next health emergency and facilitate health research.** Evident in our site visits to CIGB, IFV and the Mariel Biotech Industrial Complex was Cuba's capacity for biomedical science and innovation, despite the impact of the pandemic, a global recession and US sanctions. Nevertheless, some basic technology is lacking at the local level.

   Investing in updated heath systems technology would result in more efficient electronic records, promotion of data-sharing and facilitation of more robust epidemiological surveillance, as well as expedited reporting of adverse events and analysis of the pandemic's macro effects on Cuban population health.

4. **Cuban researchers should be encouraged to routinely publish their results more quickly and more frequently in international peer-reviewed journals.** This is essential to achieve credibility for their findings, especially for those as important and compelling as we observed during our visit for the COVID-19 vaccines. Cuban scientists completed phase 3 clinical trials of their COVID-19 vaccines a year prior to our fact-finding mission, but these results had yet to be published. Delegation members acknowledged that Cuban scientists were operating in challenging conditions but recognized that the lack of peer-reviewed publication—also considered by WHO for determining emergency use listing—is an obstacle to worldwide accessibility for these vaccines. While the dearth of publications from low- and middle-income country scientists in peer-reviewed journals is well established, Cuban scientists' track record in biotech and public health appears to position them well to confront this obstacle. In this context, we encourage primarily English-language journals to extend support and mentoring where possible, and in all cases, give due consideration to Cuban research.The delegation commends our Cuban peers for providing comprehensive and thought-provoking presentations on all aspects of the country's COVID-19 vaccine experience: the science involved in developing and testing the vaccines; production processes and facilities used for their manufacture; regulatory protocols employed to provide emergency use authorizations; and the public health strategies Cuba implemented for maximum vaccination coverage in a short period of time. Delegation members also noted the responsiveness and generosity of Cuban experts in providing thoughtful, detailed consideration of our questions and comments.

As previously noted, the delegation was not intended to provide formal vetting or certification of vaccine safety and efficacy, or to verify vaccination coverage or compliance. That said, Cuba has a well-documented international reputation for developing safe, effective vaccines, and delegation members found the science presented on the country's COVID-19 vaccines to be compelling and convincing. Like the Cuban scientists we met, we share a commitment to promoting scientific collaborations that aim to address the global gap in access to high-impact health innovations and interventions, a long-term disparity magnified by the pandemic.

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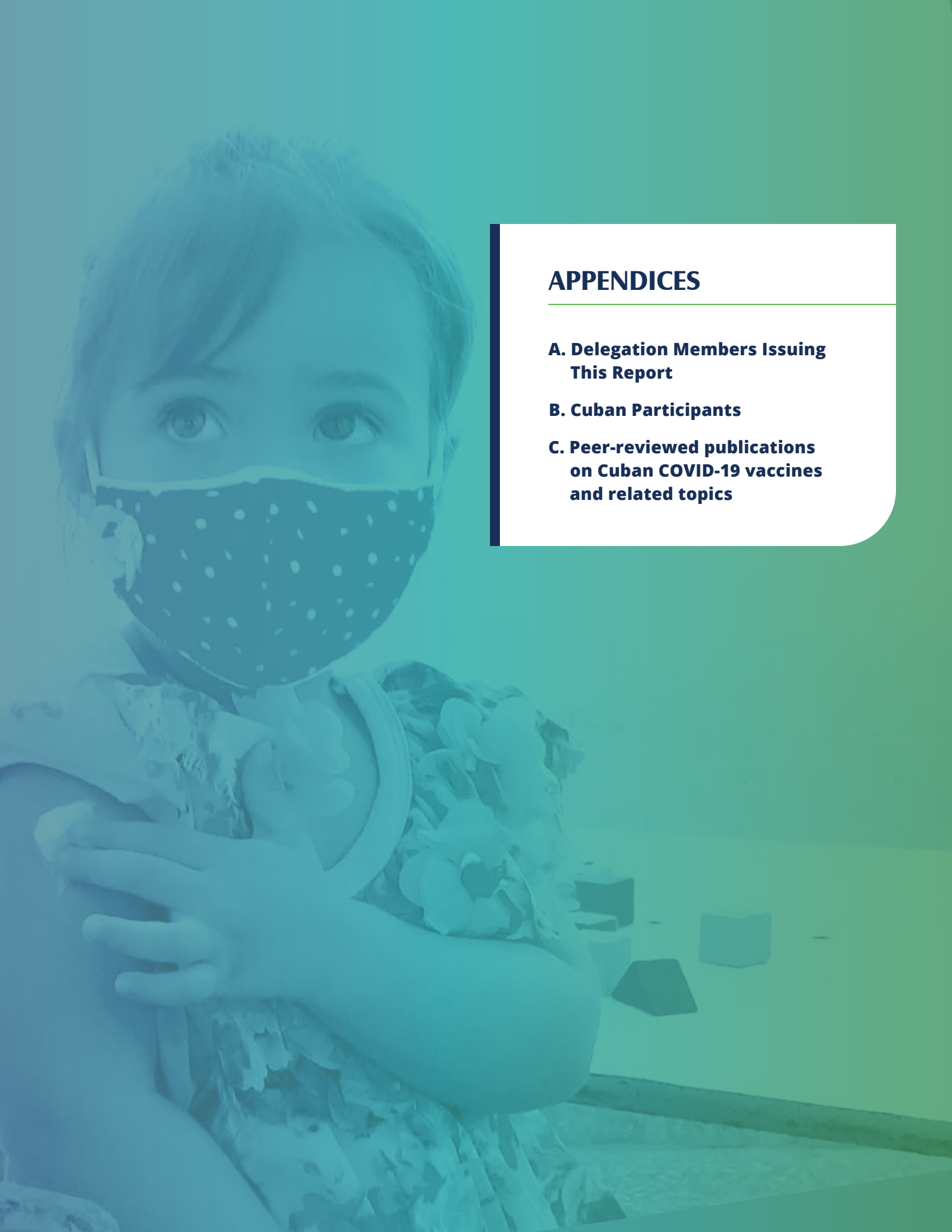

# APPENDICES

# Appendix A

## Delegation Members Issuing this Report

### Delegation Co-Leaders:

**MICHAEL T. OSTERHOLM PHD** is Regents Professor, McKnight Presidential Endowed Chair in Public Health, the director of the Center for Infectious Disease Research and Policy (CIDRAP), Distinguished Teaching Professor in the Division of Environmental Health Sciences, School of Public Health, a professor in the Technological Leadership Institute, College of Science and Engineering, and an adjunct professor in the Medical School, all at the University of Minnesota. In November 2020, Dr. Osterholm was appointed to President-elect Joe Biden's 13-member Transition COVID-19 Advisory Board. He is the author of the New York Times best-selling 2017 book, Deadliest Enemy: Our War Against Killer Germs, in which he details the most pressing infectious disease threats of our day and lays out a nine-point strategy on how to address them, with preventing a global flu pandemic at the top of the list. In addition, Dr. Osterholm is a member of the National Academy of Medicine (NAM) and the Council of Foreign Relations. He is a frequent consultant to the World Health Organization (WHO), the National Institutes of Health (NIH), the Food and Drug Administration (FDA), the Department of Defense, and the CDC. He is a fellow of the American College of Epidemiology and the Infectious Diseases Society of America (IDSA).

**CRISTINA RABADÁN-DIEHL PHARMD PHD MPH** is Associate Director for Clinical Trials at Westat, a position she assumed in 2018 after a 25-year career at the National Institutes of Health (NIH) and the U.S. Department of Health and Human Services (HHS). She is a Pharmacist, Molecular Biologist and Public Health Professional with vast experience in global health. Dr. Rabadán-Diehl provides leadership and technical expertise in the design and execution of Phase I-Phase IV clinical trials and develops partnerships with domestic and international government and nongovernment stakeholders. She was Director of the Office of the Americas in the Office of Global Affairs, Office of the Secretary, at HHS. In this role, she coordinated U.S. Government (USG) policy and functions related to HHS activities in the Americas region, in collaboration with HHS Divisions such as the NIH, CDC, FDA, and the Administration for Strategic Preparedness and Response (ASPR). During her tenure, she worked closely with PAHO in areas related to health and research in the Americas and participated in the US response to the Ebola and Zika epidemics. She also served as the HHS Secretary's Representative to the US-Mexico Border Health Commission. As a multidisciplinary scientist, she currently participates in Scientific Review Panels for the NIH and the European Union and serves as a National Advisory Council Member for SAMHSA. Dr. Rabadán-Diehl is an Adjunct Professor at George Washington University, where she teaches Global Health Diplomacy, and a Visiting Professor at the National School of Public Health, Health Institute Carlos III in Spain.

### Delegation Members:

**JOSHUA ANZINGER, PHD** is a Senior Lecturer at the University of the West Indies Department of Microbiology and Consultant Virologist at the University Hospital of the West Indies, both in Kingston, Jamaica. His research interests are primarily in the fields of HIV and arboviruses, and have recently expanded to include SARS-CoV-2 in response to the COVID-19 pandemic. He is Head of the Diagnostic Virology Laboratory at the University of the West Indies, Director of the Global Virus Network Jamaica Affiliate, Member of the Abbott Pandemic Defense Coalition, Member of the PAHO Arbovirus Diagnosis Laboratory Network (RELDA), and Member of the PAHO Caribbean Sub-regional Certification Committee for the Global Eradication of Poliomyelitis.

**MARIA ELENA BOTTAZZI PHD** is Associate Dean of the National School of Tropical Medicine, Professor of Pediatrics, Molecular Virology & Microbiology, Division Chief of Pediatric Tropical Medicine and Co-director of Texas Children's Center for Vaccine Development at Baylor College of Medicine in Houston, and Distinguished Professor in Biology at Baylor University in Waco. Dr. Bottazzi obtained her bachelor's degree in Microbiology and Clinical Chemistry from the National Autonomous University of Honduras and a doctorate in Molecular Immunology and Experimental Pathology from the University of Florida. Her post-doctoral training in Cellular Biology was completed at University of Miami and Pennsylvania, and afterwards she worked at the George Washington University before relocating to Texas. She is an internationally recognized tropical and emerging

disease vaccinologist, global health advocate and co-creator of a patent-free, open science COVID-19 vaccine technology that led to the development of Corbevax, a COVID-19 vaccine for the world. She pioneers and leads innovative partnerships for the advancement of a robust vaccine development portfolio tackling diseases that disproportionally affect the world's poorest populations, making significant contributions to catalyze policies and disseminate science information to reach a diverse set of audiences. In 2022, alongside vaccine researcher Peter Hotez, she was nominated by Congresswoman Lizzie Fletcher of Texas for the Nobel Peace Prize.

**CELIA CHRISTIE-SAMUELS MBBS DM PEDS MPH FAAP FIDSA FRCP(EDIN)** is Professor of Paediatrics (Infectious Diseases, Epidemiology and Public Health) at The University of the West Indies (UWI), Mona, Jamaica, on a recent post retirement contract. She is also a consultant paediatrician at the University Hospital of the West Indies and headed their Vaccines Infectious Diseases Centre, along with Jamaica's Perinatal, Paediatric and Adolescent HIV/AIDS Programme. She participated in several international clinical trials, including one that led to approval of a pentavalent vaccine for infant gastroenteritis. She also served on the Anti-infective Drugs Advisory Committee of the US Food and Drug Administration (FDA). She received her undergraduate training in Medicine and Surgery and postgraduate training in Pediatrics at The University of the West Indies (UWI), Mona. She also completed two Post-Doctoral Fellowships in Pediatric Infectious Diseases and in Hospital and Molecular Epidemiology at Yale University and Yale New Haven Hospital, USA and later pursued an MPH degree from Johns Hopkins University. Her work has focused on: HIV/AIDS in women, children and adolescents; implementation of clinical trials of vaccine-preventable diseases; and emerging infectious diseases. She has been recognized with awards including The International Leadership Award from the Elizabeth Glaser Pediatric AIDS Foundation, Excellence in Science Inaugural Stephen Preblud Award from the US CDC (2022), The UWI's Vice Chancellor's Award for Research Excellence (2008), and the Gold Medal for Eminence in Research from the Board of Governors of the Institute of Jamaica (2014). In 2018 she was conferred with the Jamaican national honor of Order of Distinction in the rank of Commander (CD).

**NGOZI ERONDU PHD MPH** trained as an infectious disease epidemiologist and recently joined the Global Institute for Disease Elimination (GLIDE) as Technical Director. She often provides technical support to the US CDC, WHO and governments across sub-Saharan Africa, the Middle East, and Southeast Asia to strengthen capacities under the International Health Regulations for the control of infectious diseases such as Ebola, meningitis, malaria, and polio. She also has served in an advisory capacity to the Global Preparedness Monitoring Board, Africa CDC, and the UK Government All-Party Parliamentary Group on Global Health. Dr. Erondu is also a Senior Scholar with the Global Health Policy and Politics Initiative at the O'Neill Institute, Georgetown University Law Center, where she co-chairs the Lancet-O'Neill Commission on Racism and Structural Discrimination in Global Health. She serves as a commissioner on the Lancet Infectious Diseases Commission on Preparedness for Emerging Epidemic Threats. Dr. Erondu is a former Assistant Professor at the London School of Hygiene and Tropical Medicine, where she taught disease outbreak response and epidemiology; an Associate Fellow at Chatham House and the John Hopkins University Emerging Leaders in Biosecurity Programme; and co-editor of the Health Security section for PLOS Global Health.

**JEANNE MARRAZZO MD MPH FACP FIDSA** holds the C. Glenn Cobbs Endowed Chair and is Professor of Medicine and Director of the Division of Infectious Diseases at the University of Alabama at Birmingham Heersink School of Medicine (UAB). She is a Fellow of the American College of Physicians and of the Infectious Diseases Society of America (IDSA), and was elected Treasurer of the IDSA in 2021, having served on the board since 2018. She was Chair of the American Board of Internal Medicine Council from 2015 to 2018. Dr. Marrazzo has a broad research portfolio that includes the relationships between the vaginal microbiome and female reproductive tract infections, HIV pre-exposure prophylaxis, hormonal contraception, and risk of STI/HIV acquisition. She leads UAB's participation in the RECOVER trial, funded by NIH to study post-acute sequelae of SARS-CoV-2 infection, and a large clinical trial of the meningococcal Group B vaccine rMenB+OMV NZ (Bexsero) to prevent gonococcal infection. She chairs the Biomedical Science Committee of the HIV Prevention Trial Network, the group tasked with integrating the biomedical science agenda across numerous clinical trials of antiretroviral prevention agents. She is also a Co-PI of the NIH-funded Infectious Disease Clinical Research Consortium that leads the Vaccine Treatment and Evaluation Units as well as NIH-funded STI clinical trials. She has been a leading voice in educating colleagues, the community, and the media during the COVID-19 pandemic.

**SANDRA MILAN PHD BIO** is a Vice President, Project Team Leadership, Molecular Oncology at Genentech's Research and Early Development. She is responsible for leading a group of Project Team Leaders with oversight for programs in cancer. Her group focuses on strategy and implementation of

development programs that have the potential to transform the treatment of cancer. Dr. Milan is responsible for setting multi-molecule, multi-indication franchise strategies and leading teams through significant risk and complexity. Her passion for using science to fight disease and improve patient's lives extends to developing the next generation of leaders. She serves on the Diversity and Inclusion Board of Directors and is a Founder and member of gWISE (Genentech Women in Science and Engineering). Outside of work, Dr. Milan serves on the Board of Directors for UCLA Life Science Division, Chabot Space & Science, and the Chicana Latina Foundation. Dr. Milan received her PhD in Molecular and Cell Biology from UC Berkeley, where she also completed a postdoctoral fellowship. She held a number of leadership roles at Chiron prior to joining Genentech in 2006.

**PETER KOJO QUASHIE PHD** is a specialist in molecular virology, viral enzymology, antiviral therapeutics and antimicrobial drug mechanism and resistance and Senior Research Fellow and Deputy Director in Charge of Research at the West African Centre for Cell Biology of Infectious Pathogens (WACCBIP), University of Ghana. Dr. Quashie holds a doctorate in Experimental Medicine from McGill University in Canada, backed up by post-doctoral training from the University of Toronto. He is a specialist in antiviral therapeutics with a focus on HIV, Dengue and SARS-CoV-2. He also leads the Quashie Research Group, a subunit of the Virology laboratory at WACCBIP. The group aims to understand and therapeutically target key replicative processes in viruses of pandemic concern. His previous research has spanned the areas of mechanistic enzymology, HIV drug resistance mechanisms, antiviral drug discovery and structural biology of viral proteins. Dr. Quashie coordinates much of WACCBIP's SARS-CoV-2 research initiatives spanning molecular and seroepidemiology studies, host-virus interactions and drug discovery.

**THOMAS SCHWAAB MD, PHD** is an immunologist and Chief of Strategy, Business Development and Outreach at the Roswell Park Comprehensive Cancer Center in Buffalo, New York, USA. He is also CEO, Global Biotechnology and Cancer Therapeutics (GBCT), CEO, RPCI Oncology, PC; and The Bill and Nancy Gacioch Family Endowed Chair for Translational Research. Dr. Schwaab is an Associate Professor of Oncology and Immunology and joined the Institute's faculty in 2009. In his current role, Dr. Schwaab supervises Roswell's business development and overall strategic development. He assures that business and clinical initiatives are delivered appropriately and support maximal quality, efficiency and effectiveness, and works to continually widen the Institute's scope of operations and growth potential at national and international levels.

**DAVID WILLIAMS MA MPH MDIV PHD** is the Norman Professor and Chair of the Department of Social and Behavioral Sciences at the Harvard T. H. Chan School of Public Health. He is also a Professor of African and African American Studies at Harvard University. Dr. Williams is an internationally recognized social scientist focused on social influences on health. His research has enhanced our understanding of the complex ways in which socioeconomic status, race, stress, racism, health behavior and religious involvement can affect health. He is the author of more than 500 scientific papers, and he has been invited to keynote scientific conferences in Europe, Africa, Australia, the Middle East, South America, the Caribbean and across the United States. The Everyday Discrimination Scale that he developed is the most widely used measure of discrimination in health studies. Dr. Williams has been elected to the National Academy of Medicine, the American Academy of Arts and Sciences and the National Academy of Sciences. He has been ranked as the Most Cited Black Scholar in the Social Sciences and as one of the World's Most Influential Scientific Minds. His research has been featured in the national print and television media and in his TED Talk.

# Appendix B

## Cuban Participants in Delegation Discussions

### Delegation Co-Leaders:

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Managing Director, Biotech Industrial Complex, Genetic Engineering and Biotechnology Center (CIGB Mariel)

**VICENTE VÉREZ-BENCOMO DSC**
Director, Finlay Vaccine Institute (IFV)

**PASTOR CASTELL-FLORIT MD PHD DSC**
Director of the National School of Public Health and President of the Council of Scientific Societies in Health

**ARTURO CHANG MD MS**
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**ERNESTO CHICO PHD**
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**DAGMAR GARCÍA PHD**
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Director of Biomedical Research, Genetic Engineering and Biotechnology Center (CIGB)

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Director, Center for State Control of Medicines and Medical Devices (CECMED)

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**MAYDA MAURI PHD**
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**MANUEL MONTANÉ MS**
Associate Director, Biotech Industrial Complex (CIGB Mariel)

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**VERENA L. MUZIO PHD DSC**
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**RINALDO PUGA MD**
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**MARIA DEL CARMEN RICARDO MD MS**
Saturnino Lora hospital, principal investigator, ABDALA clinical trials

**BELINDA SÁNCHEZ PHD**
Director of Tumor Immunology, Molecular Immunology Center

**MARIA EUGENIA TOLEDO-ROMANI PHD**
Pedro Kouri Tropical Medicine Institute, principal investigator, SOBERANA clinical trials

**YURI VALDÉS**
Chemist and Deputy Director, Finlay Vaccine Institute (IFV)

**MARILUZ VALDIVIESO MS**
Director, Rafael María de Mendive Primary School, Havana

# Appendix C

## Articles Published by Cuban Scientists on Cuba's COVID-19 Vaccines & Related Topics *(Peer-reviewed unless indicated by PREPRINT)*

### Abdala Vaccine

**Cuban Abdala vaccine: Effectiveness in preventing severe disease and death from COVID-19 in Havana, Cuba; A cohort study.** Pedro I. Más-Bermejo, Félix O. Dickinson-Meneses, Kenia Almenares-Rodríguez, Lizet Sánchez-Valdés, Raúl Guinovart-Díaz, María Vidal-Ledo, et al. *Lancet Reg Health Am.* Volume 16, 2022, 100366, https://doi.org/10.1016/j.lana.2022.100366.

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**Potency, toxicity and protection evaluation of PastoCoAd candidate vaccines: Novel preclinical mix and match rAd5 S, rAd5 RBD-N and SOBERANA EGFRic-RBD protein.** Hassan P.M., Ali T., Saber E., Asghar A., et al. Vaccine. 2022 May 3;40(20):2856-2868. https://doi.org/10.1016/j.vaccine.2022.03.066. Epub 2022 Apr 4. PMID: 35393148

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**Safety and immunogenicity of the FINLAY-FR-1A vaccine in COVID-19 convalescent participants: an open-label phase 2a and double-blind, randomised, placebo-controlled, phase 2b, seamless, clinical trial.** Ochoa-Azze R., Chang-Monteagudo A., Climent-Ruiz Y., Macías-Abraham C., et al. Lancet Respir Med. 2022 Aug;10(8):785-795. https://doi.org/ 10.1016/S2213-2600(22)00100-X. Epub 2022 Jun 9.

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## Disclosures

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INSIGHTS FROM

MEDICC

# Cuba's COVID-19 Vaccine Enterprise: Report from a High-Level Fact-Finding Delegation to Cuba

## EXECUTIVE SUMMARY

# Executive Summary

**In June 2022 an international delegation of scientists from the United States, the Caribbean and Africa traveled to Havana, Cuba for a three-day fact-finding mission to better understand Cuba's COVID-19 vaccine development and vaccination efforts. It was the first time in five years that a scientific delegation with significant US presence had engaged in discussions with medical researchers in Cuba. It also included African and Caribbean members to ensure there was a diversity of scientific and public health perspectives. Together, delegation members represented a broad range of expertise in public health systems, infectious diseases, biotechnology, and vaccine development.**

The visit was organized by MEDICC (Medical Education Cooperation with Cuba), a US-based non-profit that promotes health-related dialogue and collaboration. Since 1997, MEDICC has facilitated exchanges between Cuban and US health professionals, scholars, policymakers, foundations, students, and leaders of medically underserved communities. Delegation organization and travel were supported in part by a grant from the Open Society Foundations.

During our three days in Havana, MEDICC delegation members met with experts involved in the production and deployment of Cuba's COVID-19 vaccines. We also visited three vaccine production facilities, met with Cuban health professionals who led the country's national COVID-19 adult and pediatric vaccination campaigns, and visited a local elementary school where children and teachers described their experiences with vaccination efforts.

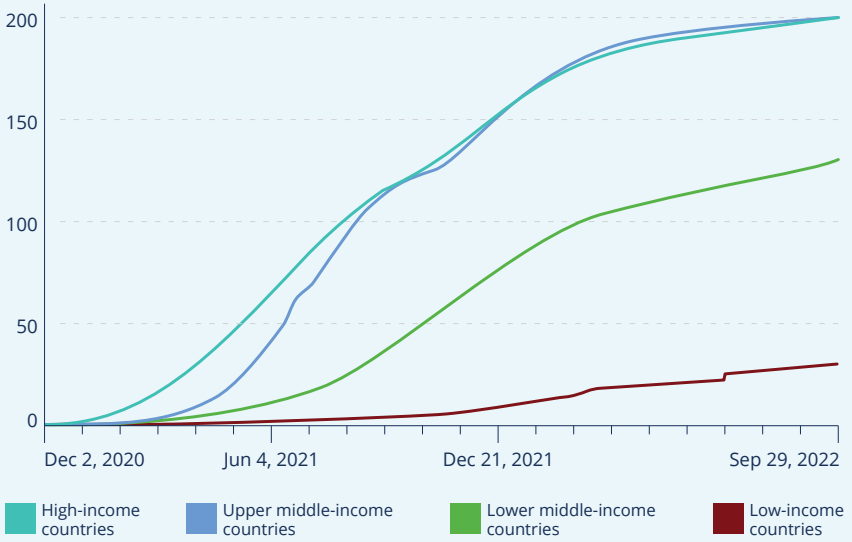
The purpose of the fact-finding mission was three-fold: first, we wanted to learn how and why a relatively small country of some 11 million people—one facing considerable economic hardships—had developed, manufactured and deployed its own vaccines, which were shown to have over 95% efficacy in preventing severe disease and death. Second, we wanted to understand Cuba's vaccine rollout strategy and preliminary results. Third, we wanted to explore Cuba's approach to science in the context of public health, with a focus on how Cuba's COVID-19 vaccine development effort and vaccination model could reveal opportunities to reduce global inequities in access to vaccines and other health innovations—especially given the country's existing network of international partnerships.

When we visited in mid-2022, globally there were daily tallies of some 843,000 new confirmed COVID-19 cases and 1874 deaths per day, with only 60% of the global population fully vaccinated.[1,2] In contrast, Cuba was reporting fewer than 20 new infections daily and zero deaths,[3] and 90% of the population, including 97.5% of children over the age of 2, had been fully immunized with the Cuban-developed vaccines. This compared to August of 2021, prior to vaccination, when infections in Cuba peaked at 10,000 a day and deaths at 100.[4]

The delegation was mindful of predictions that the world is perilously close to the next pandemic, with crossover zoonotic infections—which already account for 75% of emerging infectious diseases—on the rise amidst advancing climate change.[5] We were also alarmed by the inequitable vaccine access that has prolonged the pandemic to now—and how it highlights a broader failure of today's surge of biomedical innovation to reach billions of people in low- and middle-income countries.[6]

## **Worldwide COVID-19 Doses Administered per 100 People**, By Income Group

### All doses, including boosters, are counted individually




*Source: Official data collated by Our World in Data, World Bank*

https://doi.org/10.37757/MR2022.V24.N3-4.13

It is important to note that the MEDICC delegation was not functioning as a regulatory review or certification body. Likewise, it was outside of its scope to seek independent verification of the data presented regarding COVID-19 vaccines, vaccination coverage and voluntary vaccination compliance or their role in Cuba's overall pandemic response strategy. Rather, we sought to engage in frank, open and direct exchanges with Cuban scientists and regulatory, industry and public health experts—without involvement by high-level government officials—an aim that was fulfilled.

The following summary is based on our site visits, extensive discussions between delegation members and our Cuban peers, and relevant documents in the public record. A more detailed exploration, including technical aspects of Cuba's COVID-19 vaccines, plus extensive data from clinical trials and vaccine rollout, can be found in the full Technical Report.

## Cuba COVID-19 Vaccine Timeline

- **March 2020:** Cuba initiates COVID-19 vaccine development effort.
- **July 9, 2021:** Abdala vaccine receives Emergency Use Authorization (EUA) for use in adults.
- **August 20, 2021:** SOBERANA 02 and SOBERANA Plus vaccines receive EUA for use in adults.
- **September 3, 2021:** SOBERANA 02 and SOBERANA Plus receive EUA for use in the pediatric population.
- **October 27, 2021:** Abdala receives EUA for use in the pediatric population.

# Cuba's COVID-19 Vaccines

Concerned about the potential to procure vaccines from global suppliers, Cuban health officials decided in March of 2020 to pursue their own COVID-19 vaccines. They ultimately developed, manufactured and used two COVID-19 vaccine regimens.

One vaccine is called Abdala. It utilizes three doses of the same formulation administered intramuscularly 14 days apart. It was developed by Cuba's Genetic Engineering and Biotechnology Center. It is based on technology similar to that employed by the Center for its hepatitis B vaccine, Heberbiovac. That vaccine has been used in Cuba since 1992 and was approved in 2001 by the World Health Organization (WHO) for international use.

Cuba's other COVID-19 vaccine regimen involves two doses of a vaccine called SOBERANA 02 followed by one dose of a formulation known as SOBERANA Plus. Each is administered intramuscularly 28 days apart. The SOBERANA vaccines were developed by Cuba's Finlay Vaccine Institute. SOBERANA 02 is what is known as a conjugate vaccine. It is based on technologies developed in the 1980s that have produced vaccines now in use globally that are known to be safe and especially effective for producing a strong immune response in children.

Both the Abdala and SOBERANA vaccines generate immunity with a 'spike' protein isolated from the SARS-CoV-2 virus. The spike protein is what the virus uses to penetrate human cells. Many vaccines in use globally—including those available in the United States and Europe—rely on some aspect of the spike protein to generate immunity. Also, like those vaccines, clinical trials assessing their efficacy were conducted before the arrival of the omicron lineage of immune-escaping variants.

**Evidence of efficacy, Abdala vaccine:** Data presented from a phase 3 clinical trial indicated the vaccine's efficacy was 92.28% for preventing symptomatic disease, 100% for preventing severe disease and death, and had a good safety profile with no reports of severe adverse events.

**Evidence of efficacy, SOBERANA vaccines:** Data presented from a phase 3 clinical trial showed the SOBERANA regimen's efficacy was 92% for preventing symptomatic disease and 100% for preventing severe disease and death, and had a good safety profile with no reports of severe adverse events.

# Vaccine Regulatory Review and Emergency Use Authorization

Review of Cuba's COVID-19 vaccines—from pre-clinical development through phase 3 clinical trials—was conducted by the country's regulatory authority, the Center for State Control of Medicines and Medical Devices (CECMED). Since 2011, the Center has retained certification as a Level 4 National Regulatory Authority of Reference from WHO and the Pan American Health Organization (PAHO).

In the summer of 2021, CECMED granted emergency use authorizations (EUA) allowing the Abdala and SOBERANA vaccines to be administered to adults. In the fall of 2021, after additional trials were conducted in children, the agency granted emergency use authorization for vaccination of Cuba's pediatric population (aged 2 to 18 years).

# Cuba's COVID-19 Vaccination Strategy and Results

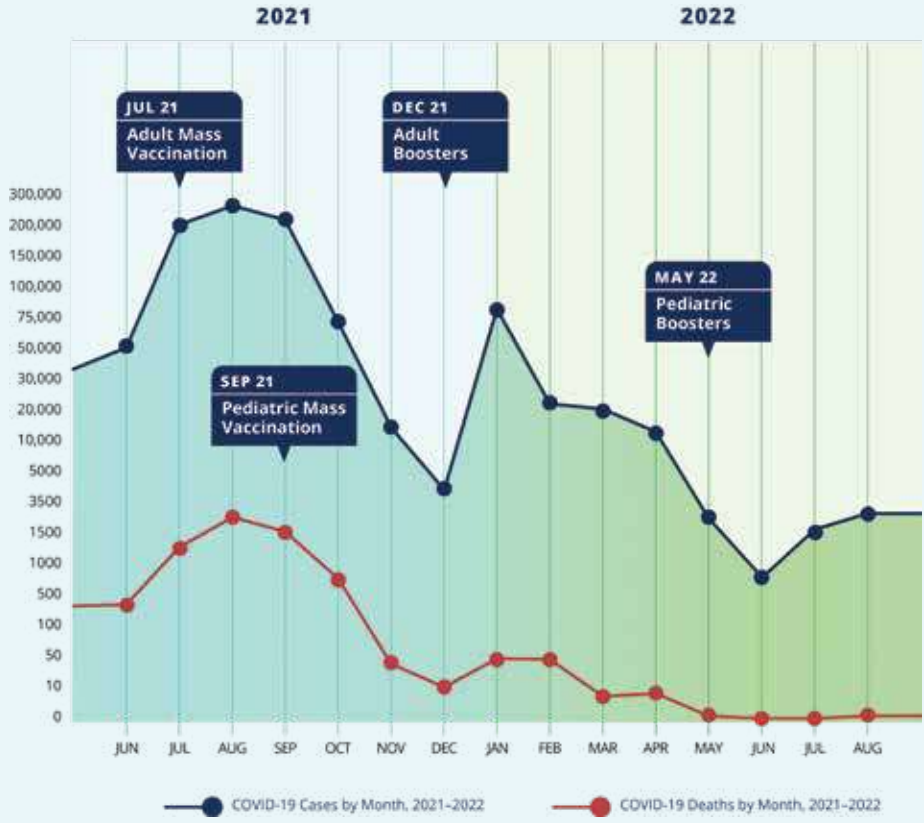
Cuba's Ministry of Public Health formulated a multi-phase strategy for achieving rapid coverage with Cuba's COVID-19 vaccines via the country's universal public health system, relying mainly on its neighborhood-based primary health care professionals and facilities. Rollout began with a series of targeted efforts to vaccinate essential health workers and scientists along with people in high-risk groups and those living in territories with particularly high infection rates. Cuba then proceeded to mass vaccination focused on adults and, later, children and adolescents aged 2–18 years. Thus, Cuba became the first country to vaccinate children as young as two years old.

Subsequent booster vaccination efforts for adult and pediatric populations were implemented via the same multi-phase approach used in the initial vaccine rollout.

Cuban health officials credited past investments in nationwide primary healthcare infrastructure—including some 11,000 neighborhood family doctor-and-nurse offices and nearly 500 community clinics—with facilitating rapid immunization of the general population.  At the peak of the campaign, Cuban health professionals were administering 300,000 COVID-19 vaccine doses per day.

> Cuban health officials credited past investments in nationwide primary healthcare infrastructure with facilitating rapid immunization of the general population.

## COVID-19 Cases and Deaths by Month Cuba, 2021-2022



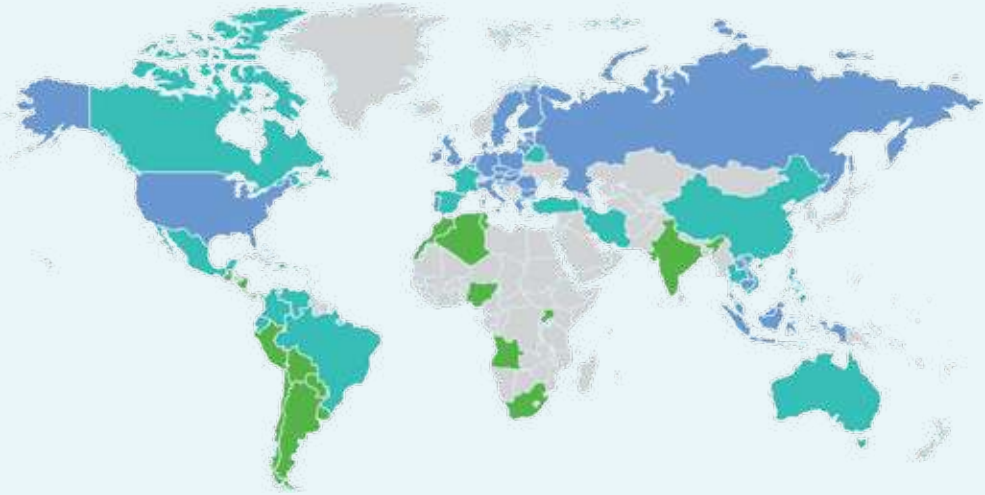

*Source: I Morales, Ministry of Public Health, Havana*

# Cuba's Biotech Sector: Foundation for COVID-19 Vaccine Development

As has been documented elsewhere and confirmed by our visit, Cuba's COVID-19 vaccine development was rooted in a decades-long effort by the Cuban government to create a biotech R&D sector, which began in 1981. Today, Cuba's biotech industry includes 32 research and development institutes and manufacturing entities operating under the umbrella of the state-owned conglomerate **BioCubaFarma**. Their collective mandate is to develop products that address health problems in Cuba and, once domestic needs are met, to engage in export and partnerships to make them available in other countries.[7] BioCubaFarma companies export products to some 40 countries in the Americas, Africa, Europe, Asia and the Middle East and are involved in product development partnerships in the USA, France, Iran, China and Viet Nam, among others.

Prior to embarking on the COVID-19 vaccine effort, the two institutions that led the work already had an international reputation for developing safe, effective vaccines. This includes a recombinant hepatitis B vaccine approved for use in Cuba since 1992; another against Haemophilus influenza type b (Hib), in use in Cuba since 2003; and the world's first effective vaccine against a deadly form of meningococcal meningitis caused by serogroup B meningococcus (MenB), in use in Cuba since 1989.[8,9] Additionally, BioCubaFarma companies produce 8 of the 11 pediatric vaccines administered through the country's national immunization program. (*See full **Technical Report** for peer-reviewed content on Cuban biotech products.*)

# Cuban Biotech in the World, 2021



| Exports | Business Modalities | | Exports & Business Modalities |
|---|---|---|---|
| Angola | Armenia | Laos | Australia |
| Algeria | Austria | Latvia | Belarus |
| Argentina | Belgium | Lithuania | Brazil |
| Bolivia | Brunei | Luxembourg | Canada |
| Chile | Bulgaria | Malaysia | China |
| Dominican Republic | Cambodia | Malta | Colombia |
| Guatemala | Croatia | Myanmar | Ecuador |
| India | Cyprus | Netherlands | France |
| Jamaica | Czechia | Poland | Iran |
| Morocco | Denmark | Portugal | Mexico |
| Nicaragua | Estonia | Republic of Korea | Philippines |
| Nigeria | Finland | Romania | Serbia |
| Panama | Germany | Russia | Singapore |
| Paraguay | Greece | Slovakia | Spain |
| Peru | Hungary | Slovenia | Thailand |
| St. Vincent & the Grenadines | Indonesia | Sweden | Turkey |
| South Africa | Ireland | United Kingdom | Venezuela |
| Tunisia | Italy | USA | Viet Nam |
| Uganda | Kazakhstan | | |
| Uruguay | Kyrgyzstan | | |

*Source: BioCubaFarma, Havana, 2022*

# Potential for International Collaboration

A new high-tech manufacturing facility at the Mariel Biotech Industrial Complex, coupled with the plants producing the SOBERANA vaccines, boosts minimum production capacities to some 15 million doses monthly, according to Cuban vaccine producers. Cuba's trade and technology transfer agreements, its joint ventures and related commercial partnerships—particularly, but not exclusively, with other developing countries—indicate that Cuba's COVID-19 vaccines could be exported to countries of the Global South. While global supplies of existing formulations of COVID-19 vaccines rose substantially in 2022, less clear is the availability of boosters that could help maintain protection. And equitable access, regardless of supply, remains a goal rather than a reality.

As of September 2022, Cuba's COVID-19 vaccines had received emergency use authorizations from several countries that have also signed commercial contracts, including Mexico, Iran, Viet Nam, St. Vincent & the Grenadines, Belarus and Venezuela. Abdala was being considered for Emergency Use Listing (EUL) by WHO, and the SOBERANAs were expected to follow suit. Cuban health professionals also have expressed consistent interest in collaborating with other health systems. Their input could be particularly valuable for developing strategies to achieve high rates of vaccination compliance, as well as rapid and equitable vaccine rollout—including for children—during public health emergencies.

Meanwhile, we also learned about international partnerships with public and private sectors for developing a number of other advanced health innovations—and efforts in Cuba to increase production capacity for therapies to treat a range of infectious and non-communicable diseases. For example, Cuba's Molecular Immunology Center is partnering with the Roswell Park Comprehensive Cancer Center in Buffalo, NY, to test a Cuban biotech treatment targeting certain types of lung as well as head and neck cancers.[10]

# Summary of Findings and Recommendations

**The following is a synthesis of the delegation's findings and related recommendations that emerged from our site visits, discussions with Cuban scientists and public health experts, and relevant documents in the public record.**

**FINDING: In a difficult social and economic environment, Cuban scientists developed, tested and manufactured two safe and highly efficacious COVID-19 vaccine regimens and used them to vaccinate more than 90% of the population.** This achievement demonstrates product development and public health capabilities urgently needed in the developing world. For example, the Abdala and SOBERANA vaccines utilize technologies that require only household refrigeration, important for low-resource settings. Cuba also has increased its capacity to manufacture vaccines and other advanced biologics targeting both infectious and non-communicable diseases. Meanwhile, Cuba's biotech sector embraces a strong public health mission. It has tight linkages to a nationwide primary healthcare system, which provides access to its innovations via a comprehensive network of health professionals and community clinics.

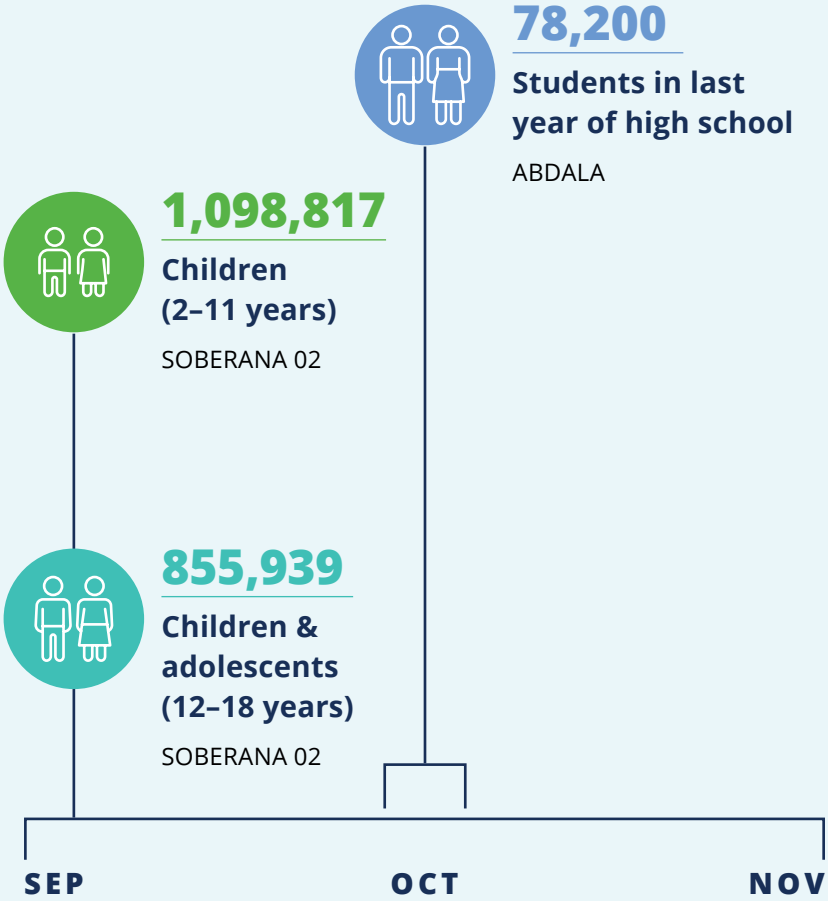
**RECOMMENDATION: External economic barriers that hamper development, production, use, or cost recovery for Cuba's biotech and pharmaceutical products or international collaboration with Cuba's research institutions, biotech firms and public health professionals should be removed, to aid in the global fight against existing and emerging threats and support equitable access to medical innovations.** The pandemic we still fight today has been greatly prolonged by shocking inequities in access to vaccines and treatments. This same disparity will impede efforts to address the next pandemic, just as it has affected fights against a number of diseases in the past. It is also impeding efforts to reduce the growing global burden of non-communicable diseases.

Cuba alone cannot close this gap, but it could be making a much greater contribution. However, current restrictions on trade and investment with Cuba are severely limiting efforts to take advantage of Cuba's considerable biotech R&D capabilities. Despite these impediments, Cuban biotech companies and research institutions have managed to establish a network of global partnerships. It includes at least one US partner and a number of institutions in Africa, Latin America and the Caribbean, and Asia. These should serve as frameworks for wider engagement with Cuba as a full partner for improving access to medical innovation, as can decades of WHO/PAHO and UNICEF partnerships with Cuba. Newer multilateral mechanisms such as the Coalition for Epidemic Preparedness Innovations (CEPI) and recent summits on global health security should be informed by Cuban expertise and experience. We also urge the new Financial Intermediary Fund for Pandemic Prevention, Preparedness and Response, hosted by the World Bank, to include Cuban vaccine developers in considerations by implementing entities.

**FINDING: Cuba's COVID-19 vaccination rate in children and adolescents 2–18 years old (97.5%) is far greater and was achieved far earlier than any country in the world.** Cuba conducted pediatric clinical trials to assess safety and efficacy in children and adolescents and sought high coverage rates once immunizations began as a way to both protect youngsters and limit infections in the broader population. In general, children often serve as vectors for accelerating the spread of infectious diseases to populations that are more at risk, such as the elderly.

**RECOMMENDATION: Cuba's high pediatric coverage merits immediate international assessment for its potential to help blunt transmission rates in the general population, especially with new variants that partially escape immunity provided by vaccines or prior infections.** Given the relatively low pediatric coverage with COVID-19 vaccines in most countries, there is a paucity of data available internationally to assess how high coverage might impact disease transmission in other populations. An international research collaboration with Cuban scientists and public health professionals can help answer that question.

## Cuba: COVID-19 Vaccination in Pediatric Ages, 2021




*Source: I Morales, Ministry of Public Health, Havana*

**FINDING: Cuban health authorities are exploring the potential of the SOBERANA Plus vaccine to serve as a universal booster, regardless of the initial vaccine series, and to boost protection provided by prior infection.**[11]

**RECOMMENDATION: International partnerships should explore whether SOBERNA Plus, either in its current form or modified to target new variants, can serve as a universal booster to support efforts globally to maintain protection against COVID-19.** The vaccine utilizes a well-established technology that could enable rapid scale-up of production—inside or outside Cuba—and the ability to adjust the formulation to target new variants of concern. Also worthy of study is Cuba's experience using SOBERANA Plus to boost immunity in COVID-19 convalescent patients.

**FINDING: Lag in publishing peer-reviewed phase 3 results has likely delayed global access to Cuba's COVID-19 vaccines.** Cuban scientists were working in very challenging conditions and were rightly focused on readying vaccines for domestic use. But peer-reviewed evidence is particularly important for the global scientific community and is considered by WHO for its emergency use approval, a crucial benchmark for guiding vaccine procurement for low-income countries.

**RECOMMENDATION: Cuban researchers should be encouraged to publish their results more quickly and more frequently in international peer-reviewed journals.** While the dearth of publications from low- and middle-income country scientists in peer-reviewed journals is well established, Cuban scientists' track record in biotech and public health appears to position them well to confront this obstacle. We encourage primarily English-language journals to extend support and mentoring where possible—and in all cases to give due consideration to Cuban research, especially given its potential contribution to health needs in the Global South.

As previously noted, the delegation was not intended to provide formal vetting or certification of vaccine safety and efficacy, or to verify vaccination coverage or compliance. That said, Cuba has a well-documented international reputation for developing safe, effective vaccines, and delegation members found the science presented on the country's COVID-19 vaccines to be compelling and convincing.

Like the Cuban scientists we met, we share a commitment to promoting scientific collaborations that seek to address the global gap in access to high-impact health innovations and interventions, a long-term disparity magnified by the pandemic. Our delegation and our Cuban colleagues benefited from open, transparent scientific engagement, a prerequisite for the bilateral and multilateral collaboration urgently needed today to effectively prevent and address global health emergencies.

> Like the Cuban scientists we met, we share a commitment to promoting scientific collaborations that seek to address the global gap in access to high-impact health innovations and interventions.

# Delegation Members Issuing This Report

## Co-Leaders

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**David Williams MA MPH MDiv PhD** is the Norman Professor and Chair of the Department of Social and Behavioral Sciences at the Harvard T. H. Chan School of Public Health . He is also Professor of African and African American Studies at Harvard University.

*(See Appendix A in the **Technical Report** for full bios.)*

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**The full Technical Report from this fact-finding delegation includes detailed findings and recommendations, a list of Cuban scientists participating in our discussions, and links to peer-reviewed content on the Cuban COVID-19 vaccines.**

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