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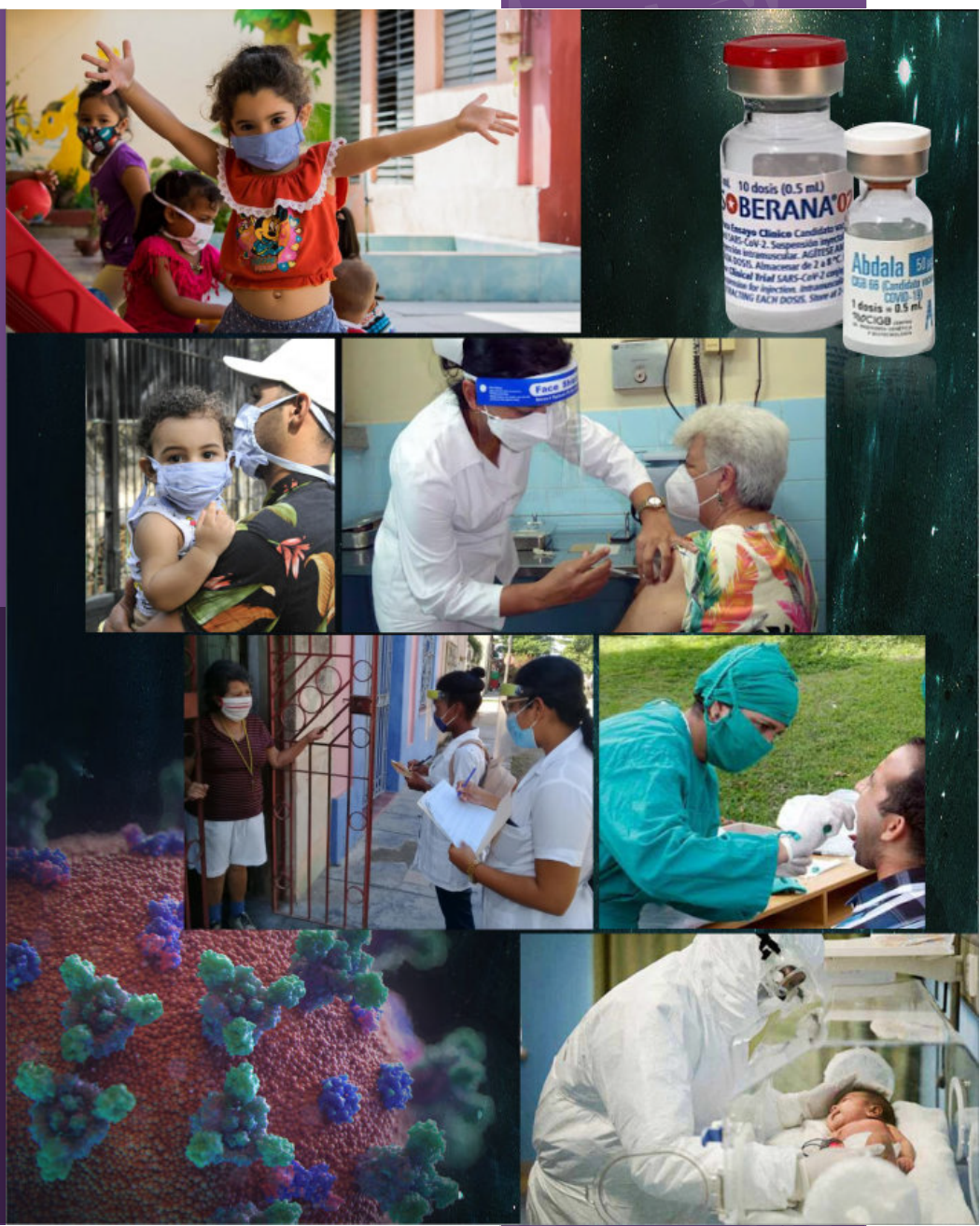
On COVID-19



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ABSTRACTS

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US Sanctions on Cuba Further Imperil Global Vaccine Equity

The results are in and deeply troubling: in the absence of rapid, comprehensive vaccination rollouts, SARS-CoV-2 runs amok, mutates into more contagious variants and, for the unvaccinated, kills at a breakneck pace. Witness the Delta variant that research suggests is more than twice as contagious as the original strain that ripped the world asunder in 2020. While vaccinated people can contract and transmit Delta, the probability of developing serious illness is significantly reduced.[1] This is good news for populations in high- and upper-middle income countries—where 80% of the global vaccine stock has been "hoarded," in the words of WHO Director-General Dr Tedros Adhanom Ghebreyesus.[2]

The news is not at all good, however, for the unvaccinated: contracting Delta means a one-way ticket to the hospital, all too often followed by the morgue. Recent data from the Centers for Disease Control and Prevention (CDC) reveal that nearly all recent COVID-19 hospitalizations and deaths in the United States are among the unvaccinated.[3] Death is even more certain for the 99% of people in low- and middle-income countries (LMICs) with still no access to vaccines.[4] Meanwhile, the world's richest nations continue to gobble up the critically insufficient supplies, not nearly offset by tardy, token donations to the most vulnerable countries.

Across Africa, despite national COVID-19 strategies in place (see Dzinamarira this issue on response in Rwanda, South Africa and Zimbabwe), a mere 1% of people have been fully vaccinated and the case fatality rate is 18% above the global average. WHO estimates deaths there from coronavirus, accelerated by the Delta variant, surged by 80% in July.[4,5]

In Latin America and the Caribbean, 16.6% of people have been fully vaccinated. Nevertheless, vaccination rates are widely uneven (more than 65% fully vaccinated in Chile, but only 1% in Honduras and Guatemala). And this is the region hardest hit by the pandemic thus far: with just 8.4% of the world's population, it accounts for 32.5% of COVID-19–related deaths.[6,7] As the region struggles to find answers, health systems threaten collapse, essential primary health care services have been interrupted and routine childhood immunizations disrupted. Not surprisingly, infection rates are rising in areas of Mexico, Guatemala, Paraguay, Colombia and elsewhere.[8]

Poverty, including extreme poverty, unequal and slow vaccine distribution, plus fragmented, segmented and underfunded health systems compound the complex situation, according to Alicia Bárcena, Executive Secretary of the UN Economic Commission for Latin America and the Caribbean (ECLAC). The picture is bleak: not enough critical-care beds, personnel, technology, medicines or money. Relying on imports for oxygen, PPE, COVID-19 tests and of course, vaccines, has drained national coffers while millions get sick and thousands die.

The way out of this grim scenario, say Bárcena and other regional experts, is through investing in primary health care systems and a "concerted regional health strategy…for reducing external dependence."[7,9] This has prompted ECLAC's Comprehensive Plan for Health Self-Sufficiency, adopted by the Community for Latin American and Caribbean States (CELAC) at their recent meeting. The Plan calls for regional vaccine procurement, a clinical trials platform, accelerated vaccine development, harmonization of regulatory authorities, access to intellectual property and an inventory of regional capacities.

Cuba, with a universal health system anchored by community-based primary care and supported by an adroit, experienced biotechnology sector, embraced the approach expressed by Bárcena and colleagues once COVID-19 was detected in the country in March, 2020. "Reducing external dependence" indeed: the first Cuban SARS-CoV-2 vaccine candidate that began clinical trials is named Soberana (Sovereign). And as the pandemic progressed, Cuba became the first in Latin America and the Caribbean to develop a proven COVID-19 vaccine.

But for Cuba, self-reliance is not a matter of choice or strategy. It's a matter of survival.

It's a matter of survival

As one of his last salvos, US President Trump dealt another blow to Cuba by placing it on the list of State Sponsors of Terrorism, providing no evidence for the move. That brought to 243 the tally of additional economic, commercial and financial sanctions heaped on the island by his administration.[10] Now, contradicting his own campaign promises, President Biden has adopted Trump's punitive gambit as policy: every one of the 243 sanctions is still in place—amidst the greatest global health crisis of our times.

The labyrinth of tightened restrictions includes blacklisting a host of state-run companies, Cuban financial institutions responsible for family remittances and even some 400 hotels on the island, the latter move targeting tourism, a major hard-currency earner for Cuban public and private businesses alike. The health sector has been especially hard hit, the US policy reportedly costing it $198.3 million between April and December, 2020.[11] Higher-priced intermediaries and farther shipping distances become the rule when US government licenses are required for export to Cuba—from anywhere in the world—of any item with as little as 10% US components. Just four banks in the world will now transfer funds to Cuban entities.

As early as April, 2020, a shipment of ventilators to Cuba was blocked when the European manufacturing companies (IMT MEDICAL AG and ACUTRONIC) were acquired the US company Vyaire Medical Inc.[12] Similarly, US policy has hindered international aid donations of face masks, PCR tests, gloves and syringes, all fundamental for controlling COVID-19.

And what is worse now, Cuban vaccine developers cite the US sanctions as hindering and even blocking purchase of dozens of equipment, supplies and ingredients for clinical trials and production—reagents, filtration tanks, potassium chloride solution, purification systems and more.[11]

Such continued punishment-as-policy is reprehensible. It is inhumane. It is also, in the context of COVID-19, lethal. The

These sanctions-on-steroids aren't just bad public health policy, they are bad statesmanship

Biden administration itself has recognized this: on June 17, 2021, it circumvented its own sanctions on three other countries by issuing general licenses permitting unfettered export and re-export of any and all pandemic-response items such as medicines and medical devices. The countries: Iran, Syria and Venezuela.[13] But not Cuba.

Support for Cuba's 11 million people during the pandemic was reduced to a July State Department Fact Sheet apparently encouraging humanitarian donations,[14] when in reality it includes nothing new, much less an easing of restrictions for exports. Calls by President Biden for vaccine equity, human rights, regional stability and integration, and easing Cubans' suffering all ring hollow while his White House champions punishment during a pandemic.

These sanctions-on-steroids aren't just bad public health policy, they are bad statesmanship.

The fact is that, despite US sanctions, Cuban scientists have taken five vaccine candidates through advanced clinical trials. The first, Abdala, received Emergency Use Authorization (EUA) from the country's national regulatory authority on July 9 after phase 3 clinical trials showed 92% efficacy; another with comparable efficacy is expected to receive EUA soon.

As of this writing, nearly 11 million doses have been administered, and more than 25% of the population has been fully vaccinated.[15] Clinical trials are also ongoing for use of the vaccines in youngsters and convalescent patients; for a nasally-administered vaccine; and for a number of COVID-19 treatments and much-needed medical equipment, such as ventilators. (See our exclusive with Olga Lidia Jacobo, Director of Cuba's National Regulatory Authority, this issue).

US policy intended to hobble the Cuban government actually takes aim at the world's poorer countries

Given the track record of Cuban biotechnology and the export potential for the new vaccines, it becomes clear that a US policy intended to hobble the Cuban government actually takes aim at the world's poorer countries, which could stand to benefit from Cuban science to address the dearth of life-saving vaccines for their populations. Several countries in Latin America, Asia and Africa have already expressed interest.

The world still needs production and equitable distribution of several billion more vaccine doses to ramp up immunity and prevent new variants from taking hold, and no one is protected unless that immunity can be achieved more evenly on a global scale. Yet, at least two main manufacturers are already boosting sticker prices: this August, Pfizer and Moderna raised prices to the European Union, despite $41 billion in net profits already accrued.[16]

Year two of the pandemic, emboldened by Delta and other variants of concern, offers us a second wakeup call. As businesses, schools and governments rush to do what they tiptoed around before—whether mask up or vaccinate—so, too, the world more squarely faces facts. We are all challenged to work together or perish, to substitute nationalism with internationalism, sanctions with collaboration.

In a sobering open letter to President Biden (in our new documents section, **Keynotes**, this issue), top Cuban scientists and vaccine developers put it this way: "during the pandemic, science reiterates that (politics aside) we are all in this together…the essential question, not only for Cuba and the US, but also for human civilization, is whether nations can respect each other enough to exist side-by-side and cooperate."[17]

Compiling this issue of ***MEDICC Review*** has been a Herculean effort, as Cuba and many in Latin America are in the throes of the worst COVID-19 wave to date. We are grateful to reviewers, authors, issue coordinators, translators and our whole team.

The Editors

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AT LEAST ONE ZOONOSIS SILENTLY SPREADS DURING COVID-19: BRUCELLOSIS

To the Editors:
Brucellosis dates back to when humans began herding goats and several reasons account for its re-emergence today. First is underestimation stemming from the erroneous notion that brucellosis was brought under control in the last century. Second is underestimation of the role that goats and goat products play in the chain of transmission to humans. And finally is the failure to consider biofilm phenotypes in research assessing *Brucella* spp.–ecosystem interactions. Thus, false results are derived, the illness's chronicity increases along with the corresponding failures in antibiotic therapies, and measures more apt to control the disease are simply not adopted.[1]

For the last 60 to 70 years in fact, many zoonotic diseases have simply been undervalued. Ironically, COVID-19 now plays a role in further sidelining them, as the challenges it poses to humanity rivet attention in one direction. However, this shouldn't keep us from preparing ourselves, aware that in the pandemic's shadow, old zoonotic threats, even some with similar symptoms, may be gaining ground and can even be lethal. A first step is to assess these in their true dimensions.[2] In the case of brucellosis, while 500,000 cases are reported globally, this is certainly an inexact figure.[1]

In this vein, it is important to consider *Brucella* spp. in its abiotic and biotic environments: as bacterial biofilms.[3,4] Although its planktonic forms cause the acute phase of disease, it is the biofilm phenotype that is expressed in the ever more frequent chronic cases. This phenotype is also responsible for antibiotic-resistant cases and other therapy failures associated with this zoonotic disease. Adopting such an understanding facilitates addressing the illness with alternatives aimed at inhibiting, destroying or at least minimizing biofilm formation.[1]

It is useful to note that 90% of the world's over one billion goats are concentrated in the poorest countries, adding another element of bias when assessing the global impact of this zoonotic disease and often of others, not to mention the associated lack of resources for diagnosis. Goats are small ruminants and excellent reservoirs of *B. melitensis*, the most virulent brucellosis strain in humans, a fact that should not be destined to the same fate of underestimation as has the impact of the disease itself.[5]

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Cuba's National Regulatory Authority & COVID-19: Olga Lidia Jacobo-Casanueva MS

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As of this writing, more than 4.6 million Cubans (over 40% of the population on the island), had received at least their first dose of Soberana 02 or Abdala, two of five vaccine candidates for SARS-CoV-2 developed and produced on the island. Late-phase clinical trial data revealed that Abdala is 92.28% effective after the full, three-dose cycle and Soberana 02 is 91.2% effective after two doses, when followed by a booster of Soberana Plus.[1] Cuban health authorities have committed to vaccinating the entire population, including children aged 3–18 years old, using these vaccines by the end of 2021. The first pre-clinical, peer-reviewed data are available,[2] with clinical trial results already submitted to various international journals.

Building on decades of biotechnology know-how developing, producing and administering 11 preventive vaccines for childhood diseases—used in the nation's universal health system and also marketed elsewhere—Cuba is the first, and to date only, country in Latin America and the Caribbean to develop its own vaccine candidates for COVID-19 (Soberana 01; Soberana 02; Soberana Plus; Abdala and Mambisa; see Box on following page). In a strategy designed to ensure comprehensive and importantly, independent solutions to the global health crisis, research institutes and manufacturing facilities coordinated by BioCubaFarma—the country's biopharmaceutical conglomerate—have also developed COVID-19 treatments and essential medical equipment.

To gain a better understanding of the regulatory process involved, ***MEDICC Review*** turned to Olga Lidia Jacobo-Casanueva, Director of the Center for State Control of Medicines and Medical Devices (CECMED), Cuba's national regulatory authority (NRA). A clinical microbiologist, Jacobo-Casanueva served as interim director throughout 2020 before becoming director in January 2021. She has spent nearly her entire career at CECMED, working her way up the ranks in a unique trajectory: from her first position in 1992 in the Center's microbiology laboratories, she has since worked in all but one of the six areas required by WHO to qualify as a National Regulatory Authority of Reference (NRAr; CECMED was certified as a Level 4 NRAr in 2011, a qualification it maintains). In short, Jacobo-Casanueva is a regulatory polymath, with hands-on experience in nearly every facet of regulation. She is also an adjunct researcher in the Faculty of Biology at the University of Havana.


E. Añé

Cuba's decision to confront the pandemic autonomously by developing preventive vaccines to control COVID-19 is deliberate and fraught with challenges. With dozens of ongoing clinical trials, coupled with the declining epidemiological and economic situation in Cuba—exacerbated by tightened US sanctions affecting all facets of COVID-19 prevention and response—we appreciate the time Jacobo-Casanueva took from her schedule to parse the complex regulatory mechanisms required to introduce Cuban and imported products into the national health system.

Editor's note: Just days after this interview was conducted in Havana, CECMED granted Emergency Use Authorization for Abdala, one of five Cuban COVID-19 vaccine candidates undergoing clinical trials since 2020.

MEDICC Review: **Can you synthesize CECMED's role as a national regulatory authority?**

Olga Lidia Jacobo: Any medicine, device or equipment—Cuban or imported—used in our health system must be approved by CECMED, the country's single regulatory authority. To ensure new products meet global standards of quality, safety and efficacy, WHO defines the six basic functions for NRAs as: medicine registration; lot-by-lot vaccine release; inspections; clinical trial authorization; laboratory access; and post-marketing surveillance. As a WHO Level 4 NRAr, CECMED undergoes regular, independent evaluations and inspections to certify compliance with standardized best practices throughout each aspect of these six functions.

By way of background: after Cuban scientists started producing new vaccines and other biotech products in the1990s, CECMED,

under the leadership of Dr Rafael Pérez-Cristiá, was restructured in 2000 to conform to international standards. My first job at CECMED was to organize the lot-release system for vaccines in the newly-established biologicals division. Once we designed the system, we began implementing it with Cuba's meningococcal BC vaccine (VA-MENGOC-BC) and the recombinant hepatitis B vaccine (Heberbiovac HB). We then set to work on re-organizing and improving other functions falling within CECMED's purview including inspections and medicine registration specific to vaccines. With the restructuring in place, we turned our sights to systematizing the remaining NRA functions.

Our researchers continued to innovate and develop novel vaccines and therapies—making CECMED's efforts to comply with international guidelines paramount. This was a priority leading up to WHO inspections and pre-qualification for the Genetic Engineering and Biotechnology Center's (CIGB) recombinant hepatitis B vaccine. Obtaining WHO pre-qualification for vaccines is an arduous, biennial process, designed to ensure the regulatory authority, production facilities and research laboratories are qualified to evaluate, register and control the entire manufacturing process.

MEDICC Review: **WHO pre-qualification for that Cuban vaccine ushered in an era of closer collaboration among Cuba, PAHO/WHO and countries in the region, correct?**

Olga Lidia Jacobo: That's right. CECMED and CIGB passed the inspections, the recombinant hepatitis B vaccine received pre-qualification and our work in the region around regulation intensified. Since then, our specialists have worked very closely with PAHO/WHO and other countries in the region to develop biological standardization guidelines, advise on technical documents and serve as experts within thematic work groups. The idea is that specialists from the eight countries with Level 4 NRAs in the Americas (Argentina, Brazil, Canada, Chile, Colombia, Cuba, Mexico and the United States) help develop guidelines and provide expertise for countries with less-developed regulatory authorities.

For example, CECMED has been part of the Pan American Network for Drug Regulatory Harmonization (PANDRH), since its formation in 1999. Within this network are groups of specialists organized by theme; I coordinated the vaccines working group for the Americas and we drafted the common technical document (CTD) establishing requirements for vaccine registration. This document, *Harmonized requirements for the licensing of vaccines in the Americas and Guidelines for preparation of application* (https://iris.paho.org/handle/10665.2/31215), was approved and now is a tool any regulatory authority in the region can use. Coordinating this was a wonderful experience allowing me to work with specialists from throughout the hemisphere.

Clinical Trials Timeline for Cuban COVID-19 Vaccine Candidates

Event	Date
2020	
CECMED authorizes parallel phase 1/2 clinical trials for Soberana 01	August 13
Soberana 01 phase 1/2 clinical trials begin in Havana	August 24
CECMED authorizes phase 1 clinical trials for Soberana 01A	October 17
CECMED authorizes phase 1 clinical trials for Soberana 02	October 27
Phase 1 Soberana 02 clinical trials begin (Havana; 40 volunteers)	November 2
CECMED authorizes parallel phase 1/2 clinical trials for Abdala	November 26
CECMED authorizes parallel phase 1/2 clinical trials for Mambisa	November 27
Phase 1 Mambisa clinical trials begin (Havana; 88 volunteers)	December 7
Phase 1 Abdala clinical trials begin (Santiago de Cuba; 132 volunteers)	December 7
CECMED authorizes phase 2 clinical trials for Soberana 02A	December 17
2021	
CECMED authorizes phase 1 clinical trials for Soberana 01B	January 5
Phase 2 Soberana 02 clinical trials begin (Havana; 810 volunteers)	January 18
Phase 2 Abdala clinical trials begin (Santiago de Cuba; 660 volunteers)	February 1
CECMED authorizes phase 3 clinical trials for Soberana 02	March 3
Phase 3 clinical trials begin (Havana; 44,010 volunteers)	March 4
CECMED authorizes phase 3 clinical trials for Abdala	March 18
CECMED authorizes health intervention study for Soberana 02	March 19
Soberana 02 health intervention study begins (Havana; 150,000 volunteers)	March 22
Phase 3 Abdala clinical trials begin (Santiago de Cuba, Granma, Guantánamo Provinces; 48,000 volunteers)	March 22
CECMED authorizes health intervention study for Abdala	March 27
Abdala health intervention study begins (Santiago de Cuba; Granma; Guantánamo Provinces; 120,000 volunteers)	March 29
CECMED authorizes phase 2 clinical trial for Soberana Plus in COVID-19 convalescent patients	April 9
Soberana public health intervention begins; Abdala public health intervention begins	May 12
CECMED authorizes Soberana Pediatría, parallel phase 1/2 trials for Soberana 02 in children	June 10
Soberana Pediatría phase 1/2 trials begin (Havana; 50 volunteers ages 3–18)	June 14
CECMED authorizes Ismaelillo, parallel phase 1/2 trials for Abdala in children	July 1
CECMED issues Emergency Use Authorization (EUA) for Abdala	July 9
CECMED authorizes parallel phase 1/2 clinical trials for Abdala and Mambisa combination vaccine in COVID-19 convalescent patients	July 9
Soberana Pediatría phase 2 trial begins (Havana; 300 volunteers ages 3–18)	July 14
Ismaelillo phase 1/2 trials begin (Camagüey; 592 volunteers ages 3–18)	July 15
CECMED authorizes Soberana Centro, parallel phase 2 non-inferiority clinical trials	July 23

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https://rpcec.sld.cu/

More recently, we consulted on the forthcoming document establishing a framework for evaluating national regulatory authorities globally. To be included in this framework requires an extraordinarily rigorous evaluation and inspection process, which CECMED is scheduled to undergo at the end of 2021. This is a complex endeavor, with module-by-module evaluations, but we have to buckle down and prepare so we can demonstrate the rigor of our regulatory authority, on par with others around the world.

MEDICC Review: **With the COVID-19 pandemic, vaccine regulatory mechanisms are being closely scrutinized—a topic covered extensively in previous issues of our journal. [3] Can you provide examples of how CECMED adapted and streamlined its process?**

Olga Lidia Jacobo: Normally a vaccine takes between five and ten years from development to licensing, but the urgency of the pandemic required regulatory authorities to adjust processes to make them more agile, without compromising science or regulatory best practices. In Cuba, we implemented a streamlined, collaborative process that has proven fundamental in shortening the clinical trial timeline while maintaining international standards. Under normal circumstances, a vaccine producer submitted to CECMED all necessary documentation, protocols, etc., to apply for clinical trial authorization. This was a very slow process because we would usually make recommendations to bring the design into line with international best practices or to otherwise improve the rigor of the trial. For this reason, we were often criticized for being too slow, but CECMED is the ultimate authority, with the highest level of responsibility—we are very meticulous and demanding as a result.

When COVID-19 was declared a global health crisis in early 2020, the Cuban Ministry of Public Health (MINSAP) convened the Innovation Committee, a multi-disciplinary work group including scientists, specialists, biopharmaceutical and technological experts and others. CECMED, in our capacity as independent regulatory agency, is also part of the Committee, though our role has not changed: we remain a non-partisan judge of clinical trial designs, ensuring their compliance with global regulatory norms.

The Innovation Committee strategy pivots on debating which products and devices should be prioritized and rather than wait for the manufacturer to submit their final trial design for authorization—which often would require changes and slow down the process—CECMED now makes recommendations earlier, limiting the back and forth and avoiding re-design of trials. We made other adjustments as well, to timeframes around pre-clinical trials and stability studies for instance, but also strengthening our surveillance system, especially around adverse events following immunization (AEFI). As an NRAr, these adaptations had to be made within a legal framework. As always, scientific rigor, proper documentation and a legal foundation drive our strategy. Since the beginning of the pandemic, CECMED has issued 13 regulatory provisions providing a legal basis to make these adjustments.

MEDICC Review: **Cuba's COVID-19 vaccine candidates were developed rapidly and completed several clinical trial phases in 2020 and 2021. How close is CECMED to issuing Emergency Use Authorization (EUA) for these vaccines?**

Olga Lidia Jacobo: We are right now evaluating the CTD and conducting inspections for the Abdala vaccine submitted for this purpose. This isn't just a question of looking over some paperwork. We have to evaluate all documentation related to the vaccine, including pre-clinical data, toxicology and animal studies, plus clinical trial results including safety and immunogenicity data, statistical analysis and more. Analyzing vaccine quality is also part of CECMED's work in terms of evaluating the entire production process for consistency, lot production, best practices compliance within the manufacturing facility and validity of the technical analyses.

Our role also includes conducting inspections throughout every phase of trials to ensure compliance with protocols and best practices. This means we visit every vaccination site including hospitals, community polyclinics and family doctor-and-nurse offices where vaccinations are administered. We also verify that the cold chain is properly maintained—if vaccines are distributed at 5:00 AM, CECMED specialists are there before then, and also at the vaccination sites to measure the temperature upon arrival to ensure there has been no break in the cold chain. We also inspect the transportation mechanisms and protocols of the Medicines Marketing and Distribution Company (EMCOMED) responsible for delivering the vaccines.

MEDICC Review: **How is it possible that Cuba vaccinated over 2 million people without having first obtained EUA for either of its two vaccines in phase 3 clinical trials?**

Olga Lidia Jacobo: The fact that Cuba launched intervention studies in high-risk groups (health and BioCubaFarma workers, followed by a public health intervention in the hardest-hit Havana municipalities that were the epicenter of the pandemic at that time) before receiving EUA has been a topic of huge debate. It's important to note that CECMED, along with BioCubaFarma, which represents the pharmaceutical industry, are independent entities, but also sit on the Innovation Committee that made that decision. It wasn't made in a vacuum, but rather using the data we had at hand. And we had a lot of data at that point—from completed phase 1 and 2 trials and ongoing phase 3 trials, after many doses had already been administered with only mild adverse events. So we had data demonstrating the safety and immunogenicity of Soberana 02 and Abdala at this point. [The public health intervention was initiated after over 145,000 Cubans had received their full vaccinations using one of these two candidates in clinical trials and the intervention study, Eds].

At that moment, Cuba was facing a very complex epidemiologic scenario, with variants of concern circulating in the country and high rates of autochthonous transmission. So the Innovation Committee convened, with CECMED presenting the vaccine data to date, epidemiologists presenting transmission data and virologists from the Pedro Kourí Tropical Medicine Institute (IPK) providing the latest information on variants of concern. Although these vaccines still had not received EUA, a cost-benefit analysis concluded that the benefits outweighed the risks of a public health intervention, plus we had safety, immunogenicity and partial efficacy results.

Let me also point out that while phase 3 clinical trials were not yet complete, trial protocols for these vaccines allowed for precisely this scenario. Used by vaccine producers around the world and known as a data cutoff date, it permits specialists to analyze vaccine efficacy using data at a defined point in the trial. The data cutoff date for both Soberana 02 and Abdala was 15 days after receiving two doses of the former and three doses of the latter (Abdala has a shorter vaccination schedule than Soberana 02—every 14 days versus every 28).

MEDICC Review: **What role does WHO play in vaccine approval? And other regulatory authorities in countries seeking access to Cuban vaccines?**

Olga Lidia Jacobo: WHO is an international organization that makes recommendations and supports national regulatory authorities but it does not approve vaccines; it isn't a regulatory authority itself. Once COVID-19 was declared a Public Health Emergency of International Concern (PHEIC), WHO began implementing streamlined mechanisms for faster delivery of vaccines to those countries needing them. One piece of this is a more agile authorization tool for vaccines known as Emergency Use Listing (EUL). WHO-recommended vaccines including Pfizer, Moderna and others, were authorized under this EUL category, following approval by the Federal Drug Administration (FDA) or the national regulatory agency of the country producing the vaccine. The process for Cuban vaccines is the same.

WHO Emergency Use Listing (EUL) is a procedure for assessing unlicensed vaccines, therapeutics and in vitro diagnostics during public health emergencies with the ultimate goal of expediting the availability of these products to people who need them. When products are not licensed yet (still in development), WHO will assess the quality, safety and efficacy (or performance) data generated during development and conduct a risk-benefit assessment to decide if they can be used outside clinical trials.

Source: WHO. Coronavirus disease (COVID-19): Use of Emergency Use Listing procedure for vaccines against COVID-19, 30 Sep 2020. https://www.who.int/news-room/q-a-detail/coronavirus-disease-use-of-emergency-use-listing-procedure-forvaccines-against-covid-19

Soberana 02 and Abdala have not yet received our own EUA, required for EUL. Our regulatory process is based on reviewing all the technical documentation for these vaccines, plus inspections of production facilities, fundamental for certifying best practices throughout the process. National regulatory authorities interested in introducing Cuban vaccines into their health systems—Mexico, Argentina and other countries beyond Latin America have signed collaboration agreements with Cuba—will apply their regulatory process to our vaccines before they are administered. CECMED has been working closely with PAHO to foster cooperation and transparency among national regulatory authorities and promote Good Regulatory Practices (GRP) and Good Reliance Practices (GReP) in the region so that when a vaccine receives EUA, the approval process can proceed quickly and confidently by individual NRAs in countries wishing to administer it.

MEDICC Review: **Will CECMED have a lighter work load once the EUA is issued?**

Olga Lidia Jacobo: Not at all. There's the entire vaccine licensure process that is usually quite long but will probably happen fairly quickly since these vaccines have amassed so much data already. And two areas of regulation are ongoing: vaccine lot release (every lot of vaccine produced must be analyzed and authorized by CECMED specialists) and post-marketing surveillance, which is critically important.

CECMED is also responsible for evaluating and authorizing the use of any medical device or equipment used in the national health system—like ventilators and SARS-CoV-2 test kits, for example. We work closely with various BioCubaFarma companies that manufacture medical equipment and once they started developing ventilators, we assumed our role as regulatory authority; after the corresponding pre-clinical and clinical trials, one of these ventilators was approved for emergency use, according to clinical protocols, in patients meeting specific criteria. All imported medicines, meanwhile, plus medical devices and equipment, are subject to CECMED oversight and authorization, as well. In short, anything entering the country for use in the health system—whether purchased or donated—has to be licensed and authorized by CECMED to ensure proper control and surveillance.

And then there are all the other ongoing clinical trials—everything from drug repositioning and treatments to COVID-19 pediatric vaccines.

MEDICC Review: **You mention drug repurposing or repositioning, which has been a strategy employed widely in the search for COVID-19 treatments. Is Cuba pursuing this? What is CECMED's role in this scenario?**

> **The severity of the pandemic—with so many people falling gravely ill and dying—prompted us to think about drug repositioning early on**

Olga Lidia Jacobo: The severity of the pandemic—with so many people falling gravely ill and dying—prompted us to think about drug repositioning early on. Several likely candidates for repositioning were presented to the Innovation Committee, activating CECMED's role as regulatory authority. This is how Jusvinza and Itolizumab entered into Cuban treatment protocols.

Jusvinza (produced by CIGB), is a peptide that while still not registered, has undergone clinical trials for treating rheumatoid arthritis. Specialists hypothesized that the effect this peptide has on the cytokine storm and hyperinflammation might be useful in treating seriously ill COVID-19 patients. They presented data from the rheumatoid arthritis trials and argued for compassionate use authorization—also known as expanded access—of Jusvinza for certain patients. This type of authorization falls within CECMED's legal framework and is used around the world for patients with life-threatening conditions when no other therapeutic options are available. Itolizumab (produced by the Molecular Immunology Center, CIM), a monoclonal antibody, also acts to mitigate hyperinflammation and was developed to treat severe psoriasis and cutaneous

T-cell lymphoma. This is another Cuban product that received expanded-access authorization for use in our health system; it is in clinical trials overseas specifically for treating COVID-19, and more data will be available soon.

Another Cuban product, Biomodulina T (produced by the National Biopreparations Center, BioCen), is an immunomodulator that supports immune system response; it is registered and approved for use in our health system for patients with recurrent respiratory infections. Since it underwent clinical trials, is legally registered and boosts the immune system, some had hoped we could just start treating COVID-19 patients with Biomodulina T. But regulation doesn't work that way. To treat a different condition—in this case COVID-19—it must go through the entire authorization process as if it were a new drug, with separate clinical trials designed for treating this disease and official, unique registration in the Cuban Public Registry of Clinical Trials.

MEDICC Review: **There has been a troubling surge in pediatric COVID-19 cases globally, but also in Cuba. You mentioned clinical trials specifically for children using Cuban vaccines…**

Olga Lidia Jacobo: First let me say that we are even more demanding when it comes to pediatric vaccine trials: the immune system of a child is not the same as an adult, so we always perform clinical trials in the adult population first. COVID-19 vaccines are no different. The pediatric clinical trial administers Soberana 02 (2 doses, 0–28 days) followed by a Soberana Plus booster shot on day 56. A full description of this *Soberana Pediatría* clinical trial is available at: https://rpcec.sld.cu/en/trials/RPCEC00000374-En

Pediatric clinical trials require even more rigor due to the ethical considerations involved

Pediatric clinical trials require even more rigor due to the ethical considerations involved. Understanding what the trial entails is part of it, but providing written, informed consent is indispensable as well. In the case of children under 12, parents or legal guardians sign the informed consent, but children and teens pre-selected for the trial have the right to say 'no, I don't want to participate.' Obviously, we all want our children to be vaccinated, so they can begin their school year and resume normal lives, but parents cannot obligate them to participate in the trial.

CECMED approved parallel phase 1/2 trials, now underway, at the Juan Manuel Márquez Pediatric Teaching Hospital in Havana. This hospital has extensive experience conducting clinical trials, including the requisite physical infrastructure, trained personnel and specialists, and adherence to clinical best practices. The first group of 25 youngsters, aged 12–18 years, received their first dose several weeks ago and were monitored for adverse effects for one week after vaccination. The safety report submitted to CECMED after this 7-day observation period indicated local injection-site reactions as the only adverse effects. With this data confirming the vaccine's safety (already demonstrated in phase 1, 2 and partial 3 clinical trials in adults), the second group of 25 children, 3–11 years old, was authorized to receive their vaccinations. To date, this clinical trial has proceeded smoothly. The other vaccine, Abdala, will also enter pediatric clinical trials this year. We are vaccinating our adult population quickly, but they can be carriers of SARS-CoV-2 and infect their children at home, which is why we are emphatic about pediatric trials and vaccination.

MEDICC Review: **Many developing countries are inspired by Cuba's capacity to produce and authorize a COVID-19 vaccine. But isn't it draining scarce resources?**

Olga Lidia Jacobo: Controlling this pandemic has been the highest priority for our country—not only public health authorities, but for our President as well. Obviously, many resources are required to properly conduct clinical trials: syringes, PCR kits, laboratory analysis to measure immunogenicity, reagents for neutralization assays and more. Plus, there's the cost of equipping and staffing all the vaccination sites. At this moment, we are facing a scarcity of certain medicines in Cuba, it's true, but we have those needed to treat COVID-patients.

MEDICC Review: **US sanctions and their extraterritorial applications, strengthened under the previous US administration and still in place, certainly don't help…**

Olga Lidia Jacobo: Where the US policy affects CECMED's work the most is in our laboratories. Materials, especially reagents, are very difficult to procure. The same holds true for any equipment that breaks: finding replacement parts or buying new equipment becomes especially problematic under the US blockade.

This policy affects every aspect of life in our country—it's not only limited to the inputs and equipment needed in labs. One indispensable part of our work involves printing reports and we've been dangerously low on paper and toner since last year. This gives you an idea of the comprehensiveness of this policy—something as simple as paper is hard for Cuba to purchase on the international market. Building maintenance is another area affected, as well as replacing broken or obsolete computers, which is not only difficult, but costly.

MEDICC Review: **It's hard to conceive working under these conditions, with such responsibility, in the midst of a global pandemic. What keeps you going?**

Olga Lidia Jacobo: It's a huge challenge, but we have a really good team at CECMED, in terms of responsibility and dedication to the work we're doing. Working together day after day has forged an environment of close collaboration where people are willing to go the extra mile. CECMED also has a solid, stable workforce—sure some people come and go, but on the whole, our staff is committed long-term and that has been an important asset and strength. We wish we could do more, much more actually, but we face certain limitations.

I couldn't do my job without the support of my family. It just would not be possible. I love how we've organized our household so that everyone has their individual duties and responsibilities—my husband does the shopping, my daughter cleans the house, I do the laundry and my son and daughter-in-law are in charge of the kitchen—which means I, thankfully, don't have to cook. Family too, is an ever-present strength in this fight against the pandemic.

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COVID-19: Comparison of the Response in Rwanda, South Africa and Zimbabwe

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ABSTRACT

The COVID-19 pandemic has had an impact worldwide with regions experiencing varying degrees of severity. African countries have mounted different response strategies eliciting varied outcomes. Here, we compare these response strategies in Rwanda, South Africa and Zimbabwe and discuss lessons that could be shared. In particular, Rwanda has a robust and coordinated national health system that has effectively contained the epidemic. South Africa has considerable testing capacity, which has been used productively in a national response largely funded by local resources but affected negatively by corruption. Zimbabwe has an effective point-of-entry approach that utilizes an innovative strategic information system. All three countries would benefit having routine meetings to share experiences and lessons learned during the COVD-19 pandemic.

KEYWORDS COVID-19, SARS-CoV-2, epidemics, pandemic, Africa, Zimbabwe, Rwanda, South Africa

INTRODUCTION

COVID-19 emerged in China in late 2019 and has spread around the globe, infecting nearly 200 million and leading to more than 3 million deaths. To date, SARS-CoV-2, the virus that causes COVID-19, has spread to almost every region of the world.[1,2] Affected countries have mounted different response strategies with the overall goal of minimizing morbidity and mortality and associated socioeconomic impacts.[3] Drawing experience from the 2014 Ebola virus disease crisis in West Africa, African leaders are keenly aware that failure to contain COVID-19 would threaten health, prosperity and security.[4]

In this manuscript, we compare COVID-19 response strategies in Rwanda, South Africa and Zimbabwe. We specifically focused on these three countries as the authors are involved in the COVID-19 response in these countries and therefore would have insights sufficient for detailed comparisons. All figures in this study, including those in Table 1, correspond to February 25, 2021, when this paper was drafted. The Rwandan COVID-19 pandemic has had over 18,500 positive cases and more than 250 deaths. South Africa has had the worst COVID-19 outbreak among the three countries with 1.5 million confirmed cases and over 49,600 deaths. As of the same date, Zimbabwe had recorded over 35,900 confirmed-positive cases with over 1450 deaths.[5]

IMPORTANCE Comparing COVID-19 responses between countries is useful as countries are learning in real time from practicing in adaptive systems; a necessity given the nature of the pandemic. This paper compares the diversity of responses to the pandemic in different African countries. This contributes to ongoing efforts to understand and adapt to the demands of the COVID-19 pandemic through shared experiences.

DEVELOPMENT

For this study, we conducted a literature review of COVID-19 response strategies across the three countries. We searched for articles published in English on: WHO's website; peer-reviewed articles on Google Scholar and PubMed; official public health websites operated by the respective governments of each country; and newspaper articles written and published within each country. We used the following keywords: COVID-19; response; Africa; Rwanda; South Africa; Zimbabwe; and other subject specific terms such as surveillance; infection prevention and control; policy. We used the Boolean operators AND and OR to separate the keywords. For instance, the search strategy used in PubMed was ("COVID-19"[All Fields] OR "COVID-19"[MeSH Terms] OR "COVID-19 Nucleic Acid Testing"[All Fields] OR "covid-19 nucleic acid testing"[MeSH Terms] OR "COVID-19 Serological Testing"[All Fields] OR "covid-19 serological testing"[MeSH Terms] OR "COVID-19 Testing"[All Fields] OR "covid-19 testing"[MeSH Terms] OR "Severe Acute Respiratory Syndrome Coronavirus 2"[All Fields] OR "coronavirus"[All Fields] OR response[All Fields] AND ("africa"[MeSH Terms] OR "africa"[All Fields]) OR ("rwanda"[MeSH Terms] OR "rwanda"[All Fields]) OR ("south africa"[MeSH Terms] OR ("south"[All Fields] AND "africa"[All Fields]) OR "south africa"[All Fields]) OR ("zimbabwe"[MeSH Terms] OR "zimbabwe"[All Fields]) OR ("epidemiology"[Subheading] OR "epidemiology"[All Fields] OR "surveillance"[All Fields] OR "epidemiology"[MeSH Terms] OR "surveillance"[All Fields]) AND ("infections"[MeSH Terms] OR "infections"[All Fields] OR "infection"[All Fields]) AND ("prevention and control"[Subheading] OR ("prevention"[All Fields] AND "control"[All Fields]) OR "prevention and control"[All Fields]) AND ("policy"[MeSH Terms] OR "policy"[All Fields]) Consistent with standard literature review methodology, some steps, such appraising evidence quality (a standard step in systematic reviews) were omitted.

To allow for a well-rounded comparison, the information gathered was structured and is presented according to ten pre-established comparison domains:

- Coordination, planning and monitoring
- Policy framework
- Risk communication and community engagement
- Surveillance, rapid response teams and case investigation
- Infection prevention and control
- Case management and continuity of essential services
- National laboratory system
- Role of private sector in the national response
- Points of entry
- COVID-19 logistics, supply and procurement implementation/ operational plan.

These comparison domains were adopted from the monitoring and evaluation framework for the COVID-19 response in WHO's

African Region.[6] Using this framework, we discuss key findings in each country's response based on the ten established domains of comparison. We also present key background information and COVID-19 related statistics for each country to provide context (Table 1).

Coordination, planning and monitoring Governments of these countries have made COVID-19 responses a national priority with each one instituting a variety of measures aimed at curbing the virus's spread. The Rwandan government quickly formed a Joint Task Force to plan, coordinate and monitor the response to the COVID-19 epidemic.[10] In fact, the Task Force was formed before the country recorded its first confirmed case. This organization was comprised of various stakeholders in the Ministry of Health (MOH) and chaired by the Prime Minister. In South Africa, literature revealed evidence of coordinating bodies. An inter-ministerial organization comprised of the Ministry of Health, Department of Defense, Ministry of Public Works, Ministry of Justice and Correctional Services, and Ministry of Basic and Higher Education, among other line ministries, was set up to coordinate COVID-19 response with assistance from an advisory board composed of medical experts.[11] As is the case with Rwanda, this organization was formed before any known cases were reported in the country.

Similarly, Zimbabwe prepared a National Preparedness and Response Plan tailored to minimizing COVID-19 morbidity and mortality and mitigating the pandemic's socioeconomic consequences.[12] It included prevention, containment and mitigation strategies for different COVID-19 transmission scenarios. Given the vast number of health-related (and non-health–related) actors and stakeholders who would be potentially involved in addressing the outbreak, it was deemed important that they all work under one framework with clearly articulated roles and responsibilities. This was designed to ensure efficient allocation of scarce resources as well as alignment by all stakeholders in the overall strategic direction of the response. Two levels of coordination were set up to ensure a robust pandemic response: first is the Inter-Ministerial Task Force (chaired by one of the vice presidents and responsible for overall response coordination). The national public health emergency management mechanisms work with all relevant ministries including education, travel and tourism, public works, environment, social protection, agriculture, trade, and industry and finance to provide coordinated management of COVID-19 preparedness and response. Implementation of the plan was rolled out to provinces throughout the country. The second level Health Sector Coordination is for activities both within the Ministry of Health itself and within the broader health sector (which includes local government, the private sector, nonprofits and other related stakeholders).

For this domain, all three countries demonstrated strong government involvement and willingness to set up institutions to lead the response. South Africa and Zimbabwe have involved more line ministries in the national coordination taskforce to improve inter-ministerial cooperation and streamline the delegation of responsibilities.

Policy framework Within a few weeks of its formation, the Rwandan Joint Task Force for COVID-19 put forward a national policy on COVID-19 prevention within communities, places prone to large gatherings of people, markets, and other crowded places such as bus stations.[13] Clinical tools and guidelines were quickly developed and shared to help heath care providers manage testing, and also offer instructions as to how to access services after exposure.[13] By March 2020, various containment and mitigation measures had been put in place.[14] These included lockdowns, restricted movement between Kigali and other provinces, reducing the number of traders in markets and closing markets with high rates of transmission, closing schools and churches, and isolating regions with transmission clusters in lockdown.

A similar response was observed in South Africa. Building on the declaration of COVID-19 as a national disaster by the Minister of Cooperative Governance and Traditional Affairs, a national policy including various clinical tools and guidelines were put in place in South Africa.[15]

At the time this article was written, our search could not find a COVID-19 policy on the Zimbabwean Ministry of Health and Child Care website. However, the Ministry of Health and Child Care had published a COVID 19 National Preparedness and Response Plan.[3] The plan includes prevention, containment and mitigation strategies for different COVID-19 transmission scenarios.[16]

The existence of a COVID-19 national policy that covers the major response areas in public domain or on the government websites is critical for guiding national responses. In this context, Rwanda moved with respective alacrity, establishing policies that allowed for rapid introduction of strict prevention and control interventions.

Risk communication and community engagement Our literature search showed that all

Table 1: Key statistics on Rwanda, South Africa and Zimbabwe

		South Africa	Zimbabwe	Rwanda
Population[7]		59,802,408	15,005,632	13,162,804
GDP/Capita in 2019 (thousands of US$)[7]		6,001.4	1,464.0	820.0
Urbanization[7] (%)		70	34	56
Demographic structure (age group in years)[7]	0–14 (%)	29.2	41.6	41.1
	15–24 (%)	19.3	20.9	19.3
	25–64 (%)	46.4	34.5	36.9
	≥65 (%)	5.0	3.0	2.8
COVID-19				
Tests /1 million population[8]		151,791	22,565	76,659
Deaths /1 million population[8]		836	97	20
Case fatality rate (%)[8]		3.3	4.2	1.4
Time from first confirmed case to lockdown initiation (days)[8]		22	4	7
Vaccination start date (in 2021)[9]		16 February	18 February	14 February
Cumulative COVID-19 vaccine doses given as of 28 February 2021[9]		>70,000	>18,000	Data not publicly available at the time of writing

Unless otherwise stated, figures correspond to information from sources obtained as of February 25, 2021.

three countries have employed the use of SMS text alerts, videos, infographics and posters to alert the public to the dangers of COVID-19. Public and private radio and television were also used to disseminate information. A compendium of key messages has been developed and these guide other partners involved in the COVID-19 response in the development of information, education and communication activities. There were no clear differences among the three countries in risk communication and community engagement strategies.

Surveillance, rapid response teams and case investigation All three countries have set up systems actively involved in case detection, quarantine and isolation. In South Africa, community health workers conduct house-to-house screening and testing, especially in vulnerable communities. In Rwanda and Zimbabwe, rapid response teams investigate suspected cases and support the surveillance task force at subnational levels through data reporting, capacity building and supportive visits.[3] However, in Zimbabwe, there were reports of rapid response teams in the first wave (July–August 2020),[17] but at the time of this writing there was no literature available which would reveal whether the number of these increased during Zimbabwe's COVID-19 resurgence from mid-December 2020 through the end of January 2021.

Infection prevention and control A major component of the COVID-19 response has been infection prevention and control (IPC). All three countries have implemented COVID-19 IPC response plans, albeit with logistical and personnel challenges.[17] In Rwanda and South Africa, various mitigation measures were put in place; including limiting visits to healthcare facilities, screening all patients for COVID-19 symptoms and patient triage. Healthcare facility staff receive routine training on COVID-19 risk reduction.[18] Similarly, in Zimbabwe, IPC reference materials for reducing COVID-19 exposure risks were developed rapidly and distributed in health facilities and within communities in an effort to better capacitate healthcare workers (HCWs) to provide safe community environments. The literature in this review revealed that the Zimbabwean IPC response was affected by staffing shortages, lack of motivation among HCWs and personal protective equipment (PPE) shortages.[17] In this regard, Rwanda and South Africa had more engaged volunteer health workers[19] and reassigned HCWs who had switched to employment outside the health sector.

Case management and continuity of essential services All three countries have active case management systems functioning at varying degrees of efficiency. Their objectives are to provide timely high-quality care for COVID-19 patients; to ensure adequate capacity for managing COVID-19 cases during all phases of the pandemic, including during case surges; and to ensure routine essential health service delivery continuity and utilization during all phases of the pandemic and beyond.

In Rwanda, clinical management guidelines were continuously updated to reflect new guidance from WHO. South Africa utilized various action plans recommended by WHO, among them mapping vulnerable populations, as well as public and private health facilities and workforces, thereby identifying alternative facilities for treatment. Similarly, Zimbabwe released guidelines for the clinical management of COVID-19 that covered testing, case management, antiviral treatment and patient discharge. While there was evidence on the existence of such guidelines in all three countries, our literature search did not reveal evidence on the quality or efficiency of case management.

Essential services in Rwanda remained functional, if depleted. However, movement restrictions and bans on public transportation impeded access to non-COVID healthcare services. For instance, one study reported that less than half of HIV–positive patients attended their antiretroviral collection clinic appointments during the first lockdown period in March–April 2020.[20] In South Africa, the healthcare facilities were continuously assessed to ensure continued capacity in delivering primary and other essential services. Furthermore, the private for-profit healthcare system in South Africa in general is very active in care management; mainly attending to patients with medical insurance.

In Zimbabwe, although essential services remained open during the lockdown period, movement restrictions and fear of contracting COVID-19 at healthcare facilities affected utilization of such services for other public health threats.[21] A report by the Zimbabwean Ministry of Health and Child Care revealed that during the period of April–June 2020 there was a 59% reduction in the number of clients tested for HIV who received their results; 15% reduction in the distribution of HIV self-test kits; 99% reduction in voluntary medical male circumcisions performed; a 49% reduction in patients tested for syphilis; 46% reduction in pregnant women booking for first prenatal appointment; 51% reduction in newly diagnosed HIV patients initiated on antiretroviral therapy and a 29% decline in viral load sample collection in Zimbabwe. One observation is that Rwanda and Zimbabwe could benefit from scaling up telehealth utilization, as reportedly used in South Africa,[22] to support essential services' continuation during the pandemic.

National laboratory system COVID-19 tests in Rwanda were initially performed at the National Reference Laboratory (NRL) before a new testing strategy was introduced to decentralize capacity through peripheral district laboratories. The Rwandan laboratory system started off (in March 2020) with the capacity to test close to 1000 samples per day but in 4 months (by July 2020), the testing capacity increased 15-fold with a shift from manual RNA extraction to an automated system providing results more quickly (pooling system).[11,23,24] This was due in part to the introduction of a mobile laboratory unit in May 2020 that doubled the country's COVID-19 testing capacity, its mobility facilitating mass testing throughout the country.

The South African COVID-19 response laboratory is led by the National Institute of Communicable Diseases (NICD). Rapid testing expansion was enabled by a large network of private and National Health Laboratory Services (NHLS) laboratories. As with Rwanda, South Africa has employed mobile laboratories to expand testing.

Zimbabwe has a national-level laboratory system whose objectives include capacitating laboratories to perform molecular diagnosis using real-time reverse transcription polymerase chain reaction (RT-PCR) with demonstrated quality and biosafety; ensuring adequate supplies of test kits and reagents; increasing access to testing at provincial level using GeneXpert (Cepheid); strengthening COVID-19 testing support systems including data collection and analysis, waste and sample management; and establishing and strengthening COVID-19 testing quality

assurance systems. Major impediments to this system are inadequate resources, specifically the lack of test kits and an ineffective sample transportation system to the few available testing centers.

The success of the Rwandan laboratory system has been attributed to population and governmental goodwill, research-based actions, optimized use of available human resources, and the use of limited resource funding models to support the established public laboratory and health system governance structures.[25] In this regard, there was very little available literature on how Zimbabwe approached scaling up research-based testing. The South African COVID-19 laboratory response relies on sufficient resources, now available at the national level, and includes routine genomic typing of the COVID-19 variants as part of surveillance.

Role of private sector in national response The COVID-19 response in all three countries has seen governments working closely with stakeholders from across the private sector, civil society, academia, professional associations, the private nonprofit sector, community-based organizations and international organizations. In all three countries, for example, a key role for the private for-profit healthcare systems has been in the provision of COVID-19–related treatment and care;[26] PPE for medical staff in under-resourced hospitals; and rapid test kits, hand sanitizers and food hampers to vulnerable communities.[27,28] There was no literature in our review showing any marked differences in private sector engagement in COVID-19 responses in all three countries.

Points of Entry All three countries are in compliance with WHO International Health Regulations (IHR).[29] The purpose of the IHR is to "prevent, protect against, control and provide a public health response to the international spread of disease in ways that are commensurate with and restricted to public health risks, and which avoid unnecessary interference with international traffic and trade." In Rwanda, a negative COVID-19 PCR test is required at any of the entrance points. A repeat test is conducted upon arrival while travelers are in a mandatory 24-hour quarantine.

South Africa and Zimbabwe require a negative COVID-19 PCR test taken 72 and 48 hours, respectively, prior to arrival. No retesting is conducted at the airport. The Zimbabwean point-of-entry approach leverages a mature strategic information (SI) system. (The Zimbabwean port-of-entry screening system has screened over 120,000 individuals over the 6 months from June through November, 2020). The festive season period saw the Zimbabwe–South Africa Beitbridge border post experiencing a huge influx of people crossing the border. There were reports of a high number of fake COVID-19 clearance certificates by travelers from Zimbabwe resulting in South African authorities resorting to testing every traveler passing through the border post before allowing them to enter the country.[30] Another threat faced by both Zimbabwean and South African COVID-19 responses are people entering both countries using undesignated entry points. The Rwandan strategy of repeat testing could help South Africa and Zimbabwe address the threat of the fake COVID-19 certificates.

COVID-19 logistics, supply and procurement implementation/operational plan We found very little literature on COVID-19 logistics, supply and procurement implementation in the three countries. The available evidence suggests that all three countries have set up systems to map available resources and supply systems in their healthcare sectors. However, just like other African countries, Rwanda, South Africa and Zimbabwe have been affected by shortages of diagnostic kits due to disruptions in the global supply chain.[31] Furthermore, reports of COVID-19 procurement-related corruption (concerning contracts for products and services related to COVID-19) have hampered the response in South Africa[32,33] and Zimbabwe.[34,35]

The COVID-19 response has exacerbated the need for South Africa and Zimbabwe to establish measures to curb corruption within their governments. In this regard, the Rwandan model could serve as an exemplar. The Rwandan government formulates and implements anti-corruption efforts mainly via homegrown initiatives, minimizing corruption by eradicating opportunities for misconduct, focusing on governance reforms and maintaining a zero-tolerance policy towards corruption.[36] Political will, strong leadership, the active role played by the anti-corruption agency and effective governmental reforms have made Rwanda's anti-corruption activities largely successful in the context of the pandemic.[36]

DISCUSSION

COVID-19 burdens in these countries vary, with South Africa experiencing the worst epidemic of the three. The ten comparison domains discussed above influence the burden of COVID-19 in each of the countries, albeit there are concerns on the reliability of reported data due to the poor surveillance systems in place in Africa.

In general, countries with strong, coordinated government responses have experienced far less severe COVID-19 epidemics than countries with more *ad hoc* or *laissez faire* approaches. While most African countries have under-resourced health systems, many of them also have very robust public health systems, an important asset in disease mitigation and containment during a pandemic.

Our findings revealed some critical response areas where the three countries could learn from each other. For instance, Rwandan response could learn from South Africa and Zimbabwe on inter-ministerial coordination and involve more line ministries in the national coordination taskforce to improve inter-ministerial cooperation and streamline delegation of responsibility. Regarding framing and implementing policies, South Africa and Zimbabwe could learn from Rwanda to improve their speeds in implementing and establishing COVID-19 policies and making them available in the public domain. The existence of a COVID-19 national policy that covers major response areas in the public domain or on government websites is critical to guiding the response in any country.

Zimbabwe could learn from Rwanda and South Africa in devising innovative ways to improve the health worker staff complement as these are critical frontline workers in the pandemic response. Rwanda and Zimbabwe could learn from South Africa's rapid expansion of telehealth services to ensure the continuation of health services during the lockdown period. Finally, South Africa and Zimbabwe could learn from Rwanda's response to corruption, which has hampered their two countries' supply chains and logistics. In Rwanda's case, political will and strong leadership, the active role played by the anti-corruption agency, and effective governance reform have prevented mismanagement of COVID-19 resources or procurement processes.

Between 2015 and 2017, the doctor–inhabitant ratio improved in Rwanda, from 1:15,428 to 1:8,592, while the nurse–inhabitant ratio improved from 1:1200 to 1:1070.[37] Rwanda is among the few countries in Africa to have achieved universal health coverage based on a vision of inclusiveness, equity, and comprehensive and integrated services, with a focus on primary health care (PHC).[37] Not surprisingly, Rwanda has been ranked first in Africa and sixth globally in managing the COVID-19 pandemic and making information about the pandemic accessible to the public.[38]

According to the World Bank, Rwanda has 0.1, South Africa 0.9, and Zimbabwe 0.2 physicians per 1000 population.[39] The same source reports that Rwanda has 1.2, South Africa 1.3, and Zimbabwe 1.9 nurses or midwives per 1000 population.[38] Regarding COVID-19 deaths per 1 million population, Rwanda has 14 (ranked 34th in Africa), South Africa 730 (ranked 1st in Africa) and Zimbabwe 77 (ranked 13th in Africa).[40] Interestingly, South Africa, with the highest proportion of physicians, also shows the highest proportion of Coronavirus deaths per 1 million. It is also worth noting that South Africa has become the first country in Africa to receive a shipment of COVID-19 vaccines.

CONCLUSIONS

Frequently, analyses about Africa are based on viewpoints formulated outside the continent. We have intentionally avoided this approach. Our perspective is based on a narrative literature review, consisting mainly of documents elaborated by African policymakers. Nevertheless, it has some limitations. Firstly, as an analysis based on a literature review, steps in systematic evidence synthesis were omitted. These include quality assessment of the findings. Second, our search was limited in scope and depth. For instance, we did not screen references in the reviewed papers. However, findings in the present study still offer important insights as to similarities and differences in COVID-19 response strategies across three countries in Africa that have experienced varying impacts from the pandemic.

The findings allow for each country's COVID-19 response leaders to learn from the others and may also serve as a guide for similar settings with limited resources on the best practices for curbing the pandemic's spread.

For example, Rwanda could learn from South Africa on strategies to ensure continuation of essential services during lockdown. South Africa and Zimbabwe could learn from Rwanda's response to corruption, a factor that has hampered the two countries' supply chains and logistics. Zimbabwe could learn from Rwanda and South Africa in devising innovative ways for strategic health worker deployment. All three countries can benefit from exchanging lessons they have been learning during the pandemic and by establishing routine meetings to share their experiences.

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Racotumomab in Non-Small Cell Lung Cancer as Maintenance and Second-Line Treatment

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ABSTRACT

INTRODUCTION Racotumomab is a therapeutic vaccine based on a monoclonal anti-idiotypic antibody developed by the Molecular Immunology Center in Havana, Cuba, that is registered in Cuba and Argentina for treatment of non-small cell lung cancer. It induces a specific humoral and cellular immune response against the N-glycolyl GM3 (NeuGcGM3) ganglioside present in tumor cells, thereby provoking the death of these cells.

OBJECTIVE Evaluate racotumomab vaccine use as switch maintenance and second-line therapy for patients with inoperable non-small cell lung cancer in routine clinical practice, outside the framework of clinical studies, and assess the overall survival, stage-specific survival and safety in these patients.

METHODS A descriptive, retrospective study was carried out in patients diagnosed with non-small cell lung cancer not suitable for surgical treatment, who received racotumomab as a part of switch maintenance or second-line treatments. Overall survival was defined from diagnosis and from the first immunization, until death.

RESULTS We included 71 patients treated with racotumomab, 57.7% (41/71) of whom were in stages IIIB and IV of non-small cell lung cancer. Of the patients, 84.5% (60/71) had no adverse events, and 15.5% (11/71) had mild adverse reactions. The median overall survival was 24.5 months, calculated from the first immunization, 17.2 months for those who received racotumomab as switch maintenance and 6.8 months for patients who had progressed after the first line of treatment.

CONCLUSIONS Racotumomab in routine clinical practice prolonged overall survival in patients with non-small cell lung cancer treated in switch maintenance, and in stage IV patients who received the treatment as second-line therapy. The vaccine was well tolerated.

KEY WORDS Racotumomab; carcinoma, non-small cell lung; lung neoplasms; Cuba

INTRODUCTION

Lung cancer is one of the most frequent neoplasms in the world. In 2020, it accounted for 11.7% of new cancer cases and 18.0% of reported cancer deaths.[1,2] In Cuba, the highest cancer incidence rates in men correspond to skin, prostate and lung cancer; and in women to skin, breast and lung cancer. In both sexes, lung cancer ranks third in incidence and first in mortality (5626 deaths in 2019; of which 3406 were men and 2220 were women).[3]

Smoking is the most common etiological factor, present in about 95% of male and 80% of female patients affected worldwide. Factors associated with lung cancer occurrence in non-smokers include environmental pollution, exposure to radon and other occupational substances, diet, lifestyle and genetic predisposition.[4,5]

Malignant epithelial lung tumors are classified into two major groups: small cell or microcytic lung carcinomas (15%–25%) and non-small cell or non-microcytic lung carcinomas (75%–85%). The second group includes three main types: adenocarcinoma (40%), squamous cell carcinoma (25%) and large-cell carcinoma (10%).[4,5] Adenocarcinoma usually occurs in young, female non-smokers with genetic alterations.

IMPORTANCE This is the first report on use of racotumomab in treatment of non-small cell lung carcinoma as part of routine clinical practice outside the context of a clinical trial.

Worldwide, the median age of lung cancer diagnosis is 70 years, with a high incidence of cases from 65 to 75 years of age. Of these, 50%–70% are diagnosed with locally advanced or metastatic disease, and 5-year survival is only 17%–18%.[4–6] For non-small cell lung carcinoma, the International Association for the Study of Lung Cancer (IASLC) reports 5-year survival for stages IIIA, IIIB, IIIC, and IV as 36%, 26%, 13%, and 6%, respectively.[7]

For patients in early stages of the disease who are unable to undergo surgical treatment or who refuse surgery, and for patients with locally advanced and metastatic disease, concurrent or sequential chemotherapy with radiotherapy is the therapeutic option of choice.[4,6] Traditionally, first-line treatment is chemotherapy with platinum derivatives (cisplatin, carboplatin) combined with other agents (such as pemetrexed, gemcitabine, vinorelbine, paclitaxel, docetaxel, bevacizumab).[5,6] However, their efficacy is limited, with little increase in survival, and high toxicity can cause discontinuation of treatment or precludes their application.

In recent years, important changes have been introduced in the treatment of non-small cell lung carcinoma, including therapies directed at targets on tumor cells' epidermal growth factor receptor (EGFR) or anaplastic lymphoma kinase (ALK)[6] and therapies that activate the immune system against the tumor. Among these are therapies that block the immune checkpoint represented by the programmed

death receptor and its ligand PD-1/PD-L1 (atezolizumab, nivolumab, pembrolizumab) and immune checkpoint inhibitors of cytotoxic T-lymphocyte–associated antigen 4 or CTLA-4 (ipilimumab).[8–10]

Understanding cell signaling mechanisms and their role in tumor formation, the use of monoclonal antibodies and recombinant proteins and the development of immunotherapy have opened new therapeutic avenues, among them immune response activation against tumor cells by means of vaccines based on tumor antigens. The Molecular Immunology Center (CIM) in Havana, Cuba, developed the racotumomab vaccine based on a murine monoclonal anti-idiotype antibody belonging to the IgG1 subclass.[11–14] Racotumomab stimulates an immune response against the tumor antigen N-glycolyl GM3 (NeuGcGM3). This ganglioside is virtually undetectable in normal cells; however, it is present in the cells of certain tumors (melanoma, breast, non-small cell carcinoma, Wilms tumor and neuroblastoma monocytes).[15,16] Its overexpression has been associated with altered cell growth, immune tolerance and tumor metastasis and angiogenesis, making it a target for cancer therapy.[14,15]

The response induced by antibodies generated after immunization with racotumomab is understood, as is its mechanism of action.[17] Most patients generate antibodies capable of binding to NeuGcGM3-expressing tumor cells, destroying them by a non-apoptotic mechanism, independent of complement activation.[17] Racotumomab's use in 20 patients with advanced-stage non-small cell lung cancer who had received 4 to 6 cycles of cisplatin/vinblastine induced an IgM and IgG antibody response against NeuGcGM3.[18]

A meta-analysis including 26 studies and 7839 patients with stage III and IV non-small cell lung cancer found that racotumomab and pemetrexed maintenance therapies were the most effective in terms of overall survival and disease-free survival.[19] A phase 2/3 trial involving 87 patients with advanced non-small cell lung cancer also showed an increase in overall survival and progression-free survival, and found local reactions at the injection site, bone pain, cough and asthenia to be the main adverse reactions, leading to the conclusion that the product is effective and safe.[20] It exhibits very low toxicity and is well tolerated, so patients sustain long-term treatment without complications, gaining control of disease symptoms, with increased survival and a substantial improvement in quality of life.[21] Racotumomab (trade name: Vaxira) is registered in Cuba[22] and Argentina[13] for treatment of patients with non-small cell lung carcinoma.

The objective of this study was to report on the use of racotumomab in early and advanced stages of the disease, as part of routine clinical practice outside the context of a clinical investigation for patients who could not receive surgical treatment. Its use as switch maintenance or second-line therapy is described.

METHODS

We conducted a descriptive, retrospective, non-probability sampling study to evaluate survival in patients with non-small cell lung carcinoma who could not be treated by surgery, and who received racotumomab as switch maintenance or second-line therapy.

Of the 86 patients seen in the Oncology Service of the Hermanos Ameijeiras Clinical-Surgical Hospital in Havana, Cuba, from January 2010 through December 2014, with cyto/histological confirmation of non-small cell lung carcinoma who did not undergo surgery, and who had received the racotumomab vaccine, 71 patients were selected who had completed first-line treatment with chemotherapy, radiotherapy or both, and whose clinical charts had the information required for this study. Of these, 36 received the vaccine as switch maintenance (new treatment given immediately following first-line treatment, requiring no evidence of disease progression) and 35 as second-line treatment (introduced after evidence of failure of first-line treatment due to disease progression).

Vaccine administration route, dosage and frequency Racotumomab (1 mg/mL, with alumina as adjuvant) was administered intradermally in 4 subdoses of 0.25 mL, in deltoid regions and anterior surface of the forearms. The first 5 doses were administered at 14-day intervals (± 7 days of tolerance), the following doses at 28-day intervals (± 15 days of tolerance), provided that the patient's general condition permitted subsequent doses.

Statistical analysis Data was obtained by reviewing medical records in hospital archives, the primary registry and the database of the Oncology Service's Functional Thoracic Tumor Unit. We created a database using SPSS version 20 in which age, sex, histologic type, smoking habit, tumor location, comorbidities, clinical stage, treatments received, vaccine immunizations and causes of immunotherapy discontinuation were recorded.

The main variables studied were: overall survival, defined as time elapsed from diagnosis or from first immunization to death or knowledge of the latest update; survival according to progressors and non-progressors at initiation of treatment with the vaccine and by stage (stages I and II were reported together). Survival estimation was made using the Kaplan-Meier method. Severity of adverse effects was assessed according to the US National Cancer Institute's Common Terminology Criteria for Adverse Events version 4.0.[23]

RESULTS

Median age of patients was 66 years. Of the total, 67.6% (48/71) presented at least one comorbidity and the vast majority were smokers. More than half of patients (57.7%, 41/71) were in locally advanced or metastatic stages. The main histologic subtype was adenocarcinoma, followed by squamous cell carcinoma. It was not possible to determine the histologic subtype in 13 patients (18.3%) (Table 1).

Most patients (73.2%, 52/71) had undergone chemotherapy in addition to radiotherapy; 94.4% (67/71) adhered to the first-line treatment schedule (chemotherapy, radiotherapy, 4–6 cycles); only 4 patients (5.6%) received less than 4 cycles, 3 due to hematologic toxicity and 1 due to nephrotoxicity.

Of the total, 98.6% (70/71) of patients adhered to the vaccine induction phase (first 5 doses administered at 14-day intervals); 38.0% (27/71) received over 14 vaccine administrations during more than one year of treatment. The most frequent cause of treatment discontinuation was disease progression (57 patients) and 2 dropped out of vaccination (Table 2). 84.5% (60/71) had no adverse events following immunization, and 15.5% (11/71) reported some mild events (Table 3).

Table 1: Patient characteristics

Variable	Categories	N (%)
Sex	Female	23 (32.4)
	Male	48 (67.6)
ECOG	0	14 (19.7)
	1	48 (67.6)
	2	9 (12.7)
Presence of comorbidities	No	23 (32.4)
	One	27 (38.0)
	Multiple	21 (29.6)
Tobacco use	Ex-smoker	9 (12.7)
	Smoker	57 (80.3)
	Non-smoker	5 (7.0)
Disease stage	IA	3 (4.2)
	IB	4 (5.6)
	IIA	4 (5.6)
	IIB	4 (5.6)
	IIIA	15 (21.1)
	IIIB	15 (21.1)
	IVA, IVB	26 (36.6)
Histological type	Non-small cell carcinoma (not otherwise specified)	13 (18.3)
	Adenocarcinoma	28 (39.4)
	Squamous cell carcinoma	22 (31.0)
	Undifferentiated large-cell carcinoma	8 (11.3)
First-line treatment	Chemotherapy/radiotherapy	52 (73.2)
	Chemotherapy	19 (26.8)
Chemotherapy regimen	Cisplatin or Carboplatin/VP16	11 (15.5)
	Cisplatin or Carboplatin/VLB	50 (70.4)
	Cisplatin or Carboplatin/paclitaxel	6 (8.4)
	Cisplatin	4 (5.6)
Response to first-line treatment	Complete tumor remission	1 (1.4)
	Partial tumor remission	22 (31.0)
	Stable illness	32 (45.1)
	Progression	16 (22.5)

ECOG: functional capacity according to the Eastern Cooperative Oncology Group scale;[24] VLB Vinblastine; VP16: Etoposide

Median overall survival from the time of diagnosis was 24.5 months, (95% CI: 19.03–30.10), with a 5-year survival rate of 17.3%. Patients who received racotumomab as switch maintenance achieved median survival from the start of immunization of 17.2 months. In patients who received racotumomab as second-line treatment, median survival was 6.8 months (Table 4 and Figure 1).

For the 41 patients in stages IIIB and IV, median overall survival from first immunization was 11.3 months, 15.8 months for patients who were non-progressors at the start of treatment with the vaccine, and 9.0 months for patients who were progressors at the start of treatment with the vaccine, for a rate of 51.2% at 2 years and 7.0% at 5 years. Stage IV patients who were progressors at treatment initiation attained 10.2 months of survival, and patients who were non-progressors at treatment initiation attained 15.1 months of survival (Table 4).

DISCUSSION

Lung carcinoma occurs more frequently in men and individuals between 65 and 75 years of age with an average age of 70 years.[4,6] In our study, most patients were over 60 years of age.

Table 2: Treatment with racotumomab vaccine: induction and immunizations

Compliance with vaccine induction phase	N (%)
Yes	70 (98.6)
No	1 (1.4)
Number of immunizations	**N (%)**
1–5	15 (21.1)
6–9	18 (25.4)
10–14	11 (15.5)
>14	27 (38.0)

Table 3: Frequency and characterization of adverse events following immunization with racotumomab

Adverse event following immunization	Frequency* N (%)
Yes	11 (15.5)
No	60 (84.5)
Type of adverse event (degree of toxicity)	
Systemic:	
Low-grade fever (I)	2 (2.8)
Arthralgia (I)	1 (1.4)
Myalgia (I)	1 (1.4)
Local:	
Redness at the injection site (I)	5 (7.0)
Pain at the injection site (I)	2 (2.8)

Degree of toxicity: according to the scale of the National Cancer Institute, USA.[23]
*Frequency: adverse event frequency of occurrence (number of patients experiencing the event)

Table 4: Survival in months since start of racotumomab immunization, by cancer stage and progressor/non-progressor status

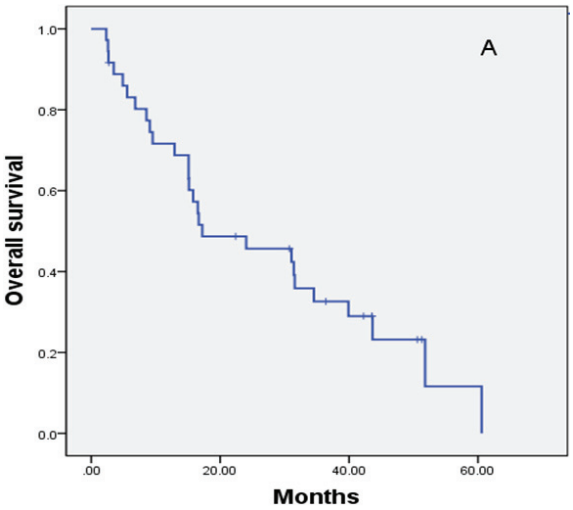
	Progressors	Non-progressors
Cancer stage	Median survival in months (CI 95%)	Median survival in months (CI 95%)
I and II	4.0 (0.00–8.07)	39.9 (10.61–69.18)
IIIA	4.7 (0.00–11.12)	12.9 (0.00–38.93)
IIIB	5.2 (2.94–7.45)	16.5 (14.39–18.67)
IV	10.2 (4.22–16.17)	15.1 (5.81–24.51)

Non-progressors: 36 patients who at the time of first immunization were progressors and who received racotumomab as switch maintenance therapy.
Progressors: 35 patients who at the time of first immunization were progressors and who received racotumomab as second-line treatment.

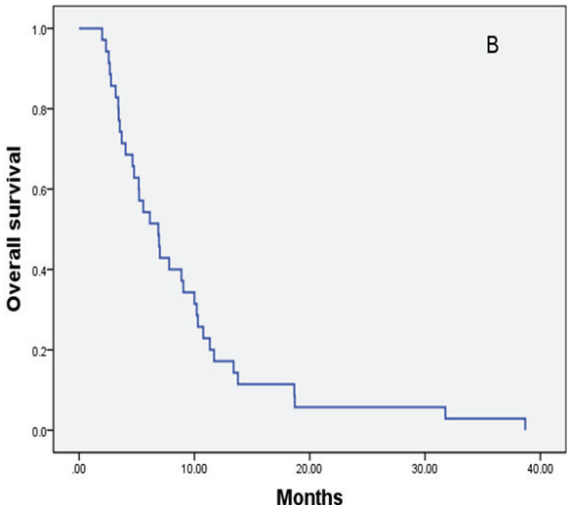
Patients at disease diagnosis had predominantly functional capacity according to the scale used by the Eastern Cooperative Oncology Group (ECOG),[24] with at least one comorbidity. Most patients (93%) were active or former smokers; these data are similar to those reported elsewhere in the literature: 85%–90% of patients with non-small cell carcinoma are or have been smokers. Tobacco use is associated with oncologic, cardiovascular, pulmonary, renal and infectious diseases.[4,25]

It has been reported that 50%–70% of patients are diagnosed at locally advanced and metastatic stages, for which there are no curative treatments.[5,6] Locally advanced and metastatic stages also predominated in this study. In 18.3% (13) of patients, the histologic subtype could not be defined and was classified as non-small cell lung carcinoma (not otherwise specified). In patients classified histologically, adenocarcinoma predominated (39.4%).

Figure 1: Overall survival since beginning immunization



A: Patients treated with racotumomab in switch maintenance (non progressors: 36 patients with stable disease, with partial or complete response after first-line treatment). Median overall survival: 17.2 months (95% CI, 0.26–34.20).
Overall survival: Proportion of surviving patients (1.0: all alive, 0.0: all dead).



B: Patients treated with racotumomab in second-line treatment (progressors: 35 patients with progressing disease). Median overall survival: 6.86 months (95%CI, 4.78–8.95).
Overall survival: Proportion of surviving patients (1.0: all alive, 0.0: all dead).

Adenocarcinoma is the most frequent subtype in non-small cell lung carcinomas.[26,27] In this subgroup, which has experienced an increase in incidence rates worldwide, there have also been important advances in diagnosis and treatment, thanks to the identification of mutations in EGFR genes and ALK gene fusion, in which treatment with tyrosine kinase inhibitors has led to longer survival than that achieved with chemotherapy plus radiotherapy.[6,28]

The most effective chemotherapy combines a platinum derivative with another antitumoral (cisplatin or carboplatin plus paclitaxel, gemcitabine, docetaxel, vinorelbine, irinotecan or pemetrexed). Most patients included in this study received cisplatin with vinblastine as first-line therapy, as was the case in the Alfonso study (176 randomized patients with stage IIIB/IV disease, a study coordinated by the Cuban National Oncology and Radiobiology Institite).[20] Almost all patients completed the prescribed cycles of chemotherapy. Prolongation of treatment up to six cycles may lead to increased toxicity with poor or no overall survival benefit.[6,29,30] For first- and second-line treatment in advanced lung carcinoma, six cycles of chemotherapy are recommended for patients who are non-progressors after the fourth cycle and who cannot receive switch maintenance or immunotherapy.[6,29,30]

At the end of first-line treatment (chemotherapy, radiotherapy, or both), stable disease and partial remission was predominant among patients. This is a tumor that responds poorly to chemotherapy and for which the treatment of choice is surgery (not applicable to these patients for various reasons).[29,31]

Clinical studies[31,32–34] have shown that the racotumomab vaccine increases survival in patients with recurrent or advanced stage (IIIB/IV) non-small cell lung cancer, compared to patients treated with best-practice supportive care. The vast majority in our research (70/71; 98.6%) completed the induction phase (the first four cycles of the vaccine), the completion of which results in a better immune response. Most (53.5%, 38/71) received 10 or more immunizations, and continued vaccination for more than one year until clinical progression of the disease, which was the predominant cause for discontinuation of treatment.

Adverse events were minimal, characterized mainly by mild local reactions (none leading to discontinuation of treatment or affecting patients' health). In earlier clinical trials, racotumomab proved to be a very well-tolerated vaccine, with a low toxicity profile.[21,29,34,35] Viada[36] evaluated adverse events in six clinical trials designed to assess the vaccine's safety profile and efficacy at various tumor sites. No serious adverse events were reported; the most frequent adverse events occurred at the injection site, and systemic reactions consisted of fever, myalgias, arthralgias, pruritus, headache and fatigue, and generally subsided in less than 48 hours. These results are consistent with ours and with those reported by Pérez,[35] who assessed safety in 86 patients with non-small cell carcinoma.

Survival

Overall survival The most informative indicators of cancer severity and treatment efficacy is overall survival rate and disease-free survival rate. The overall survival rate at 5 years in patients with non-small cell carcinoma in the United States is 18%,[4] 9%–13% with a median survival of 9–12 months, and 30%–40% at 2 years and 10% at 5 years in the European Cancer Registry in Spain.[37]

Various studies[29,32,34] have shown that the racotumomab vaccine increases survival in patients with recurrent or advanced stage (IIIB/IV) non-small cell lung carcinoma compared with patients treated with best-practices supportive care. The first study with racotumomab in patients with stage IIIB and stage IV non-small cell carcinoma reported median overall survival of 16.4 months from time of diagnosis and a median survival of 9.93 months from time of vaccination, for a 1-year survival rate of 34%.[29]

One of the most important clinical studies with racotumomab in patients with stage IIIB and stage IV non-small cell carcinoma evaluated the effectiveness of treatment in 176 randomized

patients—87 treated with the vaccine and 89 with placebo.[20] Median overall survival was 8.2 months and 6.8 months, respectively. Median progression-free survival in vaccinated patients was 5.33 months versus 3.9 months for the placebo group. This study demonstrated for the first time the superiority of racotumomab over placebo in a randomized, controlled clinical trial.

The overall survival rate in the present study was 17.3% at 5 years. In stages IIIB and IV, a survival rate of 51.2% was observed at 2 years, which falls to 7% at 5 years, with a median survival of 11.3 months.

Overall survival in our study was 24.5 months from time of diagnosis. Median survival was 17.2 months from first immunization in patients who received the vaccine as switch maintenance after first-line treatment, with a survival rate at 1 year of 69%. In the subgroup that received the vaccine as second-line therapy, median survival was 6.86 months and the survival rate at 1 year was 17.1%. In the subgroup of patients who were progressors at the start of vaccine therapy, the data coincides with that reported by Alfonso;[20,29] in patients who received the vaccine as switch maintenance, overall survival was higher. This could be explained by the fact that this study includes all cases seen in routine medical practice involving patients in early disease stages. For the 41 patients in stages IIIB and IV, median overall survival was 11.3 months from first immunization (15.8 months for patients in switch maintenance and 9.0 months for patients undergoing second-line treatment). Survival was higher in the present study than in the clinical trials conducted in Cuba.[20,29]

Survival in non-small cell lung carcinoma depends on early detection, early initiation of treatment and, especially, early surgical treatment. Survival in stage IA or IB patients undergoing surgery is 60%–80% at 5 years, while in patients receiving no treatment, survival does not exceed 6%.[7] Overall survival in advanced stages treated with chemotherapy (platinum plus a third-generation drug) and radiotherapy is only 10–12 months and the 1-year survival rate is 30%–40%.

Hardstock reported an overall survival of 11.1 months for patients without noteworthy genetic mutations treated with first-line chemotherapy, and 18.8 months for patients with mutations who were treated with a tyrosine kinase inhibitor.[38] In a European study, overall survival after first-line chemotherapy was 10.3 months.[39] The FLEX study in patients with stage III and IV non-small cell lung carcinoma reported 11.3 months overall survival for patients treated with standard chemotherapy plus cetuximab, and 10.1 months for those who received only standard chemotherapy.[40]

Our survival results in stages I and II differ from those published by Arnold.[41] In our study, no patient received surgical treatment (even in early stages) because surgery was contraindicated. Our results are better in stages IIIB and IV. The lower survival in stage IIIA, as compared to IIIB, could be due to the fact that most of these patients were already progressors at the start of immunization with the vaccine.

Switch maintenance The results in our investigation in the 36 patients who received racotumomab as switch maintenance are similar to those reported with the vaccine in previous studies and with other maintenance therapies.[19,20,31,32]

Many drugs approved as maintenance or switch maintenance therapies that have demonstrated longer overall survival and better symptomatic control also entail substantial toxicity: docetaxel, bevacizumab, pemetrexed, gemcitabine and erlotinib. Of these, pemetrexed is the best tolerated.[42–44] Maintenance with pemetrexed has demonstrated efficacy after induction therapy with a platinum doublet and after cisplatin-pemetrexed combination,[44] with similar increases in overall survival and progression-free survival in both studies (median of 13 and 4 months, respectively). In a phase 3 clinical trial of 663 patients diagnosed with non-small cell lung carcinoma, an overall survival of 13.4 months was attained in the group treated with pemetrexed as switch maintenance therapy, compared to 10.6 months in the placebo group.[45]

Immunotherapy today is one of the most important treatment avenues for lung cancer. The KEYNOTE-021 study[9] included non-progressor patients with various levels of PD-L1 expression. Longer survival (34.5 months) was attained in patients who received the humanized monoclonal antibody pembrolizumab in combination with chemotherapy as first-line treatment than in those who received chemotherapy alone. This result is superior to ours (24.5 months) in terms of overall survival from diagnosis, possibly because our study included patients at all stages as well as patients in progression at the start of vaccination, and because the population under study was not stratified by tumor molecular studies.

In the period under study, no stratification studies were performed using molecular markers, which currently allow *a priori* discrimination of potential responders from non-responders. Some patients treated with racotumomab could present genetic alterations; mutation of the epidermal growth factor receptor, ALK translocations or ROS-1–positive status, for example; it is now known that the vaccine is not effective in these patients because these signaling pathways are aberrant.

Second-line treatment Docetaxel has been standard second-line treatment for non-small cell non-squamous lung carcinoma progressing after treatment with a platin doublet, with median overall survival of 7.5 months, a median duration of response of approximately 26 weeks, and an overall one-year survival rate of approximately 37%.[46] A multicenter phase 3 clinical trial comparing atezolizumab and docetaxel in second-line treatment in patients with non-small cell carcinoma reported improved overall survival in the group treated with atezolizumab. In patients with non-small cell non-squamous carcinoma, median overall survival was 15.6 and 11.2 months for atezolizumab and docetaxel, respectively (hazard ratio of 0.73; 95% CI: 0.60–0.89) and in patients with non-small cell squamous carcinoma, overall survival was 8.9 and 7.7 months for atezolizumab and docetaxel, respectively (hazard ratio: 0.73; 95% CI: 0.54–0.98).[47] A multicenter study evaluating survival in patients with non-small cell carcinoma treated with racotumomab as second-line therapy reported an overall survival of 8.9 months.[48]

In the present study, overall survival from the start of immunization in 35 patients treated with racotumomab as second-line was 6.8 months, slightly lower than that reported by Rittmeyer and by Santiesteban,[47,48] who also did not perform molecular studies to stratify patient treatment according to mutations or immunohistochemistry. Our patients who received treatment in

stage IV attained 10.2 months of survival, exceeding that reported by Rittmeyer[47] and Santiesteban,[48] and also superior to our results for stage IIIB, although the latter sample included only 4 patients.

Our results coincide with those of Alfonso in the first study performed with racotumomab in lung cancer[29] (which obtained better survival for stage IIIB and IV patients who responded to first-line chemotherapy) and were superior to those obtained in a noninferiority clinical trial that evaluated the efficacy of racotumomab, nimotuzumab and docetaxel as second-line treatment,[49] in which median survival times of 4.8, 4.6 and 5.8 months, respectively, were obtained.

Limitations of this study Although the information provided by a retrospective study does not have the same confirmatory value as a prospective study, the rigorous selection of clinical histories and information quality control give this study, the only one of its kind in Cuba, great value as support for use of racotumomab in clinical practice. Histologic subtype could not be defined in some patients, which prevented a more refined histologic stratification. Nor were methods available to determine genetic markers to which prognostic value is currently attributed (ALK translocations, ROS-1 positive, EFG receptor mutation and other genetic disorders). Patients with these mutations currently benefit from tyrosine kinase inhibitors, permitting higher survival rates.

CONCLUSIONS

This is the first study of racotumomab use outside a clinical investigation and as part of routine clinical practice. Racotumomab is an option for switch maintenance for patients with non-small cell lung carcinoma. It is well-tolerated; adverse events do not increase with the number of immunizations, and it is safe for prolonged use. Our survival results in patients treated with second-line racotumomab who were progressors at treatment initiation were slightly lower than those reported in some clinical trials, and similar to or greater than those reported in other trials. It is important to conduct clinical trials in patient populations selected through molecular marker studies, in which racotumomab is combined with other antineoplastic agents recently introduced for specified use depending on the presence of molecular markers.

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Subtype-Dependent Co-receptor Tropism in Cuban HIV-1–Infected Patients: Implications for Maraviroc Treatment

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ABSTRACT

INTRODUCTION Unlike most high-income countries where subtype B viruses predominate, the Cuban HIV-1 epidemic is characterized by a great diversity of subtypes and circulating recombinant forms. Some studies have shown that HIV variants exhibiting a preference for the CXCR4 co-receptor (X4-tropic) could have impacts on disease pathogenesis, with clinical implications for antiviral treatment plans. Determination of HIV co-receptor tropism is crucial for clinicians in deciding whether maraviroc is an appropriate antiviral.

OBJECTIVE Characterize V3 sequence variability and its relation to viral tropism across different subtypes circulating in Cuba and explore how this may affect treatment success with maraviroc.

METHODS We designed a cross-sectional study that included 72 plasma samples obtained at the Pedro Kourí Tropical Medicine Institute in Havana, Cuba. We sequenced the C2V3 *env* region and assessed subtype based both on *env* and *pol* sequences; tropism was predicted by Geno2pheno analysis. Additionally, 35 V3-loop Cuban sequences, obtained from a previous study, were incorporated into the analysis. Statistical associations among virological, clinical and epidemiological variables were assessed by a chi-square test.

RESULTS Tropism prediction for 72 variants revealed that CRF19_cpx was associated with dual-tropic R5X4 viruses ($p = 0.034$). Moreover, when 35 sequences from a former study were added, the association was significant not only for R5X4 ($p = 0.019$) but also for X4-tropic variants ($p = 0.044$). Alignment of 107 V3-loop sequences showed wide diversity among the different HIV-1 subtypes circulating in Cuba.

CONCLUSIONS In accordance with G2P, CRF19_cpx is a genetic variant with a high proportion of X4 and R5X4-tropic viruses. The results from the present study suggest that the Cuban recombinant could be a more pathogenic variant and that maraviroc may not be suitable for patients infected with CRF19_cpx.

KEYWORDS HIV, CCR5 receptor antagonists, maraviroc, HIV envelope glycoprotein gp120, Cuba

INTRODUCTION

During entry into the host cell, HIV-1 surface glycoprotein gp120 interacts with the CD4 receptor and, generally, with one of two chemokine co-receptors: CCR5 (chemokine [C-C] motif receptor 5) or CXCR4 (CXC chemokine receptor 4). This interaction defines viral tropism in R5-tropic or X4-tropic strains, respectively.[1,2] Co-receptor selectivity is determined by genetic sequences within the HIV-1 gp120, particularly in a highly variable and structurally flexible region called the 'V3 loop', which is involved in co-receptor binding.[3,4]

Viral tropism has been proposed as an influence on HIV-1 pathogenesis and replication, with implications for treatment. R5-tropic variants have shown predominance during early stages of the disease, are generally not syncytium-inducing and have low replication capacity.[5,6] It is believed that during the course of infection, R5 viruses experience a switch in tropism and thus, X4 variants emerge, characterized by syncytium induction and higher replication capacity. In addition, a third group of viruses has been classified as dual-tropic or R5X4-tropic variants, referring to their ability to interact with both CCR5 and CXCR4 co-receptors.[2,7]

IMPORTANCE

CRF19_cpx is one of the most prevalent HIV-1 subtypes circulating in Cuba. X4- and dual-tropic viruses prevail in CRF19_cpx-infected patients, which suggest this may be a more pathogenic variant and less susceptible to treatment with maraviroc.

Determining viral tropism is important when choosing antivirals like maraviroc, the first CCR5 inhibitor approved for treatment of R5-tropic HIV-1 infection in both treatment-naive and treatment-experienced adult patients. However, maraviroc does not inhibit entry of X4-tropic or dual-tropic HIV-1 viruses and a test that predicts HIV-1 co-receptor use is highly recommended before treatment prescription.[1,8,9]

Two different methods are employed to predict co-receptor use. Phenotypic assays have greater accuracy; however, they are expensive, time-consuming, and require special facilities and trained personnel. On the other hand, genotypic assays rely on bioinformatic tools to predict co-receptor use based mainly on the sequence of the gp120's V3 loop.[10,11] These latter methods are rapid and easier to use; however, their development has been based mostly on sequences from HIV-1 subtype B isolates, without considering the viral diversity of the epidemic within countries.[1,12–14]

In Cuba, the HIV-1 epidemic is atypical when compared to the rest of the Latin American and Caribbean region. Cuba has a great diversity of HIV-1 subtypes including many recombinant forms (CRFs) such as BG recombinants (CRF20_BG, CRF23_BG and CRF24_BG) and complex CRFs, such as CRF18_cpx and CRF19_cpx.[15,16]

A Cuban study identified that in patients harboring CRF19_cpx viruses, CXCR4-using variants prevailed and disease progression to AIDS occurred more rapidly than in patients harboring subtype B viruses.[15] An association between HIV-1 subtype and co-receptor use has also been demonstrated in other studies.[2,6,17]

The objective of this study was to characterize V3 sequence variability across different Cuban HIV-1 subtypes and its relation to viral tropism in the context of the Cuban epidemic, in order to achieve a more complete understanding of HIV-1 pathogenesis and improved management of antiviral treatment for Cuban patients.

METHODS

Study Design We analyzed additional plasma samples from Cuban HIV-1–infected patients in order to extend our previous analysis on prevalent subtypes[18] and include data on viral tropism. Samples were selected from HIV-1–infected patients who were tested for antiretroviral resistance at the Pedro Kourí Tropical Medicine Institute (IPK) in Havana, Cuba, from January 2015 through July 2016. Those patients infected with the following subtypes and recombinant forms (classified regarding *pol* sequence employed in resistance testing) were selected: subtype B, CRF_BG (for the purpose of this study CRF20_BG, CRF23_BG and CRF24_BG were grouped together as CRF_BG), CRF18_cpx and CRF19_cpx.[15,16,19] After selection, a total of 72 patients were included and C2V3 sequences of the glycoprotein gp120 were obtained and employed to assess viral subtype regarding *env* region and predict co-receptor use.

An additional group of 35 V3-loop sequences from Cuban patients[18] was included for analysis of association between viral subtype and tropism, to better characterize V3 sequence variability. We decided to include these sequences because they corresponded to a similar study performed by our group from January 2014 through January 2015.[18] For sequence selection, the same criteria mentioned above were applied. We included a total of 14 sequences from subtype B, 13 from CRF_BG, 2 from CRF18_cpx and 6 from CRF19_cpx, classified according to *pol* sequence.

Epidemiological, clinical, virological and immunological information Epidemiological and clinical data were collected at sampling as well as from a screening of selected databases stored at IPK (SIDATRAT). Patient viral load and CD4+ cell counts were performed as previously described.[18]

HIV sequencing and subtype assignment RNA extraction from plasma samples, C2V3 amplification and sequencing were obtained as previously described.[18] Sequences were edited with Sequencher 4.9 (Gene Codes Coorporation, USA) employing the HXB2 strain (Genbank access number K03455.1, at https://www.ncbi.nlm.nih.gov/genbank) as the reference sequence. Subtype was assessed via COMET version 2 (Luxembourg Institute of Health, Luxembourg) and REGA version 3 (Stanford University, USA).[20,21] In addition, subtype assignment was confirmed by manual phylogenetic analysis employing PhyML/One Click software (Institut Pasteur, France).[22] Sequences and HIV-1 subtype in the *pol* region were obtained from antiretroviral resistance testing routinely performed in our laboratory at IPK.[23]

Prediction of co-receptor use Tropism prediction based on V3-loop sequence was performed using the Geno2pheno (G2P) algorithm (Max Planck Institute, Germany). The false positive rate (FPR) that defines the probability of classifying an R5 virus (incorrectly) as an X4 virus was set at a cut-off value of 5% following German guidelines.[15,24,25] Patients with FPR ≤5% were considered infected with X4 viruses; patients with FPR ≥20% were predicted to harbor R5 viruses; and patients with FPR values >5% and <20% were considered to have dual-tropic R5X4 virus infections. Additionally, V3 net charge and 11/25 rule were employed in predicting viral tropism.[26]

Statistical analysis Comparison of FPR values among groups was performed using GraphPad Prism version 5 software (GraphPad, USA) and the non-parametric Kruskall Wallis Test and Dunn Test for post-hoc comparisons. Analysis of the association between co-receptor use, subtype, clinical, virological and immunological variables was performed via a chi-square test using SPSS v.22 (IBM, USA). For all comparisons, a p-value ≤0.05 was considered statistically significant.

Ethics Study protocols were designed in accordance with the Helsinki Declaration and approved by the IPK Ethics Committee.

RESULTS

Study population characteristics The study universe consisted of 72 plasma samples corresponding to the same number of patients. Two patients lacked clinical and epidemiological information, and were therefore only included in the analysis of co-receptor tropism prediction and its association with HIV-1 subtype. For the remaining 70 patients, epidemiological, virological and immunological information demonstrated that the majority were male (74.3%), men who have sex with men (MSM, 68.6%), aged 31–45 years (41.4%) who had been diagnosed with HIV-1 >10 years ago (Table 1); 11.4% of patients had AIDS at the time of HIV-1 diagnosis and 64.3% had AIDS at the time of sampling. For 41.4% of patients, viral load at sampling was 10,000–100,000 copies/mL and 47.1% had a T CD4+ cell count ≤200 cells/mL. These patients had been assigned a previous HIV-1 subtype based on the *pol* sequence. Twenty patients were infected with subtype B, 24 with CRF19_cpx, 9 with CRF18_cpx and 19 with CRF_BG.

***Env* subtyping** Samples were processed to obtain partial *env* sequences. Of these, 12.5% (9/72) samples had a C2V3 region too short for phylogenetic subtyping (Figure 1), but were still able to be subtyped using REGA version 3 and COMET version 2 bioinformatics platforms.

After *env* subtyping, 21 sequences were subtype B, 23 were CRF19_cpx, 7 were CRF18_cpx, and 21 were CRF_BG. Considering both *pol* and *env* sequences, an overall subtype was assigned to samples, resulting in 19 classified as subtype B, 21 as CRF19_cpx, 7 as CRF18_cpx, 18 as CRF_BG and 7 as unique recombinant forms (URF) since they had different subtypes in *pol* and *env* sequences.

Co-receptor use prediction Co-receptor use was analyzed based on G2P algorithm, 11/25 rule and V3 net charge. Co-receptor prediction by G2P showed that 59.7% (43/72) of analyzed samples were R5-tropic viruses, 16.7% (12/72) were X4-tropic viruses, 23.6% (17/72) were classified as dual tropic

Table 1: Patient characteristics

Characteristics	N (%)
Total number of patients*	70 (100)
Male	52 (74.3)
Sexual orientation: MSM	48 (68.6)
Years since HIV-1 diagnosis	
≤1	9 (12.9)
>1–3	9 (12.9)
>3–5	3 (4.3)
>5–10	20 (28.6)
>10	29 (41.4)
Age (years)	
<15	2 (2.9)
15–30	17 (24.3)
31–45	29 (41.4)
46–61	20 (28.6)
62–77	2 (2.9)
AIDS at diagnosis	8 (11.4)
Evolution to AIDS after HIV-1 diagnosis (years)	
<1	19 (27.1)
1–3	8 (11.4)
>3	18 (25.7)
No AIDS	25 (35.7)
Current AIDS	45 (64.3)
ARV treated	68 (97.1)
Viral load (copies/mL)	
<1000	3 (4.3)
1,000–10,000	13 (18.6)
>10,000–100,000	29 (41.4)
>100,000	25 (35.7)
T CD4+ cell count (cells/mL)	
<200	33 (47.1)
200–350	20 (28.6)
>350–500	11 (15.7)
>500	6 (8.6)

*Two patients were not included due to lack of information.
ARV: antiretroviral MSM: men who have sex with men.

viruses and 40.3% (29/72) were predicted to be dual-tropic or X4 variants (Table 2). There was a 42.9% agreement in predicting X4-tropic viruses between G2P and the 11/25 rule for subtype B, while there was 50.0% agreement for non-B subtypes. Calculation of V3-loop net charge showed a 42.9% agreement with G2P for subtype B and 50.0% agreement for non-B subtypes.

Analysis of co-receptor use by G2P showed that CRF19_cpx viruses were more likely to be R5X4-tropic (p = 0.034; OR = 3.295; CI: 1.065–10.191) than other subtypes. This statistical difference was strengthened when we analyzed R5X4 and X4 together using variants (p = 0.015; OR = 3.526; CI: 1.253–9.921). No other statistical association was found for remaining subtypes. A comparison of FPR values across subtypes also showed that CRF19_cpx had the lowest mean value (Figure 2), even though the difference was only significant with regard to CRF18_cpx and CRF_BG.

Patient epidemiological, virological and immunological information was analyzed in relation to co-receptor use and viral *env* subtype (Table 3). Nevertheless, few statistical associations were found. X4-tropic viruses were associated with >10 years since diagnosis (p = 0.022), with a viral load at >10,000–100,000 copies/mL (p = 0.022) and with AIDS at the time of sampling (p = 0.045). R5-tropic viruses prevailed in non-AIDS patients (p = 0.002), in those with viral loads at 1,000–10,000 copies/mL (p = 0.008) and a T CD4+ cell count at 200–350 cells/mL (p =0 .031). R5X4 viruses corresponded to patients with a viral load >100,000 copies/mL (p = 0.004) and with T CD4+ cell counts <200 cells/mL (p = 0.005). CRF19_cpx prevailed in patients who were 15–30 years old (p = 0.028).

Our analysis incorporated an additional 35 HIV-1 V3-loop sequences from Cuban patients, obtained from January 2014 through January 2015 under the same criteria outlined above. Thus, this set of 107 sequences spanned a period from January 2014 through July 2016. Determination of co-receptor use in this group of viruses (Table 4) revealed an association of CRF19_cpx with X4-tropic viruses (p = 0.044), R5X4-tropic viruses (p = 0.019) or either R5X4 or X4 (p = 0.0004). Additionally, most CRF_BG were R5-tropic viruses (p = 0.032).

Genetic diversity of V3 loop After alignment of 107 V3-loop sequences, we identified some differences in aminoacidic patterns expressed among subtypes. Arginine (R) at position 13 was more prevalent in CRF_BG (p ≤0.0001) and CRF19_cpx (p ≤0.0001) than in subtype B (Supplementary Figure 3).

Valine (V) at positions 12 and 19 prevailed in CRF19_cpx compared to subtype B (p ≤0.0001). On the other hand, for non-B subtypes (CRF19_cpx, CRF18_cpx and CRF_BG), the sequence GPGQ in the tip of the V3 loop was more frequent than GPGR, characteristic of subtype B (p <0.0001). Some motifs found at the tip of the loop other than GPG were RPA/G, APG, GAG, DAG, GRG and GLG. All sequences with APG motifs were R5-tropic, while all CRF19_cpx sequences with substitution 34Y were either X4- or R5X4-tropic strains. However, these differences were not significant.

DISCUSSION

CRF19_cpx is currently one of the most prevalent subtypes circulating in Cuba. This recombinant is thought to be of African origin, resulting in a mosaic of subtypes A1, D and G; though the virus has spread most successfully in Cuba. An earlier study performed in our laboratory showed an association between infection with CRF19_cpx and rapid progression to AIDS.[15] These results led us to hypothesize that this recombinant virus may be a more pathogenic form of HIV-1. Although it has not been demonstrated whether X4 viruses are a cause or rather a consequence of immune system deterioration, many studies reported co-receptor use preference based on the subtype.[6,17] Based on G2P prediction, we found that CRF19_cpx had preferential tropism towards R5X4, which has been described as capable of using both CCR5 and CXCR4 co-receptors for entry into the host cell.[4]

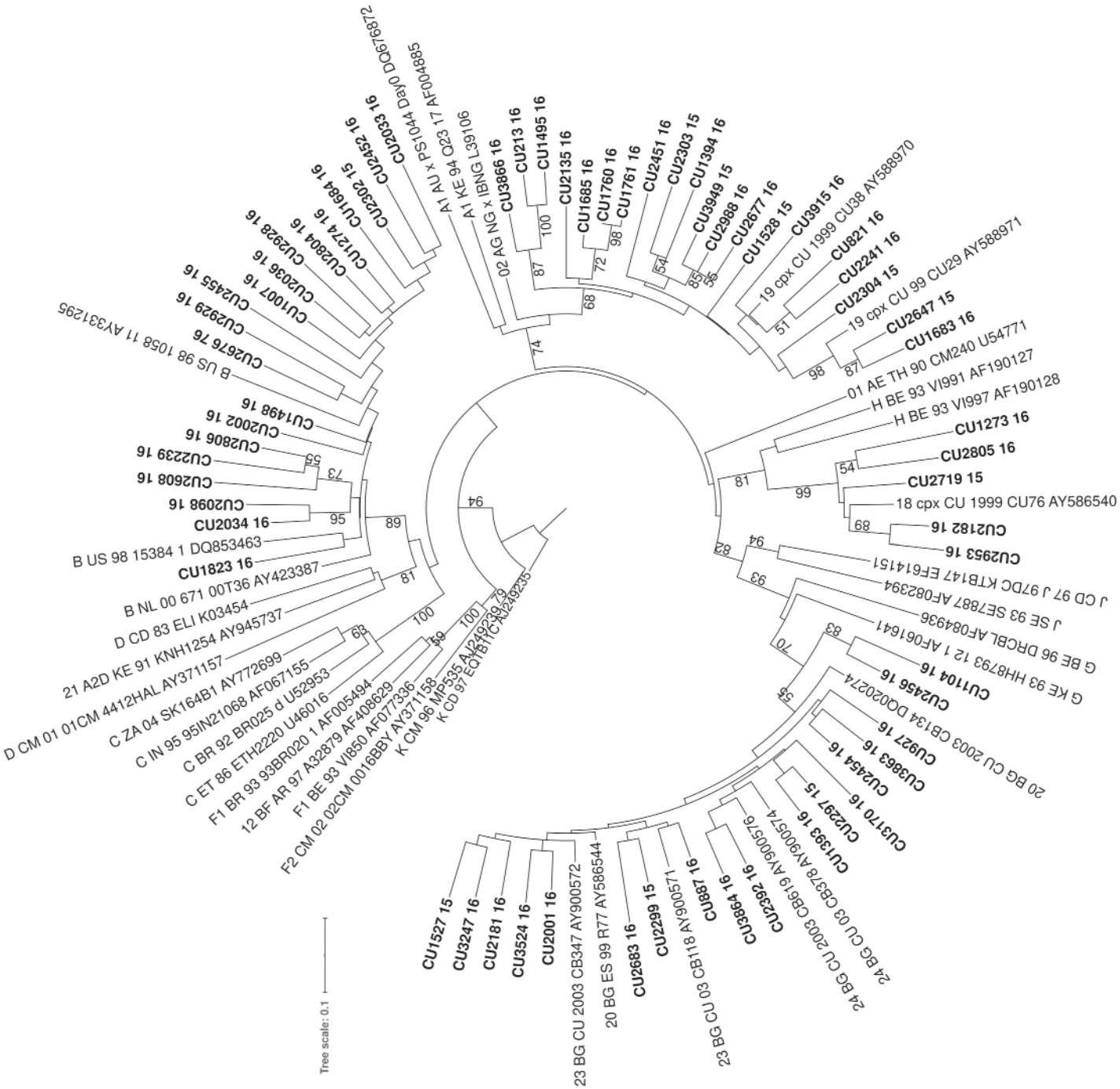
When we expanded our analysis to 107 samples, we found CRF19_cpx to be associated with dual-tropic forms of the virus and also ratified our previous observations about a link between these viruses and X4-tropic variants.

Since dual-tropic viruses have been considered an intermediate stage during the switch from R5 to X4 variants that may even result

from intrapatient recombination once X4 viruses emerge in the host, CRF19_cpx association is expected not only with X4 but also with R5X4 viruses. In addition, understanding that some patients never experience this switch in tropism and continue harboring R5 viruses,[7,27] we speculate that some HIV-1 subtypes or CRFs, such as CRF19_cpx, are likely to evolve towards dual-tropic and X4 variants during the course of infection. Whether this evolution is faster with respect to other subtypes or is actually related to HIV-1 pathogenesis has yet to be demonstrated. Furthermore, the cause of this subtype-dependent co-receptor use may be explained by intrinsic virological properties of each subtype (i.e. enhanced replicative capacity, antigenic diversity) or by the differential effects of viruses on host immune systems, which may eventually lead to expansion of the X4 virus population.[27]

Even though we measured no direct association between CRF19_cpx and disease progression stage, R5X4 viruses were related to a CD4+ cell count ≤200 cells/mL. Characteristically, X4 viruses have

Figure 1: Phylogenetic tree constructed by PhyML/One Click software[22]



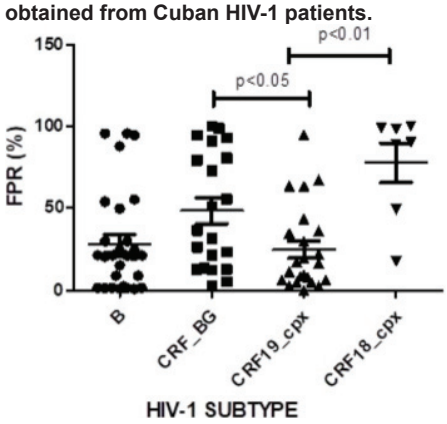
Sixty-three sequences encompassing C2V3 env region of HIV-1 (in bold) were compared with reference sequences from Genbank. Bootstrap values (as a result of 500 replicates), above 50% are shown at the nodes. The tree was rooted in strain K.CD.97. EQTB11C.AJ249235.

Table 2: Co-receptor use and subtype distribution prediction

Sample number (%)		Subtype in *env* region			
	All subtypes	Subtype B	CRF19 _cpx	CRF18 _cpx	CRF_BG (20-23-24)
	72 (100.0)	21 (100.0)	23 (100.0)	7 (100.0)	21 (100.0)
R5 Samples number (%)	43 (59.7)	13 (61.9)	9 (39.1)	6 (85.7)	15 (71.4)
R5X4 Sample number (%)	17 (23.6)	2 (9.5)	9 (39.1)	1 (14.3)	5 (23.8)
p-value R5X4			p = 0.034 OR = 3.295 CI: 1.065–0.191		
X4 Sample number (%)	12 (16.7)	6 (28.6)	5 (21.7)	0 (0.0)	1 (4.8)
R5X4/X4 Sample number (%)	29 (40.3)	8 (38.1)	14 (60.9)	1 (14.3)	6 (28.6)
p-value R5X4/X4*			p = 0.015 OR = 3.526 CI: 1.253–9.921		

*R5X4/X4: sequences with either R5X4 or X4 tropism.
CI: 95% confidence interval OR: odds ratio of CRF19_cpx vs rest of subtypes

Figure 2: Alignment of 107 V3-loop amino acid sequences obtained from Cuban HIV-1 patients.



FPR: false positive rate
Subtype B identity is indicated by dots.

Table 3: Clinical, virological and immunological variable distribution according to co-receptor prediction and subtype

Characteristics	N (%)	R5	R5X4	X4	Subtype B *env*	CRF18_cpx *env*	CRF19_cpx *env*	CRF_BG *env*
	70* (100.0)	42 (100.0)	17 (100.0)	11 (100.0)	21 (100.0)	7 (100.0)	22 (100.0)	20 (100.0)
Male sex	52 (74.3)	29 (69.0)	13 (76.5)	10 (90.9)	15 (71.4)	6 (85.7)	14 (63.6)	17 (85.0)
Sexual orientation: MSM	48 (68.6)	25 (59.5)	13 (76.5)	10 (90.9)	14 (66.7)	6 (85.7)	13 (59.1)	15 (75.0)
Years since HIV-1 diagnosis								
≤1	9 (12.9)	7 (16.7)	2 (11.8)	0 (0.0)	1 (4.8)	0 (0.0)	4 (18.2)	4 (20.0)
>1–3	9 (12.9)	6 (14.3)	2 (11.8)	1 (9.1)	2 (9.5)	0 (0.0)	4 (18.2)	3 (15.0)
>3–5	3 (4.3)	3 (7.1)	0 (0.0)	0 (0.0)	1 (4.8)	0 (0.0)	0 (0.0)	2 (10.0)
>5–10	20 (28.6)	12 (28.6)	6 (35.3)	2 (18.2)	8 (38.1)	2 (28.6)	5 (22.7)	5 (20.0)
>10	29 (41.4)	14 (33.3)	7 (41.2)	8 (72.7)	9 (42.9)	5 (71.4)	9 (40.9)	6 (30.0)
Age (years)								
<15	2 (2.9)	2 (28.6)	0 (0.0)	0 (0.0)	0 (0.0)	1 (14.3)	0 (0.0)	1 (5.0)
15–30	17 (24.3)	13 (31.0)	4 (23.5)	0 (0.0)	3 (14.3)	0 (0.0)	9 (40.9)	5 (25.0)
31–45	29 (41.4)	18 (42.9)	7 (41.2)	4 (36.4)	9 (42.9)	4 (57.1)	6 (27.3)	10 (50.0)
46–61	20 (28.6)	9 (21.4)	6 (35.3)	5 (45.5)	9 (42.9)	2 (28.6)	5 (22.7)	4 (20.0)
62–77	2 (2.9)	0 (0.0)	0 (0.0)	2 (18.2)	0 (0.0)	0 (0.0)	2 (9.1)	0 (0.0)
AIDS at diagnosis	8 (11.4)	4 (9.5)	2 (11.8)	2 (18.2)	4 (19.0)	0 (0.0)	2 (9.1)	2 (10.0)
ARV treated	68 (97.1)	40 (95.2)	17 (100.0)	11 (100.0)	21 (100.0)	7 (100.0)	21 (95.5)	19 (95.0)
Evolution to AIDS after HIV-1 diagnosis (years)								
<1	19 (27.1)	10 (23.8)	5 (29.4)	4 (36.4)	7 (33.3)	1 (14.3)	6 (27.3)	5 (25.0)
1–3	8 (11.4)	3 (7.1)	3 (17.6)	2 (18.2)	2 (9.5)	1 (14.3)	1 (4.6)	4 (20.0)
>3	18 (25.7)	8 (19.0)	6 (35.3)	4 (36.4)	5 (23.8)	3 (42.9)	6 (27.3)	4 (20.0)
No AIDS	25 (35.7)	21 (50.0)	3 (17.6)	1 (9.1)	7 (33.3)	2 (28.6)	9 (40.9)	7 (35.0)
Actual AIDS	45 (64.3)	21 (50.0)	14 (82.4)	10 (90.9)	14 (66.7)	5 (71.4)	13 (59.1)	13 (65.0)
Viral load (copies/mL)								
<1000	3 (4.3)	1 (2.4)	1 (5.9)	1 (9.1)	1 (4.8)	0 (0.0)	1 (4.6)	1 (5.0)
1000–10,000	13 (18.6)	12 (28.6)	0 (0.0)	1 (9.1)	3 (14.3)	1 (14.3)	3 (13.6)	6 (30.0)
>10,000–100,000	29 (41.4)	16 (38.1)	5 (29.4)	8 (72.7)	10 (47.6)	2 (28.6)	11 (50)	6 (30.0)
>100,000	25 (35.7)	13 (31.0)	11 (64.7)	1 (9.1)	7 (33.3)	4 (57.1)	7 (31.8)	7 (35.0)
T CD4+ cell count/mL								
<200	33 (47.1)	14 (33.3)	13 (76.5)	6 (54.5)	8 (38.1)	4 (57.1)	10 (45.5)	11 (55.0)
200–350	20 (28.6)	16 (38.1)	0 (0.0)	4 (36.4)	6 (28.6)	2 (28.6)	8 (36.4)	4 (20.0)
>350–500	11 (15.7)	8 (19.0)	2 (11.8)	1 (9.1)	6 (28.6)	1 (14.3)	2 (9.1)	2 (10.0)
>500	6 (8.6)	4 (9.5)	2 (11.8)	0 (0.0)	1 (4.8)	0 (0.0)	2 (9.1)	3 (15.0)

*Two patients were not included in the analysis due to lack of information
ARV: antiretroviral MSM: men who have sex with men

Table 4: Co-receptor use prediction distributed across different subtypes in 107 HIV-1 Cuban sequences

		Subtype in *env* region			
Sample number (%)	All Subtypes	Subtype B	CRF19 _cpx	CRF18 _cpx	CRF_BG (20-23-24)
	107 (100)	35 (100.0)	29 (100.0)	9 (100.0)	34 (100.0)
R5 Sample number (%)	66 (61.7)	22 (62.9)	10 (34.5)	8 (88.9)	26 (76.5)
p-value R5					p = 0.032 OR: 2.681 CI: 1.072–6.706
R5X4 Sample number (%)	24 (22.4)	6 (17.1)	11 (37.9)	1 (11.1)	6 (17.6)
p-value R5X4			p = 0.019 OR: 3.056 CI:1.173–7.962		
X4 Sample number (%)	17 (15.9)	7 (20.0)	8 (27.6)	0 (0.0)	2 (5.9)
p-value X4			p = 0.044 OR: 2.921 CI:1.001–8.518		
R5X4/X4 Sample number (%)	41 (38.3)	13 (37.1)	19 (65.5)	1 (11.1)	8 (23.5)
p-value R5X4/X4			p = 0.0004 OR:4.836 CI:1.945–12.025		

*OR: odds ratio of CRF19_cpx vs rest of subtype CI: 95% confidence interval

been correlated with advanced disease stage and low levels of CD4+ T cell count.[28–30] Nevertheless, it has been hypothesized that dual-tropic viruses could represent variants with the same potential as R5 viruses to evade immune response and also infect cells expressing CXCR4. The broadening in cell tropism of the viral population to include CXCR4-expressing cells would result in increased CD4+ T cell death and further immune impairment.[7]

To our knowledge, this is the first report on the association of CRF_BG viruses and R5 tropism. Previously, a link between CRF18_cpx viruses and R5 tropism had been reported.[15] We found no significant relationship between those variables in the current study.

We also observed some changes in the aminoacidic sequence of the V3 loop among different subtypes. Particularly, some motifs other than GPG were found at the tip of the loop. In accordance with previous observations, GPGR motifs prevailed in subtype B, while the GPGQ motif was predominant among Cuban recombinants.[31,32] The GPGR motif is a very important target of neutralizing antibodies in subtype B viruses, so its difference with other subtypes should be considered for assessing monoclonal antibodies and peptide vaccine design.

In 1997, Milich et al. reported a high frequency of valine (V) at position 19 in syncytium-inducing viruses. According to these authors, the interaction between 19V and phenylalanine (F) at position 20 would contribute to a switch in tropism from non-syncytium–inducing to syncytium-inducing viruses.[33] This hypothesis could also explain the association between CRF19_cpx viruses and R5X4 and X4 tropism found in the current study. Considering that almost all sequences analyzed had V19, we could ask ourselves if this recombinant is capable of evolving to a change in tropism faster than other subtypes.

This study has some limitations. The number of sequences we have obtained so far is still small for ascertaining an association between co-receptor tropism and subtype; hence results need to be confirmed by future research including a greater number of patients. Additionally, currently-employed tools for HIV-1 tropism prediction, including G2P, were developed on the basis of a restricted set of subtypes, mainly subtypes B and C.[1] Therefore, their accuracy in predicting tropism for other subtypes and recombinant forms including the ones circulating in Cuba have to be assessed in the future based on their correlation with phenotypic methods. A phenotypic test was not performed due to restricted availability.

In Cuba, maraviroc could be a treatment alternative for patients experiencing failure with other HIV-1 drugs or be considered an option in combination with other regimens; however determining HIV-1 co-receptor use is mandatory before its prescription.[1,8,34] A more thorough understanding of the possible associations between co-receptor tropism and subtypes circulating in Cuba, together with phenotypic assays and the possible creation of new bioinformatic algorithms more suitable for Cuban HIV-1 recombinants' tropism prediction would facilitate proper decisions regarding treatment choice with maraviroc or other co-receptor inhibitors.

CONCLUSION

In the current study, 72 subtypes obtained from Cuban HIV-1 infected patients were sequenced and tropism was predicted employing a G2P bioinformatic tool.

Additionally, 35 other viruses that were sequenced in a former study were included for analysis. An association between CRF19_cpx and X4 and R5X4 tropism was found, which suggests that patients infected with this recombinant are probably less suitable to receive maraviroc than those infected with other HIV-1 subtypes. The study reinforces findings from previous studies on this subject and highlights the importance of HIV-1 diversity when considering pathogenesis and treatment options.[1,35,36]

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SEQUENCE DATA AND SUPPLEMENTARY MATERIAL

Sequences obtained in the present study are deposited in Genbank under access numbers MT785557–MT785663.

Supplementary Figure 3 : Alignment of 107 V3 loop amino acid sequences obtained from Cuban HIV-1 patients. Supplementary Figure 3 is available upon request from the corresponding author.

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Influence of Inflammation on Assessing Iron-Deficiency Anemia in Cuban Preschool Children

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ABSTRACT

INTRODUCTION Anemia is a public health problem worldwide and is most prevalent in preschool children, for whom it is the most frequent cause of nutritional deficits. In turn, iron deficiency is the main cause of anemia, affecting 43% of children globally. Previous studies in Cuba show rates of iron deficiency in preschool children between 38.6% and 57.6%, higher in infants (71.2% to 81.1%). WHO recommends using serum ferritin as an indicator of iron deficiency accompanied by acute (C-reactive protein) and chronic (α1-acid glycoprotein) inflammation biomarkers.

OBJECTIVE Assess how inflammation affects measuring and reporting of iron-deficiency anemia rates in Cuban preschool children.

METHODS Data were obtained from serum samples contained in the National Anemia and Iron Deficiency Survey, and included presumably healthy preschool Cuban children (aged 6–59 months). Serum samples were collected from 1375 children from randomly selected provinces in 4 regions of the country from 2014 through 2018. We examined the association between ferritin and two inflammatory biomarkers: C-reactive protein and α1-acid glycoprotein. Individual inflammation-adjusted ferritin concentrations were calculated using four approaches: 1) a higher ferritin cut-off point (<30 g/L); 2) exclusion of subjects showing inflammation (C-reactive protein >5 mg/L or α1-acid glycoprotein >1 g/L); 3) mathematical correction factor based on C-reactive protein or α1-acid glycoprotein; and 4) correction by regression with the method proposed by the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia Group. We estimated confidence intervals of differences between unadjusted prevalence and prevalence adjusted for inflammation by each method.

RESULTS The proportion of children with inflammation according to C-reactive protein concentrations >5 mg/L was lower (11.1%, 153/1375) than the proportion measured according to the concentrations of α1-acid glycoprotein, at >1 g/L (30.8%, 424/1375). The percentage of children with high concentrations of at least one of the aforementioned biomarkers was 32.7% (450/1375). Thus, each correction method increased the observed prevalence of iron deficiency compared to unadjusted estimates (23%, 316/1375). This increase was more pronounced when using the internal regression correction method (based only on C-reactive protein) or the method based on a higher cut-off point. Adjustment using all four methods changed estimated iron deficiency prevalence, increasing it from 0.1% to 8.8%, compared to unadjusted values.

CONCLUSION One-third of preschool children had biomarkers indicating elevated inflammation levels**.** Without adjusting for inflammation, iron deficiency prevalence was underestimated. The significant disparity between unadjusted and inflammation-adjusted ferritin when using some approaches highlights the importance of selecting the right approach for accurate, corrected measurement. The internal regression correction approach is appropriate for epidemiological studies because it takes into account inflammation severity. However, other models should be explored that account for inflammation and also provide better adjusted ferritin concentrations.

KEYWORDS Anemia, iron deficiency; child, preschool; inflammation; Cuba

INTRODUCTION

Anemia is a public health problem affecting millions of persons globally. It is most frequent in preschool children,[1] with an estimated 273 million affected worldwide. Among them, 43% have iron-deficiency anemia.[2] Previous studies in Cuba show iron deficiency rates in preschool children between 38.6% and 57.6%,[3–5] while higher rates have been found in infants (71.2%–81.1%).[6] Iron deficiency occurs mainly in the first two years of life due to the low iron content and decreased bioavailability of supplementary foods that do not cover a developing child's nutritional requirements, in addition to children's rapid growth rate during their first year of life.[7]

IMPORTANCE

This is the first national study in Cuba that confirms the usefulness of two inflammation biomarkers for adjusting ferritin concentrations in preschool children, resulting in a more accurate determination of iron-deficiency anemia.

WHO considers serum ferritin concentration as the best iron level indicator.[7] Ferritin, however, is also an acute-phase protein (APP) that increases during inflammatory processes. Because inflammation levels cannot be determined through routine clinical examination in apparently healthy persons, WHO suggests confirming inflammation by measuring ferritin in addition to other APPs.[7]

Several approaches have been proposed to correct the bias in ferritin measurement introduced by inflammation,[8–13] but a consensus has not been reached on how to use APPs to adjust for inflammation's effect on ferritin concentration. The most common inflammation biomarkers used in clinical practice and nutritional research include C-reactive protein (CRP) and α-1 acid

glycoprotein (AGP).[14] In populations with high inflammation prevalence, failure to assess acute-phase proteins could greatly impact micronutrient deficiency prevalence measurements, specifically those pertaining to iron deficiency.[14]

The Cuban population is thought to have low levels of inflammation[15] since it does not have case reports of infection due to malaria and schistosomiasis and it benefits from safe drinking water and sanitation. However, assessing inflammation when analyzing the population's iron status is important, as there are other risk or morbidity factors in Cuba associated with inflammation such as obesity, asthma, acute diarrheal diseases (ADD), acute respiratory infections (ARI) and other infections.[14]

Cuba's National Hygiene, Epidemiology and Microbiology Institute (INHEM) has been in charge of the design, implementation and surveillance of varying prevention and control programs for iron status since 1987. The Comprehensive Plan for Prevention and Control of Iron-deficiency Anemia was established in 2008 by the Council of Ministers.[16] It includes programs for food supplements, fortified foods and diet diversity, plus program surveillance. In spite of this, iron-deficiency anemia prevalence remains high in preschool children.

Correctly assessing iron deficiency and the factors associated with it (inflammation; nutritional status; related comorbidities; and genetic, physiologic and socio-demographic factors) would help create more effective prevention strategies better able to respond to the causes of anemia in Cuba.

The aim of this paper is to assess how inflammation may influence the measurement and reporting of iron status in Cuban preschool children.

METHODS

INHEM carried out a cross-sectional study in Cuban preschool children during February through April annually from 2015 to 2018. Data from the National Survey of Anemia and Iron Deficiency in Cuban Preschool Children (as yet unpublished) were used for analysis.

Sample selection The study universe included children aged 6–59 months. The sample consisted of 1400 children. The sample size was calculated assuming an overall anemia prevalence of 20% in the country in this age group, a predicted sample size reduction of 10% due to non-response, a confidence level of 95%, a relative precision of <3% and a design effect of 2%.

Cuba has 15 provinces and one Special Municipality. The sample was stratified by region: west, central, east (northeast and southeast) and the province of Havana, the latter considered a region in itself. Each region was represented by either one or two provinces. Two provinces were selected at random in the western and central regions: Mayabeque Province and the Isle of Youth Special Municipality, considered here as a province, in the west; and Sancti Spíritus and Cienfuegos Provinces in the central region. For the northeast and southeast regions, we randomly selected only one province per region: Holguín Province for the northeast, and Santiago de Cuba province for the southeast. We randomly selected 30% of municipalities from each province, which included the municipal capital.

Primary health care in Cuba is organized under municipal health departments, under which multispecialty community polyclinics act as hubs and supervise the work of family doctor-and-nurse offices located in the surrounding geographic health areas. The entire population in each health area is served by both the local polyclinic and family doctor-and-nurse offices.[17] In consideration of this structure, we used two-stage cluster sampling, in which health areas were the primary selection units in each municipality, and family doctor-and-nurse offices were secondary units. Using this method, 200 children were studied in each of the selected provinces.

We selected the minimum number of doctors' offices required to reach the specific sample size, considering the average number of children served by the offices in each municipality. All children aged 6–59 months in the catchment area of the selected family doctor's office were included in the sample.

The study included presumably healthy children, free of chronic disease (sickle cell anemia, diabetes, kidney disease, epilepsy, severe or moderate asthma, or any other disease requiring treatment or specialized medical care). Based on these criteria, we enrolled 1417 children in the survey database. A total of 1375 children with complete records on serum ferritin and inflammation (CRP and AGP) were finally included.

Biochemical data Three mL of blood were taken through antecubital puncture. Samples were centrifuged the same day as extraction, and blood serum was stored at –40 ˚C for later analysis for ferritin and inflammation indicators. Iron deficiency was measured through ferritin concentration, and inflammation was measured through high-sensitivity CRP and AGP in the serum. Indicators for ferritin and inflammation were determined by the immuno-turbidmetric method using INLAB 240 equipment (CPM Scientifica Tecnologie Biomediche, Italy). The limit of detection (LOD) reported for CRP, AGP and ferritin was 0.1 mg/L, 0.04 g/L and 5.2 µg/L respectively. When the CRP, AGP or ferritin concentrations were lower than that of the LOD, they were set equal to their respective LOD's and reported as such by the laboratories. These determinations were made by trained personnel at INHEM's Nutritional Anemia Laboratory using quality control reference guides from CPM. The National Metrology Research Institute calibrated the equipment.

Defining cases Iron deficiency was defined as ferritin concentrations <12 µg/L, a cut-off point recommended by WHO.[8] Inflammation was declared when CRP concentrations were >5 mg/L, AGP concentrations were >1 g/L, or both.[18]

Statistical analysis Data management and statistical analyses used R software version 3.5.3 (Free Software Foundation, USA).[19] Simple statistics and histograms were used to study distribution of biochemical variables. All variables showed some type of positive asymmetry and were transformed logarithmically.

We used four approaches to eliminate inflammation's influence on ferritin concentration: 1) an increase in the cut-off point;[7] 2) exclusion of subjects with inflammation from the analysis, as explained;[20] 3) an inflammation correction factor;[18] and 4) correction by linear regression via the method proposed by Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA).[20]

To evaluate the influence of each inflammation biomarker (CRP and AGP) on ferritin concentration, all analyses were made using both individual and combined biomarkers. We estimated iron deficiency prevalence and referenced these estimates as non-proportional estimations on sampling. We calculated 95% confidence intervals (CI) of the differences between the unadjusted prevalences and those adjusted for inflammation, by each method.

To identify potential relationships between inflammation and age, the study also obtained separate results for two age groups: children aged 6–23 months (<2 years) and children aged 24–59 months (≥2 years).

Inflammation Correction Approaches

Increasing the cut-off point This approach classifies those individuals with inflammation (concentrations of CRP >5 mg/L or AGP >1 g/L), as having iron deficiency when serum ferritin concentrations are <30 g/L.

Subject exclusion This discards individuals with high levels of inflammation biomarker concentrations (concentrations of CRP >5 mg/L or AGP 1 g/L) and calculates the prevalence of iron deficiency for individuals without inflammation.

Correction factor The internal correction factor (ICF) approach, proposed by Thurnham,[18] uses mathematical correction factors. Individuals are classified into four groups by states of inflammation: 1) reference (CRP ≤5 mg/L and AGP ≤1 g/L); 2) incubation (CRP >5 mg/L and AGP ≤1 g/L); 3) early convalescence (CRP >5 mg/L and AGP >1 g/L); and 4) late convalescence (CRP ≤5 mg/L and AGP >1 g/L).

Additionally, we calculated ICF for two groups (those with inflammation and those without inflammation) for each biomarker (CRP and AGP), separately.

Internal Correction Factors Based on internal survey-specific data, internal correction factors were then generated by dividing the geometric mean (GM) of ferritin values of the non-inflammation group by GM ferritin values of each inflammation group:

$$ICF = GM_{ref} / GM_{inflam}$$

Where *ref* and *inflam* are the reference and the inflammation groups, respectively.

Correction factors are defined as the ratio between the ferritin GM of the reference group and the ferritin GM for each inflammation group.

Ferritin values for those in the groups with high inflammation biomarker concentrations are multiplied by the ICF corresponding to their inflammation group to obtain adjusted ferritin values. To compare ferritin concentrations between the subgroup without inflammation and each subgroup with inflammation, we used the Student t-test to compare geometric means, and we obtained 95% CI for the ratio of geometric means.

Correction by regression This approach (internal regression correction, IRC) is based on linear regression models used to adjust for ferritin concentrations in the presence of inflammation. Following methodology proposed by BRINDA,[20] we used regression coefficients of models where the response variable is the logarithm of the ferritin concentrations, and where the transformed variables ln(CRP) and ln(AGP) are included as continuous predictors.

Multivariate regression that considers both biomarkers (AGP and CRP) was used to show different stages of inflammation. To adjust for individual concentrations of ferritin, in the multivariate case, the influence of CRP and AGP is subtracted:

$$\ln ferritin_{adj} = \ln ferritin_{unadj} - \beta_1[\ln CRP_{obs} - \ln CRP_{ref}] - \beta_2[\ln AGP_{obs} - \ln AGP_{ref}],$$

Where the subindexes *adj* and *unadj* refer to adjusted and unadjusted values of ferritin concentration, and β_1 and β_2 are regression coefficients for CRP and AGP, respectively. The subindex *obs* refers to the values observed for the independent variables and the subindex *ref* refers to the inflammation reference values assuming that these mark the cut-off points for the CRP and AGP biomarkers, which show the increase in ferritin concentrations.

The reference value is defined as the maximum value of the lowest decile of the specific marker (AGP or CRP). The reference values we obtained were CRP = 0.10 mg/L and AGP = 0.54 g/L. Adjustments were applied only in the case of CRP >$\ln CRP^{ref}$, AGP >$\ln AGP^{ref}$, or both.

A multi-colinearity test was performed between ln(CRP) and ln(AGP), on the basis of a tolerance test (>0.1) and a variance inflation factor (<5) to determine whether it was appropriate to include both variables in the model. To avoid over-adjustment, the correction was applied only to the ferritin concentrations that corresponded to individuals with values of CRP or AGP that were greater than the reference values.

The analysis also eliminated individuals with censored data (CRP <LOD), taking into account that adjustment of the regression models with censored variables may produce bias when estimating the model's coefficients.[21]

Ethics The study was authorized by the Maternal–Child Division of the Ministry of Public Health, after reviewing the research's ethical aspects. All mothers gave informed consent to include their children in the study. Our work was conducted according to principles for conducting research in human subjects outlined by the Helsinki Declaration,[22] and the protocol was approved by the research ethics committee assigned to this project.

RESULTS

Participant characteristics The sample distribution was as follows, by age and sex: 36.4% (500/1375) aged <2 years; 63.6% (875/1375) aged ≥2 years; 51.1% (703/1375) male; 48.9% (672/1375) female.

The median CRP value was 0.91 mg/L (quartile 1 = 0.52; quartile 3 = 1.83), far below the cut-off point. The median AGP value was 0.86 g/L (quartile 1 = 0.69; quartile 3 = 1.07), indicating that more than 25% of children had values above the cut-off point.

Inflammation prevalence assessed by CRP concentrations >5 mg/L was less (at 11.1%, 153/1375) than that assessed by AGP concentrations >1 g/L (30.8%, 424/1375). The proportion of children with high concentrations of at least one biomarker was 32.7% (450/1375).The highest inflammation prevalence was found in those aged <2 years (n = 500) for all 3 inflammation conditions (CRP 11.8%, 59/500; AGP 33.4%, 167/500; or both 34.6%, 173/500), with no significant differences between age groups (CRP p = 0.610, AGP p = 0.156, both p = 0.325).

Inflammation correction approaches

Increasing the cut-off point Iron deficiency prevalence increased in the entire sample from 23% (316/1375) to 31.8% (437/1375). For children aged <2 years, it increased by 9.8 percentage points and in those aged ≥2 years, it increased by 8.3 percentage points (Table 1).

Subject exclusion Eliminating cases where inflammation was present resulted in a 32.7% (450/1375) reduction in cases. Ferritin values before and after these exclusions yielded a slight reduction in median concentrations, both in the whole sample and in the two age groups, with no significant differences between the two age groups. The decrease in concentration was about 3 µg/L (Table 2).

Iron deficiency prevalence values ranged from 23% (316/1375) before excluding cases, to 26% (240/925) for the entire sample. Iron deficiency increased from 25% (125/500) to 28.4% (93/327) for the group aged <2 years and from 21.8% (191/875) to 24.6% (147/598) for those aged ≥2 years, after excluding subjects with inflammation (Table 1).

Internal Correction Factor Ferritin concentration in the reference group was compared to each of the three inflammation groups in each age category. The geometric mean of ferritin was lower in children without inflammation compared to those with inflammation in all groups (Table 3).

Considering the entire sample, the ratio of the ferritin concentration geometric means for each inflammation group and the reference group produced values >1 for the five comparison scenarios. In other words, the quotient of ferritin's geometric mean was higher in the corresponding inflammation group than in the reference group (Table 3). Specifically, the geometric mean of ferritin was 84% (39.76/21.57) higher in the incubation group than in the reference group, 67% (36.09/21.57) higher in the early convalescence group than in the reference group, and only 19% (25.68/21.57) higher in the late convalescence group. The ratio of geometric means was

Table 1: Prevalence of iron deficiency and confidence interval by age group, without adjustment and after adjusting for ferritin concentrations in the presence of inflammation

Approach	All		<2 years old		≥2 years old	
	n/N	% (95% CI)	n/N	% (95% CI)	n/N	% (95% CI)
Unadjusted	316/1375	23.0 (20.8–25.3)	125/500	25.0 (21.4–29)	191/875	21.8 (19.2–24.7)
Higher cut-off ferritin <30 µg/L						
With inflammation defined as:						
CRP >5 mg/L	347/1375	25.2 (23.0–27.6)	140/500	28.0 (24.2–32.1)	207/875	23.7 (21.0–26.6)
AGP >1 g/L	430/1375	31.3 (28.9–33.8)	173/500	34.6 (30.6–38.9)	257/875	29.4 (26.5–32.5)
CRP >5 mg/L or AGP >1 g/L	437/1375	31.8 (29.4–34.3)	174/500	34.8 (30.8–39.1)	263/875	30.1 (27.1–33.2)
Exclusion defined as:						
CRP >5 mg/L	300/1222	24.6 (22.2–27.0)	118/441	26.8 (22.8–31.1)	182/781	23.3 (20.5–26.4)
AGP >1 g/L	242/951	25.5 (22.8–28.3)	94/333	28.2 (23.7–33.3)	148/618	24.0 (20.8–27.5)
CRP >5 mg/L or AGP >1 g/L	240/925	26.0 (23.2–28.9)	93/327	28.4 (23.8–33.6)	147/598	24.6 (21.3–28.2)
Internal Correction Factor						
ICF–CRP	333/1375	24.2 (21.9–27.5)	134/500	26.8 (23.0–30.7)	199/875	22.7 (20.2–25.5)
ICF–AGP	336/1375	24.4 (22.2–26.7)	136/500	27.2 (23.3–31.1)	200/875	22.9 (20.1–25.6)
ICF–CRP + AGP	342/1375	24.9 (22.9–27.2)	138/500	27.6 (23.7–31.5)	204/875	23.3 (20.5–26.1)
Internal Regression Correction						
Including observation with CRP ≤LOD						
IRC–CRP	409/1375	29.8	148/500	29.6	258/875	29.5
IRC–AGP	317/1375	23.1	125/500	25.0	194/875	22.2
IRC–CRP + AGP	394/1375	28.7	143/500	28.6	248/875	28.3
Excluding observation with CRP ≤LOD						
IRC–CRP*	337/1210	27.9	134/429	31.2	199/781	25.5
IRC–CRP + AGP*	294/1210	24.3	114/429	26.6	182/781	23.3

AGP: α-1 acid glycoprotein CI: confidence interval CRP: C-reactive protein ICF: Internal Correction Factor IRC: Internal Regression Correction LOD: limit of detection

n: number of individuals with inflammation; N: number of total individuals in the group. The increase in the cut-off point approach (higher cut-off; AGP + CRP) considers individuals with high concentrations of inflammation biomarkers (CRP >5 mg/L and AGP >1 g/L) to be iron deficienct if ferritin concentrations are <30 µg/L. The exclusion approach (exclusion, AGP + CRP) discards individuals with high inflammation biomarker concentrations (CRP >5mg/L and AGP >1 g/L) and calculates the prevalence of iron deficiency for individuals without inflammation. The correction factor approach (ICF-AGP + CRP) uses correction factors. Correction factors are defined as the ratio between the geometric mean of the ferritin values of the reference group (CRP and AGP are not increased) and ferritin's geometric mean for each inflammation group. The correction by regression approach (IRC-AGP + CRP) uses linear regression to adjust serum ferritin concentrations using CRP and AGP concentrations as continuous predictors.
* The exclusion approach excludes individuals with censored CRP values (CRP <0.1 mg/L than the LOD). Regression coefficients depend on the sample so they are different for each study group and for the sample in its entirety, therefore the sum of number of individuals with iron deficiency after adjusting for inflammation using ICR on each study group does not necessarily equal the number of individuals with iron deficiency after adjusting for inflammation using ICR on the entire sample. Values indicate prevalence, % (95% CI, where applicable); 95% CIs are not reported for the internal correction regression method because they do not take into account variability in the estimates for the regression coefficients of the slopes used to derive adjusted ferritin values.

Table 2: Median ferritin concentration (µg/L) before and after excluding subjects with high CRP/AGP concentrations

Age group	Median ferritin concentration (Quartile 1–Quartile 3)							
	All subjects		After excluding subjects					
	n	Unadjusted	n	Concentrations of CRP >5 mg/L	n	Concentrations of AGP >1 g/L	n	Concentrations of CRP >5 mg/L or AGP >1 g/L
<2 years	500	23.6 (12.1–50.7)	441	21.9 (11.3–48.3)	333	20.7 (10.5–46.9)	327	20.0 (10.3–46.7)
≥2 years	875	26.6 (13.7–47.7)	781	25.0 (13.0–44.2)	618	24.0 (12.7–43.6)	598	23.6 (12.5–42)
Total	1375	25.4 (13.1–48.8)	1222	24.1 (12.4–45.9)	951	23.0 (11.7–44.4)	925	22.8 (11.6–43.6)

AGP: α-1 acid glycoprotein CRP: C-reactive protein

Table 3: Ferritin adjusted for inflammation using the correction factor approach

	Children		Ratio[1] (95% CI)		p	CF
	With inflammation n	Without inflammation n				
CRP[1]						
<2 years old	59	441	1.54	(1.20–2.00)	0.001	0.65
≥2 years old	94	781	1.68	(1.41–2.02)	<0.001	0.59
All	153	1222	1.63	(1.41–1.89)	<0.001	0.61
AGP[1]						
<2 years old	167	333	1.30	(1.08–1.55)	0.004	0.77
≥2 years old	257	618	1.30	(1.15–1.47)	<0.001	0.77
All	424	951	1.29	(1.17–1.43)	<0.001	0.77
Incubation vs Reference[2]						
<2 years old	6	327	1.78	(0.82–3.83)	0.143	0.56
≥2 years old	20	598	1.85	(1.27–2.69)	0.0014	0.54
All	26	925	1.84	(1.31–2.59)	0.0005	0.54
Early Convalescence vs Reference[2]						
<2 years old	53	327	1.60	(1.22–2.10)	0.0008	0.62
≥2 years old	74	598	1.74	(1.42–2.13)	<0.001	0.58
All	127	925	1.67	(1.42–1.97)	<0.001	0.60
Late Convalescence vs Reference[2]						
<2 years old	114	327	1.19	(0.98–1.47)	0.085	0.83
≥2 years old	183	598	1.19	(1.03–1.37)	0.016	0.84
All	297	925	1.19	(1.06–1.33)	0.003	0.84

AGP: α-1 acid glycoprotein CF: correction factor CI: confidence interval
CRP: C-reactive protein
The ratio column indicates the ratio of the geometric means of ferritin concentrations (95% CI) in µg/L for: [1] groups with and without inflammation as indicated by CRP or AGP concentration
[2] inflammation group versus reference group comparisons; reference: CRP concentration ≤5 mg/L and AGP concentration ≤1 g/L; incubation: CRP concentration >5 mg/L and AGP concentration ≤1 g/L; early convalescence: CRP concentration >5 mg/L and AGP concentration >1g/L; late convalescence: AGP concentration >1 g/L and CRP concentration ≤5 mg/L; p: p values associated to a test for comparing geometric means

significantly higher than 1 (p <0.05) in all variants, except for the group aged <2 years in incubation and late convalescence groups.

Estimated iron deficiency prevalence for the entire sample with ICF increased from 23% (316/1375) to 24.4% (336/1375), adjusted only for AGP, while estimated prevalence for AGP and CRP increased from 23% (316/1375) to 24.9% (342/1375) (Table 1).

Internal Regression Correction Results of this analysis are shown in Table 4. For CRP, the reference value calculated (0.1 mg/L) was the same in each group. For AGP, the reference values were 0.51 g/L, 0.55 g/L and 0.54 g/L for the two age groups and the entire sample, respectively. Excluding children with censored values of CRP (CRP <LOD) produced an increase in the value of the slope between concentrations of ln(ferritin) and ln(CRP) in both models (univariate analysis for all: 0.105 vs. 0.158; multivariate analysis for all: 0.132 vs. 0.189) indicating that exclusion of censored data may bias coefficient estimation, and increase the effect of inflammation on ferritin. The exclusion reduced sample size by 12% from 1375 to 1210.

Exploratory analysis and descriptive statistics (not considered in this paper) show that children with undetected CRP values tend, on average, to be less iron deficient.

The estimated regression coefficient in multivariate analysis for the association between ln(ferritin) and ln(CRP) concentrations was higher than the corresponding values in the univariate analysis (the entire sample, excluding children with CRP <LOD: 0.158 vs. 0.189). This indicates that this relationship is better adjusted by including ln(AGP) in the model, which, because of its negative effect, tends to balance the effect of ln(CRP).

Estimating prevalence Use of this methodology produced an increase in estimated iron deficiency prevalence in the entire sample, from 23% (316/1375) to 28.7% (394/1375) when the censored values were included and to 24.3% (294/1210) when they were excluded (Table 1). Prevalence ranges obtained from models with significant coefficients varied between 24.3% (294/1210) (IRC–CRP + AGP, excluding children with CRP <LOD), and 29.8% (409/1375) (IRC–CRP), which represents an underestimation (from 1.3% to 6.8%) of the prevalence observed without adjustment.

Before making decisions based on these results, it is important to consider the margin of error in relation to a specific estimation of differences between unadjusted prevalences and those adjusted for inflamation. Figure 1 summarizes the CI of such differences, as estimated by each method. For example, in studies considering all children in the sample, the specific estimation of the difference between the unadjusted prevalence and the prevalence adjusted by the higher cut-off (CRP) approach is 0.02 and a 95% CI ensures us that the population difference is in the range of **–**0.01–0.056.

Intervals corresponding to the higher cut-off point approach, where AGP is considered either alone or combined with CRP, do not contain the zero value. This indicates that the unadjusted iron deficiency prevalence is significantly different from the adjusted prevalence (95% CI). In all intervals corresponding to differences between unadjusted and adjusted prevalences by exclusion or ICF approaches, the range of values includes differences that are not significant. Differences between the unadjusted prevalence

Table 4: Univariate and multivariate regression models of ln(ferritin) concentrations and inflammation markers, by subpopulation

	n	Intercept	lnCRP	p	lnAGP	p	Adjusted R^2
Univariate analysis (by age group)							
<2 years	500	3.136	0.077	0.011	-	-	0.011
≥2 years	875	3.190	0.123	<0.001	-	-	0.035
All	1375	3.171	0.105	<0.001	-	-	0.025
<2 years	500	3.131	-	-	<0.011	0.907	0.002
≥2 years	875	3.197	-	-	0.046	0.521	0.001
All	1375	3.172	-	-	0.020	0.718	0.001
Excluding children with CRP <LOD <2 years	429	3.075	0.153	<0.001	-	-	0.030
Excluding children with CRP <LOD ≥2 years	781	3.167	0.162	<0.001	-	-	0.044
Excluding children with CRP <LOD All	1210	3.131	0.158	<0.001	-	-	0.038
Multivariate analysis (by age group)							
<2 years	500	3.107	0.106	0.003	<0.169	0.108	0.014
≥2 years	875	3.156	0.147	<0.001	<0.177	0.026	0.039
All	1375	3.137	0.132	<0.001	<0.178	0.005	0.030
Excluding children with CRP <LOD <2 years	429	3.033	0.19	<0.001	<0.205	0.079	0.034
Excluding children with CRP <LOD ≥2 years	781	3.129	0.186	<0.001	<0.152	0.079	0.046
Excluding children with CRP <LOD All	1210	3.094	0.189	<0.001	<0.177	0.011	0.043

AGP: α-1 acid glycoprotein CF: correction factor CI: confidence interval CRP: C-reactive protein
LOD: limit of detection

The values of the columns named intercept, ln(CRP, mg/L) and ln(AGP, g/L) are specific estimations of each model's coefficients. AGP (g/L); CRP (mg/L); LOD of the CRP (0.1 mg/L). CRP and AGP were included in the models as log-transformed covariables. The regression coefficients (except for the intercept), indicate the change in ln(ferritin) associated with the change in ln(CRP) or ln(AGP) per one unit, since the rest of the variables remain constant. Column p contains the values associated with the significance tests of the regression coefficients.

and prevalences adjusted by the IRC method (whether considering CRP alone, or CRP + AGP, as explanatory variables) are significant.

Stratified analyses yield very wide CIs for the whole sample and for both age groups.

DISCUSSION

Chronic inflammation (assessed by AGP) was more frequent than acute inflammation, although with concentrations only slightly above the cut-off point. The highest inflammation rates diagnosed by AGP have been reported in Asian and African countries by the BRINDA study.[23]

Nonetheless, even though high CRP levels were found only in about 10% of children in the study, some had concentrations much higher than the cut-off point. Chronic inflammation, assessed by AGP, is associated with a higher frequency of obesity and asthma in children[18] and high CRP values are associated with systemic bacterial infections, tissue damage and allergies.[14] Asymptomatic infections in children can also increase CRP and AGP.[18]

Cuba is a Caribbean island country in the tropics, with high temperatures and high relative humidity, which favors development of infectious disease.[24,25] Children aged <5 years show the highest rates of ADD and ARI.[26] The main causes of ADD in Cuba are rotaviruses and noroviruses;[27] for ARI the main cause is rhinovirus, and human sincitial respiratory virus in children aged <1 year.[28]

The norovirus as a cause of inflammation was described in a longitudinal study in infected adults.[29] A paper on preschool children in Gambia[30] observed that iron-deficiency anemia was associated even with the low degree of inflammation produced by respiratory infections. These viral infections should therefore be considered in assessing inflammation and estimating iron deficiency.

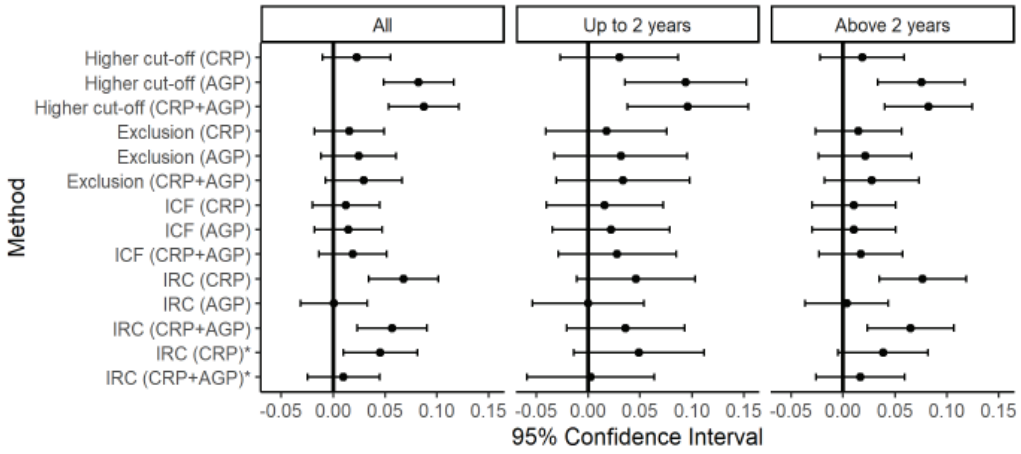
Iron deficiency rates estimated in this study, without adjusting for inflammation, are lower than in previous studies. This may be due to the effect of food and nutritional intervention programs existing in Cuba.[31,32] In studies of preschool children in Havana[3] and in Cuba's eastern provinces[4,5] iron deficiency was found to be one of the main risk factors associated with anemia (ferritin <10 mg/L in 41.8% of those in Havana, 38.6% in the provinces of Santiago de Cuba, Holguín, Granma and Las Tunas, and 57.6% in Guantánamo). However, the cut-off point used to define iron deficiency through ferritin was lower, and in those studies inflammation was not assessed as a factor influencing increased serum ferritin levels.

Approximately 50% of main acute-phase proteins (such as ferritin) are related to transportation of nutrients or regulation of nutrient concentration.[18] At times or conditions in which there is asymptomatic inflammation, a protective mechanism involving nutrient sequestering has been proposed. In this case, increased ferritin levels will limit the availability of iron for pathogen growth or function.[33] Evaluating the relationship between inflammation and ferritin is fundamental for more accurate estimations of iron deficiency in the population.

Inflammation-adjusted ferritin concentrations increased iron deficiency prevalence in Cuban preschool children. Adjusted prevalence showed high variability among different approaches, ranging from 0.1 to 8.8 percentage points, similar to that obtained in a previous study.[34] As discussed in BRINDA, each approach has advantages and disadvantages.[20]

We found that correcting ferritin levels for inflammation using an increased cut-off point yielded an important change in the estimation of iron deficiency prevalence as compared to unadjusted estimations. In this approach, AGP exerts the highest influence, since almost one third of the sample had high AGP values. Note that increasing the cut-off point may lead to a reduction in biomarker specificity by increasing the proportion of false negatives.[35]

Figure 1: Diferences in proportions and confidence intervals (95%) between the methods with unadjusted values. Cuba 2015–2018



Footnote: Differences in proportions and confidence intervals (95%) to test the proportion comparison hypothesis for the prevalence of iron deficiency without adjustment and after applying the methods used to adjust the ferritin concentrations in the presence of inflammation. The approach using the increase of the cut-off point (Higher cut-off; AGP + CRP) considers individuals with high concentrations of the inflammation biomarkers (concentrations of CRP >5 mg/L and AGP >1 g/L) with iron deficiency if the ferritin concentrations are <30 mg/L. The exclusion approach (Exclusion; AGP + CRP) discards the individuals with high concentrations of inflammation biomarkers (concentrations of CRP >5 mg/L and AGP >1 g/L) and it calculates the prevalence of iron deficiency for individuals without inflammation. The internal correction factor (ICF-AGP+CRP) uses mathematical correction factors. The correction factors are defined as the ratio between the geometric mean of the values of ferritin in the reference group (CRP and AGP are not high) and the geometric mean of ferritin of each inflammation group. The correction through regression approach (IRC-AGP + CRP) uses linear regression to adjust the concentration of serum ferritin with the concentrations of CRP and AGP as continuous predictors. The asterisk (*) denotes the exclusion from the analysis of the individuals censored by CRP (Values of CRP <0.1 mg/L, LOD).

The exclusion approach increased iron deficiency prevalence estimation but resulted in a loss of precision due to an almost one-third reduction in sample size (by excluding children with high CRP + AGP). Iron deficiency rates obtained by using either CRP or AGP alone yielded very similar prevalence estimates to those obtained when using the biomarkers together.

ICF has the advantage of using correction factors calculated specifically for Cuban preschool children. Sample sizes in the study separated by inflammation group (or by groups in which CRP and AGP were analyzed separately) were large enough to accurately estimate correction factors for inflammation.

Ferritin concentrations increased during periods of inflammation, mainly during the incubation period and early convalescence. During late convalescence, ferritin concentration decreased slightly when compared to the group where there was no inflammation. Hence, in the ICF approach, AGP has a smaller effect on iron deficiency prevalence adjustment.

IRC uses linear regression to adjust ferritin according to CRP and AGP concentrations on a continuous scale. It also has the advantage of using internal reference values calculated from the sample of Cuban preschool children. This approach led to an important decrease in ferritin concentration estimations. The IRC approach yielded higher estimates of iron deficiency prevalence than the ICF approach.

In contrast, the IRC approach requires more careful analytical interpretation. The quality of the models should be evaluated by how well they describe the relationship between inflammation biomarkers (CRP and AGP) and ferritin values.

Coefficient (slope) estimations of univariate regression models with AGP as a single predictor were all non-significant, suggesting that AGP does not have a linear effect on ferritin concentration. When AGP is included in the multivariate model (IRC, CRP and AGP), iron deficiency prevalence differs by only 1.1% compared to the univariate model (IRC–CRP) (29.8%). This result differs from the literature concerning the expected positive relationship between ferritin and AGP as an indicator of inflammation.[20,21] Before making decisions that may lead to erroneous judgments, non-linear regression models must be assessed in future studies, as the effect of inflammation biomarkers on ferritin measurements could change when AGP values increase.

Iron deficiency prevalence estimates were lower when censored data was excluded, but slightly higher than the unadjusted value. This contrasts with other findings,[21] where ignoring censored data of CRP <LOD produced a substantial bias in iron deficiency estimates.

The difference between unadjusted and adjusted prevalences for inflammation by the highest cutoff approach showed statistically non-significant differences when ferritin concentrations were adjusted for CRP alone. When considering AGP in the analysis, the intervals for AGP and CRP + AGP are practically equal, therefore including CRP will not provide any benefit to the adjusted prevalence. Using the exclusion approach, no significant differences were found in any case, but before making decisions based on the statistical results, it should be taken into account that the interval corresponding to the CRP + AGP case includes positive differences with important practical implications that must be evaluated.

In the IRC approach, differences between adjusted and unadjusted prevalences are statistically significant, both when considering CRP alone or with CRP + AGP. However, significant results would not justify the use of adjusted prevalences or efforts to measure and include AGP. It should be noted that when both biomarkers were included in the linear regression model, both coefficients were significant, but AGP's effect turned negative.

As shown in other studies,[14,18] this research supports the hypothesis that the magnitude of ferritin concentration change depends on inflammation stage. From these results we concluded that it was necessary to adjust ferritin concentrations according to inflammation, since, if unadjusted, iron deficiency prevalence may be underestimated.[34] However, the results also support the conclusion that we should search for other models that enable the study of the effects of inflammation at higher ferritin concentrations. This study is the first time that measurements of

both inflammation biomarkers (CRP and AGP) and iron deficiency (by ferritin) in Cuban preschool children are included. Information on the prevalence of these factors in Cuba provides new insights that could allow decision-makers to adjust Cuba's childhood nutrition programs.

One study limitation is that data were taken from a national sample from selected provinces. In addition, its cross-sectional nature precludes the analysis of any seasonal influence of inflammation on ferritin concentrations .

CONCLUSIONS

One third of preschool children in the sample had elevated indicators of inflammation. This study confirms that inflammation is a confounding factor in estimating iron status during the first four years of life. Ignoring inflammation would result in underestimating iron deficiency prevalence in Cuban preschool children. The IRC approach would be appropriate in epidemiological studies because it takes into account inflammation severity. This study is a first step in the search for models that could better account for the effects of inflammation in estimating ferritin concentrations.

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Improved Recovery Protocols in Cardiac Surgery: A Systematic Review and Meta-Analysis of Observational and Quasi-Experimental Studies

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ABSTRACT

INTRODUCTION Improved recovery protocols were implemented in surgical specialties over the last decade, which decreased anesthetic and surgical stress and the incidence of perioperative complications. However, these recovery protocols were introduced more slowly for cardiac surgeries. The most frequent complications in cardiac surgery are related to patient clinical status and the characteristics of the surgical procedures involved, which are becoming more varied and complex every day. The first version of the enhanced recovery program for cardiac surgery was published in 2019, but its recommendations were based on only a few studies, and scant research has evaluated its implementation. Randomized and controlled clinical trials for these protocols are scarce, so research that summarizes the results of studies with other methodological designs are useful in demonstrating their benefits in cardiovascular surgery services in Cuba and in other limited-resource settings.

OBJECTIVE Estimate the effectiveness of improved recovery protocols in the perioperative evolution of patients undergoing cardiac surgery.

METHODS We performed a systematic review and meta-analysis according to the guidelines of manual 5.1.0 for reviews of the Cochrane library. We included observational and quasi-experimental studies published from January 2015 through May 2020 that compared enhanced recovery protocols with conventional treatments in patients older than 18 years, and used a quality score to evaluate them. We used the following sources: the Cochrane Library, PubMed, LILACS, SciELO, EBSCO, Google Scholar, Web of Science, Clinical Key, ResearchGate and HINARI. The following keywords were used for the database searches in English: ERAS, protocols and cardiac surgery, enhanced recovery after cardiac surgery, ERACS, clinical pathway recovery and cardiac surgery, perioperative care and cardiac surgery. We used the following search terms for databases in Spanish: *protocolos de recuperación precoz* and *cirugía cardiaca, protocolos de recuperación mejorada* and *cirugía cardiaca, cuidados perioperatorios* and *cirugía cardiaca, programas de recuperación precoz* and *cirugía cardiovascular*. Methodological quality of included investigations was evaluated using the surgical research methodology scale. Meta-analyses were performed for perioperative complications, intensive care unit and hospital stays, and hospital readmission within 30 days of surgery. We calculated effect sizes of the interventions and the corresponding 95% confidence intervals. We used mean differences and confidence intervals for continuous variables, and for qualitative variables we calculated relative risk (RR). Random effects analysis was used. Heterogeneity of the studies was assessed using the Q statistic and the I^2 statistic.

RESULTS We selected 15 studies (a total of 5059 patients: study group, n = 1706; control group, n = 3353). The average quality score for the 15 articles included was 18.9 (out of a maximum of 36 according to the scale) and 66.6% had a score ≥18. With improved recovery protocols in cardiac surgery, the incidence of perioperative complications decreased (RR = 0.73; 95% CI 0.52–0.98) as did hospital readmission within 30 days after surgery (RR = 0.51; 95% CI 95% CI: 0.31–0.86). Differences in extubation time, hospital stay and length of stay in intensive care units were less marked, but always favored the group in which the enhanced protocols were implemented.

CONCLUSIONS Improved recovery protocols in cardiac surgery increase quality of care evidenced by reductions in perioperative complications and decreased incidence of hospital readmission in the month following surgery.

KEYWORDS Enhanced recovery after surgery, rehabilitation, perioperative care, thoracic surgery, cardiac surgical procedures, systematic review, meta-analysis, Cuba

INTRODUCTION

In the last decade, improved recovery protocols were introduced in the surgical clinics of various specialties, which decreased anesthetic and surgical stress, as well as incidence of perioperative complications and morbidity; but their use in heart surgery has been slower despite the obvious advantages. In cardiac surgical procedures, the most frequent complications are related to patient clinical status, including comorbidities, and to increasingly complex and varied surgical procedures. The multimodal, multidisciplinary and continued-care approach of these protocols—which are applied before, during and after surgery—aim to improve quality of care and perioperative evolution, and to aid in early recovery.[1]

IMPORTANCE This study provides evidence pointing to benefits of improved recovery protocols in cardiac surgery, which may lead to their implementation in Cuban heart surgery units and those of hospitals in limited-resource settings.

Patients who undergo cardiac surgery are exposed to events and procedures that can become risk factors for increased morbidity and mortality, including but not limited to: progressive deterioration of nutritional status due to decreasing daily intake and preoperative

fasting; anticoagulation procedures during the intraoperative period; prolonged periods of aortic clamping and cardiac arrest; extracorporeal circulation including the potential development of an inflammatory response syndrome; blood transfusions; intensive pharmacological support or mechanical support for low-output syndrome; and late postoperative nutritional support.[1–3] Improved recovery protocols propose comprehensive treatment with actions that cover the entire perioperative period and are designed to ameliorate the negative effects of these factors, and hence they are recommended for implementation in cardiac surgery units.

In 2002, Henrik Kehlet introduced the concept of enhanced recovery protocols (ERAS), and from his work the international non-profit society Enhanced Recovery After Surgery Society (ERASS) was created.[3–6] These programs were applied first in colorectal surgery, and later extended and adapted to other surgical specialties.[4,6–11] The main objective of ERAS protocols is that patients arrive at the surgical procedure in the best clinical conditions possible and that they remain so during and after surgery until discharge via preoperative, intraoperative and postoperative interventions.[7,8,11–15]

ERAS was slow to be introduced into cardiac surgery compared to some other surgical specialties due to the complexity of procedures, differences in conditions required for each intervention, and wide diversity of patient clinical characteristics.[3,16] The first enhanced recovery programs in cardiovascular surgical procedures were the so-called fast-track and ultra-fast track programs, introduced in the 1990s.[17–19] These proposed shortening the duration of orotracheal extubation and postoperative ventilation mechanics, which are risk factors for respiratory complications, as well as shortened stays in hospitals and intensive care units (ICUs). But these actions were focused on a single stage of the perioperative period and were not multidisciplinary. In cardiac surgery, such fast-track and ultra fast-track programs are not applied to all cardiac surgical procedures or to all patients.[17,19–26]

Between 2017 and 2019, publications on the results of ERAS programs in cardiac surgery increased.[6,14,19,23,27–33] World leaders in the specialty recognized the need to adapt the original ERAS programs to cardiac surgery patient characteristics and to each type of intervention, and to generalize a protocol based on the best scientific evidence.[2,14,34,35] The first cardio-surgical symposium for development, evaluation and control of enhanced recovery protocols was held in 2017, whereas ERAS experts published the first ERAS guidelines for cardiac surgery in March 2019,[34,36,37] collectively known as 'ERACS protocols or guidelines'.

These ERACS guidelines have the following characteristics: in the preoperative stage, they propose to educate patients and family members, stratify and control nutritional status, estimate blood glucose levels using glycosylated hemoglobin, eliminate risk factors (tobacco and alcohol), treat infections with prophylaxis, administer carbohydrates two hours before surgery, detect kidney dysfunction early and decrease fasting time (six hours for solids and two to four hours for clear liquids). For the intraoperative period, they propose performing antifibrinolytic therapy with tranexamic acid or Epsilon aminocaproic acid, using multimodal anesthetic and analgesic techniques involving minimal opioids, administering fluids according to hemodynamic variables, controlling hypothermia, maintaining glycemic control, implementing prophylaxis of acute kidney injury and of infections, and using a plate for rigid sternal fixation. For postoperative recovery, they recommend intensively controlling blood glucose levels via continuous infusion, removing dressings from wounds at 48 hours, maintaining thromboprophylaxis, preventing hypothermia, treating pain with minimal use of opioids, stratifying and controlling postoperative delirium, treating acute kidney injury prophylactically, and extubating within the first 6 hours after surgery.[37]

Despite progress in introducing these programs for heart surgery, the authors of the first guidelines concluded that there was not enough published research on the subject, and not enough sound evidence such as that provided by randomized controlled clinical trials (RCTs) and systematic reviews or meta-analyses. Guidelines were issued when there were enough studies to support the introduction of therapeutic measures and diagnostic means.[37]

Evidence-based clinical practice is related to better quality of patient care and improvements in major hospital indicators, and so systematic reviews have gained more followers than detractors and have come to be seen in recent decades as essential tools in developing evidence-based medicine. The validity of individual studies is increased through systematic reviews and areas of controversy are identified where it is necessary to update information and build consensus.[38,39]

At the cardiac unit of the Hermanos Ameijeiras Clinical–Surgical Hospital (HHA) in Havana, Cuba, the first RCT (retrospective record dated 06/09/2012, code RPCEC00000131) was carried out on enhanced recovery in cardiac surgery, with fast-track and multimodal anesthetic methods (association of spinal regional anesthetic techniques with general anesthesia) in myocardial revascularization surgery without extracorporeal circulation. As a result of this RCT,[18] our practice experienced better results during perioperative analgesia, lower doses of systemic opioids were used, the time of mechanical ventilation in the postoperative period was reduced to less than four hours, and incidence of perioperative complications and postoperative stays in hospitals and ICUs decreased. This was the first step in implementing anesthesia strategies based on the best clinical evidence for optimizing patient recovery.[40]

Controversies persist on the benefits of multimodal anesthesia methods that include spinal regional anesthetic techniques in cardiac surgery, because some studies show that these methods do not reduce morbidity in the 30 days following surgery.[41] The authors of the first international version of the ERACS protocol stated that these methods require further evidence and expert evaluation before formal inclusion in the recommendations.[37]

Currently, data is scarce on the benefits of introducing improved recovery protocols in the perioperative clinical practice of cardiac surgery, so we set out to estimate the effectiveness of applying these protocols in the perioperative evolution of patients older than 18 years of age undergoing cardiac surgery, compared with the conventional protocol, based on the primary results of perioperative complications, length of stay in ICUs and hospitals, and hospital readmission within 30 days after the procedure, through a systematic review of observational and quasi-experimental studies, and a meta-analysis.

These programs are useful in focusing on surgical patient care in a comprehensive manner and improving patient care quality by establishing best practices based on documented evidence.

METHODS

This study is a first approximation based on observational and quasi-experimental methodological designs. We carried out a systematic review according to the recommendations outlined in version 5.1.0 of the Cochrane Handbook for Systematic Reviews of Interventions, and the evaluation criteria of the international guide "Preferred Reporting Items for Systematic Reviews and Meta Analyses" (PRISMA).[42,43]

The protocol for this systematic review has been approved by HHA's Scientific Commission (version 0.0, number 2657, May 2018), but it was not registered in electronic databases with national or international access, as is suggested by the PRISMA evaluation guides.[44]

Different meta-analyses were performed for variables of interest whose data were available in three or more of the included studies and whose summary measures were compatible for processing with the EPIDAT 3.1 and Review Manager 5.3 (RevMan 5.3) programs, because all studies did not include the same variables and they needed to be grouped to evaluate those that were both available and of interest.

Search strategy for identifying studies We use the Cochrane Library, PubMed, LILACS, SciELO, EBSCO, Google Scholar, Web of Science, Clinical Key, ResearchGate and HINARI as sources for studies in humans published from January 2015 through May 2020, in both Spanish and English.

The following search terms were used: For databases in English, ERAS; protocols and cardiac surgery; enhanced recovery after cardiac surgery; ERACS; clinical pathway recovery and cardiac surgery; perioperative care and cardiac surgery. For databases in Spanish: *protocolos de recuperación precoz* and *cirugía cardiaca; protocolos de recuperación mejorada* and *cirugía cardiaca; cuidados preoperatorios* and *cirugía cardiaca; programas de recuperación precoz* and *cirugía cardiovascular.*

The search syntax in PubMed, the database that contributed the most references, was as follows:
1. Enhanced recovery AND cardiac surgery
2. Cardiac surgery AND perioperative care
3. Heart surgery AND clinical pathway
4. Perioperative care AND heart surgery
5. # 1 or # 2 or # 3 or # 4

During the first stage, we reviewed titles and abstracts of articles with the potential of meeting study requirements that appeared in the abovementioned search engines. In the second stage, we searched and examined the full texts of the articles selected by title and abstract. Two independent evaluators were used in both stages and discrepancies were discussed. We screened the reference lists of selected articles (a 'search for pearls') to find studies that might be included in the systematic review. We were unable to contact the authors of articles with incomplete information or who presented their information in the form of graphics. An operational model was designed to select studies that included explicit criteria for collecting information. Search results were processed using Zotero 5.0 for Windows bibliographic reference manager.

Criteria for evaluating studies
Study type
1. Observational
2. Quasi-experimental

Participants
Patients aged >18 years scheduled for cardiac surgery with or without extracorporeal circulation (ECC)

Intervention
1. Enhanced recovery protocols or ERACS protocols
2. Conventional protocols

Main outcome measures
Primary or critical outcomes that directly influence decisions
1. Perioperative complications
2. Length of stay in the ICU
3. Length of stay in hospital
4. Hospital readmission within 30 days after surgery
5. Patient satisfaction

Secondary, important, non-critical outcomes that can influence decisions
1. Extubation time
2. Administration of inotropic drugs
3. Early enteral nutrition
4. Early mobilization
5. Total water balance

We did not define these results in the methodology, as definitions may differ between studies, and thus for each study reviewed, we used the same definitions as the researchers.

Exclusion criteria RCTs were excluded, as the purpose of this study was to carry out a systematic review of observational and quasi-experimental investigations for which there were no previous reviews. Studies that did not answer the review questions were also excluded.

Data collection and analysis Two observers collected information independently and selected studies according to the established criteria based on intervention type, participants and outcome measures. When there were discrepancies, a third evaluator was consulted until a consensus was reached. This procedure was followed in the order set forth in the search strategy.

Methodological quality This was assessed for each article using the Methodology of Research in Surgery (MINCIR) scale[45] validated for studies of therapy or therapeutic procedures. This scale consists of three domains: the first assigns scores 1–12 for design type, with the highest score for RCTs, particularly multicenter ones; the second evaluates sample size regardless of the method (or lack thereof) of calculation, and the third is composed of four items, assigning scores of 1–3 to each, which are: quality of the objectives, mention of or justification of the study's design, sample selection criteria (inclusion and exclusion) and whether or not the sample size is justified. The score's total is then 6–36 points. The cut-off value for methodological quality

was 8 points, because RCTs were not included, and studies were observational and quasi-experimental. Studies that obtained a score ≥18 were assessed as having good methodological quality, and studies with a score ≤17 points were assessed as having poor methodological quality.

Procedures for meta-analysis Magnitudes of the interventions' effects with their respective 95% confidence intervals (CI) were calculated for the qualitative response variables using relative risk (RR) as a measure of effect, calculated as *risk of event in the ERACS group/risk of event in the control group,* so that higher risks of the event presenting in the control group (CG) produced RRs lower than would have been the case had the two groups been combined. For quantitative variables, the difference in means between the ERACS group and the CG was used as a measure of effect, so that values <0 implied a favorable effect for the intervention. Random effects analyses were used for all variables, since fixed-effect meta-analyses ignore non-random sources of variation between studies. The heterogeneity of the studies was assessed using Q and I^2 statistics. Sensitivity was estimated by the change in the global effect when articles with inadequate or poor methodological quality were eliminated (score ≤17). Publication bias was assessed using the Egger t-test statistic.[42]

RESULTS

The study selection process' exclusion criteria are shown in a flow chart (Figure 1).

The 15 selected articles contributed 5059 patients (ERACS group: n = 1706; CG: n = 3353). The methodological quality of the studies was good. Ten of the 15 articles (66.7%) scored ≥18 points out of a maximum of 36 possible in the MINCIR guide (Table 1).

Meta-analytic comparisons were made between ERACS and conventional interventions for primary outcome variables: perioperative complications, ICU stay, hospital stay, and hospital readmission within 30 days after surgery.

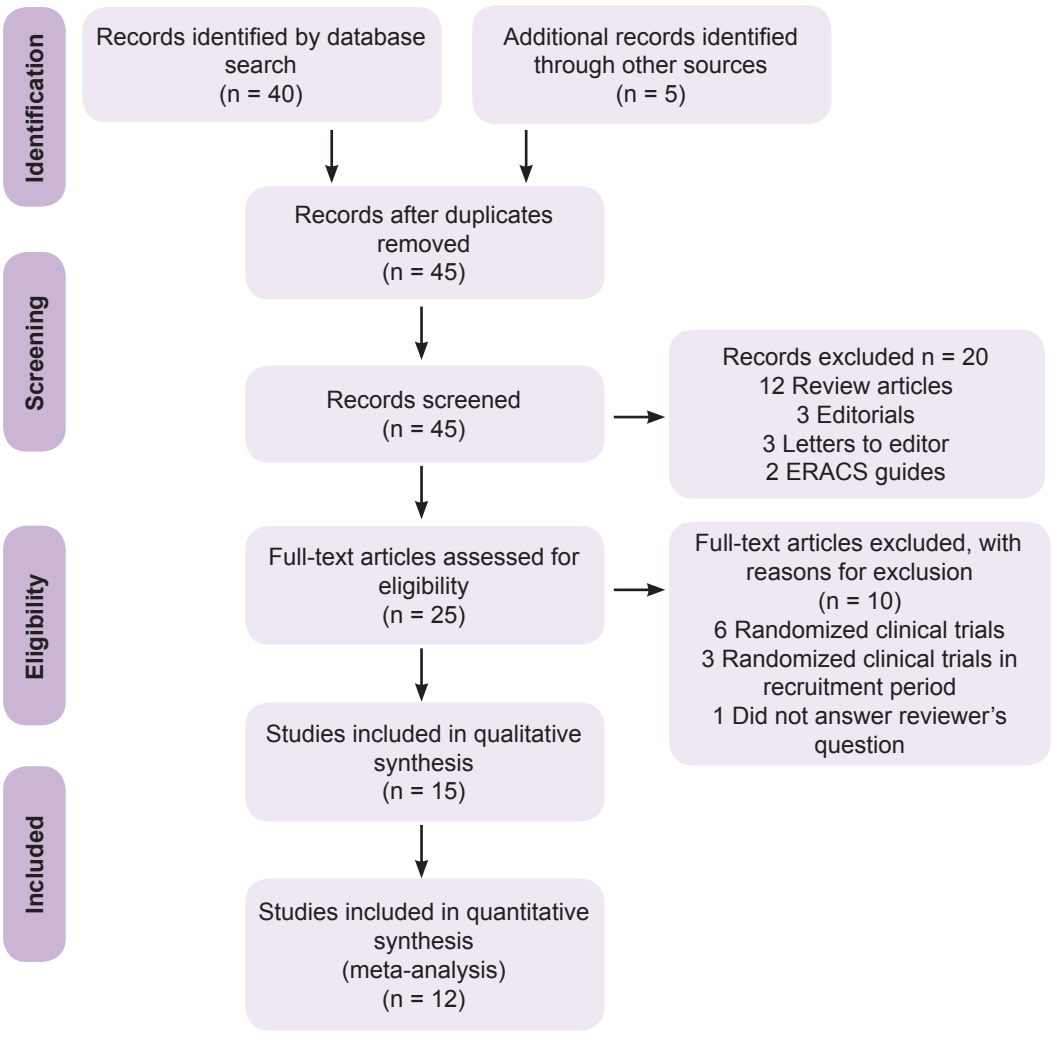
Meta-analysis was performed for perioperative complications in the 12 studies that contained information on this variable (total: n = 937 patients; ERACS: n = 290; CG: n = 647).

A tree graph shows the studies included in this meta-analysis, as well as the overall estimate of the hazard ratios for the randomized studies (Figure 2). ERACS protocols were associated with a lower incidence of complications with a RR <1 in the random effect analysis (RR = 0.72; 95% CI: 0.52–0.98). Heterogeneity was significant (p <0.001; Q statistic = 43.30; I^2 statistic = 75%). Publication bias was significant (p = 0.04; Egger's test, Z statistic = 2.10).

We analyzed the 3 of 14 studies (21.4%) that evaluated the variable of average ICU stay (Gimpel 2018, Motwani 2019 and Chen 2020; total: n = 1935; ERACS: n = 278; CG: n = 1657). There were no significant differences in the random effects analysis (mean difference = –3.52; 95% CI: –7.16–0.11) although the direction of the effect remained favorable to the ERACS group. Heterogeneity was not significant (p = 0.76; chi-square Q statistic = 468.28) and there was no publication bias (p = 0.76; Egger's test).

For hospital stay, the two groups were compared using 3/15 studies (20.0%) that contained information for this variable (Motwani 2019, Kowalski 2019 and Chen 2020; total n = 880; ERACS n = 441; CG n = 439). The results were similar to those obtained in the ICU stay analysis. No significant differences

Figure 1: Flow diagram according to PRISMA



ERACS: enhanced recovery after cardiac surgery PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses

Table 1: Basic data and methodological quality of studies

Authors	Year	Total n	ERACS n	Control n	Surgery type	Quality	Reference
Zaouter C, et al.	2015	71	38	33	MRV	20	[27]
Fleming IO, et al.	2016	106	53	53	MRV / VR / MRV+VR	23	[35]
van der Kolk M, et al.	2017	243	81	162	MRV / VR / MRV+VR	28	[30]
Martinos C, et al.	2017	100	100		MRV / VR / MRV+VR	11	[46]
Motwani SK, et al.	2019	133	63	70	MRV/VR/Myxomas	21	[47]
Gimpel D, et al.	2018	1717	168	1549	MRV /VR / MRV+VR	18	[48]
Markham T, et al.	2019	50	25	25	RVM	19	[49]
Williams JB, et al.	2019	973	443	530	MRV / VR	20	[50]
Grant MC, et al.	2019	315	84	231	MRV / VR/ MRV+VR	18	[51]
Zaouter C, et al.	2019	46	23	23	AVR. Min. Invasive	20	[52]
Varelmann D, et al.	2019	280	107	173	MRV /VR/ MRV+VR	17	[53]
Zammert M, et al.	2019	212	115	97	----------	17	[54]
Kowalski S, et al.	2019	662	331	331	MRV / VR / MRV+VR	16	[55]
Borys M, et al.	2020	57	28	29	MRV w/out ECC	14	[56]
Chen L, et al.	2020	94	47	47	MRV w/out ECC	22	[57]
Total		5059	1706	3353		18.93	

*Type of surgical procedure was not reported.
AVR: aortic valve replacement; ECC: extracorporeal circulation; ERACS: enhanced recovery after cardiac surgery; MRV: myocardial revascularization; VR: valve replacement

were found between the groups with the random effects analysis, but despite this, the direction of the measured effect favored the ERACS group (combined mean difference = –0.81; 95% CI: –2.13–0.51). Heterogeneity was significant (p <0.001; chi-square Q statistic = 95.19). There was no publication bias for this outcome according to Egger's test (p = 0.49).

We performed a meta-analysis using 6 of the 7 studies that addressed hospital readmission in the first 30 days after surgery (van der Kolk 2017, Gimpel 2018, Motwani 2019, Zaouter 2019, Zammert 2019 and Kowalski 2019; n = 3195; ERACS n = 881; CG n = 2314) (Figure 3). In the random effects analysis there were significant differences in favor of the ERACS group (RR = 0.51; 95% CI: 0.31–0.86). Heterogeneity was not significant (p = 0.27, Q chi-square statistic = 6.41, I^2 statistic = 22%), but publication bias was significant according to Egger's test (p = 0.01).

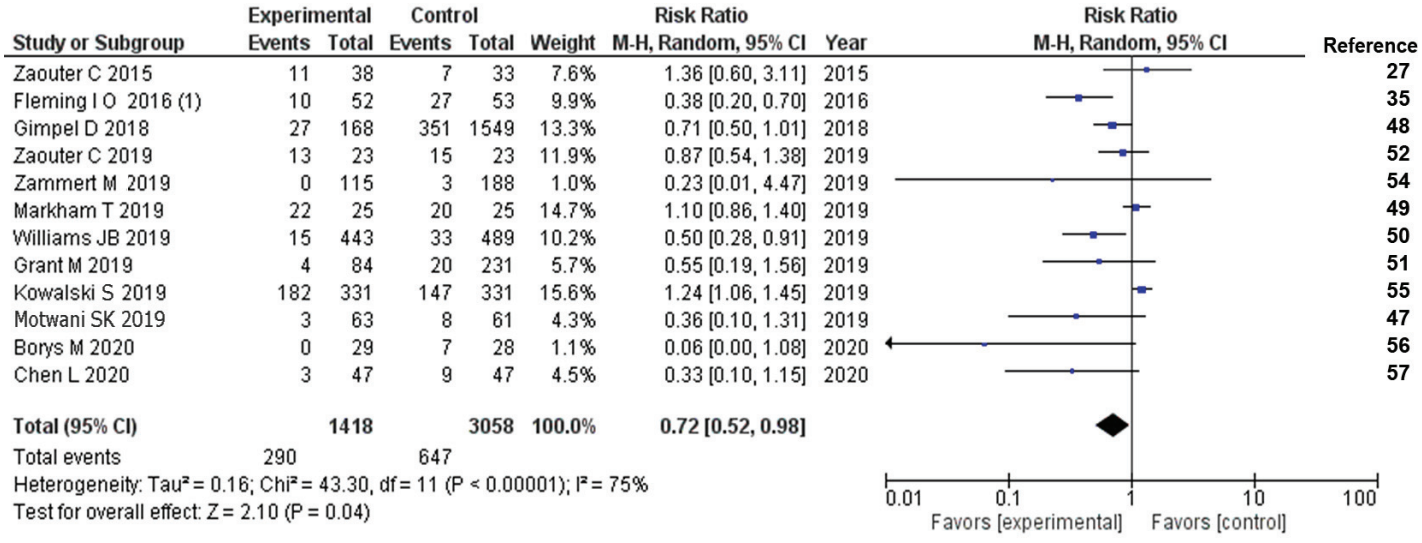
Meta-analytic comparisons were made with secondary endpoints for extubation time and inotropic drug administration.

For extubation time, a meta-analysis was performed using 3/11 studies that reported the variable (Zaouter 2015, Motwani 2019, Chen 2020; n = 289; ERACS n = 148, CG n = 141) (Figure 4). There were no significant differences in the random effect analysis; even so, the direction of the mean effect favors the ERACS group (mean difference –114.98; 95% CI: –278.74–48.78). There was heterogeneity, demonstrated in the graph with high chi-square values and very low p values, in addition to the value of the I^2 statistic. Egger's test for detecting of publication bias was not significant at p = 0.02.

There were no significant differences between the groups when assessing administration of inotropic drugs (RR = 1.34; 95% CI: 0.87–2.07). Heterogeneity was significant (p = 0.04; Q chi-square statistic = 6.37), but publication bias was not (p = 0.29).

There were no substantial changes in significance in the sensitivity analysis for the six meta-analytic comparisons, and confidence intervals were of very similar widths.

Figure 2: Perioperative complications, random effect



Footnotes
(1) Forest plot. Perioperative complications. Random efect.

Figure 3: Hospital readmission, random effect

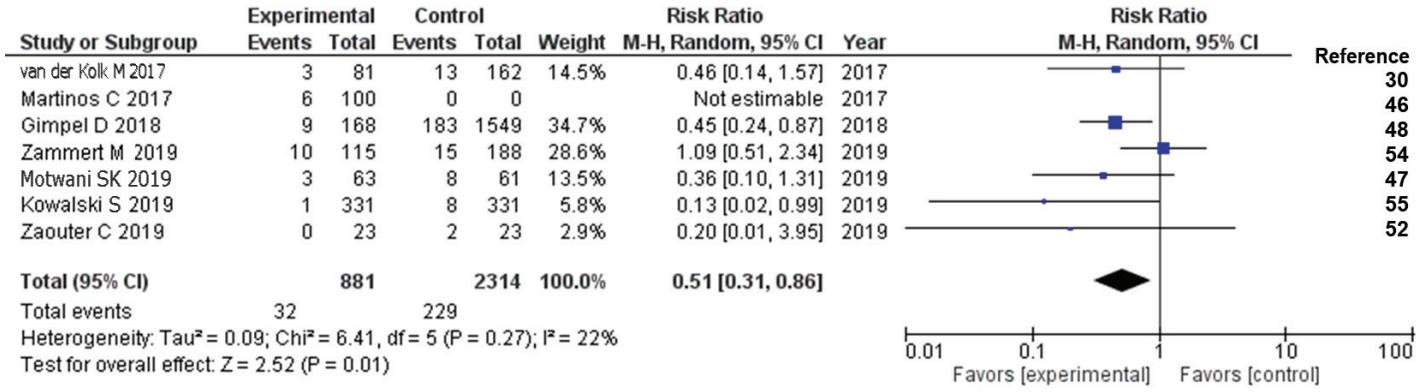
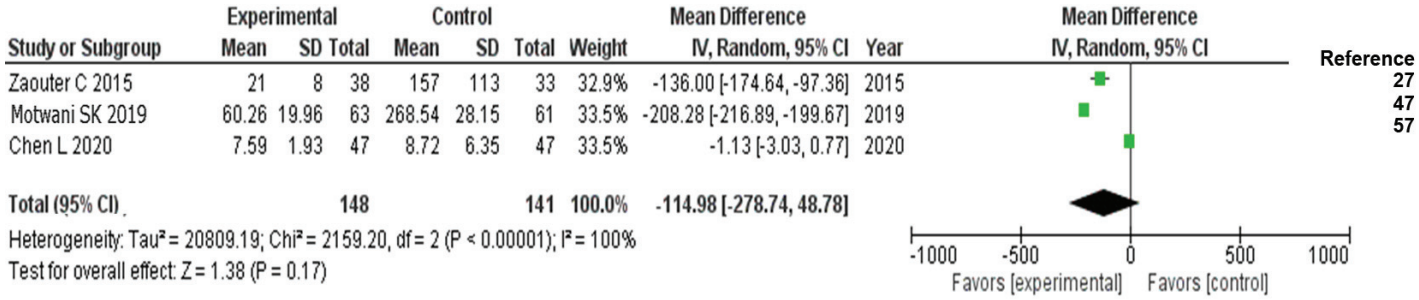


Figure 4: Extubation time (in minutes), random effect



DISCUSSION

Enhanced recovery and success of complex surgeries like cardiac surgery depend on interventions guided by evidence-based protocols on the reduction of perioperative complications, length of stays in hospitals and ICUs, and readmission to the hospital after discharge.

Our analysis, which reviews studies from varied settings, confirms that ERACS program quality depends primarily on provider experience and patient selection and preparation (as outlined in ERACS protocols), and not necessarily on available material resources of individual cardiac surgery units, which allows these programs to be implemented in limited-resource settings.

The choice of including only observational or quasi-experimental studies in this review that commonly incorporate a greater number of response variables and that had not been included in previous systematic reviews may have decreased the sensitivity of some statistical contrasts, but the exclusion of RCTs did not lead to publication bias.

There are no reports of systematic reviews accompanied by meta-analyses comparing ERACS protocols with conventional procedures for cardiac surgery. One study, published in 2018,[58] evaluates the effectiveness of fast-track programs in cardiac surgical procedures. Through individual analysis of seven investigations, the authors concluded that these programs reduce postoperative mechanical ventilation time, ICU stays and costs when implemented in well-selected patients.

In the studies we included, incidence of perioperative complications decreased with ERACS protocols compared to traditional methods of preparing patients for general anesthesia, also associated with a decrease in hospital readmission in the 30 days after surgery, indicating the advantages of these protocols in improving surgical patient quality of care. The differences for ICU and hospital stays and for extubation time after surgery were smaller, but always favored the ERACS group. No improvement was seen in the ERACS group for inotropic drug administration.

Heterogeneity tests were significant in half of the meta-analyses performed, which can be attributed to different procedures (valve replacement or repair, excision of intracardiac tumors) and surgical techniques (cardiac surgery with and without extracorporeal circulation) included in the analysis. This made it difficult to integrate evidence from studies conducted in different settings, with varied designs, and that included subjects with different clinical diagnoses and different surgical procedures and techniques.[42] The sensitivity analysis showed that eliminating studies with lower evidence quality (higher risk of bias) did not change the basic results, which lends them more credit.

A limitation of the research is publication bias in some of the meta-analyses. This may be due to the fact that few studies were included in the meta-analyses, due to strict inclusion criteria. Another limitation was the incompatibility of the metric criteria in several of the studies with those commonly used in software available for meta-analyses.

Although the scales recommended to assess methodological quality of articles[43,44] are the Newcastle–Ottawa scale,[59] the Strengthening the Reporting of Observational studies in Epidemiology (STROBE),[60] or the Quality Assessment Tool for Systematic Reviews of Observational Studies guide

(QATSO),[61] we opted for the MINCIR scale,[45] which is not limited to assessing presence or absence of attributes in articles, but also assesses quality of information presented.

Several confidence intervals for parameters of interest were considerably wide, and therefore unreliable, due to small sample sizes.[62] This circumstance does not call into question the results but reveals the need for more original studies on the implementation of ERACS protocols in cardiac surgery.

CONCLUSIONS

Improved recovery protocols in cardiac surgery reduce perioperative complications in patients and decrease the incidence of hospital readmission in the 30 days after surgery, and also reduce the length of stays in intensive care units and hospitals. The study is an important, although preliminary, step to establish the usefulness of ERAS protocols in anesthesiology and cardiac surgery, as it summarizes variables that are hospital system indicators that relate to hospital performance and quality of care.

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Hypoxemia and Cytokine Storm in COVID-19: Clinical Implications

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ABSTRACT

One of the most dreadful complications that can occur during the course of COVID-19 is the cytokine storm—also known as cytokine release syndrome—a form of systemic inflammatory response syndrome triggered by SARS-CoV-2 infection.

The cytokine storm is an activation cascade of auto-amplifying cytokines, which leads to excessive activation of immune cells and generation of pro-inflammatory cytokines. It occurs when large numbers of white blood cells are activated and release inflammatory cytokines, in turn activating even more white blood cells, finally resulting in an exaggerated pro-inflammatory–mediated response and ineffective anti-inflammatory control, leading to tissue damage, multiorgan failure, acute respiratory distress syndrome and death. Although cytokine storm pathogenesis is multifactorial, we hypothesize there is a close association between hypoxemia and cytokine storms in COVID-19, although it is difficult to establish the direction of this relationship. Most probably they coexist and, given enough time, one triggers the other in a chain reaction. Careful analysis of the day-to-day clinical evolution of COVID-19 indicates that there are short and slight periods of hypoxemia (confirmed by pulse oximetry and arterial gasometry), even on the day of the onset of persistent cough and/or shortness of breath.

We propose the use of continuous positive airway pressure in early stages of COVID-19, at the onset of respiratory symptoms. This non-invasive ventilation method may be useful in individualized treatments to prevent early hypoxemia in COVID-19 patients and thus avoid triggering a cytokine storm.

We believe such an approach is relevant everywhere, and in Cuba in particular, since the country has initiated national production of mechanical ventilation systems, including non-invasive ventilators. Moreover, as Cuba's COVID-19 protocols ensure early patient admission to isolation centers or hospitals, clinicians can prescribe the early use of continuous positive airway pressure as soon as respiratory symptoms begin, averting early hypoxemia and its triggering effect on cytokine storm development, and consequently, avoiding acute respiratory distress syndrome, multi-organ failure, and death.

KEYWORDS COVID-19, SARS-CoV-2, cytokine release syndrome, respiratory distress syndrome, noninvasive ventilation, continuous positive airway pressure, Cuba

INTRODUCTION

One of the worst complications during the clinical course of COVID-19 is the cytokine storm (CS), also known as cytokine release syndrome, a type of systemic inflammatory response syndrome that can be triggered by a variety of factors, including infections and certain drugs. In COVID-19, CS occurs when large numbers of white blood cells are activated and release inflammatory cytokines, in turn activating even more white blood cells, and is finally associated with exaggerated pro-inflammatory–mediated response and ineffective control by the anti-inflammatory system, leading to tissue damage, multiorgan failure, acute respiratory distress syndrome (ARDS) and death.[1]

Although CS pathogenesis is multifactorial, in this essay we hypothesize on hypoxemia as a main CS trigger in COVID-19. The mechanisms of lung damage and hypoxemia in COVID-19 include ventilation/perfusion mismatch, loss of hypoxic vasoconstriction and increased coagulopathy.[2,3]

IMPORTANCE

We discuss hypotheses positing a close interrelationship between hypoxemia and cytokine storm in COVID-19, as well as the importance of preventing intermittent periods of hypoxemia using continuous positive airway pressure in the early clinical stages of the disease to avert hypoxemia's triggering effect for cytokine storm and development of acute respiratory distress syndrome, organ failure and death.

Although COVID-19's clinical progression varies from patient to patient, ranging from asymptomatic to severe disease,[4] the disease's day-to-day clinical evolution can be described generally as in Table 1.[4−6]

COVID-19 presents mildly in most patients, commonly beginning with fever followed by a dry cough that dissipates without medical intervention, and flu-like symptoms like headache, malaise and muscle pain, which may develop early in symptomatic persons. Mild cases have been associated mainly with younger patients, but in some cases, these symptoms may progress to shortness of breath that usually starts within a seven-day period before appearance of a more severe form of the disease.[5,6] While many patients recover in about a week, a significant number enter a very nasty second week of illness. Usually, after onset of initial symptoms, the course of the disease plateaus and patients may even improve. This improvement is sometimes followed by an additional round of worsening symptoms. According to the Cuban Protocol for COVID-19,[4] severe complications, like ARDS, most often occur after the second week of clinical evolution.[4−6]

Hence, in considering a careful analysis of the day-to-day clinical evolution, short and slight periods of hypoxemia start even at the first manifestation of persistent cough and/or shortness of breath, as reported by authors using pulse oximetry and arterial gasometry.[6,7] Afterwards, breathing difficulty might worsen, leading to augmented periods of hypoxemia.[8] Persistent cough,

Table 1: Frequent timeline of symptoms in COVID-19 patients, day-by-day*

Day 1	Fever, fatigue, muscle pain and dry cough
Day 2–4	Fever and cough persist
Day 5	Breathing difficulty begins
Day 6	Breathing difficulty, fever and cough
Day 7	Patients who still have trouble breathing are admitted to the hospital
Day 8	Acute respiratory distress syndrome (ARDS) may develop
Day 10	If breathing worsens, patient is admitted to the ICU
Day 12	Fever ends in patients who are improving
Day 13–14	Shortness of breath stops in patients who are improving
Day 18.5	In the worst-affected patients, ARDS worsens and death occurs Illness ends in patients who are improving

*Although the clinical picture of COVID-19 patients can vary from asymptomatic to severe disease, in most patients the day-by-day clinical evolution can be described as in this table.[4]
Note that the first respiratory symptoms begin on the first day of the clinical evolution. Acute respiratory distress syndrome occurs approximately at day 8.

among other symptoms, is considered a strong predictor of poor progression.[9] Nonetheless, some asymptomatic patients present pulmonary lesions in computerized tomography (CT) scans, and therefore the Cuban Protocol proposes early imaging studies, even in patients who remain asymptomatic.[4]

Oxygen saturation (SpO_2) measured by pulse oximetry may be useful in estimating blood oxygen saturation, at onset of first respiratory symptoms, although SpO_2 should be carefully interpreted in COVID-19. The sigmoid-shaped oxyhemoglobin dissociation curve shifts to the left, due to induced respiratory alkalosis, characterized by a drop in carbon dioxide partial pressure in arterial blood ($PaCO_2$), because of hypoxemia-driven tachypnea and hyperpnea. During hypocapnic periods, the affinity of hemoglobin for oxygen, and thus oxygen saturation, rises for a specified degree of partial pressure of oxygen (PaO_2), explaining why SpO_2 can be well preserved despite profoundly low PaO_2, suggesting the need to monitor blood gases by arterial gasometry.[10]

Therefore, it is important to note that acute lung injury, hypoxemia, systemic inflammatory response syndrome and ARDS can occur after SARS-CoV-2 infection. Hence, CS in COVID-19 patients is intrinsically involved in aggravating symptoms and spurring on disease progression, and denotes a key factor contributing to ARDS and death.[11]

Many therapeutic strategies have been used to treat COVID-19 patients, including steroids, non-steroidal anti-inflammatory drugs, antiviral drugs, etc.[12] Moreover, current treatment approaches under investigation are targeting the overactive cytokine response with anti-cytokine therapies or immunomodulators, but these must be balanced by maintaining adequate inflammatory response for pathogen clearance.[13]

Two main strategies might be followed to prevent CS onset in COVID-19.[14] The first is development of new drugs that inhibit pathways in the cascade of uncontrolled cytokine production (for example, the Cuban Protocol for COVID-19 uses Jusvinza and Itolizumab to stop hyperinflammatory reactions in COVID-19).[4] The second is early prevention of hypoxemia.

Here, we delve into the second strategy, since within the CS multifactorial pathogenesis, our objective is to address our hypothesis on the close interrelationship between hypoxemia and CS in COVID-19 patients, and highlight the importance of preventing intermittent periods of hypoxemia in the disease's early clinical stages.[15–18]

EARLY PREVENTION OF HYPOXEMIA TO AVERT CYTOKINE STORM

Cytokine storm and hypoxemia The immune system is a complex mechanism capable of responding to innumerable pathogens. Normal antiviral immune response includes activation of the immune system's inflammatory pathways. A crucial part of this inflammatory process includes cytokines and chemokines produced by several cells of the innate immune system (macrophages, dendritic cells, natural killer cells), and adaptive T and B lymphocytes.[19]

CS is an activation cascade of auto-amplifying cytokine production due to unregulated host immune response to different causes, and is therefore recognized as a systemic inflammatory response to drugs and infections, which leads to excessive activation of immune cells and generates pro-inflammatory cytokines. The term CS calls up images of an out-of-control inflammatory response and an immune system run amok.[19,20]

CS after SARS-CoV-2 infection is considered to be an overreaction of the body's immune system, which releases immune messengers—cytokines—into the bloodstream out of proportion to the threat or long after the virus is no longer a threat. When this happens, the immune system attacks the body's own tissues, causing significant harm.[19]

Moreover, inflammation during CS induces a defective procoagulant–anticoagulant balance predisposing patients to develop microthrombosis and disseminated intravascular coagulation. This requires use of anticoagulants, particularly heparin, which has been recommended by expert consensus for patients with severe COVID-19.[21] This exaggerated inflammatory response may damage the liver, blood vessels, kidneys and lungs, and increase the formation of blood clots throughout the body, leading to multiorgan failure. Ultimately, CS may cause more harm than SARS-CoV-2 itself.[22]

The immune responses induced by SARS–CoV-2 infection consist of two clinical phases. During early incubation and non-severe stages, a specific adaptive immune response is initiated to exterminate the virus and halt disease progression. The second phase is often related to increased severity of the disease and is characterized by potentially deadly lung inflammation and the advent of systemic symptoms with anomalous and unrestrained production of cytokines, known as CS.[19]

Hypoxia is a common feature of SARS-CoV-2 infection, impacting COVID-19 clinical evolution, including induction of factors such as hypoxia inducible factor-1α (HIF-1α). HIF-1α is activated during the immune response and induces pro-inflammatory cytokine production through immune cells, supporting our hypothesis that hypoxemia triggers CS cascade.[19,20]

During the early stages of SARS-CoV-2 infection, innate immune response leads to hypoxia at inflammation sites. Hypoxia is a microenvironmental feature of chronically inflamed tissues that can impact inflammatory process progression in several ways. HIF-1α and nuclear factor-κB (NF-κB) are two hypoxia-responsive transcription factors which, in addition to controlling independent cohorts of adaptive and inflammatory genes, are highly interdependent. HIF-1α regulates significant cellular processes (cell proliferation, metabolism and angiogenesis) and induces pro-inflammatory cytokine production through immune cells including IL-6 and tumor necrosis factor-alpha (TNF-α), in addition to activating the signal transducer and activator of the transcription 3 (STAT3) pathway.[15,23–25] Figure 1 summarizes our hypothesized relationship between the evolution of hypoxemia and cytokine storm.

SARS-CoV-2 binds and infects cells through the angiotensin-converting enzyme 2 (ACE2) receptor, widely dispersed through mammalian tissue. The two types of ACE receptors (ACE1 and ACE2) act as opposites in the pulmonary endothelium: ACE2 functions as a vasodepressor whereas ACE1 is a vasoconstrictor. Under normal physiological conditions, ACE1 and ACE2 exist in a dynamic equilibrium. However, in hypoxemic conditions, like those found in SARS-CoV-2–infected patients, ACE1 is upregulated by HIF-1α, while the expression of ACE2 is regulated bidirectionally, increased during the early stages of hypoxemia and decreased to near-baseline levels during later disease stages.[3,24,26]

The lungs seem to be the main target, although other organs with high ACE2 expression—such as the intestines, heart, brain and kidneys—may also be vulnerable.[19] The first immune cell types to find viral antigens in the respiratory tract are alveolar and interstitial macrophages, which are capable of eradicating many different pathogens from the lungs by phagocytosis. Damaged cells in the lungs provoke a sturdy innate immune response that appears to be mediated mainly by pro-inflammatory macrophages and granulocytes, which are responsible for CS.[2,20,24,25]

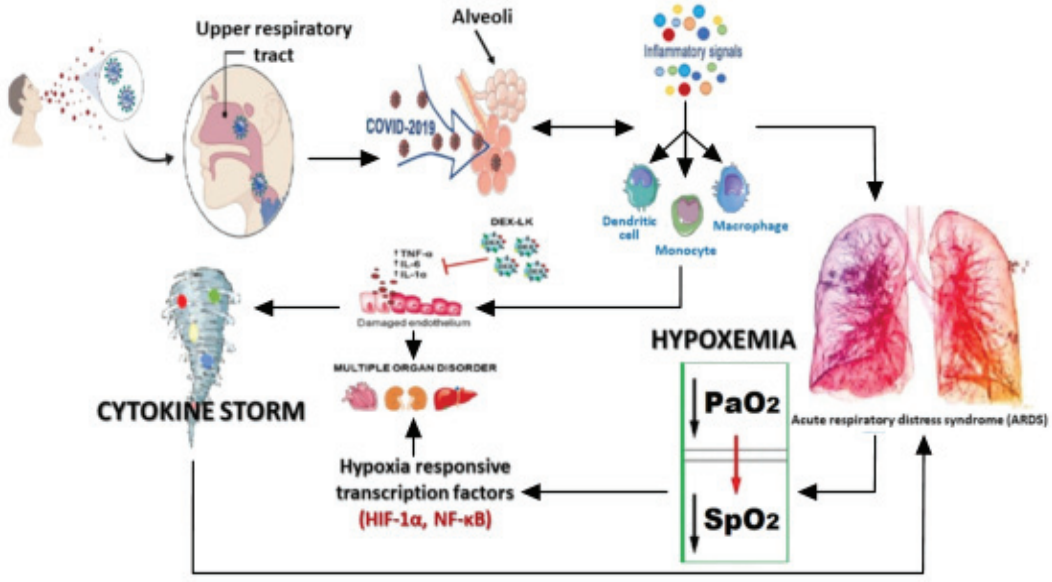
Another conceivable explanation for such severe hypoxemia occurring in otherwise compliant lungs is hypoxic vasoconstriction and loss of perfusion regulation. Several authors contend that diffuse pulmonary microvascular thrombosis might be the cause of hypoxemia in early SARS-CoV-2–induced pneumonia. Histologic and immunohistochemical studies suggest that catastrophic, complement-mediated thrombotic microvascular injury happens in severe SARS-CoV-2 infection, with continuous activation of the lectin pathway cascade, leading to the recommendation that anticoagulants, specifically low molecular-weight heparin, be employed early in disease progression.[26,27]

COVID-19 respiratory distress pathophysiology has been described as inflammation-induced pulmonary vasculitis, causing varying degrees of lung collapse secondary to edema and microthrombosis, which is characterized by bilateral ground-glass opacities on CT scan, resulting in ventilation-perfusion ratio (V/Q) mismatching and a significant shunt fraction.[28]

Cytokine-mediated lung endothelial and epithelial cell injury may damage the integrity of the blood–air barrier, thus promoting vascular permeability in addition to alveolar edema, infiltration by inflammatory cells, and hypoxemia. In COVID-19, as tissue breaks down, the walls of the lungs' tiny air sacs become leaky and fill with fluid, causing pneumonia and starving the blood of oxygen. These phenomena also lead to a lack of oxygen supply in tissues and organs due to blood hypoperfusion. Moreover, proinflammatory cytokines overwhelm mitochondrial oxygen utilization, resulting in a change of metabolic pathway from oxidative phosphorylation to glycolysis, consequently triggering cells to change their mode of metabolism to glycolytic or anaerobic.[29]

Experimental rat models demonstrated that in hypoxemic microenvironmental conditions favored by enhanced HIF-1α activity, suppression of HIF-1 transcription or inhibition of its activity are perhaps effective in ameliorating inflammation caused by viral infection, such as in the lungs of COVID-19 patients.[24]

Figure 1: Proposed hypoxemia and cytokine storm relationship in COVID-19 patients



We hypothesized there is a close association between hypoxemia and cytokine storm in COVID-19 patients, but it is difficult to establish the direction of this relationship. Most probably they coexist, and in time, one triggers the other as in a chain reaction, favored by hypoxia-responsive transcription factors HIF-1α, and NF-KB. There are multiple cytokines involved in the cytokine storm. In this figure, only some important cytokines are presented: IL-1α, and IL-6, and TNF-α.

PaO^2: Partial pressure of arterial oxygen; SpO^2: Oxygen saturation measured by pulse oxymetry

(This figure shows some elements taken from Castelli V, Cimini A and Ferri C (2020) Cytokine Storm in COVID-19: "When You Come Out of the Storm, You Won't Be the Same Person Who Walked in". Front. Immunol. 11:2132. doi: 10.3389/fimmu.2020.02132)

Systemic intermittent hypoxia is a life-threatening condition that happens in many diseases and situations, including chronic obstructive pulmonary disease, congestive heart failure and obstructive sleep apnea syndrome, all of which may coexist with SARS-CoV-2 infection in some patients.[30]

As previously discussed, hypoxemia can appear in early stages of COVID-19. Hypoxia is a common feature in inflammatory sites, which can eventually result in hypoxemia, leading in turn to induction of factors like HIF-1α. HIF-1α regulates important cellular processes, including cell proliferation, metabolism and angiogenesis. HIF-1α is activated during the immune response and plays an important role at the inflammation site by inducing pro-inflammatory cytokine production, leading to CS.[10,20,23–25,30] All the evidence connecting hypoxemia with CS highlights the importance of avoiding the former in COVID-19 patients as early as possible, to prevent development of the latter, by using non-invasive ventilation (NIV), as described in the following section.

Invasive or non-invasive ventilation in COVID-19 patients Over the past decade, NIV use has gained popularity in acute manifestations of chronic obstructive pulmonary disease (COPD).[31] Treatment for severe respiratory failure in COVID-19 patients has included early intubation and invasive ventilation, as this was deemed preferable and more effective than non-invasive options. Nevertheless, evolving evidence has shown that NIV may have a more significant and helpful role than first thought. NIV avoids the need for sedation, allows easier communication with patients and requires less intensive nursing care.[31]

There are three types of NIV: high-flow nasal oxygen (HFNO), BiPAP (bi-level positive airway pressure) and continuous positive airway pressure (CPAP).[31]

High-flow nasal oxygen HFNO therapy, administered through nasal cannulae, is a technique whereby heated and humidified oxygen is delivered at high flow rates. Such rates generate low levels of positive pressure in the upper airways, and the fraction of inspired oxygen (FIO_2) levels can be attuned by varying the oxygen amount in the driving gas. High flow rates may also reduce physiological dead space by flushing expired CO_2 from the upper airway, a process that may possibly explain the observed decline in respiration. In patients with acute respiratory failure, high-flow oxygen has been shown to result in better comfort and oxygenation than standard oxygen therapy delivered through a face mask.[32] This method may be useful to assure oxygenation during the time needed to prepare a ventilator.[4]

The 2012 Berlin definition of ARDS provided validated support for three levels of initial arterial hypoxemia that correlated with mortality in ventilated patients.[32] Since 2015, HFNO has become widely used as effective therapeutic support for ARDS, most recently in patients with severe COVID-19. It is noteworthy that the Berlin criteria propose the use of HFNO to treat ARDS, and not to prevent hypoxemia in the early stages of the disease.[32]

Nonetheless, HFNO use remains controversial in suspected and confirmed severe cases of COVID-19. As a result, the current national guidance in the UK does not recommend HFNO in COVID-19, citing lack of evidence proving its efficacy, high oxygen usage and risk of infection.[33]

Bilevel positive airway pressure BiPAP is commonly used in the care of patients with chronic respiratory disease, so it may be useful in COVID-19 patients. In COVID-19, BiPAP may have clinical use in improving the work of breathing. However, it carries the risk that inappropriate settings may allow the patient to take an excessively large tidal volume causing baro- and volutrauma. BiPAP allows for high driving pressure coupled with low driving pressure. Before commencing BiPAP, the patient must be assessed for pneumothorax, ideally by a chest x-ray or ultrasound. Due to the need for chest auscultation in COVID-19 patients, BiPAP is not recommended as it increases the risk of viral transmission to healthcare personnel.[34]

Continuous positive airway pressure CPAP is a simple and cost-effective intervention. Its use has been established for the care of other respiratory disorders but not for COVID-19 respiratory failure. At the beginning of the pandemic, international guidelines, including those from WHO, did not address the use of CPAP in COVID-19 patients, focusing instead on HFNO and invasive mechanical ventilation following intubation. In contrast, the UK National Institute for Health and Care Excellence guidance recommended the use of CPAP on April 9, although admitting evidence was lacking on its effectiveness.[33]

Nowadays, with CPAP equipment improved and commercially available, there is growing indication that it may be advantageous for avoiding hypoxemia in COVID-19 patients and thus halting disease progression, while reducing the need for invasive ventilation.[35,36] Ventilation systems have been developed in Cuba, CPAP among them, with the devices necessary for preventing aerosol dissemination, thus significantly reducing the possibility of contaminating health workers and other patients.[4]

Brusasco has recommended using CPAP in all patients presenting with signs of a severe intrapulmonary shunt (PaO_2/FiO_2 <200 or PaO_2 <60/mmHg on Ventimask 50%) or increased effort in breathing (BF >30/min or dyspnea) before considering invasive ventilation.[37]

Furthermore, negative results found for CPAP patients are not directly related to its use, as shown by evidence that reports that the failure of CPAP to avert death or invasive mechanical ventilation was related to amplified blood levels of thrombo-inflammatory and cardiac injury/dysfunction biomarkers occurring in the intensive care unit (ICU).[38]

CPAP is usually initiated at a higher level than normal intrinsic pressure (approximately 5 cm H_2O). For most ARDS patients, it is secondary to conditions that either collapse the alveoli or widen the gap between alveoli and surrounding blood vessels, thereby reducing gaseous exchange. Application of positive end expiratory pressure (PEEP) assists in maintaining patient airway pressure, thus preventing alveolar collapse and, in turn, increasing lung volumes and distending the lungs, reducing distance between alveoli and blood vessels, and improving gaseous exchange.[37] In severe COVID-19, initial CPAP settings have been suggested to begin at 10 cm H_2O and 60% oxygen.[39,40]

Some authors have demonstrated that the use of CPAP through a helmet mask in ARDS has prevented intubation in a significant number of patients,[41] although this methodology is a more complex use of CPAP.

Nonetheless, an important caveat is the possibility of aerosol dissemination, spreading the virus to health workers and other patients.[41] Two European countries heavily affected by the pandemic, Spain and Italy, tried to quickly develop safe NIV systems to treat COVID-19 patients, and created an emergency CPAP mask. This device has a positive end-expiratory pressure (PEEP) valve and a Venturi connector fitted to a facial snorkel interface.[42,43] Other alternatives have been proposed such as simple face masks, Venturi masks, non-rebreather (NRB) masks, and masks with reservoir bags. It has been argued that CPAP, as non-invasive ventilation (delivered by a mask with air diffusers), has low risk of aerosolization, provided that there is good mask fit. Of course, it is important to follow step-by-step instructions on cleaning CPAP devices and masks.[18,23,43,44] These guidelines for health worker protection are documented in detail in the Cuban Protocol for COVID-19.[4]

Persistent cough and shortness of breath in early COVID-19 stages can lead to periods of hypoxemia and subsequently to CS. This may cause worsening in a substantial proportion of patients over a short period, leakproof clinical states, and lead to death from ARDS. In the case of COVID-19 patients suffering ARDS in ICUs, invasive ventilation is preferred and recommended.[4] Some authors have proposed that CPAP is a reasonable and effective therapeutic strategy that may potentially delay or even avert the need for intubation in many patients.[44]

As has already been discussed, there is a close interrelationship between hypoxemia and CS. CS generates additional hypoxia in tissues and organs, leading to a chain reaction between hypoxemia and CS.[15–18] Hence, our proposal is the use of CPAP as an NIV method, outside the ICU, as early as possible in the disease's clinical evolution, when the first respiratory symptoms begin, to prevent periods of hypoxemia.[6,7,23] In Cuba, particularly, it is possible to use CPAP for these purposes, as COVID-19 protocols assure early admission of patients to isolation centers or hospitals, even when they are still asymptomatic,[4] and moreover, national production of ventilation systems has been initiated, including production of NIV equipment. Hence, clinicians can promptly detect the onset of respiratory symptoms, and are able to prescribe early CPAP use in these patients. This NIV method can be individually adjusted, depending on the intensity and frequency of respiratory symptoms, but it is crucial to consider times when patients are sleeping. According to the Cuban Protocol for COVID-19, the main complications of the disease generally occur after the second week of clinical evolution,[4] so there is time to prevent hypoxemia's triggering of CS.

CONCLUSIONS

Based on the relationship between hypoxemia and CS, we recommend evaluating the use of CPAP in the early stages of COVID-19 disease, at the onset of first respiratory symptoms (persistent cough and/or shortness of breath) as a personalized treatment to avert hypoxemia in patients evaluated by pulse oximetry or arterial gasometry. This would prevent hypoxemia's triggering effect on CS, thus potentially avoiding ARDS, multi-organ failure and death.

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Improving Ketosis-Prone Type 2 Diabetes Diagnosis in Africa

Dagoberto Álvarez-Aldana MD MS

Diabetes mellitus (DM) is a serious health problem with high—and increasing—prevalence and incidence around the world. Africa, with a considerable communicable disease burden, is not exempt and is facing greater DM risk due to rapid demographic, sociocultural, economic and nutritional changes. According to the International Diabetes Federation, Africa will experience the largest jump in DM prevalence (143%) of all regions over the next 25 years.[1,2]

As a member of the Cuban medical team working in Luanda, Angola, I've repeatedly seen male patients presenting with new-onset diabetic ketoacidosis (DKA) without evident precipitating cause. Medical records show these patients are between 40 and 50 years old; during ambulatory follow-up, they sometimes maintain good metabolic control despite having discontinued insulin therapy due to dramatic decline in glycemia. These clinical findings suggest the presence of ketosis-prone type 2 diabetes (KPD). Unfortunately, this variant of type 2 diabetes is often not considered during diagnosis and can lead to misclassification and incorrect treatment protocols.

Acute and chronic complications from diabetes require continual, long-term medical care and have significant economic and social impact on health systems, patients and their families

Acute and chronic complications from diabetes require continual, long-term medical care and have significant economic and social impact on health systems, patients and their families. DKA is a complex metabolic disorder caused by insulin deficiency occurring in type 1 diabetes, although under certain circumstances it is currently being more frequently ascribed to type 2 DM. While DKA is on the decline in high-income countries, in Africa it is steadily increasing among type 2 diabetics, though data is still sorely limited.[3] There, this hyperglycemic emergency translates into high mortality (26%–29%), where diagnostic delay and resource scarcity are commonplace.[4]

In the 1960s in Africa, KPD was reported as "temporary diabetes." Thirty years later, this syndrome was reported in a small cohort of young African Americans. The scientific literature has described this type of diabetes in a variety of ways: idiopathic type 1 diabetes, atypical diabetes, Flatbush diabetes and type 1.5 diabetes.[5] In 2019, WHO included it in its new classification of diseases, within the hybrid forms of diabetes.[6] In the United States, the estimated prevalence of KPD is between 20% and 50% of African Americans and Hispanics with newly-diagnosed diabetic ketoacidosis.[7]

Clinically, DKA typically presents as recently-diagnosed diabetes with short-lived, but acute, hyperglycemia symptoms. Most patients report less than four-week cycles of polyuria, polydipsia and weight loss. This is most frequently seen in middle-aged overweight and obese men who also exhibit other physical signs typical of DM including acanthosis nigricans and abdominal adiposity. In 80% of cases, there is a family history of diabetes, with an onset of acute hyperglycemia and ketosis or diabetic ketoacidosis. Autoimmune studies are negative in the majority of cases. Patients usually present with markedly high glucose levels (>500 mg/dL), mean glycated hemoglobin (A1c) >10% and a blood pH <7.30, accompanied by ketoacidosis.[5]

Hypotheses regarding implicated physiopathological mechanisms focus on β cell dysfunction. Although underlying factors are unknown, researchers posit that β cells are more susceptible to deterioration when there are prolonged high levels of blood glucose (glucotoxicity) or free fatty acids (lipotoxicity). With ketoacidosis, the presence of reduced β-hydroxybutyrate oxidation, together with greater branched-chain amino acid catabolism, leads to ketogenesis.[5,7]

In my clinical experience with both hospitalized and ambulatory patients in Luanda, I've seen recently diagnosed diabetics presenting clinical factors similar to those described in the international literature that suggest KPD. Nevertheless, KPD is rarely considered as a diagnosis. This oversight has important clinical implications since it increases the risk of ketoacidosis. In my opinion, we need to conduct a comprehensive analysis of clinical findings—particularly in those populations where access to genetic, immunological and hormonal testing is extremely limited—to improve diagnosis and correctly classify diabetes. Analysis of this type would be especially helpful to health professionals working in resource-scarce settings in Africa and those without clinical experience with this type of diabetes.

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Unnecessary Referrals to Pediatric Immunology Services

Jesús S. Burón-Hernández MD

Immunology is highly specialized and utilized in prophylactic vaccines, laboratory tests and treatments for patient care. It is also an essential tool for cutting-edge biomedical research designed to deepen scientific understanding and produce new therapies and technologies.[1]

At the William Soler Pediatric University Hospital in Havana, as pediatric immunologists, we work to improve our patients' quality of life and enhance their families' confidence and peace of mind. Nevertheless, we too often receive patients—referred by attending pediatricians—who don't require specialized immunology services. In other instances, immunologists are convened to evaluate hospitalized children who do not fit clinical criteria for diseases of the immune system. I consider these unnecessary referrals.

Necessary referrals are when patients fulfill clinical criteria for immune system diseases established by clinical immunological societies and national groups.[2,3] Criteria and guidelines set by these specialized societies include: patients suspected of having primary or secondary immunodeficiencies (PIDs and SIDs), autoimmune diseases or allergic diseases that are difficult to control and manage—such as chronic urticaria, atopic dermatitis or eczema, bronchial asthma, food and medicine allergies, and others. If patients do not fit criteria for diseases of the immune system, they should not be referred to an immunologist but rather to another specialist.

In my personal experience in the hospital's Immunology Department, approximately 80% of patients referred to our service do not fulfill the aforementioned criteria. These patients present with allergic rhinoconjunctivitis and allergies resulting from insect bites that should be treated by an allergist; acute rhinopharyngitis without complications; and other pediatric respiratory infections that should be treated by a pediatrician.

I've attended patients with multiple, simple-to-diagnose diseases including SIDs as a result of prior infections or use of immunosuppressants like steroids, and certain PIDs related to antibody deficiencies, which are easily diagnosed through a clinical exam and by measuring immunoglobulin levels. I've also seen patients with more complicated clinical pictures that are harder to diagnosis, including suspected PID associated with an autoimmune disease and others related to genetic disorders.

Unwarranted referrals are a problem for patients and their families, as well as for doctors. Patient satisfaction can be affected by overloaded specialized services, while the time lost and diagnostic/treatment delay resulting from unnecessary or non-urgent referrals can cause anxiety. Meanwhile, specialists spend time evaluating cases erroneously referred as immunological in source, which interrupts the clinical process and robs time from those patients really needing an immunologist. It is important to add that unnecessary referrals also affect the economy of health systems: once a patient is led to believe their condition is immunological in nature and sees a specialist, they may be sent for complementary analyses that are costly and unwarranted.

The Cuban health system is integrated and comprehensive. Community-based primary care is offered at neighborhood family doctor-and-nurse offices, linked to multispecialty polyclinics responsible for a health catchment area incorporating between 15 and 40 of these offices (20,000–40,000 people, depending on population density).[4] Patients requiring care not available at the primary level are referred to hospitals and institutes comprising secondary and tertiary levels of care. Each polyclinic has a Basic Work Group that includes, among others, an internist, obstetrician-gynecologist and pediatrician; this team attends patients referred for specialist services by their family doctor. If the pediatrician from the Basic Work Group determines a patient needs an immunologist, the youngster is referred to the corresponding pediatric hospital; care moving forward is coordinated between that facility and the polyclinic. Hospitalized children are supposed to be evaluated by their pediatrician before referral to an immunologist as well, but this process isn't observed satisfactorily.

> **Pediatricians at the polyclinic level may not recognize the clinical manifestations and remission criteria for immunological disorders**

I believe several reasons explain why we receive unnecessary immunology remissions. Pediatricians at the polyclinic level may not recognize the clinical manifestations and referral criteria for immunological disorders and there's a tendency to want to placate parents with a specialist consultation who say their child 'gets sick too often.' Although every Cuban doctor takes pathological anatomy in their second year of medical school where they learn the basics of immunological diseases, complemented by third-year clinical rotations, it's possible the information imparted is insufficient, unclear or too general. What's more, themes related specifically to immunology were only included within the pathological anatomy medical school curriculum after 1985—pediatricians still practicing who graduated prior may not have the information they need.

At our teaching hospital, we impart basic and clinical immunology courses with updated protocols and information, but I'm unsure if this is done systematically for primary care physicians at other pediatric hospitals or medical schools. Since 2013, one program helping update pediatricians and other primary care doctors about immunological diseases is the National Program for Comprehensive Care for Patients with Primary Immunodeficiencies. Training, led by Provincial Immunology Groups, focuses on recognizing PID warning signs at the primary care level; early diagnosis; clinical care; and schooling for patients in isolation.

And yet, unnecessary referrals persist. One recommendation is to clearly and systematically disseminate modifications to diagnostic and referral protocols—developed by the Cuban Immunology Society with the Ministry of Public Health's Primary Care Division—to polyclinics, family doctors and pediatricians. Placing a more specific emphasis on the diagnosis of immune system diseases throughout medical students' clinical rotations

would also help stem unwarranted referrals. Lastly, primary care pediatricians should conduct a preliminary interconsultation with specialists—in this case immunologists—to determine whether diagnostic criteria warrant referral; polyclinic directors should be apprised of specialist interconsultations that go unfulfilled, evaluating the process with the referring physician, as well.

Unnecessary referrals to immunology departments in pediatric hospitals adversely affect the care process, clog services, are time consuming for specialists who need to attend patients who really need them, and contribute to burnout. As a result, immunologists can see fewer patients, while accruing unnecessary costs in confirmatory diagnostic tests. In my opinion, this is an ongoing problem that can be addressed by considering the abovementioned alternatives. Our job is to deliver accurate, timely diagnoses and quality care—we owe it to our children to provide them.

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A Year in the COVID-19 Epidemic: Cuba and Uruguay in the Latin American Context

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ABSTRACT

INTRODUCTION One year after WHO declared COVID-19 a pandemic, we found it useful to carry out a diagnosis of the situation in Latin America.

OBJECTIVES Examine the prevailing epidemiological panorama in mid-March 2021 in 16 countries in Latin America and the performance, over time, in the two countries with the best responses to their respective epidemics.

METHODS Using morbidity and mortality data, we compared the relative performance of each country under review and identified the two countries with the most successful responses to the pandemic. We used five indicators to analyze the course of each country's performance during the pandemic throughout 2020: prevalence of active cases per million population; cumulative incidence rate in 7 days per 100,000 population; positivity rate over a 7-day period; percentage of recovered patients and crude mortality rate per 1,000,000 population.

RESULTS According to the performance indicators, Cuba was ranked highest, followed by Uruguay. Although figures remained within acceptable margins, both nations experienced notable setbacks in the first weeks of 2021, especially sharp in Uruguay.

CONCLUSIONS Any characterization of the situation is condemned to be short-lived due to the emergence of mutational variants; however, this analysis identified favorable sociodemographic characteristics in both nations, and in their health systems, which may offer possible explanations for the results we obtained.

KEYWORDS COVID-19, infodemic, Latin America, Uruguay, Cuba

INTRODUCTION

At the start of 2021, the world continues to experience dramatic effects associated with the emerging disease COVID-19 caused by the novel coronavirus SARS-CoV-2. Much was learned in 2020 regarding the virus's behavior, both in the body and society. Today there are well-defined, specific protocols for patient treatment[1,2] which has made it possible to mitigate deaths attributable to the virus, and dozens of vaccine candidates are in experimental stages, in the hope that they may prove effective and safe in preventing infection.

Personal hygiene—particularly handwashing—physical distancing, avoiding crowds in closed spaces, and the use of masks, were quickly identified as the most effective means of avoiding contagion.[3] Collectively, other measures have been implemented, including border closures and isolation. These are preventive measures that are not without controversy, but which became almost universally accepted since the beginning of the 20th century, as can be seen in historical studies.[4] This is also attested to by an article published in the US newspaper Douglas Island News more than a century ago,[5] on the occasion of the misnamed 'Spanish flu.'

Currently the pandemic is exhibiting aggressive dynamics and, according to the Pan American Health Organization (PAHO), we are far from reaching an endemic stage.[6] Since May 2020, the World Health Organization (WHO) had stated that endemicity was a possible outcome of the current pandemic. More recently, WHO authorities have reiterated that even given the existence of one or more effective and safe vaccines, it is possible that COVID-19 will remain an endemic disease in the world, both due its great diffusion worldwide, and because of the potential it has to survive in an animal reservoir.[7]

New knowledge occurs at high speeds during emergencies and results in operational challenges for all affected countries. The wealth of data attempting to characterize the pandemic is remarkable. Identifying those data that are truly valuable, condensing them, and, above all, translating them into possibly useful community actions for decision-makers and citizens, is a continuous and pressing need.

EndCoronavirus is a coalition of scientists that came together in response to the pandemic.[8] Based at the New England Complex Systems Institute (NECSI), it manages an open platform where it shares analyses and data from all over the world. Analogous instruments have been created, among others, by the John Hopkins University Resource Center,[9] the Brown School of Public Health[10] and the World Health Organization.[11]

A tour of these sites allows a panoramic look at COVID-19 data at the global level and at differing patterns at the national level. The pandemic has expanded over months with little or no containment in some countries (such as Brazil, the United States and the United Kingdom) and other countries that had initially achieved promising favorable scenarios experienced late and frequent outbreaks (as was the case with Germany, Malaysia and Belarus). Other countries currently show signs of effective control (including Iceland, New Zealand and Singapore).

Informational spaces of this type, however, usually offer temporal characterizations related to disease distribution, without delving deeply into the other central aspect of epidemiology: determinations of health or disease.

In every epidemic, what could have been thought 'merely medical' attains deeply social connotations. In this context, epidemiological

science, especially critical epidemiology, unravels not only the distribution of the disease but also its determinative processes, which recognize the importance of the social framework. In journalistic or digital media, not only is the social analysis of the problem often hijacked, but it is occasionally trivialized or contaminated with sensationalism, misrepresentation and political bias.[12]

In this framework, epidemiology is urged to make contributions based on its most important mandates: identification of spatial and temporal patterns of the pandemic, on the one hand, and on the other, its uncertain and changing evolution. At the same time, it must deepen critical examination of the results attained as a function of response actions deployed in different contexts.

Now, after 12 months of struggling to contain the epidemic since its arrival in Latin America, it is time to characterize the prevailing situation in the region, analyze the course of the epidemic through to the current situation, and evaluate how the epidemic has been handled by the media. We know that any characterization is condemned to be ephemeral or provisional because it concerns an ever-changing and constantly developing process. However—even with necessarily provisional results—this analysis can help us understand the determinative processes in this new phenomenon full of uncertainty. Added to this is the methodological value derived from the exercise consisting in illustrating some avenues of analysis that transcend the mere phenomenological exposition during a given period of the epidemic process. For the above reasons, we propose to examine the prevailing epidemiological panorama in mid-March 2021 in 16 countries of the region and the performance, over time, of the two countries that achieved the best results.

METHODS

This is a descriptive study where an examination of the prevailing situation in most Latin American countries was carried out one year after the outbreak of the epidemic in the region. We examined data corresponding to 16 Latin American countries. Some nations were excluded due to the dubious reliability of the data they provide. This was attributed to the relative weakness of their statistical systems (the case of Haiti);[13] to the fact that the official data fail to conform to standards dictated by international organizations (the case of Nicaragua);[14,15] or to the fact that the validity of the reported figures has aroused suspicions and been called into question, as were the cases of Venezuela[16] and El Salvador.[17,18] Although there are numerous indicators that can be used in this endeavor—related to prevention, health services, community participation and surveillance, among others—we have concentrated on morbidity and mortality due to their socio-epidemiological and public health importance.

For this initial analysis, the respective classic descriptive epidemiology indicators were used: mortality rate (R_1), and cumulative incidence rate of detected cases (R_2), both per million population. Both the definitions of the rates and the data used are those that appear at https://ourworldindata.org/coronavirus

Let us call R_{ij} the i^{th} rate (i: 1,2) corresponding to the j^{th} country (j: 1, ⋯, 16), $\min(R_i)$ is the lowest value among the R_i rates of the 16 countries considered and $\max(R_i)$ is the highest. The relative risk of dying and becoming ill was computed for each country in relation to the one that exhibited the lowest rate[A]. That is, it was computed as:

$$RR_{ij} = \frac{R_{ij}}{\min(R_i)}$$

To establish an order among the countries regarding the impact of the epidemic based on two indicators that concern conceptually different dimensions, a single impact index was constructed. First, a relative impact index was calculated for each of the rates and for each country, which we will call the "relative rate" ($RRate_{ij}$):

$$RRate_{ij} = \frac{\max(R_i) - R_{ij}}{\max(R_i) - \min(R_i)}$$

Where $RRate_{ij}$ reaches the maximum value equal to 1 for the country with the lowest value of R_i and the minimum value equal to 0 for the country with the highest R_i. Finally, the index $WMRR_j$ is computed for each country through a weighted average of the two relative rates; the formula gives more weight to mortality than to morbidity (weights 0.6 and 0.4, respectively): $WMRRate_j = (0.6)(RRate_{1j}) + (0.4)(RRate_{2j})$.

Second, based on the results obtained above, the two countries with the best indicators to date in the analysis (concluded on March 10, 2021) were Cuba and Uruguay. Their results were examined in detail throughout the period since the initial outbreak of the epidemic in the region. Emphasis was placed on daily performance of the following five indicators.

1. Prevalence rate of active cases (PRAC) per million population

$$PRAC = \frac{Number\ of\ active\ cases\ present\ on\ a\ single\ day}{Population\ size} 10^6$$

where active cases in a day is the total number of persons diagnosed up until that day, minus the total number of deceased and recovered individuals.

2. Average cumulative incidence rate (ACIR7) in 7 days per 100,000 population

This is the calculation, for each day, of the average number of new cases detected during the previous week, also known as the Harvard P7 index.[19]

$$ACIR7 = \frac{Average\ number\ of\ cases\ in\ the\ past\ seven\ days}{Population\ size} 10^5$$

3. Positivity rate for each day and the preceding six days (PR7)

Occasionally absolute thresholds are used to monitor the course of the epidemic. One of them, promoted by WHO,[20] is the so-called 'positivity rate' in a period determined by two moments t_1, t_2, defined as:

$$PR(r_1; r_2) = \frac{Number\ of\ positive\ cases\ in\ period\ (r_1; r_2)}{Number\ of\ diagnostic\ exams\ performed\ during\ period\ (r_1; r_2)} 100\%$$

We also calculated the PR corresponding to seven consecutive days. That is, for each day, the numerator is the sum of cases

detected that day (r_1) and the previous six days ($r_2 = r_1 - 6$), where the denominator is the sum of the tests carried out in those seven days. To underscore that this is the chosen period, we will call it PR7 from now on.

4. Percentage of patients who recovered (RP) to date

$$RP = \frac{Number\ of\ recovered\ infected\ cases}{Numbers\ of\ diagnosed\ infected\ cases} 100\%$$

Recovery criteria is not the same in all countries. In particular, this is the case in Cuba and Uruguay: while in Cuba a negative RT-PCR (real-time polymerase chain reaction) test has always been considered a recovery criterion, Uruguay, since October 2020, uses clinical and evolutionary criteria to grant hospital discharge without requiring a negative RT-PCR test.

5. Crude mortality rate (CMR) per million population

$$CMR = \frac{Number\ of\ deaths\ to\ date}{Population\ size} 10^6$$

We used the daily official reports provided by the National Emergency System of Uruguay[21,22] and by the Ministry of Public Health of Cuba[23] for all calculations. The article is therefore based entirely on secondary data from publicly accessible sites. Consequently, there are no potential ethical problems pertaining to data collection or analysis.

RESULTS

The situation in Latin America The table contains relevant data on COVID-19 mortality and morbidity in the 16 Latin American countries included in the study.

Cuba is used as a reference for the purposes of calculating relative risks (columns 3 and 6). This is due to the fact that it occupies the best position for both indicators in mid-March 2021. In terms of mortality, Uruguay follows, although with an appreciable difference: a crude mortality rate 6.2 times higher, which is still appreciably distant from the rest. In terms of morbidity, after Cuba, there are several countries with similar rates.

For the weighted average of relative rates (column 7), which condenses the impact of the epidemic in terms of mortality and morbidity, Cuba and Uruguay occupy the best places (in that order).

The epidemic's evolution in Cuba and Uruguay The comparison of Cuba and Uruguay is useful because they are the two countries with the best results, as well as because of their similarity in some areas that are either directly or indirectly related to the epidemic.

They are relatively isolated nations—Uruguay due to its southern latitude and Cuba due to its insularity—and they are relatively small countries that have large neighbors (Brazil and the United States, respectively) with very high levels of SARS-CoV-2 dissemination. Both have quality health and primary care systems. Cuba and Uruguay have the oldest populations in the region: the median ages are the highest (43.1 and 35.6 years, respectively) and they also have the highest percentages of people over 70 years (9.7% and 10. 4%). Their populations have a high educational level in the context of Latin America; exhibit the lowest infant mortality rates in the region (4.7 deaths per thousand live births in Cuba and 7.0 in Uruguay); and rate very highly on the UN Development Program's Human Development Index (HDI) in the regional context (the value for Uruguay is 0.817 and Cuba's is 0.783, according to the 2019 Report).[24]

They are also relative equals regarding the equitable distribution of income as measured by the Gini Coefficient (GC). In Uruguay it is 0.42. In Cuba, although the last known measurement is from 1999 (GC 0.41), it is estimated that in successive years it has remained at the same level.[25] With these values, Cuba and Uruguay occupy the best places in Latin America and the Caribbean for this indicator.

One notable difference lies in the political system. Cuba is a socialist country, while Uruguay is governed by a coalition of the right and center-right, although it is the successor to a leftist government that ruled for 15 years and ended just before the start of the pandemic, on March 1, 2020.

Another similarity, now in reference to the epidemiology of COVID-19, is the sustained growth that both countries have presented in the number of active cases throughout the past quarter, after several months of very favorable evolution, until they reached what can be considered the worst moments of the epidemic in both countries.

The successes of both Uruguay and Cuba in the first months of their respective epidemics have been progressively and seriously compromised during 2021. In the first months of this year, the number of active cases in the same day skyrocketed and broke records in both nations: for Cuba, this number rose to 5800 (February 1) while for Uruguay it reached 9261 (March 11). In just the first 10 weeks, Uruguay accumulated 74% of all deaths and 72% of all diagnosed cases. For Cuba, these data are similarly disturbing (61% of the deaths and 82% of all cases).

Figure 1 shows that shortly after the beginning of 2021, both countries (analyzed separately), show an epidemic trend that could well be described as 'alarming'. The two curves reflected there record the epidemic's evolving dynamics in the two countries. A critique has been raised that these judgments are established based on absolute numbers and not on rates;[26] for example, statements like the "epicenter of the epidemic" is located in a certain country or region, which are deemed questionable because they are based on numbers of this type (accumulated cases or registered deaths) instead of using the corresponding rates. Consequently, in order to establish adequate comparisons between countries, we calculated the rate of active cases per million population. Using the aforementioned rates, it can be seen that the situation in Uruguay on March 10, 2021 was 6 times more critical than that of Cuba (rates of 2505.1 and 410.8 respectively).

Note (Fig. 1) that the growth of Cuba's PRAC curve becomes much less pronounced when placed in the context of both countries.

The curves show a marked similarity during the first eight months of the epidemic, including the absence of a 'first wave' indicating a high incidence of new cases, which affected other countries in the region in 2020, but not Cuba or Uruguay. However, that

marked similarity disappears during the last semester; a pattern repeated when examining other selected indicators. And this trend allows us to glimpse what may be galloping growth over the next few weeks for Uruguay.

In relation to the ACIR7 Index, which measures the immediately prior situations experienced every day in terms of new cases, the similarity between the two countries is notable until mid-November, when the Uruguayan rates begin to take off (Fig. 2).

It should be noted that the fewer tests carried out, the fewer cases will be detected. Consequently, a country with a lower testing rate would 'benefit' when countries are compared using indicators that increase as said rate increases, as occurs with the PRAC and the ACIR7. This problem, however, does not affect our analysis, as the testing rates performed per 1000 population in Cuba and Uruguay in the period in which the differences were most marked are quite similar: 100.6 and 135.7, respectively, throughout 2021.

Another indicator analyzed is the positivity rate in the diagnostic tests performed for seven successive days (TP7). Until mid-November, this rate fluctuates below 2% for both countries; after which the index increases in both countries. However, while in Cuba the PR7 remains below 5%, the threshold considered the maximum acceptable by WHO,[27] in Uruguay it fluctuates at around 10% (Fig. 3).

The recovery rates of previously diagnosed patients have been high and remained similar throughout the entire period (Fig. 4). Cuba has exhibited better results in this area for much of the period, but since mid-October, the percentages have tended to equalize and remain at very high levels in both countries.

Mortality is, in our opinion, the most important of all indicators for obvious reasons. Once again, after exhibiting remarkable similarity until the middle of 2020, the mortality rate in Uruguay begins to take off very notably (Fig. 5), until it reaches the current situation—as of March 12, 2021: Cuba has experienced 361 deaths and 688. Uruguay's population is one third that of Cuba's (3,461,734 vs. 11,333,483), resulting in a mortality rate

Figure 1: Prevalence rates of active COVID-19 cases per million population, Cuba and Uruguay; March 11, 2020–March 10, 2021

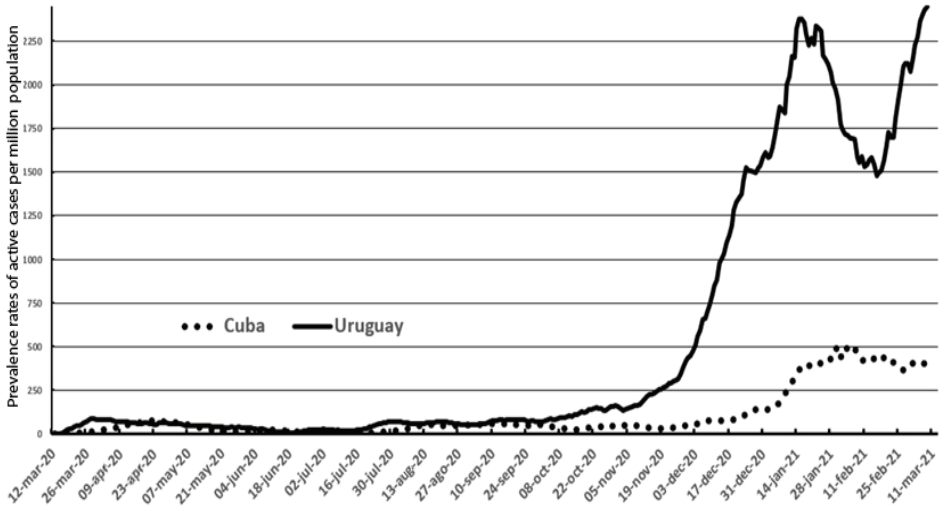


Figure 2: Moving average of accumulated COVID-19 cases in last 7 days per 100,000 population, Cuba and Uruguay; March 11, 2020–March 10, 2021

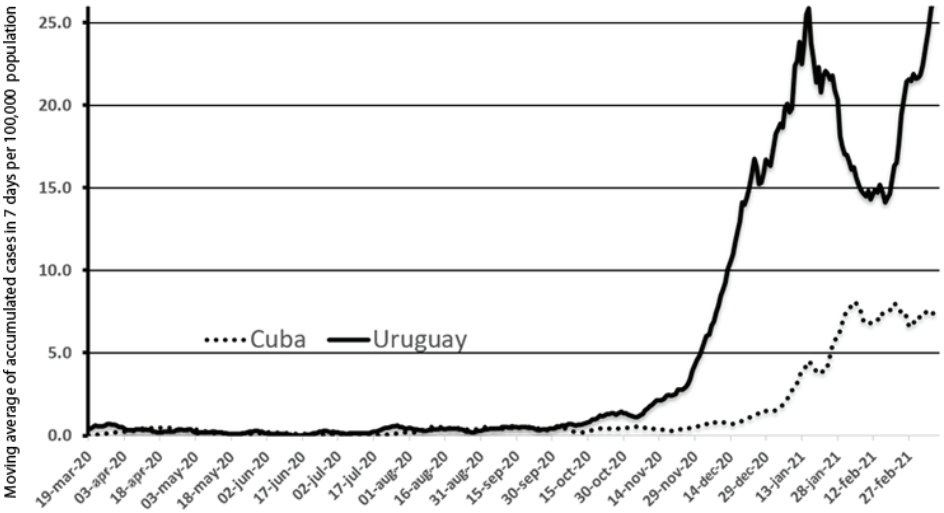


Figure 3: Seven-day moving COVID-19 positivity rate, Cuba and Uruguay; March 11, 2020–March 10, 2021

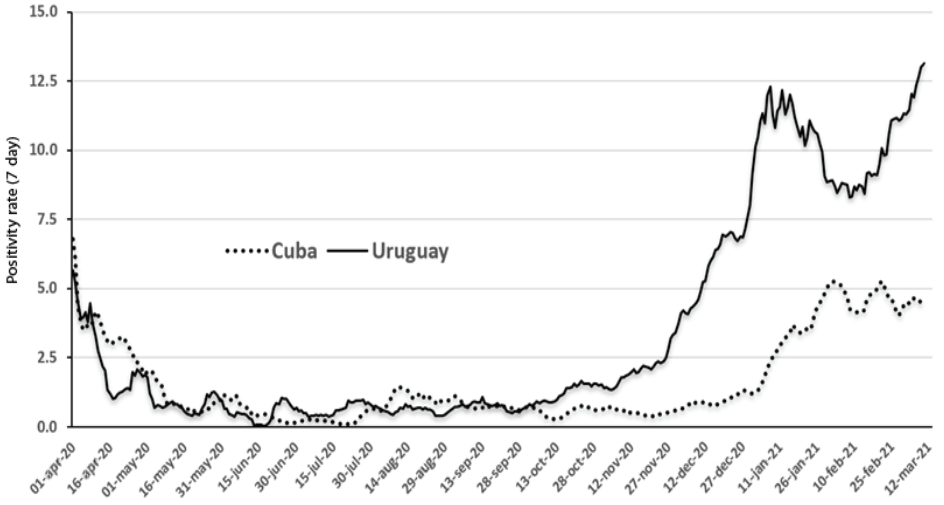


Figure 4: Percentage of people diagnosed with COVID-19 who have since recovered, Cuba and Uruguay; May 1, 2020–March 10, 2021

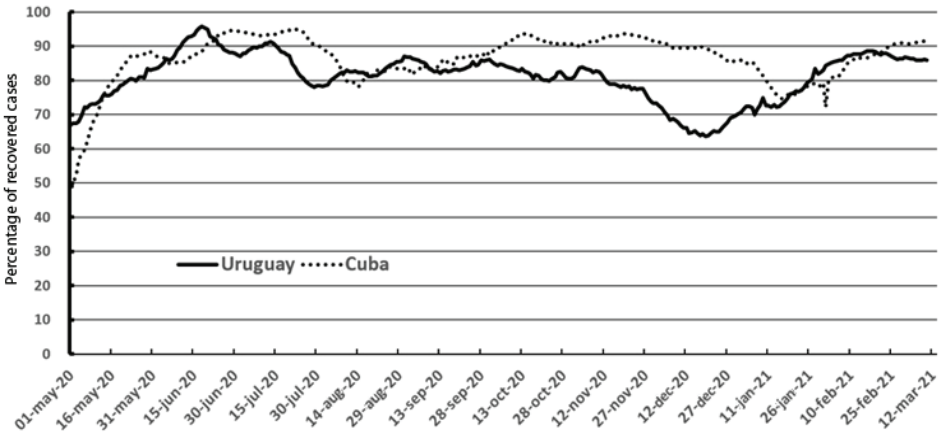
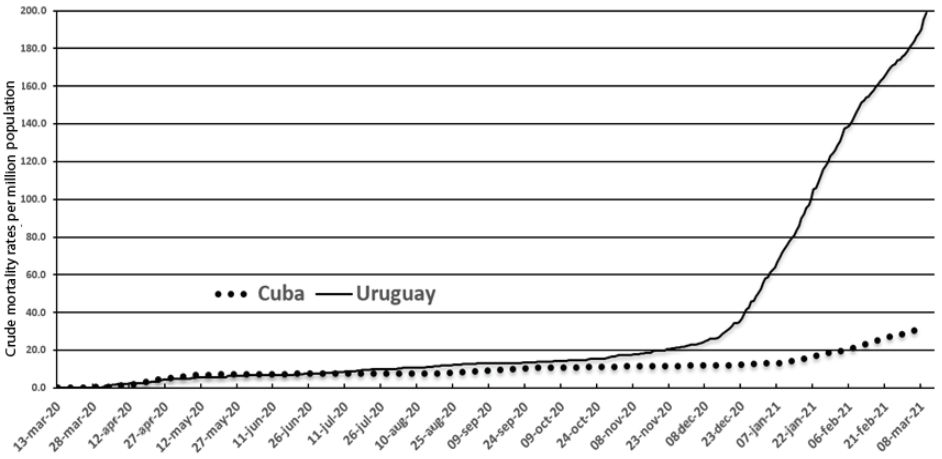


Figure 5: Crude COVID-19 mortality rates per million population, Cuba and Uruguay; May 1, 2020–March 10, 2021



that is over six times higher, as is seen in the table. The latter country has a population 3 times smaller (3 461 734 vs 11 333 483 population), which produces a mortality rate per million population more than 6 times higher in Uruguay (Table).

It should be noted, however, that in both countries most deaths correspond to persons who died 'with' COVID, and not strictly 'from' COVID in the sense that, for the most part, they were elderly patients who, at the time of death, suffered from important comorbidities such as chronic obstructive pulmonary disease (COPD), cardiovascular diseases, diabetes, chronic kidney disease, cancer and obesity, among others.

The average age of the deceased has been high and almost the same in the two countries: approximately 75 years. There have been no deaths in pediatric age groups. Except for 4 Uruguayan citizens and 1 Cuban, none of the remaining 1,044 deceased to date was under 35 years old.

The media's treatment of the epidemic

In the context of the comparison between Cuba and Uruguay proposed by this study, the information that has been provided on the epidemic exhibits some unique features. For example, Cuba and Uruguay were the only countries in the region that welcomed an international cruise ship shortly after the start of the pandemic.

Table: COVID-19 morbidity and mortality in 16 Latin American countries; March 11, 2020–March 10, 2021

Rank	Country	Total deaths	Crude mortality rate per million population	Relative risk of dying from COVID-19 (RR_1)*	Total cases	Accumulated incidence rate per million population	Relative risk of becoming ill from COVID-19 (RR_2)*	Weighted average of the two relative rates (MWRR)
1	Cuba	357	31.5	1.0	58,379	5154.1	1.0	1.000
2	Uruguay	678	195.2	6.2	66,484	19,139.1	3.7	0.858
3	Guatemala	6,522	364.0	11.6	180,393	10,069.1	2.0	0.837
4	Dominican Republic	3,198	294.8	9.4	244,168	22,508.3	4.4	0.799
5	Honduras	4,301	434.2	13.8	175,442	17,713.2	3.4	0.768
6	Paraguay	3,387	474.9	15.1	174,013	24,397.1	4.7	0.715
7	Costa Rica	2,848	559.1	17.7	207,832	40,798.5	7.9	0.594
8	Ecuador	16,105	912.8	29.0	296,841	16,824.8	3.3	0.576
9	Bolivia	11,884	1018.1	32.3	256,462	21,970.5	4.3	0.505
10	Chile	21,206	1109.3	35.2	867,949	45,403.8	8.8	0.343
11	Mexico	192,491	1493.0	47.4	2,144,486	16,632.6	3.2	0.339
12	Colombia	60,773	1194.4	37.9	2,285,960	44,925.9	8.7	0.311
13	Argentina	53,359	1180.6	37.5	2,169,694	48,006.6	9.3	0.300
14	Brazil	270,656	1273.3	40.4	11,202,305	52,702.0	10.2	0.237
15	Peru	48,163	1460.7	46.3	1,380,023	41,854.6	8.1	0.218
16	Panama	5,957	1380.6	43.8	346,301	80,259.5	15.6	0.046

*Relative risk of dying from COVID-19 (rate ratio): COVID-19 mortality rate in the country / COVID-19 mortality rate in Cuba; ** Relative risk of becoming ill from COVID-19 (rate ratio): in-country COVID-19 incidence rate/ COVID-19 incidence rate in Cuba. Source: Table prepared based on data from: https://www.ourworldindata.org/coronavirus

Amidst great anxiety provoked worldwide by the still little-studied threat, on March 13, 2020, the *MS Braemar* cruise ship, with numerous sick passengers, floated through the Caribbean with no country willing to receive it and host its passengers, as requested by the British government. Only Cuba assumed the enormous dangers posed by receiving travelers and facilitating their return by air to London.[28] BBC World News ignored the story. It is difficult to believe that, for an event of such extraordinary significance, the omission was the result of distraction. A similar gesture by the Uruguayan government a month later with the Australian cruise ship Greg Mortimer prompted high praise from the same service.[29]

As is well known, the 'virus' of distorted, tendentious or completely fabricated information—the so-called 'infodemic'—appeared across the world as soon as this health emergency began. Indeed, the novel coronavirus pandemic has been an opportunity to manufacture stories inspired by extra-scientific interests. In addition to promoting certain stereotypes, we see efforts to conceal those truths that would call them into question. That is to say, the dissemination of false information in news reports and social media, occasionally is accompanied by deliberate omission of facts.

Notable in this context is the repeated absence of Cuba when references are cited showing good management of the epidemic.

For an extended time, the media highlighted the situation of some countries, such as Uruguay, Costa Rica and Paraguay, that were considered the three countries 'winning' against COVID-19,[30] while omitting all mention of Cuba, a country that shared that privileged position in the COVID-19 epidemiology in the region. The politicization of discourse complements information bias. By way of illustration, take as an example the following text published by CNN in May: "The success of Paraguay, Costa Rica and Uruguay in the fight against the pandemic seems to contradict the generalized belief that dictatorships are more successful than democratic governments in the fight against these pandemics."[31] In some media outlets, even in late October, Uruguay and Paraguay are exalted, but Cuba is omitted, as if it did not exist and as if it were not experiencing the greatest success in handling the pandemic in the region. This led to the general opinion that: "with the exception of Uruguay and Paraguay, mortality from COVID-19 in Latin America is very high" or "except for Uruguay and Paraguay, Latin American countries have fared considerably worse than the European countries and the United States."[32]

Recently, on January 27, 2021, the Lowy Institute in Sydney released a report[33] that reflects very clearly the distortions to be found both in academic analyses and their impact in the media. The study places 100 countries, a list not including Cuba, along a ranking based on an index comprised of six indicators that involve (in a very confusing way) cases, deaths and tests performed. Despite the opaque methodology used to construct this index and the fact that a convincing explanation is not given for the exclusion of certain countries, thousands of journalistic and digital media worldwide (Google contains more than 300,000 entries with this information) reported the results as if it were a global 'barometer' that merited no objection. For example, Uruguay's media used it to proclaim that "Uruguay is the best-positioned country in the entire American continent"[34] or "Uruguay is the best in America."[35]

DISCUSSION

The results inspire both discussion and reflection. On the one hand, we have the favorable position shared by Cuba and Uruguay in the regional context. On the other hand, there is the similar development of the epidemic in the two countries during the first eight months, and the marked distancing of their indicators in the final period analyzed, although they continue to maintain certain parallels in their trends.

From the Latin American worldview, Cuba has maintained a leading position in its response to the pandemic, although, as in any other enclave on the planet, there is a risk that the epidemic—which at one point seemed completely cornered—could fly out of control. This has occurred dramatically in some countries, such as Ireland, which accumulated in just one month as many cases as it had in the previous nine months, or in the Czech Republic where the crisis has been overcome again and again and yet shortly thereafter record-breaking figures emerge. In Latin America, the countries that seemed to be 'on the right track' (notably, Costa Rica and Paraguay) now exhibit indicators several dozen times more disadvantageous (Table).

In hindsight, the triumphant exaltations of the press suggest the need to maintain a more cautious profile. Framing certain achievements as if they were immutable can generate excesses of confidence that, in the end, can be counterproductive.

A full understanding of the dynamics of a pandemic like COVID-19 will not be achievable for some time. But an analysis of what happened over the first 12 months can establish some provisional explanations. Understanding the processes that lead to the currently evolving issues experienced both in Cuba and Uruguay, is as challenging as explaining their favorable evolutions in the first months of the pandemic.

The processes underlying the epidemic's production and reproduction are of different origins and interact in complex ways. Structural aspects like social organization and relative economic situations, applied policies, the health system and demographic structures interact with aspects that arise from ways of life and lifestyles deeply rooted in social and cultural dimensions, influenced in turn by categories such as social class, gender, age and ethnicity. Fully deciphering this latticework and its multiple combinations, which can produce different expressions of epidemics caused by the same virus, transcends the possibilities of this paper. Even knowing that more questions than answers may be raised, some of the aforementioned features and corresponding responses to the epidemic in each country are examined below, which may contribute to understanding the pandemic's evolution.

The demographics and human development indicators in Cuba and Uruguay reflect several similarities that, although they favor the countries' advantageous epidemiological situations in the region, are insufficient to explain this shared success all by themselves. On the other hand, the course of the epidemic exhibited different patterns. Consequently, we propose the following dimensions in this discussion: a) available resources and the health system, b) social and cultural support for the country's pandemic response.

Undoubtedly the strengths of the Cuban health system have been behind its achievements regarding the COVID-19 pandemic.

Possessing a powerful and free-of-charge public National Health System, with universal access and coverage, Cuba has some 500 polyclinics throughout the nation, with 12,000 family doctor-and-nurse offices in communities and nearly 500,000 workers in the health sector. There is one nurse for every 133 population (75 nurses per 10,000 population) and one doctor for every 116 population which means that the rate rises to 87 physicians per 10,000 population, the highest in the world. It also has a vast network of health institutions for secondary and tertiary care, and numerous centers for epidemiological surveillance articulated with primary care, as well as prestigious centers for public health, medical and biotechnological research. Additionally, it has managed to develop and implement flexible, advanced protocols for patient care in accordance with the best existing knowledge.[36–38]

The Cuban health system's ability to adapt to new challenges is also noteworthy. In the words of Dr Carissa Etienne, Director General of the Pan American Health Organization: "Cuba expanded the extremely strong health system that it already had, further expanded this network to include more health workers and medical students, incorporating digital tools to improve contact- and case-tracing. They used a very well-established health system that now includes new elements from this pandemic."[39] Cuba has managed to articulate intersectoral action, essential to configure responses that are both agile and socially organized with an aim of developing activities to prevent infections and deaths. Cubans have seen, day by day, how all the ministries, information sources (with no private radio or TV channels in the country) and social actors have mobilized around a National Plan for Prevention and Control of SARS-CoV-2, for the defense and care of the population threatened by the virus.

Last century, a scholar noted "when we are facing a sudden disastrous event, such as a cyclone, an earthquake, or flooding, various features of the affected societies become apparent. The stress it causes puts social stability and cohesion to the test."[40] It is well known that the periodic hurricanes passing through the Caribbean, Mexico and the United States often leave a trail of deaths in their wake, which is nevertheless unfamiliar to Cubans. This is not a matter of luck: it represents defense capacities organized by the State, and, above all, actively supported by the population. The spread of a highly contagious virus is more insidious than the impact of a cyclone and represents a more lasting and complex challenge, but the social cohesion evident in the Cuban response has also been vital in the face of this health emergency.

Uruguay has a National Integrated Health System (SNIS). Its initiation in 2008 made it possible to overcome the system's fragmentation and optimize its financing, as well as guarantee practically universal comprehensive healthcare coverage. With 44 public and private providers, the financing and management of the SNIS is carried out by the State.[41] Its government agencies are supported by the social participation of workers and users. Three main axes supported the health reform. Two of them—changes to management and financing models—advanced and were consolidated throughout the first decade of the system; but the third—transformation of the healthcare model itself—has been slow, incomplete, and is not yet consolidated.

Many of the system's providers lack sufficiently developed work at the primary healthcare level and in the community context. Although progress has been made in infrastructure, organization of work in this area has not been prioritized by institutions, inclusion of specialists in family and community medicine is insufficient, there is a deficit of nursing and mental health professionals, and remunerations are not attractive. There is also no functional career path at this (primary care) level; that is, no institutional or material progression is foreseen for these professionals. The hospital-centric imprint that the SNIS tried to overcome still survives.

An event that occurred at the beginning of the epidemic in Uruguay clearly illustrates these problems: most primary care health services in the State Health Services Administration (ASSE) in Montevideo, Canelones and the country's other departments were closed in March and their personnel were redistributed to make them available for face-to-face or telephone consultations in other spaces. The reactions of the professionals involved, particularly those of the Uruguayan Society of Family and Community Medicine (SUMEFAC), supported by the Uruguayan Medical Union (SMU) and the Users' Movement, managed to reverse the situation and reestablish health teams within their communities.[42]

Additionally, primary care is not adequately prioritized in protocols designed to address the COVID-19 epidemic, especially regarding epidemiological surveillance and information systems. In this crucial area, the contrast between Uruguay and Cuba is noteworthy.

Uruguay maintains a centralized surveillance mechanism. For the COVID-19 epidemic, a tracing system was established whose capacity was quickly exceeded—even when it doubled in number—during the month of November, when the virus began to spread rapidly.[43]

It is not our intention to draw conclusions based on these realities, but the 'inability' (or exceeded capacity) of Uruguay's centralized epidemiological surveillance system should not be overlooked, nor its insufficient assignment of a leading role to primary care for monitoring the epidemic. Both deficiencies, absent in Cuba, could partially explain the lower levels of epidemic control in Uruguay.

Broadly speaking, it can be said that in Uruguay no mandatory restrictions on movement have been imposed, although measures—very strong at the beginning of the epidemic, more tenuous in recent months—have been established to reduce mobility linked to work, childcare and recreation (closure of schools, encouragement to telework, suspension of public shows, reduced capacity in interdepartmental buses, and prohibitions related to gatherings, among others). Mandatory masking was established in some settings as the epidemic advanced in 2020.

In general terms, the limitations imposed in Cuba have been similar, although flexibly adjusted depending on the epidemic's geography. Perhaps the most important distinguishing measure lies in Cuba's hospitalization of all infected persons (including asymptomatic patients) and the isolation of both the contacts of diagnosed cases and of suspected cases detected by the primary care system. Initially, there were strict limitations on travelers' entry into the country, which were relaxed in September. However, due to outbreaks linked to incoming travelers, these limitations were re-established at the end of the year. This evolution reflects the delicate balance between measures that favor economic recovery and those that hinder the pathogen's spread.

The participation of scientific and academic communities in both countries is worth highlighting, as is the early establishment of [interdisciplinary] collaboration.

It is plausible that the favorable mortality figures on record can be attributed to high quality of both health systems and services, as well as to the application of COVID-specific care protocols. Additionally, the very low case fatality rate in both countries, 12 months after the first cases (0.61% in Cuba and 1.02% in Uruguay), supports this hypothesis.

Domestic manufacture of COVID-19 diagnostic technology enabled adequate coverage of this key aspect in the pandemic, independent of the international market. Diagnostic testing capacities increased progressively throughout 2020, without interruption in Cuba and with only a few intermittent interruptions in Uruguay, guaranteeing availability of the number of tests needed for each stage of the epidemic. The non-proportional increase in the number of tests with respect to the accelerated increase in cases during December–March has translated into increased positivity (Fig. 4) and leads us to wonder if need has exceeded capacity and if this constitutes a critical point for controlling the epidemic in Uruguay at its current stage.

One result of this scientific-academic collaboration in Uruguay was the formation in April 2020 of the Honorary Scientific Advisory Group (GACH), a group of academics, teachers and researchers established as a consultative body at the request of the national government, to serve as interlocutors for decision-making and analysis of pandemic management measures. The GACH has been functioning since its creation.

The setbacks observed in the two countries require more in-depth examination, which goes beyond the scope of this study. Not having been able examine these setbacks constitutes a limitation of this paper, but we identified areas that should complement such an analysis in the future.

For example, all countries have experienced community circulation of SARS-CoV-2. However, an examination of the degree to which community organization has affected spread is still pending, using methodological approaches that consider each locality's unique characteristics. The participation of the health system's community-based entities and of communities themselves in the management of the epidemic been different in Cuba and Uruguay. It makes sense to think that high degree of social involvement played a role in the favorable evolution of the epidemic in Cuba, although this is conjecture at this point and requires more intense scrutiny.

In both countries, a greater role for the social sciences could be of assistance,[44] an idea called for by, among others, by the Spanish Political Sciences and Administration Association (AECPA). Despite the difficulties, the exigencies posed by COVID-19 should prompt all countries in the region, without exception, to go beyond biomedical sciences in our response, as was recently recommended by WHO.[45] The crisis demands the attention of public health professionals and epidemiologists, complemented by the work of historians, virologists, clinicians, philosophers, geographers, theologians and behavioral scientists, among others, to understand and address the problem.

Actions must appeal to the wisdom of community leaders and not be reduced to the sometimes chimerical demand to fulfill norms of behavior that ignore singularities unique to each locality, nor should health systems be made to shoulder the exclusive responsibility for prevention. An examination of the future of the epidemic in Latin America in general, and in Uruguay and Cuba in particular, seems to advocate for such an approach.

Two fundamental lessons follow from this study. The first and most important is that given what we know of SARS-CoV-2 it is not possible to happily 'declare victory,' since what seems a very favorable situation can be abruptly reversed. The second is that the most fruitful comparative analyses between countries or regions must consider sociodemographic and political factors (especially population size) influencing the ways in which the epidemic unfolds, as well as be cautioned against information biases induced by the media.

Various issues affecting multiple territories in Latin America, including of course both Cuba and Uruguay, merit continued attention. This paper offers a modest contribution in this direction, but the pandemic has opened numerous avenues for study today and in the future.[46] Examples include: the impact of the contempt that some statesmen hold for science, individuals who routinely contradict and undermine experts leading the response to COVID-19; the extent to which inequality has catalyzed tragedy; and the impact the pandemic has had in the deepening of inequalities by race, gender and class.

Finally, we conclude that any characterization of the situation is condemned to be ephemeral due to the ever-changing nature of the epidemic and its viral mutations; however, this analysis allowed us to identify favorable sociodemographic characteristics in both nations, as well as those of their health systems, and to provide possible explanations for each country's relatively favorable outcomes.

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Open Letter to President Biden about COVID Vaccines for Cuba

Document presented by BioCubaFarma scientists in Havana on August 10, 2021. Spanish and English versions available at: https://www.cienciacubana.cu/es

President Biden:

You recently referred to Cuba at a White House saying: "I would be prepared to give significant amounts of vaccines if… an international organization would administer those vaccines and do it in a way that average citizens would have access to those vaccines." You also called Cuba a "failed state".

These statements surprised many, including those in the U.S. who have first-hand exposure to Cuba's health system. It also rankled frontline Cuban health workers risking their lives to contain the COVID epidemic in our country. They do not reflect Cuban reality, and we deplore that disinformation by malicious actors is influencing your policy decisions. As scientists, doctors, and concerned citizens, we believe it's worth fact-checking three assumptions implicit in what you said.

Assumption one: International intervention is needed to ensure all Cubans receive vaccines.

Assumption two: Cuba's response to the pandemic has been dismal, symptomatic of a "failed state".

Assumption three: U.S.-supplied vaccines are the only route to guarantee COVID-19 immunization for Cuba's 11 million people.

Let's take these one by one: the first assumption—that intervention is needed to guarantee vaccine access for all Cubans—suggests that vaccine rollout in Cuba is inefficient and discriminatory. But the data does not support this. In fact, as both UNICEF and the World Health Organization have confirmed, childhood vaccination rates are over 99%. Immunization is part of our country's universal public health system, free to all Cubans regardless of socioeconomic status, politics, religion, sex, or race.

The national immunization program, created in 1962, covers the whole country. Since 1999, all Cubans have been protected against 13 potentially fatal diseases, including diphtheria, tetanus, and pertussis. Eight of these vaccines are manufactured in Cuba.

As a result of high vaccination rates, we have not had a single case of measles. In contrast, the CDC confirmed 1282 measles cases in the United States in 2019, with only 74% of children receiving all CDC-recommended vaccines.

The Finlay Vaccine Institute in Havana developed the world's first effective vaccine against/for meningitis B (meningococcal disease) in 1989. The annual incidence of meningococcal disease in Cuba dropped from 14.4/100,000 population before vaccination to less than 0.1/100,000 since 2008—eliminating the illness as a public health problem in the country.

Several factors explain the success of Cuba's national vaccination program: people trust the easily accessible neighborhood family doctors and nurses, and the health professionals at their community polyclinics—making vaccine hesitancy very rare. In turn, the health system's organizational capacities make vaccine rollout fast and dependable. Finally, Cuban biotechnological research and production centers are well integrated with the needs of the public health system.

Working partnerships on vaccination have developed with the World Health Organization and UNICEF. But none of these has ever suggested the need to step in to administer vaccines in Cuba. Rather, Cuban vaccine experts have been called upon to assist in global efforts to eliminate polio, and our production facilities have been tapped by WHO to export urgently needed vaccines to the "meningitis belt" in sub-Saharan Africa.

Assumption two: Cuba's "failed" pandemic response. It is puzzling why, with so many real COVID catastrophes in the Western Hemisphere, only Cuba is labelled a "failed state". Cuba has indeed seen a recent spike in cases that threatens to overwhelm the health system in parts of the country. However, its response has been more effective than many other nations that have not received this harsh criticism from the U.S.

All countries are now challenged with new COVID variants, such as Delta, often driving sharp increases in cases. Cuba is no exception. What makes Cuba unique is the need to manage the epidemic under a crippling financial, trade and economic embargo enforced by the U.S. government for the last six decades. The 243 additional restrictions slapped on by the Trump administration—every one still in place under your presidency—were intended to close the blockade's few remaining loopholes, and thus choke off revenues to Cuba. This reduces the cash available to buy medical supplies and food, and delays the arrival of materials to the country.

Assumption three: the only route to COVID immunity in Cuba is through U.S.-supplied vaccines. This ignores the fact that more than two million Cubans, or nearly 30.2% of the population, have already been fully vaccinated with Cuban developed vaccines.

The Abdala vaccine received emergency use authorization from the Cuban regulatory authority on July 9, making it the first vaccine to achieve this status in Latin America. Abdala achieved 92% efficacy in Phase III clinical trials, while the Soberana Vaccine achieved 91% and is also close to emergency use authorization. At the current rate of vaccination, the entire population could be reached by October or November. Difficulties in rollout, including imports of vital vaccine ingredients, are due primarily to the financial squeeze of U.S. sanctions.

If the U.S. government really wanted to help Cubans, it could roll back the 243 Trump-era measures—possible with the stroke of the president's pen. Congress could also lift sanctions altogether, as demanded each year by overwhelming votes at the UN General Assembly by the nations of the world.

During the pandemic, science reiterates that (politics aside) we are all in this together. All of us are threatened not only by disease but also by the unprecedented challenge of climate change. In this context, health systems of all countries should be supported, not undermined; and collaboration should be the order of the day. More so, taking into consideration the alarming dearth of vaccines worldwide, especially dangerous for middle- and low-income countries. A number of them have already shown an interest in acquiring the Cuban vaccines, and we would argue that such a Cuban contribution to vaccine equity should be applauded by the Biden administration, not stifled. The Cuban Democracy Act of 1992 (Part II.6) explicitly bans exports to Cuba from the U.S. in cases where: "the item to be exported could be used in the production of any biotechnological product", which includes vaccines.

We had a glimpse of [what] both countries could have done together during the Western African Ebola virus epidemic (2013–2016), when both countries strove to contain disease and save lives. Obviously, the U.S. and Cuban governments differ on fundamental issues. Yet the world is full of such discrepancies. The essential question, not only for Cuba and the U.S., but also for human civilization, is whether nations can respect each other enough to exist side-by-side and cooperate.

President Biden, you can do much good if you move in the right direction and take into consideration what most Cubans living in Cuba desire. This does not include bypassing and weakening its public health system but does include respect for the nation's achievements. Let us hope that the shared threats posed by the COVID pandemic will lead to more collaboration, not more confrontation. History will be the judge.

Signed by scientists, doctors and concerned citizens from Cuba and the world.

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