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Empowering Cuban Women 6

Cuban Diabetic Foot Ulcer Medicine 10

Cuba's Meningitis B-C Vaccine at Work 22

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MEDICC Review

July 2018, Vol 20, No 3

EDITORIAL

3 Out of the Box: Needs-Driven Passion Meets Population Health

ABOUT THE CONTRIBUTORS

4

LETTERS

5

INTERVIEW

6 Empowering Cuban Women:
Marta Núñez MS PhD
Sociologist & Consulting Professor, University of Havana
Conner Gorry MA

ERRATA

9

ORIGINAL RESEARCH

10 Heberprot-P's Effect on Gene Expression in Healing Diabetic Foot Ulcers
Hanlet Camacho-Rodríguez MS, et al.

15 HIV-1 Antiretroviral Resistance in Cuba, 2009–2014
Yoan Alemán-Campos MS, et al.

22 Effectiveness of a Serogroup B and C Meningococcal Vaccine Developed in Cuba
Rolando F. Ochoa-Azze MD PhD, et al.

30 Cuban Application of Two Methods for Analyzing Multiple Causes of Death
Armando H. Seuc PhD, et al.

36 Estimating Need, Demand and Supply in Primary Health Care Services:
A Local Application in Argentina
María E. Elorza PhD, et al.

45 Researchers' Perceived Challenges in Studying
Chronic Kidney Disease of Nontraditional Etiology in Mesoamerica
Emily M. Wright

VIEWPOINT (REPRINT)

52 Family Planning as a Human Right:
Knowledge for Better Decision-making by Cuban Adolescents
Sara Más
Revista Mujeres

ABSTRACTS

Cuban Research in Current International Journals

Cover photo: *E. Añé. Anonymous photo of Dr Laura Martínez de Carvajal (1869–1941), Cuba's first woman doctor. Courtesy Cuban Academy of Sciences.*

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Out of the Box: Needs-Driven Passion Meets Population Health

An editorial in a recent double issue of *Seminars in Oncology* dedicated to Cuba commented that, in contrast to most current therapeutic research that provides relatively little new information, Cuban research represents " . . . truly out of the box thinking, crafted in a country where resource limitations have clearly incentivized novel approaches." Indeed, this is a hallmark of Cuba's scientific sector, forging ahead in a developing island nation under six decades of US embargo and battered by the vicissitudes of global, regional and national economic fluctuations.

But "out of the box" may only be the entry point into a broader conceptualization that calls upon science to generate discoveries not for its sake alone, but in response to the critical needs of population and planetary health and wellbeing. Today, in addition to medicine and vaccine development and production, priorities for Cuban science include food security, sustainable energy, climate adaptation and society-wide incorporation of information technologies. Several of these notions are in fact incorporated into draft constitutional reforms approved by parliament this month and now subject to a national referendum.

What place, in scholarship for passion? Is it truly 'neutral' to remain dispassionate before unnecessary suffering? Or is such studies neutrality really a concession to the inevitability of inequalities of outcome?
—Paul Farmer, Infections and Inequalities: The Modern Plagues

In other words, this approach argues that science, although evidence-based, is not neutral. New knowledge, excellence in research and improved outcomes result from scientists' expertise, environment and persistence…but also from the passion derived from how they view their role in society. And passion for equitable growth and sustainable development put science squarely at the center of any fight for social justice. In one way or another, all the articles selected for Editors' Choice in this issue, as well as several others, touch upon this theme.

This July, our journal inaugurates a new series profiling outstanding women in Cuban health, science and technology. Cuba's scientific sector today employs more than 86,000 people, more than half of whom are women. Their contribution to scientific and social development over time has been extraordinary, as we have come to observe. One outstanding example is pictured on our cover: Dr Laura Martínez de Carvajal (1869–1941), Cuba's first woman doctor. The eldest daughter of a wealthy Spanish family, she defied the social conventions of the day regarding women's roles and entered the University of Havana at age 14. She graduated medicine at 19, on July 5, 1889, going on to also become Cuba's first woman ophthalmologist. Today, the original photo of this pioneering woman hangs in the halls of the Cuban Academy of Sciences. To contextualize this ***MEDICC Review*** series, our first interview (and an Editors' Choice) is with sociologist Marta Núñez, who shares insightful reflections on the history and current status of women in Cuba in every aspect of life.

Two other Editors' Choice articles highlight research in Cuba's burgeoning biotech sector, which has already made important contributions to population health at home and abroad. Cuban research institutions employ a "closed-loop" approach, identifying important problems, developing and testing new products to address them, and manufacturing such products in sufficient quantity, not only to cover domestic needs, but for export (completing the sustainability loop). In his article, Camacho-Rodríguez describes the likely mechanisms through which Cuba's Heberprot-P prevents amputations due to diabetic foot ulcers. Now eligible for clinical trials in the USA, Heberprot-P has been shown effective in healing such ulcers, reducing morbidity, disability and deaths among the increasingly numerous diabetic population. And one of Cuba's most eminent immunologists, Ochoa-Azze, presents an assessment of the field effectiveness of a Cuban vaccine against meningitis serogroups B and C. VA-MENGOC-BC, was developed by Cuba's Finlay Vaccine Institute in the 1980s in response to a nationwide epidemic of meningitis B, particularly affecting youngsters. The vaccine became the world's first effective vaccine against this particular serogroup, and its use in Cuba and abroad is documented and analyzed here.

A fourth Editors' Choice, by Wright, explores researchers' perceptions of obstacles to studying chronic kidney disease of nontraditional origins (CKDnt). Readers may remember the 2014 ***MEDICC Review*** special issue on this new form of nephropathy that has ravaged poor agricultural communities in Central America and elsewhere. Prior to that, we published the first major epidemiological study of the problem in El Salvador, an article which El Salvador's then Minister of Health, Dr María Isabel Rodríguez, presented as evidence to the UN High-level Meeting on Non-Communicable Diseases (NCD), successfully arguing that CKDnt should be included in WHO's strategy for reducing the burden of NCDs worldwide, enunciated in the 2011 Political Declaration on the Prevention and Control of NCDs.

The Viewpoint by Mas, a reprint from the Cuban magazine *Mujeres,* deserves special attention*:* Since 1989, World Population Day (WPD) has been celebrated on July 11 to raise public and political awareness of urgent population issues. Appropriately, the WPD 2018 theme is *Family Planning is a Human Right,* and Más's Viewpoint addresses these issues (particularly informed decision-making) in Cuba, inspired by a workshop held by Cuba's Center for Demographic Studies.

Finally, we at ***MEDICC Review*** are devastated by the death of Dr José Baudilio Jardines Méndez, eminent public health physician, medical educator and codeveloper of Cuba's Virtual Public Health Campus (an indispensable tool for online learning in public health). He was Cuba's Vice Minister of Health at a time when few believed US–Cuba cooperation was possible, and was a prime mover in the creation of our publisher, Medical Education Cooperation with Cuba (MEDICC). We have lost a great humanitarian, colleague and friend.

The Editors

PS Access online articles on the brand new ***MEDICC Review*** website, premiering with this issue at **www.mediccreview.org** Your opinions welcome on our new look.

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Rapid Response for Diphtheria Control in the Dominican Republic

To the Editors:

Corynebacterium diphtheriae is a toxin-producing bacterium that causes respiratory or cutaneous manifestations and, without treatment, can lead to mortality. Key to diphtheria control is prevention by vaccination, which can be a challenge for health leaders in low-resource settings.

WHO reported stark differences in 2015–2016 three-dose diphtheria (DTwP) vaccine coverage rates across the island of Hispaniola, which is shared by the Dominican Republic (DR) and Haiti. In the DR, with 4 reported cases, national vaccination coverage with the third dose was estimated at 86%, while almost 60% of DR municipalities reached 80% coverage.[1] In Haiti, with 37 reported cases, national third-dose coverage was estimated at 60%, while almost 30% of Haitian municipalities reached 80%. [1] Since the two nations share a political border, characterized by active binational commerce and cross-border movement, robust emergency preparedness for natural hazards or disease outbreaks is essential to enhance population health.

On March 27, 2018, the DR's Ministry of Public Health (MSP) mandated increased epidemiologic surveillance capacity, because of the diphtheria death of a visiting Haitian child. Vulnerable groups, such as children under five, school-aged children, health care workers, military service members, prisoners and persons with high-risk occupational exposure, have been prioritized. MSP also recommends multiple strategies to strengthen diphtheria surveillance of public and private health institutions for improved disease control: 1) strengthen capacity for early diagnosis and treatment; 2) provide health care workers with continuing education about diphtheria; 3) maintain reports and case notifications by geographic area; 4) guarantee prompt laboratory analysis of samples for case confirmation; and 5) promote clinical and epidemiological research as well as community interventions.[2]

MSP's prompt national response echoes the successful control of a DR outbreak in 2004–2005, alerting health authorities to the value of national vaccination coverage and robust surveillance programs as primary prevention strategies.[3] Strengthening DR emergency preparedness for infectious disease outbreaks requires strong political will, a national health system that prioritizes population health, and a prepared workforce for effective health service delivery.

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2. Ministry of Health (DO). Lineamientos para proteger contra la difteria poniendo al día esquema nacional de vacunación. Circular No. 000967. 22 Mar 2018. Santo Domingo: Ministry of Health (DO); 2018 Mar. Spanish.
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Empowering Cuban Women
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After nearly 60 years of universal education and health, coupled with national policies supporting women's rights and advancement, the results are in: according to recent data, more than half of Cuban scientists and almost 60% of all professionals in Cuba are women. Moreover, women's representation in government is rising, including at the highest levels such as parliament, where they constitute 53.2% of members.

Digging deeper, we find a story richer than national statistics or political representation. It's the story of the collective achievements of female professionals on the island. For example, the clinical research team responsible for developing CIMAvax-EGF, Cuba's novel biotech therapy for non-small cell lung cancer, was headed by a woman. Likewise, the lead scientist of the Cuban team that developed the world's first effective meningitis B vaccine is a woman. And the cofounder of the country's clinical trials coordinating center and registry is a woman, as is the founder of the National Center for Agricultural Animal Health. Yet, as in any country, there is more to be done to achieve true gender parity and release the full potential of women.

To begin our series profiling outstanding Cuban women professionals, ***MEDICC Review*** spoke with sociologist Dr Marta Núñez, who has devoted decades to research on gender relations and the role of women in Cuba. She provides an overview and framework for contextualizing the advancement of Cuban women—including the challenges still to overcome.

MEDICC Review: **Gender equality doesn't happen by chance or overnight. Can you discuss specific policies supporting the advancement of women in Cuba?**

Marta Núñez: In my mind, it began in 1961 with the literacy campaign and subsequent policy declaring all levels of education universal and free. I call this moment the 'feminization of education' in Cuba.

Of the more than 260,000 volunteers who fanned out across the country to teach every citizen to read and write, 70% were women—the majority of them young women [Cuba was declared illiteracy free following the one-year campaign—Eds.]. I was one of them. I was 14 years old and it was the first time I saw real poverty. This experience showed many women for the first time, especially in the countryside, the importance of education. In turn, they passed this appreciation for education on to their children, encouraging them to go to school when universal education was implemented that year. This gave women and girls—no matter their location or financial possibilities—the opportunity to pursue professional goals. All international studies show that if girls (regardless of context, whether in the developed or developing world) successfully enroll and stay in school, they perform better academically than boys and we began to see this in Cuba.

With this culture of education came the expectation that girls excel scholastically—but due to our patriarchal society and traditional gender ideology, girls are also expected to attend to domestic chores and be 'feminine.' While boys are given time to play and ride their bikes after school and finishing their homework, girls are helping mom clean or cook. This gender construct can become problematic once a woman enters the workplace and starts her own family.

Universal health care, which came shortly after universal education, has also contributed greatly to the advancement of women in Cuba. This meant free services, including all antenatal checkups, more than two dozen well-baby and maternal consults during the baby's first year of life, effective family planning and safe, accessible abortion. A national network of nursery schools—at very low cost—was also established in 1961, allowing working women to leave their children in a positive, educational setting with qualified caregivers during their work week. Such policies support mothers and children and create a culture of health and wellbeing that permeates our society. Of course, equal pay for equal work is another national policy supporting women's progress.

In short, there are various reasons why so many professionals in Cuba are women. By 1978, there were more women professionals than men, and the proportion has climbed slowly ever since.

MEDICC Review: **You mentioned traditional gender roles being a potential area of conflict for working women. Can you elaborate?**

Marta Núñez: Gender norms in Cuba hold that girls do housework and caregiving, while boys are typically not called on to share these duties. So a professional woman, who needs a nutritious diet and a good night's rest to be able to perform at her job, comes home and her husband or male partner expects her to assume these tasks as well; usually the women in his life (grandmother, mother, sisters, aunts) have always done the cooking, cleaning, child rearing and caretaking of elders.

The latter is especially pressing now, since over 20% of our population is 60 years old or more, and Cuba has a culture of aging at home, rather than placing seniors in nursing homes. Sooner or later, working women get worn down and exhausted by this 'double shift.' Further exacerbating this problem is our economic context: salaries are too low, food procurement can be difficult and Cuban women in general have few home appliances, making all housework that much harder. I think lack of resources, financial and material, together with macho gender roles are the greatest obstacles to further advancement of Cuban women.

MEDICC Review: **How can Cuba—or any country for that matter—transform this traditional gender paradigm to further empower women?**

Marta Núñez: There are many components and strategies, but further gains for Cuban women depend on creating synergy between government policies and grassroots initiatives. I call this top-down/bottom-up: if you have a viable grassroots movement but don't have the support of policy makers, it's not going to result in substantive change. Likewise, top-down policies without popular support or taking into account public opinion can only be so effective. Decision-makers have to consult with, listen to, observe and respond to what the public wants and needs. They have to work together to forge solutions.

Maternity leave in Cuba is a great example. In 1974, three months' maternity leave became national policy. In 1993, it was increased to six months and now it's one year. Most recently, maternity leave was extended to grandparents or another family member to provide even more flexibility for the family to decide as a unit how best to balance child care with work and other responsibilities. This was a top-down (governmental) response to what policy makers were hearing from their base and is an example of the two working together towards women's advancement. Paternity leave is also an option, but since it was instituted in 2002, only 104 families around the country have chosen this alternative.

Culturally, machismo is very much a reality here and I've spent a lot of time analyzing the images of women portrayed in music, on TV and in the media. In 2017, this line of research prompted me to initiate a dialogue with five of Cuba's top band leaders—musicians who are among the most popular today. I wanted to get their opinions on racist, sexist, homophobic and other discriminatory content in music videos and lyrics. It was a fascinating conversation and I was pleased to learn that not a single one of them wants to use this type of language or imagery. Unfortunately, they told me, it's what the public wants and what the market demands. Lo and behold, a few weeks later, one of the participants in this conversation, who spoke very eloquently and intelligently about incorporating a gender perspective and not using sexist or racist language in his music, premiered a new video—which was shown on Cuban TV—that was one of the most sexist, racist, and consumerist music videos I've seen. So the private dialogue might be towards rights and respect but the product being sold can be completely the opposite.

I'm continuing these dialogues (my next one is with a hip-hop musician) to see how we might progress towards lyrics and imagery that fight against misogyny, gender violence and the like, rather than promoting this kind of bias. And program directors can be more discriminating about what they show on television, which influences trends and tastes around the country.

MEDICC Review: **You noted low salaries as a roadblock to further progress for women. How so?**

Marta Núñez: Cuban women are excelling—more than half our scientists are women for example and 8 of the 15 provinces in the country are led by women—but we need to resolve practical problems so working women have a chance to get their heads above water and aren't in this constant, day-to-day struggle to provide for their families. Low salaries, poor public transportation, the housing crisis—all of these are stressors that can even affect life expectancy and incidence of chronic disease. Cuba just held its national labor union congress and the issue discussed in every meeting around the country was low salaries. We need to forge sustainable solutions to these problems, especially in the public sector, or young women are going to leave our public institutions or leave the country altogether. [Cuba's public sector includes all workers in public administration, major industries, state companies, health, education, utilities and research institutes, as well as most in public transportation, the environment, tourism, communications and culture, among others.—Eds.]

MEDICC Review: **Brain drain—whether from the public to state sector or through emigration—is particularly topical as Cuba undergoes an historic social and economic transformation. Can you expand on how this looks through a gender lens?**

Marta Núñez: Consider that 85% of Cuba's export revenue—the funds used for all national programs and subsidies—comes from the public sector. And who are the majority working in the public sector? Women. And when they can't make ends meet with their public-sector salaries, what do they do? They migrate to the private sector. Or they leave Cuba altogether. Both are happening quite a bit, and this has the potential to destabilize the country economically and socially. Luckily, several policies were recently introduced to stem this drain. For instance, our new economic model allows people to work in both the public and private sectors at the same time—you can be a researcher at a biotechnology institute, for instance and rent a room in your home. It's not easy—you have to have applicable managerial and administrative skills and renting a room requires a lot of physical and mental strength. But as we say here: though it isn't easy, it *is* possible.

Another policy adjustment under the new economic model allows us to travel internationally more easily, with less bureaucracy. Many young women still pursue careers in the public sector because they know an alternative for supplementing their low

salaries is to travel abroad—on scholarships, to attend or present at professional congresses or to give classes as visiting professors. This is what I do: I was a visiting professor at Harvard University's David Rockefeller Center for Latin American Studies, for example. Health professionals, meanwhile, have the opportunity to serve in medical missions overseas, which is another way to augment their public sector income. Nevertheless, as I've said, public sector salaries are too low, and this has to change.

MEDICC Review: **The opening of Cuba's private sector is talked about every day—on the news, in the street, among friends and family. Do you have a sense of women's participation in the emerging private sector economy?**

Marta Núñez: In 2014, I published a study on exactly this topic [La cara de género del cuentapropismo habanero, *Revista Temas*. 2014;80:79–87]. While my research was limited to Havana and examined only 3 of the more than 200 activities permissible at that time, it provides a baseline for further investigation. In my sample of 61 owners of small businesses, only 17% were women. Statistics were just published this July stating that 33% of the more than 590,000 workers in the private sector are women. However, these data don't separate owners from contracted workers—an important distinction—nor do they specify in what service or activity women are working. For example, many women work as subcontractors selling cell phone cards for ETECSA, Cuba's telephone/Internet service provider. But this reaps minimal earnings, so even for these workers in the private sector, making ends meet is still very difficult.

My research revealed that where Cuban women excel as small business owners is in renting rooms in their homes. Some of my Cuban colleagues in gender and society research have told me, "Yes, but running a *casa particular* (as room rentals are known here) is women's work after all. It makes sense that they would choose and thrive in this type of private business." I disagree. It takes a wide array of managerial, organizational, marketing, accounting and now technological skills to run a successful room-rental business. But this example illustrates that even within the gender studies field, gender-based perceptions about division of labor persist.

MEDICC Review: **Along with the new economic model, Cuba has a new president, Miguel Díaz-Canel. Do you see further advances for women under his leadership?**

Marta Núñez: President Díaz-Canel has a different style of governing—different from both Raúl Castro and Fidel Castro. This is logical and implicit when you have a new president, especially when he's only 58. I like that he uses new technologies to obtain information and make decisions efficiently. During Subtropical Storm Alberto last May, for example, he convened regular video conference meetings with the heads of the most affected provinces and directors of different sectors to get constant updates. Then he went to those provinces, met with local authorities and talked to the populace to see and hear for himself about the situation on the ground.

And like the 'feminization of education' that Cuba underwent in the 1960s and 1970s that I mentioned, we're now experiencing the 'feminization of government.' Cuba has successfully increased—by appointment in ministries and other institutions, by election to the National Assembly and at the regional and local levels—female representation. There are dozens of women vice ministers in different sectors. Also, a number of ministries are headed by women, who are each members of the Council of Ministers (See table). The majority of provinces are headed by women, and in 2012, voters in the Caibarién Municipality (Villa Clara Province) elected a transgender representative to the National Assembly [parliament].

But here we have to take an analytical approach. Just because women are in policy or decision-making positions does not mean they approach their responsibilities with a gender perspective. Perhaps they've experienced sexual harassment in the workplace but haven't spoken out for fear of professional repercussions—something we see in both the private and public sectors incidentally, which are equally obligated to abide by national labor laws. Or maybe they oppose same-sex marriage on moral grounds or because the couples don't procreate.

Right now, sexual harassment and gender identity are in the news and part of national dialogues. But these issues were not raised formally here until 2013 when Mariela Castro, Director of the National Center for Sex Education and a member of parliament, voted against proposed labor law reforms because they didn't include language specific to workplace discrimination based on gender identity and HIV status. Her dissenting vote came after decades of cross-sectoral work with local, regional and national authorities in collaboration with Cuba's LGBTQ community to combat homo- and transphobia and to sensitize Cubans about issues of sexual and gender diversity—something that not everyone here supports at the grassroots level.

So while top-down and bottom-up is the ideal for promoting substantive change—that synergy I mentioned between grassroots opinion and policy making—sometimes policymakers are able to take a longer view on issues of civil rights and justice. And they have the responsibility to broaden those rights, even if not everyone is in agreement.

So, now we're seeing this in action with same-sex marriage. It is coming under this new presidency and leadership, there's no doubt about it. By and large, Cuba has a homophobic culture and

Female Ministers & Presidents of National Institutions, Cuba

Ministry	Minister (year designated)
Labor & Social Security	Margarita González Fernández (2009)
Education	Ena Elsa Velázquez Cobiella (2008)
Science, Technology & the Environment	Elba Rosa Pérez Montoya (2013)
Domestic Trade	Betsy Díaz Velázquez (2018)
Finance & Prices	Lina Olinda Pedraza Rodríguez (2009)
Food Industry	Iris Quiñones Rojas (2018)
Central Bank of Cuba (Minister and President)	Irma Martínez Castrillón (2017)

Note: All are also members of the Council of Ministers. Inés María Chapman Waugh, designated Minister and President of the National Institute of Hydraulic Resources in 2011, was promoted as one of five vice presidents of the Council of Ministers in July, 2018. The complete composition of the current Council is available from http://www.granma.cu/cuba/2018-07-21/nuevo-consejo-de-ministro-es-aprobado-por-el-parlamento-cubano

some are protesting against it, but we're going to see a top-down policy change that permits same-sex unions—although it must pass a citizen-wide referendum as well. This will affirm the rights of couples not falling within the traditional 'man–woman' scheme and make for a happier, more peaceful society as a whole—even though there are those who maintain moral, prejudicial or religious arguments against it.

MEDICC Review: **Among all these complexities and difficulties, what do you think the future holds for further advancement of Cuban women?**

Marta Núñez: Despite the material difficulties and traditional gender constructs, Cuban women are empowered. For instance, some 75% of divorces are initiated by women [Cuba has one of the world's highest divorce rates, with over 60% of all Cuban unions ending in divorce—Eds.] and women are encouraged to pursue careers in math, science and technology. Male colleagues are accustomed to having women bosses and coworkers and generally experience a spirit of collaboration, rather than competition and discrimination. During my research in the private sector, I've heard several opinions recently from people who were ready to emigrate, but now with more possibilities to improve their standard of living, have decided to stay and raise their families here.

Obviously, women's advancement—and how to continue making strides—isn't a clear-cut issue. It's peppered with grey areas where many doubts and contradictions lurk. Nevertheless, I assure you, Cuban women have it better than women in many other contexts and cultures. And we intend to keep the gender lens trained on our society, to keep making progress.

ERRATA

Chapman HJ, Armas-Pérez LA, Lauzardo M, González-Ochoa ER. Moving Closer to Tuberculosis Elimination through Institutional Scientific Collaboration: Opportunities for Cuba and the USA. MEDICC Rev. 2018;20(2):59–63.

Page 60, Table 1, rows 9 and 10: row labels "New cases (%)" and "Previously treated cases (%)" should be indented, to make clear that they are subsets of MDR-TB cases.

González-Quevedo A, Santiesteban-Freixas R, Eells JT, Lima L Sadun AA. Cuban Epidemic Neuropathy: Insights into the Toxic–Nutritional Hypothesis through International Collaboration. MEDICC Rev. 2018 Apr;20(2):27–31.

Page 30, first complete paragraph, line 7, "Two models were developed independently by Cuban researchers" should read "Two models were developed independently by AAS and AGQ."

Heberprot-P's Effect on Gene Expression in Healing Diabetic Foot Ulcers

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ABSTRACT

INTRODUCTION Diabetic foot ulcers are a chronic complication in patients with diabetes mellitus. They appear as a result of the combination of diabetic polyneuropathy and angiopathy, and in many cases require amputation of the affected extremity. Clinical trials have demonstrated that repeated local infiltration with Heberprot-P can improve healing of chronic diabetic foot ulcers. Although there is evidence of its effects as a granulation stimulator and on cell migration and proliferation, genetic control mechanisms explaining its anti-inflammatory and oxidative stress reduction properties are not yet thoroughly understood.

OBJECTIVE Analyze changes in expression of genes involved in healing after treatment of diabetic foot ulcers with Heberprot-P.

METHODS Biopsies were collected from diabetic foot ulcers of 10 responding patients before and after 2 weeks' treatment with Heberprot-P (75-µg applied intralesionally 3 times per week). Total RNA was obtained and quantitative PCR used to determine expression of 26 genes related to inflammation, oxidative stress, cell proliferation, angiogenesis and extracellular matrix formation. Genetic expression was quantified before and after treatment using REST 2009 v2.0.13.

RESULTS After treatment, there was a statistically significant increase in expression of genes related to cell proliferation, angiogenesis and formation of extracellular matrix (PDGFB, CDK4, P21, TP53, ANGPT1, COL1A1, MMP2 and TIMP2). A significant decrease was observed in gene expression related to inflammatory processes and oxidative stress (NFKB1, TNFA and IL-1A).

CONCLUSIONS Our findings suggest that Heberprot-P's healing action on diabetic foot ulcers is mediated through changes in genetic expression that reduce hypoxia, inflammation and oxidative stress, and at the same time increase cell proliferation, collagen synthesis and extracellular matrix remodeling. The kinetics of expression of two genes related to extracellular matrix formation needs further exploration.

KEYWORDS Epidermal growth factor, EGF, diabetic foot ulcer, wound healing, quantitative real-time PCR, gene expression, Cuba

INTRODUCTION

Diabetes mellitus (DM) is a chronic disease with increasing global prevalence over recent decades; according to WHO, DM affects 8.5% of the global population.[1] One of its main complications is lower-extremity ulceration, known as diabetic foot ulcer (DFU), which often leads to amputation.[2,3] Recent reports on DM in Cuba suggest an overall prevalence of 58.3 per 1000 population.[4]

Diabetes-induced hyperglycemia activates four biochemical pathways: the polyol, hexosamine, protein-kinase C (PKC) and advanced glycation end products (AGE). Together, these cause inflammation and oxidative stress (OS).[5,6]

Endothelial cells in the vasculature, neurons and Schwann cells in peripheral nerves contain only high-affinity glucose transporter proteins (GLUT1 and GLUT3).[7] Thus, in hyperglycemic conditions, a massive and unregulated amount of glucose enters these cells, which makes them targets for inflammation and OS, and explains the occurrence of long-term complications such as diabetic angiopathy and polyneuropathy—the main causes of DFU.[8,9]

IMPORTANCE This research increases our understanding of the complex mechanisms of action by which Heberprot-P speeds wound healing in diabetic foot ulcers, reducing related amputations and mortality.

Wound healing is the process by which damaged tissue is replaced by healthy connective tissue, forming a scar. This process can be divided into four dynamic, overlapping phases: vascular response, inflammatory response, proliferation and maturation (or remodeling).[10]

According to estimates from Berlanga in 2013, 3000 to 5000 amputations are performed annually in Cuba due to DFU. To treat DFU, the Genetic Engineering and Biotechnology Center in Havana developed Heberprot-P, based on human recombinant epidermal growth factor (EGF).[11]

Local conditions resulting from hyperglycemia in DM include:

- decreased vascularization from a reduction in expression of genes regulating angiogenesis, namely vascular endothelial growth factor (VEGF) and angiopoietin-1. This causes hypoxia and cytoplasmic membrane rupture leading to release of cellular content, increased inflammation and OS.
- increased chronic inflammation and OS. These are linked to diabetic angiopathy and polyneuropathy and are a consequence of increased expression of proinflammatory cytokine genes, including tumor necrosis factor alpha (TNFA), interleukin 6 (IL-6), and interleukin 1 alpha (IL-1A). There is also increased expression of genes for the receptor for advanced glycation end products (AGER), related to OS, as well as the gene that regulates their expression, NF-kappa B transcription factor (NFKB1).
- reduced bioavailability of growth factors due to the excess of proteases released by active neutrophils. There are five families of growth factor: EGF, platelet-derived growth factor

(PDGF), transforming growth factor beta (TGFB), insulin-like growth factor (IGF) and fibroblast growth factor (FGF). They all play a part in healing and the processes of chemotaxis, cell proliferation induction, angiogenesis stimulation, synthesis regulation and extracellular matrix (ECM) degradation. Prolonged inflammation prevents progression to the proliferation phase and causes delayed or incomplete healing. [12,13]

Although gene expression can be controlled at various levels, it is widely accepted that it generally happens in DNA transcription, and evidence of degree of a gene's expression can be observed by measuring the quantity of messenger RNA corresponding to the gene's DNA.[14,15] To study gene expression variation, real-time PCR is routinely used in molecular biology to amplify products transcribed from messenger RNA. Quantification of such variation may be relative (based on target gene expression relative to that of a reference gene) or absolute (based on an internal or external calibration curve). With relative quantification, change in RNA expression is shown as the fold change between two sample groups using normalization, a process that compares the degree of expression of the genes being studied with two or more reference genes that have unchanging expression levels, regardless of cell type and treatment being investigated.[16]

It has been reported that treatment with Heberprot-P leads to a 77% cure rate in cases of DFU,[17] and that EGF stimulates proliferation of epithelial cells, fibroblasts and vascular endothelial cells.[13] However, there is little information regarding which changes in gene expression could lead to ulcer healing in patients with DFU treated with EGF.

This study's goal was to analyze changes in gene expression involved in processes affecting DFU healing (inflammation and OS, cell proliferation, angiogenesis, and ECM formation and remodeling) after treatment with Heberprot-P and clinical evidence of patient response.

METHODS

Design Ulcerous tissue was biopsied in 156 patients included in clinical trial code IG/FCEI/PD/0911 in the Cuban Public Registry of Clinical Trials, prior to treatment (T0) with Heberprot-P (75-µg dose applied intralesionally, 3 times per week). Another biopsy was taken after 2 weeks of application (T1) in granulation tissue. At the end of the study, 29 patients met the following criteria: they had been treated for Wagner grade 3–4 diabetic foot ulcers, they responded to treatment with Heberprot-P,[2] and their RNA samples were of optimal quality for differential expression studies.[18] Of the 29 patients who met study criteria, 10 were chosen at random, the minimum sample size able to detect a 1.5-fold difference with 80% statistical power and a maximum of 5% type I error. [19] Patients were considered responders if they had complete wound closure at end of treatment with Heberprot-P.

Relative expression of genes of interest was measured by comparing expression levels in biopsies taken at T1 vs. T0. The experiments were normalized using previously validated reference genes as internal controls, each group with a total of 10 biological replicates; 3 technical replicates were used for each gene. A significance threshold of $p = 0.05$ was chosen.

RNA purification Extracted samples were stored in Ambion RNAlater (AppliedBiosystems, USA) at −20°C for one week. Tissue was processed in a Tissue Lyser unit (Qiagen, Germany). Total RNA was extracted with the RNeasy Plus reagent kit (Qiagen GmbH, Germany) using the Quiacube platform (Qiagen, Germany).

RNA quality control Quantity, purity and integrity of RNA was assessed using the Nano Drop spectrophotometer (Thermo Fisher Scientific, USA) and the Bioanalizador Agilent 2100 with the Eukaryote RNA 6000 Nano Chip (Agilent Technologies, USA). RNA integrity values greater than seven are considered acceptable for differential gene expression studies.[18]

Complementary DNA synthesis The complementary DNA chain was synthesized from 1 µg of total RNA using Superscript III First-Strand Synthesis Supermix for qRT–PCR (Invitrogen Technologies, USA), per manufacturer's instructions.

qPCR and bioinformatics tools Gene sequence expression was obtained from the US National Center for Biotechnology Information database (Table 1).[20] Specific primers were designed for amplification of genes of interest, using the web application Primer3.[21] Reference genes were selected from a group of candidate genes using the geNorm tool.[22] qPCR reactions were incubated in an optical detection rotor (Capital Bio Co., China) and prepared using the Thermo Scientific ABsolute QPCR SYBR Green Mix reagent case (Thermo Fisher Scientific, USA), per manufacturer's instructions. The qPCR data was analyzed using the Capital Bio RT-Cycler analysis program, version 2.001 (Capital Bio Co. Ltd., China) and relative quantification of genetic expression was performed using REST 2009 v2.0.13.[23] Differences were expressed as fold changes.

Ethics Samples used in this study were from a clinical trial (code IG/FCEI/PD/0911, approved by Cuba's Center for State Control of Medicines and Medical Devices, registration number Reg/10/002/Z/SAEC/01, results not yet published). Participating patients gave written informed consent according to Declaration of Helsinki principles.[24]

RESULTS

Quality control performed with Bioanalyzer and Nanodrop complied with accepted parameters for RNA sample use in differential gene expression studies.[18] Average RNA concentration was 468.96 ng/uL (SD 308.57) at T0 and 669.08 ng/uL (SD 365.24) at T1. RNA integrity was 8.22 (DS 0.82) at T0 and 8.2 (DS 0.9) at T1.

Comparing DFU patients' biopsies at T1 to those at T0 revealed an increase in expression of genes related to cell proliferation (CDK4, CDKN1B, P21, TP53 and FOS); differences were statistically significant for CDK4, P21 and TP53 (Table 2). There was also increased expression of genes involved in collagen synthesis and ECM remodeling, (COL1A1, MMP2, MMP7, MMP9, TIMP1 and TIMP2). Increases were statistically significant for COL1A1, MMP2 and TIMP2 (Table 2). Decreases were detected for genes related to inflammation and OS (IL-1a, IL-6, IL-17, TNFA, NFKB1 and AGER), statistically significant for NFKB, TNFA and IL-1A, but not for IL17, IL6 and AGER (Table 2).

Expression increased for another group of genes related to proliferation and cell migration—protein-3 insulin-like growth factor-

Table 1: Genes analyzed by qPCR

Gene	Access #	Biological function
AGER	NM_001136.4	AGE receptor
ANGPT1	NM_001146.3	Transcription factor
CDK4	NM_000075	Cell cycle
CDKN1B	NM_004064.4	Cell cycle
COL1A1	NM_000088.3	Collagen protein
CTGF	NM_001901.2	Growth factor
FOS	NM_005252.3	Transcription factor
HIFA1	NM_001243084.1	Transcription factor
IGFBP3	NM_001013398.1	Cell proliferation
IL-17	NM_002190.2	Proinflammatory cytokine
IL-1A	NM_000575.4	Proinflammatory cytokine
IL-6	NM_000600.4	Proinflammatory cytokine
MMP2	NM_004530.5	Tissue remodeling
MMP7	NM_002423.4	Tissue remodeling
MMP9	NM_004994.2	Tissue remodeling
NFKB1	NM_003998.3	Transcription factor
P21 (WAF1)	NM_000389.4	Cell cycle
PDGFB	NM_002608.2	Growth factor
PHB	NM_002634.2	Transcription factor
PLCG1	NM_002660.2	Membrane associated enzyme
TGFB1	NM_000660.3	Growth factor
TIMP1	NM_003254.2	Tissue remodeling
TIMP2	NM_003255.4	Tissue remodeling
TNFA	NM_000594.3	Proinflammatory cytokine
TP53	NM_000546.4	Transcription factor
VEGFA	NM_001025366.1	Growth factor
GAPDH	NM_001256799.2	Reference gene
MAP2K5	NM_001206804.1	Reference gene
MAPK6	NM_002748.3	Reference gene
RPL13A	NM_001270491.1	Reference gene
YWHAZ	NM_001135699.1	Reference gene

AGE: advanced glycation end product
Source: US NCBI[20]

binding factor (IGFBP3) and PDGFB—but the increase was only statistically significant for PDGFB. Prohibitin (PHB) expression decreased, but not significantly.

There was increased expression of VEGFA and ANGPT1—genes related to angiogenesis and ischemia—the latter statistically significant, while there was reduced expression of hypoxia-inducible factor 1, alpha subunit (HIF1A). There was also decreased expression of TGFB 1 and connective tissue growth factor (CTGF), genes related to ECM formation; and of phospholipase C, gamma 1 protein, (PLCG1), genes related to the PKC pathway (Table 2).

DISCUSSION

A proposed conceptual model of Heberprot-P's mechanism is displayed in Figure 1.

Biochemical mechanisms suggested for diabetic neuropathy's etiology include nonenzymatic glycosylation with AGE formation and activation of the PKC pathway, which cause both inflammation and OS. They also contribute to damage in nerve, glial and

Table 2: Change in gene expression after treatment with Heberprot-P

No.	Gene	Fold change	P-value	Direction of change*
1	AGER	−1.20	0.190	
2	ANGPT1	1.45	0.001	↑
3	CDK4	1.48	0.009	↑
4	CDKN1B	1.04	0.568	
5	COL1A1	1.67	0.005	↑
6	CTGF	−1.28	0.302	
7	FOS	1.10	0.740	
8	HIF1A	−1.25	0.088	
9	IGFBP3	1.28	0.220	
10	IL17A	−2.17	0.079	
11	IL-1A	−13.70	0.000	↓
12	IL-6	−1.78	0.207	
13	MMP2	2.21	0.000	↑
14	MMP7	1.07	0.886	
15	MMP9	1.69	0.090	
16	NFKB1	-1.37	0.002	↓
17	P21	1.54	0.009	↑
18	PDGFB	1.68	0.002	↑
19	PHB	−1.20	0.073	
20	PLCG1	−1.08	0.325	
21	TGFB1	−1.08	0.540	
22	TIMP1	1.08	0.652	
23	TIMP2	1.43	0.007	↑
24	TNFA	−1.96	0.001	↓
25	TP53	1.99	0.000	↑
26	VEGFA	1.38	0.227	

*where significant

vascular endothelial cells, causing diabetic angiopathy and polyneuropathy.[25]

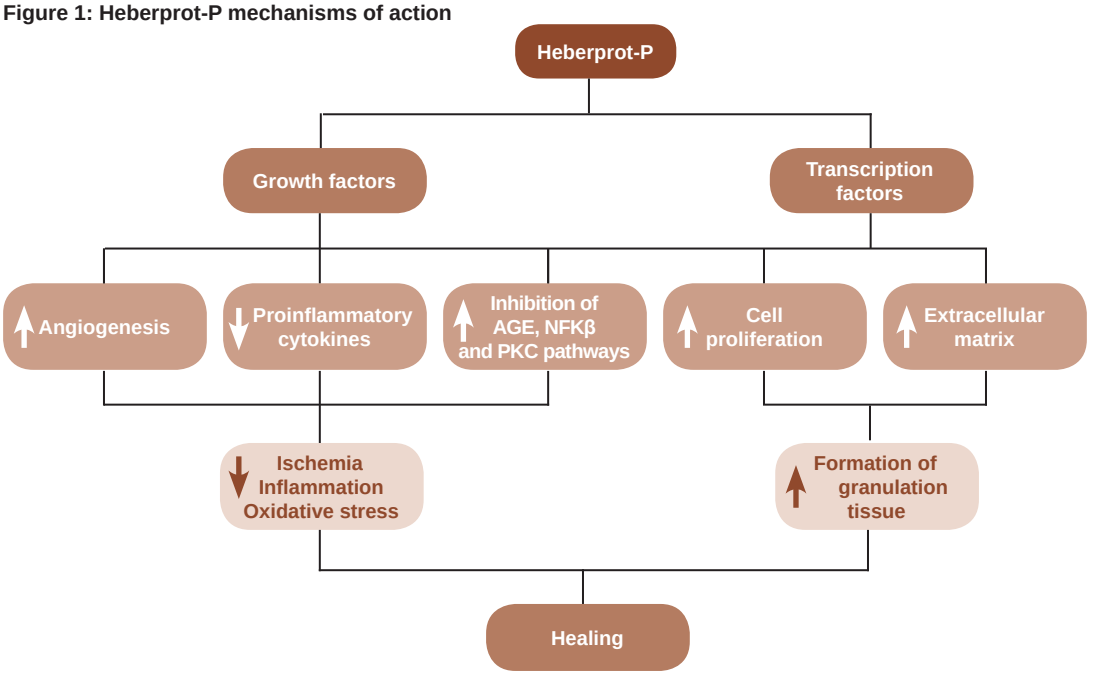
AGE molecules can spread outside cells and modify blood proteins such as albumin. By binding to specific AGERs, these modified proteins activate the NFKB1 pathway, which induces expression of proinflammatory cytokines and increases production of reactive oxygen species. NFKB1 also controls AGER expression.[25,26]

The significant decrease at T1 in expression of the transcription factor NFKB1 is associated with reduced expression of proinflammatory cytokines and AGER genes. This implies, in turn, less damage from inflammation and OS. The lack of statistical significance for the reduced expression of proinflammatory cytokines genes IL 6 and IL17 can be explained by data dispersion.

The PLCG1 enzyme catalyzes formation of diacylglycerol (DAG), a PKC pathway activator.[27] Therefore, the observed decrease in PLCG1 expression (Table 2) may have prevented activation of the PKC pathway, an important mechanism in the physiopathology of diabetic complications.

Increased expression of VEGFA and ANGPT1 genes (the latter significantly) favors angiogenesis, and is related to decreased expression of HIF1A, a transcription factor expressed in tissue hypoxia (Table 2). This increased blood flow may promote DFU healing.

Figure 1: Heberprot-P mechanisms of action



Increased expression of PDGFB, a potent cell proliferation stimulator[28,29] and the resulting decrease of PHB, a negative regulator of proliferation,[30,31] may explain the increased expression of genes related to the cell cycle. We also observed an increase in expression of IGBP3, which according to Ferry, regulates bioavailability of another growth factor, IGF.[32]

The heightened expression in responders of genes involved in ECM formation and remodeling (Table 2), specifically MMP genes, may seem to contradict Liu's findings; Liu suggests that increased MMP9 predicts poor DFU healing through its association with inflammation.[33] However, it has also been reported that MMP2 and MMP9 can be produced by fibroblasts and keratinocytes, which are noninflammatory cells, and their functions could be different in a repair microenvironment.[34]

Healing goes through several phases. At T0, the DFU is in the inflammation phase, when increased expression of MMP genes (which degrade components of ECM and basement membrane proteins) causes serious tissue damage, suppressing reepithelialization. Increased MMP expression thus implies a poor prognosis for ulcer healing. At T1, when there is decreased expression of inflammatory genes and formation of granulation tissue, increased expression of MMP genes could suggest that healing is at a more advanced stage, because MMP acts to remodel the scar tissue being formed,[35] and thus its increase could be interpreted differently than as proposed by Liu.[33]

The decreased expression of TGFB1 and CTGF, which stimulate expression of genes related to ECM formation, is paradoxical,[36,37] and could be due to the fact that both genes are expressed transiently, with maximum expression in early healing.[38,39] At T0, there is inflammation and therefore there should be heightened expression of TGFB1 and CTGF. At T1, there is resolution of inflammation and healing is in the proliferation and remodeling phase. Therefore, one might well expect a relative decrease in TGFB1 and CTGF gene expression at T1.

One limitation of this study is that analysis of gene expression was performed with biopsies at only two points in the ulcer healing process, insufficient to detect early expression of genes. Despite this, and limited sample size, our results offer a clearer view of transcriptional activity induced by Heberprot-P in responders with DFU.

CONCLUSION

Our findings suggest that Heberprot-P's DFU healing action is mediated through changes in genetic expression that reduce hypoxia, inflammation and oxidative stress, and increase cell proliferation, collagen synthesis and ECM remodeling. The kinetics of expression of two genes related to ECM formation needs further exploration.

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HIV-1 Antiretroviral Resistance in Cuba, 2009–2014

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ABSTRACT

INTRODUCTION By the end of 2017, there were more than 28,000 individuals living with HIV in Cuba, over 80% receiving antiretroviral therapy, which dramatically reduces viral replication, improves immune status and decreases risk of transmission. These results could be jeopardized by emergence of HIV-1 drug resistance. In 2009, a test for HIV-1 genotypic resistance was introduced in routine clinical practice in Cuba.

OBJECTIVE Investigate antiretroviral resistance and its relation to subtype distribution in HIV-1 treatment-naïve and previously treated patients in Cuba.

METHODS Resistance and HIV-1 subtype distribution were determined in 342 antiretroviral treatment-naïve patients and 584 previously treated for HIV-1 whose blood specimens were sent to the Pedro Kourí Tropical Medicine Institute during 2009–2014. Transmitted drug resistance was determined using the Calibrated Population Resistance Tool v.6. Drug resistance analysis was conducted using the algorithm Rega v9.1.0.

RESULTS Prevalence of transmitted drug resistance was 11.4%, and 41% of mutated viruses exhibited dual-class resistance to nucleoside reverse transcriptase inhibitor and non-nucleoside reverse transcriptase inhibitor. Overall, 84.9% of patients had ≥1 resistance mutation, 80% had ≥1 nucleoside reverse transcriptase inhibitor mutation, 71.4% had ≥1 non-nucleoside reverse transcriptase inhibitor mutation and 31.7% had ≥1 protease inhibitor mutation. K65R and K101E mutations were significantly more frequent in subtype C, L210W in CRF19_cpx, and M47V/I in CRF BGs (20, 23, 24). Full class resistance to nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, protease inhibitors and multidrug resistance were detected in 21.2%, 32.4%, 8% and 4.1% of patients, respectively. Average percentage resistance to nucleoside reverse transcriptase inhibitor, protease inhibitor, full class resistance to nucleoside reverse transcriptase inhibitor, protease inhibitor and multidrug resistance increased in patients failing two or more regimens. Nevertheless, after 2011, a declining trend was observed in the frequency of multidrug resistance and full class resistance to nucleoside reverse transcriptase inhibitors and protease inhibitors.

CONCLUSIONS Detected levels of transmitted drug resistance highlight the need for a national surveillance study in treatment-naïve patients. Resistance prevalence is high in previously treated patients but appears to be decreasing over time. The frequency of resistance mutations in recombinant forms of HIV in Cuba needs further study.

KEYWORDS Antiretroviral therapy, highly active antiretroviral therapy, HIV, anti-HIV agents, drug resistance, multiple drug resistance, Cuba

INTRODUCTION

There is a global and regional commitment to reach the Joint United Nations Programme on HIV/AIDS' 90–90–90 target in 2020, and to end AIDS by 2030.[1] The 90–90–90 target is that 90% of all people living with HIV will have been diagnosed, 90% of all people with known HIV infection will be receiving antiretroviral therapy (ART), and 90% of all people receiving ART will have a suppressed viral load. Latin America and the Caribbean region face major challenges in meeting this target. PAHO has reported substantial progress (continuing decline in AIDS-related deaths and mother-to-child HIV transmission, increasing numbers of people who know their HIV status and receive treatment), but the annual number of new infections in the Caribbean has remained static since 2012 and HIV incidence remains high in key populations, mainly men who have sex with men (MSM) and transgender women.[1,2]

IMPORTANCE This study shows high levels of resistance to antiretroviral drugs used in Cuba up to 2014, indicating an urgent need for changes in first-line therapy. It also reinforces the necessity of resistance testing for all patients failing antiretroviral therapy.

In 2001, Cuba's Ministry of Public Health (MINSAP) decided to produce generic drugs for treatment of HIV. Efforts to provide access to ART have accelerated since then and have resulted in decreased AIDS mortality and incidence of opportunistic infections.[3,4] By the end of 2017, >28,000 individuals were living with HIV in Cuba, >90% of infected individuals were aware of their HIV status and approximately 80% were on ART. However only half of patients in treatment were virally suppressed (information from MINSAP's National HIV Registry, 2016), a major gap for Cuba in meeting the third 90–90–90 target.

ART dramatically reduces viral replication, improves immune status and decreases risk of HIV transmission, but these outcomes could be jeopardized by HIV-1 resistance. A 2004 study that explored ART resistance in Cuba found low levels of resistance.[4] In 2009 the Pedro Kourí Tropical Medicine Institute (IPK) introduced an in-house HIV-1 genotyping system for routine assessment of drug resistance in Cuban patients.[5]

The aim of this research was to investigate the frequency and profile of antiviral drug resistance in HIV-1 treatment-naïve and previously

treated patients and estimate the prevalence of specific resistance mutations among HIV-1 variants circulating in Cuba.

METHODS

Population IPK is the reference center for HIV care and therapy in Cuba, thus samples from all over Cuba are sent to IPK for genotypic drug resistance testing. A total of 926 viral sequences were collected of all HIV-1 genotypic drug resistance testing carried out at IPK's laboratory as part of routine clinical care from April 2009 to December 2014. One sample per patient was analyzed from 584 previously treated patients and 342 treatment-naïve individuals. Only epidemiologic, demographic, clinical, virological and immunological data were collected; no patient identifying information was retained.

Viral load and CD4 count Plasma HIV-1 viral loads were determined using the Nuclisens Easy Q HIV-1 kit v2.0 (Biomérieux, France) or COBAS Ampliprep/COBAS Taqman HIV-1 test v2.0 for use with the High Pure System (Roche, Germany). CD4 cell counts were determined using a Becton Dickinson counter (Bio-Sciences, USA).

Genotypic drug resistance testing For HIV-1 genotyping, 1 mL plasma was ultracentrifuged and the suspended pellet extracted using QIAamp Viral RNA Kit (QIAGEN, Germany) manually, or automatically on QIAcube (QIAGEN, Germany), per manufacturer's protocol. HIV-1 RNA reverse transcription, amplification and population-based bidirectional Sanger sequencing of pol fragments were carried out as described elsewhere.[5] Sequences obtained covering a fragment of 1302 bp that overlaps with codons 1–99 of protease and 1–335 of reverse transcriptase were edited and assembled using Sequencher, v4.1 (Gene Codes Corporation, USA).

Data analysis HIV subtype was determined using Rega subtyping tool version 3 and confirmed by manual phylogenetic analysis, using MEGA v6 (Kimura's 2-parameter correction, bootstrap 1000).[6]

Therapeutic failure was defined as ART failure to reduce and maintain viral load at <200 copies/mL. Information about treatment compliance was unavailable.

Prevalence of genotypic drug resistance mutations in treatment-naïve patients was analyzed using the Calibrated Population Resistance Tool v6 and based on WHO's 2009 surveillance of drug-resistant mutations.[7]

Drug resistance interpretation in previously treated patients was conducted using the resistance interpretation algorithm Rega v9.1.0. Resistance to drug classes was calculated by averaging the percentage of resistance (R) and intermediate resistance (I) for each drug class. Full-class resistance (FCR) was defined as lack of full susceptibility to any antiviral drug in a given drug class.[8] Multidrug resistance (MDR) was scored if the virus strain was susceptible to no more than one drug belonging to the three commonly available drug classes in Cuba.[8] For statistical analysis, chi square with Yates correction, Fisher exact test and odds ratios (OR) were calculated using Epidat v3.0.10.[9]

Ethics The study was approved by the IPK Ethics Committee and complies with the Declaration of Helsinki.[10] At time of collection, all subjects included in the study gave written informed consent for their specimens to be used for research purposes.

RESULTS

Study population Participants were predominantly male (83.3%), MSM (76.8%) and resided in Havana (66.1%). Median age was 32.4 years (interquartile range, IQR: 24.6–41.3) and 40.5 years (IQR: 33.6–46.6) for treatment-naïve and previously treated patients, respectively. Median CD4 cell count in treatment-naïve patients was higher than in previously treated patients (349 cells/mm^3 vs 208 cells/mm^3), but viral loads were similar in both groups (18,966 copies/mL and 21,264 copies/mL, respectively) (Table 1).

Mean time since ART initiation was 3 years (IQR: 1.1–5.6). All patients had received nucleoside reverse transcriptase inhibitors (NRTI); 90.1% had received ≥1 non-nucleoside reverse transcriptase inhibitors (NNRTI) and 62.7% had received ≥1 protease inhibitor (PI). Only 12.8% of patients had received mono- or dual therapy regimens. At the time of drug resistance testing, the most commonly prescribed drugs were lamivudine (3TC), 92.5%; zidovudine (AZT), 44.7%; and nevirapine (NVP), 44.2%.

Subtype distribution In the study period, 30.9% of HIV-1 strains were subtype B, 22% were BG recombinants (CRF20_BG, CRF23_BG and CRF24_BG), 18.3% CRF19_cpx, 9.8% CRF18_cpx, 6.5% URF, 5.5% subtype C, 2.3% subtype G and 4.7% were other subtypes with frequencies <1% (subtypes A, F, J, H; CRF02_AG, CRF06_cpx, CRF14_BG and CRF31_BC). There were no significant differences between HIV-1 subtypes identified in samples from treatment-naïve and those from treatment–experienced patients (Table 1).

Drug resistance in treatment-naïve *patients* Overall, 11.4% (39/342) of treatment-naïve HIV-1 patients showed evidence of transmitted drug resistance (TDR). The frequency of single TDR against NRTI was 20.5%, against NNRTI 12.8% and against PI 17.9%, for a total of 51.2% single drug class resistance. High prevalence of dual-class resistance was observed (43.6%), mainly to NRTI+NNRTI (41%). Triple drug class resistance was observed in 2 patients (5.1%) (Table 2).

The most common mutations related to NRTI resistance were M184V/I (46.2%), T215Y/I/S/D (25.6%) and K219Q/E/N/R (20.5%); for NNRTI were K103N (23.1%) and Y181 C/I (28.2%) while for PI was M46I/L (15.4%) (Table 2).

No significant differences were observed in overall TDR mutation frequency between chronically infected patients (48.7%) and recently diagnosed individuals (51.3%). However, TDR to NRTI was higher in chronically infected individuals. In contrast, TDR against NNRTI and PI was higher in recently diagnosed individuals. Mutation M184V/I was more frequently detected among chronically infected individuals ($p = 0.0390$, OR 4.0, 95% CI 1.0–15.2) (Table 2).

Drug resistance mutations in previously treated patients Overall, 84.9% of patients had ≥1 resistance mutation, 80% had ≥1 NRTI mutation, 71.4% had ≥1 NNRTI mutation and 31.7% had ≥1 PI mutation. The most frequent NRTI mutations were M184V/I (75.9%), T215Y/F (37.3%), and M41L (25.7%). The most frequent NNRTI mutations were K103N/S (28.6%), Y181C/I/V (26.4%) and G190S/A (21.7%). The most common PI mutations were L90M (16.3%), M46I/L (15.9%) and V82A/T/F/S (10.3%) (Table 3).

Frequency of drug resistance mutations to any drug class was significantly higher in patients who had undergone ≥3 therapy regimens ($p = 0.0149$, OR 2.1, 95% CI 1.1–3.8) compared to those with fewer regimens. Mutations associated with NRTIs, NNRTIs and PIs were observed in 74.4%, 69.9% and 9.7% of first-line failures, respectively. In patients failing second-line therapy, the respective frequencies were 79.2%, 72.2% and 31.9%. In patients exposed to ≥3 ART regimens, these values increased to 84.1%, 72% and

Table 1: Characteristics of patients with HIV-1

Characteristic	Total	Treatment naïve	Previously treated
Patients [n (%)]	926 (100)	342 (36.9)	584 (63.1)
Age [median years (IQR)]	37.8 (30.0–45.0)	32.4 (24.6–41.3)	40.5 (33.6–46.6)
Male [n (%)]	771 (83.3)	290 (82.6)	481 (82.4)
Transmission route MSM [n (%)]	711 (76.8)	270 (76.9)	441 (75.5)
CD4 [median cell count/mm^3 (IQR)]	241 (138–382)	349(201–479)	208 (111–305)
Viral load median RNA copies/mL (IQR)	20,000 (3966–80,458)	18,966 (3794–84,768)	21,264 (4052–80,458)
HIV status [n (%)]			
Recent diagnosis[a]	199 (21.5)	178 (52.0)	21 (3.6)
Chronic infection[b]	727 (78.5)	164 (48.0)	563 (96.4)
Therapy history			
Years since therapy initiation [median years (IQR)]	3.0 (1.1–5.6)	—	3.0 (1.1–5.6)
Previous therapy exposure [n (%)]			
Mono or dual	75 (12.8)	—	75 (12.8)
NRTI	584 (100.0)	—	584 (100.0)
NNRTI	526 (90.1)	—	526 (90.1)
PI	366 (62.7)	—	366 (62.7)
ART at time of resistance testing [n (%)]			
NRTI			
3TC	540 (92.5)	—	540 (92.5)
ABC	93 (15.9)	—	93 (15.9)
AZT	261 (44.7)	—	261 (44.7)
D4T	129 (22.1)	—	129 (22.1)
DDI	6 (1.0)	—	6 (1.0)
FTC	17 (2.9)	—	17 (2.9)
TDF	93 (15.9)	—	93 (15.9)
NNRTI			
EFV	54 (9.2)	—	54 (9.2)
NVP	258 (44.2)	—	258 (44.2)
PI			
ATV/r	5 (0.9)	—	5 (0.9)
FPV/r	71 (12.2)	—	71 (12.2)
IDV/r	37 (6.3)	—	37 (6.3)
LPV/r	63 (10.8)	—	63 (10.8)
NFV	40 (6.8)	—	40 (6.8)
SQV/r	52 (8.9)	—	52 (8.9)
TPV/r	5 (0.9)	—	5 (0.9)
HIV-1 subtype			
B	286 (30.9)	104 (30.4)	182 (31.2)
C	51 (5.5)	12 (3.5)	39 (6.7)
G	21 (2.3)	2 (0.6)	19 (3.3)
CRF 18_cpx	91 (9.8)	35 (10.2)	56 (9.6)
CRF 19_cpx	169 (18.3)	65 (19.0)	104(17.8)
CRF_BGs (20, 23, 24)	204 (22.0)	91 (26.6)	113 (19.3)
URF	60 (6.5)	17 (5.0)	43 (7.4)
Other	44 (4.7)	16 (5.2)	28 (4.8)

[a]sampling <1 year after HIV-1 diagnosis (recent infections included)
[b]sampling >one year after HIV-1 diagnosis
3TC: lamivudine ABC: abacavir ART: antiretroviral therapy ATV: atazanavir AZT: zidovudine CRF: circulating recombinant form D4T: stavudine DDI: didanosine EFV: efavirenz FPV: fosamprenavir FTC: emtricitabine IDV: indinavir IQR: interquartile range LPV: lopinavir MSM: men who have sex with men NFV: nelfinavir NNRTI: non-nucleoside reverse transcriptase inhibitor NRTI: nucleoside reverse transcriptase inhibitor NVP: nevirapine PI: protease inhibitor /r: ritonavir SQV: saquinavir TDF: tenofovir TPV: tipranavir URF unique recombinant form

46.2%, respectively. For each specific NRTI and PI mutation, significant differences were observed between patients exposed to 1 or 2 regimens compared to those exposed to ≥3 regimens ($p < 0.05$). The number of patients harboring viruses with NNRTI mutations did not significantly increase in those exposed to ≥2 regimens, but the frequency of K103N/S and V108I was higher ($p = 0.0402$ and $p = 0.0049$, respectively) in patients exposed to ≥3 than in patients failing the first regimen. Dual-class resistance mutations to NRTI+NNRTI were more frequently observed in patients exposed to 2 therapies ($p = 0.0017$, OR 2.0, 95% CI 1.3–3.2) compared with first therapy failures. The same was observed for dual-class resistance to NRTI+PI ($p < 0.001$, OR 14.2, 95% CI 6.6–30.5) and for triple class resistance ($p = 0.0067$, OR 2.0, 95% CI 1.2–3.4) (Table 3).

Prevalence of resistance mutations among different subtypes in patients on active ART As shown in Table 4, NRTI resistance mutation K65R was significantly more frequent among subtype C isolates from patients treated with 3TC whereas L210W was present in higher proportions among CRF19_cpx isolates from individuals failing AZT or stavudine (D4T) regimens. NNRTI resistance mutation K101E was more frequent in subtype C isolates from patients failing NVP therapy. PI mutation M47V/I was more frequent among recombinant forms CRF_BGs (20, 23, 24) isolates from patients failing LPV/r therapy.

Drug resistance prevalence and trends in previously treated patients The highest drug resistance levels against NRTI were detected for 3TC/FTC (76.9%) and ABC (50.2%); against NNRTIs were for NVP (71.2%) and EFV (70.9%); against PI were NFV (31.8%) and SQV/r (26.4%) (Figure 1a).

The average proportions of patients harboring NRTI, NNRTI and PI resistance were 52.7%, 54.7% and 21.4%, respectively. This average significantly increased in patients failing ≥2 regimens for NRTI ($p < 0.0001$) and PI resistance ($p < 0.0001$). FCR to NRTI, NNRTI and PI was observed in 21.2%, 32.4% and 8%, respectively. FCR to NRTI and PI also significantly increased after two regimens failures ($p = 0.0001$ and $p < 0.0001$, respectively). MDR was present in 4.1% of studied patients and significantly increased after two regimens failures ($p = 0.0017$) (Figure 1b).

From 2009 to 2014, a significant declining trend in MDR prevalence was noticed. In 2009 12.6% of patients harbored an MDR virus, whereas in 2011 prevalence fell to 2.3% (OR = 0.80, 95% CI 0.69–0.93; $p = 0.003$) (Figure 2a). Furthermore, a significant decline

Table 2: HIV-1 drug resistance mutations in treatment-naïve patients

Mutation	Total n (%)	Recent diagnosis n (%)[a]	Chronic infection n (%)[b]	OR (95% CI)
Any	39 (100)	20 (51.3)	19 (48.7)	
NRTI[c]				
Single TDR against NRTI	8 (20.5)	2 (10.0)	6 (31.6)	4.2 (0.7–24.0)
Any	27 (69.2)	12 (60.0)	15 (78.9)	2.5 (0.6–10.3)
M41L	6 (15.4)	3 (15.0)	3 (15.8)	–
D67N/G	7 (17.9)	2 (10.0)	5 (26.3)	3.2 (0.5–19.1)
M184V/I	18 (46.2)	6 (30.0)	12 (63.2)	4.0 (1.0–15.2)
T215Y/I/S/D	10 (25.6)	4 (20.0)	6 (31.6)	1.8 (0.4–8.0)
K219Q/E/N/R	8 (20.5)	5 (25.0)	3 (15.8)	1.8 (0.4–8.8)
NNRTI[c]				
Single TDR against NNRTI	5 (12.8)	3 (15.0)	2 (10.5)	1.5 (0.2–10.1)
Any	18 (46.2)	10 (50.0)	8 (42.1)	1.4 (0.4–4.9)
K103N	9 (23.1)	5 (25.0)	4 (21.1)	1.3 (0.3–5.6)
Y181C/I	11 (28.2)	6 (30.0)	5 (26.3)	1.2 (0.3–4.9)
G190A	7 (17.9)	6 (30.0)	1 (5.3)	7.7 (0.8–71.7)
PI[c]				
Single TDR against PI	7 (17.9)	5 (25.0)	2 (10.5)	2.8 (0.5–16.8)
Any	10 (25.6)	6 (30.0)	4 (21.1)	1.6 (0.4–6.9)
M46I/L	6 (15.4)	4 (20.0)	2 (10.5)	2.1 (0.3–13.2)
Dual or triple TDR				
NRTI+NNRTI	16 (41.0)	9 (45.0)	7 (36.8)	1.4 (0.4–5.1)
NRTI+PI	1 (2.6)	0 (0.0)	1 (5.3)	—
NRTI+NNRTI+PI	2 (5.1)	1 (5.0)	1 (5.3)	–

[a]sampling <1 year after HIV-1 diagnosis (recent infections included)
[b]sampling >one year after HIV-1 diagnosis
[c]NRTI mutations K70R, L210W; NNRTI mutations L100I, K101P/E, Y188L, P225H and PI mutations V32I, I47V/A, I54L/M/V/T/A/S, G73C/S/T/A, L76V, V82A, I85V, N88D/S, L90M were observed <15% of patients.
NNRTI, non-nucleoside reverse transcriptase inhibitor
NRTI: nucleoside reverse transcriptase inhibitor PI: protease inhibitor
TDR: transmitted drug resistance

was observed for FCR NRTI and FCR PI. Statistical analysis demonstrated that FCR NRTI is significantly decreasing over time, from 37.6% in 2009 to 9.5% in 2014 (OR = 0.74, 95% CI 0.70–0.82; p <0.001). For FCR PI, a significant decrease was also observed between 2009 and 2012, from 24.7% to 0.8% (OR = 0.80, 95% CI 0.71–0.89; p <0.001). When this analysis was performed to include any drug in each drug class, PI resistance showed a similar declining trend (p <0.001) (Figure 2b).

DISCUSSION

These results describe circulating subtypes and prevalence of drug resistance for HIV-1 infections in Cuba during 2009–2014. The finding that HIV-1 non-B subtypes were more frequent is consistent with previous studies[6,11–14] and in contrast with the high proportion of subtype B reported in the Caribbean.[15,16] The broad genetic diversity of HIV-1 in Cuba is thought to be due to its originating from contacts in Central Africa.[14,17,18]

Cuba has made great strides in decreasing HIV-related morbidity and mortality by providing universal free access to ART.[11] Because of economic constraints, the most common drug combinations for first-line ART are restricted to nationally manufactured generic drugs.[12] Drug resistance testing was not available until May 2009, so a substantial number of patients may have been treated with failed virological regimens.[6]

The high overall TDR prevalence detected confirms previous reports in Cuba,[11,19] and is higher than reported in other Caribbean countries, Mexico and Central America.[20–26] Particularly alarming is the frequent detection of dual-class resistance to NRTI+NNRTI, since these classes of drugs constitute the backbone of first-line therapy in Cuba.

Table 3: HIV-1 drug resistance mutations in previously treated patients

Mutation[a]		Previous regimen exposure[b] n (%)						
	Total n (%)	1 regimen			2 regimens			≥3 regimens
		Total	NRTI+NNRTI	NRTI+PI	Total	PI/NNRTI	NNRTI/PI	Total
NRTI[c]								
Any	467 (80.0)	131 (74.4)	112 (63.6)	19 (10.8)	114 (79.2)	64 (83.1)	46 (74.2)	222 (84.1)
M41L	150 (25.7)	31 (17.6)	27 (15.3)	4 (2.3)	39 (27.1)	19 (24.7)	18 (29.0)	80 (30.3)
D67N	140 (24.0)	22 (12.5)	17 (9.7)	5 (2.8)	33 (22.9)	19 (24.7)	12 (19.4)	85 (32.2)
K70R/E	124 (21.2)	25 (14.2)	22 (12.5)	3 (1.7)	32 (22.2)	22 (28.6)	7 (11.3)	67 (25.4)
M184V/I	443 (75.9)	126 (71.6)	108 (61.4)	18 (10.2)	107 (74.3)	60 (77.9)	44 (71.0)	210 (79.5)
T215Y/F	218 (37.3)	48 (27.3)	43 (24.4)	5 (2.8)	59 (41.0)	29 (37.7)	29 (46.8)	111 (42.0)
NNRTI[c]								
Any	417 (71.4)	123 (69.9)	110 (62.5)	13 (7.4)	104 (72.2)	68 (88.3)	34 (54.8)	190 (72.0)
K103N/S	167 (28.6)	42 (23.9)	38 (21.6)	4 (2.3)	38 (26.4)	28 (36.4)	10 (16.1)	87 (33.0)
Y181C/I/V	154 (26.4)	44 (25.0)	40 (22.7)	4 (2.3)	39 (27.1)	23 (29.9)	15 (24.2)	71 (26.9)
G190S/A	127 (21.7)	33 (18.8)	28 (15.9)	5 (2.8)	35 (24.3)	28 (36.4)	7 (11.3)	59 (22.3)
PI[c]								
Any	185 (31.7)	17 (9.7)	7 (4.0)	10 (5.7)	46 (31.9)	14 (18.2)	30 (48.4)	122 (46.2)
M46I/L	93 (15.9)	9 (5.1)	4 (2.3)	5 (2.8)	14 (9.7)	3 (3.9)	10 (16.1)	70 (26.5)
L90M	95 (16.3)	9 (5.1)	3 (1.7)	6 (3.4)	20 (13.9)	7 (9.1)	12 (19.4)	66 (25.0)
Any	496 (84.9)	138 (78.4)	117 (66.5)	21 (11.9)	124 (86.1)	70 (90.9)	50 (80.6)	234 (88.6)
Any NRTI+NNRTI	388 (66.4)	116 (65.9)	105 (59.7)	11 (6.3)	94 (65.3)	50 (64.9)	26 (41.9)	178 (67.4)
Any NRTI+PI	182 (31.2)	17 (9.7)	7 (4.0)	10 (5.7)	46 (31.9)	0 (0.0)	0 (0.0)	119 (45.1)
Any NRTI+NNRTI+PI	139 (23.8)	10 (5.7)	5 (2.8)	5 (2.8)	32 (22.2)	0 (0.0)	0 (0.0)	97 (36.7)

[a]amino acid changes at positions included in HIV genotypic drug resistance interpretation algorithm Rega v9.1.0
[b]NRT+NNRTI, NNRTI-based first-line regimen; NRTI+PI, PI-based first-line regimen; NNRTI/PI, NNRTI-based first-line regimen, followed by PI-based second-line regimen; PI/NNRTI, PI-based first-line regimen followed by NNRTI-based second-line regimen
[c]NRTI mutations K65R/E/N, V75I, F77L, Y115F, F116Y, Q151M; NNRTI mutations L100I, V179L, Y188C/L/H, P225H, F227C, M230I/L; and PI mutations D30N, V32I, I47V/A, G48V, I50L/V, Q58E, T74P, L76V, V82A/T/F/S/L, N83D, N88S were observed in <15% of patients.
NNRTI: non-nucleoside reverse transcriptase inhibitor NRTI: nucleoside reverse transcriptase inhibitor PI: protease inhibitor

The most frequent mutations found for NRTI and NNRTI in previously treated patients were expected because, for over a decade, AZT+3TC+NVP has been the most common combination used in Cuba for first-line therapy.[12] Worrisome is the high prevalence of V82A mutation which is selected by ritonavir and produces treatment failure with most PI.[27]

In Cuba, HIV-1 patients can only receive ART if it is prescribed by authorized HIV specialists; thus, our observation of NNRTI and PI resistance mutations in patients never exposed to these drug classes (Table 2) supports previous reports that drug-resistant strains are in circulation.[11,19] Subtype B viruses played a major role in the earliest ARV resistance studies, most of which reported that existing ARVs are equally effective at treating subtype B and non-B viruses. However, protease and reverse transcriptase sequence data from non-B subtypes isolated from previously treated patients have shown several drug resistance mutations that preferentially occur in certain HIV-1 subtypes. Most of these subtype-specific differences in drug resistance mutation distribution are attributed to differences in codon usage.[28]

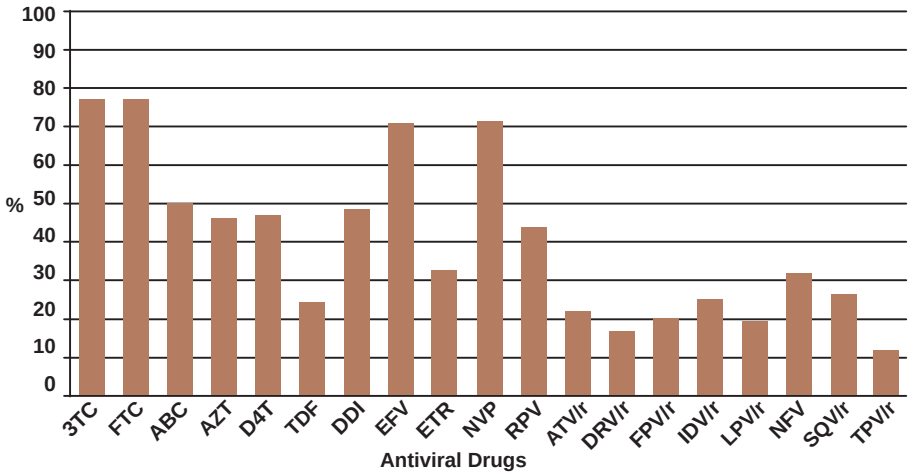
Table 4: Resistance mutations with HIV-1 viral variant in previously treated patients on active ART at time of testing

Subtype (n)	ART	Mutation (n)	p Value*	OR (95% CI)
		NRTI		
C (34)	3TC	K65R (5)	0.001	10.733 (3.303–34.873)
CRF 19_cpx (68)	AZT o d4T	L210W (15)	0.007	2.479 (1.260–4.878)
		NNRTI		
C (6)	NVP	K101E (3)	0.037	6.636 (1.285–34.267)
		PI		
CRF_BG (10)	LPV/r	M47V/I (4)	0.029	6.4 (1.338–30.606)

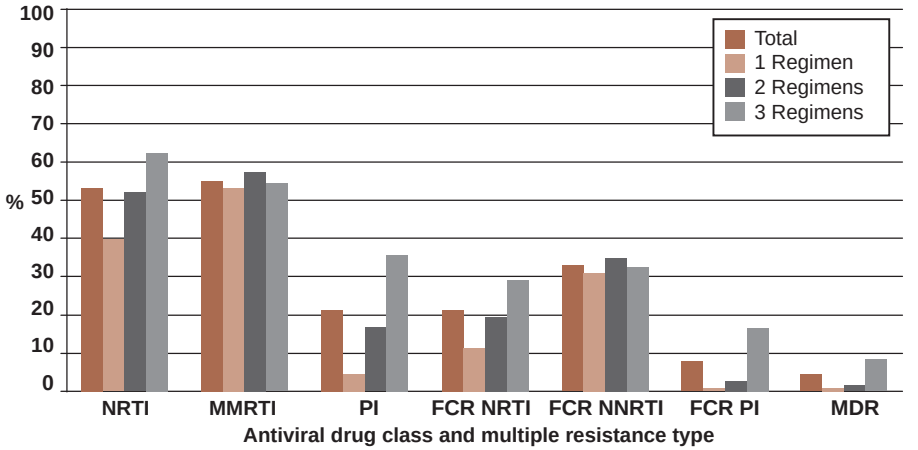
*chi square with Yate's continuity correction
ART: antiretroviral therapy NNRTI: non-nucleoside reverse transcriptase inhibitor
NRTI: nucleoside reverse transcriptase inhibitor OR: odds ratio PI: protease inhibitor

Figure 1: Antiviral resistance in previously treated patients, Cuba, 2009–2014

a. By drug



b. By drug class

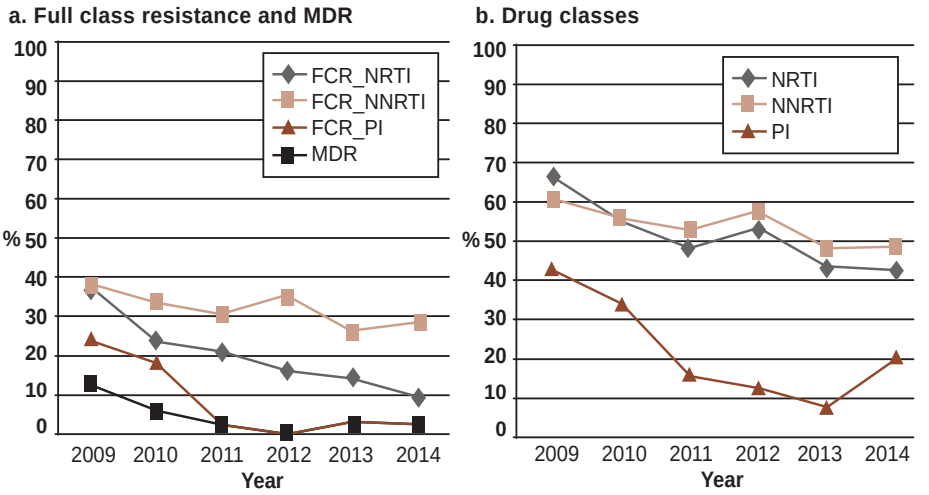


3TC: lamivudine ABC: abacavir ATV: atazanavir AZT: zidovudine D4T: stavudine DDI: didanosine
DLV: delavirdine DRV: darunavir EFV: efavirenz ETR: etravirine FCR: full class resistance
FPV: fosamprenavir FTC: emtricitabine IDV: indinavir LPV: lopinavir MDR: multidrug resistance
NFV: nelfinavir NNRTI, non-nucleoside reverse transcriptase inhibitor
NRTI: nucleoside reverse transcriptase inhibitor NVP: nevirapine PI: protease inhibitor RPV: rilpivirine
/r: ritonavir SQV: saquinavir TDF: tenofovir TPV: tipranavir

ART susceptibility of different HIV-1 subtypes is currently the subject of much attention and hence, further research on this topic is encouraged. Our finding that K65R resistance mutation was more likely detected in subtype C is consistent with previous reports.[29–32] The higher prevalence of NRTI mutation L210W in the viral strain CRF19_cpx, has important implications for NRTI-based ART regimens in Cuba, because CRF19_cpx is the third most frequent strain in the Cuban HIV-1 epidemic,[11–13] and has recently been associated with rapid progression to AIDS.[33] Moreover, the higher prevalence of PI mutation M47V/I among Cuban recombinants represents a hazard for PI-based ART.[34] CRF19_cpx and CRFs BGs circulate almost exclusively in Cuba,[11–13] so there are no previous prevalence studies of resistance mutations among these CRFs. Further studies are required to confirm our findings.

Overall, drug resistance to NRTI, NNRTI and PI in the sample studied is high, probably due to the combination's lack of potency, acquisition of resistant virus[12,35,36] and lower frequency of viral load testing. Despite overall high resistance, our analysis showed a significantly declining trend over time for FCR NRTI, FCR PI and MDR. This might be due to changes in patient selection for resistance testing. In the first years after implementation of the test, samples were selected mainly from patients failing multiple therapy regimens; after 2011, all patients failing first therapy regimen were tested. It might also reflect better clinical management of HIV ART, greater experience of clinicians, and virologists' assistance in interpreting genotypic resistance assays, resulting in increasing ART effectiveness.[8] The declining resistance observed in Cuba is in line with a trend observed in recent years in high-income countries in Western Europe and North America.[35,37,38]

Figure 2: Drug resistance trends, Cuba, 2009–2014



FCR: full class resistance MDR: multidrug resistance
NNRTI: non-nucleoside reverse transcriptase inhibitor
NRTI: nucleoside reverse transcriptase inhibitor PI: protease inhibitor

The study's main limitation is that it does not meet WHO standards for a national surveillance study, which require a nationally representative sample.[39]

CONCLUSIONS

TDR levels observed reinforce the need for a national surveillance study of Cuban treatment-naïve patients. Despite the high prevalence of resistance in patients failing ART, its frequency seems to be decreasing over time. The frequency of specific drug resistance mutations in recombinant forms of HIV in Cuba needs further attention.

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Effectiveness of a Serogroup B and C Meningococcal Vaccine Developed in Cuba

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ABSTRACT

INTRODUCTION Serogroup B meningococcal outer membrane vesicle vaccines have been effective against vaccine-type strains, but their effectiveness against heterologous strains has been controversial. The Cuban VA-MENGOC-BC vaccine is of this type, but also includes meningococcus C capsular polysaccharide.

OBJECTIVES Assess the effectiveness of VA-MENGOC-BC in reducing meningococcal disease caused by homologous or heterologous serogroup B strains and its serological effectiveness against meningococcus C.

METHODS A review of studies of VA-MENGOC-BC's application in Cuba, Brazil, Uruguay and Colombia was carried out to examine the vaccine's effectiveness in reducing meningococcal disease during serogroup B outbreaks. Serological effectiveness against serogroup C determined in these studies (indicated by bactericidal antibody titers before and after vaccination) was also analyzed.

RESULTS VA-MENGOC-BC's effectiveness against homologous serogroup B strains has consistently been greater than 80% in all age groups. Effectiveness in heterologous contexts was also above 80% in individuals aged >4 years. Lower effectiveness in heterologous contexts was found in Brazilian children aged <2 years, although still >50%. Effectiveness increased when assessed based on mortality rates, as well as in cases of clinically severe meningococcal disease. The carrier-state pattern was modified after vaccination with reduction of hypervirulent lineages. Some 60% of infants (aged <1 year) attained protective bactericidal antibody titers against serogroup C. Higher protection rates were achieved in older children.

CONCLUSIONS In addition to prevention of meningococcal disease caused by homologous serogroup B strains, VA-MENGOC-BC should be considered for heterologous contexts. It is protective against serogroup C in all age groups.

KEYWORDS *Neisseria meningitidis,* meningococcal disease, meningococcal vaccines, serogroup B meningococcus, serogroup C meningococcus, immunogenicity, bacterial outer membrane proteins, heterologous effects of vaccines, acellular vaccines, Cuba

INTRODUCTION

Meningococcal disease is an important global public health concern because of high rates of serious sequelae and mortality. It is endemic worldwide, and outbreaks and epidemics have occurred in all continents, most caused by 5 (A, B, C, Y and W135) of the 13 meningococcal serogroups.[1–6]

Since the introduction of polysaccharide vaccines against serogroups A, C, Y and W135, serogroup B has emerged as the most important cause of meningococcal disease in the Americas, Europe, as well as in Australia and New Zealand.[1,6,7] Effective overall protection against meningococcal disease cannot be achieved without routine use of vaccines against *Neisseria meningitidis* (Nm) serogroup B.

The serogroup B capsule elicits a poor immunogenic response because its structural similarity to human tissue leads to immunological tolerance. Concerns about autoimmunity have therefore shifted research attention to subcapsular antigens. Several subcapsular proteins have been studied, but vaccines based on outer membrane vesicles (OMV) have been the most widely used.[1–3,8]

Protection induced by serogroup B OMV vaccines has been discussed in various scientific fora. Many researchers believe that these vaccines' effectiveness is limited to specific strains (thus describing them as "tailor-made" vaccines), concluding that new vaccine generations are required to ensure broad cross-reactivity, such as those that include recombinant outer membrane proteins (OMP).[1,3,8–10]

IMPORTANCE The article assesses and synthesizes available evidence regarding the effectiveness of the world's first vaccine effective against *Neisseria meningitidis* type B, Cuba's VA-MENGOC-BC.

VA-MENGOC-BC is a bivalent vaccine based on Nm serogroup B OMVs and the capsular polysaccharide of Nm serogroup C attached to OMP.[2,3] It was developed by Cuba's Finlay Vaccine Institute in Havana in the 1980s and has been extensively used in several countries. The US CDC acknowledged that it was available commercially (but not in the USA) and had been used to control epidemics in South America.[11] However, the vaccine's effectiveness against heterologous serogroup B strains (different phenotype) is controversial, since outside Cuba, it has been used in countries where circulating strains differ from the original strain for which it was developed.[1–3]

VA-MENGOC-BC OMVs are obtained from a hypervirulent strain (B:4:P1.19,15) that caused an extensive epidemic in Cuba in the 1980s, when incidence rose as high as 14.4 per 100,000 population overall and 120 per 100,000 among infants.[2] Conceptually, it could be considered a tailor-made vaccine, since the B:4:P1.19,15 strain caused the majority of cases. From the beginning, however, vaccine researchers addressed the need for cross-reactive antigen expression to induce protection against heterologous strains (not just homologous ones) for vaccine strain selection and development of production methods.[2,12]

More than one hundred proteins have been detected. A bioinformatics analysis of known components resulted in identification of 31 predicted OMPs. The contribution of major proteins

(Por A, Por B, Opa, OpcA, RmpM, FetA) to total protein content is <65%. Vesicles are especially lacking in Por A, <20%. Conserved proteins (NadA, fHbp, NMB0088, NMB1796, NMB0928) and other important minor proteins (Tbp, NspA, FbpA, HrpA, PilQ, ATP synthetases, bacterioferritins, heat shock proteins and ribosomal proteins, among others) have also been identified.[12–14]

A study published in 2014 by University of Southampton (UK) researchers and GlaxoSmithKline Pharmaceuticals identified novel antigens in VA-MENGOC-BC OMVs, such as exopolyphosphatase and gamma-glutamyltranspeptidase enzymes, and a putative cell-binding factor protein. They also demonstrated that VA-MENGOC-BC induces widely cross-reactive bactericidal antibodies.[15]

In this paper, VA-MENGOC-BC effectiveness in reducing meningococcal disease caused by homologous and/or heterologous serogroup B strains was analyzed by age group, as was its serological effectiveness against meningococcus C.

METHODS

Study design We reviewed results from all published postmarketing studies on VA-MENGOC-BC application in different epidemiological contexts, as well as from the phase III clinical trial in Cuba, to integrate results and derive conclusions on the vaccine's effectiveness. Effectiveness against serogroup B meningococcal disease was defined as the degree of protection attributable to the vaccine when administered under field conditions.[16] VA-MENGOC-BC's serological effectiveness against Nm serogroups B and C was defined by bactericidal antibody titers.

Data sources Scientific papers on VA-MENGOC-BC composition and vaccine efficacy from the Cuban prelicensure phase III clinical trial were reviewed, as were postmarketing observational studies on VA-MENGOC-BC application in Cuba, Brazil, Colombia and Uruguay. The phase III efficacy trial was designed to assess whether VA-MENGOC-BC produced the expected results under ideal circumstances, that is, in a randomized, double-blind, placebo-controlled trial based on a clinical disease endpoint.

We reviewed analytical observational studies (case–control and cohort studies) performed in Cuba, Brazil and Colombia that assessed effectiveness against serogroup B, and studies in Cuba and Uruguay that assessed the impact of vaccination on meningococcal disease burden. Serological effectiveness against serogroups B and C, and Nm strains isolated from patients with meningococcal disease and carriers was also investigated in postmarketing studies.

Study variables Age: grouped by <2; 2–4; <4, >4 years. Nm serogroup B strains: Homologous strains of Nm serogroup B are those similar to vaccine-type strains. Heterologous strains are meningococcal B strains with different phenotypes from the vaccine strain. Bactericidal antibody levels: Seroconversion was defined as a ≥4-fold increase from baseline in titers of antibodies against serogroups B and C. Seroprotection was defined as bactericidal titers ≥1:4 for serogroup B and ≥1:8 for serogroup C. When whole-blood assay was used, seroprotection against serogroup C was defined as >50% killing of meningococci inoculated into whole blood.[3,10]

Data collection and analysis VA-MENGOC-BC's protective effectiveness against homologous and heterologous serogroup B strains was assessed in case–control and cohort studies carried out in Cuba, Brazil and Colombia. Vaccine effectiveness was assessed by age group, taking age four years as the primary breaking point, with additional analyses, when data were available, of groups aged less than two years and two to four years. Bactericidal titers against serogroup B were analyzed before vaccination and one month after the second dose.

Case–control studies compared rates of vaccinated and unvaccinated individuals with meningococcal disease (cases) versus rates of vaccinated and unvaccinated individuals without disease (controls) from the same population. Cohort studies compared rates of meningococcal disease in vaccinated and unvaccinated persons from the same population. The main variable of interest was effectiveness in reducing risk of meningococcal disease, which was reported as odds ratios (OR) in case–control studies and relative risk (RR) in cohort studies; effectiveness was estimated as (1−OR) x 100 or (1−RR) x 100, respectively.

Vaccination impact on meningococcal disease burden in Cuba and Uruguay was assessed taking into account incidence rates of meningococcal disease before and after introducing vaccination. In Uruguay, vaccination effectiveness was calculated by the following formula: VE = (P−C)/[P(1−C)] x 100, where P is the proportion of the population vaccinated and C the proportion of cases among vaccinees.[16]

Phenotypic and genetic structures of Nm populations in Cuban patients and carriers were analyzed during pre- and postvaccination periods. Phenotypic characterization was also carried out of Nm strains in Uruguayan, Colombian and Brazilian patients.

Serological effectiveness against Nm serogroup C was assessed by means of serum bactericidal antibody assay (gold standard for meningococcal polysaccharide vaccines) or whole blood assay. Bactericidal activity against the ATCC C11 strain was assessed in postmarketing studies a month after finishing the 2-dose vaccination schedule (with an interval of 6–8 weeks). It was also assessed a year post vaccination (with 3 consecutive doses at ages 3, 5 and 7 months).

Effectiveness against meningococcal disease caused by homologous or heterologous strains of Nm serogroup B in children aged <2 years, 2–4 years, and >4 years was compared using the Kruskal–Wallis test, with 95% confidence intervals usually estimated for measures of effect. Analyses were performed using Statgraphics Plus for Windows v. 3.1 (Statistical Graphics Corporation, USA).

Ethics The Finlay Vaccine Institute's scientific and ethics committees approved the study (code: 002-10-03-2017). Data were collected from published papers and no patient files or electronic medical records were accessed at any stage.

RESULTS

Vaccination impact on meningococcal disease in Cuba In 1987, a Phase III efficacy trial of VA-MENGOC-BC was conducted.

It was a controlled, double-blind, randomized trial involving 106,251 boarding school students, aged 10–16 years, using a 2-dose schedule with a 6–8-week interval. This 16-month trial was carried out in the 7 Cuban provinces with highest meningitis incidence. Estimated efficacy was 83% (CI 44%–93%). A similar point estimate of 81% was achieved in a cohort study performed in children aged <4 years (886,148 children, 85% of whom were vaccinated).[2,3,12]

Following VA-MENGOC-BC's study and subsequent approval by national regulatory authorities, Cuba's Ministry of Public Health (MINSAP) used a two-stage strategy to control meningococcal disease. First, a nationwide mass vaccination campaign was launched to curb increasing incidence. The 1989–1990 campaign targeted the highest-risk population (aged 3 months to 24 years), reaching more than 3 million people, for 95% coverage. The second stage, still ongoing, began in 1991 with VA-MENGOC-BC's inclusion in the National Immunization Program (PNI), using a two-dose schedule (first dose at age three months, second at five months), to protect children born after the mass campaign and prevent further epidemics. VA-MENGOC-BC has also been used in other age groups.[2,3,12]

The strategy has succeeded in reducing overall incidence to 0.1 per 100,000 population in recent years (from 14.4 per 100,000 at the height of the epidemic in 1983–1984). Vaccine effectiveness ranged from 80% to 100% in infants (aged <1 year), children, adolescents and adults. Mean effectiveness in infants was 84% between 1997 and 2008, and reached 93% in preschool children.[2,3,12,17]

Phenotypic and genetic structure of Nm populations in Cuba before and after vaccination Phenotypic characterization of strains isolated in carriers during epidemic and postepidemic stages is important to assess the role of vaccination as an intervention. Martínez characterized Nm strains for 20 years and showed that during the epidemic stage in Cuba, serogroup B (67.6%), serotype 4 (70.5%) and subtype P1.19,15 (61.9%) predominated, and during the postvaccination period, nonserotypable (NT) (70.8%), nonserosubtypable (NST) (34.4%) and nonserogroupable strains (NG) (79.7%) were more frequently found. Thus, the predominant phenotype during the epidemic was B:4:P1.19,15, which was favorably modified in the postvaccination period to phenotype NG:NT:P1.NST.[18]

It is also important to examine, using new tools of molecular biology, the impact of vaccine application on strain distribution in patients and carriers in pre- and postvaccination periods. To that end, Climent isolated strains from 12 clonal complexes in Cuba. The main strain that caused the 1980s' outbreak belonged to the ST-32 complex (58.6% of isolates). VA-MENGOC-BC was found to be effective not only against ST-32 complex, but also ST-41/44, ST-8 and ST-11, among others. Furthermore, the carrier-state pattern was modified, with reduction of hypervirulent lineages. The ST-53 complex, common in asymptomatic carriers, became predominant.[19] A similar conclusion was reported in Climent's study, in which experts from Oxford University (UK) also participated.[20]

VA-MENGOC-BC's effectiveness for controlling serogroup B outbreaks in Brazil and Colombia VA-MENGOC-BC has been widely used to control serogroup B outbreaks in various countries with epidemiological profiles different from Cuba's, in many cases with wide circulation of heterologous strains.

In Brazil, de Moraes performed an ambispective case–control study to estimate VA-MENGOC-BC effectiveness against meningococcal disease in children aged 3 months to 6 years at the beginning of the 1990s: 112 cases and 409 controls were assessed. Only positive or probable cases of serogroup B meningococcal disease were analyzed.[21]

Most cases (83%) were confirmed by isolation of Nm serogroup B from cerebrospinal fluid. Effectiveness was 73% for children aged >4 years; 53% in children aged 2–4 years, and 5% in those aged <2 years.[21] However, the authors identified biases in selection of cases and controls in their research, which could lead to underestimates of degree of protection, mainly in the retrospective arm. Sources of selection bias were the low vaccination coverage estimated in this arm, as well as the small proportion (17%) of patients reported with meningococcal disease who met diagnostic criteria for study enrollment: identification of Nm by culture or antigen detection. On the other hand, for the prospective arm, estimated effectiveness was 55% in children aged <2 years.[21] Meningococcus strains different from the vaccine-type strain were responsible for about 60% of cases of meningococcal disease in all age groups, suggesting that the vaccine does provide some protection against strains other than the vaccine type.[21] Table 1 shows the great diversity of strains causing disease in the unvaccinated control group.

Table 1: Serotype and subtype classification of *Neisseria meningitidis* serogroup isolates, Sao Paulo, Brazil, June 1990–June 1991[21]

Serotype:subtype	n (%)
B:4:P1.15	39 (41.9)
B:4:P1.NST	16 (17.2)
B:NT:P1.NST	7 (7.5)
B:8:P1.NST	4 (4.3)
B:NT:P1.15	3 (3.2)
B:2b:P1.NST	2 (2.2)
B:15:P1.15	2 (2.2)
B:2b:P1.2	1 (1.1)
B:4:P1.2	1 (1.1)
B:8:P1.15	1 (1.1)
B:15:P1.NST	1 (1.1)
Unknown	16 (17.2)
Total	93 (100.0)

NT: nonserotypable NST: nonserosubtypable

However, other analytical observational studies conducted in Brazil during those years showed greater effectiveness. Cases of meningococcal disease were defined by presence of one or more of the following criteria: a) Nm isolation, b) meningococcal antigens demonstrated by immunological tests, c) gram-negative diplococci identified by gram staining of cerebrospinal fluid, d) patients with a clinical picture compatible with meningococcal disease.[22,23]

In Rio de Janeiro, Noronha's retrospective case–control study in children aged 6 months to 9 years (275 cases, 279 controls) demonstrated high effectiveness in preventing meningococcal disease in children aged >2 years.[22] Effectiveness was lower in younger children, but still higher than reported by de Moraes (Table 2).[21]

Table 2: VA-MENGOC-BC's effectiveness in analytical observational studies of Cuban, Brazilian and Colombian children

Location (Date)	Age group	Study type	Age-specific effectiveness Age group, %
14 Provinces/Cuba (1989–1994)[3,12]	3 months–4 years	Case–control	All, 81
Santa Catarina, Brazil (1990–1992)[a][23]	3 months–7 years	Cohort	<2 years, 55 2–4 years, 62 <4 years, 59 >4 years, 78
Rio de Janeiro, Brazil (1990–1992)[b][22]	6 months–9 years	Case–control	<2 years, 53 2–4 years, 77 <4 years, 64 >4 years, 80
Antioquia, Colombia (1991–1994)[24]	3 months–4 years	Cohort	All, 98

[a]cases confirmed as meningococcal disease by laboratory tests
[b]cases of meningococcal disease diagnosed by laboratory or clinical criteria

Noronha's research revealed effectiveness against confirmed serogroup B strains slightly lower than effectiveness for cases defined by all criteria in children aged <4 years. It was 69% in children aged 2−4 years and 47% in children aged <2 years old. Vaccine-induced protection reached 67% (28%–85%) in children aged <2 years with severe clinical symptoms.[22]

In the Brazilian state of Santa Catarina, Costa carried out a retrospective cohort study in children aged 3 months to 7 years (400,482 vaccinated children; 89,610 unvaccinated).[23] The reported effectiveness against laboratory confirmed cases of meningococcal infections was similar to the effectiveness found in Rio de Janeiro (Table 2).[22] Effectiveness increased when analysis was restricted to cases that could be classified as Nm serogroup B, 66% (CI 5%–89%) in children aged 3 months to 4 years and 88% (CI 54%–97%) in those aged >4 years.[23] Effectiveness in children aged <4 years reached 68% (CI 51%–79%) in cases defined by all criteria; specific data were not available for age groups 2–4 and <2 years. On the other hand, when effectiveness assessment was based on mortality rates, it rose to 76% in the younger children (CI 41%–91%).[23]

In Santa Catarina, only a small proportion of cases were serogrouped. In Rio de Janeiro, 57% of patients enrolled were serogroup B, but only 6% were serotypable or serosubtypable. Although the circulating strains were not specified in these studies, they were likely similar to those isolated in Sao Paulo, with wide circulation of heterologous strains.[23,25]

When Brazil's Ministry of Health assessed the impact of vaccination with VA-MENGOC-BC in 6 Brazilian states, including those referred to here, it reported effectiveness against serogroup B as 72% (CI 63%–80%) in children aged 6–83 months.[25] The effectiveness found in Brazil is high, especially considering the country's geographical and epidemiological characteristics. The great diversity of meningococcal serosubtypes supports the contention that VA-MENGOC-BC induced cross-reactive protection in the age groups assessed.

VA-MENGOC-BC was also used in Colombia during an epidemic in Itaguí, Antioquia, in children aged 3 months to 4 years, in which effectiveness was assessed by examining laboratory-confirmed cases of meningococcal disease in a cohort of 16,762 children (92% vaccinated). The effectiveness calculated by different methods was >98%, exceeding the effectiveness estimates of Cuban and Brazilian researchers.[24] These results are important, given that this age group is the most vulnerable and not fully mature immunologically. Although the strains responsible for the outbreak were not precisely determined in the study, both the vaccine strain and the heterologous B:8:P1.NST strain were isolated.[24,26]

VA-MENGOC-BC's impact on a meningococcal disease outbreak in Uruguay The vaccine was used to control a serogroup B meningococcal disease outbreak in Uruguayan children and adolescents aged 4–19 years, the most affected group. Incidence and mortality rates in Canelones Department dropped dramatically after vaccination. Incidence decreased from 7.4 per 100,000 in the epidemic period, to 0 after vaccination in that age group.[27] While the vaccine serosubtype prevailed in the most severe cases, other strains causing meningococcal disease were detected in unvaccinated individuals (Table 3).

In Montevideo Department, incidence declined slightly in the group aged 4–19 years: from 4.6 per 100,000 in the epidemic period, to 3.4 per 100,000 after vaccination.[27] Heterologous strains were isolated in most patients with meningococcal disease (83.9%) during the epidemic period (Table 4).

In Canelones Department, estimated vaccination coverage was 81%. Meningococcal disease was not detected in vaccinees during the postvaccination period. Vaccination coverage was slightly low-

Table 3: Serotypes and subtypes of *Neisseria meningitidis* serogroup B isolates, Canelones, Uruguay, April 2000–March 2003[27]

Serotype:subtype	Pre–epidemic period n (%)	Epidemic period n (%)	Prevaccination Total n (%)	Postvaccination period* n (%)
B:4,7:P1.15,19	3 (43)	7 (47)	10 (46)	3 (38)
B:4,7:P1.NST		1 (7)	1 (5)	
B:4:P1.14				1 (13)
B:19:P1.NST		1 (7)	1 (5)	
B:19:P1.14	1 (14)		1 (5)	
B:19,14:P1.17,1	1 (14)	1 (7)	2 (9)	
B:15:P1.16		2 (13)	2 (9)	
B:15:P1.7,16				1 (13)
B:2b:P1.10	1 (14)		1 (5)	
B:NT:P1.5,2				1 (13)
B:1:P1.NST				1 (13)
B:1:P1.9	1 (14)		1 (5)	
Not classified		3 (20)	3 (14)	1 (13)
Total	7 (100)	15 (100)	22 (100)	8 (100)

*cases identified in the postvaccination period were not in the vaccinated age groups
NT: nonserotypable NST: nonserosubtypable

Table 4: Serotype and subtype classification of *Neisseria meningitidis* serogroup B isolates, Montevideo, Uruguay, April 2000–March 2003[27]

Serotype:subtype	Pre-epidemic period n (%)	Epidemic period n (%)	Prevaccination Total n (%)	Postvaccination period n (%)
B:4,7:P1.15,19	4 (20)	5 (16)	9 (18)	4 (17)
B:4,7:P1.22	2 (10)	2 (6)	4 (8)	
B:4:P1.4		3 (10)	3 (6)	2 (8)
B:19:P1.16				1 (4)
B:4,7:P1.7,1		2 (6)	2 (4)	1 (4)
B:4,7:P1.NST	1 (5)		1 (2)	1 (4)
B:4:P1.NST				2 (8)
B:4,10:P1.NST		1 (3)	1 (2)	
B:19,14:P1.10	1 (5)		1 (2)	
B:19,8,7:P1.19	2 (20)	1 (3)	3 (6)	
B:19,1:P1.NST	2 (20)		2 (4)	
B:19,7:P1.NST		1 (3)	1 (2)	
B:15:P1.16	1 (5)	2 (6)	3 (6)	6 (25)
B:15:P1.14		2 (6)	2 (4)	
B:15:P1.NST		1 (3)	1 (2)	1 (4)
B:14,14:P1.1,7	1 (5)	1 (3)	2 (4)	
B:1:P1.NST	1 (5)	3 (10)	4 (8)	1 (4)
B:NT:P1.NST	5 (25)	5 (16)	10 (20)	5 (21)
B:NT:P1.14		2 (6)	2 (4)	
Total	20 (100)	31 (100)	51 (100)	24 (100)

NT: nonserotypable NST: nonserosubtypable

er (73%) in Montevideo. Most cases (75%) were detected in individuals who had not received the two scheduled VA-MENGOC-BC doses. Furthermore, a significant mortality reduction was observed in the vaccinated group, which has also been observed in other studies. In the postvaccination period, disease incidence increased in persons aged >20 years, an unvaccinated age group, indicating that outbreak strains continued circulating.[27]

Although the authors did not report vaccine effectiveness,[27] it can be estimated at 88% in Montevideo and 100% in Canelones, using Orenstein's formula.[16] Since most strains isolated in Montevideo were heterologous, we can infer that vaccine protection is not strictly limited to the vaccine strain.

Serum bactericidal antibody titers against serogroup B Bactericidal activity against serogroup B was not assessed in most analytical observational studies, except for the Colombian cohort study. Seroconversion in a subsample of 213 children was 84% (CI 72%–96%), higher than reported in prelicensure clinical trials (CI 50%–78%), but lower than effectiveness based on clinical endpoint (98%). Seroprotection, defined as bactericidal antibody titers ≥1:4, was not analyzed in this study.

However, all seroconverted individuals were obviously seroprotected, because seroconversion was defined as greater than fourfold increase in antibody titers from baseline.[24,26] It is worth noting that seroconversion rates observed in phase II clinical trials found were lower than efficacy in phase III clinical trials (that is, seroconversion underestimated the degree of protection conferred).[2,12]

Statistical analysis of VA-MENGOC-BC's effectiveness against homologous and heterologous serogroup B strains Overall, the vaccine's effectiveness against homologous strains has been >80% in all age groups, with no significant differences by age (p = 0.772). Its effectiveness in heterologous contexts was similar to the one found against homologous strains in individuals aged >4 years (p = 0.434). Lower effectiveness was detected in Brazilian children aged <2 years; although there was no statistically significant difference by age in heterologous contexts (p = 0.067).[21–23,25]

The cohort of Colombian children aged <4 years was not subdivided, but it is unlikely that differences would have been found, because overall effectiveness was so high (98%).[24]

VA-MENGOC-BC's serological effectiveness against Nm serogroup C This vaccine has only been used to control outbreaks of serogroup B meningococcal disease, so clinical effectiveness against serogroup C has not been estimated. However, bactericidal activity against Nm serogroup C has been assessed. High seroconversion and seroprotection rates have been detected in all age groups against this serogroup (Table 5).

Morley's study of Cuban infants assessed seroprotection rates by whole-blood assay. Seroprotection against serogroup C was 60% a year after vaccination with doses at ages 3, 5 and 7 months.[28] Mean seroprotection in young Cuban adults 12 years after vaccination and with high background levels was 66%, higher than seroconversion.[29]

Notably, high seroconversion rates were found in Colombian toddlers and preschool children (88%); most seroconverted

Table 5: Bactericidal activity induced by VA-MENGOC-BC against *Neisseria meningitidis* serogroup C in infants, children, teenagers and young adults

Country (year)	Age group (years)	Seroconversion (%)	Seroprotection (%)
Colombia (1995)[a][30]	1–5	88	86
Belarus, Ukraine, Russia (1995)[a][2]	5–11 12–18	74 67	na na
Iceland (1998)[a][2]	20–22	96	na
Cuba (2001)[b][28]	<1	na	60
Cuba (2004)[a][29]	15–18	58	66

[a]Bactericidal antibody levels measured by serum bactericidal antibody assay
[b]Bactericidal antibody levels measured by whole blood assay
na: data not available
Seroconversion: (bactericidal antibody titers postvaccination / bactericidal antibody titers prevaccination) ≥4
Seroprotection (serum bactericidal antibody assay): bactericidal antibody titers ≥1:8
Seroprotection (whole blood assay): >50% killing of meningococci inoculated in whole blood

children had bactericidal titers ≥1:8, and were therefore protected against serogroup C.[30]

DISCUSSION

OMV vaccine candidates have been developed to control meningococcal disease outbreaks, but few vaccines have been licensed by regulatory agencies.

MenBvac (National Institute of Public Health, Norway) is a tailor-made vaccine prepared from strain B:15:P1.7,16. The phase III controlled trial carried out in the 1990s demonstrated an efficacy of 57% in teenagers aged 14–16 years. This vaccine was not widely used in the Norwegian and French outbreaks, and its effectiveness in these homologous contexts was not estimated.[1,31]

The vaccine MeNZB (Chiron, USA) was a vaccine specifically developed to control an epidemic produced by New Zealand strain B:4:P1.7b,4. No phase III clinical trials were undertaken; therefore, no direct estimate of efficacy was calculated. However, this tailor-made vaccine was administered to >100,000 individuals aged 6 months to 19 years in 2004 and 2005, with vaccine effectiveness estimated at 73% by statistical modeling and 80% (CI 52%–92%) in an observational cohort study of children aged <5 years.[1,31,32]

VA-MENGOC-BC's effectiveness in homologous contexts has ranged from 80% to 100% in all age groups, higher than other OMV vaccines.[2,3,12,17] This is interesting, considering that efficacy was established in the phase III clinical trial under ideal circumstances, while effectiveness was evaluated under field conditions.

The effectiveness of MenBvac and MeNZB against heterologous serogroup B strains has not been sufficiently explored. However, VA-MENGOC-BC's effectiveness in heterologous contexts has been extensively studied in epidemiological research carried out in Brazil, Uruguay and Colombia.

It is noteworthy that the effectiveness of VA-MENGOC-BC in heterologous contexts was >80% in individuals aged >4 years, 60%–80% in children aged 2–4 years, and 50%–60% in younger children. However, results in Colombia suggest that effectiveness might be higher in preschool children and infants in heterologous contexts.[24,26]

The lowest effectiveness was found in Brazilian children aged <2 years, though it was usually >50%. On the other hand, estimates of effectiveness increased when based on mortality rates or clinically severe meningococcal disease.[22–25]

The lower protective effectiveness for children aged less than four years reported by Moraes could be due to the selective effect of analyzing only laboratory-confirmed cases. Exclusion of cases not confirmed by laboratory analysis decreased effectiveness estimates because it missed severe cases in nonvaccinated children who died before reaching the hospital. Vaccinated children who developed the disease had milder clinical presentations, allowing them to reach the hospital and undergo laboratory diagnosis.[21,25]

On the other hand, the lower effectiveness found by Moraes' study, compared with other Brazilian studies, was also apparently related to retrospective and prospective enrollment of cases to increase sample size, which may have biased results. Retrospective analysis estimated vaccine coverage at 5%, but actual population coverage was 12%; thus, data for the controls underestimated vaccine coverage. As a result, odds ratio increases because of the large number of unvaccinated control children (130) and the small number of vaccinated children (7), decreasing estimated effectiveness. For prospective analysis, estimated and actual coverage were 93% and 92% respectively, and effectiveness increased to 55%.[21,25]

Higher effectiveness against heterologous strains in older age groups could be related to immune system maturation, but one must also consider increasing exposure to Nm through close contact with carriers at home or at school (and at workplaces in adults). In this situation, immune response could be greater because of a booster effect against shared OMV components. However, protection levels achieved in children aged less than four years should not be undervalued.

It should be noted that a two-dose immunization schedule with a six-to-eight–week interval was used, and other studies have demonstrated that three doses of OMV vaccines, similar to the VA-MENGOC-BC protocol in Cuba, increase immunogenicity against heterologous strains, which could be necessary for children aged less than two years.[28,33]

Morley's study at the Imperial College School of Medicine in London showed VA-MENGOC-BC immunogenic against various strains of meningococcus B in children aged less than one year, supporting the conclusion that the vaccine-induced immune response in this age group is not limited to the vaccine strain.[28]

Some researchers contend that changes in meningococcal epidemiology and isolate phenotypes should not be attributed solely to the vaccine, because meningococcal disease occurs in cycles typically associated with switches between different genotype lineages.[1,4,6,7] However, no switches to hypervirulent lineages have been detected in >25 years of VA-MENGOC-BC application in Cuba.[18–20,34]

On the other hand, the Uruguayan study found that meningococcal disease incidence increased in unvaccinated age groups, while incidence was reduced significantly in the vaccinated age groups, regardless of whether outbreak strains were still circulating.[27] We therefore infer that changes in meningococcal disease epidemiology and carrier-state patterns in Cuba can be attributed to the vaccine, with sustained and massive vaccination campaigns essential to achieve these results.

Recently, other vaccines have been introduced on the market. Bexsero (GSK, UK) was developed by reverse vaccinology and contains the recombinant proteins NadA, fHbp and NHBA, combined with OMVs of the New Zealand strain.[35,36] Trumemba (Pfizer, USA) is a recombinant vaccine containing fHbp A and B.[37] Both vaccines were designed to achieve broad cross-reactivity. Phase II clinical trials have proven their immunogenicity, but controlled, randomized phase III clinical trials based on clinical endpoints have not been carried out and effectiveness has not yet been thoroughly assessed.[35–38]

Bexsero was used by special FDA approval to control an outbreak of meningococcal disease at a US university in 2013. Immunogenicity was assessed by means of serum bactericidal antibody assay. Seropositivity, defined as an increase in serum bactericidal antibody titers of >1:4, was 66%, although no cases of meningococcal disease were reported among vaccinated students.[38] On the other hand, correlation between carriage and postvaccination serum bactericidal antibody titers has not been established.[39] Trumemba has been also used against university outbreak strains in the USA. Subjects with serum bactericidal antibody titers >1:4 have ranged from 20% to 100%.[37]

Despite the appearance of these new vaccines, VA-MENGOC-BC should not be disregarded. This vaccine has proven high effectiveness not restricted to preventing meningococcal disease caused by homologous serogroup B strains. This leads us to suggest that it should also be considered an option in heterologous contexts.

No data on OMV immunogenicity are available in most postmarketing effectiveness studies. In any case, one must consider that the serum bactericidal antibody assay, gold standard for meningococcal polysaccharide vaccines, underestimates the protection elicited by VA-MENGOC-BC OMVs. In addition, the vaccine induces other protective mechanisms more important than antibody-dependent complement mediated lysis and should be factored in: T-cell mediated responses (Th1 pattern), stimulation of neutrophils and other phagocytic cells, antibody opsonophagocytosis, interference with bacterial metabolism, and mucosal immunity.[40]

The reactivity against heterologous serogroup B strains induced by VA-MENGOC-BC could be due to deficiency in immunodominant proteins, in particular Por A, and a higher expression of cross-reactive antigens identified on the vaccine's OMV. These include not only well-studied antigens, but also novel antigens that could be useful for developing a new generation of meningococcal vaccines.[13–15] On the other hand, although OMVs are deficient in Por A, they are sufficiently present for homologous bactericidal activity.

VA-MENGOC-BC's clinical effectiveness against Nm serogroup C outbreaks has not been assessed, but the elevated levels of bactericidal antibodies it elicits indicate protection against this serogroup in all age groups, even infants. Furthermore, serogroup C strains have not been isolated in patients or carriers after the vaccine's massive application and inclusion in Cuba's PNI. These results support the serological effectiveness of the vaccine polysaccharide.[2,28–30]

The adequate immune response in children aged less than two years vaccinated with VA-MENGOC-BC, the absence of hyporesponsiveness after several doses of the vaccine, and the long-lasting immune response induced by the vaccine, support the thymus-dependent nature of the vaccine's C component.[28–30] On the other hand, the vesicles' adjuvant capability could considerably improve serogroup C polysaccharide immunogenicity, which should be considered for new combinations of the other meningococcal polysaccharides with OMVs.[40]

Finally, it is important to emphasize that modification of carrier-state patterns with reduction of hypervirulent lineages should be a consideration in designing vaccination strategies. The carrier-state pattern modification in vaccinated persons can interrupt the transmission chain, even protecting unvaccinated young children. This interesting strategy should be explored in greater depth.

CONCLUSIONS

In addition to prevention of meningococcal disease caused by homologous serogroup B strains, Cuba's VA-MENGOC-BC should be considered for heterologous contexts. It is protective against serogroup C in all age groups.

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Cuban Application of Two Methods for Analyzing Multiple Causes of Death

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ABSTRACT

INTRODUCTION Mortality analysis based on a single cause of death is not, in most cases, fully informative. There are several more illuminating procedures using a multiple cause of death approach; these are little known and rarely used in Cuba. The simplest of these methods, while methodologically limited, consists of summing all deaths from a specific cause mentioned on death certificates, regardless of whether the cause is listed as underlying or contributing.

OBJECTIVE Using Cuban data, critically assess and implement two of the most recognized approaches to analyzing multiple causes of death.

METHODS Multiple causes of death in Cuba were assessed for the years 2005, 2010 and 2015, employing death records from the National Medical Records and Health Statistics Bureau of Cuba's Ministry of Public Health. With the example of diabetes mellitus as underlying cause, we explored connections between underlying and associated (antecedent and contributing) causes on death certificates using two approaches from the international literature: the simple method and the cause-of-death association indicator.

RESULTS The study identified main trends in multiple causes of death identified in 2005, 2010 and 2015, overall and by age group and sex. We observed a trend to increasing mean number of causes of death per death certificate between 2005 and 2015. The number of causes reported showed no substantial differences by age group or sex. Diseases of the arteries, arterioles and capillaries were by far the most frequently associated with diabetes mellitus as underlying cause.

CONCLUSIONS The multiple causes of death approach affords more nuanced understanding of patterns of disease, comorbidity and death in the Cuban population. The indicators used fulfill different roles: the simple method brings to light the full range of ways in which a given cause contributes to mortality, and the cause-of-death association indicator enables exploration of links between different causes of death, not possible with the simple method.

KEYWORDS Mortality, multifactorial causality, causes of death, diabetes mellitus, Cuba

INTRODUCTION

Historically, mortality analysis uses a single underlying cause. But this one-dimensional, single-cause approach has long been considered inadequate for studying mortality patterns, especially when analysis spans geography and time.[1–3] A more nuanced mortality analysis would provide important information about population health status and priorities for prevention and treatment options, as well as projections for the mid and long term.

Other areas of epidemiology approach etiological problems in a more comprehensive and multidimensional fashion.[4] For example, in the case of causal attribution for a health event such as morbidity from cause *j,* it is standard procedure to consider various (*k*) risk factors "j_1, j_2", …, j_k" as causal.[5] In the case of mortality, unicausal analyses can be justified by the ease and convenience of the ICD-10 algorithm, which identifies the underlying cause as the most important one,[6] but the fact that the underlying cause may eventually be determined to be the most important does not mean that it is the sole cause. Nor is it advisable to ignore the others.

Various methods have been used for analyzing mortality in terms of multiple causes of death (MCD), all of which make use of death certificate entries of causes besides the underlying one.[7–10] Some, known as intermediate or antecedent causes, are part of the chain of events leading to death initiated by the underlying cause and are included in Part I. Others are contributing causes unrelated to the causal chain and are listed in Part II of the death certificate.[2,7] Information in Parts I and II about whether causes are intermediate parts of the causal chain or merely contributing causes may be omitted when death certificates are digitized, which means that, in practical terms, all causes of death—underlying and intermediate—are lumped together. Hereafter, when intermediate and contributing causes are referred to together, they will be described as associated or non underlying causes.

IMPORTANCE The article demonstrates the utility of a multidimensional approach to analyzing mortality data to better understand the complex associations among conditions leading to death.

The most frequently used MCD method, and one of the simplest, consists of calculating mortality from cause *i* by counting all death certificate mentions of cause *i*, whether underlying or associated.[9] An important limitation of this method (which we will call the simple method), is that deaths with *k* associated causes are counted *k* times, which distorts the total number of deaths for a given time period and place (that is, deaths are counted for every associated cause mentioned, so a death with six conditions mentioned on the death certificate would look like six deaths when causes are summed).

One can avoid this limitation by assigning weighted values (summing to unity) to different causes of death within a single death certificate.[10] The main virtue of weighted methods is that they bring to light causes that are rarely reported as underlying causes but are frequently reported as intermediate or contributing causes. In contrast, analysis based solely on underlying causes completely ignores intermediate and contributing causes.

The MCD approach presents as-yet-unexplored possibilities.[11] Two of the most promising are described below:

- Cluster analysis to identify mortality patterns.[2,7,9] Such analyses can be designed in different ways. One consists of considering cause *i* as a fixed underlying cause.[12] This analysis can be repeated for different years, provinces, or municipalities, to assess pattern stability over time and place.
- Regression models to assess diseases as risk factors for given underlying causes.[13,14] Death records provide sufficient information on multiple causes for this approach to derive inferences regarding which diseases constitute important risk factors for a given underlying cause
- The cause of death association indicator (CDAI),[8] one of the most interesting among various MCD approaches.[2,7,8,12] Important correlations between causes of death provide information about causal patterns, for example a pattern in which a particular group of causes is shared by a relatively large number of deaths. If deaths in a specific place and time could be grouped into a small number of patterns, health interventions could be organized around these patterns, which would facilitate the work of health decision-makers.

CDAI is interesting because it assesses the "true" impact of a disease associated with an underlying cause of death, since it quantifies the observed relative to the expected association. This correction is common in statistical indicators, as in the cases of relative risk and the Kappa coefficient, the former adjusted to a reference risk category/value of reference,[15] and the latter to random inter-rater agreement.[16]

Repeated application of CDAI to a potentially long list of diseases a_1, a_2, …, a_k associated with an underlying cause *b* would enable detection of those that correlate most strongly with *b*, and perhaps, under specific conditions, those that best "explain" deaths by underlying cause *b*. In this context, it would undoubtedly be useful to employ a multivariate procedure to complement CDAI and somehow adjust the effect (association) of a_i (with *b*) to the effect (association) of a_j (with *b*), for *i*, *j* = 1, …, *k*; $i \neq j$. To date, we do not know of any such multivariate procedure.

MCD approaches to analyze death certificates are rare internationally and quite limited in Cuba. The usual practice in Cuba is to analyze underlying and contributing causes separately, without exploring their connections.[17,18]

This study aimed to review and implement two of the most important MCD procedures, using Cuban data from death certificates listing diabetes mellitus (DM) as a cause of death. DM is a chronic disease of increasing impact internationally and in Cuba,[19–21] where it has ranked among the 10 main causes of death for all ages since the end of the 1960s.[22] We focused on DM because it is most frequently listed on death certificates as a contributing cause of death and can thus be used to illustrate the impact of employing MCD methods versus the traditional approach using only underlying causes.

METHODS

A descriptive study was conducted using data from the mortality database of the National Medical Records and Health Statistics Bureau of Cuba's Ministry of Public Health (DNE/MINSAP) for 2005, 2010 and 2015 (84,817; 91,060 and 99,684 deaths respectively). Deaths for which no age was reported were excluded (7, 5 and 7 in 2005, 2010 and 2015, respectively). Sex was recorded on all death certificates.

Multiple causes of death were described in terms of the proportion of deaths for which >1 cause was reported, and mean number of causes reported per death, overall and by age group (<1 year, 1–4 years, 5–9 years . . . up to 85–89 years, ≥90 years). These indicators are widely used in the MCD literature.[13]

The simple method, which counts all deaths for which a given associated cause is mentioned (whether underlying or contributing), was applied to DM mortality utilizing ICD-10 codes E10–E14.[6] Among the more sophisticated methods available that combine underlying and intermediate or contributing causes[23] is CDAI, proposed by Désesquelles in 2010 to quantify the relative association between a contributing cause *a* and an underlying cause *b*, and between the contributing cause *a* and any underlying cause.[8] In formal terms, it is defined as the ratio between two standardized proportions:

- prevalence at time of death of a specific combination between a contributing cause *a* and an underlying cause *b*, among all deaths with *b* as underlying cause; and
- prevalence at time of death of the same contributing cause among all deaths by any cause, expressed as follows:

$$IACM(b,a) = \frac{\sum_x \frac{m^x_{b,a}}{m^x_b} * \overline{m_x}}{\sum_x \frac{m^x_a}{m^x} * \overline{m_x}} * 100$$

where

1. $m^x_{b,a}$ = number of observed deaths in age group *x* with underlying cause *b* and contributing cause *a*
2. m^x_b = number of observed deaths with underlying cause *b* in age group *x*
3. m^x_a = total number of observed deaths in age group *x* with contributing cause *a* (independent of underlying cause)
4. m^x = total number of observed deaths in age group *x*
5. $\overline{m_x}$ = mean deaths in age group *x*, in locations (countries, regions, zones, etc.) used to calculate the indicator, used as a standardizing reference.[8]

Given that this study did not compare CDAI for different regions, places or time periods (except 2015) with mortality patterns by age, standardization was not necessary. Thus, in expression (1) the proportions were calculated overall and not by age group, and the factor m^x was not applied.

To illustrate this, CDAI was applied to the four contributing causes that most frequently appear with deaths citing DM as the underlying cause:[1,5] circulatory diseases (essential hypertension, code 110X); diseases of arteries, arterioles and capillaries (DAAC, code 179.2); respiratory diseases (codes J15.9 and J18.2), and glomerular and renal diseases (codes N08.3 and N18.9).[6]

Ethics This study was a component of the Disease Burden and Risk Factors in Cuba:1990–2015 project and was approved by the Scientific Council and Ethics Committee of the National Hygiene, Epidemiology and Microbiology Institute, as part of

a collaboration between the latter and the Institute of Tropical Medicine (Antwerp, Belgium).

RESULTS

Table 1 shows the mean numbers of causes noted on 2005, 2010 and 2015 death certificates overall. A total of 42.7% of certificates noted only the underlying cause in 2005, the corresponding proportions for 2010 and 2015 being 39.8% and 38.5%, respectively. There was a slight increase in the number of causes reported over the period: the mean number of causes recorded was 1.77 in 2005, 1.8 in 2010, and 1.82 in 2015.

Table 2 displays the proportions of each age group for which a given number of causes were reported on death certificates in all three years studied. In 2005, the reporting of two causes of death was slightly more frequent for ages 5–49 years than for other age groups, in which a single cause predominated. Tables 2b and 2c (2010 and 2015) indicate that over time the number of age groups with two causes of death increased.

Table 3 presents a cross tabulation of DM as a single underlying cause (traditinal approach) and DM as including both contributing and underlying causes (simple MCD method), both as dichotomous variables. Using the simple MCD method, DM deaths for 2015 were almost twice the proportion obtained by the traditional, single-cause approach, 4.7% of all deaths vs. 2.4%, respectively.

Finally, Table 4 presents the results using CDAI to adjust for nonunderlying causes' baseline

Table 1: Deaths by number of causes (underlying and associated*): Cuba 2005, 2010, 2015

No. of causes	Deaths								
	2005			2010			2015		
	n	%	Cumulative %	n	%	Cumulative %	n	%	Cumulative %
1	36,225	42.7	42.7	36,263	39.8	39.8	38,369	38.5	38.5
2	34,539	40.7	83.4	39,056	42.9	82.7	43,859	44.0	82.5
3	11,952	14.1	97.5	13,490	14.8	97.5	14,827	14.9	97.4
4	1,805	2.1	99.7	2,010	2.2	99.7	2,270	2.3	99.6
5	269	3.0	100.0	219	0.2	100.0	321	0.3	100.0
6	23	0.0	100.0	20	0.0	100.0	34	0.0	100.0
7	3	0.0	100.0	2	0.0	100.0	4	0.0	100.0
8	1	0.0	100.0	0	0.0	100.0	0	0.0	100.0
Total	84,817	100.0		91,060	100.0		99,684	100.0	
	No. of causes								
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
	1.77	1.0	8.0	1.80	1.0	7.0	1.82	1.0	7.0

*includes intermediate and contributing

Table 2: Number of causes of death by age group: Cuba 2005, 2010, 2015

a. 2005[a]

Age group	Number of causes of death % of group						Total	Mean no. of causes
	1	2	3	4	5	≥6		
<1	50.4[b]	32.4	13.7	2.9	0.5	0.0	100.0	1.7078
1–4	53.4[b]	43.8	2.3	0.5	0.0	0.0	100.0	1.4977
5–9	41.1	52.1[b]	5.5	0.7	0.7	0.0	100.0	1.6781
10–14	33.5	63.0[b]	3.5	0.0	0.0	0.0	100.0	1.7000
15–19	29.4	58.6[b]	11.6	0.2	0.2	0.0	100.0	1.8337
20–24	24.1	54.7[b]	19.5	1.2	0.4	0.0	100.0	1.9899[c]
25–29	28.0	52.2[b]	18.4	1.3	0.2	0.0	100.0	1.9344[c]
30–34	33.0	50.7[b]	16.1	0.2	0.0	0.0	100.0	1.8351
35–39	34.7	50.8[b]	13.2	1.1	0.2	0.0	100.0	1.8128
40–44	41.6	44.0[b]	13.1	1.2	0.1	0.0	100.0	1.7437
45–49	43.6	44.7[b]	10.3	1.2	0.3	0.0	100.0	1.6988
50–54	45.4[b]	41.9	10.9	1.7	0.1	0.1	100.0	1.6946
55–59	45.7[b]	41.0	10.9	2.1	0.3	0.1	100.0	1.7072
60–64	44.5[b]	40.4	12.1	2.4	0.5	0.0	100.0	1.7408
65–69	43.8[b]	39.7	13.4	2.8	0.3	0.0	100.0	1.7616
70–74	42.7[b]	40.4	13.7	2.6	0.5	0.0	100.0	1.7777
75–79	43.6[b]	39.5	14.1	2.4	0.3	0.0	100.0	1.7644
80–84	42.7[b]	39.6	14.8	2.5	0.4	0.0	100.0	1.7833
85–89	41.5[b]	39.6	16.5	2.0	0.3	0.0	100.0	1.8012
≥90	42.7[b]	39.2	16.2	1.6	0.2	0.0	100.0	1.7735
Total	42.7[b]	40.7	14.1	2.1	0.3	0.0	100.0	1.7672

[a]deaths at unknown age not included [b]for each age group, number of causes with greatest relative frequency
[c]age group with highest mean of number of causes of death

b. 2010[a]

Age group	Number of causes of death by age group % of group						Total	Mean no. of causes
	1	2	3	4	5	≥6		
<1	46.0[b]	33.2	13.8	5.7	1.2	0.2	100.0	1.8348
1–4	58.6[b]	36.8	4.6	0.0	0.0	0.0	100.0	1.4605
5–9	32.3	63.8[b]	3.9	0.0	0.0	0.0	100.0	1.7165
10–14	43.8	50.6[b]	5.6	0.0	0.0	0.0	100.0	1.6173
15–19	28.9	57.1[b]	12.8	1.1	0.0	0.0	100.0	1.8609
20–24	27.3	55.3[b]	15.8	1.1	0.2	0.2	100.0	1.9222[c]
25–29	29.2	51.7[b]	17.0	1.8	0.2	0.0	100.0	1.9209[c]
30–34	34.3	49.5[b]	14.7	1.1	0.3	0.0	100.0	1.8365
35–39	36.7	50.9[b]	11.2	1.0	0.1	0.1	100.0	1.7714
40–44	37.7	49.2[b]	11.8	1.0	0.3	0.1	100.0	1.7728
45–49	42.5	45.9[b]	10.2	1.4	0.1	0.0	100.0	1.7059
50–54	42.5	45.5[b]	10.5	1.4	0.1	0.0	100.0	1.7093
55–59	43.3[b]	43.1	11.4	1.9	0.3	0.0	100.0	1.7265
60–64	43.2[b]	41.7	12.8	2.1	0.2	0.0	100.0	1.7439
65–69	42.7[b]	40.9	13.6	2.5	0.2	0.0	100.0	1.7669
70–74	41.4[b]	41.1	14.5	2.7	0.3	0.0	100.0	1.7930
75–79	39.0	42.9[b]	15.4	2.4	0.3	0.0	100.0	1.8220
80–84	38.6	42.6[b]	15.9	2.5	0.3	0.0	100.0	1.8345
85–89	37.8	42.2[b]	17.4	2.3	0.2	0.0	100.0	1.8516
≥90	37.5	42.8[b]	17.6	1.9	0.2	0.0	100.0	1.8459
Total	39.8	42.9[b]	14.8	2.2	0.2	0.0	100.0	1.8023

[a]deaths at unknown age not included [b]for each age group, number of causes with greatest relative frequency
[c]age group with highest mean of number of causes of death

c. 2015[a]

Age group	Number of causes of death % of group						Total	Mean no. of causes
	1	2	3	4	5	≥6		
<1	45.4[b]	34.2	12.7	7.1	0.4	0.2	100.0	1.8336
1–4	55.4[b]	37.5	6.5	0.5	0.0	0.0	100.0	1.5217
5–9	40.9	53.6[b]	5.5	0.0	0.0	0.0	100.0	1.6455
10–14	35.8	61.3[b]	2.9	0.0	0.0	0.0	100.0	1.6715
15–19	30.1	57.3[b]	11.8	0.7	0.0	0.0	100.0	1.8315
20–24	29.8	52.1[b]	16.8	0.8	0.5	0.0	100.0	1.9016[c]
25–29	28.1	50.6[b]	19.6	1.4	0.3	0.0	100.0	1.9520[c]
30–34	34.0	48.1[b]	16.5	1.0	0.3	0.0	100.0	1.8545
35–39	36.4	48.1[b]	13.7	1.4	0.2	0.1	100.0	1.8126
40–44	39.9	44.7[b]	12.2	2.6	0.5	0.1	100.0	1.7920
45–49	40.8	45.2[b]	12.3	1.5	0.2	0.0	100.0	1.7531
50–54	42.4	44.3[b]	11.5	1.5	0.2	0.0	100.0	1.7287
55–59	43.2[b]	42.8	11.6	2.2	0.2	0.1	100.0	1.7378
60–64	42.5	42.6[b]	12.5	2.2	0.3	0.0	100.0	1.7520
65–69	41.5	42.3[b]	13.4	2.5	0.2	0.0	100.0	1.7771
70–74	40.0	42.6[b]	14.4	2.6	0.4	0.0	100.0	1.8087
75–79	38.8	43.3[b]	14.9	2.5	0.4	0.1	100.0	1.8253
80–84	36.2	45.1[b]	15.8	2.4	0.4	0.0	100.0	1.8583
85–89	36.0	44.5[b]	16.9	2.4	0.3	0.0	100.0	1.8650
≥90	34.4	45.1[b]	18.1	2.0	0.3	0.0	100.0	1.8888
Total	38.5	44.0[b]	14.9	2.3	0.3	0.0	100.0	1.8206

[a]deaths at unknown age not included
[b]for each age group, number of causes with greatest relative frequency
[c]age group with highest mean of number of causes of death

association with deaths overall. Results are displayed for the four causes listed most frequently as associated with DM when DM is recorded as the underlying cause of death: a) circulatory diseases; b) diseases of arteries, arterioles and capillaries; c) respiratory diseases; and d) glomerular and renal diseases.

DISCUSSION

The percentages for the association of each of these disease groups with DM as the underlying cause (second column) are potentially biased. According to these figures, it could be concluded that respiratory diseases, and glomerular and renal diseases, are the groups that most strongly correlate with deaths from DM (as the underlying cause), and that diseases of arteries, arterioles and capillaries have a low correlation. Under specific assumptions, these correlations could be interpreted as if the first two disease groups are those that best explain or predict mortality attributed to DM (as the underlying cause) and that DAAC is the group that explains or predicts the least.

This is an erroneous interpretation in the case of respiratory diseases, as it ignores the fact that this disease group appears very frequently as a contributing cause overall, independent of underlying cause of death. It is also erroneous in the case of DAAC, insofar as it ignores that these diseases rarely appear as a contributing cause of death independent of the underlying cause.

The last column of Table 4 is the one that brings us closest to understanding the "true" association between the four disease groups and DM as underlying cause of death. CDAI is close to

Table 3: Multicausal mortality from diabetes using the simple method employing a single underlying cause[a] and both (underlying and associated[b])[c]

DM underlying cause	DM associated cause		Total n (%)
	No n (%)	Yes n (%)	
No	95,037 (97.6)	2,295 (98.7)	97,332 (97.6)
Yes	2,322 (2.4)	30 (1.3)[d]	2,352 (2.4)
Total	97,359 (100.0)	2,325 (100.0)	99,684 (100.0)

[a]2352 deaths with diabetes as underlying cause
[b]includes intermediate and contributing
[c]2352 deaths with diabetes as underlying and 2295 as associated
[d]30 deaths citing diabetes (ICD-10: E10–E14)[6] as both underlying and contributing, counted among underlying causes to avoid duplication

Table 4: Selected diseases associated with death from DM as underlying cause, Cuba 2015

Selected diseases associated with death from diabetes mellitus as the underlying cause	Overall association[a] (%)	Association with DM[b] (%)	CDAI[c]
a) Circulatory diseases[d]	3.2	13.4	4.2
b) Diseases of arteries, arterioles and capillaries	0.2	9.9	49.5
c) Respiratory diseases[f]	22.5	22.3	1.0
d) Glomerular and renal diseases[g]	1.5	31.0	20.7

[a]prevalence of association with death by any of these causes (a, b, c or d, independent of underlying cause)
[b]prevalence of association with DM as underlying cause
[c]cause of death association indicator = (association with DM)/(overall association)
[d]circulatory diseases (ICD I05–I52), using only code I10X code (essential hypertension)[6]
[e]diseases of arteries, arterioles and capillaries (ICD i70–i79), using only code I79.2[6]
[f]respiratory diseases (ICD J09–J18), using only codes J15.9 and J18.2[6]
[g]glomerular and renal diseases (ICD N00–N19), using only codes N08.3 and N18.9[6]
DM: diabetes mellitus

50 for DAAC, which suggests that, on the rare occasions when DAAC is associated with an underlying cause of death, it is almost always DM. Stated another way, DAAC is associated with DM as underlying cause of death 50 times more often than it is associated with deaths overall.

Studies using MCD approaches to analyze mortality patterns are rare,[3,8,24,25] and even more so in Cuba.[17,18,26]

The numbers of causes reported in death certificates in Cuba for the years studied were significantly lower than reported in a 1998 Cuban study,[26] and there was a higher proportion of death certificates citing a single, underlying cause compared to results from developed countries.[27] Some authors hold that death certificates reporting multiple causes of death generate more useful information.[27] From that vantage point, our observation of a trend toward reporting more causes over the later of the three years studied is encouraging.

International authors have proposed that the number of causes of death reported in death certificates should be higher for older adults than for people dying at younger ages,[11,17,27] given the complexity of disease processes leading to death in older adults.[12] We did not observe this pattern in Cuba. On the contrary, in all three years analyzed, the mean number of causes reported for people aged 20–29 years was slightly higher than

for other age groups. To our knowledge, this pattern has not been reported.

It should be noted that traditional mortality analysis based on one underlying cause is in fact a weighted MCD approach, in that it assigns a value of 1 to the underlying cause and 0 to the remaining causes.

The simple MCD method is one of the most frequently used.[28] However, it is essentially univariate, because it counts a single cause regardless of where it is mentioned on the death certificate. A truly multivariate approach would look for patterns of association and clustering.

Proponents of the simple method would argue that it "retrieves" the 2295 deaths where DM is not listed as an underlying cause but is listed as a contributing cause. However, the total appropriation of these 2295 deaths ignores the fact that some or many of these would be double counted, since some or many of these would also be counted as associated with other causes. Thus this may lead to biased estimations of the relative impact of associated causes and hence does not permit a summary assessment of the relative impact of individual causes on all deaths.

CDAI has been used to explore the impact of cancer as the underlying cause.[9] A 2010 study used CDAI to identify subgroups of diseases according to their relative position in the causal chain. In particular, it stated the hypothesis that there are different morbidity processes at work when DM is the underlying cause and circulatory diseases are contributing causes, than when heart disease is the underlying cause and DM is a contributing cause.[8]

It should be noted that CDAI's utility is independent of the absolute frequency of a particular contributing cause among deaths from the underlying cause under study (DM, in this case). In other words, analysis could well have included more than the four groups of causes we chose.

As far as we know, no other study to date has used CDAI to reassess the roles of contributing causes when DM is the underlying cause.

This study did not aim to exhaust the topic but reviewed two multicausal methods that respond to different objectives. It also considered a limited number of causes associated with DM as an underlying cause. In our opinion, this is sufficient to demonstrate the importance of the CDAI method. However, a more thorough approach to DM would contextualize it within a broader group of causes associated with DM as an underlying cause and include analysis of other noncommunicable chronic diseases.

CONCLUSIONS

The multiple causes of death approach affords more nuanced understanding of patterns of disease, comorbidity and death in the Cuban population. The indicators used fulfill different roles: the simple method brings to light the full range of ways in which a given cause contributes to mortality, and the cause-of-death association indicator enables exploration of links between different causes of death, not possible with the simple method.

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Estimating Need, Demand and Supply in Primary Health Care Services: A Local Application in Argentina

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ABSTRACT

INTRODUCTION To provide equal access, health care provision should be distributed across geodemographic space based on need. In Argentina, the social security, publicly funded health care and private health care subsectors are responsible for delivering health services. In the public subsector, which is responsible for providing primary and secondary care mainly to the uninsured population, supply of services is not always associated with need. The lack of coordination between levels and subsectors makes it difficult to transform need into demand.

OBJECTIVE Design a methodology to systematically estimate need, demand and supply of primary health care services based on secondary data sources in order to assess potential mismatches in any geographical area.

METHODS An ecological analysis was conducted based on outpatient visits in primary care in Bahía Blanca, Buenos Aires Province, Argentina. A mathematical approach was proposed to systematize data collection by census tract regarding estimated need (number of outpatient visits needed, by specialty, according to age- and sex-specific care protocols and the area's demographics), demand (actual outpatient visits by specialty in each primary health care center), and supply (visit capacity or available appointment slots, taking into account number of personnel hours worked, by specialty).

RESULTS Demand for outpatient visits exceeded need (299,731) by 24% while available visit capacity (993,903) could have covered more than twice the number demanded (370,881). Analysis of the three variables grouped by area found that supply correlated more closely with demand ($\rho = 0.90$) than with need ($\rho = 0.68$), while spatial analysis showed that supply distribution responded to need. Areas with greater need had a health facility relatively close by, although supply was often located in areas of lower need, and some areas struggle with relatively high need and insufficient supply.

CONCLUSIONS Results suggest the need for some reconfiguration of primary health care in the study area. The proposed mechanism for estimating relationship among supply, demand and need is a useful tool to support decision-making.

KEYWORDS Health services needs and demand; access to health care; health care accessibility; health care quality, access, and evaluation; health care inequalities; primary health care; Argentina

INTRODUCTION

Primary health care resources should be distributed differentially to ensure equitable population access according to need. To do so requires taking into account many differences (demographic, epidemiological, geographic, sociocultural, economic and political) between groups[1] using information from population censuses, vital statistics, epidemiological studies, studies of individual perceptions, clinical measurements, as well as facility and specialty utilization rates and costs.[2]

Indicators of need must be considered separately from utilization/demand indicators, because not all needs are perceived, and when they are perceived, may not lead to attention at health services because of barriers to access (lack of money, distance, cultural differences between providers and consumers, etc.).[3] Thus, when evaluating access to health care, the dimension of need must be considered along with interactions between supply and demand. An integrated analysis can help identify whether supply adjusts to need and demand, whether need translates into demand, and whether supply falls short of demand.[4]

Methodologies available for these studies include those based on health economics, health planning, and operations research. The first type applies econometric models to study utilization based on observational data. The second applies needs indicators and assesses relative need per capita for services at various government levels by type of service. The third examines optimal use of resources by applying tools such as mathematical programming, simulation and systems dynamics, and, unlike economic or planning methodologies, involves decision-makers.[5]

IMPORTANCE The proposed methodology supports systematic estimation of a population's health service need, demand and supply in a relatively simple and low-cost way.

This last methodology's main strength is that it provides input for designing decision-making support tools based on simultaneous analysis of information about the three dimensions of interest (need, demand and supply), information which can be obtained free of cost from available secondary sources. This approach turns out to be highly useful for decision-makers in the health sector who want to optimize resource allocation.

For example, this approach has been used to determine optimal locations for community health care facilities.[6–8] It is especially important to assess where to locate primary health care (PHC) facilities, in Argentina called Primary Health Care Centers (CAPS).[9] CAPS provide less-complex services in public health systems intending to strengthen primary health care.[10] In Argentina, the health system's subsectors (public, private, and social security) are poorly integrated, leading to many situations of unequal access.[11] Some 38% of the population is uninsured and this group mostly uses the free services provided by the public subsector.[12]

Selection of CAPS locations responds to multiple factors (historic, cultural, economic, political) that can skew supply away from need. This mismatch can be observed in Buenos Aires Province where PHC provision has been delegated to local governments,

which has led to extremely unequal access to health services among regions; access ends up responding to differences in economic standard of living rather than differences in need.[13] Nevertheless, available evidence does not suggest such inequitable access within each locality.

Most studies on access to PHC services examine supply and demand through the ratio of providers to population, which can help identify resources available in a given area but does not reveal variation within it.[14–16]

Methodologies need to be designed that can answer these two questions: Is supply in each locality geographically distributed in such a way as to meet demand? To what extent does demand in each area reflect real need? Such analysis calls for information that is difficult to collect, especially in poorly coordinated decentralized health systems or those with substandard information systems. A mechanism needs to be designed to systematically estimate these figures in order to implement decision-making support tools.

Health systems that lack information systems need a protocol for deriving indicators of supply, demand and need that would enable progress toward implementation of systematic decision-making processes based on knowledge of these variables.

In the Bahía Blanca *Partido* (a subdivision of Buenos Aires Province), public health system managers have expressed concerns about matching PHC supply to need.[17] The Bahía Blanca case study is interesting for several reasons:

1. While each CAPS can treat on average about 5500 residents (1500 uninsured)—in line with widely accepted potential access conditions in relation to the estimated national average (1 CAPS for every 3184 uninsured individuals)[18]—it remains unclear whether geographic service distribution responds to regional variation in need.
2. In many cases, CAPS operate out of neighborhood development associations (nonprofit organizations that carry out activities to provide community services in the neighborhood and its surroundings). These associations opened their premises to house the CAPS and are opposed to any relocation.
3. The partido's complex situation—with a large population widely dispersed over extensive territory, and many differences evident among census tracts (socioeconomic conditions, demographic features, health and epidemiological risks, etc.)—calls for a mathematical approach.

The purpose of this study was to design a methodology based on secondary data sources to help systematically estimate supply, demand and need in PHC services in order to assess potential mismatches within geodemographic areas. Specific objectives include: design a method to estimate need for PHC outpatient visits based on specific population features; determine demand for PHC visits; quantify supply based on available personnel; and assess the relationship between supply, demand and need for PHC visits in Bahía Blanca.

METHODS

An ecological analysis was conducted to examine supply, need and demand for outpatient visits in seven basic PHC services (nursing, general family medicine, pediatrics, gynecology, obstetrics, mental health and dentistry) in Bahía Blanca Partido in 2015.

Study area Bahía Blanca Partido in Buenos Aires Province, Argentina includes three urban areas, Cabildo, General Daniel Cerri and the city of Bahía Blanca (in the remainder of the text, if Partido is not specified, Bahía Blanca refers to the city). According to Argentina's National Statistics and Census Institute, Bahía Blanca Partido's 2010 population was 301,572, 27% without health insurance.[19]

Bahía Blanca Partido's health system has facilities in the public health subsector at three government levels: municipal, provincial and national. The provincial level is responsible for the Dr José Penna Interzonal General Acute Care Hospital, and the municipal level is responsible for Dr Leónidas Lucero Municipal Acute Care Hospital and 56 CAPS. The CAPS treat less complex health problems and carry out health promotion; they treat mostly uninsured low-income individuals. Of the 56 CAPS, 5 are health centers (HC), which offer a wide range of specialties, and 51 are health units (HU) with reduced services and operating hours. The CAPS are grouped in 11 Program Areas (AP) (based on geographic, demographic, health and technical or administrative factors), each run by a management team.

Figure 1 shows the geographic distribution of CAPS in the study area, and the APs to which they belong.

Case study conception, design and application *Conception* The analysis unit for need was the census tract, a geographically defined space that includes a given number of households. Census tracts can be urban, rural or mixed, depending on population dispersion.[20] Estimates of annual outpatient visits needed per specialty in each census tract were based on size of population requiring checkups and annual frequency recommended for the population group in accordance with clinical practice guidelines and medical protocols established in Argentina.[21–30] Given that these recommendations represent the healthy population's need for routine care, the estimate was adjusted for conditions in the home in order to capture the potentially greater need associated with social determinants of health.

The CAPS was the analysis unit for quantifying supply and demand. Supply was estimated by measuring the number of visits potentially available per specialty based on annual hours worked by each professional and number of visits per hour that can be accommodated by specialty. Demand was determined by the actual number of visits by specialty in each CAPS.

Although mismatches among need, supply and demand do not necessarily occur in geographic zones and may be due to other factors, this approach (which focuses on geographic accessibility) was used to diagnose the need to relocate CAPS. Data were presented according to AP, since each CAPS belongs to an AP, which comprises a set of census tracts. To assess the relationship among dimensions—supply, demand, and need—the Pearson correlation coefficient, ρ, was calculated for all visits by specialty for demand and supply (by CAPS and AP) and for supply and need (by AP). Correlation thresholds were $\rho = 0.9$ for very strong, $\rho = 0.75$ for strong, $\rho = 0.5$ for moderate, $\rho = 0.25$ for weak, and $\rho = 0.1$ for very weak.[31] SPSS 15 software was used. Analytical distribution maps were compiled by geographical zone (for need)

and facility (for supply and demand); form, color and size were used as visual representation. The shade of each census tract represented level of need for visits. We used SIG software[32] for classification using the natural breaks method,[33] which identifies maximum homogenization within each classification and maximum difference among classifications, so limits can be established when a relatively significant jump occurs between values.[34,35] CAPS were represented by concentric black and grey circles proportionate to size of the facility's supply of and demand for visits, respectively.

Design Need. Annual need per census tract for each specialty was estimated based on size and requirements of different population groups (PG) according to age and sex. To capture health needs, the following PGs were defined: aged<1 year, aged 1 year, aged 2 years, aged 3–4 years, aged 5–9 years, girls aged 10–14 years, boys aged 10–14 years, women aged 15–19 years, men aged 15–19 years; women aged 20–39 years, men aged 20–39 years, women aged 40–64 years, men aged 40–64 years, women aged ≥65 years, and men aged ≥65 years. Estimates of need were made with the following equation, in which each census tract's needs depend on number of individuals in the different population groups ($P_{p,r}$) and a set of variables and parameters:

$$N_{r,j} = \sum_p \{(pn_{p,j} * fn_{p,j}) * SC_p * (1 + DS_r) * P_{p,r}\} (1)$$

$N_{r,j}$ = specialty *j* visits needed in one year in census tract *r*

$P_{p,r}$ = number of individuals belonging to $\mathrm{PG}p$ in census tract *r*

SC_p = uninsured proportion of $\mathrm{PG}p$

DS_r = proportion of population exposed to adverse living conditions in census tract

$pn_{p,j}$ = proportion of $\mathrm{PG}p$ needing specialty *j*

$fn_{p,j}$ = annual frequency of visits in specialty *j* needed by $\mathrm{PG}p$

SC_p represents the proportion of uninsured individuals in a PG and is significant because this is generally the only population that demands PHC services in the publicly funded health care subsector. The parameters $pn_{p,j}$ and $fn_{p,j}$ represent proportions of the population that would use each specialty according to age and sex, respectively, and number of annual checkups recommended for each PG by specialty. These factors are fundamental inputs to obtain the number of visits needed based on each census tract's demographic data. Their values are established according to evidence-based medical guidelines for routine care in healthy populations.[21–30] To replicate the study in another area, parameters should be adapted to outpatient visits needed by specialty according to health authorities' criteria in each jurisdiction, determined by adapting sound international scientific evidence to the local context to derive minimum requirements.

Finally, assuming that adverse socioeconomic conditions can swell the need for health services beyond scientifically based recommended levels for healthy populations, the number of outpatient visits needed in each census tract was affected by parameter DS_r, which measures general environmental quality of life. Each census tract's housing shortage was estimated by the proportion of critically overcrowded households (indicated by the variable *overcrowding* recorded in the national census: households with >3 individuals per room). Household overcrowding was included as a surrogate for the social determinants of health.[36]

Demand. Annual demand by specialty ($D_{c,j}$) was obtained for each CAPS from number of visits registered by the health authority ($R_{c,j}$). Since these figures can vary, the following calculation is proposed:

$$D_{c,j} = \epsilon_{c,j} * R_{c,j} (2)$$

in which:

$D_{c,j}$ = specialty *j* visits demanded in one year in CAPS c

$R_{c,j}$ = specialty *j* visits registered in one year in CAPS c

$\epsilon_{c,j}$ = correction factor for specialty *j* demand in CAPS c

$0 \leq \epsilon_{c,j} \leq 1$

With the addition of factor $\epsilon_{c,j}$, the formula is adjusted for potential over-registration, capturing situations in which the target population's demand for outpatient visits does not match the number of visits registered by the health authority. Such differences can occur when individuals with health coverage or residents of other municipalities use PHC services in the study area. This factor can vary from one CAPS to another. For example, facilities with greater response capacity and facilities located near the partido's borders are more likely to attract individuals who are insured or who live in neighboring areas. This factor could also vary by service. It is difficult to obtain in poorly computerized systems and can be estimated based on surveys of CAPS personnel and client population or obtained from health authority registries.

Supply. Annual supply in each CAPS by specialty ($O_{c,j}$) was calculated based on time available per year and each specialist's productivity:

$$O_{c,j} = \beta_j * (Hs_{c,j} * Sa) (3)$$

in which:

$O_{c,j}$ = specialty *j* visits provided in one year in CAPS *c*

β_j = specialty *j* visits provided per week

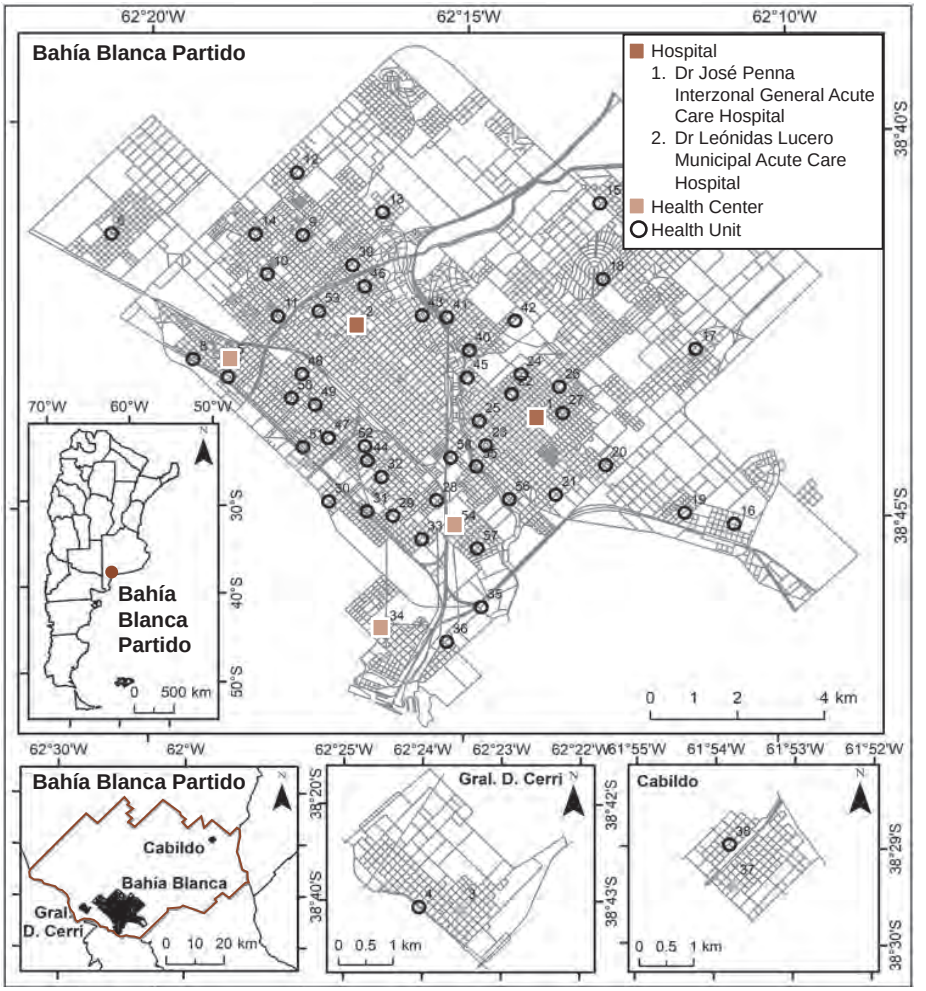
$Hs_{c,j}$ = specialty *j* hours available per week in CAPS *c*

Sa = number of weeks per year

To determine the supply (visit capacity), the annual number of available hours was multiplied by a parameter measuring the specialty's productivity (β_j) approximated by maximum possible number of visits per hour. According to a literature review, the average time for a clinical outpatient visit is 10–15 minutes,[37] which would give β_j values of 4–6. This figure may differ from one CAPS to another.

Hours per week by specialty in each CAPS ($Hs_{c,j}$) were obtained by multiplying total number of health personnel (nurses, physicians,

Figure 1:Study area and PHC facility locations, Bahía Blanca Partido, Argentina (2015)



Program Area 1	Program Area 5	Program Area 9
3 A. Menghini Health Center	22 Anchorena Health Unit	39 San Cayetano Health Unit
4 Cem Health Unit	23 Barrio Obrero Health Unit	40 Bella Vista Health Unit
Program Area 2	24 Sanchez Elias Health Unit	41 La Falda Health Unit
5 L. Piñeiro Health Center	25 Villa Mitre Health Unit	42 Miramar Health Unit
6 Villa Bordeau Health Unit	26 Villa Amaducci Health Unit	43 Naposta Health Unit
7 Villa Nocito Health Unit (Ext)	27 Don Bosco Health Unit	44 Pedro Pico Health Unit
8 Maldonado Health Unit (Ext)	**Program Area 6**	45 Tiro Federal Health Unit
Program Area 3	28 Centenario Health Unit	46 Universitario Health Unit
9 Avellaneda Health Unit	29 Enrique Julio Health Unit	**Program Area 10**
10 Estomba Health Unit	30 L. Paraguaya Health Unit	47 Colon Health Unit
11 Lujan Health Unit	31 Villa Delfina Health Unit	48 Mariano Moreno Health Unit
12 N. Belgrano Health Unit	32 Villa Ressia Health Unit	49 Noroeste Health Unit
13 Villa Floresta Health Unit	33 Villa Rosas Health Unit	50 Pampa Central Health Unit
14 Barrio Latino Health Unit	**Program Area 7**	51 San Dionisio Health Unit (Ext)
Program Area 4	34 Menor I. White Hospital	52 San Martin Health Unit
15 Aldea Romana Health Unit	35 Saladero Health Unit	53 Kilometer 5 Health Unit
16 Grünbein Health Unit	36 San José Obrero Health Unit	**Program Area 11**
17 Villa H. Green Health Unit	**Program Area 8**	54 Spurr Community Integration Center
18 Patagonia Health Unit	37 Cabildo Health Center	55 Rosario Sur Health Unit
19 Villa Gloria Health Unit	38 S. Claire Health Unit (Ext.)	56 Villa Esperanza Health Unit
20 12 de Octubre Health Unit		57 Villa Serra Health Unit
21 Villa Muñiz Health Unit		58 Rivadavia Health Unit

Ext: extension of a larger health center or unit
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technicians, etc.) assigned to CAPS *c* ($RH_{c,j}$) by hours contracted per week (C_j):

$$Hs_{c,j} = \alpha_{c,j} * C_j * RH_{c,j} (4)$$

$RH_{c,j}$ = specialty *j* professionals in CAPS *c*

C_j = weekly hours contracted by specialty *j* professionals

$\alpha_{c,j}$ = correction factor for specialty *j* supply

$0 \leq \alpha_{c,j} \leq 1$

The value C_j assumes a homogeneous format for contracts in which all professionals in a certain specialty are contracted for the same number of hours per week in the CAPS where they work. A more general model could consider the weekly number of hours contracted, which differs from one CAPS to another or even among individual professionals.

Parameter $\alpha_{c,j}$ was also incorporated as a correction factor for outpatient visit capacity per year to consider the amount of time specialists allot to work activities not directly involved in patient care (teaching, research and/or administration) and to more precisely estimate the amount of time effectively available for patient visits. The parameter may vary according to specialty and to CAPS. Its estimated values should be determined based on interviews with health personnel.

Application in Bahía Blanca Partido Need estimates were made based on population data from the 369 census tracts of the 2010 national census (excluding nine rural census tracts with a total population of 7340).[19] Populations per PG in 2015 were estimated based on 2010 population per census tract adjusted by the average municipal annual population growth rate according to projections for 2015[38] and proportions represented by each PG in 2010. Health coverage for each PG was estimated from municipal and provincial data.[39,40] With respect to nursing services, the maternal and child population was not affected by the correction factor, since it is mandatory for all pregnant women, as well as new mothers and children, to receive services at a CAPS facility for vaccinations (in accordance with the country's official immunization schedule). Table 1 presents proportions of each PG in total population and percentages of uninsured.

Supply was estimated based on number of professionals assigned to each CAPS, ac-

Table 1: Health coverage by population group, Bahía Blanca Partido, 2010

Age (years)	Sex	Proportion of total municipal population	Percentage uninsured or using public health services (%)
<1	both	0.01	33
1	both	0.01	33
2	both	0.01	33
3–4	both	0.02	33
5–9	both	0.07	31
10–14	M	0.03	32
	F	0.03	33
15–19	M	0.04	35
	F	0.03	34
20–39	M	0.15	30
	F	0.15	31
40–64	M	0.12	27
	F	0.14	23
≥65	M	0.05	5
	F	0.07	3

Source: 2010 National Population and Household Census, Argentina

cording to whether they have 24- or 40-hourweek contracts with an average visit session lasting 16.7 to 18.5 minutes,[41] an adjusted figure based on expert opinions in the specialty. Finally, due to lack of information on supply and demand correction factors, these were considered to be equal to 1. Data were provided by the Health Secretariat of Bahía Blanca Partido.

Need for outpatient visits by specialty was estimated based on the values of $pn_{g,j}$ and $fn_{g,j}$ as determined from a literature review (clinical practice guidelines, population studies, utilization rates, etc.). In Table 2, each specialty has two columns: indication for visit and frequency of visit.

All children aged <1 year, for example, should be brought to CAPS for 12 visits with a nurse and 10 pediatric checkups annually. This group does not require treatment from other specialties at PHC levels.

Nursing services included vaccinations according to Argentina's national schedule.[21] For children and adolescents, information from pediatric and dental services was used as recommended by the Argentinean Pediatrics Society;[22] and for adults, from the regularly scheduled routine medical care of healthy adults.[23,24] For mental health, prevalence of psychiatric disorders was used, as well as proportion of the national population aged ≥15 years affected by mental and behavioral disorders.[25,26] For obstetrics, we assumed the early adolescent (ages 10–14 years) and late adolescent (ages 15–19 years) pregnancy rates for Buenos Aires Province and the mean age-specific fertility rates for women aged 20–39 years; and estimated needed visit frequency according to health authorities' recommendations for Buenos Aires Province and Argentina regarding antenatal care in low-risk pregnancies.[27–30]

RESULTS

According to 2010 projections, an estimated 299,731 outpatient visits with all PHC specialists treating Bahía Blanca's uninsured population were needed in 2015 (with the exception of nursing services, which provide treatment for the entire maternal and child population). The population in each census tract needed an average of 812 visits. The census tract with the least need registered 81 visits and the one with the most 3095.

Distribution of need for outpatient visits by specialty included: nursing services (41%), dentistry (25%), pediatrics (17%), gynecology (35%), mental health (4.6%), general family medicine (3%) and obstetrics (2%). However, need for services varied by neighborhood and locality.

Table 2: PHC outpatient visits by specialty, Bahía Blanca Partido, Argentina (2015)

Population group		Nursing*		General family medicine		Gynecology		Obstetrics		Pediatrics		Mental health		Dentistry	
		Ind	Freq	Ind	Freq	Ind	Freq	Ind	Freq	Ind	Freq	Ind	Freq	Ind	Freq
Age (years)	Sex														
<1	both	1.00	12	—	—	—	—	1.00	—	1.00	10	1.00	—	1.00	—
1	both	1.00	7	—	—	—	—	1.00	—	1.00	4	1.00	—	1.00	—
2	both	0.00	—	—	—	—	—	0.00	—	1.00	2	0.00	—	1.00	1
3–4	both	0.00	—	—	—	—	—	0.00	—	1.00	1	0.00	—	1.00	1
5–9	both	0.20	3	—	—	—	—	0.00	—	1.00	1	0	—	1.00	2
10–14	M	0.19	1	—	—	—	—	—	—	1.00	1	0.2	1	1.00	1
	F	0.19	2	—	—	0.00	—	0.00	5	1.00	1	0.2	1	1.00	1
15–19	M	0.00	—	—	—	—	—	—	—	1.00	2	0.2	1	1.00	1
	F	0.06	3	—	—	1.00	0.5	0.06	5	1.00	2	0.2	1	1.00	1
20–39	M	0.00	—	1.00	0.2	—	—	—	—	—	—	0.2	1	1.00	1
	F	0.10	3	1.00	0.2	1.00	0.5	0.1	5	—	—	0.2	1	1.00	1
40–64	M	1.00	0.1	1.00	0.2	—	—	—	—	—	—	0.2	1	1.00	1
	F	1.00	0.1*	1.00	0.2	1.00	0.5	0.00	—	—	—	0.2	1	1.00	1
≥65	M	1.00	1.1	1.00	1	—	—	—	—	—	—	0.2	1	1.00	1
	F	1.00	1.1	1.00	0.5	0.00	—	0.00	—	—	—	0.2	1	1.00	1

*vaccination according to national schedule Freq: frequency (number of visits recommended annually)
Ind: indication (proportion of population in need of service; 0 = no one 1 = everyone) PHC: primary health care

Demand was quantified at 418,214 outpatient visits. There were 370,881 visits with specialists (89% of total). Differences among CAPS were significant: CAPS that provide specialties handled 36% of visits and 4 of the 5 HCs accounted for 20% of total visits. Average number of visits per CAPS was 4302 in nursing, 1111 in general family medicine, 518 in pediatrics, 251 dental appointments, 151 in mental health services, 147 in gynecology, and 116 in obstetrics (values varied among CAPS).

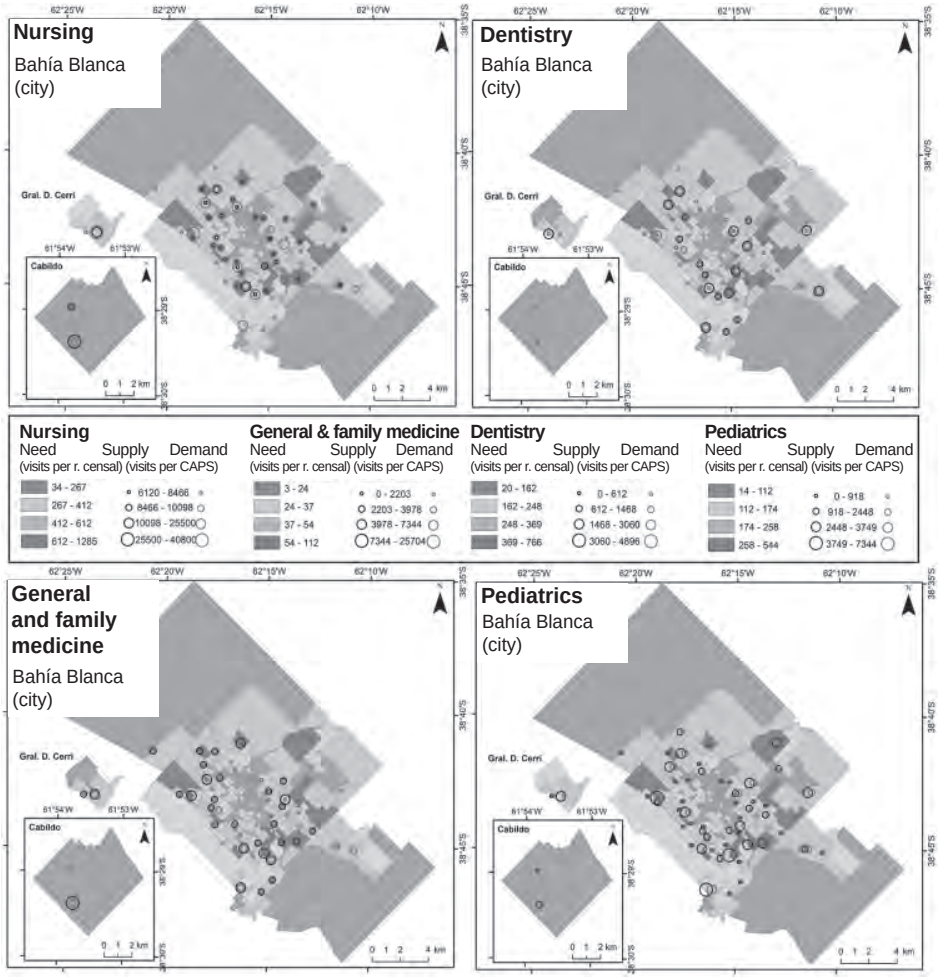
Regarding supply, available municipal PHC person-hours in 2015 were sufficient for a total of 993,903 outpatient visits. CAPS provided an average of 10,162

appointments in nursing, 3287 in general family medicine, 1508 in pediatrics, 819 in gynecology, 761 dental appointments, 624 in obstetrics and 558 in mental health services. Important differences were observed among CAPS in outpatient visits by specialty: an average of 3638 pediatric visits in each CAPS providing all specialties (4 HCs and 7 HUs), but only 1110 in CAPS with pediatric services but not all the other specialties (1 HC and 26 HUs).

Based on this protocol, it was estimated that in 2015 Bahía Blanca saw demand exceed need (299,731 visits) by about 24%, while theoretical supply (993,903 visits) was more than twice the number of visits demanded (370,881).

Distribution of need, supply and demand was mapped spatially for each service, For reasons of space, results are presented for only four services (nursing, dentistry, general family medicine, and pediatrics), selected because they revealed different types of results: nursing services accounted for greatest need (41%), supply (57%) and demand (65%); general family practice services accounted for the next greatest supply (19%) and demand (17%) but not need (4%); dentistry presented greater need among services (25%) with insufficient supply to cover it, and pediatrics presented somewhat high need (17%) totally covered by supply.

Figure 2: PHC outpatient visit need, demand, and supply by service, Bahía Blanca Partido, Argentina (2015)



CAPS: primary health care centre PHC: primary health care
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Figure 2 shows that spatial distribution of need is similar among services. The census tracts needing more annual visits coincide for all services and are generally located on the periphery of Bahía Blanca. Supply dispersion increases the further a CAPS is located from the center of the city. The CAPS with greatest supply, however, are not necessarily located in the census tracts with the greatest need.

Most census tracts with greater need had a CAPS nearby, and all five HCs were located in the areas with greatest need. On the other hand, for all services, there were CAPS in low-need census tracts. For all services there were no CAPS nearby in census tracts with mid-level needs.

Table 3 presents distribution of supply, demand, and need for outpatient visits by AP for each specialty.

Nursing services were the most readily available and provided 57.3% (569,058) of total visit supply (993,903), followed by general family practice with 18.5% (184,059) and pediatrics with 8.5% (84,441). Over half of total PHC supply was concentrated in five APs: AP #4 with population of 108,304 (10.9%), AP #9 with 107,222 (10.8%), AP#5 with 102,485 (10.3%), AP #2 with 100,929 (10.2%), and AP#10 with 98,430 (9.9%). Each of these APs contained at least one CAPS that provided all specialty services.

With respect to distribution of demand by AP and specialty, it was observed that nursing, general family medicine, and pediatric services accounted for 90% of visits and 65% (240,890), 16.8% (62,189) and 7.8% (29,038) of demand (370,881), respectively. Four APs (AP #9 with population of 56,994 or 15.4%, AP #3 with 50,035 or 13.5%, AP #4 with 42,885 or 11.6%, and AP #10 with 40,500 or 10.9%) accounted for over half of total visits. The CAPS of Cerri (AP #1); White (AP #7) and Cabildo (AP #8) accounted for smaller percentages of visits in all specialties (5.8%, 4.9%, and 2.8%, respectively).

The correlation assessment indicated a positive and strong relationship between supply and demand when analyzed by CAPS as well as by AP, except for general family medicine. In both CAPS and AP analyses, the highest correlation coefficients corresponded to pediatrics (0.929 and 0.951, respectively).

Distribution by AP of outpatient visits needed indicated that those with greater need also presented greater demand. These were: AP #9 with a need for 82,371 visits (27.5%), AP #10 with 42,024 (14%), AP #3 with 38,907 (13%) and AP #4 with 34,805 (11.6%), which together covered 66% of total need (299,727) and 52% of demand (370,881). A strong positive correlation was found be-

Table 3: PHC outpatient visit supply, demand and need by specialty and AP, Bahía Blanca Partido, 2015

Program area	Nurse			General family medicine			Gynecology			Obstetrics			Total		
	Supply	Demand	Need	Supply	Demand	Need	Supply	Demand	Need	Supply	Demand	Need	Supply	Demand	Need
β	4	—	—	3	—	—	3	—	—	3	—	—	—	—	—
1	32,640	17,025	2,795	8,262	2,933	250	3,672	287	331[a]	1,836	0	200[a]	54,366	21,685	6,760
2	65,280	24,653	5,969	12,240	4,309	529	4,590	945	709	4,820	1,560	428	100,929	36,083	14,414
3	46,920	30,449	16,083	20,426	9,313	1,439	6,197	2,773	1,907	0	10	1,151[a]	91,877	50,035	38,907
4	57,630	24,244	14,396	17,626	7,555	1,284	4,590	1,219	1,708[a]	4,590	1,159	1,031	108,304	42,885	34,805
5	66,300	24,067	12,873	17,060	4,395	1,153	3,672	752	1,526[a]	4,590	817	921[a]	102,485	33,407	31,147
6	57,120	20,107	8,937	15,422	5,674	799	3,672	892	1,060[a]	4,590	531	640[a]	94,513	29,273	21,614
7	32,640	8,138	4,303	12,852	3,548	385	3,672	112	510[a]	3,672	668	308	68,748	18,157	10,408
8	43,248	4,816	831	26,622	4,186	75	918	401	98	0	0	59	74,154	10,532	2,012
9	66,504	45,455	34,032	11,016	3,125	3,053	7,344	1,542	4,032[a]	4,590	506	2,433[a]	107,222	56,994	82,371[a]
10	57,120	26,120	17,372	21,114	10,155	1,554	2,754	552	2,059[a]	3,672	65	1,243[a]	98,430	40,500	42,024[a]
11	43,656	15,816	6,319	21,420	6,996	562	4,774	271	750[a]	3,534	1,206	453	92,876	31,330	15,268
Total	569,058	240,890	123,910	184,059	62,189	11,081	45,854	9,746	14,690[a]	35,894	6,522	8,866[a]	993,903	370,881	299,727

Program area	Pediatrics			Mental health			Dentistry			Total		
	Supply	Demand	Need	Supply	Demand	Need	Supply	Demand	Need	Supply	Demand	Need
β	2	—	—	1	—	—	1	—	—	—	—	—
1	3,672	846	1,179[a]	1,224	0	314[a]	3,060[b]	594	1,691[a]	54,366	21,685	6,760
2	8,109	2,332	2,522[a]	3,341	702	666	2,550[b]	1,582	3,592[a]	100,929	36,083	14,414
3	10,175	4,217	6,784[a]	2,550	1,084	1,806[a]	5,610[b]	2,189	9,738[a]	91,877	50,035	38,907
4	14,688	5,704	6,076[a]	4,284	1,645	1,613	4,896[b]	1,359	8,698[a]	108,304	42,885	34,805
5	6,885	1,636	5,428[a]	1,938	898	1,447[a]	2,040[b]	842	7,800[a]	102,485	33,407	31,147
6	7,344	1,578	3,770[a]	1,469	134	1,003[a]	4,896[b]	357	5,407[a]	94,513	29,273	21,614
7	7,344	2,968	1,815	3,672	1,191	483	4,896[b]	1,532	2,604[a]	68,748	18,157	10,408
8	1,836	581	350	1,530	548	93	0[b]	0	504[a]	74,154	10,532	2,012
9	9,180	3,850	14,345[a]	3,692	1,158	3,829[a]	4,896[b]	1,358	20,646[a]	107,222	56,994	82,371[a]
10	4,590	1,348	7,327[a]	4,284	410	1,950[a]	4,896[b]	1,850	10,517[a]	98,430	40,500	42,024[a]
11	10,618	3,978	2,669	3,978	697	706[a]	4,896[b]	2,366	3,810[a]	92,876	31,330	15,268
Total	84,441	29,038	52,266[a]	31,962	8,467	13,909[a]	42,636[b]	14,029	75,006[a]	993,903	370,881	299,727

[a]need > demand [b]supply < demand < need AP: Program Area β: number of visits per hour
Source: Health Secretariat, Bahía Blanca Partido

tween demand and need by AP (ρ = 0.90), while the correlation between need and supply was still positive but weaker (ρ = 0.68) and varied according to specialty: considerable for nursing services (ρ = 0.754) and weaker for general family medicine (ρ = 0.252).

Comparison of 2015 visit numbers showed that, except for nursing and general family medicine services, some APs presented greater need than demand. This was observed in all APs for dentistry, the only service in which supply did not match total coverage needed overall, nor by AP (except for AP #7). For total services, AP #9 and AP #10 were the only ones where need for visits exceeded demand. In AP #10, supply exceeded need, which was not true for AP #9.

DISCUSSION

Various methodologies have been used to examine the relationship between supply, need and demand in health services. A study in Canada assessed whether distribution of PHC nurses and physicians matched population needs (measured by standardized mortality rate).[42] A study in Mexico determined that the need for PHC physicians and nurses per inhabitant (estimated based on expert opinions and utilization data) was greater than supply.[43] Murphy estimated drug shortages using a needs-based simulation model, keeping in mind age, sex, health status and service-use rates.[44] Teerawattananon looked at burden of disease associated with reproductive health as a measure of need in order to design a basic package as part of a universal coverage plan.[45] De Graaf-Ruizendaal estimated need for outpatient visits based on census and service utilization data.[46] Barber Pérez assessed need for medical specialists by region, applying international standards for ratios of human resources to population.[47]

A study in Argentina analyzed each CAPS' geographic accessibility using effective demand (measured by prescriptions filled) and potential demand (estimated by population density of each census tract in the CAPS catchment area), revealing that some areas had unmet need.[48]

Unlike these studies, we proposed to differentiate need from demand by applying mathematical models to enable estimates based on secondary data sources. Mathematical modeling for optimal positioning of health services has antecedents in the literature. In Cuba, a study of physician demand and distribution used an information tool based on the center of gravity method[49] (which minimizes travel distance) to characterize physician demand by assigned population and evaluate allocation improvements. Griffin implemented an optimal allocation model based on health conditions and utilization rates[50] to estimate visits needed in services provided by HCs. But these studies do not consider differences between need and demand,[42,51] or if they do use some concept of need, it is with broad strokes based on population statistics and some characteristics that modify need.[52]

A study in Argentina to quantify the difference between demand and need examined public provision of medications for uninsured diabetic patients. The results indicated that total public supply covers only about one quarter (25%) of treatment need.[53]

The main innovation of our contribution is including medical recommendations in estimates of need for outpatient visits and not utilization rates, in recognition that utilization indicators do not take into account potential obstacles to access. The method can potentially be extended to all specialties and/or practices where it is possible to identify recommendations by PG. The proposed methodology is also easy to implement via spreadsheets and can be replicated in other regions by updating the data sources.

However, several constraints can be identified:

1. While in the effort to prevent information bias it would be appropriate to use datasets recorded in the same year, this was not possible due to lack of annual population statistics and unavailability of information on supply and demand in 2010. The decision was thus made to use population projections for 2010 to 2015 (the year of the study), information which is available at the national level.
2. Need may be underestimated since only scheduled checkups are considered, while a significant proportion of outpatient visits in CAPS are spontaneous visits associated with common health problems requiring quick solutions. The difference between need and demand can be expressed by including a correction factor for demand based on estimates of the proportion of this type of outpatient visit.
3. Estimates of need are based on PHC clinical practice guidelines for checkups for the healthy population in the study area. Although it was proposed that visit indication and frequency for each specialty follow recommendations based on independently developed evidence, it is recognized that their applicability to other geodemographic contexts would require adapting care protocols to local features, such as, for example, the local epidemiological profile.
4. Although CAPS are expected to serve as the entry gateway to the health system, a proportion of the population seeks out a hospital for a problem that could be appropriately resolved in a PHC facility. This factor could be captured by including as units of analysis hospital ambulatory care clinics that provide these specialty services.
5. Estimates of service supply should take into account the amount of time professionals are available to see patients and the amount of time dedicated to additional activities (such as administration) in the CAPS. This factor could explain the wide mismatch observed between supply and demand.
6. Application of supply and demand correction factors that have theoretically been determined would require fieldwork that exceeds the scope of the present study. For this reason, these factors were applied with a value of 1. However, they could be estimated based on secondary data sources or information from health authorities. This article is the partial result of a larger study to develop a mathematical model for redesigning PHC.

CONCLUSIONS

The proposed methodology enables relatively simple, systematic, low-cost estimation of a population's health care needs, supply and demand. Such information is essential for making decisions about PHC design in the community and is often difficult to obtain directly in large or poorly computerized systems.

In the case of Bahía Blanca, where some areas were observed to have relatively high need yet no CAPS nearby, the findings indicated the appropriateness of redesigning supply of several PHC services to more closely match distribution to estimated need.

The tool can be replicated in any geodemographic area and applied over time as the variables that determine need, supply and demand change. It has the capacity to predict future discrepancies and can therefore be widely used in PHC services planning. A future line of investigation is to modify the methodology to calculate precise values for the proposed correction factors.

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Researchers' Perceived Challenges in Studying Chronic Kidney Disease of Nontraditional Etiology in Mesoamerica

Emily M. Wright

ABSTRACT

INTRODUCTION Despite growing research interest in the epidemic of chronic kidney disease of nontraditional etiology (a distinct form of chronic kidney disease disproportionately affecting agricultural populations across Mesoamerica—Central America and southern Mexico), its etiology remains poorly understood.

OBJECTIVE Elucidate factors that impact researchers' efforts to understand the epidemic of chronic kidney disease of nontraditional etiology.

METHODS Semistructured interviews were conducted with 39 international researchers, selected based on their publications and participation in conferences about chronic kidney disease of nontraditional etiology. Interviews were conducted from May through September of 2015 in English or Spanish by video conference, telephone or in person. Interviews were audio recorded, transcribed, and analyzed iteratively using content analysis.

RESULTS Of 39 researchers interviewed, 30.8% were women, 84.6% had a medical and/or doctoral degree and 74.3% had ≥6 years' experience carrying out research on chronic kidney disease of nontraditional etiology. Three major themes were identified related to factors affecting research progress. The first, influence of state and private interests, concerned perceptions that sugar industry and some governments in Mesoamerica dismissed, hindered, intimidated and inaccurately represented research on chronic kidney disease of nontraditional etiology. The second, limited material and human resources, had to do with scarcity of stable, impartial funding and adequate in-country research infrastructure. Researchers were largely funded by nontraditional sources (charitable organizations, private donations, sugar industry in Mesoamerica, personal funds) or not funded at all. The third, logistical challenges across study lifetimes, referred to barriers such as unwieldy approval mechanisms, gang interference and publication hurdles.

CONCLUSIONS Producing high quality and clinically relevant studies to address chronic kidney disease of nontraditional etiology in the resource-scarce Mesoamerican research climate has been fraught with challenges. These findings contextualize the progress that has been made in understanding chronic kidney disease of nontraditional etiology to date and highlight the need for public health and biomedical organizations to support researchers' ongoing efforts to engage all stakeholders in addressing the epidemic, disseminate their research findings and identify feasible strategies for addressing the community-wide suffering caused by chronic kidney disease of nontraditional etiology.

KEYWORDS Chronic kidney disease, chronic renal insufficiency, chronic renal failure, chronic kidney failure, interstitial nephritis, qualitative research, epidemiology, occupational health, Costa Rica, El Salvador, Guatemala, Mexico, Mesoamerica, Nicaragua

INTRODUCTION

Chronic kidney disease of nontraditional etiology (CKDnt), also called chronic interstitial nephritis of agricultural communities and Mesoamerican nephropathy, among other names, is a distinct form of chronic kidney disease that disproportionately affects young male agricultural laborers in communities along the Pacific coast of Central America.[1,2] Although historical data are lacking, this epidemic is estimated to have caused at least 20,000 deaths in this region in the last 2 decades.[1,3,4] Furthermore, other countries, notably Sri Lanka and India, are experiencing what many researchers argue are clinically and etiologically similar outbreaks of CKDnt.[2,5,6]

Since the first peer-reviewed article about this phenomenon in Mesoamerica (which includes Central America and Southern Mexico) was published by a team of Salvadoran investigators in 2002, a small but growing group of researchers—along with local governments, scientific societies, PAHO and WHO—has mobilized to investigate this notion further.[7–9] They have found, among other key insights, that CKDnt disproportionately affects sugarcane harvesters. Although the disease has been shown to also affect men not employed in agriculture, women and children, much research has focused on agricultural workers and, specifically, sugarcane harvesters, given the disproportionate burden in this population. Sugarcane in Mesoamerica today is mostly produced in expansive monoculture plantations owned by powerful conglomerates.[10] Sugar plantations in these countries rely on local manual labor to sustain their large-scale production. Sugarcane cutters are the men and (less often) women who use machetes to cut down ripe sugarcane by hand, so it can be processed in mills. These cutters are often exposed to intense heat, pesticides, long shifts and other hazardous working conditions, and are paid meager, piecework wages, both keeping workers poor and incentivizing overexertion.[11,12] However, as the amount of land used for sugarcane production has grown in many places across Mesoamerica and many young, undereducated and impoverished individuals in these areas may have few other employment options, many become sugarcane harvesters out of necessity.

IMPORTANCE Findings suggest opportunities for facilitating progress toward nuanced understanding of the epidemic of chronic kidney disease of nontraditional etiology in Mesoamerica, which may have positive implications for affected populations.

CKDnt's etiology remains poorly understood, despite mounting research interest and efforts by ministries of health to engage national and international experts with the support of international public health agencies.[13] For example, although researchers agree that CKDnt is multifactorial, involves complex macro-level social determinants of health and is not associated with diabetes or hypertension—leading causes of chronic kidney disease globally—more specific causes have yet to be identified.[1,3,4,8] Previous research has focused on investigating risk factors and causal

hypotheses (including recurrent heat stress and dehydration, pesticides, infectious agents and heavy metals) to address these gaps in basic and clinical understanding.[14–19] Some investigators have begun developing targeted interventions to protect affected communities, based on findings from this etiological research.[10] Given that renal replacement therapies are often scarcely available and prohibitively expensive for patients in this region, such intervention efforts are especially important.[20]

To date, no studies have assessed the challenges of carrying out CKDnt research from researchers' perspectives. In light of discussions of the challenges of CKDnt research in some editorial commentaries and news media coverage of the role of the sugar industry and national governments in responding to the epidemic, this research explores how these and other factors may have shaped CKDnt research.[21–23] Understanding such influences may help researchers advance toward a comprehensive, unified understanding of CKDnt, with positive implications for affected populations.

This qualitative study involved semistructured interviews with a diverse sample of leading international researchers studying CKDnt in Central America and Mexico. The primary aim was to elucidate factors that have impacted researchers' efforts to understand the disease. Better understanding the context in which CKDnt research has been conducted to date can help identify specific strategies to facilitate high-quality, clinically relevant CKDnt research.

METHODS

Design and population A multidisciplinary team, including a medical anthropologist and an environmental sociologist, developed an interview guide for this project. This guide included questions and probes about participants' understanding of CKDnt, their own CKDnt research and perceptions of the CKDnt research environment. Experts in qualitative methodology on our team provided training for conducting semistructured interviews and data analysis, as well as input and feedback on all aspects of the study.

We selected participants purposively, with the objective of sampling individuals extensively familiar with and involved in CKDnt research from across demographic and relevant, expertise-related variables. We began this process by conducting a review of peer-reviewed literature and publicly available attendance lists for international conferences about CKDnt. We identified a list of 101 researchers involved in such work who had attended at least one conference and/or been a coauthor on multiple peer-reviewed articles about CKDnt. We narrowed this list down by focusing on senior and active members of research teams when multiple individuals from one research group were captured and selecting individuals with distinct training (medical, doctoral, or master's degree), expertise, length of involvement in CKDnt research, geographical location and institutional affiliation(s). We initially invited 69 researchers to participate.

Data collection and processing Of the 69 researchers we initially invited to participate, 54 (78.3%) responded, 1 of whom declined to participate due to perceived lack of relevant experience to contribute. We interviewed 39 of the 53 (73.6%) who agreed to participate, from May through September 2015. The remaining 14 were not interviewed, either because they did not respond to attempts to schedule an interview or because other members of their research teams with similar roles and training had already been interviewed. Researchers who did not respond to our initial invitation to participate or who did not participate for other reasons did not differ in gender, expertise, or country from those who participated.

Interviews were conducted by video conference, telephone or in person and were audio recorded. One participant participated via Qualtrics survey based on the interview guide because establishing an internet connection reliable enough for live contact was not feasible. Recruitment continued until thematic saturation was reached. Interviews were transcribed verbatim, deidentified and (if in Spanish) translated into English by the study team.

Given the small population of CKDnt researchers, we took rigorous precautions to deidentify participant data and allowed participants to review deidentified quotes prior to inclusion in this publication. All participants directly quoted here were given an opportunity to remove their quote(s) to ensure they did not feel identified.

Analysis Transcripts were analyzed iteratively and collaboratively by inductive content analysis.[24] We began designing a coding structure by open coding three transcripts. A study team member read and annotated them to distill participants' points, record impressions of them and attend to unifying ideas as well as variations over participant type. After grouping these annotations, we developed a provisional codebook. Our study team reviewed this initial set of codes, providing feedback to address ambiguities and redundancies in the codes. We then applied the codebook to all transcripts. Coding discrepancies were resolved by consensus. New codes were added as they were identified. When a section of a transcript addressed multiple concepts, it was marked with all applicable codes. We also examined whether interview responses differed by participant location (local: from Central America, Mexico, or Cuba; international: from other than Central America, Mexico or Cuba; expatriate: from a country outside of Central America, Mexico, or Cuba, but now living and working on CKDnt in the region). Although Cuba is not within Mesoamerica, we categorized participants from Cuba as "local" given cultural and political similarities between Cuba and Mesoamerica, relative to other "international" countries where participants resided (the USA and European countries), and our interest in the influence of these factors on participant responses. Emergent themes were identified inductively through abstraction of data collected from coding as it progressed and organized into major themes and subthemes.

Ethics This study was exempt from review by the Institutional Review Board (IRB) of Brown University (where the author was studying), but study procedures were conducted in accordance with IRB guidelines for informed consent, participant safety and data quality. All participants provided written informed consent (consent form provided in both English and Spanish).

RESULTS

We interviewed 39 researchers from 14 countries, with diverse expertise and experience in CKDnt research from clinical, laboratory, academic and/or field settings (Table 1). Interviews were conducted in English (29/39, 74.4%) and Spanish (10/39, 25.6%) and lasted an average of 46 minutes (range 16–87 minutes). Thematic analysis generated three themes, each with associated subthemes (Table 2).

Table 1: Participant characteristics

Characteristic	Participants (n=39) n (%)
Sex	
Women	12 (30.8)
Men	27 (69.2)
Country of origin	
USA	16 (41.0)
El Salvador	4 (10.3)
Sweden	4 (10.3)
Costa Rica	3 (7.7)
Cuba	2 (5.1)
Mexico	2 (5.1)
England	1 (2.6)
Guatemala	1 (2.6)
Nicaragua	1 (2.6)
Sri Lanka	1 (2.6)
Other*	4 (10.3)
Degree(s)	
MD or equivalent	18 (46.2)
MD and PhD or equivalents	8 (20.5)
PhD or equivalent	7 (17.9)
BA and/or MA, MS or MPH	6 (15.4)
Discipline	
Medicine	
Nephrology	11 (28.2)
Internal medicine	5 (12.8)
Pathology	1 (2.6)
Other	3 (7.7)
Public health	
Epidemiology	9 (23.1)
Policy	4 (10.3)
Environmental health	2 (5.1)
Occupational health	1 (2.6)
Industrial hygiene	1 (2.6)
Other	2 (5.1)
Years of CKDnt research	
1–5	10 (25.6)
6–10	16 (41.0)
>10	13 (33.3)

*countries grouped as "other" to protect researcher identities
CKDnt: chronic kidney disease of nontraditional etiology

Theme 1: Influence of private and state interests *Sugar industry interference and government intractability* Although a few instances were identified in which sugar industry and national governments cooperated with or spearheaded responses to CKDnt, participants more often reported that these stakeholders had stymied CKDnt research. Regarding industry, participants said that sugarcane companies had largely refused to participate in studies of CKDnt. Only two research teams had been engaged in long-term work on plantations in the region at the time interviews were conducted. Researchers reported that some sugarcane harvesters who participated in CKDnt research had been fired as a result. Participants said the sugar industry had also misrepresented research findings in company publications. Participants also felt that industry had sometimes targeted CKDnt researchers directly. For example, two participants described being followed, approached or watched by strangers, attributing it to their work on CKDnt. Additionally, three participants reported having received threats (one participant explicitly described them as death threats) because of their work studying links between sugar industry working conditions and CKDnt. Expatriates living and working in Central America expressed concerns about being targeted directly because of their work on CKDnt more often than did national and international researchers.

Governments in Mesoamerica, participants said, had also largely been intractable. Participants' attention to government roles centered on the lack of monetary and institutional support for CKDnt research and related policy-change efforts. Most participants agreed that Costa Rica and El Salvador had, at least recently, taken a more proactive, coordinated approach to addressing CKDnt. Conversely, many researchers, both from Nicaragua and elsewhere, lamented that the Nicaraguan government continued to dismiss the epidemic. Participants said it had dissolved the Ministry of Health's ethics review committee (preventing approval of research proposals), issued formal directives to local agencies to not engage in CKDnt research and suppressed government employees who did not support the government stance on the issue.

Theme 2: Limited material and human resources *Funding* Securing substantial, stable, impartial funding for CKDnt research was widely described by participants as one of the major challenges. Although participants recognized funding challenges were not unique to CKDnt science, high-caliber funding was perceived as acutely scarce in this arena. Most participants' work was funded by nontraditional sources such as charitable foundations, sugar industry in Mesoamerica, or private donations from friends, family or religious groups. In addition, many participants described using their own personal resources to fund their work on CKDnt. Several of them received no funding at all for their CKDnt research. Suggested reasons for this difficulty obtaining funding included major charitable foundations preferring to fund work to eradicate diseases rather than understand them and Latin America having relatively low priority on the geopolitical agenda for international aid and research support. Many participants linked the slow pace and overall limited scope and quality of CKDnt research to their reliance on piecemeal and limited funding.

Mesoamerican research capacity A majority of participants, from both Mesoamerica and elsewhere, also noted the paucity of infrastructure and high-quality biomedical equipment necessary for CKDnt research in Mesoamerica. For example, electricity can be unreliable in many parts of Mesoamerica, making it difficult to properly refrigerate biological samples. Furthermore, few in-country laboratories are equipped to handle and process these samples. As a result, research teams often rely on point-of-care biological assays, which can be less accurate.

Ten participants, all of whom work from outside Mesoamerica, commented on the availability of highly trained researchers in Mesoamerican countries. Their consensus was that there were too few researchers in Mesoamerica available and/or interested in studying CKDnt in the context of limited funding. Those who did engage in CKDnt research were perceived as overextended and overloaded with other responsibilities, to a greater degree than their US or European counterparts.

Collaborations with international researchers. In part to help address this limited local research capacity, researchers from other regions including the USA and Europe have begun studying CKDnt,

Table 2: Representative quotes of major themes and subthemes

Theme and subtheme	Quotation
State and private interests	
Sugar industry interference and government intractability	"The second [challenge] is . . . the intractability of industry . . . The effort and the investment that has gone into discrediting research, or distracting research, or buying research, it's a huge challenge because introducing doubt where there is none or shouldn't be any is the ultimate battle that you have to fight because once doubt is introduced it's really hard to fight against [as a researcher] . . . The strength and the financial brute force and bullying and thuggishness that sometimes emerges from the industry—not all the players of course, we have found some really good ones—but from the general industry has been a major challenge." (Interview 11)
	"El Salvador is still a unique state actor in Central America when it comes to making [CKDnt] a priority. Although the Zika outbreak and the violence in the region seem to have assumed primacy and [CKDnt] seems to be a secondary concern despite its impact once again . . . in Mesoamerica, apart from El Salvador, the other countries still seem to be in the "deny and delay" game or just unwilling to fund [CKDnt] research. Costa Rica has acknowledged it, but they are trying to figure out what to do. It's not moving quickly there, but maybe something will come of it. El Salvador remains underfunded and they face a lot of challenges. However, the Salvadoran government put this disease on the map in many ways. But Mexico, Guatemala, Honduras, Nicaragua—nothing. Nothing worth noting all these years later. It's troubling." (Interview 17)
Limited material and human resources	
Funding	"There's no high-profile money here. The research grant, it's a lottery. It's not like the NIH or the Wellcome Trust will fund you . . . " (Interview 19)
	"In a way you could describe it as involvement because of personal interest. The reason is that I can't do research on that [CKDnt] or participate in research on that within my normal work. At our clinic there is no funding. So what I can do, I have to do on the side." (Interview 22)
CKDnt research capacity	"And then you have the whole capacity, infrastructure [problem]. Is there a lab that can handle this? No. We decided to send all of our samples to Sweden. There's not a single freezer in the whole country that is secure, that has a backup power system. So we had to fix that ourselves." (Interview 19)
Collaborations with international researchers	"Very . . . if I needed to pick a word, it [collaborating] is difficult. Difficult not in terms of them being very difficult people to relate to, but them being people who are from abroad, who have never lived here, who don't understand the language, who don't get many of the details and the little things that happen, or they don't get the cultural discourse, the cross-cutting things that makes this look the way it looks . . . So, it's frustrating sometimes. Sometimes I feel a little bit lost. And a little bit alone." (Interview 5)
Logistical challenges	
Study approval	"In Nicaragua, for example . . . the IRB board . . . was disintegrated last year and the last time we checked, which was a couple months ago, they hadn't invited more people to [participate]. They dissolved it and then they didn't rebuild it. So, right now, it is—or so they're saying—it's impossible to get a chronic kidney disease related research project approved by the Ministry of Health's ethical review board because the ethical review board doesn't exist." (Interview 11)
Study implementation	"There's also just the geography and the logistics, trying to keep samples cold and get out to the villages before people go to work. People are leaving town at three o'clock in the morning [to go work in the sugarcane fields] so you've got to keep the samples cold and get back to the lab before the end of the day. The logistics are a huge challenge and thankfully I haven't had to actually do that [by] myself. I've got [to work with a team of] people who, yes, we've discussed it and how to do things, but I couldn't do that [kind of study] in that environment [without them]. I could run a study here, but I couldn't run a study in Nicaragua." (Interview 35)
Working with communities	"And there was a real problem at the beginning of the zafra [harvest season] last year with the gangs on the coast. Which is hard, we had armed military or police with us the whole time, a police force of four men with automatic rifles sitting around us, accompanying us to the field, and then sitting with us until they accompanied us back to safe territory. So it's not what you would say very comfortable." (Interview 15)
Publishing	"It's a question of: all of the ways in which we study these chronic diseases were developed in the developed world and require developed world level infrastructure and this is affecting people way out in the rural areas. So it just makes it really, really hard to do studies that your colleagues in the USA consider to be up to par." (Interview 14)

CKDnt: chronic kidney disease of nontraditional etiology IRB: institutional review board

a development which drew mixed responses. Several participants, across countries of origin, said that collaborations were productive and that the resources, expertise and media attention contributed by international collaborators had helped improve CKDnt research quality. Others warned that the influx of funding and investigators from abroad may distract from an emphasis on national institutions, funding and researchers. Expatriate researchers working both with international counterparts and national team members sometimes described having difficulty navigating cultural and language boundaries and distinct interests among collaborators.

Theme 3: Logistical challenges across study lifetimes According to participants, carrying out studies in Mesoamerica presents distinct, nontrivial logistical challenges. These factors affected the feasibility and quality of all phases of CKDnt research, from design to data collection and publication.

Study approval Obtaining study approval was often slow and laborious. Researchers affiliated with institutions outside Mesoamerica must get approval at their own institutions and Mesoamerican ones, which may have very different procedures. Three participants described extensive difficulty getting study approval from the Nicaraguan Ministry of Health. One participant described waiting at least two years for study approval.

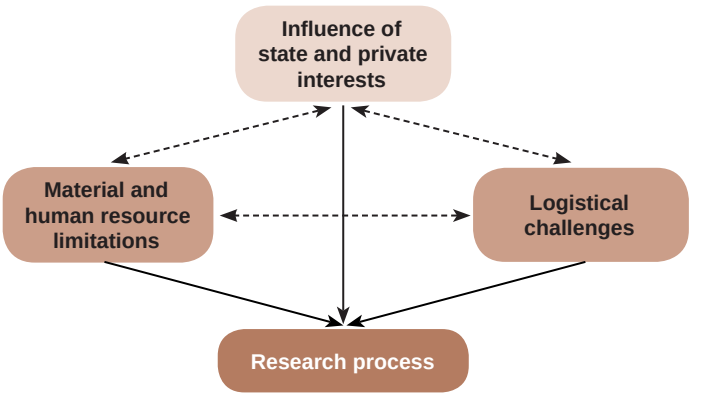
Study implementation If a project was approved, transporting and supporting equipment and researchers presented another set of obstacles, participants said. Given limited national research infrastructure, some participants described trying to bring laboratory equipment from their home countries to be able to process biological samples. Others had opted to ship samples to the USA or Europe for analysis. For studies examining sugarcane cutters during the harvest season, researchers had to transport themselves and their equipment to the fields to assess workers before the work day begins, as early as three in the morning. Community-based studies required commuting to remote locations by sometimes impassable roads. Furthermore, researchers reported that homes rarely have addresses or internet accessibility, preventing mail or email contact with participants.

Working with communities Researchers described numerous complexities in engaging and studying Mesoamerican communities related to their social and political context. In El Salvador, where gang violence is a threat, researchers had to be accompanied by teams of armed policemen. Participants detailed how studies had gone awry because of gang influence, a phenomenon that prompted some investigators to seek permission from gang leaders for study staff to enter communities. In other areas, communities' distrust of foreign researchers and health professionals was a significant barrier, which several international and expatriate researchers recognized. Some community members were wary of participating in CKDnt research because sugarcane workers who had done so before had been fired. More broadly, participants said that, although communities at the epicenters of the CKDnt epidemic understand the importance of etiological research in developing prevention strategies to address CKDnt, some had experienced substantial research fatigue.

Publishing According to participants, disseminating CKDnt research was also challenging. First, some researchers required language support to publish their findings in widely disseminated biomedical journals. Second, as one clinician discussed, publishing findings about CKDnt etiology was difficult if findings challenged current accepted knowledge in the field. Finally, a few participants discussed what may be the most dismaying of challenges: despite researchers' ingenuity in the face of these complexities of CKDnt science, their studies may not be accepted for publication because they do not match methodological standards that are expected in higher resource settings.

A proposed conceptual model of CKDnt research challenges Participants' perceptions of factors influencing CKDnt research were used to develop a conceptual model of CKDnt research challenges (Figure 1). This model illustrates that the three classes of challenges described by researchers—state and private interests, material and human resource limitations, and logistical challenges—are distinct, but interrelated. For example, limited availability of high-quality laboratory resources and clinical equipment locally in Mesoamerica in turn creates logistical challenges for transporting samples abroad for maintenance and analysis. Similarly, attempts by the sugar industry to intimidate CKDnt researchers and limited access to sustainable, substantial funding have likely exacerbated the perceived shortage of researchers interested in carrying out CKDnt research. According to participants, these challenges affect CKDnt research at every stage, from obtaining funding to publication. Our proposed model accounts for both the interconnectedness of challenges facing CKDnt research and their influence on the overall research process.

Figure 1: Conceptual model of CKDnt research challenges



CKDnt: chronic kidney disease of nontraditional etiology

DISCUSSION

In this qualitative study involving leading CKDnt researchers, participants described the CKDnt research process and environment. We found that numerous factors have hindered, delayed, threatened and complicated the CKDnt research process. Although many of these challenges, from the influence of private interests to resource limitations, have been explored in detail in other scientific arenas and resource-limited settings,[25–27] this study is the first to elucidate the complexities of carrying out CKDnt science in particular. It is also one of only three existing CKDnt studies that have employed qualitative methodology[10,28] and the first to examine CKDnt researchers' perspectives.

Our participants described that although some sugar plantations in Mesoamerica are responding to concern about CKDnt, others—at least at the time these interviews were conducted—continued to stymie CKDnt research. Numerous examples exist of corporations (including in the tobacco, mining and sugar industries) making similar

efforts to protect themselves from potentially damaging research in high-, middle- and low-income countries.[27,29,30]

The CKDnt research community has already made considerable progress toward establishing transparent, productive relationships with the sugar industry, focused on public health protection and scientific understanding of CKDnt. They have begun building networks among researchers, sugar plantations, affected workers, national governments and other key stakeholders, taking economic and political pressures felt by sugarcane plantations into account in workplace interventions and other studies, and collaborating with researchers who have experience studying industry-related health problems.[10,31–33] Participants also commented on the reactions of some government bodies to this issue, though largely from a regional stance. Despite participants' comments about the intractability of some government bodies in some countries at the time these interviews were conducted, several publications have resulted from collaboration between national specialists and experts appointed by international cooperation institutions, as well as multiple meetings establishing CKDnt as a public health priority and specific governmental actions taken in response to the problem.[9,13,16,34–38] As policies, legislation and reforms that protect at-risk communities such as sugarcane workers are enacted, it will be important to ensure that plantations and local governments are implementing these new protections.

We also elucidated the difficulties of working in a resource-poor setting in which high-tech laboratories and substantial funding are scarce. A minority of international researchers additionally commented on the relatively limited availability of highly trained researchers and clinicians to study CKDnt in Mesoamerica, in comparison with the USA or Western Europe. Rather than being meant as a slight to the competence of national professionals, these opinions were expressed in the context of broader comments about the importance of national clinicians, researchers and public health workers in this field, as well as concerns about the impact of limited CKDnt funding on national and international researchers' involvement.

There was no debate that national experts were among the first to draw attention to the epidemic, have made numerous significant contributions to our understanding of CKDnt epidemiology and etiology in Mesoamerica, and are essential to carrying out CKDnt research. Efforts to build nephrology workforce and research capacity, strengthen research networks that enable pooling of resources, expertise and data, and establish CKDnt research priorities are already under way.[8,9,39–41] However, research on other diseases of public health importance in low- and middle-income countries (LMIC), such as HIV/AIDS, has shown that some research "collaborations" can be exploitative of in-country researchers, resources and patient populations as well as insensitive to the nuances of carrying out research in that particular context.[42] Recognizing and emphasizing existing in-country expertise, including that of affected communities, will only become more important in the field of CKDnt as more researchers, funding and institutions from outside Mesoamerica mobilize to address this public health crisis.

We have found that carrying out CKDnt science presents significant challenges across study lifetimes. Together, these challenges make it difficult for CKDnt research that reaches the publication stage to meet methodological standards derived from and adjudicated in more generously resourced settings. This significant barrier to publication has been documented, yet there are no realistic, practical strategies to address it.[43] Expanding the role of scientists from these countries as editors and reviewers in high-impact scientific publications, offering journal-initiated mentorship to submitting authors, and addressing potential biases in review of manuscripts from LMICs would support researchers from resource-limited settings such as Mesoamerica in publishing their work, without negating other initiatives to improve research quality and methodological soundness.[44–46]

Limitations Several limitations should be considered concerning our findings. First, because we interviewed a purposive sample of leading researchers studying CKDnt in Central America and Mexico in particular, conclusions drawn here may not be generalizable to the entire population of CKDnt researchers or those studying CKDnt in other regions. Second, since this study involved one-time interviews, opinions and experiences communicated should be considered in the context of the timeframe during which interviews were conducted, May through September of 2015. Third, we analyzed what CKDnt researchers articulated in semistructured interviews, not direct observations. Fourth, we were unable to validate all reported concerns, given the sample size and subjective perspectives in this study. Finally, since this is the first study of its kind in the field of CKDnt research, these results should be viewed primarily as hypothesis generating.

CONCLUSIONS

Public health and biomedical research in low-resource, marginalized communities often present distinct complexities and pitfalls. These challenges are magnified when the public health problem under study is an etiologically novel non-communicable disease, as is the case with CKDnt. We have shown that these circumstances create a unique constellation of challenges to researchers attempting to address the CKDnt epidemic. Our findings identified important considerations for contextualizing progress in understanding CKDnt and underscored the need to enact and support efforts to facilitate CKDnt science and address the community-wide suffering it causes. Further studies reexamining CKDnt research challenges in other samples, time points and settings will help capture shifting and distinct factors not addressed in this single study. Finally, continued study of CKDnt's biomedical and epidemiologic characteristics is essential. Scientific understanding of and consensus about these aspects of the epidemic will have important implications for what kinds of treatments and protections can be put into place and enforced for affected workers and their communities.

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Family Planning as a Human Right: Knowledge for Better Decision-making by Cuban Adolescents

Sara Más

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Family as well as individual behavior play an important part in the decision to have a child [in Cuba], according to research from the University of Havana's Center for Demographic Studies (CEDEM).

Sexual initiation often takes place without contraception, said CEDEM researcher, Maydee Vázquez, during a recent panel entitled *Family planning and reproductive rights from the perspectives of demographics and sexuality*. The gathering was convened on July 9 by CEDEM, the Population Studies Network and the Cuban Society for Multidisciplinary Sexuality Studies (SOCUMES), to mark World Population Day (July 11), whose theme this year was *Family Planning is a Human Right*.

Several studies [in Cuba] have revealed that many women are unaware of their monthly fertile period, never mention male sterilization and rarely speak of emergency contraception. In addition, contraceptive usage is sporadic or interrupted, a behavior pattern associated with fluctuating understanding of their use and intermittent market availability.

Research also identifies deficiencies in sex education and awareness, as well as sexual and reproductive health services that focus almost exclusively on women, thus limiting opportunities for involving men, who may also be participants in the decision-making process.

Vázquez noted that adolescents often copy the reproductive behavior of their elders and women are the main ones who decide on family planning methods. These may be used irregularly, and when they fails, women resort to abortion or decide to have the child.

Thus, in the arena of gender and sexual rights [in Cuba], she described the pending challenges as: irregular contraceptive use, predominance of female contraceptive methods, higher rate of female sterilization compared to male, low acceptability and promotion of the latter, and the descending mean age of sexual initiation in adolescent girls.

A case study of pregnant adolescents in San Miguel del Padrón Municipality in Havana found that these young women tended to repeat familial patterns, in which mothers and grandmothers were also adolescent mothers, and were ill informed about family planning methods.

[Adolescents] may protect themselves in their first sexual encounter, but then use contraception only intermittently, and it's not a theme discussed by their families, which also often have misconceptions about family planning methods, commented CEDEM researcher Gabriela Dujarric. The majority of adolescents interviewed [by CEDEM researchers] had had at least one abortion and it was clear that often their mothers had been involved in decisions about continuing or interrupting the pregnancy.

What's more, those who decided to continue their pregnancy at that time distanced themselves from the study, some withdrawing completely, also avoiding activities such as parties, games and excursions, many of which were the setting for the relations that led to their pregnancy. Rather, in such cases, watching television, and chatting with partner and family become the routine for them, and they tend to leave school to take on domestic tasks and child care. Thus, commented Dujarric, they suddenly go from a girlfriend/boyfriend relationship to one that calls for child-rearing, with the resulting loss of outside interests, in a scenario that keeps them from recognizing the situation in which they find themselves.

> **Family planning is now recognized as a right for women and families, although education is still not where it needs to be...**

The family transmits reproductive values, from grandmother to mother to daughter, and with vague communication about sexuality and little educational oversight. [In this context], Dr Gabino A. Alessandrini noted: "Family planning is now recognized as a right for women and families," although education [in that respect] is still not where it needs to be, and adolescents still get most of their information from their peers. He reflected that such education is the responsibility of the schools and public health system, which should accompany them as subjects with rights and transformation agents in their journey to autonomy, so that they can realize their life goals.

The gathering was dedicated to Professor Sonia Catasús Cervera, an outstanding demographer and population researcher at CEDEM, who died in June. Catasús carried out landmark studies for Cuba and Latin America, notably research on marriage and families. Colleagues and friends praised her scholarship, human qualities and approach to life, as well as her teaching and professional practice.

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