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MEDICC Review

Editorial Board

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The Long March for Science in Defense of Population Health

Rare grand initiatives and discoveries aside, population health and its supporting science usually advance by the slow accretion and sharing of a myriad of capacities, findings and interventions across the global scientific community. In the best of cases, ordinary people benefit. But in the worst cases, science is shunted aside, ignored or twisted to fit interests that actively oppose the right to health.

Among those threatened today are indigenous health, reproductive rights, quality health care for marginalized populations and entire continents, planetary health, and certainly health in all policies. A version of "racial and economic profiling" turns patients into clients or turns them away altogether. These are some reasons why health professionals and researchers have taken to the streets with entire communities, defending their work to improve medicine and health equity: "I can't believe we have to march for science…yet we do," one prestigious scholar put it.

The first step along this march is often getting the word out. This is the case of the chronic kidney disease (CKD) epidemic in Central America and elsewhere, until recently a silent killer of over 20,000 subsistence farmers and their families in El Salvador alone. ***MEDICC Review*** is proud to be among the journals that first called attention to this disease of nontraditional causes that fells people often too poor and too sick to join any march. In this issue we carry research that raises important hypotheses on the causes of the epidemic, as Orantes' paper analyzes findings from a national Salvadoran survey, implicating both working conditions and agrochemical exposure in this CKD etiology.

Yet, when it comes to getting the word out, population health researchers from the Global South are often at a distinct disadvantage, especially publishing in international peer-reviewed journals, ever more so if their native language isn't English. They are also hampered by the dearth of scientific writing courses in their medical and health sciences curricula. Thus, their research may be flawless, but their work is like the proverbial light hidden under a bushel basket. And the whole world suffers as a result, particularly those who most need their research.

MEDICC Review is pleased in this regard to collaborate with Cuba's National School of Public Health and PAHO/WHO in an annual Scientific Writing Course offered in Havana. The course's fourth edition this year enrolled another 50 participants, manuscripts in hand, who attended interactive lectures, workshops and tutorial sessions. We…and indeed other journals…look forward to receiving their papers.

While we are dedicated primarily to publishing work from Cuban professionals in health and related fields, ***MEDICC Review*** is committed to raising the profile of research and opinion from the Global South as a whole. In this issue, we're glad to see a variety of countries represented among our authors, including Chile, Colombia, El Salvador, Panama, and the Dominican Republic—as well as Cuba, the USA and UK.

All have one thing in common: their goal is to apply their discoveries, intelligence and commitment to produce scientific evidence that addresses big population health issues, and uses modern as well as traditional tools to do so. Thus, our pages reflect progress in genetic, statistical and biological analyses, allowing for greater precision in diagnosis and treatment plans, earlier disease detection, or more accurate prediction of disease appearance and progression. Gari-Llanes' study of pediatric hypertension examines its relationship to the biochemical risk markers most associated with cardiovascular disease in adults. The importance of her research can't be over-emphasized, since cardiovascular disease is expected to take a much greater toll in middle- and low-income countries over the next decades.

> ***MEDICC Review*** **is committed to raising the profile of research and opinion from the Global South**

Using a novel statistical approach to dengue data, López-Montenegro of Colombia develops a model to forecast dengue outbreaks, allowing for more precise and effective control strategies. In the sphere of pharmacology, Garcia-Blanco of Cuba looks at antiretroviral therapy for HIV through the prism of genetics, reviewing the latest literature from around the world to discover reasons for adverse drug reactions.

Other contributions turn their sights on the implications of population aging, a fact of life and longevity in Latin America and many other regions. Hirmas-Adauy assesses the effectiveness of a public policy in Chile designed to provide assistive devices to older adults, and Villareal provides the first study of cognitive impairment in Panama's aging population, serving as a baseline for further research on early detection. Women of science are represented in a preponderance of authorship in this issue, and in the intersection of race and gender explored in Gorry's interview of Cuban anthropologist Lourdes Serrano.

Recent news of measles outbreaks and emergencies across the USA, as well as Ebola's leap across the border from Congo to Uganda, are potent reminders that progress towards universal and planetary health need both science in the public interest and a public interested in science. Not only interested, but also placing their trust in fruits of rigorous science. This is why ***MEDICC Review*** and other peer-reviewed journals publish, but also why we encourage scientists to go beyond our pages to make the argument to a wider audience. This wider audience, after all, will cast the deciding vote on whether health, their own and others', is worth marching for.

The Editors

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ERRATUM

Raines N, et al. Risk Factors for Reduced Glomerular Filtration Rate in a Nicaraguan Community Affected by Mesoamerican Nephropathy. MEDICC Rev. 2014;16(2):16–22.

Page 16, in authors, line 2, "Eugenia Marcas" should read "Eugenia Márquez."

Educating Well-rounded Physicians for the 21st Century

To the Editors:

In ***MEDICC Review***,[1] Natalia Orihuela presents her four-year journey at the Latin American School of Medicine (ELAM), Havana, Cuba, describing her didactic coursework and community rotations in vulnerable communities where access to healthcare is a major priority. Using a team-oriented approach, ELAM's educational program focuses on the value of primary care, where medical students strengthen their clinical knowledge and skills through community interactions with patients. As such, applying these insights to other nations can provide a framework about communities serving as learning powerhouses and their significant contribution to medical training.

As a fourth-year medical student in the Dominican Republic (DR), I have experienced the value of community-based medical education (CBME), which encourages future physicians to immerse themselves in diverse social contexts.[2] These direct patient interactions promote the biopsychosocial approach, examining patients' illnesses from within their communities. By understanding the social determinants that influence patients' health, hand in hand with the development of clinical skills, these environments can provide strong learning opportunities.

During my medical education, I have served as an active leader in the Dominican Medical Students Organization (Organización Dominicana de Estudiantes de Medicina–ODEM), a non-governmental organization that represents DR medical students within the International Federation of Medical Students' Associations (IFMSA) network. By serving as a platform that promotes health education, medical training, scientific research and community service, ODEM fosters teamwork principles and empowers medical students to develop community health campaigns for vulnerable populations. Through my ODEM participation, I have observed first hand how multidisciplinary health teams in community settings contribute to collaborative and comprehensive healthcare service delivery that focuses on a multidimensional approach for disease management.

My active involvement in CBME and ODEM has provided me with an essential global vision of doctors *"de ciencia y conciencia" ("with science and a conscience")*. As future health leaders, we should acknowledge health as a public good and human right; conceive medicine as a profession ruled by humanism and altruism; and deliver high-quality health services in our communities. The community-centered learning programs and activities described can strengthen medical education and training, with potential to enhance healthcare delivery and doctor-patient communications.

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Lilian Teresa Pimentel *(lilianteresap@gmail.com), fourth-year medical student; Vice-President of External Affairs, ODEM-Dominican Republic, Santo Domingo, Dominican Republic.*

Gender & Race in Cuba: An Anthropological Perspective

Lourdes Serrano Peralta PhD

Professor, University of Havana
Former Director, Cuban Anthropology Institute*

Conner Gorry MA

How does a developing island nation, beleaguered by climatic challenges and 60 years of adverse geo-political pressures become a beacon of scientific innovation, medical services and applied research—all on a shoestring budget? What's more, how does such a country, rooted in a traditional patriarchal paradigm, overcome barriers to create a scientific and medical community where the majority of researchers and professionals are women? These are some of the questions that motivated *MEDICC Review* to publish this series on Cuba's women in STEM (science, technology and math).

E Añé

Spanning a variety of themes and disciplines exploring the history of women and science; the role of female protagonists in the development of Cuba's public health and biopharmaceutical sectors; and results produced by women professionals and their colleagues, these interviews illuminate lessons learned and what strategies might be applicable, adapted and replicable in other contexts. This time, we explore the intersection of gender and race in Cuba, a country with the world's third-highest percentage of female parliamentarians—many of them women of color.

To help us better understand this complex topic, we spoke with Dr Lourdes Serrano, who served as Director of the Cuban Anthropology Institute* (under the aegis of the Ministry of Science, Technology and the Environment and the Cuban Academy of Sciences) from 1991 to 2005. During her tenure there, Dr Serrano's research focused almost exclusively on gender and race, including the impact of structural and policy changes since 1959; the manifestations of discrimination and bias in contemporary Cuba; and the role of women in economic, cultural and political life. A lifelong scholar and teacher, Dr Serrano is currently professor at the University of Havana in the Cuban History and Caribbean Studies Departments, and also a coordinator of the Afro-Descendant Caribbean Women's program in the University's Caribbean Studies Department.

MEDICC Review: **What awakened in you the call to teach?**

Lourdes Serrano: More than a call, I'd say it is a passion. I had my first inkling when I went into the countryside as part of Cuba's 1961 literacy campaign. This nationwide initiative saw tens of thousands of young volunteers, mostly women and girls, fanning out in towns across the country to teach every Cuban to read and write. I was just 14 years old and went to live with a family in Remate de Guane, a small, remote town in Pinar del Río Province. Today it makes me wonder: would I let my teenage daughter leave home, move to the countryside in another province, to live with a family of strangers to teach them to read and write! That would be a hard decision.

Nevertheless, my mother—who wasn't an educated woman, who never went to university and wasn't a professional—allowed me to go. The excitement and effervescence of that time infected her, like all of us. It marked our entire generation; we weren't only teachers but social activists. This experience awoke in me the calling to teach and I've been doing it now for over 45 years. I love teaching and will be doing it until my dying day.

Once education became free and universal—another early policy instituted by the [post-1959] revolutionary government—I took advantage of scholarships for women, completing degrees in history and pedagogical sciences at the University of Havana. Afterwards I

obtained my doctorate from the Academy of Sciences in the former Soviet Union. My life as a researcher began when I was promoted to Director of the Cuban Anthropology Institute and became interested in how structural and political changes were transforming the role of women in the country. From there, I began drilling down further, researching how these changes were affecting Cuban women of color. Since then, I've been researching gender and race through an anthropological lens. I began looking at structural changes and their impact and asking what's working? What isn't? Where can we improve and provide better support for Cuban women of color?

See the full series of interviews with Cuba's Women of Science at www.mediccreview.org

MEDICC Review: **Given your experience as director of a major research center, do you believe men and women have different management styles?**

Lourdes Serrano: I've had a lot of conversations about this because my management style is not based on male models. I'd ask colleagues: why do women in decision-making positions have to adopt traits considered masculine? Women don't have to wear pants or direct and delegate from a position of force to be effective. In my center, the majority of researchers were men, but I led speaking softly, with what might be considered a more feminine style. I believe women can lead any center or group—including a military installation—just like this. Look at Vilma Espín, who founded and directed the Federation of Cuban Women (FMC) and fought in the Rebel Army [that defeated Fulgencio Batista in 1959—Eds.]. Is there any model of leadership more feminine than hers?

For me, it's about the work, being rigorous and gaining the respect of your team; this transcends gender. We see this in the classroom with professors and students as well—if you earn the respect of your students, the teaching style can be your own, it doesn't have to mimic a man or another professor, because you're teaching from a position of mutual respect.

MEDICC Review: **Before jumping into the structural changes, can you describe historical representation, participation and marginalization of women in Cuba?**

Lourdes Serrano: There's a tradition of female participation in Cuban politics, activism, even war: *Las Mambisas* were women who fought in Cuba's independence wars, including Mariana Grajales, a mulatta woman and mother of independence hero General Antonio Maceo. This had an impact and by the latter half of the 1800s, Cuban women were fighting for gender equality, emancipation and representation.

In 1923, the First Women's Congress was held in Cuba. Though this was a watershed for Latin America, the Congress was organized by upper-class white women, so neither women of color nor working class women were represented. In 1933, Cuban women won the right to vote. The Constitution of 1940—considered the most progressive in Latin America at the time—prohibited gender-based discrimination; and by 1949, we had the first black, female parliamentarian.

The root of the problem isn't women's membership in parliament, however, it's women's *representation* in parliament and there's a fundamental difference. Women need to be an important share of voting members, but these parliamentarians must also be an *empowered voice*, bringing women's problems, with their optics, to bear on policymaking. We can't just hear from parliamentarians—male or female—who are replicating traditional patriarchal views. Another vital aspect to being represented is integrating more working women, women who wake up every morning to go to work, get their kids to school and make sure they are fed even when food is sometimes hard to find. These women, living the day-to-day reality, who struggle to resolve daily needs despite scarcity, are also transmitting values. All this is very powerful and we need to hear from them.

So this is also why it is important to educate and prepare women to actively contribute to policies with a gender focus. The literacy campaign made sure every woman could read and write, and universal education opened the halls of learning to all Cubans regardless of gender, skin color or financial resources. It's true that women worked before the structural changes instituted by the revolutionary government, but it was typically in traditional "women's" jobs like teaching, nursing or as maids and other domestic service roles. But when higher education became accessible to everyone, women became lawyers, engineers, scientists, and doctors—they were represented in all sectors. Importantly, they also began to be represented at all levels. The current Rector of the University of Havana is a woman of color, for example, and we have many ministers and vice ministers who are women, as well.

Table 1: Status of Women in Cuba, Selected Data

Variable	Value
University graduates (%†)	60.5
Teachers, professors & scientists (%)	81.9
Health & science sectors (%)	60.0
Working in paid labor force (%)	45.1
Self-employed (%)	34.0
Parliamentarians	322 (53.2%)
Council of State (%)	48.4
Council of Ministers (%) (Includes Vice President of Council of Ministers & Council of State; 5 Ministers; and President of Central Bank of Cuba)	26.9
Presidents of Provincial Assemblies (of 15)	8
Vice Presidents of Provincial Assemblies (of 15)	8
University Rectors (exclusive of medical sciences universities) University of Havana; Villa Clara; Cienfuegos; Matanzas; Oriente	5
Maternal mortality (direct and indirect, per 100,000 live births)	38.3
Life expectancy (in years)	80.8
Adult women reporting psychological violence by an intimate partner in past 12 months (%)	25.7
Adult women reporting physical violence by an intimate partner in past 12 months (%)	2.4
More hours women dedicate to household work as compared to men (per week)	14

†Unless otherwise indicated, % is percent of total.

Sources: Encuesta Nacional sobre Igualdad de Género, 2016; Anuario Estadístico de Salud, 2017; Anuario Demográfico de Cuba 2017

***MEDICC Review:* What were some of the major structural changes made to combat gender and race-based discrimination after 1959?**

Lourdes Serrano: From the first structural changes, like the founding of the FMC in 1960, to the recent new constitution, our government has made it clear that there is no room for discrimination of any kind in our society. But eliminating it doesn't happen overnight and isn't a linear process: sometimes we've swerved from our goal, but the results so far are measurable. And as concepts evolve, these structural changes evolve. We have national programs for early development and learning that focus on new concepts and constructs related to gender and race, for example. If we want to build a society free of sexism, we need to reach kids at a very young age, teaching them that we are all equal. Very simple concepts like pink clothes aren't only for girls and that boys can play with dolls, help break down traditional gender constructs.

The new constitution, approved by a public referendum in February of this year, strengthens articles against discrimination in terms of property ownership, elimination of inequalities, and so forth. Gender and gender identity, sexual orientation, age, ethnic origin, disability and territorial origin have been added to the list of categories of rights protected under the constitution. And women's reproductive and sexual rights are now constitutionally guaranteed as well. Some of this was in place already, but I think needed to be strengthened nonetheless; for example, our 1976 constitution had already codified and put into practice anti-discrimination articles, especially as pertain to skin color.

All these articles are enforceable under complementary laws, including those contained in our criminal code, such as Article 265, which penalizes racial discrimination by imposing fines or even prison sentences. The recent constitutional reform incorporated legal mechanisms for prosecuting gender-based violence as well, so we're making even more progress towards equality in legal terms.

***MEDICC Review:* Of course, structural or legal changes aren't enough…**

Lourdes Serrano: Right. These structural changes have to be complemented by cultural changes to achieve lasting impact. What's more, breaking down these cultural barriers to gender equality has to be an interdisciplinary, cross-sectoral strategy, as we see with skin color, too. Since 2002, the sociocultural audiovisual group *Proyecto Palomas* has contributed to educational efforts around gender roles and equity, and decades of work on this issue by the National Sex Education Center (CENESEX) is also producing results. Mariela Castro, who directs CENESEX, takes the approach that any kind of prejudice or discrimination is damaging to health, as was recently quoted in *The Lancet*.[1]

As pertains to race, *Ser Cubano*, a research project headed by the National Union of Cuban Writers and Artists (UNEAC), identified a problem with racial representation in the Cuban media, specifically women of color in Cuban films and television shows. The problem was identified as needing more women of color portrayed in the media. And the classic response was a movie or soap opera about slavery. But *Ser Cubano* concluded that this isn't the answer and doesn't get to the root of the problem! The answer has to be much more profound, nuanced and empowering—with women of color directors and producers and characters that are three dimensional, professionals, career women, as well as mothers and wives.

***MEDICC Review:* A recent *New York Times* editorial[2] described real, sustainable empowerment as "transforming gender subordination," breaking down "other oppressive structures," and encouraging "collective political mobilization"—as you observe, empowerment goes beyond representation. Can you discuss this in the Cuban context?**

Lourdes Serrano: Cuba sprang from a powerful patriarchal tradition that continues today. The barriers to gender and racial equality today aren't structural, they're cultural—and cultural barriers are much harder to deconstruct. We're talking about changing a national identity with deeply embedded roots. This is a process that takes time. But even though it's a slow, hard process—or precisely because it is so slow and difficult—we have to keep fighting and educating and doing everything we can to shift that mentality.

The programs mentioned above and the constitutional reforms are helping move us towards that goal, and our long tradition of co-education plays a role, too. Look, as a professional woman of color, I've had my share of run-ins with this kind of cultural construct. I'd be attending conferences as the Director of the Cuban Anthropology Institute and colleagues would mistake me for the translator/interpreter, the assumption being that a woman of color could only reach so high on the professional ladder and couldn't possibly be the head of a research center.

This kind of racial bias—prejudices based not on how we talk or dress or behave, but strictly on skin color—happens all the time, often unconsciously, which is why it makes it a very difficult change to make, especially society-wide. The media, as I mentioned, plays a role in both context and content, reflecting ordinary people, taking on more of an ombudsman role, and tackling issues in a respectful informed manner that were previously taboo—including sexual diversity and racism.

I believe the younger generation has different optics when it comes to gender and race and we have to incorporate these voices as much as possible. It's also true that sometimes young people replicate learned gender and racial bias, often without knowing it. We have to work to transmit values to break down these patterns of prejudice and provide mentoring and guidance—and give them the space they need to grow into leaders. We older folks have to give up this idea that we are the ultimate authority. We also make mistakes and learn from those mistakes. We have to trust our young people and extend them the same opportunities we had. Experience with a young outlook: this is the ideal combination.

***MEDICC Review*: What role do—or can—Cuban men play in moving towards greater gender equality?**

Lourdes Serrano: I believe many Cuban men have evolved towards understanding what constitutes gender bias and how it manifests. Thanks to educational efforts about how to combat these cultural patterns of patriarchy and *machismo*, we've seen this evolution. But we want more. Of course we want more!

One thing is certain: to attain true equality, we need participation of the entire population—men and women. And this holds true for whatever sector of the population is fighting for equality—you can't reduce it to 'this is a problem for people of color, this is a problem for women to address, this is a problem for poorer people to address.' This approach divides us; we have to work together, across society and these lines. Nor can true equality be achieved by dictate. You can't just say 'okay, tomorrow we'll have an equal society.' So, we've gained substantial ground with the hard work put in by both men and women. And this process continues, as it must.

*Formerly National Anthropology Center

NOTES & REFERENCES

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Biochemical Markers and Hypertension in Children

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ABSTRACT

INTRODUCTION Hypertension is one of the most studied risk factors for cardiovascular disease in adults; in children and adolescents, its global prevalence changes with age, from 1%–3% in children to 3.2% in adolescents. In adults, in addition to hypertension, several biochemical markers of cardiovascular risk have been identified. Confirming an association between these and hypertension in childhood and adolescence would allow for more timely diagnosis and monitoring of cardiovascular disease, since the presence of both the markers and hypertension would imply increased risk.

OBJECTIVE Confirm an association between biochemical risk markers of cardiovascular disease and hypertension in children aged 8 to 11 years.

METHODS A cross-sectional study of 373 children aged 8–11 years was conducted in 3 primary schools in the city of Santa Clara in central Cuba. The variables examined were age, sex, height, blood pressure, cholesterol, triglycerides, lipoproteins and apolipoproteins. The children were classified as normotensive, prehypertensive or hypertensive, based on blood pressure readings and percentiles for age, sex and height. Descriptive statistics were calculated for quantitative variables. A bivariate analysis, tests of independence for qualitative variables and a means comparison for quantitative variables (ANOVA and its nonparametric alternative, the Kruskal Wallis test) were performed. Fisher's F-test and its associated probability value were employed.

RESULTS Some 32.2% of the children were prehypertensive and 5.1% hypertensive. Cholesterol and triglyceride values were significantly higher in hypertensive than in normotensive children ($p = 0.028$ and $p = 0.047$, respectively). HDL numbers were higher in normotensive children ($p = 0.001$), and LDL numbers and the LDL/HDL ratio were higher in the hypertensive children, with differences between groups ($p = 0.001$ for both variables). There were differences between the three blood pressure categories for lipoprotein(a) and ApoA ($p < 0.001$ and $p = 0.001$), for ApoB and for the ApoB/ApoA ratio ($p < 0.001$ for both variables), with lower ApoA values and higher ApoB and ApoB/ApoA values in the hypertensive children.

CONCLUSIONS The biochemical risk markers most strongly associated with hypertension in children are ApoB values, LDL, lipoprotein(a), and LDL/HDL and ApoB/ApoA ratios.

KEYWORDS Adolescent, child, hypertension, apolipoproteins, cardiovascular diseases, risk factors, Cuba

INTRODUCTION

Globally, cardiovascular disease (CVD) is a major cause of illness and the leading cause of death, accounting for more than 17.9 million deaths per year in 2015. This number is expected to grow to more than 23.6 million by 2030.[1] CVD is the main cause of death in Cuba and in high-income countries, although it is also on the rise in other middle- and low-income countries.[2]

Atherosclerosis is a process characterized by abnormal deposits of lipids, cholesterol and plaque, leading to coronary artery disease and other cardiovascular problems. Its origin is multifactorial, and many of its main risk factors are known.[3] It is increasingly found in children at younger ages. The importance of determining their health status in relation to its main causal factors facilitates the adoption of preventive and therapeutic interventions to avert or minimize its consequences.

A risk factor for a condition is causal if in its absence, the condition in question would not have occurred at all or would not have occurred until later.[4,5] Cardiovascular risk factors include hypertension, diabetes, dyslipidemia, obesity, smoking, genetic factors and family history.[6]

IMPORTANCE The results provide evidence for greater attention to hypertension in children and adolescents, and for adding study of biochemical markers to conventional risk indicators to ensure timely CVD risk assessment.

The presence of a cardiovascular risk factor is influenced by the presence and extent of coexisting risk factors. Epidemiologic and clinical studies have shown that individuals with multiple risk factors are at higher risk for cardiovascular disease than individuals with just a single risk factor.[7] Concurrence of lipid disorders (as evidence of atherosclerosis) with hypertension increases risk and makes the determination of related biochemical markers particularly important.

Many risk factors are determined by behaviors and attitudes acquired in childhood and continued throughout life. Thus, timely prevention of cardiovascular risk should begin in childhood and adolescence.[8] In recent decades, interest has grown in identifying factors that predispose children and adolescents to developing cardiovascular disease as part of public policies aimed at primary prevention.[9,10]

Hypertension (HT) is one of the world's most common disorders in adults; its prevalence increases with age, and it is a risk factor for cardiovascular disease. HT is a serious health issue, because its consequences include heart, blood vessel, kidney and retina damage. HT in children is more common than might be expected; globally, its prevalence ranges from 1%–3% in children to 3.2% in adolescents.[11] In Cuba, the prevalence in youngsters aged 5–14 years was 1.8 per 1000 population in 2017.[12]

It is currently recognized that onset and progression of atherosclerosis are related to an inflammatory process in the blood vessels,[13] and recent studies have focused on identifying a number of biochemical markers such as angiogenic growth factors, plate-

let activation and thrombosis related to lipids and other factors.[14,15] Available data show that these local and systemic inflammatory markers play a key role in development and progression of the atherosclerotic process. Several of them are considered independent risk markers for atherosclerosis and CVD and can therefore be used for screening, diagnostic and monitoring purposes.[5]

Oxidation of low-density lipoproteins (LDL) in the subendothelium is a key factor in vascular damage. LDL molecules are carried through the endothelial cells and accumulate in the vascular intima, where they can undergo oxidation, glycosylation, acetylation and triglyceride enrichment, thus increasing their atherogenicity.[15]

Oxidized LDL particles have atherogenic effects, although other lipoproteins, such as very low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL) and lipoprotein(a), do as well. Atheroma plaque formation is a complex inflammatory process involving various cells and mediators. Oxidized lipoproteins infiltrated into the subendothelial space stimulate intima's cells to produce proinflammatory cytokines and chemokines that activate circulating monocytes, which then enter tissues, becoming inflammatory macrophages and later, foam cells, resulting in intracellular accumulation of cholesterol esters and fatty streak formation.[15]

The purpose of this study is to draw attention to the relatively high HT prevalence in Cuban children and adolescents, frequently accompanied by abnormal values of biochemical markers that are predictors of CVD risk. A particularly important motivation is to prevent early-onset CVD and reduce mortality from this cause in Cuba.[1,16]

METHODS

Design and sample Between April 1, 2014 and March 31, 2015, a cross-sectional study was conducted of 373 students aged 8 to 11 years from schools in the city of Santa Clara in central Cuba, chosen for ease of access. All children from the third through fifth grade were included whose parents provided written informed consent.

Variables

Blood pressure For HT classification, criteria of the 4th Report of the American Academy of Pediatrics[11] and the recommendations of the European Society of Hypertension of September 2009[17] were used. Children were classified as normotensive if average systolic and diastolic pressure were both less than the 90th percentile; prehypertensive if average systolic and/or diastolic pressure were greater than or equal to the 90th and less than 95th percentile; and hypertensive if average systolic or diastolic pressure was above or equal to the 95th percentile. All percentiles were age-, sex-, and height-dependent.

Weight was measured with an analog scale and expressed in kilograms.

Height was measured using a height rod attached to the scale and expressed in centimeters.

Lipid panel biochemical markers Cholesterol, triglycerides, high-density lipoprotein (HDL), LDL, lipoprotein(a) [Lp(a)], apolipoprotein A (ApoA), and apolipoprotein B (ApoB) were measured. Cholesterol/HDL, LDL/HDL and ApoB/ApoA ratios were calculated.

Data collection and management Data included a clinical history and measurements as well as laboratory tests. Blood pressure, weight, height and lipid marker results were recorded. Blood pressure was taken five times over the course of three days.

For laboratory tests, children were instructed to fast for 12 hours before venous blood draw. Their parents were also informed to help guarantee compliance. Blood was drawn from the antecubital vein, and serum was obtained following coagulation after centrifuging at 3000 rpm for 15 min. Serum concentrations of total cholesterol (TC) (Cholesterol liquicolor, CHOD-PAP Method, Human, Germany), triglycerides (TG) (Triglycerides GPO liquicolor, GPO-PAP Method, Human, Germany), and HDL cholesterol (HDL-c) (HDL-Cholesterol, Human cholesterol liquicolor test kit, Germany) were then determined. LDL cholesterol (LDL-c) was calculated using the Friedewald-Fredrikson formula: LDL-c = TC - (HDL-c + (TG/5)). Apolipoproteins B-100 and A1 were determined by immunoturbidimetry. The values were expressed in standard international units as mmol/L.

Lipid reference values (mg/dL) are presented in Table 1.[18]

Normal values for the remaining lipid markers are shown in Table 2.[19]

Table 1: Lipid reference values[18]

Parameter	Ideal (mg/dL)	Normal range (mg/dL)	Dyslipidemia (mg/dL)
Triglycerides			
0–9 years	<75	75–99	≥100
10–19 years	<90	90–129	≥130
TC	<170	170–199	≥200
LDL-c	<110	110–129	≥130
HDL-c	>45	40–45	<40

TC: total cholesterol LDL-c: low-density lipoprotein HDL-c: high-density lipoprotein

Table 2: Normal values for lipid markers[19]

Markers	Normal values
TC/HDL ratio	≤3.5
LDL/HDL ratio	≤2.2
Apolipoprotein - A1 (ApoA)	>130 mg/dL
Apolipoprotein B-100 (ApoB)	90–140 mg/dL
ApoB/ApoA ratio	0.8–1.2
Lipoprotein(a)	>25 mg/dL

TC: total cholesterol LDL: low-density lipoprotein HDL: high-density lipoprotein

Analysis Descriptive statistics, means and standard deviations in the lipid panel biochemical markers were used for each group. Tests for independence based on the chi-square statistic and its associated probability were used for qualitative variables, and mean comparison tests (ANOVA and its Kruskal Wallis nonparametric alternative) for quantitative variables. In all cases, the significance threshold was $p = 0.05$.

Ethics Once the purpose of the study and the risks and benefits of participation had been explained to the children's parents, they were asked to provide written consent in the

presence of the investigator and a witness, in accordance with Declaration of Helsinki and WHO guidelines.[20,21] Since schoolchildren were involved, Ministry of Education authorization for the study was also obtained as required by Cuban law. All information obtained remained confidential, and in no case was the identity of a child disclosed. Choice of diagnostic media was based on material accessibility, care to maximize benefits for participants, the ethical norm of "do no harm," and international and domestic guidelines established for good clinical and laboratory practices.

Results of physical examinations and diagnoses based on clinical and biochemical data obtained were provided to parents of participating children. When the examinations revealed health problems, appointments for medical followup were arranged with specialists, at the José Luis Miranda Pediatric Teaching Hospital in Santa Clara.

RESULTS

Although most of the children were classified as normotensive, a substantial percentage were hypertensive or prehypertensive (37.3%) (Table 3).

There were significant differences among groups (normotensive, prehypertensive and hypertensive) with respect to classic biochemical markers (Table 4). With the exception of triglycerides—which were higher in normotensive than in prehypertensive children—all indicators showed a monotonically decreasing pattern for HDL and an increasing pattern for the rest of the indicators, as expected.

All biochemical indicators based on the structural proteins of the lipoproteins also exhibited significant differences. With the exception of ApoA, higher values were found in the hypertensive children (Table 5).

DISCUSSION

The proportion of hypertensive and prehypertensive children in the sample exceeds the HT prevalence reported in Cuba for 2017.[11] The high percentage of prehypertensive children is a matter of concern. The global literature reports that progression of prehypertension to HT is approximately 7% per year and that prehypertension poses a high risk for development of HT in children,[22] making it a major challenge for the health system. Lipid alterations found in the prehypertensive children magnify the importance of

Table 3: Distribution of studied children by blood pressure group

Blood pressure category	Frequency	Percentage
Normotensive	234	62.7
Prehypertensive	120	32.2
Hypertensive	19	5.1
Total	373	100.0

Table 4: Comparison of classic biochemical markers by blood pressure group

Variable	Category	n	Mean	Standard deviation	Fisher's F	p
Cholesterol mg/dL	Normotensive	234	134.1	23.5		
	Prehypertensive	120	137.5	24.7		
	Hypertensive	19	149.5	39.8		
	Total	373	136.0	25.1	3.61	0.028
Triglycerides mg/dL	Normotensive	234	85.0	36.3		
	Prehypertensive	120	78.4	37.1		
	Hypertensive	19	100.5	62.8		
	Total	373	83.7	38.0	3.08	0.047
HDL mg/dL	Normotensive	234	45.2	9.2		
	Prehypertensive	120	44.4	6.9		
	Hypertensive	19	37.4	6.3		
	Total	373	44.5	8.6	7.60	0.001
LDL mg/dL	Normotensive	234	73.1	20.8		
	Prehypertensive	120	85.7	27.4		
	Hypertensive	19	104.4	36.6		
	Total	373	78.7	25.4	22.22	<0.001
Cholesterol/HDL	Normotensive	234	2.96	0.02		
	Prehypertensive	120	3.09	0.01		
	Hypertensive	19	4.00	0.03		
	Total	373	3.05	0.02	14.27	<0.001
LDL/HDL	Normotensive	234	1.7	0.7		
	Prehypertensive	120	2.0	0.8		
	Hypertensive	19	2.9	1.2		
	Total	373	1.9	0.8	25.72	<0.001

LDL: low-density lipoprotein HDL: high-density lipoprotein

Table 5: Comparison of biochemical markers based on structural proteins, by blood pressure group

Variable	Category	N	Mean	Standard deviation	Fisher's F	p
Lipoprotein(a) mg/dL	Normotensive	234	11.6	8.5		
	Prehypertensive	120	13.1	8.1		
	Hypertensive	19	23.6	8.9		
	Total	373	12.7	8.7	18.27	<0.001
ApoA mg/dL	Normotensive	234	135.9	9.9		
	Prehypertensive	120	134.7	8.8		
	Hypertensive	19	127.3	11.8		
	Total	373	135.1	9.8	7.12	0.001
ApoB mg/dL	Normotensive	234	75.2	40.3		
	Prehypertensive	120	88.4	40.0		
	Hypertensive	19	117.7	46.2		
	Total	373	81.6	41.7	12.11	<0.001
ApoB/ApoA	Normotensive	234	0.6	0.3		
	Prehypertensive	120	0.7	0.3		
	Hypertensive	19	1.0	0.4		
	Total	373	0.6	0.3	12.91	<0.001

ApoA: Apolipoprotein A ApoB: Apolipoprotein B

this condition, underscoring the need to study lipid markers in these children and provide guidance on lifestyle changes to reduce risk of HT and CVD.

The association between serum cholesterol and blood pressure and between both variables and CVD has been confirmed by epidemiologic studies.[23,24] The higher cholesterol values we found in hypertensive and prehypertensive children are consistent with these results and alert to risk of atherosclerosis and CVD in early adulthood.

A close relationship between total serum cholesterol and CVD risk has been reported, along with the finding that changes in cholesterol concentrations through pharmacologic or lifestyle interventions are accompanied by changes in CVD incidence.[25,26] Based on these findings, clinicians and epidemiologists agree that total plasma cholesterol is a useful marker for predicting CVD.[24,27]

Although to a lesser extent than other classic biochemical indicators, triglycerides differed significantly among the groups, with higher concentrations in the hypertensive children. The lower triglyceride numbers in prehypertensive children may have been due to fat distribution, a characteristic not examined in this study. Some studies have shown that hypertriglyceridemia is more strongly associated with body fat distribution and diets rich in saturated fats than with alterations in blood pressure values.[28,29] High triglyceride values have been considered an independent CVD risk factor, although this remains a controversial issue and some authors question the independent nature of the relationship.[23,26]

The hypertensive and prehypertensive children had lower HDL values; according to the literature, therefore, they should be at higher risk of developing atherosclerosis and CVD. High HDL values are considered protective in the presence of hypertension.[28,30] Studies since the 1950s note that persons with high HDL concentrations are less likely to suffer from CVD. Coronary risk is estimated to decrease 2%–3% with a 1 mg/dL increase in HDL concentration.[23,25]

There were wide differences among the groups in terms of both LDL and the LDL/HDL ratio. These results are consistent with those of several studies showing an association between HT and high LDL, and reveal a direct association between high LDL values and CVD.[31,32]

High LDL concentrations in young adults predict appearance of CVD later in life; thus, the relationship between changes in LDL and the development of CVD is considered a continuous process that begins at an early age.[33,34] High values or an increase in values of this lipid marker in the hypertensive and prehypertensive children indicate higher risk of developing CVD in adulthood than in the normotensive children; therefore they need increased medical attention to control blood pressure and dyslipidemia.

Several studies show that high total cholesterol and LDL levels and the total cholesterol/HDL ratio are independently associated with a higher incidence of HT and CVD.[35–38] The prehypertensive and hypertensive children with elevated values for these markers in our study are at greater risk of developing CVD—one more reason to offer them specialized consultations for monitoring and followup.

Lp(a) values were higher in the hypertensive and prehypertensive children. While this is unrelated to HT in children, several studies[39–42] indicate that determining this marker helps identify children and adolescents at future risk of vascular disease, since high values are associated with higher thrombogenic capacity.

These studies also report that high Lp(a) concentrations are an independent CVD risk factor, even in the presence of normal concentrations of cholesterol and triglycerides.[40] Dalmau Serra[42] contends that the risk is higher for Lp(a) concentration in plasma above 30 mg/dL and is 2 to 3 times greater when it exceeds 50 mg/dL. Lp(a) concentrations normally show little variation throughout life, and in adulthood remain at levels similar to those in childhood. High Lp(a) concentrations have been reported in children with familial cardiovascular risk factors.[40] Determining this marker is useful, especially in children with an adverse lipid profile.

ApoA is the main constituent of high-density lipoproteins and higher levels are therefore associated with lower CVD risk.[39] ApoB is found in VLDL, LDL, IDL and lipoprotein remnants, all with atherogenic properties. Thus, high values in hypertensive and prehypertensive children are relevant when estimating the risk of CVD.[39] It has been suggested that low levels of ApoA1 and high levels of ApoB in young people, as found in the hypertensive and prehypertensive children in this study, could predict intimal thickening of the carotid artery in early adulthood and thus be associated with cardiovascular risk.[43] The ApoB/ApoA ratio accurately reflects the balance between atherogenic and antiatherogenic lipoproteins.[44,45] In the hypertensive and prehypertensive children studied, the balance favored atherogenic lipoproteins, denoting greater CVD risk.

An increase in ApoB levels and the ApoB/ApoA1 ratio, as well as a decrease in ApoA1, are better predictors of cardiovascular events than any other known risk factor, including high LDL concentrations.[46–49] Recent prospective studies show an association between ApoA1 and ApoB lipoprotein plasma levels and coronary artery disease, mainly in adults,[48,49] and suggest that the ApoB/ApoA ratio is the strongest predictor of this condition.[48,50] Studies in children are more scarce, but some supporting these results were conducted in Venezuela in 2004 by Fernández and in Spain in 2007 by Garcés and de la Oya.[51,52] This suggests that the hypertensive children in our study are at high risk of developing CVD in early adulthood.

CONCLUSIONS

The proportion of hypertensive and prehypertensive children is higher than previously reported, and their blood pressure values are associated with high values of lipid markers usually considered predictive of CVD. The most important biochemical markers associated with HT in these children were high values for LDL, LDL/HDL and ApoB/ApoA ratios and lipoprotein(a) concentrations. Our results point to the need to ensure monitoring and control of prehypertensive and hypertensive children and adolescents in Cuba.

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Normal Values of T, B and NK Lymphocyte Subpopulations in Peripheral Blood of Healthy Cuban Adults

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ABSTRACT

INTRODUCTION Quantification of lymphocyte subpopulations is useful for evaluating immune response in states of health and disease, including immunodeficiencies, autoimmunity, infections and cancer. Studies have found that concentrations and proportions of different cell subpopulations vary with geographic location, age, sex and ethnicity. Knowing the normal values of these cells and their variation in healthy populations will contribute to improved clinical practice and scientific research.

OBJECTIVE Estimate normal absolute concentrations and percentages of the most abundant lymphocyte subpopulations in peripheral blood and their relation to sex and age.

METHODS A cross-sectional analysis was conducted in 129 healthy adults, 61 men and 68 women aged 18–80 years; 89 aged <50 years and 40 ≥50 years. We included individuals who agreed to participate by written informed consent. Exclusion criteria were chronic disease, or use of tobacco, alcohol or medications that can alter immune system cell numbers and functions.

Through dual platform flow cytometry, we determined absolute and percentage values for T lymphocyte subsets CD3+, CD3+/CD4+T, CD3+/CD8+T, CD19+ B cells and CD3-/CD56+ natural killer cells in peripheral blood, using an 8-color flow cytometer. We estimated medians and the 2.5 and 97.5 percentiles and calculated the Pearson correlation coefficient to evaluate associations. Significance tests were also used to compare groups. The significance threshold was p = 0.05 in all cases.

RESULTS Ranges of absolute values and percentages (%) were: total lymphocytes: 1200–3475 cells/μL (20.2–49.3); CD3+ T cells: 880–2623 cells/μL (56.5–84.7); CD3+/CD4+ T cells: 479–1792 cells/μL (30.3–55.7); CD3+/CD8+ T cells: 248–1101 cells/μL (13.2–42.9); CD19+ B cells: 114–1491 cells/μL (5.4–49.5); CD3-/CD56+ natural killer cells: 70–652 cells/μL (3.7–28.0); and the CD4+:CD8+ index: 0.80–3.92. Absolute numbers—but not percentages—of lymphocytes and CD3+ T cells were higher in those <50 years (p = 0.025 and 0.020, respectively). Absolute values and relative percentages of CD3+/CD8+ and relative values of CD3+/ CD4+ T cells were significantly higher in the younger subgroup (p = 0.004 and p = 0.047). Age was not associated significantly with B lymphocytes or natural killer cells. Absolute and relative values of CD3+/CD4+ T lymphocytes were significantly higher in women (p = 0.009 and 0.036, respectively).

CONCLUSIONS. Absolute numbers of total lymphocytes and T and CD3+/CD8+ T lymphocytes are higher in younger individuals. In percentage values, CD3+/CD4+ T lymphocytes are lower in older persons. Absolute and percentage values of CD3+/CD4+ T phenotype are higher in women. These differences justify adjusting clinical analyses to different values by age and sex.

KEYWORDS T lymphocytes, B lymphocytes, normal values, flow cytometry, age, sex, Cuba

INTRODUCTION

Quantification of lymphocyte subpopulations by flow cytometry is considered the most exact and reliable procedure to assess immunocompetence, which allows the body to maintain homeostasis against invading pathogens and the body's own damaged cells that would otherwise have adverse health effects. Numerous diseases are associated with alterations in peripheral blood lymphocyte subpopulations, including primary or congenital immunodeficiencies, in which certain lymphocyte subpopulations are absent or reduced; secondary immunodeficiencies including HIV infection, which destroys the CD4+ T cell subpopulation; systemic autoimmune diseases; infections; and cancer. Quantification of lymphocyte subpopulations is necessary in these clinical conditions to establish diagnosis, and as a criterion of disease progression and treatment optimization.[1–3]

Normal or reference values of lymphocyte subpopulations obtained in several countries show association with ethnicity, sociodemographic factors (including age and sex) and environmental factors such as exposure to infectious agents.[4–6] Interpretation of lymphocyte subpopulation data for clinical practice requires establishing normal ranges for local populations. These ranges have not been defined for the Cuban population.

IMPORTANCE This study takes a first step toward establishing normal values of the most abundant lymphocyte subpopulations in peripheral blood of healthy Cuban adults using flow cytometry as a basis for more accurate clinical diagnosis of immune disorders in Cuba.

The study objective was to estimate absolute and relative values of peripheral lymphocyte subpopulations of CD3+, CD3+/CD4+, CD3+/CD8+ T cells, CD19+ B cells, CD3-/56+ natural killer cells, the CD4+:CD8+ index and the variation in their distribution by age and sex in healthy Cuban adults.

METHODS

Design, subjects and sample A cross-sectional study was conducted from January through April 2017 in 129 healthy adults, 61 men and 68 women, aged 18–80 years, of whom 89 were <50 and 40 were ≥50 years. Average age was 43.5 for men and 37.8 for women. The proportion of men and women was similar in both age groups (p = 0.172). Participants were hospital workers and patient companions recruited in the clinical laboratory of the Hermanos Ameijeiras Clinical-Surgical Teaching Hospital in Havana (HCQHA), Cuba. After agreeing to participate on the basis of written informed consent, they completed a questionnaire requesting information on demographic characteristics (age, sex, and schooling), lifestyle, medication consumption and personal medical history. Subjects with the following characteristics were excluded: present tobacco or al-

cohol use, treatment with immunosuppressive and immunomodulatory drugs in the previous six months, pregnant women, and those with acute or chronic infectious diseases such as HIV, hepatitis B, hepatitis C and tuberculosis; chronic metabolic diseases; and cardiac, pulmonary, renal, cerebral, systemic, allergic and neoplastic autoimmune diseases.

Peripheral blood samples were obtained by antecubital venous puncture, collected in vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA) as anticoagulant, and processed by flow cytometry within four hours following best laboratory practices. Absolute and differential blood cell count was calculated for all samples using an automated hematology analyzer (Horiba Medical, France).

Flow cytometry Lymphocyte subpopulations were determined in whole peripheral blood, lysed using a Versalyse buffer (Beckman Coulter, France) without washing. For immunophenotyping using fluorochrome conjugated monoclonal antibodies anti-CD45 AA750 (Clone J33), anti-CD19-PC7 (Clone J3-119), anti-CD3 FITC (Clone UCHT1), anti-CD4 PC5.5 (Clone 13B8.2), anti-CD8 AA700 (Clone B9.11) and anti-CD56 PE (Clone N901) (NKH-1), 100 µL of blood were dispensed. Sample preparation was carried out according to manufacturer guidelines.

Lymphocyte subpopulations were characterized by combining monoclonal antibodies for T, B and NK cells:
T cells: (CD45+, CD3+); (CD45+, CD3+, CD4+); (CD45+, CD3+, CD8+)
B cells: (CD45+, CD19+)
NK cells: (CD45+, CD56+, CD3-).

Sample collection was carried out with an 8-color Gallios flow cytometer (Beckman Coulter, France), using Kaluza Acquisition software for Gallios, Version 1.0 (Beckman Coulter, France). For each sample, a minimum of 10,000 events were collected (referring to number of cells in the sample of lysed red blood cells passing the cytometer's laser beam for counting and analysis).

In each case, more than 4,000 events were obtained in the lymphocyte gate, characterized by high expression of CD45 and low side-scatter complexity (SSC). Figure 1 shows the gate strategy with a dot plot, which starts by eliminating doublets (cells passing the interrogation point in groups), followed by a plot removing artifacts and detritus (forward scatter vs. side scatter) and from which the lymphocyte population was selected. Plots were generated from this lymphocyte region by combining two of the antigens expressed on the cell surface with separation by quadrants, allowing identification and determination of percentage of subpopulations.

Data were analyzed with Kaluza Analysis software V 1.2 (Beckman Coulter, France). Absolute counts of lymphocyte subpopulations were calculated by dual platform, from percentages obtained by flow cytometry and lymphocyte counts obtained by hematological analyzer (Horiba Medical, Montpellier, France) using the formula:

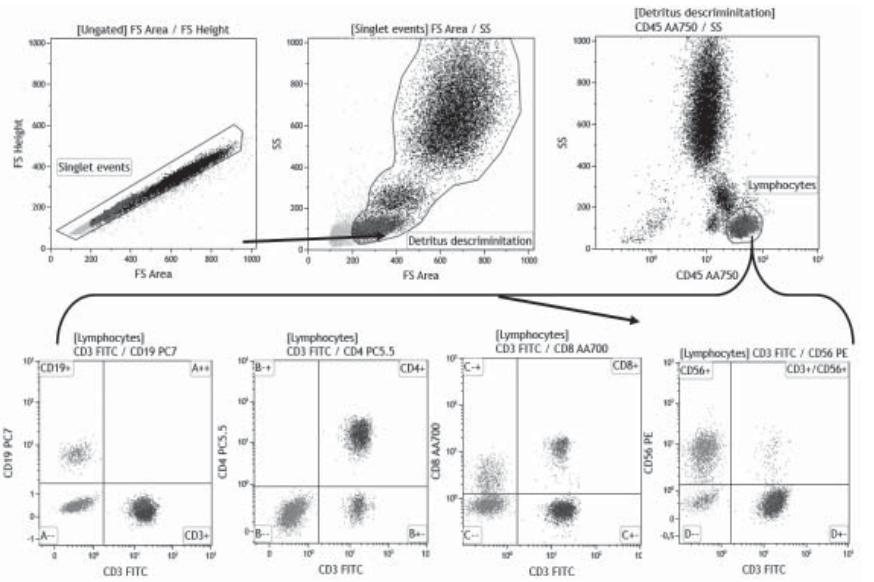
Absolute count (cells/µL) = Lymphocyte count (cell number/µL of the blood count) x proportion of the cell subpopulation of interest ÷ 100.

Internal quality control was performed daily by checking the cytometer's optical detector and aligning lasers and fluid systems using Flow-SET Fluorospheres and Flow-Check Fluorospheres (Beckman Coulter, France), respectively, according to manufacturer guidelines.

Statistical analysis Descriptive statistics were calculated for absolute and relative frequencies, means, medians, standard deviations (SD) and reference ranges of percentage and absolute values for each cell marker. The Kolmogorov-Smirnov test was used to test the normal distribution of variables. Ref-

Figure 1. Gate strategy

a) Protocol design for T, B and NK phenotypes Starting point must be the dot plot, from which coinciding events are eliminated and another plot is prepared to eliminate detritus. This one, in turn, is used to construct the dot plot in which the lymphocyte region is defined. From this region, plots are constructed to define the four subpopulations: T, B, CD4 +T, CD8 +T and NK lymphocytes.



b) Hierarchical matrix for reporting percentage distribution of lymphocyte subpopulations

Control 063 M63-2017-02-02-122648-8

Gate	% Gated
All	100.0
Singlet events	98.4
Detritus descriminitation	81.5
Lymphocytes	28.1
CD19+	8.0
CD3+	55.7
CD4+	41.4
CD56+	27.5
CD8+	13.7

(available in color online at: www.mediccreview.org/gate-strategy)

erence ranges were defined as 2.5–97.5 percentiles. The Student t test was applied for variables with Gaussian distribution and the two-tailed Mann Whitney U test for independent samples to evaluate the influence of sex and age on peripheral lymphocyte concentrations. Age association with cell values was calculated using the Pearson correlation coefficient. The significance threshold was p = 0.05. Statistical analysis was done with SPSS v 20.0 software for Windows XP.

Ethics The project was approved by the HCGHA research ethics committee. All participants were asked if they were willing to participate and joined the study only after providing written informed consent, in compliance with the Helsinki Declaration.[7] The consent form explained the importance of participating, characteristics of the research, and possible risks and benefits. Data storage adhered to principles of confidentiality and data were de-identified. The selection of diagnostic means followed principles of maximum benefit, the ethics rule of do no harm, and best laboratory practices.

RESULTS

Means, standard deviations, medians and reference ranges of absolute values and percentages of lymphocyte subpopulations are shown in Table 1. Absolute values are expressed in number of cells per microliter and percentages refer to relative frequencies of each subpopulation in relation to total lymphocytes.

Table 2 shows ranges of lymphocyte subpopulations in absolute numbers, age ranges and sex composition obtained for Cuban adults in this study and in studies conducted in five countries, where differences can be observed in cell concentrations as well as technologies and platforms used.

Table 1: Ranges for peripheral blood lymphocyte subpopulation values in healthy Cuban adults (N = 129)

Parameter	Mean (SD)	Median	Percentile range 2.5–97.5
Lymphocytes (cells/µL)	2177 (573)	2200	1200–3475
%	33.8 (7.5)	32.3	20.2–49.3
CD3+ T cells (cells/µL)	1579 (446)	1508	880–2623
%	72.6 (7.3)	73.0	56.5–84.7
CD3+/CD4+ T cells (cells/µL)	945 (292)	904	479–1792
%	43.6 (7.0)	43.8	30.3–55.7
CD3+/CD8+T cells (cells/µL)	583 (232)	540	248–1101
%	26.7 (7.3)	26.0	13.2–42.9
CD19+ B cells (cells/µL)	559 (368)	452	114–1491
%	25.0 (12.8)	25.6	5.4–49.5
CD3-/CD56+ NK cells (cells/µL)	297 (166)	256	70–652
%	13.7 (6.5)	12.7	3.7–28.0
CD4+:CD8+ index	1.81 (0.73)	1.73	0.80–3.92

SD: standard deviation

Table 2: Lymphocyte subpopulation values (percentile 2.5–97.5, cells/µL) in selected populations of healthy adults, by country

	Cuba (this study)	Brazil (8)	Peru (9)	Korea (11)	Germany (4)	Tanzania (10)
n	129	641	318	294	100	274
Men/women (n)	61/68	404/237	197/121	139/155	50/50	143/131
Age (years: mean)	41	33	30	47	43 [a]	29; 26 [b]
Age range (years)	18–80	19–56 [c]	18–55	21–80	19–85	19–48
Technology/ platform	Gallios/ dual	FACS Calibur/ single	Cytomics/ single	FACS Calibur/ single	FACS Calibur/ dual	FACS Calibur/ dual
Lymphocytes	1200–3475	1257–4104	na	1178–3262	1140–3380	1168–3320
CD3+ T cells	880–2623	1025–2904	818–2838	708–2294	780–2240	683–2106
CD3+/CD4+ T cells	479 –1792	540–1731	436–1510	394–1574	490–1640	406–1392
CD3+/CD8+ T cells	248–1101	263–1189	236–982	188–830	170–880	188–990
CD19+ B cells	114–1491	na	151–730	57–461	80–490	109–637
NK cells	70–652	na	139–789	91–682	80–690	123–801
CD4+:CD8+ index range	0.80–3.92	na	0.84–3.06	0.77–4.42	0.9–5.0	0.83–3.19

a: median; b: median of men and women respectively; c: Interquartile range; na: not available

Percentages and absolute values of cell subpopulations were related to age. Regression analysis showed that absolute lymphocyte counts (r = -0.265; p = 0.002), CD3+ T cells (r = -0.314; p = 0.0001) and CD3+/CD8+ (r = -0.326; p = 0.0001) significantly decrease with age; while the CD4+:CD8+ index significantly increases (r = 0.263, p = 0.003)

The total number of lymphocytes and other cells in the T cell compartment were associated with age, with lower absolute values of CD3+ T and CD3+/CD8+ T cells in the older age group. The CD3+/CD4+ T cell fraction had higher concentrations in participants aged ≥50 years. CD19+ B lymphocyte and CD3-/CD56+ NK cell values did not change with age (Table 3).

Absolute numbers and percentages of CD3+/CD4+ T cells were higher in women (p = 0.009 and p = 0.036, respectively). The CD4+:CD8+ index had higher values in women (p = 0.043); there were no differences by sex for CD3-/CD19+ B lymphocytes and CD3-/CD56+ NK cells (Table 4).

DISCUSSION

This is the first study reporting normal absolute and percentage values for T lymphocytes and their subpopulations, as well as B lymphocytes and NK cells, in peripheral blood of healthy Cuban adults. Because clinical diagnoses based on measures drawn from local populations are more reliable, the study's results are a first step in the application of domestic data to diagnosis and clinical management of Cuban adults with diseases causing alterations of peripheral lymphocyte subpopulations.

Some authors have demonstrated the influence of ethnicity and certain sociodemographic characteristics on the composition of

Table 3: Absolute values and percentages of lymphocyte subpopulations in healthy Cuban adults by age groups

Parameter	Mean (SD)		Percentile range 2.5–97.5		
	<50 years	≥50 years	<50 years	≥50 years	p
	n = 89	n = 40	n = 89	n = 40	
Lymphocytes (cells/µL)	2257 (531)	2000 (626)	1300–3475	1200–3886	0.025
%	33.9 (7.4)	33.6 (7.9)	20.2–51.0	18.0–49.6	NS
CD3+ T cells (cells/µL)	1642 (433)	1441 (449)	859–2795	912–2625	0.020
%	72.7 (7.5)	72.5 (6.9)	56.5–85.0	55.3–83.3	NS
CD3+/CD4+ T cells (cells/µL)	959 (265)	913 (346)	472–1766	475–2125	NS
%	42.7 (6.7)	45.5 (7.6)	29.9–54.0	31.0–65.9	0.047
CD3+/CD8+ T cells (cells/µL)	620 (238)	501 (198)	248–1110	224–960	0.004
%	27.1 (7.2)	25.6 (7.5)	14.6–43.8	12.8–39.4	NS
B CD19+ cells (cells/µL)	578 (377)	518 (349)	126–1491	83–1640	NS
%	24.9 (13.2)	25.3 (12.2)	5.4–50.0	4.6–54.7	NS
NK cells CD3-/CD56+ (cells/µL)	305 (169)	281 (161)	67–652	76–760	NS
%	13.6 (6.5)	14.0 (6.7)	3.7–27.0	3.0–31.6	NS
CD4+:CD8+ index	1.72 (0.64)	2.0 (0.88)	0.75–3.56	0.79–4.70	NS

SD: standard deviation; NS: not significant

Table 4: Absolute values and percentages of lymphocyte subpopulations in healthy Cuban adults by sex

	Mean (SD)		Percentile range 2.5–97.5		
	Men	Women	Men	Women	p
	n = 61	n = 68	n = 61	n = 68	
Lymphocytes (cells/µL)	2093 (597)	2251 (543)	1200–3445	1173–3755	NS
%	32.6 (7.5)	34.9 (7.5)	19.4–47.9	20.9–52.9	NS
CD3+T cells (cells/µL)	1521 (487)	1632 (402)	863–2766	872–2695	NS
%	72.5 (7.8)	72.7 (6.8)	55.2–85.9	59.1–83.9	NS
CD3+/CD4 T cells (cells/µL)	875 (263)	1007 (304)	484–1683	446–1977	0.009
%	42.2 (6.2)	44.8 (7.6)	28.7–54.7	30.0–61.9	0.036
CD3+/CD8+T cells (cells/µL)	594 (267)	574 (197)	202–1194	259–1031	NS
%	27.8 (7.6)	25.7 (6.9)	13.3–42.6	1 2.4–44.3	NS
CD19+ B cells (cells/µL)	570 (409)	550 (330)	84–1664	128–1229	NS
%	25.6 (12.8)	24.5 (13.0)	5.4–55.5	5.1–48.6	NS
NK cells CD3/CD56+ (cells/µL)	304 (146)	291 (184)	63–612	74–856	NS
%	14.9 (6.6)	12.7 (6.3)	3.1–29.8	3.4–28.3	NS
CD4+:CD8+ index	1.67 (0.61)	1.93 (0.81)	0.76–3.39	0.78–4.54	0.043

SD: standard deviation; NS: not significant

lymphocyte subpopulations. Regional differences have been reported within large countries such as Brazil, China and India. [12–14] When we compare lymphocyte values between our study and those of other populations in America, Europe, Asia and Africa, differences between countries are observed which could be attributed not only to biological or sociodemographic factors such as age, sex and ethnicity, but also to environmental factors such as exposure to infectious agents, air pollution and lifestyles.[13–17] Taking into account the influence of environmental and sociodemographic factors and their changes over time, specific ranges of lymphocyte subpopulations should ideally be determined by geographically localized populations and updated through periodic re-evaluation.[11–18]

Average CD3+, CD3+/CD4+ and CD3+/CD8+ T lymphocyte subpopulations in our study are closer to those of other countries and areas of the American continent, such as south Florida (US),[17] Brazil,[8] and Peru,[9] than to those of Korea,[11] Germany, [4] Tanzania,[10] and China[14], which confirms the role of geographic, ethnic and lifestyle factors and exposure to infectious agents in modulating the values of peripheral lymphocytes and the immune system.[14,15,17,18] Determination of reference values for lymphocytic subpopulations by region and country makes for more reliable data interpretation in clinical cases.[8,12–14,18] The reliability of normal CD3+/CD4+ lymphocyte subpopulation values takes on special clinical importance in prognosis and treatment strategies for patients infected with HIV-1.[19]

Average absolute and percentage values of CD19+ B cells in our study were higher than those reported in south Florida (US),[18] Korea,[11] Germany,[4] Tanzania,[10] and China.[14] One explanation for differences in lymphocyte subpopulation distribution is variations in infectious agent exposure, even within the same geographic region.[15,18] The greater expansion of peripheral B cells in our study participants could be associated with the presence of arboviral diseases, especially dengue virus, in this area. Dengue virus is endemic in most tropical and subtropical regions and the vast majority of infections are asymptomatic.[20] It has been reported that individuals exposed to dengue virus have abnormally high levels of activated B cells,[21] not only due to the clonal expansion of virus specific B cells, but also to virus-induced nonspecific polyclonal activation.[22]

Change in the number and composition of different lymphocyte subpopulations in peripheral blood is a distinctive characteristic of immune system aging. Numerous studies have shown the relationship between age and lymphocytic subpopulation concentrations.[4,5,11,14,23,24] Several authors have reported lower levels of CD3+ and CD3+/CD8+ T lymphocytes in the elderly.[4,5,11,14, 24,25] Decreased effectiveness of senescent immune response reflects degeneration of lymphoid cell development and function at various levels. A lower potential of hematopoietic cells for lymphoid differentiation decreases the formation of immune cells.[26] T cell-mediated immunity has been shown to be especially susceptible to aging due to decline in the number and diversity of naïve T cells affected by involution of the thymus, a primary lymphoid organ crucial for T lymphocyte generation and maturation.[27]

Reasons for decline of CD3+ and CD3+/CD8+ T cells in younger persons are still unknown; unlike CD3+/CD4+ T cells, whose values remained stable in older individuals in this study.[24] In each T cell subpopulation, naïve cell production decreases and memory-cell accumulation increases with age. But the combinations of decreased naïve lymphocyte production and the accumulation of memory cells may be different within the CD3+/CD4+ and CD3+/CD8+ subpopulations depending on total lymphocyte production and interactions with the environment over time.[28,29]

Depletion of a T cell compartment may occur at senescence in response to persistent infections. Stervbo found higher numbers of CD4+ T cells specific for cytomegalovirus (CMV) in individuals

aged 53–67 years, whereas presence of CMV-specific CD8+ T cells was not modified by age.[28] The differences in stability of the CD3+/CD4+ and CD3+/CD8+ T cells in relation to age could be attributed to these two subpopulations being regulated by different growth factors and cytokines.[30]

Previous studies reported a reduction in the CD4+:CD8+ index with increasing age, which constitutes the Immune Risk Profile associated with morbidity and mortality in old age.[31,32] Some investigations attribute the reduction in the CD4+:CD8+ index to immune response against CMV and have shown a negative association between the CD4+:CD8+ index and CMV seropositivity in old age.[33] Others observe no decrease in the CD4+:CD8+ index with respect to age, but do report a negative correlation between this ratio and CMV seropositivity.[29] In this study we did not find reduction of the CD4+:CD8+ index in older individuals, so the decline reported in the literature could be a characteristic of older adults infected with CMV.

Comparison of lymphocyte subpopulation levels between men and women found CD3+/CD4+ T cells were more numerous in women. Higher levels of this subpopulation in Cuban women in relation to men were more marked in absolute values than in the fraction of CD3+CD4+ T cells. Higher values of CD3+/CD4+ cells have been observed in women compared to men in different populations of Brazil,[8] Spain,[17] Korea, [11] France,[34] Israel,[23] and India.[6] Although no differences were found in lymphocyte subpopulations between men and women in a study of the south Florida (US) population, the CD4+:CD8+ index was higher in women.[18] For this to occur there should be more CD4+ cells or fewer CD8+ cells.

Absolute values of activated T cells within the CD3+ subpopulation (CD3+/HLA-DR/CD25) were also higher in Cuban women.[35] Unequal distribution of lymphocyte subpopulations between men and women and in particular the higher frequency of CD4+ T cells in women may explain their greater resistance to infectious diseases and greater likelihood of developing autoimmune diseases.[36,37] The difference in values of CD3+CD4+ cells can be attributed to the effect of sex hormones on their specific receptors expressed in T and B lymphocytes.[38]

Sex chromosome genes and sex hormones such as estrogen, progesterone and androgens contribute to male/female differences in immune response.[39] Estrogen is known to enhance both cell-mediated and humoral immune responses, while androgens suppress the differentiation of Th1 cells, and reduce gamma interferon (IFN-γ) production and B-cell antibody responses.[40,41] In general, women develop stronger humoral and cellular responses than men, associated with higher levels of antibodies and other immune mediators such as IL-1, IL-4 and IFN-γ.[42] Nutrition and microbiome composition also determine differences in development and functioning of immune response in men and women.[39]

We note several limitations of our study. The sample included only residents of Havana and none from other Cuban provinces. Additional serologic studies were not carried out, leaving open a possible sub-diagnosis of chronic and infectious diseases. Absolute values were calculated on a dual platform, which could be the source of inter-laboratory variability. However, no difference has been demonstrated between single or dual platform results.[43]

It would be advisable to study peripheral B cell numbers in a larger sample of healthy Cuban individuals including different areas of the country to determine the possible relationship with regional and individual factors that may vary according to genetic ancestry, living conditions and lifestyles (e.g., nutrition, smoking) and air pollution. The composition of B cells in terms of memory cells and plasmablast proportions in Cuban individuals with high and low peripheral B cell levels should also be determined.

CONCLUSIONS

Absolute and percentage concentrations of lymphocyte subpopulations found in the Cuban sample were different from those obtained in other countries. Absolute and percentage values of the CD3+/CD4+ T phenotype are higher in women. Absolute values of total lymphocytes and of CD3+/CD8+ T lymphocytes are higher in younger participants. In percentage values CD3+/CD4+ T lymphocytes have lower values in older subjects. These differences justify adjusting clinical studies to different values by age and sex. The study supports improved diagnosis in Cuba, since it provides a new, albeit preliminary, set of reference values.

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Corrected QT-Interval Dispersion: An Electrocardiographic Tool to Predict Recurrence of Myocardial Infarction

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ABSTRACT

INTRODUCTION Many clinical settings lack the necessary resources to complete angiographic studies, which are commonly used to predict complications and death following acute coronary syndrome. Corrected QT-interval dispersion can be useful for assessing risk of myocardial infarction recurrence.

OBJECTIVE Evaluate the relationship between corrected QT-interval dispersion and recurrence of myocardial infarction in patients with ST-segment elevation.

METHODS We conducted a prospective observational study of 522 patients with ST-segment elevation myocardial infarction admitted consecutively to the Camilo Cienfuegos General Provincial Hospital in Sancti Spiritus, Cuba, from January 2014 through June 2017. Of these, 476 were studied and 46 were excluded because they had other disorders. Demographic variables and classic cardiovascular risk factors were included. Blood pressure, heart rate, blood glucose, and corrected and uncorrected QT-interval duration and dispersion were measured. Patients were categorized according to the Killip-Kimball classification. Association between dispersion of the corrected QT-interval and recurrence of infarction was analyzed using a binary logistic regression model, a regression tree and receiver operator characteristic curves.

RESULTS Patients with recurrent infarction (56; 11.8%) had higher average initial blood glucose values than those who did not have recurrence; the opposite occurred for systolic and diastolic blood pressure and for left ventricular ejection fraction. Dispersion of the corrected QT-interval was a good predictor of infarction recurrence according to a multivariate analysis (OR = 3.09; 95% CI = 1.105–8.641; p = 0.032). Cardiac arrest is the variable that best predicts recurrence. No recurrence of infarction occurred in 97% of patients without cardiac arrest, left ventricular ejection fraction >45% and corrected QT-interval dispersion <80 ms.

CONCLUSIONS Risk of infarction recurrence is low in patients without cardiac arrest, with left ventricular ejection fraction >45% and with dispersion of corrected QT-interval <80 ms. Patients with corrected QT-interval dispersion ≥80 ms have greater risk of recurrence of infarction, which suggests that this variable could be used for stratification of risk following ST-segment elevation myocardial infarction.

KEYWORDS ST-elevation myocardial infarction, myocardial infarction, electrocardiography, chronic disease, risk assessment, Cuba

INTRODUCTION

Cardiovascular diseases are the most frequent cause of death worldwide. Eighty percent of deaths due to heart attacks occur in middle- and low-income countries.[1] In Cuba, the heart disease mortality rate was 241.6 per 100,000 population in 2017; the rate for ischemic heart disease was 156.7, including mortality of chronic cases and from acute episodes; mortality rate from myocardial infarction (MI) was 71 per 100,000 population. In 2017, Sancti Spíritus Province had a crude mortality rate from heart disease of 231 per 100,000 population, age adjusted to 100.8 per 100,000 population.[2]

Among patients with acute coronary syndrome (ACS) the proportion with ST-segment elevation myocardial infarction (STEMI) ranges from 29% to 47%. Moreover, STEMI is the most severe of MI.[3] Although STEMI frequency is generally decreasing,[3] risk of death and complications following a STEMI is high despite diagnostic and treatment advances. In-hospital fatality varies from 4% to 12% for European Union countries, where 1-year mortality among STEMI patients is 10%.[4,5] Post-STEMI readmission rates are high, about 15.4%, with 26.6% of readmissions due to recurrent ischemia.[6]

IMPORTANCE Easily measured with equipment readily available even in low-resource clinical settings, corrected QT-interval dispersion following ST-elevation myocardial infarction offers a reliable and simple predictive tool for assessing myocardial infarction recurrence risk.

Prognosis for STEMI patients relates to the probability of developing short- or long-term complications and depends more on conditions upon admission than on prior coronary risk factors.[7–10] According to international MI treatment guidelines, conditions associated with poor outcomes are advanced age, development of some degree of heart failure, decreased ventricular function, diabetes, treatment strategy and type of hospital where the patient is treated.[5,11] Brogan[12] describes multiple models for stratifying risk of death and complications following MI that include variables such as troponin levels and coronary angiography data, but these are not always available in internal medicine and cardiology services in middle- and low-income countries.[13,14]

Acute myocardial ischemia changes QT-interval (QTi) duration. Although the causal mechanisms are controversial, a rise in repolarization heterogeneity of the ventricular myocardium increases the difference between maximum and minimum QTi, referred to as QT-interval dispersion (QTd).[15,16] QTi is measured by electrocardiogram (ECG) from initiation of the QRS complex to the point where the T wave returns to the isoelectric line. This interval corresponds to potential action duration, and includes ventricular depolarization and repolarization.[16–18] Corrected QTi (QTc) is the duration of this parameter, adjusted for heart rate.[17]

The dispersion of corrected QT-interval (QTdc) measures severity of coronary artery damage.[19–22] Values >59 ms have been associated with myocardial viability,[23] which makes QTdc a plausible predictor of MI recurrence. Higher QTdc is related to complications such as malignant ventricular arrhythmias,[24–26] but its relationship to recurrent ischemia has been less studied.

In a 2007 study, Kenigsberg[27] modified the classic ischemic cascade concept by demonstrating that the first indication of coronary occlusion is QTc prolongation. Acute ischemia causes an increase in potassium concentrations and shortening of repolarization time that leads to slow conduction and decreased excitability. Response to this damage is greater in the subepicardium than in the subendocardium and causes repolarization dispersion. Lack of homogeneity and increased spatial dispersion of repolarization results in increased QTdc in patients with ischemic heart disease.[16,28] Such physiological and pathological effects of acute ischemia support using QTdc as an important predictor of MI recurrence. Moreover, QTdc is obtained from surface ECG and is a simple, low-cost tool that can be useful in assessing risk of MI recurrence in STEMI cases, especially in low- and middle-income countries.

Recurrent MI, defined as a repetition of the signs and symptoms of acute heart failure in the first 28 days following an initial MI, carries a worse prognosis, including increased risk of death.[29] In recurrent MI, there is reocclusion of the affected artery, whether from initial non-reperfusion or associated with thrombosis from an implanted stent. Nowinski[30] demonstrated that when inflating the balloon during percutaneous coronary intervention (PCI) in patients with myocardial ischemia, immediate changes occur in ventricular repolarization and QTi is prolonged. Such changes persist for minutes and even hours. These findings suggested that QTi could be used as an early marker of acute and transitory myocardial ischemia, easily detected on a surface ECG, and useful for prognosis in middle- and low-income countries where therapeutic and diagnostic alternatives described in international guidelines are not always available.[4,11]

The objective of this paper is to evaluate the relationship between QTdc and MI recurrence in patients with ST-segment elevation myocardial infarction.

METHODS

Design and study population We conducted a prospective observational study of all STEMI patients admitted to the coronary care unit of Camilo Cienfuegos General Provincial Hospital (HGPCC) in Sancti Spíritus, Cuba, January 1, 2014 through June 30, 2017. The study enrolled 522 patients, of which 46 were excluded later for the following reasons: 13 for left bundle branch block; 11 for previous atrial fibrillation; 14 receiving pharmacological treatments that prolong QTi; and 8 with life expectancy less than 1 year due to non-cardiac conditions that could trigger MI recurrence. The final study group consisted of 476 patients with an average age of 67.4 years (SD = 13.8); 304 (63.9%) were men.

STEMI was diagnosed by pain typical of heart failure with new ST-segment elevation >0.2 mV, measured from point J on ≥2 precordial leads, or 0.1 mV on ≥2 standard leads.[4,11,29] Recurrence was diagnosed by the same criteria within the first 28 days following initial MI.[29]

Variables Demographic variables were age, sex and skin color (white, mestizo/mixed or black). Cardiovascular risk factors were hypertension (HT), prior ischemic heart disease, lipid metabolism disorders (cholesterol >6.71 mmol/L and triglycerides >1.60 mmol/L in women and >1.88 mmol/L in men, according to established reference values), smoking, history of diabetes, and obesity (defined as body mass index >30 kg/m^2). Clinical variables were systolic and diastolic blood pressure (BP) and heart rate on admission.

The Killip-Kimball[31] classification was used to assess the degree of acute heart failure according to the following criteria:

1) Class I. No heart failure. No clinical signs of cardiac decompensation.
2) Class II. Heart failure. Diagnostic criteria include rales, third heart sound gallop and pulmonary venous HT, and pulmonary congestion with wet rales in the lower half of the lung fields.
3) Class III. Severe heart failure. Obvious pulmonary edema with rales in all lung fields.
4) Class IV. Cardiogenic shock. Clinical signs include hypotension (systolic BP <90 mmHg) and evidence of peripheral vasoconstriction, such as oliguria, cyanosis and sweating.

Laboratory variables were hemoglobin, blood glucose, leukogram, creatinine and creatine kinase (CPK); CPK was repeated at 6, 12, 24 and 48 hours, and the maximum value was used. Venous blood samples taken within 24 hours of patient admission during initial MI were processed with the High Technologies COBAS c311 automated analyzer (Hitachi,Tokyo, Japan).

Reperfusion strategy was thrombolysis with 1,500,000 IU intravenous Heberkinasa (recombinant streptokinase, Heber Biotec SA, Cuba).[32] In no case was primary coronary intervention performed as established by international guidelines for MI treatment, as no hemodynamic service was available.[4,11] Infarction location was determined by admission ECG and classified using Bayés de Luna's criteria (large anterior, mid-anterior, apical anterior, septal, inferior, inferolateral and lateral wall).[33] Complications studied were new-onset atrial fibrillation determined by surface ECG, cardiac arrest on admission, and death. Once hemodynamic stability was attained without signs of hypotension, extreme bradycardia or arrhythmias that could endanger the patient's life, a transthoracic echocardiogram was performed using the ProSound Alpha 5 (ALOKA, Japan), and left ventricular ejection fraction (LVEF) was determined by the biplane Simpson method.[34]

Electrocardiographic variables A 12-lead ECG was performed upon admission before thrombolysis, repeated after 90 minutes and then every hour for the first 6 hours. Electrocardiographic variables were based on the first ECG in non-thrombolysed cases and on the 90-minute ECG in the other patients. ECG was recorded at a sweep speed of 25 mm/s with 10 mm/mV standardization using a CardiocidBB electrocardiograph (Central Digital Research Institute, Cuba)[35] with a band-pass filter that restricts frequencies to a spectrum of 0.05–150 Hz, and a comb filter for hum at 60 Hz. Two observers manually and independently measured the following parameters on all ECG leads with a magnifying lens:[19,20,36]

1) QTi: QT interval, corresponding to the time in milliseconds from initiation of QRS complex to T wave termination, defined as the point when the T wave returns to the isoelectric line, or the nadir between T and U waves whenever the latter was

present.[16,17] It was measured for all leads and the average calculated;
2) QTc: QT interval corrected following Bazett's formula;[18]
3) QTd: Difference between the maximum and minimum QTi measured on 12 ECG leads; and
4) QTdc: Difference between corrected maximum and minimum QTi measured on the 12 ECG leads.

Data collection settings and procedures Patient assessment and followup were carried out by cardiologists. Hospital stays lasted five to seven days. Followup was done for one month after discharge, with hospital outpatient visits on days 15 and 28. MI recurrence was diagnosed during this period. Data were collected on forms that included study variables.

Data analysis A database was created in SPSS statistical software, version 21.0 for Windows. Continuous data were summarized with means (m) and standard deviations (SD). Absolute numbers and percentages were used for categorical data. The Kolmogorov-Smirnov test was used to verify distribution normality. Comparison of quantitative variables among groups, when these followed a normal distribution, was done with the Student t test for independent samples; if distribution was not normal, the non-parametric Mann–Whitney U test was used.

To verify strength of association among qualitative variables (sex, skin color, risk factors, Killip-Kimball class, reperfusion strategy and complications), the non-parametric Pearson chi square test was used. To measure association between a continuous quantitative variable (QTdc) and an ordinal qualitative variable (Killip-Kimball class), the Spearman correlation coefficient was used. For all statistical tests, a significance threshold of p = 0.05 was applied.

To obtain the QTdc cutoff point with best metric properties (sensitivity and specificity), a receiver operator characteristic curve was constructed. With these results, a value of 80 ms was determined and used to dichotomize the variable and include it in a logistic regression model together with other binary predictors (cardiac arrest, systolic BP ≤100 mmHg, LVEF ≤45%, Killip-Kimball Class II- IV, blood glucose ≥11 mmol/L, and large anterior MI). Epidat 3.1 statistical software was used to calculate sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for MI recurrence of a QTdc greater or lesser than 80 ms.

To assess the independent role of QTdc in prediction of recurrent MI, a binary logistic regression model was fitted, in which MI recurrence was considered the dependent (dichotomous) variable. Estimated coefficients were expressed as odds ratios (OR) with their respective 95% confidence intervals (95% CI). For inclusion of covariates in the logistic regression model together with QTdc, three criteria were applied: clinical or anatomic functional significance, statistical significance in the prior bivariate analysis and a principle of parsimony (Occam's razor) to prevent inclusion of redundant variables. The variables included were cardiac arrest on admission, QTdc, systolic BP, LVEF, Killip-Kimball class, location of infarction and blood glucose. To identify whether QTdc contributes predictive capacity when cardiac arrest has occurred and LVEF values are <45%, a regression tree model was constructed using variables included in the logistic model.

Ethics The study design respected the Helsinki Declaration principles[37] and was approved by the HGPCC's ethics committee. Each patient was informed of the study details and their written consent was obtained; in extremely serious cases or loss of consciousness, an immediate relative provided written informed consent. The study design did not include manipulation of variables, and the hospital's established MI treatment protocol was observed. To respect privacy and confidentiality, databases used coded information, without names or patient identifiers.

RESULTS

MI recurrence was seen in 56 (11.8%) patients; 17 (30.4%) had recurrence before hospital discharge, and the remainder within the following 28 days. Average age and sex distribution were similar in both groups. Relative frequency of recurrence did not differ by skin color. Nor did cardiovascular risk factors (HT, diabetes, dyslipidemia, tobacco use, prior ischemic heart disease and obesity) differ between patients with and without MI recurrence.

Reperfusion by thrombolysis was performed in 82.1% of patients without MI recurrence and in 76.8% of patients with recurrence. Thrombolysis was not used in 88 patients for various reasons: 41 (46.6%), prolonged ischemia; 12 (13.6%), recent use of Heberkinasa; 11 (12.5%), transient ischemic attack in the preceding 6 months; 9 (10.2%), cerebrovascular accident; 6 (6.8%), refractory cardiogenic shock; 5 (5.7%), known bleeding disorders; and 4 (4.5%), gastrointestinal bleeding in the previous month. Although not included in Table 1, this distribution was similar in both patient groups.

Mean blood glucose on admission was higher in patients with recurrence of acute coronary syndrome (ACS). Mean BP and mean LVEF recorded during admission were lower in this group of patients. In patients with recurrent MI, atrial fibrillation and cardiac arrest were frequent complications and mortality was significantly higher. The most frequent initial infarction location for patients with recurrent MI was the large anterior myocardium, while the inferior myocardium was the most frequent location for those without recurrent MI.(Table 1).

A positive correlation was observed between degree of heart failure (Killip-Kimball class) and QTdc (Spearman rho 0.697, p ≤0.001). Patients with cardiac arrest had higher QTdc means (m = 94.7, SD = 30.9) compared to the others (m = 63.9, SD = 29.5) with p = 0.001. Cases with recurrent ischemia and cardiac arrest also had higher QTdc means (m = 105.0, SD = 22.3) when compared to those without cardiac arrest (m = 75.7, SD = 21.2).(Table 2).

Calculation of covariate-adjusted ORs based on the logistic regression model showed a significant association between QTdc and MI recurrence (OR = 3.09; 95% CI = 1.105 – 8.641; p = 0.032) which, although much lower than that attributable to cardiac arrest history (OR = 51.22; 95% CI = 16.72–156.97), suggests a marginal predictive effect for QTdc.(Table 3).

The QTdc cutoff point had a sensitivity of 66.1% and specificity of 68.1%. (Table 4) The probability of infarction not recurring in patients with QTdc <80 ms is higher (NPV = 93.8%) than the probability of recurrence in patients with QTdc ≥80 ms (PPV = 21.9%).

Table 1: Baseline characteristics of patients

Variable	Recurrent MI 56 (11.8%)	No recurrent MI 420 (88.2%)	p
Demographic variables			
Age	69.8 (SD = 13.6)	67.1 (SD = 13.8)	0.166
Male sex	34 (60.7%)	270 (64.3%)	0.603
Female sex	22 (39.3%)	150 (35.7%)	
Skin color: white	37 (66.1%)	279 (66.4%)	0.958
Skin color: mestizo/mixed	13 (23.2%)	103 (24.5%)	0.829
Skin color: black	6 (10.7%)	38 (9.0%)	0.692
Cardiovascular risk factors			
Hypertension	44 (78.6%)	328 (78.1%)	0.935
Diabetes mellitus	21 (37.5%)	121 (28.8%)	0.19
Dyslipidemia	11 (19.6%)	64 (15.2%)	0.408
Smoking	30 (53.6%)	186 (44.3%)	0.191
Previous ischemic heart disease	30 (53.6%)	176 (41.9%)	0.100
Obesity	3 (5.4%)	30 (7.1%)	0.609
Clinical variables			
Heart rate	80.3 (SD = 24.1)	78.8 (SD = 23.0)	0.657
Systolic BP	97.5 (SD = 36.3)	118.6 (SD = 37.2)	<0.001
Diastolic BP	57.3 (SD = 24.8)	71.6 (SD = 23.6)	<0.001
Killip-Kimball class			
Class I	16 (28.6%)	219 (52.1%)	0.001
Class II-IV	40 (71.4%)	201 (47.9%)	
Reperfusion strategy			
Thrombolysis	43 (76.8%)	345 (82.1%)	0.332
None	13 (23.2%)	75 (17.9%)	
Laboratory variables			
Hemoglobin g/L	11.6 (SD = 1.8)	11.4 (SD = 1.7)	0.432
Blood glucose mmol/L	11.2 (SD = 2.3)	9.3 (SD = 2.7)	0,000
Leukogram x 10^9/L	10.1 (SD = 2.2)	10.1 (SD = 2.0)	0.884
Creatinine µmol/L	96.6 (SD = 25.7)	90.6 (SD = 25.8)	0.422
Total peak CPK UI/L	1929.2 (SD = 590.4)	1934.9 (SD = 528.8)	0.941
Electrocardiographic variables			
Measured QTi	436.9 (SD = 57.6)	394.8 (SD = 49.7)	<0.001
Corrected QTi	493.2 (SD = 63.7)	444.1 (SD = 66.3)	<0.001
Measured QTd	78.4 (SD = 21.3)	55.8 (SD = 25.2)	<0.001
QTdc	88.8 (SD = 26.0)	62.9 (SD = 29.8)	<0.001
Other variables			
LVEF	40.4 (SD = 11.8)	48.6 (SD = 10.6)	<0.001
Complications			
Newly appearing atrial fibrillation	16 (28.6%)	33 (7.9%)	<0.001
Cardiac arrest on admission	25 (44.6%)	5 (1.2%)	<0.001
Deaths	14 (25.0%)	47 (11.2%)	0.008
Location of infarction			
Large anterior	17 (30.4%)	48 (11.4%)	0.001
Apical anterior	4 (7.1%)	50 (11.9%)	
Mid-anterior	10 (17.9%)	86 (20.5%)	
Inferior	14 (25.0%)	192 (45.7%)	
Inferior plus right ventricle	2 (3.6%)	8 (1.9%)	
Inferolateral	5 (8.9%)	24 (5.7%)	
Lateral	3 (5.4%)	10 (2.4%)	
Septal	1 (1.8%)	2 (0.5%)	

BP: blood pressure; CPK: creatine kinase; LVEF: left ventricular ejection fraction; QTd: QT-interval dispersion; QTi: QT interval; QTdc: corrected QT-interval.

Table 2: QTdc means and standard deviations in patients with and without MI recurrence according to Killip-Kimball classification and occurrence of cardiac arrest

Variable	QTdc	
	Recurrent MI	No recurrent MI
Killip-Kimball Class		
Class I	65.1 (SD = 21.6)	41.8 (SD = 18.6)
Class II	94.9 (SD = 11.0)	83.1 (SD = 19.3)
Class III	107.9 (SD = 24.1)	85.3 (SD = 20.2)
Class IV	76.7 (SD = 3.2)	93.1 (SD = 29.4)
Spearman rho: 0.697[a], p <0.001		
Cardiac arrest		
Cardiac arrest	105.0 (SD = 22.3)	43.5 (SD = 4.6)
No cardiac arrest	75.7 (SD = 21.2)	63.1 (SD = 29.9)
Student t test	5.0 p <0.001 DM = 29.4 95% CI = 17.7 to 41.0	-7.7 p <0.001 DM = -19.6 95% CI = -25.3 to -13.9

DM: difference between means QTdc: corrected QT interval
[a] Spearman correlation between QTdc and left ventricular ejection fraction

Table 3: Logistic regression model results

Variable	OR	p values	95% CI*	
			Lower	Upper
Cardiac arrest on admission	51.22	<0.001	16.71	156.96
QTdc >80 ms	3.09	0.032	1.10	8.64
Systolic BP ≤100 mmHg	1.17	0.688	0.54	2.56
LVEF ≤45%	2.70	0.019	1.18	6.18
Killip-Kimball Class II-IV	0.48	0.229	0.15	1.58
Extensive anterior location	1.40	0.454	0.58	3.42
Blood glucose ≥11 mmol/L	0.84	0.662	0.38	1.83
Constant	0.04	<0.001		

BP: blood pressure; LVEF: left ventricular ejection fraction; QTdc: corrected QT-interval dispersion
*Regression parameter estimates: B, p values, odds ratios (OR) and 95% confidence interval (CI)

Table 4: Sensitivity, specificity and predictive values of QTdc cutoff point for STEMI recurrence

QTdc	Recurrent MI	No recurrent MI	Total
>80 ms	37 (66.1%)	132 (31.4%)	169 (35.5%)
≤80 ms	19 (33.9%)	288 (68.6%)	307 (64.5%)
Total	56 (100%)	420 (100%)	476 (100%)

Sensitivity: 66.1%
Specificity: 68.6%
Positive predictive value: 21.9%
Negative predictive value: 93.8%

The regression tree model showed that cardiac arrest is the variable with greatest predictive capacity for MI recurrence. In cases that did not experience cardiac arrest (446 patients, 93.7%), LVEF >45% was an important predictor of non-recurrence. Ninety-seven percent of patients without cardiac arrest, with LVEF >45% and QTdc <80 ms did not have MI recurrence. This model correctly classified 92.4% of cases overall (sensitivity 44.6%; specificity 98.8%).(Figure 1).

DISCUSSION

QT is an electrocardiographic indicator of regional differences and their heterogeneity during cardiac repolarization.[16,18] QTdc is a predictor of ventricular arrhythmias,[24–26] an indicator of myocardial viability[23,38-40] and more recently, it has been considered an indicator of successful reperfusion and associated with greater severity of coronary artery disease (CAD).[19–22,41]

Since myocardial ischemia occurs in viable tissue with significant CAD, QTdc should be a good predictor of MI recurrence. However, no studies were found assessing its prognostic capacity.

Jensen[39] demonstrated a QTdc decrease following recanalization of the affected artery and George[40] found a greater reduction of this electrocardiographic parameter following PCI as compared to fibrinolysis. Eslami[41] also demonstrated a significant QTdc reduction following PCI (5.8 ms mean compared with 3.6 ms, p <0.001). These studies showed that when the artery is successfully opened through primary coronary intervention—the suggested treatment in international guidelines—[1,9] ventricular repolarization homogeneity is reestablished between the affected myocardium's different zones. QTdc values found in this study suggest absence of flow reestablishment in the artery responsible for infarction.

This study's results show high QTdc values, which were higher in patients with MI recurrence. This coincides with Pekdemir,[42] who demonstrated a relationship between QTd >40 ms and appearance of new ACS and death, despite a normal initial ECG. Furthermore, Machín[43] found infarction recurrence within 30 days following initial MI in 26% of cases, of which 86% presented increased QTd with a significant association (p = 0.009).

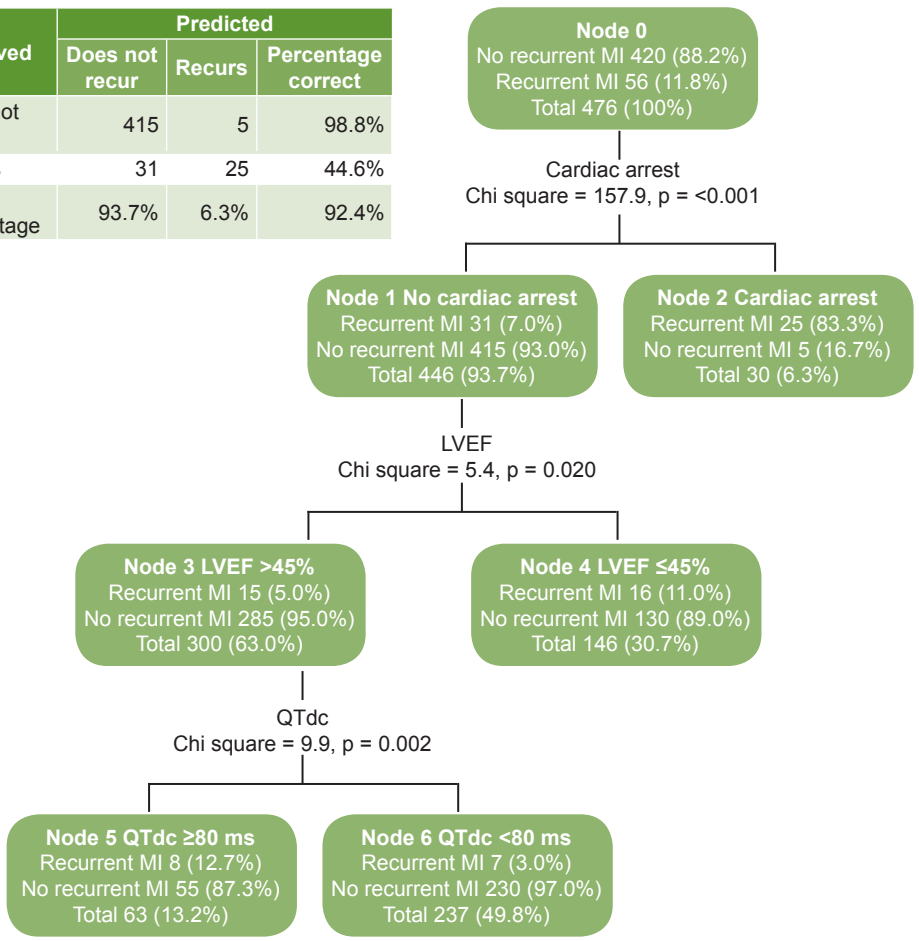
QTdc has also been associated with myocardial viability, a necessary condition for recurrence of angina. Once the necrotic scar forms, recurring ischemic episodes are very unlikely. Using low-dose dobutamine (10 mg), Moreno[23] found significant differences in QTdc between patients with viable and nonviable myocardium (m = 86.1, SD = 30.8 and m = 60.0, SD = 20.1 ms respectively; p = 0.013) and concluded that a QTdc >59 ms predicts greater myocardial viability. Ikonomidis[44] and Lancellotti[45] also found higher QTd in patients with viable myocardium. If these results are considered, it can be assumed that STEMI patients with QTdc ≥80 ms also presented viable myocardium.

High QTdc values have been associated with greater severity of coronary disease. Akgumus found significantly higher QTdc in patients with 3-vessel disease than in patients with 1-vessel disease (m = 68, SD = 32 and m = 50, SD 32 ms; respectively; p = 0.001).[20] In another study, however, relating severity of CAD to this electrocardiographic parameter in patients with chronic ischemic heart disease, Stankovic found higher values in patients with affected vessels as compared to those with three affected vessels.[19]

Several factors have been associated with greater QTdc during acute ischemia. Thus, it is uncertain whether higher QTdc predicts higher risk for ischemic patients or is the expression of other cardiovascular risk factors such as hyperglycemia, obesity and left ventricular hypertrophy.[19] The results of this study show an association between Killip-Kimball class and greater QTdc, both

Figure 1: Classification tree for identification of recurrence predictors

Observed	Predicted		
	Does not recur	Recurs	Percentage correct
Does not recur	415	5	98.8%
Recurs	31	25	44.6%
Overall percentage	93.7%	6.3%	92.4%



MI: Myocardial infarction, QTdc: Corrected QT interval difference, LEVF: Left ventricular ejection fraction

of which are evidence of a greater degree of heart failure. In a retrospective study, Chávez-González[46] found the variables most associated with a QTdc >50 ms were ischemic heart disease (OR 4.2; 95% CI 1.84–10.13; p = 0.001), hypertension (OR 3.56; 95% CI 1.73–7.34; p = 0.001) and diabetes mellitus (OR 3.21; 95% CI 1.46–7.05; p = 0.002), which supports a hypothesis of association of greater morbidity with greater repolarization dispersion.

Mortality in our study population was higher in patients with MI recurrence, consistent with findings by Jiménez-Candil[47] who included patients with non-ST-segment elevation ACS. This author found that QTc ≥450 ms was a predictor of independent risk of death or recurrent ischemia (adjusted OR 3.8; 95% CI 2.5–6.5; p <0.001). Another study conducted in Santa Clara, Villa Clara Province, Cuba by Rodríguez González[48] found QTdc >50 ms associated with greater mortality and incidence of a new ACS within 30 days of hospital discharge. Our results suggest the importance of evaluating QTdc with risk stratification following STEMI, especially in patients without cardiac arrest on admission and with LVEF >45%, which characterized most patients in this study. A study limitation was that primary percutaneous coronary intervention was not performed and therefore it was not possible to correlate QTdc values with the severity of coronary disease. Nevertheless, these results could be useful for low- and middle-income countries in need of quality, low-cost medical care alternatives.

CONCLUSIONS

Risk of infarction recurrence is low in patients without cardiac arrest, with left ventricular ejection fraction >45% and with dispersion of corrected QT-interval <80 ms. Patients with QTdc ≥80 ms have a greater risk of MI recurrence, which suggests the utility of this parameter for risk stratification after STEMI in settings with limited resources.

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The Chronic Kidney Disease Epidemic in El Salvador: A Cross-Sectional Study

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ABSTRACT

INTRODUCTION Chronic kidney disease has reached epidemic levels in several Central American countries since the early years of this century. In El Salvador, it is the second cause of death in men, the fifth in persons over 18 years old and the third cause of hospital deaths in the adult population. Its features, especially those of a subtype unassociated with traditional risk factors such as diabetes and high blood pressure, are only partially understood.

OBJECTIVE Estimate the magnitude of chronic kidney disease in the adult population of El Salvador, considering both prevalence of the disease in its diverse forms as well as presence of potential risk factors nationally and in major subpopulations.

METHODS A descriptive, cross-sectional analysis was conducted on data obtained from the Survey of Chronic Non-communicable Diseases in Adults in El Salvador, completed in 2015. The original data (interviews and measurements) were collected between October 2014 and March 2015 from 4817 adults employing a two-stage probabilistic cluster sample, with stratification of primary sampling units. Our analysis, using 20 of the 118 primary variables included in the original survey, focused on point estimation of prevalence rates and means, related to both traditional biological risk factors and nontraditional ones, such as insufficient hydration, strenuous working conditions and exposure to toxic agents. A separate analysis was performed to estimate prevalence of chronic kidney disease from nontraditional causes. Corresponding confidence intervals were calculated with proper weighting.

RESULTS The general prevalence of chronic kidney disease in El Salvador was 12.8% (men 18.0%; women 8.7%). Of the chronically ill kidney patients, 13.1% were between 20 and 40 years of age. Among biological risk factors, the most frequent was high blood pressure (37.0%). Among nontraditional risk factors, high levels of sugary drink consumption (81.0%), insufficient hydration (65.9%) and high levels of exposure to agrochemicals in the work environment (12.6%) were also observed. Prevalence of chronic kidney disease from nontraditional causes was 3.9% (men 6.1%; women 2.2%).

CONCLUSIONS Chronic kidney disease has reached epidemic proportions in El Salvador. The data confirm a health tragedy that, although especially striking older men, also takes a severe toll on young men and women. The results confirm findings of previous research in several Salvadoran agricultural communities. The relatively high level of population exposure to agrochemicals is important and alarming, especially in rural areas, meriting health-impact studies that include and go beyond possible impact on chronic kidney disease.

KEYWORDS Kidney, renal insufficiency, chronic, risk factors, epidemics, El Salvador

INTRODUCTION

According to a 2018 report, the crude global prevalence of chronic kidney disease (CKD) rose by 87% between 1990 and 2016 and mortality from this cause doubled. In 2016, CKD was eleventh on the list of leading global causes of death.[1]

Non-communicable diseases have become the main causes of premature and preventable death in the Americas.[2] CKD is one of the most important, involving irreversible damage to kidney function and thus representing a serious health problem. Once established, the disease tends to progress to chronic kidney failure (CKF) or end-stage renal disease, requiring expensive renal replacement therapies (dialysis) or organ transplantation.[3] In the absence of such interventions, death is inevitable.[4]

Following a 2002 report on CKD in El Salvador,[5] various publications have described the emergence of a CKD epidemic in Central America starting early in this century.[6–9] It is estimated that the disease has caused at least 20,000 premature deaths in men across the region.[9] PAHO notes that between 1997 and 2013 in Central America more than 60,000 deaths were due to kidney problems (KDN18 according to the *International Statistical Classification of Diseases and Health Related Problems, Tenth Edition, ICD-10)*.[10] Of those deaths, 41.0% corresponded to persons aged ≤60 years.[11]

IMPORTANCE For the first time by means of a national probabilistic sample, this analysis reveals a chronic kidney disease epidemic of massive and unprecedented proportions in El Salvador.

These values are extremely high in the context of the Americas.[11] Although El Salvador and Nicaragua have the highest mortality rates (47.4 and 33.7 per 100,000 population, respectively), the situation is also alarming in areas of Guatemala, Honduras and Costa Rica.[12] According to a study carried out under PAHO auspices,[13] the greatest contrast in mortality rates for 1997–2003 is between El Salvador (47.4 per 100,000) and Cuba (2.7 per 100,000).

Epidemiological knowledge of CKD is fragmentary. It is a serious health problem in El Salvador, where CKD is the second cause of death in men and the fifth in persons aged >18 years. The CKD death rate increased by 153% between 1997 and 2012.[14] In its annual report for 2012–2013, the Salvadoran Ministry of Health (MINSAL) declared CKD as the third leading cause of hospital deaths in the adult population: the first for men and the fifth for women, with a fatality rate of 12.6%.[14]

For over a decade, a severe and premature form of CKD has reached epidemic proportions in Central America's agricultural com-

munities, especially in Nicaragua and El Salvador.[11,13,15–18] Among other labels, it has been termed "chronic kidney disease of nontraditional etiology" (CKDnT),[13] as its causes are unrelated to the traditional risk factors for kidney disease—mainly diabetes mellitus (DM) and high blood pressure (HBP), but also proteinuria, glomerular diseases, systemic erythematous lupus, polycystic kidneys and obstructive uropathies.[19]

Although it has also been denominated CKD of unknown etiology (CKDu), in our analysis it will be called CKDnT to stress that not knowing the causes of a disease is not the same as knowing what does not cause it. While the exact cause of the disease cannot be known for a specific patient, in this epidemic we do know what has not caused CKD for a large number of patients. Valuable information is available from epidemiologic and clinical studies in Salvadoran farming communities.[20–22] Their results show high presence of CKDnT in rural areas, especially in male farmers aged <60 years and, to a lesser extent, in women, children and adolescents.[23] Among the main risk factors found in these studies, exposure to agrochemicals and insufficient hydration under exhausting working conditions stand out, as do the occupation of "farmer", as well as male sex, age, family history of CKD, and consumption of non-steroid anti-inflammatory drugs.

High-level meetings have been convened to address this health tragedy. In the San Salvador Declaration of 2013,[24] the Council of Ministers of Health of Central America and the Dominican Republic (COMISCA)[25] recognized CKDnT as a serious public health problem requiring urgent and coordinated action. In September of that year, the 52nd meeting of PAHO's Directing Council adopted a resolution on CKD in Central American agricultural communities in support of the San Salvador Declaration.[26] Ranking medical journals have echoed the concerns raised in these pronouncements.[27] Since then, greater attention has been focused on epidemiological studies of the problem's magnitude and location.

With the aim of providing a more complete assessment of CKD prevalence, the present study uses a CKD-specific methodological framework to estimate the magnitude of the epidemic in El Salvador's adult population, considering prevalence of the disease in its various forms and possible risk factors nationally and in selected subpopulations.

METHODS

The most complete dataset available for epidemiological research on CKD in El Salvador is from the National Survey of Chronic Noncommunicable Diseases in Adults in El Salvador (ENECA-ELS 2015).[28] With a sample of nearly 5000 subjects, representative of the entire adult population (≥20 years of age), this research effort was distinguished by its methodological rigor, especially because all measurements were performed using internationally recommended methods. Due to its importance and its bearing on the present study, we briefly describe the ENECA-ELS 2015 sample design and some of its key methodological details first, followed by a similar description of design and methods for the present study.

ENECA-ELS 2015[28] A two-staged cluster sample design was employed with stratification of first-stage units. Primary sampling units (PSU) almost always corresponded to census tracts, though sometimes several tracts were joined to yield PSUs of similar size. Each sample segment included 150–250 households. Stratification prior to PSU selection was based on urban/rural residency, as well as geographic distribution throughout the country's five regions according to the latest national population and households census conducted in 2007. In selected PSUs within each of the ten strata formed by the crossing of these two axes, households were selected (secondary sampling units) and, finally, all individuals corresponding to the target population residing in them were included in the sample. In each of its phases, selection was carried out applying probabilistic techniques.

Inclusion criteria All persons aged ≥20 years, residing in each selected household and providing written informed consent to participate in the study.

Exclusion criteria Persons with limitations preventing them from understanding the contents of the questionnaire and/or the research objectives.

Sample Of the 9097 persons originally included in the selected households, it was possible to study 6150, although some only partially. Both interviews and measurements, including serum and urine laboratory tests, were completed for 4817 participants, representing a response rate of 67.6%.

Data collection ENECA-ELS 2015 included epidemiologic and clinical data obtained through a structured individual questionnaire and biometric tests, completed from October 2014 through March 2015. A questionnaire adapted to the research purposes was prepared, using as references the WHO STEPwise methodology[29] and the US National Health and Nutrition Examination Survey (NHANES) carried out by the US Centers for Disease Control.[30]

Measurement of physical and laboratory variables

Blood pressure was measured with a digital sphygmomanometer (RIESTER model RI-Champion, Germany) with a precision of ± 3 mm Hg (cuff pressure) and ± 5% pulse frequency.

Blood biochemical determinations of creatinine, glycaemia, total cholesterol, HDL, LDL and triglycerides Measurements were taken after an 8-hour fast. They were performed in blood using calibrated state-of-the-art dry chemistry analyzers (REFLOTRON PLUS, Switzerland) under strict quality controls. Creatinine measurement was not recalibrated by isotope dilution mass spectrometry (IDMS).

Albuminuria and creatininuria were determined by the albumin-to-creatinine ratio (ACR) (CLINITEK Microalbumin 2, USA).

Quality control, standard procedures and data validation All medical instruments and supplies were calibrated. Laboratory analyses included the appropriate controls and were performed according to manufacturers' specifications. Specialized, certified personnel were in charge of measurements and analyses. The interviewer and then the fieldwork supervisor coded and examined information obtained from questionnaires. Whenever possible, data were collected using electronic devices to minimize transcription and typing errors;[31] data cleaning was performed daily by questionnaire supervisors and through random checks on 20% of cases.

Ethical considerations The study was designed and carried out according to the Helsinki Declaration of the World Medical Association.

[32] Written informed consent was obtained from all participants, who agreed to use of their data under conditions of confidentiality. All participants with detected abnormalities received clinical followup. The study was conducted at the request of MINSAL and the research protocol was approved by the National Clinical Research Committee of El Salvador, of the Higher Council of Public Health.

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THE CKD STUDY The present analysis draws upon the descriptive data collected by ENECA-ELS 2015.[28]

Variables Of the 118 primary variables in the full dataset, our analysis used a subset of 20 selected for their relevance to CKD diagnosis or characterization, or because they provided stratification criteria for subgroup estimations (Table 1). The criteria for CKD presented in Table 1 are those listed in the International Society of Nephrology's guidelines for improving global CKD outcomes (KDIGO, Kidney Disease Improving Global Outcomes).[33,34] A study's adherence to these standards should be taken into account when assessing reported prevalence rates. PAHO[13] and NHANES[30] follow KDIGO criteria to identify CKD.

CKD is established in a single measurement whenever the glomerular filtration rate (GFR) is <60. When the GFR is ≥60 and the ACR is ≥30, another ACR measurement of ≥30 separated by at least 3 months is required for CKD diagnosis, thus discarding subjects with circumstantial acute kidney disease. Studies that fail to take a second ACR measurement risk including persons with circumstantial acute kidney dysfunction in the CKD category, and will consequently report higher CKD prevalence. Consistent with International Society of Nephrology guidelines, we defined chronic kidney failure (CKF) as GFR <60 mL/min/1.73m²SC (or >CKD 2).

Statistical analysis Relevant information from the ENECA-ELS was analyzed

Table 1: Study variables

Variable	Description
Age	By age group in years (20–40, 41–60, >60)
Sex	Male, female
Educational level completed	No schooling or less than primary, primary, secondary, higher
Urban-rural residence	Urban, rural
Current occupation	Farmer, not farmer
Chronic kidney disease (CKD)[34]	CKD if one or both of the following characteristics is present: 1) GFR <60 mL/min/1.73m²SC 2) GFR ≥60 mL/min/1.73m²SC and ACR which reveals persistent A2 or A3 albuminuria level
Family history of chronic kidney disease (CKD)	Present if the father or mother had suffered the disease
Water consumption	Sufficient: self-reported consumption of ≥2 liters a day Insufficient: otherwise
High consumption of non-steroidal anti-inflammatory drugs (NSAID)	Yes: self-reported daily consumption of any of the following medications for at least one month in the past year: aspirin, ibuprofen, naproxen sodium, desketopropoxifen, indomethacin, ketorolac, diclofenac, celecoxib, meloxicam No: otherwise
Consumption of sugary drinks	Yes: self-reported consumption of one or more of the following beverages: hydrating beverages, soft drinks and processed juices, energy drinks, light beverages, processed tea No: otherwise
Intense occupational exposure to agrochemicals	Yes: self-reported occupation as at least one of the following occupations: pesticide applicator (or exterminator), mixer or formulator of pesticides, flagman No: otherwise
High blood pressure (HBP)	Hypertensive: known high blood pressure (self-reported or diagnosed by a physician before the study) or HBP diagnosed during the study (BP ≥140/90) Not hypertensive
Diabetes mellitus (DM)	Diabetic: known DM (self-reported or diagnosed by a physician before the study) or DM diagnosed during the study (glycemia ≥126 mg/dL at random or ≥200 mg/dL with symptoms) Not diabetic
Albumin-to-creatinine ratio (ACR)	A1: <30 mg/g (normal or slightly increased) A2: 30–300 mg/g (moderately increased) A3: >300 mg/g (severely increased)
Glomerular filtration rate (GFR)	GFR (mL/min/1.73m²) was calculated from serum creatinine (SC) using the dry chemistry method and applying the CKD-Epi mathematical formula.[33]
CKD stage[34]	Stage 1: GFR ≥90 mL/min/1.73m²SC and ACR A2 or A3 Stage 2: GFR 60 to ≤89 and ACR A2 or A3 Stage 3a: GFR 45 to ≤59 Stage 3b: GFR 30 to ≤44 Stage 4: GFR 15 to ≤29 Stage 5: GFR <15
Chronic kidney disease of nontraditional causes (CKDnT)	Presence of CKD without HBP or DM
CKDnT stage	Stage 1: GFR ≥90 mL/min/1.73m²SC and ACR A2 or A3 Stage 2: GFR 60 to ≤89 and ACR A2 or A3 Stage 3a: GFR 45 to ≤59 Stage 3b: GFR 30 to ≤44 Stage 4: GFR 15 to ≤29 Stage 5: GFR <15
Chronic kidney failure (CKF)	CKD at stage >2
Chronic kidney failure of nontraditional causes (CKFnT)	CKDnT at stage >2

with SPSS version 24 statistical program (Complex Samples module) for Windows (SPSS Inc, Illinois, USA) and EPIDAT 4.2.[35]

In keeping with the descriptive nature of a cross-sectional study, analysis focused on point estimations of prevalence rates and means, and calculation of respective confidence intervals. Since definitions of CKD and CKDnT used in this study adhere to KDIGO guidelines (Table 1), all CKD prevalence estimates included a second ACR measurement. This allowed for valid comparisons of results with those of other studies.

All estimates (point and interval) were made considering adequate weightings (derived from the non-equiprobabilistic nature of the sample and the need to calibrate "non-response") [36,37] as well as the complex structure of the sample design. Estimates were made both for overall population and according to characteristics of selected population groups (according to urban/rural setting, occupation, age and sex). All results previously obtained by MINSAL[28] were recalculated after conducting a final database validation.

RESULTS

Sociodemographic characteristics of the sample Mean age of interviewees was 44.9 years (95% CI 44.2–45.7) and slightly fewer than half (45.4%) were aged 20–40 years. Education level was predominantly low (70.8% had completed primary school or less). Most resided in urban areas (58.6%) and about 1 in 5 worked as farmers (Table 2).

Prevalence of CKD risk factors Table 3 summarizes prevalence estimates by sex, age group, urban/rural residence and current occupation. Among the traditional, biological risk factors, the most frequent was HBP (estimated prevalence 37.0%), followed by DM (12.5%). Prevalence was similar for men and women, and higher in the older age groups. Family history of CKD was found in 8.7% of the study population. All biological risk factors were more prevalent among urban residents and non-farmers, with a greater difference in the case of hypertension in the first group and DM in the second.

Among the nontraditional CKD risk factors, high prevalence of sugary drink consumption (81.0%) and insufficient water consumption (65.9%) were observed. Insufficient water consumption was approximately 10 percentage points higher in those aged >60 years relative to the other two age groups, much higher in women (75.3%), and noticeably lower in farmers. Sugary drink consumption showed an opposite pattern: lower in the older age groups and higher in men and farmers. Consumption of NSAID's was low and fairly similar among stratifying variables.

Regarding toxic exposure, it was estimated that more than 1 in 10 persons were intensely exposed to agrochemicals (12.6%) at work. Level of exposure was higher in men (23.0%), farmers (14.7%) and rural residents (16.5%) and increased moderately with age.

Prevalence of CKD and CKDnT Estimated CKD prevalence in the Salvadoran adult population, considering the two ACR measurements, was 12.8%, representing an estimated population of nearly half a million adults (Table 4). Considering only one ACR measurement, prevalence reached 28.1% (95% CI 26.1–30.1), or over 1 million adults.

Estimated CKD prevalence in men was more than double that in women and increased with age, reaching one in every three Salvadorans of either sex aged >60 years. However, most noteworthy is the high prevalence of the disease in the younger adult population (<60 years), at 7.1% (95% CI 5.8–8.4). Overall national prevalence of CKF was 8.5%, notably higher in those aged >60 years and markedly higher in men. Higher levels of CKD and CKF were observed in farmers, relative to those in other occupations (Table 4). Of the CKD cases detected, 30.5% (95% CI 25.9–35.5) classified as CKDnT, 33.9% in men (95% CI 28.1–40.2) and 25.1% in women (95% CI 19.2–32.1).

CKDnT national prevalence was 3.9%, representing an estimated population of some 140,000 adults. Among those aged >60 years, CKDnT prevalence was very high, affecting 1 out of every 11 Salvadorans. CKDnT and CKFnT prevalences among farmers surpassed those found among non-agricultural workers (Table 4). The mean age of those with CKD (61.3, 95% CI 59.3–63.4) was almost 6 years higher than those with CKDnT (55.5, 95% CI 51.8–59.2).

In general, more advanced stages of CKD had lower prevalence. The lowest was stage 5—requiring renal replacement therapy—at 0.7%, representing ±26,000 adults (Table 5). A similar pattern of disease stages was observed for CKDnT (Table 6).

Data shown in tables are slightly different from those previously reported by MINSAL,[28] because all estimates were recalculated after a thorough database validation.

Table 2: Sociodemographic characteristics of the sample studied. ENECA-ELS 2015[a]

Variable		n	Weighted percentage	95% CI
Sex	Female	3111	56.8	54.9–58.7
	Male	1706	43.2	41.3–45.1
Age group (years)	20–40	2235	45.4	43.0–47.8
	41–60	1605	34.4	32.4–36.5
	>60	977	20.2	18.5–22.1
Urban/rural residence	Urban	2551	58.6	53.7–63.4
	Rural	2266	41.4	36.6–46.3
Education level completed	No schooling	1045	19.3	17.3–21.3
	Primary	2418	51.5	48.9–54.1
	Secondary	890	20.1	18.6–21.6
	Higher	348	9.1	6.8–11.4
Current occupation	Farmer	877	18.9	16.6–21.2
	Not farmer	3 940	81.1	78.8–83.4

ENECA-ELS: National Survey of Chronic Non-communicable Diseases in Adults in El Salvador[28]

[a]Some data are slightly different from previously reported by MINSAL[28] because they were recalculated following a subsequent database validation

DISCUSSION

Comparison of CKD prevalence estimates across diverse temporal or geographic contexts should take into account the criteria adopted to define CKD and the methods used to assess kidney

Table 3: Prevalence* of biological and nontraditional risk factors for CKD in the Salvadoran adult population, by selected variables. ENECA-ELS 2015[a]

Variable		Biologic			Nontraditional			
		Family history of CKD % (95% CI)	Diabetes mellitus % (95% CI)	High blood pressure % (95% CI)	Insufficient water consumption % (95% CI)	Intense consumption of NSAIDs % (95% CI)	Consumption of sugary drinks % (95% CI)	Intense work exposure to agrochemicals % (95% CI)
National		8.7 (7.6–9.9)	12.5 (11.2–13.8)	37.0 (35.0–39.1)	65.9 (63.9–67.9)	8.1 (7.0–9.1)	81.0 (79.1–82.9)	12.6 (11.0–14.3)
Sex	Female	8.3 (6.9–9.8)	13.9 (12.3–15.4)	38.0 (35.6–40.4)	75.3 (73.3–77.3)	8.7 (7.3–10.1)	78.1 (75.8–80.4)	4.8 (3.8–6.0)
	Male	9.2 (7.5–11.0)	10.6 (8.8–12.4)	35.8 (32.8–39.0)	53.5 (50.2–56.8)	7.3 (6.0–8.6)	84.8 (82.6–86.9)	23.0 (20.0–26.2)
Age group (years)	20–40	8.4 (6.8–9.9)	3.2 (2.4–4.0)	18.3 (16.4–20.3)	64.4 (61.7–67.1)	6.1 (5.0–7.3)	89.2 (87.2–91.2)	10.3 (8.4–12.3)
	41–60	10.3 (8.2–12.4)	18.4 (16.1–20.7)	44.8 (41.7–47.9)	62.3 (59.3–65.4)	9.9 (8.2–11.6)	77.9 (75.4–80.3)	13.3 (10.8–15.7)
	>60	6.7 (4.9–8.5)	23.1 (19.8–26.5)	66.0 (62.0–69.8)	75.2 (72.2–78.2)	9.4 (6.9–11.8)	67.7 (64.2–71.3)	16.8 (13.2–20.4)
Urban/rural residence	Urban	8.8 (7.3–10.3)	14.2 (12.5–16.0)	40.1 (37.4–42.9)	68.6 (66.5–70.8)	7.5 (6.1–8.9)	80.9 (78.5–83.2)	9.9 (7.9–12.0)
	Rural	8.6 (6.9–10.4)	10.0 (8.3–11.6)	32.7 (29.7–35.8)	62.0 (58.0–65.8)	8.9 (7.4–10.5)	81.1 (77.9–84.3)	16.5 (13.8–19.2)
Current occupation	Farmer	8.1 (6.1–10.2)	6.4 (4.2–8.5)	33.2 (28.7–37.7)	46.5 (41.8–51.3)	7.8 (6.0–9.6)	84.0 (81.3–86.8)	14.7 (10.7–18.6)
	Not farmer	8.9 (7.6–10.1)	13.9 (12.4–15.3)	38.0 (35.7–40.1)	70.3 (68.5–72.3)	8.1 (7.0–9.3)	80.3 (78.3–82.2)	12.2 (10.4–14.0)

* Weighted estimates

CKD: chronic kidney disease; ENECA-ELS: National Survey of Chronic Non-communicable Diseases in Adults in El Salvador;[28] NSAIDs: Non-steroid anti-inflammatory drugs

[a]Some data are slightly different from those previously reported by MINSAL[28] because they were recalculated after a subsequent validation of the database

Table 4: Prevalence* of CKD, CKF, CKDnT and CKFnT by selected variables in the Salvadoran adult population. ENECA-ELS 2015

Variable		CKD % (95% CI)	CKF % (95% CI)	CKDnT % (95% CI)	CKFnT % (95% CI)
National		12.8 (11.2–14.4)	8.5 (7.2–9.9)	3.9 (3.1–4.8)	2.4 (1.7–3.0)
Sex	Female	8.7 (7.2–10.3)	5.0 (3.9–6.2)	2.2 (1.6–2.8)	1.0 (0.5–1.4)
	Male	18.0 (15.2–20.7)	13.2 (10.7–15.6)	6.1 (4.5–7.6)	4.2 (2.9–5.5)
Age group (years)	20–40	3.7 (2.5–4.9)	1.3 (0.7–1.9)	2.4 (1.5–3.2)	0.7 (0.3–1.1)
	41–60	11.7 (9.6–13.8)	7.3 (5.5–9.1)	3.0 (2.0–4.0)	1.9 (1.1–2.6)
	>60	35.5 (31.0–40.0)	26.9 (22.9–30.1)	8.9 (6.3–11.6)	7.0 (4.6–9.3)
Urban/rural residence	Urban	11.4 (9.2–13.6)	8.2 (6.4–10.0)	3.2 (2.1–4.3)	2.2 (1.3–3.0)
	Rural	14.7 (12.2–17.1)	9.0 (7.0–10.1)	4.9 (3.4–6.3)	2.6 (1.5–3.8)
Current occupation	Farmer	18.4 (14.8–22.0)	12.9 (9.5–16.3)	7.5 (4.9–10.1)	5.0 (2.7–7.3)
	Not farmer	11.4 (9.8–13.1)	7.5 (6.2–8.8)	3.0 (2.3–3.8)	1.7 (1.2–2.3)

* Weighted estimates

CKD: Chronic kidney disease; CKF: Chronic kidney failure; CKDnT: nontraditional chronic kidney disease; CKFnT: nontraditional chronic kidney failure; ENECA-ELS: National Survey of Chronic Non-communicable Diseases in Adults in El Salvador[28]

All rates were estimated taking into account the two ACR measurements carried out to confirm chronicity

function.[38,39] Despite the Kidney Disease Outcomes Quality Initiative (K/DOQI) CKD guidelines published in 2002 and updated in 2012,[34,39] definitions of CKD and the cut-off values for GFR and albuminuria vary noticeably. A recent systematic review examining 48 papers published between January 1, 2003 and November 1, 2014, reporting CKD prevalence for the general adult population in 20 European countries, found that no study used the KDIGO specificities for confirming chronicity.[38]

The CKD epidemic in the adult population of El Salvador is characterized by very high prevalence, if we consider that the definition of CKD includes confirmation of chronicity as prescribed in KDIGO guidelines.[34] However, this figure is lower than that found in studies carried out in El Salvador's agricultural communities by INS/MINSAL, in which chronicity of kidney disease was also verified.[20] In those cases, CKD prevalence of 15.4%–21.1% was reported and, when diagnosis was confirmed after 3 months, a CKF prevalence of 8.8%–13.3%.[8,16,21,40] When prevalence in our study was estimated using a single measurement, the value obtained was even higher (28.1%). Regarding other countries of the region, it should be noted that in Nicaragua, prevalence rates of up to 42% in men and 9.8% in women have been reported in some areas.[41] High rates elsewhere in the region also point to an outbreak of the epidemic.[42]

Table 5: Prevalence* of chronic kidney disease stages by sex, age group, urban/rural and current occupation, in El Salvador's adult population. ENECA-ELS 2015

Variable		Prevalence of chronic kidney disease by stage % (95% CI)					
		CKD 1	CKD 2	CKD 3a	CKD 3b	CKD 4	CKD 5
National		1.8 (1.3–2.4)	1.6 (1.2–2.1)	4.4 (3.6–5.4)	2.7 (2.1–3.5)	1.3 (0.9–1.8)	0.7 (0.4–1.1)
Sex	Female	1.5 (1.0–2.2)	1.6 (1.1–2.1)	3.0 (2.0–4.0)	1.4 (1.0–1.9)	0.9 (0.4–1.4)	0.2 (0.0–0.4)
	Male	2.2 (1.3–3.1)	1.7 (1.1–2.3)	6.3 (4.8–7.8)	4.5 (3.3–5.8)	1.9 (1.1–2.7)	1.4 (0.7–2.0)
Age group (years)	20–40	1.9 (1.1–2.7)	0.4 (0.1–0.6)	0.8 (0.3–1.2)	0.2 (0.0–0.4)	0.3 (0.0–0.6)	0.2 (0.0–0.4)
	41–60	2.2 (1.4–3.1)	1.4 (0.8–2.1)	3.7 (2.5–4.9)	1.9 (0.9–2.8)	1.5 (0.8–2.3)	0.9 (0.3–1.6)
	>60	1.0 (0.3–1.7)	4.9 (3.4–6.5)	14.2 (11.1–17.3)	10.3 (7.7–13.0)	3.5 (2.1–4.9)	1.6 (0.7–2.5)
Urban/rural residence	Urban	1.3 (0.7–1.9)	1.1 (0.6–1.5)	5.2 (3.8–6.5)	2.2 (1.4–3.0)	1.0 (0.6–1.4)	0.7 (0.3–1.1)
	Rural	2.6 (1.6–3.6)	2.4 (1.6–3.2)	3.5 (2.5–4.5)	3.6 (2.5–4.7)	1.8 (1.0–2.7)	0.7 (0.2–1.3)
Current occupation	Farmer	2.5 (1.4–3.6)	2.2 (1.1–3.2)	6.2 (4.3–8.1)	4.1 (2.5–5.8)	2.5 (1.2–3.9)	0.9 (0.0–1.8)
	Not farmer	1.7 (1.1–2.2)	1.5 (1.1–2.0)	4.0 (3.1–5.0)	2.5 (1.7–3.2)	1.1 (0.7–1.5)	0.7 (0.4–1.0)

* Weighted estimates

CKD: Chronic kidney disease; ENECA-ELS: National Survey of Chronic Non-communicable Diseases in Adults in El Salvador[28]

Table 6: Prevalence* of chronic kidney disease of nontraditional causes for each stage by sex, age group, urban/rural and current occupation, in El Salvador adult population. ENECA-ELS 2015

Variable		Prevalence of chronic kidney disease of nontraditional causes by stage % (95% CI)					
		CKDnT 1	CKDnT 2	CKDnT 3a	CKDnT 3b	CKDnT 4	CKDnT 5
National		0.8 (0.5–1.1)	0.5 (0.2–0.7)	1.3 (0.9–1.8)	0.7 (0.4–1.0)	0.4 (0.1–0.6)	0.1 (0.0–0.3)
Sex	Female	0.7 (0.4–1.1)	0.3 (0.0–0.5)	0.5 (0.2–0.9)	0.3 (0.0–0.6)	0.2 (0.0–0.4)	–
	Male	0.8 (0.3–1.3)	0.7 (0.3–1.3)	2.4 (1.4–3.3)	1.3 (0.7–1.9)	0.6 (0.2–1.1)	0.3 (0.0–0.6)
Age group (years)	20–40	1.3 (0.7–1.9)	0.3 (0.1–0.6)	0.4 (0.1–0.7)	0.1 (0.0–0.3)	0.2 (0.0–0.4)	0.1 (0.0–0.2)
	41–60	0.3 (0.1–0.5)	0.5 (0.1–1.0)	1.3 (0.6–1.9)	0.3 (0.0–0.5)	0.5 (0.0–1.0)	0.1 (0.0–0.2)
	>60	0.5 (0.0–1.0)	0.8 (0.2–1.5)	3.6 (2.0–5.3)	3.0 (1.7–4.3)	0.7 (0.0–1.3)	0.4 (0.0–1.0)
Urban/rural residence	Urban	0.5 (0.2–0.8)	0.3 (0.0–0.5)	1.5 (0.8–2.2)	0.4 (0.1–0.7)	0.3 (0.0–0.5)	0.2 (0.0–0.5)
	Rural	1.1 (0.5–1.7)	0.8 (0.3–1.3)	1.1 (0.6–1.7)	1.2 (0.6–1.7)	0.6 (0.1–1.0)	0.0
Current occupation	Farmer	1.1 (0.3–1.9)	1.0 (0.2–1.8)	2.6 (1.3–3.9)	1.6 (0.6–2.5)	0.9 (0.1–1.7)	0.3 (0.0–0.9)
	Not farmer	0.7 (0.4–1.0)	0.4 (0.1–0.6)	1.0 (0.6–1.5)	0.5 (0.3–0.8)	0.3 (0.0–0.5)	0.1 (0.0–0.2)

* Weighted estimates

CKDnT: Chronic kidney disease of non-traditional causes; ENECA-ELS: National Survey of Chronic Non-communicable Diseases in Adults in El Salvador[28]

Comparing our findings with national statistics from other countries, mortality data lead us to assert that CKD is a health problem of epidemic magnitude in the adult population of El Salvador. Though El Salvador's CKD prevalence (12.8%) was lower than other rates obtained by national studies conducted in the USA (16.8%),[43,44] Japan (26.7%)[45] and Australia (20.4%),[46] none of these studies used the KDIGO definition for CKD chronicity, which prescribes a second measurement after 3 months. Nevertheless, other investigations that have not adhered to this criterion have found prevalence rates lower than those estimated for the Salvadoran population.[47–49]

This study brings to light the remarkable presence of CKDnT in the Salvadoran population: about 3 out of 10 adults diagnosed with CKD do not suffer HBP or DM. That is, CKDnT constitutes approximately one third of all CKD in El Salvador. The ratio CKDnT:CKD is similar for men and women: CKDnT rate for men is 6.0% (95% CI 4.6%–7.8%) and for women 2.1% (95% CI 1.6%–2.9%), while CKD rates were estimated at 18.8% and 8.7% respectively.

Although the highest CKD and CKDnT prevalences were found in persons aged >60 years, the difference between those above and below that age threshold is much smaller for CKDnT. This points to earlier onset of this form of the disease. This possibility, combined with high levels of nontraditional CKD risk factors (insufficient water consumption, high consumption of sugary drinks and intense occupational exposure to agrochemicals), should be considered when designing subsequent research aimed at identifying possible causal factors of El Salvador's CKD epidemic. Insufficient hydration is a general phenomenon, but the study shows that it is more pronounced among women than men, which calls for further scrutiny.

We note the very high prevalence of exposure to agrochemicals in the Salvadoran population (12.6%). This risk factor was nearly 5 times higher in men than in women and 1.7 higher in rural areas. Though not surprising, these figures are alarming. Many results

thus far published for both Central America[50–54] and countries in other regions, including Mexico,[55,56] the USA[57,58] and Sri Lanka,[59,60] show that populations in agricultural communities most affected by the epidemic are directly or indirectly exposed to pesticides, herbicides and numerous toxic substances (heavy metals and metalloids) contained as impurities in certain agrochemicals and fertilizers (e.g. glyphosate, paraquat, carbofuran, deltamethrin, organophosphates). Many of these substances are banned in the countries where they are produced, and yet are in general use in others, occasionally mixed and frequently applied with little or no protection for those who handle them.[61]

The CKDnT epidemic has appeared in other parts of the world,[62] particularly in agricultural areas of Sri Lanka,[63,64] where it has been estimated that at least 400,000 persons suffer from the disease in the northern region[65] and prevalence could reach 21%.[66] A similar pattern has been reported in southern India[67,68] and in other countries such as Saudi Arabia,[69] Egypt[70] and Senegal.[71] The health tragedies in Sri Lanka and El Salvador show pronounced similarities in practically all relevant parameters,[72–74] suggesting that the countries are facing the same epidemic.

This study has limitations; among them, that exposure data were based on study participants' reports, which often entails underreporting and recall bias. In addition, interviewing and measuring nearly 5000 persons under the highly hostile circumstances prevalent in El Salvador partially explains the anticipated substantial rate of non-response. However, from the quantitative point of view, the confidence intervals are narrow enough to produce reliable point estimates. While this loss of information could theoretically bias the estimates, non-response as well as the non-equiprobability of the sample were considered when constructing the weightings. On the other hand, this non-response can be considered unrelated to the real value of variables, since it was largely due to rejection of hundreds of cases—in some instances because informed consent was not unambiguously recorded, and in others because informal groups blocked access to households in the areas where they operated.

Despite these limitations, the importance of this paper lies in the fact that it reports the results of the first epidemiological investigation on the prevalence of CKD and CKDnT based on a nationally representative sample of adults. Its findings may serve as a valuable benchmark for present and future studies addressing the epidemic appearance of the disease, both in El Salvador and in the Central American region.

One of the principal strengths of the study is the application of the CKD definition accepted by the International Society of Nephrology[34] to a national-scale study conducted in a socially conflicted and violent area.

CONCLUSIONS

The results confirm previous research in El Salvador's agricultural communities: that CKD constitutes a health problem of epidemic magnitude in the adult population. This health tragedy especially strikes older men but is also present in young people of both sexes.

An important and alarming fact is that 1 out of 10 persons surveyed attested to being exposed to agrochemicals at work. This finding merits more detailed study, beyond its possible impact on CKD.

Reversing the epidemic can only be achieved through practical measures. Their identification and implementation require knowledge of the disease and its associated risk factors, which is the aim of this article. The topic is far from exhausted and, as argued in a recent paper,[75] demands further research.

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Dengue Cases in Colombia: Mathematical Forecasts for 2018–2022

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ABSTRACT

INTRODUCTION Dengue is a disease caused by any one of five virus serotypes and transmitted to humans by the *Aedes aegypti* mosquito. Climate change and health conditions have combined to make dengue a global public health problem. The situation is especially serious in Colombia, where by week 36 of 2018, dengue incidence was 96 cases per 100,000 population, with a total of 111 deaths. Different mathematical and statistical models have been proposed to understand the dynamics of transmission and consequently to apply control strategies to reduce the number of dengue cases.

OBJECTIVE Forecast the number of dengue cases expected in Colombia from 2018 through 2022 with the stochastic Auto-Regressive Integrated Moving Average (ARIMA) model and use the results to adjust the parameters of an ordinary differential equations model in order to determine the disease's basic reproduction number in the year presenting the highest number of dengue cases.

METHODS An ecological time series study was conducted to forecast dengue incidence in Colombia from 2018 through 2022. The data were compiled from Colombia's National Health Institute series on dengue cases reported by epidemiological week from 2009 to 2017. The stochastic ARIMA time series model was applied. Forecasts were then analyzed, and the year with the highest number of predicted cases was used to adjust the parameters of an ordinary differential equations model (ODE) through nonlinear least squares regression to calculate the vectorial capacity of the transmitting mosquito.

RESULTS Forecasts of the total number of dengue cases per year in Colombia for the following five years were: 32,411 (2018); 88,221 (2019); 56,392 (2020); 47,940 (2021); and 77,344 (2022). The highest number of cases was forecast for 2019. Values for the parameters affecting dengue transmission that year (by the year's four quarters), such as recovery rate (0.0992, 0.0838, 0.1177, and 0.1535, respectively), vectorial capacity of the transmitting mosquito (0.1720, 0.1705, 0.1204, and 0.2147, respectively) and the basic dengue reproduction number (1.73, 2.03, 1.02, and 1.40, respectively) were estimated, indicating that most cases would occur in the second quarter and, since the basic reproduction number values were >1, the disease would persist in the country throughout the entire year.

CONCLUSIONS ARIMA model forecasts for 2018 through 2022 predicted the highest incidence of dengue cases in Colombia would occur in 2019. Comparison of ARIMA model forecasts and the ODE model allowed projections of possible variations in dengue cases reported, and the basic reproduction number predicted that dengue would persist throughout 2019.

KEYWORDS Arboviruses, climate, dengue, models, theoretical, basic reproduction number, prognosis, Colombia

INTRODUCTION

Arboviral diseases have become a global public health problem due to factors such as climate change, population growth, waste accumulation, pollution, inadequate recycling, and insufficient vector control.[1] Dengue is an acute viral disease transmitted to humans by its principal vector, the *Aedes aegypti* mosquito. Dengue virus (DENV) comprises five serotypes (DENV1, DENV2, DENV3, DENV4, DENV5);[2] the first four circulate simultaneously in Colombia.[3] The different serotypes do not confer cross immunity; an individual who recovers from one acquires permanent immunity against that serotype but only partial and temporary immunity against the other four types.[4]

Several studies have shown that one of dengue's greatest dangers is the increased severity of the infection when multiple serotypes are simultaneously present. That is, a secondary infection with another dengue serotype has a greater probability of causing severe acute infections than the primary disease, due to formation of autoimmune complexes that can attack the body.[5,6]

IMPORTANCE Forecasts of dengue cases in Colombia from 2018 through 2022 based on a time-series study design, combined with an ordinary differential equations model, can help health systems and institutions take more effective, precise preventive and control measures to reduce dengue infection, a serious health problem in the country.

Application of different theoretical and mathematical models has helped us understand the epidemiological dynamics of dengue. Nonlinear models based on systems of ordinary differential equations (ODE) describe variations in numbers of cases in a specific population. However, many factors and conditions are involved in dengue transmission dynamics, and any variation in one of them can lead to significant changes in the shape of the curves that describe growth of the infected population. For example, the model proposed by López[7] contains the following variables: average numbers of healthy, infected, and recovered individuals and average number of mosquitos in the environment; classified as larval (aquatic) or aerial (adult) phase, with adults also classified as female or male.

Through a series of calculations, this model was simplified to include only two variables (proportion of healthy individuals and proportion of infected individuals), thus making it easier to analyze while preserving the hypothetical viral transmission factors of the original version.

Amaku's model considers the virus's state of latency in the mosquito population, its vertical transmission, and how variations in climate factors can influence transmission dynamics.[8] Koiller proposes a population growth model for the *Aedes aegypti* mosquito that considers all phases and states in its life cycle.[9] The model then incorporates a biological control measure through use of *Wolbachia* bacterium. This mosquito-growth dynamic is included in a model to determine the dynamics of transmission to humans under these assumptions, considering the number of individuals exposed.

Each factor involved in the complex dynamics of the disease (presence of asymptomatic individuals and infected individuals, dispersion of the vector and infected individuals, application of one vector control or another, resistance to controls, reinfection by a different serotype, etc.) opens new modeling options. No single model can include all the real factors involved in dengue transmission, so selection of a forecasting model often depends on availability of reliable information on the variables and parameters involved, and on the specific objective of the projection.

Time-series modeling, particularly with ARIMA models[10]—which benefit from repeated autocorrelations to make extrapolations—allows reasonable estimates of future effects of an infectious outbreak on a specific population. In combination with other predictive nonlinear models, ARIMA models have been extremely useful in epidemiological investigations to prevent and control infectious diseases.[11–14]

In Colombia, temperature changes caused by climate phenomena and the growing population living in unhealthy conditions have caused intense propagation of dengue virus and the appearance of new viruses in different regions.[15–17] The community epidemiological bulletin published by the National Epidemiological Surveillance System (SIVIGILA) reported a total of 13,427 confirmed dengue cases at the national level by week 23 of 2018,[15] and by week 36, an incidence rate of 96 cases per 100,000 population, with 111 deaths.[16] The Ministry of Health and Social Protection advised health sector units to monitor and control virus transmission and its effects on the population, which reconfirms the importance of projections to support decision-making and financial resources for preventive actions in the face of such a serious health problem.

The purpose of this study was to apply mathematical models to forecast the average number of dengue cases per year from 2018 through 2022 in Colombia, and to determine dengue's basic reproduction number (BRN) in the year with the highest number of predicted cases in order to identify possible outbreaks.

METHODS

Type of study A time-series study was conducted using Colombia's National Institute of Health routine surveillance system database. The study was conducted from February 2017 through March 2018 by researchers in San Juan de Pasto, Nariño Department, Colombia.

Data sources The database from which the reports on incidence of dengue were taken as time series from 2009 to 2017 was obtained from the National Health Institute's SIVIGILA web portal.[15] Specifically, the data correspond to dengue cases reported by the country's departments (principal administrative divisions) and municipalities as "confirmed" cases of dengue or severe dengue. Data were compiled in XLS-formatted files and included records of dengue cases for each epidemiological week of the respective year.

Variables The study considered two variables: 1) number of dengue cases in Colombia and 2) number of epidemiological weeks.

Mathematical models

ARIMA model This model effectively combines three components: autoregressive (AR), integrated (I) and moving average (MA). It is used to analyze stochastic time series and takes into consideration the correlation between the data and the errors corresponding to the preceding periods.[10] It is generally represented as $ARIMA(a,d,b)$, where a is the number of autoregressive terms, d is the number of differences, and b is the number of moving average terms. Mathematically it is expressed as:

$$y_t^4 = c + \alpha_1 y_{t-1}^d + \cdots + \alpha_a y_{t-a}^d + \vartheta_1 \varepsilon_{t-1}^d + \cdots + \vartheta_b \varepsilon_{t-b}^d + \varepsilon_t^d$$

where $\alpha_i, i = 1, \ldots, a$ and $\vartheta_j, j = 1, \ldots, b$ are the parameters to estimate and ε_t^d is a white-noise process.

The mathematical processes and algorithms to analyze time series and their respective predictive process with ARIMA follow four steps:[18]

1) Definition of the model, which includes the assumption that forecast errors will be distributed normally around the mean and time-independent variance (white noise) and application of simple and partial autocorrelation functions (ACF and PACF, respectively).
2) Estimation of parameters with the orders of the fitting process, according to ACF and PACF functions.
3) Evaluation and validation of the tentative ARIMA model.
4) Forecasting of time series.

To carry out these four steps, researchers used R software,[19] a free programming language and statistical computing and graphics tool, together with a series of algorithms and packages related to the models under consideration.

Step 1: The time series was generated from the data to be analyzed. Trend and seasonality could be identified and removed through differentiation. A density histogram was also constructed to determine whether the series is stationary, which means forecast errors normally distributed, with zero mean and constant variance.

Step 2: ACF and PACF analyses were performed along with the application of several functions related to ARIMA in the R statistical package; respective graphics were plotted indicating the appropriate order of the lag in the linear $ARIMA(a,d,b)$ model, and respective parameters of the polynomial coefficients were estimated for the tentative model.

Step 3: The Ljung-Box[20] contrast was used to determine the dependency relation among residuals, enabling validation of the model's forecasting capacity.

Step 4: The previously validated $ARIMA(a,d,b)$ model and periodogram were used to forecast future incidence of dengue.

ODE model These equations relate a dependent variable with one or more of its derivatives with respect to an independent variable.[21] In infectious diseases such as dengue, ODEs are generally used to represent variations in time of average numbers of susceptible individuals, infected individuals and recovered individuals, providing a basis for fitting models with parameters that weight features associated with disease transmission. These models are used to estimate the disease prevalence or to predict its disappearance in a particular setting.[22,23]

This study is based on a system formulated by López[7] presented below and adjusted to fit the reported data on dengue in Colombia. The authors have shown that the new model, a simplified version of a more complex one that includes a larger number of variables and parameters, nevertheless preserves the properties of the original model. The rationale for the use of this simplified system

is that the only data available for Colombia from SIVIGILA and the National Statistical Administration Department (DANE)[24] are life expectancy and the number of dengue cases reported per epidemiological week. The transformed model is the simplest possible without affecting the close approximation of estimated case numbers.

The original model was rewritten as:

$$\begin{cases} \dot{p} = \mu(1-p) - Bpq \\ \dot{q} = Bpq - (\mu+\theta)q \end{cases}$$ MODEL 1

Where

p and q are the proportion of susceptible and infected individuals, respectively;

$\dot{p}$ is the variation in the proportion of susceptible individuals over time (measured in weeks);

$\dot{q}$ is the variation in the proportion of infected individuals over time.

In this model, 1 (100%) represents the total human population under consideration, so the sum of p and q (proportions of susceptible and infected individuals) will never be >1; i.e., $p + q \leq 1$.

Given that μ represents the population growth minus death rate, it can be interpreted as the proportion of people entering weekly into the susceptible population, while μp and μq represent the proportion of susceptible individuals and infected individuals who die each week, respectively. Considering that B is the dengue recovery rate, θq represents the proportion of infected individuals who recover each week.

Now, if $B = \beta\psi\frac{\sigma}{\delta+\sigma}$

Where

β is the probability of mosquito-to-human viral transmission;

ψ is human-to-mosquito contagion rate;

σ is development rate of immature mosquitos into female adults; and

δ is development rate of immature mosquitos into male adults;

Then

Bq is infectious rate of susceptible individuals as a result of contact with a previously infected female adult mosquito (infected with the virus due to prior contact with an infected human).

Bpq is the proportion of susceptible individuals who become infected with the virus each week.

In this case, B represents the vectorial capacity of the transmitting mosquito (*Aedes aegypti*), i.e., the number of secondary infections per week following the entry of an infected individual into a susceptible setting.[25,26]

Nonlinear least squares regression This method is used to estimate the value of one or more parameters in a function in order to fit the function's graph more closely to the data distribution in the Cartesian plane.[27] Values of parameters B and in B Model 1 were estimated weekly to better fit the real-time data of dengue cases in Colombia.

According to the National Statistics Administrative Department,[24] average life expectancy in Colombia is 74 years (3848 weeks), so μ was defined as $\mu = \frac{1}{3848}$ per week. Estimated values of B and θ minimize the function:

$$z = \sum_{i=1}^{n}(q_i - \tilde{q})^2$$ MODEL 2

Where

n is the number of data to be fitted;

q_i ($i = 1, \ldots, n$)are the proportions of confirmed cases from week 1 to week n); and

$\widetilde{q_i}$ (i = 1,...,n) are estimated values of q for each week using Model 1.

Data collection and processing A time series was generated with SIVIGILA data using R statistical software to analyze the trend and seasonality of dengue cases. To generate the number of individuals infected with dengue for each week of 2009 through 2017 and determine the time series for the respective forecasting analysis, a filter was applied to the files reported annually, to select only dengue cases per week. This process was repeated for each year (2009 through 2017). The stochastic ARIMA model was also applied to generate annual forecasts of dengue cases for 2018 through 2022.

Then, totaling all cases reported in all epidemiological weeks of each year, data were taken from the 52 weeks of the year in which the most cases were reported. To determine the disease's BRN, mathematical software Matlab was used to fit a mathematical model based on a system of nonlinear ODEs.[28]

RESULTS

Figure 1a shows the time series of classic dengue cases reported by epidemiological week from 2009 through 2017 in Colombia. Week 1 corresponds to the first week of 2009 and week 469 to the last week of 2017. The time series shows the occurrence of several dengue outbreaks; for example, week 59 (7th week of 2010) when 1787 cases were reported, week 224 (16th week of 2013) with 3231 cases, and week 370 (5th week of 2017) with 3301 cases.

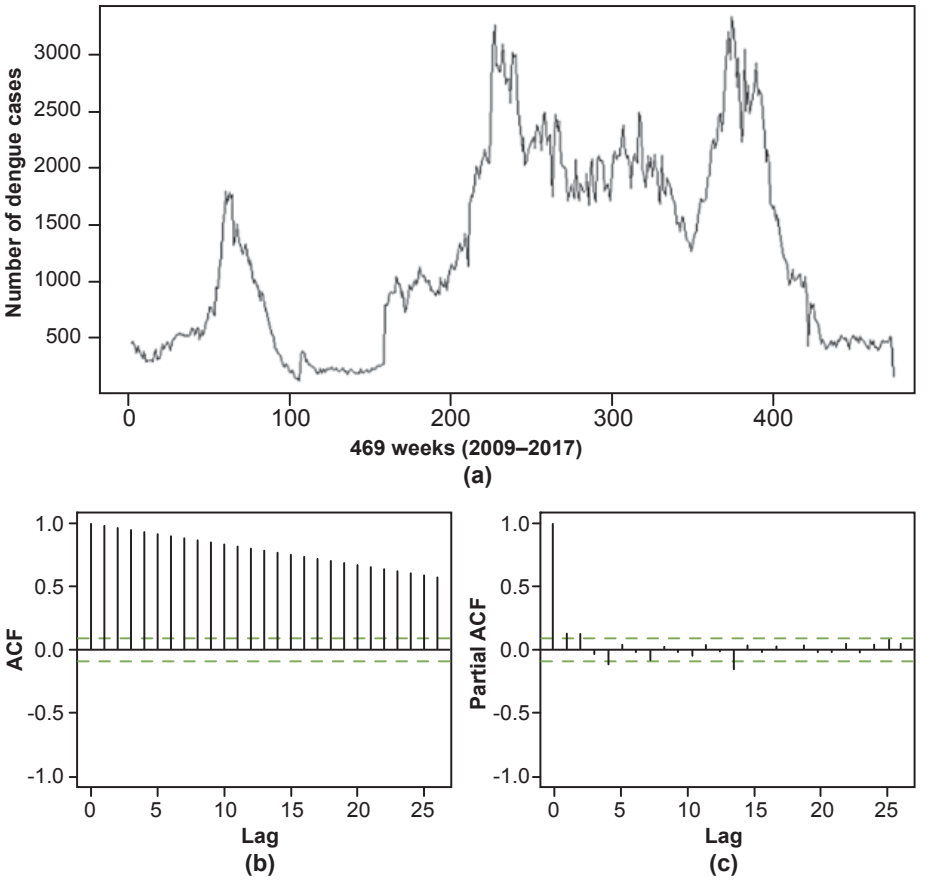
Figures 1b and 1c show the existence of simple and partial autocorrelations every 5 terms in the data, which suggests an autoregressive prediction of order 5, moving average of order 1; and order of integration 1. Thus, the appropriate method to project dengue cases in Colombia would be $ARIMA(5,1,1)$, whose polynomial model is given by the equation:

$$y_t = -0.89y_{t-1} - 0.26y_{t-2} - 0.02y_{t-3} + 0.25y_{t-4} + 0.16y_{t-5} + 0.67\varepsilon_{t-1} + \varepsilon_t$$

Application of the Ljung-Box test in software R found the chi-square value to be $\chi^2 = 0.27607$ and p value 0.5993>0.05. These differences showed that there was no autocorrelation in the residuals and the tentative model was valid for forecasting.

The normality test and the periodogram (shown in Figures 2a and 2b, respectively) helped determine that the adjusted residuals had a constant mean and variance, and enabled identification of periods with the highest peaks of dengue cases. Figure 2a shows that the difference between adjacent data of the series approaches a normal curve and Figure 2b shows the occurrence of three peaks; the first is presented in the logarithmic scale of 0.00625 which suggests selecting a cycle period of $\frac{1}{0.00625} = 160$ weeks to make forecasts. Considering the 160-week period and time-series data, a process to validate the model was undertaken for which

Figure 1: Number of dengue cases. (a) Number of cases. (b) Simple autocorrelations. (c) Partial autocorrelations. Colombia, 2009–2017



ACF: Autocorrelation functions

data from the 52 weeks of 2017 were used as real observations and the forecast was generated with the ARIMA(5,1,1) model of the same year. Figure 3 shows a good fit with a root mean squared error (RMSE) of 122.84 and Theil index (Theil's U) of 0.037. The model's fit for 2017 was validated and the stochastic model was used to generate a long forecast of potential dengue cases through 2022.

Weekly forecasts are shown in Figure 4a, which extends the series presented in Figure 1a to Week 729 (week 52 of 2022) with a margin of error of 0.5%. The forecast shows two other possible dengue outbreaks: one in 2019 and another in 2022. The first peak can be observed in Week 544 (week 23 of 2019) with an approximate total of 2624 cases. The second peak occurs in Week 690 (week 13 of 2022) with approximately 2336 cases. Figure 4b shows a bar chart with data on predicted cases per year. According to those projections, the highest number of cases will occur in 2019 (approximately 88,221) and in 2022 (approximately 77,344 cases).

Since the ARIMA forecasts of total dengue cases are highest for 2019, an adjustment was made to parameters B and θ of Model 1 through the nonlinear least squares regression given in Model 2 for data obtained for that year. Figure 5 shows data estimated by the ARIMA model projections and the solution for q of the ODE system presented in Table 1, showing the results of the two different analytic models (one stochastic and the other deterministic). Although the estimates differ, the deterministic model (the ODE system) approximates the stochastic model (ARIMA) after calibration of parameters via the nonlinear least squares regression.

In Model 1, q is the proportion of infected individuals. To adjust the model without loss of generality, the data yielded by ARIMA were normalized per 10,000 population; for example, the initial number of 1099 dengue cases (Week 1) was given as: $q(1) = q_1 = 0.1099$. To better adjust q, the 52 weeks of 2019 were divided into four epidemiological quarters. The first quarter is composed of weeks 1–15, and the second, third, and fourth, of weeks 16–28, 29–43, and 44–52, respectively.

The initial conditions for the first quarter are given by $q(1) = 0.1099$ (1099 infected individuals). The nonlinear least-squares regression estimates were $B \cong 0.1720$ per week and $\theta \cong 0.0992$ per week. For the second quarter, the initial condition was set as the final number of cases in the first quarter, i.e., $q(16) = 0.1802$ and after the adjustment, the following estimates were obtained: $B \cong 0.1705$ and $\theta \cong 0.0838$ per week. Similarly, for the third quarter, the initial condition was: $q(29) = 0.2433$, and $B \cong 0.1204$ and $\theta \cong 0.1177$ per week. Finally, for the fourth quarter, the initial condition was: $q(44) = 0.1342$, and $B \cong 0.2147$ and $\theta \cong 0.1535$ per week.

Using the values of parameters estimated for each quarter in 2019, dengue's BRN values were calculated. Vectorial capacity was recalculated as $B = R_0(\mu + \theta)$ where R_0 represents the BRN, defined by López[7] as the number of secondary infections produced after introduction of an infected individual into a susceptible setting. Unlike vectorial capacity, BRN is not measured in units of time, since it measures secondary infections produced during an individual's entire infectious period, while vectorial capacity represents the mosquito's capacity per unit of time to infect humans due to the original infected individual. When the BRN value is >1, the disease will persist in the setting. But when BRN is <1, dengue will disappear.[7]

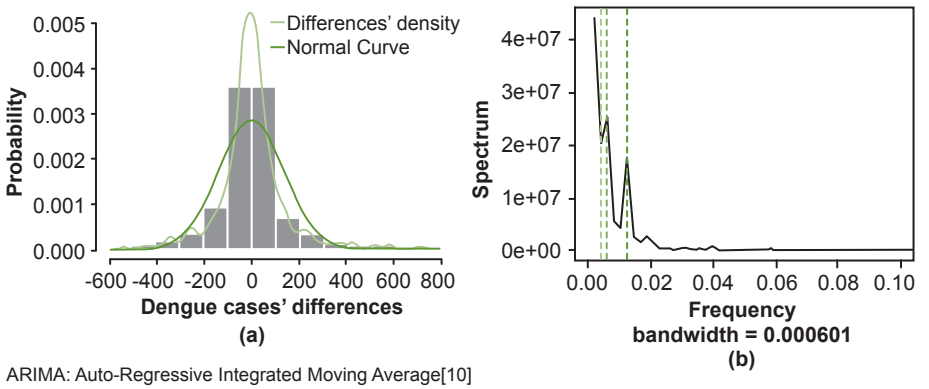
R_0 values estimated for each period in 2019 were:

- 1st quarter: $R_0 = 1.73$
- 2nd quarter: $R_0 = 2.03$
- 3rd quarter: $R_0 = 1.02$
- 4th quarter: $R_0 = 1.4$

During all four quarters of 2019, R_0 value was >1, indicating that the disease will persist in Colombia throughout the entire year. Since the second quarter had the highest value, the number of cases will be higher during those weeks, as observed in Figure 5.

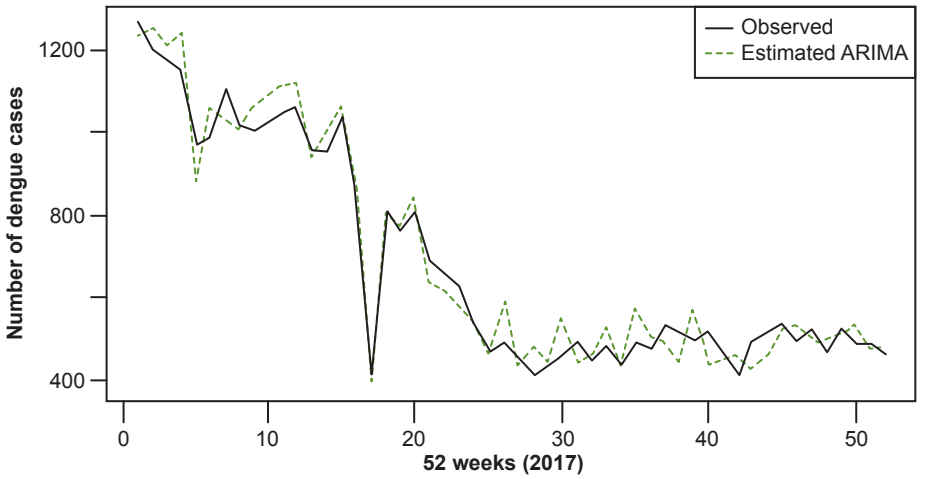
Table 1 summarizes the forecasts for 2019 by epidemiological week, with both the ARIMA and ODE models. The two models' forecasts presented only minor differences, except in the second

Figure 2: ARIMA model. (a) Normality test of residuals of adjusted model. (b) Periodogram of frequencies of peaks in which time-series cycles are presented



ARIMA: Auto-Regressive Integrated Moving Average[10]

Figure 3: Real values (observed) and estimated values (obtained via ARIMA model), Colombia, 2017



ARIMA: Auto-Regressive Integrated Moving Average[10]

and third weeks of May, when the numbers of predicted cases differed by more than 350.

DISCUSSION

No prior studies for Colombia have used adjusted numbers for forecasting dengue cases via time series using the ARIMA model, so there are no comparable results to examine. However, time-series analysis to forecast incidence of dengue has been the subject of research in other countries. In Brazil, for example, Cortes[29] applied and adjusted an ARIMA model, which he used to detect peak incidence and distribution patterns of the disease in two different areas of the country. In Sri Lanka, in the Gampaha District, Withanage[30] developed a forecasting model for dengue using multiple time-series regressions and successfully modeled the effects of climatic factors on imminent outbreaks. Both studies predicted the disease's distribution and incidence in a population, but unlike the present study, they did not determine other factors or measurements of the disease such as BRN and vectorial capacity (via integration with a determinist model such as ODE). Although this study's forecasts do not exactly mirror reality, application of a time-series model and a dynamic system to forecast disease incidence can produce a mathematical modeling trajectory that can be further applied and improved.

Several ODE systems-based mathematical models describe dengue transmission in the population and in general make it possible to determine the BRN and vectorial capacity based on parameters included in the model. Sardar,[23] for example, obtained the BRN based on an ODE system, and Liu-Helmersson[26] generated both the BRN and vectorial capacity. However, neither study identified a specific value for parameters in a given region. Other studies have taken hypothetical parameters or parameters obtained in laboratory studies in countries with very different climatic and health conditions from the country of application. Sepúlveda-Salcedo,[31] for example, adjusted the mathematical model parameters to the data reported in one year, and based on that, obtained forecasts of dengue cases in subsequent periods, the forecasts preserving the properties and values of the previous period's parameters.

In this study, parameter values were estimated for 2019 based on Colombian data and on forecasts for that same year. Though similar to other studies in expressing vectorial capacity and BRN under the parameters of the ODE system, the ARIMA model forecasts and subsequent adjustment of ODE parameters through nonlinear least-squares regression applied here enabled projection of specific BRN values and vectorial capacity specific to Colombia in 2019. Thus, ODE forecasts do not depend on parameters from other countries or time periods. The predicted numbers of dengue cases and BRN provide information on the disease's behavior in upcoming years and specifically predict the weeks that could present more severe outbreaks. Since the ARIMA-model forecasts extend through 2022, adjustments can be made over time to ODE parameters for subsequent periods to find the corresponding R_0.

To the best of our knowledge, no previous studies compared the application of ARIMA and ODE methods for viral diseases, or specifically for dengue. In addition to forecasting possible variations in the number of cases, the importance of this approach is that the ARIMA method allows forecasting of potential cases based solely on data already reported; with this forecast in mind, the ODE parameters can then be adjusted to provide information on which qualities (ODE parameters) are affecting the rise or fall in the number of cases. While it is important to predict occurrence of high numbers of cases, it is also important to identify the factors causing the increase, such as BRN, vectorial capacity, and other relevant parameters.

One shortcoming of the study is that it was based on data for past and present conditions, and results could change significantly if, among other things, vector controls are applied or climatic and environmental conditions change; in the transmission of infectious diseases, a small change in conditions can lead to totally different behaviors than those previously forecast. Another limitation is that since the ARIMA model is not recommended for long-term forecasting, a four-year projection is likely to yield less accurate estimates. However, the 2019 forecasts in this study are in the range of close approximation. It is recommended that followup to the proposed forecasts be considered in order to establish them

Figure 4: Number of dengue cases forecast (ARIMA), Colombia, 2018–2022.
(a) Forecasts with time series. (b) Forecasts with bar chart

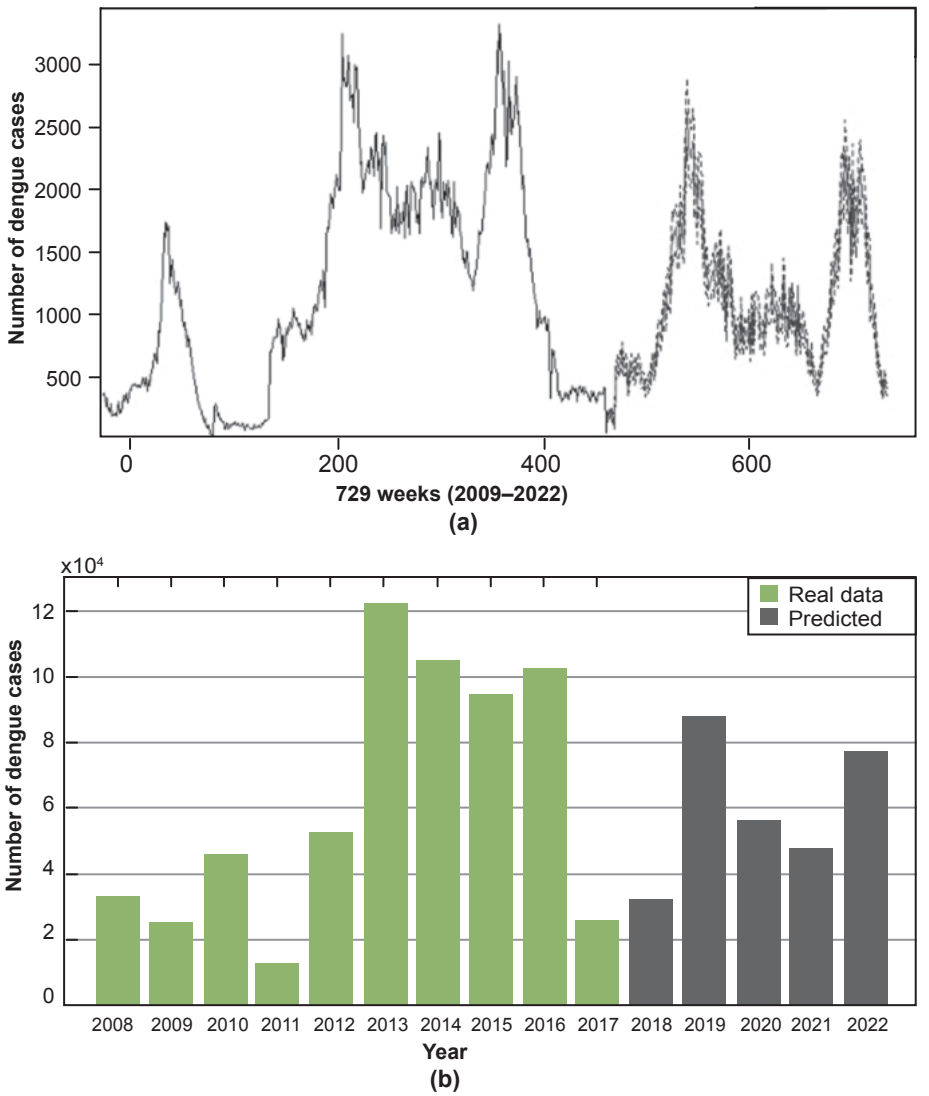
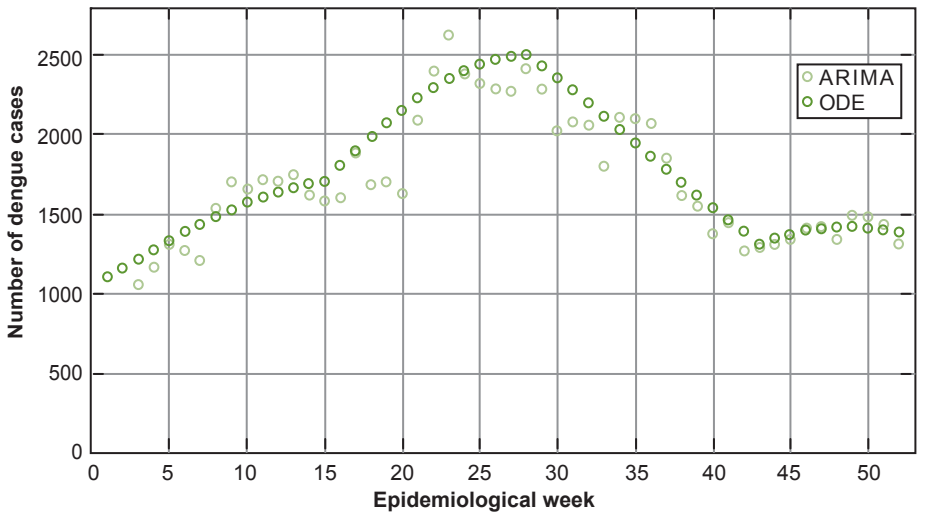


Figure 5: Number of dengue cases forecast, Colombia, 2019



ARIMA: Auto-Regressive Integrated Moving Average[10]
ODE: Ordinary differential equations[21]

Table 1: Number of dengue cases forecast, Colombia by week for 2019 based on two mathematical models (ARIMA and ODE)

Month	Week	ARIMA	ODE	Month	Week	ARIMA	ODE
January	W1	1099	1099	February	W1	1306	1329
	W2	1159	1158		W2	1265	1383
	W3	1053	1216		W3	1211	1435
	W4	1166	1273		W4	1528	1483
	TOTAL	4476	4746		TOTAL	5308	5630
Month	**Week**	**ARIMA**	**ODE**	**Month**	**Week**	**ARIMA**	**ODE**
March	W1	1698	1528	April	W1	1618	1688
	W2	1655	1569		W2	1577	1705
	W3	1713	1606		W3	1606	1802
	W4	1703	1638		W4	1884	1896
	W5	1745	1665				
	TOTAL	8515	8006		TOTAL	6684	7090
Month	**Week**	**ARIMA**	**ODE**	**Month**	**Week**	**ARIMA**	**ODE**
May	W1	1683	1987	June	W1	2624	2352
	W2	1697	2073		W2	2391	2401
	W3	1626	2154		W3	2320	2442
	W4	2086	2228		W4	2287	2472
	W5	2398	2294				
	TOTAL	9491	10736		TOTAL	9622	9667
Month	**Week**	**ARIMA**	**ODE**	**Month**	**Week**	**ARIMA**	**ODE**
July	W1	2273	2493	August	W1	2081	2279
	W2	2408	2505		W2	2059	2198
	W3	2283	2433		W3	1801	2115
	W4	2023	2358		W4	2114	2031
					W5	2100	1947
	TOTAL	8988	9789		TOTAL	10156	10570
Month	**Week**	**ARIMA**	**ODE**	**Month**	**Week**	**ARIMA**	**ODE**
September	W1	2070	1863	October	W1	1373	1539
	W2	1848	1780		W2	1448	1462
	W3	1618	1698		W3	1267	1388
	W4	1550	1618		W4	1291	1307
	TOTAL	7087	6960		TOTAL	5378	5697
Month	**Week**	**ARIMA**	**ODE**	**Month**	**Week**	**ARIMA**	**ODE**
November	W1	1310	1342	December	W1	1493	1418
	W2	1341	1370		W2	1479	1413
	W3	1401	1392		W3	1431	1402
	W4	1414	1408		W4	1310	1385
	W5	1338	1416				
	TOTAL	6803	6928		TOTAL	5713	5619

ARIMA: Auto-Regressive Integrated Moving Average[10] ODE: Ordinary differential equations[21]

as a viable methodology that could be applied to other infectious diseases.

Despite its limitations, the study shows the usefulness of synergy between two models which, although with different rationales, complement one another to make forecasts useful for timely preventive actions.

CONCLUSIONS

Our forecasts for dengue in Colombia for 2018 through 2022 using the ARIMA model show that most cases will likely occur in 2019. The ARIMA forecasts enabled adjustment to ODE parameters and subsequent BRN estimates for dengue for that year, including possible variations in different quarters, and revealed the disease's persistence.

Despite the BRN's importance (since it predicts the disappearance or persistence of a viral disease in a population), there are no reports on BRN for dengue. These estimations are essential for health institutions particularly before seasons with high predicted BRN values. These methods can be applied to other vector-transmitted diseases such as Zika and chikungunya.

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Assistive Devices for Older Adults: A Longitudinal Study of Policy Effectiveness, Santiago, Chile, 2014–2016

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ABSTRACT

INTRODUCTION Population aging is a worldwide phenomenon. It is estimated that by 2050, one of five persons will be aged ≥60 years. In Chile, 15.8% of the population is now aged ≥60 years, and this figure will reach 30.7% by 2050. In 2006, a national program was implemented to provide assistive devices to older adults aged ≥65 years with limited mobility or difficulty performing activities of daily living. To date, there have been no assessments of the program's effectiveness.

OBJECTIVE Assess the effectiveness of an assistive devices policy in Chile on improving functional capacity of older adults aged ≥65 years, and beneficiaries' perceptions of the services received, including changes in their quality of life.

METHODS This was a before–after longitudinal study. A cohort of 309 persons was recruited, consisting of patients who received care at a public hospital in Santiago, Chile during 2014–2015. They were assessed before delivery of assistive devices, then followed for seven months, with repeated evaluations made in their homes. The following indicators were measured: functional capacity (Tinetti scale and Barthel Index); changes in perceived quality of life related to use of assistive devices; and other sociodemographic, clinical and protocol-compliance variables. A longitudinal analysis of before–after progress was carried out, as well as a description of service delivery and medical followup.

RESULTS Sixty-eight percent of those surveyed were women; median age was 74 years, average schooling was 6 years, and 93% had low income (monthly income <US$398). Assistive devices increased independence in activities of daily living, improved mobility and perceived quality of life, and decreased fall risk and pain. One hundred percent felt satisfied with the service received, 91% were trained in use of the device, and delivery deadlines were met in 83% of cases, but only 2% were followed up. One negative aspect is that the program covers only 25% of estimated need.

CONCLUSIONS This assistive device program helps improve functional capacity and perceived quality of life in vulnerable patients who are able to access it. It addresses a real need and is highly valued by patients. Although delivery schedules were fulfilled, followup care schedules were not.

KEYWORDS Aging, mobility limitation, assistive devices, activities of daily living, health care system, health care reform, quality of life, Chile

INTRODUCTION

Population aging is a worldwide phenomenon. It is estimated that by 2050, there will be 2 billion persons aged ≥60 years (22%).[1] In Chile, this group now represents 15.8% of the population,[2] and is expected to reach 30.7% by 2050.[3] In addition, the percentage of those aged ≥80 years (very old age) in this group will grow substantially.[3,4]

The aging process manifests as declines in physical, mental and psychosocial health, and inability to perform activities of daily living.[5] In Chile, although 94.7% of persons aged 60–64 are classed as functionally independent, with only 0.9% severely dependent; in persons aged ≥80 years these figures change to 60.4% and 12.9%, respectively.[6] In persons aged ≥60 years, 10.4% show some cognitive impairment, and 4.5% have disability-associated cognitive impairment.[7] Functional impairment, along with environmental conditions, can affect life performance and alter gait, resulting in the need for assistive devices (ADs) in some older adults to maintain their independence and autonomy.[8]

IMPORTANCE This study is the first to assess effectiveness of the national assistive devices program for older adults in Chile, a public policy implemented since 2006.

In 2006 in Chile, the provision of ADs for persons aged ≥65 years was introduced in the Explicit Health Guarantees Regime (GES) [9] to improve functional abilities. Created in the aftermath of a 2005 health care reform,[9] the GES lists priority health conditions qualifying for access guaranteed by law (legal right to health care), timeliness (specified maximum waiting times for services), financial protection (maximum copay of 20%) and quality (accredited providers). Its main objective is to reduce health care disparities for a group of services, in both the public and private healthcare systems.

The Chilean health system is mixed. Public health insurance through a national health fund (FONASA) covers about 80% of the population with care mainly by providers within the public National Health Services System. FONASA classifies its members in categories A, B, C or D, based on income. Categories A and B are for lowest-income segments (≤US$423 per month). Another parallel, private health subsystem and a separate health system within the armed forces complete the spectrum of coverage offered to Chileans. Less than 5% of the Chilean population is not covered by any health insurance system. [10,11]

Through its AD program for persons aged ≥65 years, GES ensures provision of canes, walkers and wheelchairs to promote recovery of function, improve gait and stability, and foster independence in activities of daily living.[12] Medical AD prescription is based on a functional assessment using the Barthel Activities

of Daily Living Index and the Tinetti Falls Risk Scale.[5,12–14] From the time of medical prescription, the legally established period for timely delivery is a maximum of 20 days for canes and 30 days for walkers and wheelchairs. The clinical guidelines specify that all patients and/or caregivers must receive training and be evaluated one month after AD delivery. In addition, further followup must be performed by the patient's primary care provider.[12]

In 2017, the public health system delivered 133,356 ADs to its members throughout the nation, at an estimated annual cost of US$5.5 million. The numbers of ADs delivered increased 39% from 2012 to 2017,[15] with an associated 47% increase in financial costs.[15,16] It is estimated that 19.3% of older FONASA members need an AD.[16]

This study was carried out with FONASA beneficiaries living within the service area of the Padre Hurtado Hospital (HPH), which belongs to the Southeast Metropolitan Health Service in Santiago, Chile. HPH serves the municipalities of San Ramón, La Pintana and La Granja, and is institutionally accredited in compliance with GES.

At the time of the study, no assessment was available regarding the effect of the GES AD program on recovery of functional capacity, nor had there been any previous study of beneficiaries' perceptions of the delivery process or of changes that ADs had made in their quality of life. For this reason, in 2014, HPH, in conjunction with the National Service for Older Adults, the National Disability Service, and the Epidemiology and Health Policy Center of the Medical School, Clínica Alemana, Universidad del Desarrollo (CEPS-CAS-UDD), began a study with two objectives: to assess effectiveness of the AD provision policy in improving functional capacity in persons aged ≥65, and to understand the care process and members' perceptions of the service they received, as well as resulting changes in their quality of life (QoL).

METHODS

Study design This before–after longitudinal, analytical observational study involved persons aged ≥65 living in the HPH service area, who participated in the GES AD program and received a cane, walker or wheelchair from September 2014 through July 2015. Each participant was followed over 7 months with 4 assessment points: T0 or baseline, when participants were enrolled and received their AD; and T1, T2 and T3, at 40, 130 and 220 days after enrollment, respectively.

Population and sample size calculation Eligible persons were aged ≥65 years, who were members of FONASA and received their AD through GES at HPH. The size of the universe was estimated at 1645 persons, based on the number of ADs delivered the year before the study (2013). With a repeated sampling design and assumptions of a mean 5-point improvement from baseline to final score for dependence in carrying out activities of daily living (Barthel Index), a 95% confidence level, 90% power and expected standard deviation of 20 points at baseline and 15 points at the end, a minimum sample size of 265 persons was estimated to be monitored through study completion. Since older-adult participants were to be assessed 4 times in the 7-month period, an approximate natural attrition rate of 5% between each measurement was estimated; thus, the study began with 309 persons.

ADs delivered the year before the study to HPH included wheelchairs (35%), canes (46%) and walkers (19%). For purposes of sample distribution and subsequent analysis, these percentages were adjusted to 30% for wheelchairs and walkers, and 40% for canes. Sample size was calculated using the EPIDAT 4.1 epidemiology program, with a paired-sample average comparison design.

Inclusion and exclusion criteria, recruitment Persons aged ≥65 years who received ADs under the GES regime and did not have cognitive impairment (Mini-Mental exam ≥14 points) were included.[5,12] Persons hospitalized at the time of the study, those who received a temporary assistive device, and people diagnosed with dementia were excluded.

Recruitment took place at HPH. Every person who had been prescribed an AD from September 2014 through July 2015 was contacted to request his or her voluntary participation in the study. A Mini-Mental exam was performed to assess exclusion criteria. Participants were invited to enroll based on written informed consent. Enrollment continued until the estimated sample size was reached. A baseline functional assessment (T0) was made at hospital and subsequent assessments (T1, T2, T3) were made in participants' homes.

Variables and data analysis A description of participants was made at the time of enrollment (T0), which included: age in years; sex male/female); indigenous group membership (yes/no); educational level completed (none/primary education/secondary school/higher education, including technical, trade, normal schools or university); employment status (active/inactive); health insurance (FONASA A or B/FONASA C or D); participant's monthly income considering wages, pensions and other income; monthly per capita household income, calculated using number of dependent persons; reduced income due to health condition (yes/no); partner status (living with a partner/not living with a partner); presence of caregiver (has caregiver/needs caregiver, but does not have/does not need help); chronic diseases due to multiple causes per ICD10; chronic pain (yes/no); fall reported in the previous six months (yes/no); and type of AD delivered (cane, walker, wheelchair, and wheelchair plus another aid).

Percentages were calculated for the above variables, as well as median age and age range. To analyze the number of persons lost to followup, rates were used that took months of observation per person into account. For participants who died, the cumulative mortality rate for the followup period (seven months) and mortality incidence were used, considering the months of observation per person.

To assess effectiveness of the AD provision policy on improvement of functional capacity, the following variables already mentioned above were analyzed according to study objectives: functional capacity, fall frequency, presence of pain and mobility problems. The operationalizing, measurement and analysis of these variables is described below.

Functional capacity Two tools were used. One is the Barthel Index,[5,12–14] which measures capacity to perform ten activities

of daily living. These activities can be scored as 0, 5, 10 or 15 points, depending on degree of independence. With this method, a total score from 0 to 100 is obtained—which will always be a multiple of 5—the higher the score, the greater the independence. Scores are grouped into categories according to dependence levels: <20 totally dependent, 20–35 severely dependent, 40–55 moderately dependent, ≥60 but <100 mildly dependent, and 100 independent (90 if using wheelchair).The second tool applied was the Tinetti scale,[12–14] which measures fall risk with a score of 0–28. On this scale, the higher the score, the lower the risk. There are 3 risk levels: <19 high risk, 19–24 medium risk, and 25–28 low risk. Both variables were analyzed as continuous and categorical variables.

Means and standard deviations of the Barthel and Tinetti scores were calculated for each followup point. In addition, longitudinal analysis of these scores was performed using a General Linear Model for repeated measurements with p = 0.05 as significance threshold. Categorical analysis for both indices was performed by calculating proportions of participants at each followup point. Dichotomous variables were generated to assess progress from T0 to T3. The Barthel Index was dichotomized as "independent" and "dependent," using a score of 60 as the cutoff point.[17,18] This score classifies those who show "independence" or "mild dependence" on the original index as independent, and those with "moderate dependence," "severe dependence" or "total dependence" as dependent. The Tinetti score was measured for those able to walk at the time of testing, and a score of 18 (upper limit of the high-risk category) was taken as the cutoff point, generating the categories "high fall risk" and "medium and low fall risk." Progress of participants from beginning to end of followup was analyzed using these dichotomous variables.

Moving from dependence to independence or remaining independent were considered a positive course on the Barthel Index, while moving from independence to dependence or remaining dependent were both classified as a negative course. For the Tinetti score, moving from high fall risk to medium or low fall risk or remaining in the medium or low fall risk category were both classified as a positive course, while moving from medium or low risk to high risk, or remaining a high fall risk were both considered a negative course. The McNemar-Bowker test was used to assess the statistical significance of these changes, comparing assessments from T0 to T3 using dichotomous categories, with a significance threshold of p = 0.05.

Sample size variations were larger in the Tinetti test because of participants lost to followup and exclusion of those unable to walk at T0 or T3, as well as those who received only a wheelchair, since the test did not apply in such cases. The before–after course could not be analyzed for any of these participants, since both measurements were not always available.

Fall frequency Upon recruitment, participants were asked about number of falls in the previous six months, and the proportion of participants reporting falls as well as the total and average number of falls were calculated. Participants were also asked for the number of falls between each followup point. Fall incidence was calculated using observation time per person (months/person).
Pain The study considered two variables: "new pain with AD use" and "chronic pain that decreased with AD use." Both are dichotomous (yes/no) variables. The presence of pain for more than six months from baseline was considered chronic pain. The proportion of participants in both categories was analyzed.

Mobility problems Upon recruitment and at the last observation point, participants were asked about mobility problems both inside and outside the home, and the number of persons reporting these problems was analyzed. The number of persons reporting fear of falling when leaving home was also studied. The McNemar test was run on contingency tables, with p <0.05.

To understand the characteristics of the care process and members' perception of care and changes in their QoL with the AD, data were collected on timeliness and consistency of delivery, training in AD use, monitoring one month after delivery, and followup in primary care. These variables were analyzed according to GES clinical guideline recommendations.

Timeliness of delivery Percentage of participants receiving ADs within the period specified in GES guidelines. For time elapsed, the median number and range of days were calculated.

Consistency of delivery Percentage of participants receiving the same AD prescribed in primary care, and percentage of supplied ADs matching the degree of dependence and fall risk found at the time of recruitment (T0).

Training in use Percentage of participants and/or caregivers who reported having received training at the time of delivery.

Checkup one month after delivery to assess participant AD use and make adjustments Percentage of participants who reported being contacted for checkup one month after AD delivery, as indicated in GES guidelines.

Followup in primary care Percentage of participants who went for their regular primary care checkup and percentage of checkups in which the care provider inquired about participant's AD use. During the first year of the study, we also calculated AD deliveries as a percentage of the total number of FONASA older adults requiring them, according to national estimates (19.3%). This indicator served as a performance measure of access guarantee.

Satisfaction with care Based on participant declaration, satisfaction at time of delivery (very satisfied/satisfied/unsatisfied/very unsatisfied). Percentage of participants in each category was also calculated.

Perceived quality of life effects A tool designed in 2011 by the National Disability Service (SENADIS) was used to study changes in perceived QoL attributable to AD delivery and use. The tool, based on a questionnaire, had two dimensions: well-being (nine questions on wellbeing with AD use in education, employment, free time and leisure activities) and satisfaction (four questions about users' perception of AD related to their performance in daily life, family life, relationships with others, physical condition and emotional state). Questions were answered using the Likert scale, with responses from 1–5, grouped as "negative" (1–3) or "positive" (4–5). For each dimension, a perception was considered positive when more than half the questions received positive responses. Finally, if there was a positive perception in at least one of these two di-

mensions, this was considered a positive change in perceived AD-attributable QoL. For practical reasons, this measurement was not performed at T2.

Change in perception was also classified as positive or negative, measured from the baseline to the last assessment. Direction was considered positive if participants maintained a positive perception of their QoL, or if QoL changed from negative to positive; and was considered negative if perception remained negative from baseline to last assessment or changed from positive to negative.

Data collection A data collection card was created containing the variables and tools previously mentioned. Before recruitment, the card was tested on 25 persons not included in the sample. Data were collected on site (in hospital and participants' homes) from September 2014 through February 2016 by previously trained kinesiologists. For quality control of data collection, researchers supervised 10% of participants on site and by telephone, finding no inconsistencies. Data were uploaded to a tablet using the SurveyToGo program[19] and the database was exported to SPSS v25.0, updated and validated weekly.

Ethical aspects The study was approved by the ethics committee of the Southeast Metropolitan Health Service (SSMSO). Participants were invited to enroll based on written informed consent. Participation involved no risk or cost for them, and a conduct protocol was established to provide for situations that might compromise participants' health or safety. Data were de-identified and treated as confidential by the research team.

RESULTS

Description of participants Contact was made with 542 older adults who had been prescribed an AD, of whom 233 (43%) were excluded due to an abnormal score on the Mini-Mental exam. The remaining 309 were recruited for the study. Forty-two participants (13.6%) were lost to followup: 20 died, 8 could not be located, 7 moved to other communities, and 7 decided not to continue followup. The final sample size was 267 participants (1954 person-months) of observation. Those who died contributed 43 person-months, and the rest of the lost participants contributed 42 person-months, translating into a 9.7% loss of observation time per person. The cumulative mortality rate for the followup period was 6.5 per 100 persons, and the mortality incidence was 1.02 per 100 persons per month.

Regarding delivered ADs, 124 participants (40%) received a cane, 52 (17%) a walker, 70 (23%) a wheelchair, and 63 (20%) a wheelchair plus another AD (cane or walker).

The median age was 74 years (range of 65–99) and 68% were women. Participants who reported membership in an indigenous group were 6.1%, all Mapuche. Seventy percent reported having completed an elementary or primary level of education; 94% were not actively employed; and 89% belonged to the two lowest public health insurance income categories. Concerning personal monthly income, 93% (284/306) were receiving less than US$391, and when the number of persons depending on that income was considered, for 72.2% of cases per capita income was less than US$156 (Table 1). Participants who stated that their income had been reduced due to their health problem made up 73.8% (228/309) of the total.

The main chronic conditions reported were: diseases of the circulatory system (88.3%), musculoskeletal and connective tissue (73.5%), endocrine, and nutritional and metabolic system (62.5%), as well as vision or hearing loss (61.2%) (Table 1). Conditions leading to prescription of an AD were: arthritis/arthrosis (218/304, 72%), altered gait/fall risk (54/304, 18%) and stroke (33/304, 11%). Of the participants, 44.7% were living with a partner, 74.1% reported having a caregiver, and 3.6% needed a caregiver but did not have one.

Table 1: Sociodemographic characteristics, health problems and type of assistive device provided, Santiago, Chile, September 2014–February 2016

Characteristic	Total
Age (median and range in years) (n = 309)	**74 (65–99)**
Sex (n = 309)	
Male	99 (32.0%)
Female	210 (68.0%)
Membership in indigenous group (n = 309)	
Yes	19 (6.1%)
No	290 (93.9%)
Education level completed (n = 309)	
No schooling	21 (6.8%)
Elementary, primary	216 (69.9%)
Secondary school	57 (18.4%)
Higher education (technical, trade, normal schools or university)	15 (4.9%)
Employment status	
Active	290 (93.9%)
Inactive	19 (6.1%)
Health insurance (n = 309)	
FONASA A or B	275 (89.0%)
FONASA C or D	34 (11.0%)
Monthly per capita household income (n = 306)	
<US$156	221 (72.2%)
≥US$156 or more	85 (27.8%)
Partner status (n = 309)	
Living with a partner	138 (44.7%)
Not living with a partner	171 (55.3%)
Presence of caregiver (n = 309)	
Has caregiver	229 (74.1%)
Needs caregiver, but does not have	11 (3.6%)
Does not need help	69 (22.3%)
Chronic condition or disease (n = 309)	
Circulatory system	273 (88.3%)
Musculoskeletal and connective tissue system	227 (73.5%)
Endocrine, nutritional and metabolic	193 (62.5%)
Vision or hearing loss	189 (61.2%)
Mental and behavioral disorders	75 (24.3%)
Respiratory system	73 (23.6%)
Genitourinary system	33 (10.7%)
Neoplasms	32 (10.4%)
Nervous system	25 (8.1%)
Digestive system	21 (6.8%)
Type of assistive device delivered (n = 309)	
Cane (1 or 2)	124 (40%)
Walker	52 (17%)
Wheelchair	70 (23%)
Wheelchair plus another aid	63 (20%)

Assessment of AD policy effectiveness on improved functional capacity, fall frequency, presence of pain and mobility problems The dependence-level score improved significantly during followup, from 79.7 at T0 to 84.7 at T3 (p <0.001) (Table 2), showing a positive course in 252 of 267 participants assessed, representing 94.4% (p = 0.005) (untabulated data). The dependence profile also showed an increase in percentage of independent persons.

At baseline, 173 participants (56%) reported having fallen in the prior 6 months, with a total of 510 falls (averaging 2.9 per person). This is equivalent to an incidence of 27.5 falls per 100 person-months. During the 7 months of followup, incidence was 4.8 falls per 100 person-months, representing an approximate fivefold decrease (untabulated data).

Although the mean fall-risk score increased during followup from 19.4 at T0 to 20.4 at T3, the variation was not statistically significant (p = 0.091) (Table 2). The fall-risk profile from T0 to T3 showed a positive course in 85 of the 116 participants assessed, or 73.3% (p = 0.007) (untabulated data).

Concerning pain, at baseline 49.2% of participants mentioned chronic pain that had lasted more than 6 months. At final followup, 65.8% reported this pain had decreased with AD use (Table 3). On the other hand, approximately 14% (T1: 38/277; T2: 27/242; T3: 33/218) indicated that AD use caused them pain that they did not have previously.

With respect to mobility, a significant decrease was noted in percentage of participants who reported mobility problems at home (the same trend occurs with fear of falling outside the home). There was no significant decrease for persons reporting mobility problems outside the home (Table 3).

Table 2: Functional dependence and fall risk at followup points, Santiago, Chile, September 2014–February 2016

Followup point	T0	T1	T2	T3
Severity level (degree of dependence)	n (%)	n (%)	n (%)	n (%)
Number of cases	309	296	276	267
Totally dependent	3 (1.0)	4 (1.4)	0	0
Severely dependent	11 (3.6)	9 (3.0)	7 (2.5)	7 (2.6)
Moderately dependent	32 (10.4)	14 (4.7)	9 (3.3)	8 (3.0)
Mildly dependent	199 (64.4)	195 (65.9)	166 (60.1)	152 (56.9)
Independent	64 (20.7)	74 (25.0)	94 (34.1)	100 (37.5)
Average (SD)	79.7 (SD 17.7)	82.3 (SD 16.6)	84.3 (SD 15.8)	84.7 (SD 16.1)
Fall risk[a]	**n (%)**	**n (%)**	**n (%)**	**n (%)**
Number of cases	172	183	156	142
High risk	72 (41.9)	79 (43.2)	65 (41.7)	50 (35.2)
Medium risk	80 (46.5)	97 (53.0)	80 (51.3)	79 (55.6)
Low risk	20 (11.6)	7 (3.8)	11 (7.1)	13 (9.2)
Average (SD)	19.4 (SD 5.32)	19.8 (SD 4.04)	19.9 (SD 4.08)	20.4 (SD 4.49)

[a]Those with wheelchairs excluded, since Tinetti does not apply in such cases.

Table 3: Pain, mobility problems and fear of falling at followup after assistive device use, Santiago, Chile, September 2014–February 2016

Followup point	T0	T3	p value
Chronic pain			
Persons reporting chronic pain at start	152/309 (49.2%)	N/A	N/A
Persons reporting decreased chronic pain with AD use at followup	N/A	100/152 (65.8%)	N/A
Mobility problems			
Inside the home	49/214 (22.9%)	19/214 (8.9%)	<0.001
Outside the home	53/152 (34.8%)	39/152 (25.7%)	0.059
Fear of falling			
Fear of falling if leaving home	151/177 (85.3%)	65/177 (36.7%)	<0.001

AD: assistive device N/A not applicable

The care process and participant perception of changes in their lives attributable to AD use Timely delivery guarantee was met for 83% (256/309) of participants. A second AD was prescribed during followup for 41 participants. For these persons, the timely delivery guarantee was met in 80.5% (33/41) of cases.

Of total participants who received an AD, 87.4% were using the one prescribed in primary care (Table 4). In the remaining 12.6% (39/309), the AD was changed by the kinesiologist after assessment at time of delivery. When type of AD provided was studied according to degree of dependence assessed at T0, it was noted that 100% of persons with total or severe dependence (14) received a walker or wheelchair. Regarding fall risk at time of delivery, 79.1% (159/201) of those at high risk or who were unable to walk received a walker or wheelchair.

Most participants (90.9%) were trained at baseline in AD use (Table 4). Participants who received wheelchairs represented 75% of those who received no training (28/309). Of the caregivers who accompanied participants during AD delivery, 85.8% (205/239) were taught how to use and adjust it.

According to participant reports, only 2.3% were contacted by their primary care provider for a checkup appointment one month after AD delivery (Table 4), and of those who received a new AD due to the kinesiologist's prescription, none were contacted for a checkup appointment. Concerning followup in primary care, 92% (285/309) of participants went to a primary care checkup during the study period, and in 46.3% of these checkups, the care provider inquired about their use of the AD (Table 4).

Perception of care and change in quality of life At the time of delivery, all participants (309) were satisfied or very satisfied with the care provided both during and prior to delivery.

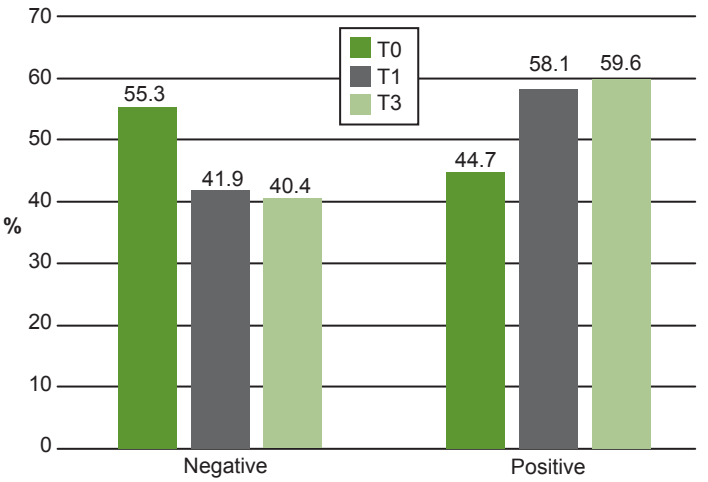
Percentage of participants who positively described their quality of life increased during followup (Figure 1). From T0 to T3, a positive change occurred in 159 (59.6%) of the 267 persons assessed (untabulated data). Differences in the total number of those assessed at each followup point are due to participants lost to followup. Those who showed a positive change in ability

to perform activities of daily living also had improved quality of life (61.1%; 154/252). A similar improvement (80%; 68/85) occurred in those who showed a positive change in fall risk (untabulated data).

Table 4: Care process for use of assistive devices, Santiago, Chile, September 2014–February 2016

Characteristic	Total
Timely delivery guarantee met (n = 309)	256 (83.0%)
Prescribed device delivered (n = 309)	270 (87.4%)
Training in use (n = 309)	281 (90.9%)
One-month followup (n = 309)	7 (2.3%)
Followup at primary care level (n = 285)	132 (46.3%)

Figure 1: Change in perceived quality of life at followup points after use of assistive devices, Santiago, Chile, September 2014–February 2016*



*Quality of life self-perception was not assessed at followup time T2.

DISCUSSION

The study shows outcomes of a public policy in a population at risk due to advanced age, lower socioeconomic status and comorbidities. It confirms that for those who accessed the program, the GES regime fosters recovery of functional capacity and improved performance of daily living activities, reduction of fall risk, and better perceived QoL. In 2014, of a total 6756 persons who required an AD according to public insurance estimates, only 1686 ADs were delivered. This means that the program can meet only 25% of the estimated need of its intended beneficiaries aged ≥65.[20]

Concerning the GES regime's other legally guaranteed standards, timeliness is being met in 83% of cases. It is important that ADs be delivered as soon as possible, since this leads to better health outcomes.[21–23] The financial protection and quality standards are being met, since the ADs are free for this population group and the hospital has institutional accreditation.

Followup and subsequent monitoring were poor. According to GES clinical guidelines, all patients must be assessed one month after AD delivery and receive subsequent followup in primary care. Only 2% of study participants received a primary care checkup one month after AD delivery, and fewer than half of those who go for regular health checkups were asked by the attending health care professional about aspects related to their AD. Since these are high-risk individuals, especially those with progressive cognitive impairment or pre-existing mental health conditions, followup is especially important and should meet GES guideline specifications. Such followup allows providers to identify new needs, assess patient experiences with the AD, understand the context in which the device is used, and make necessary adjustments.[12,24–27] To address this, a budget for followup activity should be built into the program.

More than half of participants in our study who received an AD reported falls in the previous six months. This percentage is higher than that reported by Iñaki, who predicted 30%–40% of those aged ≥65 will fall at least once a year.[28] Our percentage is also higher than the fall prevalence level of 33.3% reported by Silva for the 6 months preceding that study.[29] This finding should be duly considered, since falls contribute to higher morbidity and mortality rates, increased health care costs, loss of mobility and independence, general health declines and greater need for long-term care.

In Chile, it has been estimated that one-third of persons aged ≥65 years living at home will suffer a fall during the course of a year. This estimate increases to 50% for persons living in institutions and those aged ≥80 years.[30] Thus, the AD provision program assessed in this study represents a preventive public policy that makes a major contribution, allowing older adults to avoid falls and live longer in better health.

One surprising finding is the increase in participants with a fall risk 40 days after AD delivery. This may be related to how long it takes them to learn optimal use of the AD. Although there are few mentions in the literature of a specific learning period, experts report that adaptation should take about one month with proper training and followup, which participants in this study did not receive.[31] The eight-week study by Leving[31] in older adults using wheelchairs describes a rapid improvement in use after three weeks of guided training followed by a lesser but sustained improvement after four weeks, compared to a control group without training. Other studies of wheelchair and cane users report shorter times, but with intensive weekly training.[32,33]

The precarious social and economic circumstances of the study population make participants especially vulnerable, since they live in communities exhibiting many indicators of decay. Difficult living conditions in addition to poor health status could explain why fewer than half the participants had a positive outlook on their quality of life at the beginning of the study; however, this improved substantially after AD delivery.

This improvement is still below that found in Chile's 2013 National Socioeconomic Description Survey, which included questions on life satisfaction,[34] and in which 65.8% of older persons stated that they were completely or highly satisfied with their lives. This figure is consistent with findings of the National Survey on Quality of Life in Old Age, in which 63% indicated that they were satisfied or very satisfied.[35]

Another finding that underscores the importance of the surrounding environment for QoL are problems with mobility outside the home, which did not improve after AD delivery. Comprehensive, intersectoral policies need to be implemented to accommodate

aging. These policies should consider safe and "aging-friendly" physical and social environments; allow participation by all stakeholders; involve older adults in policy formulation, development, and implementation; and address the inequalities that affect their health.[8,21,36,37]

Those who had better functional capacity at the beginning of the study significantly improved their independence in performing daily living activities, decreased their fall risk, and improved their perceived QoL. This merits consideration for implementing proactive early-needs surveys and timely AD delivery. Both self-reliance and independence in performing daily activities as well as low fall risk are associated with better perceived quality of life. In this study, those who reported a decrease in their dependence level regarding daily living activities and fall risk also reported significantly improved QoL. Similar findings have been reported in international and national studies relating different dimensions of health to QoL.[38–44]

Although it was not among the study objectives, a collateral finding with implications for public policy was the high percentage of persons with cognitive impairment. The 2017 National Health Survey reported cognitive impairment in 16.9% of older adults with low educational level.[45] In the recruitment process for this study, the rate was 2.5 times higher (42%). This was a limitation of the study, since the research protocol excluded these patients and as a result, no information was obtained from this group. This high percentage of cognitive impairment reinforces the need for followup with systematic checkups after AD delivery.

As in all longitudinal designs, some participants were lost to followup in this study, although the number was lower than predicted.

CONCLUSIONS

The results confirm that the GES assistive device program addresses a real need in older adults, especially in the most vulnerable, and is highly valued by them. After AD delivery, perceived QoL and independence for performing daily living activities improved and fall risk decreased. This policy has proven to be an important benefit for older adults and is in line with recommendations for implementation of comprehensive policies on aging that can meet the needs of this population. It is important to conduct periodic GES outcome evaluations, and to identify and address the causes of low access and coverage.

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The Panama Aging Research Initiative Longitudinal Study

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ABSTRACT

The Panama Aging Research Initiative is a cohort study of 423 adults aged ≥65 years recruited from an outpatient geriatric department of Panama's largest public hospital, the Social Security Fund's Dr Arnulfo Arias Madrid Hospital Complex (Complejo Hospitalario Dr Arnulfo Arias Madrid de la Caja de Seguro Social). The study provides the first reports of modifiable and non-modifiable risk factors of cognitive impairment and dementia, as well as various health conditions common among older adults in Panama, including chronic illnesses, polypharmacy and rates of comorbidity. The initial study, conducted September 2012–May 2016, included a clinical interview; physical assessments of body mass index and handgrip strength; and cognitive testing, plus non-fasting blood draws for measurements of genetic (Apolipoprotein E genotype) and blood-based biological markers. Information was collected regarding limitations in activities of daily living, symptoms of depression and fall incidents. A subsample of participants provided cerebrospinal fluid to measure proteins related to Alzheimer's disease; another subsample underwent ultrasonography and electroencephalography.

This report describes the general study design and highlights lessons learned and future directions. In particular, drawing on lessons learned from this clinical research, a community-based prospective cohort study is currently under way among older adults in Panama to validate a blood-based biomarker profile for detecting mild cognitive impairment and Alzheimer's disease, as well as risk factors for cognitive decline.

KEYWORDS: Dementia, biomarkers, Latin America, aging, cognition, chronic disease, Panama

INTRODUCTION

Population aging projections suggest that by 2025 about three-quarters of the world's population aged ≥60 years will reside in developing countries.[1] This later stage of the demographic transition, resulting from declining fertility and infant mortality combined with increased life expectancy, is accompanied by significant social and economic challenges associated with caring for an increasingly older adult population. In Panama with a population of 4 million (2017), little emphasis has been placed on programs to prevent and manage risk factors for the most prevalent health problems associated with aging. In light of a growing older adult population—persons aged ≥60 years are expected to reach 23.5% of Panama's population by 2050[2]—it is urgent to address aging-related health issues.

The Panama Aging Research Initiative (PARI) is the first prospective study of age-related health factors in older Panamanians. Due to the rapid growth of Hispanic populations in the Americas and the extant literature documenting a significant impact of race and ethnicity on disease status,[3] this line of research contributes not only to national but also international aging-related research. PARI aims to (1) characterize demographic, clinical and genetic factors associated with cognitive impairment; (2) describe associated risks, including demographic, genetic and health-related factors, such as Apolipoprotein E (ApoE) genotype and non-communicable chronic diseases; (3) examine the utility of non-invasive techniques such as electroencephalography (EEG) and ultrasonography to determine risk of cognitive impairment; and (4) examine links between blood-based biomarkers and dementia using a longitudinal approach. To date, PARI has found various risk factors associated with dementia and cognitive impairment and provided evidence of the feasibility of applying methods to identify individuals at risk for these conditions. This report describes the general research study design, and highlights lessons learned and future directions.

IMPORTANCE: As the first evidence of social, behavioral and biological factors related to the aging process in Panama, the study cohort of older adults provides a baseline for prospective research on cognitive impairment and biomarkers useful in early detection of dementia. Due to the worldwide trend towards aging populations and the rapid growth of Hispanic populations throughout the Americas, this line of research may provide useful results for both national and international health strategies.

INTERVENTION

The methodology published previously was based on standard protocols for conducting longitudinal studies of cognitive health in older adults.[4–8] Participants were recruited from the outpatient geriatric services of the Social Security Fund's Dr Arnulfo Arias Madrid Hospital Complex (Complejo Hospitalario Dr Arnulfo Arias Madrid de la Caja de Seguro Social, CHAAM-CSS), the largest public hospital in Panama City. Inclusion criteria were age ≥65 years and willingness to participate in baseline and followup assessments. Exclusion criteria were any medical condition requiring hospitalization and/or participation in an ongoing clinical study at the time of enrollment. In total, 423 participants were enrolled from September 2012 through January 2013; followup assessments were conducted in participant subsamples from February 2013 through January 2016. Table 1 summarizes the variables and instruments employed at baseline and in followup studies. The protocol was approved by the National Bioethics Committee of the Gorgas Commemorative Institute of Health Studies (Instituto Conmemorativo Gorgas de Estudios de la Salud), the only competent national bioethics committee at that time. All participants (or their caregivers) provided written informed consent in accordance with the Declaration of Helsinki.[9]

Participant interviews and clinical exams Interviews were conducted by health professionals as well as medical, undergraduate and graduate students trained by a multidisciplinary group of specialists. Sociodemographic data collected included age, sex, years of education completed, marital status and household income. Chronic conditions, medications and performance in basic

Table 1 Variables and instruments by study phase

	Baseline	Followup assessments
Sociodemographic factor		
Age, sex, marital status, educational level completed	X	X
Household income	X	
Current or past occupation	X	
Clinical interview		
Chronic conditions	X	
Number of medications	X	
BADL and IADL limitations[4,7]	X	X
Tobacco use	X	
Neuropsychiatric status (n = 204)	X	
EQ-5D-3L		X
FAQ		X
Lawton-Brody IADL Scale		X
GDS-30[11]	X	X
Physical assessments		
Weight, height, pulse/blood pressure	X	X
Handgrip strength (kg)	X	X
Electroencephalography (n = 60)		X
Ultrasonography (n = 159)		X
Cognitive function[4,8]		
MMSE		X
TMT A and B		X
Clock drawing test: order		X
Clock drawing test: copy		X
Wechsler Memory Scale: Digit Span		X
Semantic Verbal Fluency		X
Boston Naming		X
Phototest		X
Poppelreuter figure visual perceptual function test		X
Object use test: imitation and verbal request		X
Verbal comprehension		X
10-word free recall		X
Global Deterioration Scale		X
Biological Assays		
Blood chemistry	X	X
Apolipoprotein E genotype (n = 267)[5,6]		X
Blood based biomarkers (24-protein panel; n = 267)[5]		X
CSF biomarkers (T-tau, P-tau, Ab 1-42; n = 51)		X

Aβ 1-42: amyloid β 1-42; BADL: Basic activities of daily living; COPD: Chronic obstructive pulmonary disease; CSF: Cerebrospinal fluid; EQ-5D-3L: European Quality of Life,EuroQol Questionnaire five-dimensional three-level version; FAQ: Functional Activity Questionnaire; GDS-30: Geriatric Depression Scale (30-item); IADL: Instrumental activities of daily living; MMSE: Mini Mental State Examination; P-tau: phosphorylated tau protein;TMT A and B: Trail Making Test A and B; T-tau: total tau protein.

24-protein panel: Eotaxin 3 and Adinoponectin; A2M: alfa-2 Macroglobulin; B2M: beta-2-microglobulin;CA-125: Cancer Antigen-125; CRP: C-reactive Protein; FABP: Fatty Acid-Binding Protein; FVII: Factor VII; I309: T-Lymphocyte-Secreted Protein I-309; IL-1β: Interleukin-1 Beta; IL-5: Interleukin-5; IL-6: Interleukin-6; IL-7: Interleukin-7; IL-10: Interleukin-10; IL-18: Interleukin-18; MIP-1α: Macrophage Inflammatory Protein-1 Alpha; PPY: Pancreatic Polypeptide Y; SAA: Serum Amyloid Protein A; sICAM1: Intercellular Adhesion Molecule; sVCAM1: Vascular Cell Adhesion Molecule; TARC: Thymus and activation-regulated chemokine; THPO: Thrombopoietin; TN-C: Tenascin-C; TNF-α: Tumor Necrosis Factor alpha.

and instrumental activities of daily living (BADL and IADL, respectively) were evaluated through self-report as published previously.[4,7] Clinical exams measured body mass index (BMI; kg/m^2), handgrip strength (kg), pulse rate and blood pressure.

Cognitive, psychiatric and other assessments Cognitive (n = 328) and psychiatric (n = 204) assessments were conducted during the first followup screening (M = 4.5 months after baseline, SD = 1.9), and a non-fasting blood draw was performed. Global cognition was assessed using the 30-item Spanish version of the Mini-Mental State Examination (MMSE)[10] adjusted for age and education. To screen for depressive symptoms, the Spanish version of the 30-item Geriatric Depression Scale (GDS-30)[11] was administered by an interviewer. Additional health measures included current or past tobacco use and frequency of physical exercise.

A subsample of participants (n = 93) was evaluated with a complete cognitive test battery approximately 17 months (M = 16.8, SD = 3.4) after baseline evaluations.[8] The assessment included clinical and demographic factors and cognitive/functional tests, summarized in Table 1. The protocol also included a non-fasting blood draw. In this sample, diagnoses of Alzheimer's disease (AD), mild cognitive impairment (MCI), no cognitive impairment or other, were made by a consensus committee according to published criteria.[12,13] Participants were classified as controls if they performed within normal limits in the neuropsychological assessment and scored below the threshold for symptoms of depression in the GDS-30. The Global Deterioration Scale[14] was used to provide information on the stage of illness or condition. The tests in the battery assessed the following cognitive domains: global cognition, language, visuospatial ability, memory, executive function, attention, praxis and gnosis.

EEGs were obtained in a subsample of participants (n = 60) selected at random using a standard 10–20 system (Natus Medical Incorporated, Canada). Records were classified as normal or abnormal by an experienced clinician blind to all other participant information. In another subsample (n = 159), also selected at random, high resolution B-mode ultrasonography was used to measure volume and speed of blood flow, intima media thickness (IMT), and presence of stenosis in the left carotid artery. The procedure for obtaining and classifying these measures is described in a recent report.[8]

Biological assays Serum samples were obtained from 63% of participants and assayed in duplicate via a multiplex biomarker assay platform using electrochemiluminescence. In order to pursue studies in biomarker discovery, we partnered with Dr Sid O'Bryant at the University of North Texas Health Science Center, whose team has a long history of conducting aging studies with Mexican-Americans.[15–17] Our current aim is to identify blood biomarkers that could be used to select potential clinical trial subjects, improve early diagnosis and enable longitudinal tracking of various disease indicators over extended periods of time. For comparative purposes, subjects were divided into three age groups: 65–74 years, 75–84 years and ≥85 years.

Cerebrospinal fluid samples (CSF; 5–10 mL) were obtained from participants who provided additional written informed consent (n = 51). Standardized lumbar puncture procedure was performed during morning hours by experienced professionals using a 20- or 22-gauge

needle after local lidocaine application. Samples were centrifuged at low speed to discard any cellular material, then aliquoted into individual polypropylene tubes (400–500 µL) without preservative, and frozen at –80 °C until analysis. Protein concentrations for Aβ1–42, T-tau, and P-tau181 were measured using standardized commercially available ELISA methods [INNOTEST β-AMYLOID(1–42), INNOTEST hTAU Ag and INNOTEST PHOSPHO-TAU(181P)], according to manufacturer specifications (Fujirebio Europe NV, formerly Innogenetics NV, Ghent, Belgium). Samples were run in duplicate along with the calibration controls and validation solutions.

LESSONS LEARNED FROM INTERVENTION RESULTS

Mean age of participants was 79.1 years (SD = 7.7). Female participants outnumbered male by a ratio of 2:1. Mean level of education was 7.3 years (SD = 4.4) and inversely related to age, with the oldest participants having the lowest levels of education. Participants, on average, were taking 3.9 medications (SD = 2.0) and levels of polypharmacy did not differ among age groups. As expected, hypertension was the most frequent chronic condition followed by diabetes and ischemic heart disease. In our sample, 59.7% of individuals aged ≥85 years were classified as cognitively impaired, consistent with other studies in the region in individuals with low levels of education.[18] Increased age combined with low educational level were found to be primary risk factors for cognitive impairment. Among the cognitive, physical function and depression variables, participants aged ≥85 years scored lower on cognitive tests, and had more BADL and IADL limitations and depressive symptoms.[4,7]

For Panama, PARI provided the first data regarding health-related factors in older adults, a line of research that has been active in most high-income countries for decades. In the initial cohort, we collected information on a wide range of variables and reported age-related impairments in cognition and everyday function, many of which were expected based on the international literature. At the same time, we established a line of research in blood-based biomarkers of AD, a relatively novel but rapidly growing area of global AD research. First, we demonstrated that a 21-protein panel could reliably distinguish AD individuals from healthy controls.[5] In subsequent studies analyzing biomarker data across cohorts, we showed that pooled data of a 24-protein panel of blood biomarkers to screen for AD had excellent diagnostic accuracy and positive and negative predictive power.[16]

Importantly, a blood screen as a first step in the diagnostic process in primary care can significantly reduce costs that represent a major barrier in health and social services in low-resource settings such as Panama. In our current (community-based) cohort, we continue to collect blood samples periodically in order to assess if previous findings are repeated. We are also extending our analysis to include the utility of the biomarker profile to indicate risk for incident cognitive impairment. Based on our findings to date and considering the time- and cost-effectiveness of blood tests in primary care, we consider this a promising line of research.

We found that age and APOE4 are the strongest risk factors associated with AD and MCI. This is one of our most relevant results concerning cognitive impairment and other age-related conditions.[6] Notably, 32.6% of participants tested (n = 267) had at least one copy of APOE4. Table 2 summarizes the distribution of APOE genotypes by age group. Our published results have revealed that APOE4 is a strong predictor of AD and MCI[4] and underscore the potential of blood-biomarker screening to identify those at highest risk for dementia.[15] Our studies also confirm the feasibility of applying common biomarkers to clinical evaluations of cognitive impairment (APOE, CSF and blood).[5,6]

It is important to take into account other measures that could contribute to the development of minimal requirements for detecting risk in primary care (screening for depressive symptoms, ADL limitations, handgrip strength, etc.).[4,7] We showed that the GDS-30 has a high negative predictive value and thus serves as a useful tool in primary care to identify individuals who should be referred for a full psychiatric evaluation.[19]

Table 2: Distribution of APOE genotype by age group

Age group	e2/e2	e2/e3	e2/e4	e3/e3	e3/e4	e4/e4
65-74 years	0.0%	5.8%	2.3%	57.0%	30.2%	4.7%
75-84 years	0.9%	6.8%	0.9%	61.5%	27.4%	2.6%
≥85 years	0.0%	6.3%	1.6%	57.8%	34.4%	0.0%
Total (n = 267)	0.4%	6.4%	1.5%	59.2%	30.0%	2.6%

The study has important limitations. The sample is not representative of the entire older-adult population. Specifically, because it consists of individuals who utilize public health services, results may underestimate the extent of cognitive impairment and age-related chronic conditions among older Panamanians, and comparisons of our results with others should be made with caution. In addition, due to time constraints imposed by the hospital setting in which we collected our data, we could not schedule all tests in a single visit. Consequently, we experienced significant losses to followup assessments. This was beyond our control, although with the exception of EEG tests, we were able to evaluate an acceptable number of participants in most followup tests, resulting in published reports.[4,8]

Building upon lessons learned in the initial cohort, we are currently conducting a longitudinal study in a community-based cohort and have been successful in enrolling participants from diverse backgrounds using a variety of strategies (social and print media). In our current protocol, we conduct the clinical interview, physical exam, fasting blood draw and cognitive testing in one visit and repeat them on a followup visit 12–18 months later, thus reducing the dropout rate significantly. Upon the advice of clinicians, we also include measures of frailty, which are highly effective in detecting patients at risk and are cost- and time-effective in primary care settings.

We also simplified our research protocol to retain cognitive and functional measures and eliminate EEG or ultrasound markers related to cognitive impairment and the collection of CSF samples. The main reason for this modification was that students could not be trained to conduct these tests, which require paid specialists.

Another important lesson learned was to provide study participants access to their cognitive test results, which has resulted in greater participant interest and retention in the study. In fact, as a result of this initial research, we concluded that all persons should have the opportunity to discuss clinical study findings with their health care providers to determine the appropriate course of action, as is done in many other countries.

IMPLICATIONS FOR RESEARCH AND POLICY DECISION-MAKING

One of the most common health concerns in older adults is cognitive decline. Recently published population-based studies confirm that rates of AD and MCI in developing countries are similar to those of developed countries[20] and are expected to increase in the coming decades due to high rates of modifiable risk factors such as diabetes, obesity and illiteracy. Thus, identifying individuals with MCI who are more likely to develop AD or other dementias is one of the primary challenges in cognitive aging research. Importantly, evidence indicates that AD-associated neuropathology is likely to appear decades before clinical symptoms,[21] suggesting that treatments that can potentially modify disease course will likely be most effective during pre-clinical disease stages. In this regard, the search for reliable biomarkers that can aid in early detection of dementia is essential for reducing the burden of cognitive impairment.

In rapidly aging countries such as Panama where more people are living with various chronic diseases—their treatment accounting for a significant portion of the country's health care expenditures—clinical studies like PARI are urgently needed to understand how modifiable and non-modifiable risk factors interact to produce common health-related conditions in older adults, such as functional disability and cognitive impairment.

Older adults commonly experience mental health conditions, yet most healthcare professionals in Panama receive no training in their assessment and treatment. Perhaps related to this, cognitive impairment is rarely diagnosed using published diagnostic criteria and in many cases is seen as a normal part of the aging process. Complicating this scenario, family members of affected individuals rarely seek formal medical services until advanced stages of impairment, which increases the burden of aging on individuals, family members and health systems.

PARI has helped to draw attention to the importance of comprehensive (cognitive, functional and psychiatric) evaluations of older adults. The proven predictive value of GDS-30 in primary care settings has already been used by the Ministry of Health to support the screening of depressive symptoms in this population. Our program has trained an average of ten research assistants annually, most of them students in social and biomedical sciences, which in turn has increased the number of students pursuing advanced degrees focused on health and aging.

PARI's collaboration with academic institutions has been indispensable to the program's day-to-day activities and to capacity building in areas directly impacting geriatric health. We have also built a strong alliance with international collaborations and the Ministry of Health, which looks to the PARI program as the first source of metadata collected from older adults in Panama. We continue to build relationships in local communities, and to increase recruitment and retention of participants, centering on characteristics such as age, socioeconomic factors, urban-rural demographics and cognitive status (healthy, asymptomatic and MCI).

Our study adds to the effort to document the cognitive status of older adults in Panama and constitutes a contribution to our understanding of the risk factors of cognitive outcomes among Hispanic populations in general. Its ultimate aim is to provide a body of evidence to support the development of public policies to improve geriatric public health care and reduce the burden of aging on health systems and individuals.

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Pharmacogenetic Markers: A Path toward Individualized HIV Therapy

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ABSTRACT

INTRODUCTION Approximately 73% of persons with HIV who receive antiretroviral therapy in Cuba are in viral suppression. The non-response of the remaining 27% could be due to several factors including adverse drug reactions and HIV resistance to antiretroviral drugs, as well as social factors and idiosyncratic characteristics of each patient. Genetic information explains from 20% to 95% of a drug's effects and variations in response. Considering optimization of therapeutic efficacy in our country, genetic factors of the host should be identified.

OBJECTIVE Identify polymorphisms affecting genetic variability of responses to antiretroviral drugs.

EVIDENCE ACQUISITION A literature review was conducted (of original articles, published theses, clinical reports and bibliographic review studies, from 2000 to 2018, in Spanish and English listed in MEDLINE/PubMed, SciELO, LILACS, PharmGKB and Google Scholar) with the following key words: pharmacogenetics, human immunodeficiency virus, anti-retroviral agents, genetic polymorphism, genetic techniques, pharmacogenomic variants.

DEVELOPMENT The review identified 77 relevant publications meeting specific quality criteria. A summary table was built with data collected on antiretroviral drugs, genes and proteins involved in polymorphic variations, their associated effects and relevant scientific references. Information was included on polymorphisms related to 12 antiretroviral drugs used in HIV therapy. Polymorphisms determine variations in proteins involved in drug transport and metabolism and in elements of immunity. Relevant pharmacogenetic biomarkers recognized by drug regulatory agencies were identified.

CONCLUSIONS The study identified genetic variations (single-nucleotide polymorphisms) associated with 12 antiretroviral drugs. In most cases, no statistically significant causal association was found. Identifying polymorphic variations is a medium- and long-term objective that requires statistical support and adoption of strategies to optimize antiretroviral therapy. An approach combining plasma-level monitoring and pharmacogenetic analysis is recommended to optimize therapy for HIV patients.

KEYWORDS Pharmacogenetics, HIV, anti-retroviral agents, antiretroviral therapy, genetic polymorphism, genetic techniques, pharmacogenomic variants.

INTRODUCTION

HIV Infection has been a global health problem since 1983. At the end of 2016, approximately 36.7 million people worldwide were HIV-positive. In mid-2017, approximately 20.9 million people were receiving antiretroviral (ARV) drugs.[1] AIDS represents the infection's advanced clinical stage. To treat HIV, numerous ARVs have been developed that act on different phases of viral replication and are used in combination as antiretroviral therapy (ART).

In Cuba, ARV drugs of all classes are used, although some of the latest-generation ARVs are only available in limited quantities and use of others has been discontinued due to their toxicity. Cuba's ARV drugs are generic and produced domestically, procured by nongovernmental organizations through projects with The Global Fund to Fight AIDS, Tuberculosis and Malaria (Global Fund) or through personal donations. ART in Cuba meets national and international therapeutic standards. First-line treatment combines nucleoside reverse-transcriptase inhibitors (NRTIs) with non-nucleoside reverse transcriptase inhibitors (NNRTIs). Thanks to ARV use, incidence of HIV/AIDS-related deaths has fallen since 2005.[2] By December 31, 2018, in Cuba 31,118 persons had been diagnosed with HIV, 25,497 of whom were alive.[3]

IMPORTANCE: This study is an updated review of the pharmacogenetics of antiretroviral drugs. There have been few studies in Cuba on the subject of pharmacogenetics and no studies examining its effects on antiretroviral therapy. The study identified single nucleotide polymorphisms associated to 12 antiretroviral drugs. To optimize therapy, pharmacogenetic and plasma drug monitoring is recommended for HIV patients.

The Joint United Nations Programme on HIV/AIDS (UNAIDS) and its partners have set global goals for HIV diagnosis, treatment and viral suppression (known as 90–90–90): by 2020, at least 90% of people living with HIV should know their serological status, 90% of those diagnosed should be receiving sustained ART, and 90% of those receiving ART should have attained viral suppression (viral load <1000 copies/mL). Cuba shares these goals and in 2018 the country attained 83%–87%–73%,[3] meaning that 27% of patients receiving therapy did not attain viral suppression.

Factors responsible for non-response could include resistance to first-line ARV drugs (22% of patients), which has increased for NRTIs, NNRTIs, and protease inhibitors (PIs), to 52.7%, 54.7%, and 21.4%, respectively.[4] Adverse reactions (both moderate and severe) also can affect regimen adherence.[5] Alterations in blood bioavailability were reported in 25% of patients receiving the three classes of ARVs within their respective therapeutic windows.[6] The most common reason described in Cuban patients for changing an ART regimen was the appearance of an adverse drug reaction (ADR).[7]

Among the most serious ADRs reported in Cuba from 2003–2012 were two hepatotoxicity reactions associated with nevirapine and lamivudine, and a case of anemia due to zidovudine. No fatal reactions were reported during the period studied.[8] There is scant literature in Cuba on adverse reactions to ARV drugs, due partly to the low number of ADRs reported by health professionals.[8, 9] As the number of cases studied is small, ADR statistics may be underestimated. Cuba's 2017 Annual Drug Safety Report,[2] lists 14 cases of ARV adverse reactions in a tertiary care institution, which represents 0.08% of all ADRs reported that year. The drugs cited in the drug safety reports were nevirapine (50% of reports), zidovudine (25%), abacavir (16.7%), and tenofovir (8.3%). Adverse drug reactions reported included headache, Stevens-Johnson syndrome, rash, vomiting, itchy skin and acute kidney failure.[2]

Current Cuban strategies to prevent therapeutic failure include surveillance of progress markers (viral load and T CD4+ lymphocytes) in clinical labs, and in some cases therapy monitoring, but the patient's genetic factors are not addressed. Recent advances in pharmacogenetics (PGx) have made it possible to identify genetic variations that modulate response to these drugs and their toxicity. The main source of variability in human genomes are variations in a single nucleotide—adenine (A), thymine (T), cytosine (C), guanine (G)—known as single-nucleotide polymorphisms (SNPs).[10]

This study is an updated review of the PGx of ARV drugs. There have been few studies in Cuba on the subject of PGx and no studies examining its effects on ART. From the perspective of therapy optimization, we proposed to compile information on the main genetic polymorphisms described in the literature affecting response to ART and to identify useful strategies for their implementation in HIV therapy.

Evidence Acquisition Terms included in the information search: pharmacogenetics, HIV, anti-retroviral agents, antiretroviral therapy, genetic polymorphism, genetic techniques, pharmacogenomic variants. Bibliographic databases consulted: MEDLINE/PubMed, SciELO, LILACS and PharmGKB. Search engines: Google and Google Scholar. Types of documents: original articles, published theses, clinical reports and bibliographic reviews. Languages: Spanish and English. Dates of publication: 2000 to 2018. Exclusion criteria: no free access to complete text due to financial constraints, studies on genetic variations unrelated to HIV or presenting inadequate scientific evidence.

DEVELOPMENT

Of 1143 reports identified in the information search, 77 met the above-mentioned criteria. Table 1 summarizes information compiled on ARV drugs, genes and proteins associated to polymorphic variations and effects.

There are six classes of ARV drugs: nucleoside reverse-transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), entry inhibitors (EIs), fusion inhibitors (FIs), and integrase inhibitors (INIs). HIV therapy also uses post-attachment inhibitors (ibalizumab) and pharmacokinetic enhancers (cobicistat). Despite advances in patient survival and reduction of HIV-associated morbidity/mortality, adverse events and disorders associated with ARVs persist, which limit their benefits and contribute to drug resistance. The following is a discussion of updated information on the pharmacogenetics of ARV drugs based on literature review.

Nucleoside reverse-transcriptase inhibitors (NRTIs)

Abacavir (ABC): hypersensitivity reaction Since its introduction on the market, ABC has shown high efficacy and an acceptable toxicity profile for HIV treatment. About 5% of ABC-treated patients develop a hypersensitivity reaction (HSR) characterized by potentially fatal multisystemic effects. Symptoms generally appear within the first six weeks of treatment and range from fever, exanthema, and gastrointestinal symptoms to lethargy or general malaise. Symptoms generally worsen as treatment continues and improve within 24 hours of interruption.[11] One study reports this HSR occurring in 3.7% of ABC-treated patients and in up to 14% in some clinical trials.[12]

Although clinical symptoms are nonspecific and difficult to differentiate from other reactions, a close association has been described with presence of allele HLA-B*5701,[13,14] which can lead to activation of cytotoxic lymphocytes T CD8+, provoking secretion of two inflammation mediators—tumor necrosis factor (TNF)-alpha and interferon (IFN)-gamma—associated with appearance of delayed HSR.[13] The HLA-B*5701 allele is more prevalent in the Caucasian population (5% to 8%), less common among Japanese, Chinese and Koreans (< 1%), and rare in Sub-Saharan Africans. In South American Caucasians, prevalence is 5% to 7%.[14]

A 2002 study supporting the clinical value of identifying the HLA-B*57:01 allele found genotyping for HLA-B*5701 conducted in predominantly Caucasian populations had a positive predictive value of 100% for hypersensitivity to ABC, and absence of this allele combination had a negative predictive value of 97%.[13] In 2008, a prospective clinical trial revealed that identifying this allele (and the resulting modification of ARV therapy) was associated with a decline in incidence of hypersensitivity to ABC from 7.8% to 2.7%.[14] The HLA-B*5701 allele is identified through flow cytometry or sequencing with molecular amplification.[15] A skin test has been developed that has shown a satisfactory correlation between presence of HLA-B*5701 allele and risk of presenting HSR,[16] but as false negatives are not uncommon, the test's use is not recommended in clinical practice.[17] This is among the earliest examples of PGx applied to clinical diagnosis in order to prevent drug-associated toxicity.

Some studies consider HLA-B*5701 genotyping cost-effective, while others argue that it is expensive, time consuming and requires a specialized laboratory, and thus propose another genetic marker, HCP5, instead.[18,19] Polymorphism 335T>G, located on gene P5 in the HLA complex HCP5, is in linkage disequilibrium with HLA-B*5701[20, 21] and its identification is easier, more economical and quicker. HCP5 SNP genotyping is being used more frequently as a simple method to detect possible hypersensitivity to ABC, although it presents some disadvantages.[22] In a study of 245 HIV patients, a close correlation was observed between HLA-B*5701 and HCP5 (negative and positive predictive values of 100% and 93%, respectively).[20]

Tenofovir disoproxil fumarate (TDF): renal toxicity TDF is generally well tolerated, with an excellent metabolic profile and no potential mitochondrial toxicity. Its most common ADR is renal toxicity, which is enhanced if co-administered with other drugs. The mechanism through which TDF causes renal tubular damage is not well understood; toxicity could be due to mitochondrial damage or interference with normal cell functions associated with intracellular drug accumulation.[23]

TDF is a substrate of transport proteins involved in its renal excretion, which occurs by both glomerular filtration and active tubular secretion. TDF enters proximal tubular cells by organic anion transporter (OAT) proteins. Its excretion in urine is mediated by multidrug-resistant protein MRP4, the main transporter of TDF at the apical level, which regulates TDF levels inside tubular cells.[24] Intervention of MRP7 and MRP2 proteins has also been described, although their role is unclear, as is the mechanism by which polymorphisms in MRP2 affect development of tubular dysfunction.[23] Polymorphisms in the genes encoding these proteins could alter their expression and activity, affecting TDF pharmacokinetics and favoring its accumulation in renal tubular cells. TDF-associated toxicity can produce proximal tubular dysfunction and acute tubular necrosis, which can progress to chronic kidney disease. Toxicity tends to reverse when the drug is withdrawn, but recovery may not be complete.[25] Nephrotoxicity in TDF patients is uncommon (about 2.5%),[26] but it must be taken into consideration given the growing use of this drug.

Table 1: Polymorphisms related to antiretroviral drugs

ARV	Gene (Protein)	Variation	Effect	Reference
Nucleoside reverse transcriptase inhibitors				
Abacavir (ABC)	HLA-B	HLA-B*5701[a,b]	HSR	[12–16]
	HLA complex P5 (HCP5)	335T>G (rs2395029)[a]	Alternative marker to determine risk of HSR to ABC	[20–22]
Tenofovir (TDF)	ABCC4 (MRP4)	3463A>G (rs1751034)	Affects TDF pharmacokinetics leading to renal toxicity	[74]
		669C>T (rs899494)[a]		[28]
		4131 T>G o G>G	Associated with increased TDF levels	[25]
	ABCC2 (MRP2)	"CATC" haplotype 24C>T, (rs717620) 1249G>A[a] (rs2273697) 3563T>A (rs8187694) 3972C>T (rs3740066) 24C (rs717620)	Higher risk of renal tubular disorders	[28,29,74]
Lamivudine (3TC)	ABCC4 (MRP4)	4131T>G	Increased plasma levels	[32]
Zidovudine (AZT)	ABCC4 (MRP4)	3724G>A		
Non-nucleoside reverse transcriptase inhibitors				
Efavirenz (EFV)	CYP2B6 (CYP2B6)	516 G>T[b] (rs3745274)	Neurotoxicity of CNS Associated with extremely high plasma levels	[35–38]
		CYP2B6 *6/*6[b]	Prolonged QTc interval	[42]
	CYP2A9	*9B T>G (rs28399433)	Lower EFV metabolism	[75,76]
	ABCC1	3435 C>T[a] 2677 G>T/A[a]	Associated with increased plasma levels. Significant association with decreased probability of virologic failure	[40,43]
Nevirapine (NVP)	CYP2B6	516 G>T (rs3745274)	Presence of polymorphism associated with varying degrees of neuropsychological toxicity	[37]
		983 T>C (rs28399499)	Associated with decrease in NPV clearance	[45]
	HLA-DR	HLADRB1* 0101	Risk of HSR and hepatotoxicity	[48]
	HLA-C	HLA-Cw*8	Higher risk of HSR	[49,50]
	HLA-B	HLA-B*14		
	ABCB1 (MDR1/P-gp)	3435 C>T[a] (rs1045642)	Associated with lower risk of hepatotoxicity	[46,47]
Protease inhibitors				
Atazanavir (ATV)	UGT1A1 (UGT1A1)	UGT1A1 *28 (rs887829)	Hyperbilirubinemia Gilbert's syndrome	[52,53]
	ABCB1 (P-gp)	3435 C>T[a] (rs1045642)	C/C carriers have higher ATV plasma levels than patients with C/T or T/T genotypes	[54]
Indinavir (IDV)	UGT1A1 (UGT1A1)	UGT1A1*28[a] (rs8175347)	Associated with increased risk of hyperbilirubinemia	[56,57]
		UGT1A1*6[a]	Increased risk of hyperbilirubinemia. Diagnosed only in Asian patients	[57]
	CYP3A5	CYP3A5*3 (A6986G)	Affects pharmacokinetics of IDV	[32,59]
Nelfinavir (NFV)	CYP2C19	681G>A	Possible effect on plasma levels	[40]
	ABCB1 (P-gp)	3435C>T	Elevated plasma levels	[43,60]
Lopinavir (LPV)	SLCO1B1 (OATP1B1)	521 T>C (rs4149056)	Higher plasma levels of LPV	[61,63]
Ritonavir (RTV)	APOE	APOE, APOC3, APOA5, CETP, y ABCA1	Increased risk of severe hypertriglyceridemia	[64,65,77]
	APOC3	482 C>T, 455 T>C	Toxicity – hyperlipidemia	[64]
Integrase inhibitors				
Dolutegravir (DTG)	ABCG2	421 C>A (rs2231142)	Variations in plasma levels	[67]

[a] Studies with significant causal association
[b] Causal associations recognized by regulatory agencies and incorporated in directions inserted in prescription drug packaging
The rs numbers are the access numbers in the database of single nucleotide polymorphisms (SNP) in the National Center for Biotechnology Information, NCBI
For updated information visit https://www.pharmgkb.org/ and https://www.fda.gov/drugs/scienceresearch/ucm572698.htm

Abbreviations:
ABCB1: ATP-binding cassette subfamily-B, member 1;
ABC: ATP-binding cassette subfamilies-C (members 2 and 4); G (member 2);
APOE: apolypoprotein E;
APOC3: apolypoprotein CIII;
CNS: central nervous system;
CYP-450: cytochrome P-450; isoform; 2B6, 2A9, 3A5, 2C19;
HCP5: human leucocyte antigen complex P5;
HLA-B: human leukocyte antigen-B;
HLA-C: human leukocyte antigen-C;
HLA-DR: human leukocyte antigen-DR;
HSR: hypersensitivity reaction;
MRP2: Multidrug resistance-associated protein 2;
MRP4: Multidrug resistance-associated protein 4;
OATP1B1: organic anion transporter 1B1;
P-gp: P-glycoprotein (multidrug resistance protein 1 (MDR1);
SLCO1B1: solute carrier organic anion transporter 1B1;
UGT1A1: uridine 5'-diphospho-glucuronosyltransferase 1A1

In gene ABCC4, which codes the MRP4 protein, three genetic polymorphisms were identified. Studies have shown that polymorphism 3463A>G is related to increased plasma and intracellular TDF levels as well as decreased renal clearance.[27] These results need to be confirmed, however, due to the small number of patients studied (n = 30). Polymorphism 669C>T in this gene was also identified; it is commonly found in patients who present renal tubular damage.[28] Another study concluded that after controlling for body weight, estimated glomerular filtration rate (GFR) and concomitant use of a PI reinforced with ritonavir, the average TDF plasma level in patients carrying genotype TG or GG ABCC4 4131 was about 30% higher than in patients who were carriers of TT.[25]

Polymorphisms in the ABCC2 gene that codes MRP2, specifically the CATC haplotype (combination of polymorphisms in positions -24C>T, 1249G>A, 3563T>A and 3972C>T) and -24C allele, have been associated with higher risk of tubulopathy.[28] According to Manosuthi, HIV patients who carry gene ABCC2 * genotype CC in position -24 or who present a high tenofovir plasma level have a higher risk of diminished GFR.[29] According to Izzedine,[28] genotype ABCC2-24CC and allele ABCC2-1249-A have been found more often in patients with tubular dysfunction than in patients with normal function. However, Rungtivasuwan[25] and Álvarez Barco[23] did not find that association. The most commonly observed SNPs in MPR2 play an indirect role: they affect transport of other substances that would influence TDF toxicity or by being in linkage disequilibrium with other SNPs which, when directly associated with expression of proteins involved in TDF transport, can lead to drug accumulation inside the cell, causing mitochondrial damage and/or interference with normal cell function. The most widely accepted hypothesis on the mitochondrial toxicity mechanism holds that it is due to inhibition of DNA polymerase γ, with a resulting decline in mitochondrial DNA required to maintain the electron transport chain proteins. This mitochondrial toxicity has been described for cidofovir and adefovir. Due to TDF's structural similarity to these nucleotide analogues, it is speculated that TDF may also cause this type of cellular damage.[30]

Toxicity associated with TDF has been shown to be dependent on concentration, which would justify monitoring plasma TFV levels for pharmacokinetic studies of kidney function. However, there are drawbacks to doing so (intraindividual variability, lack of defined therapeutic range for TDF, etc.). Available information on genetic polymorphisms in renal toxicity risk with TDF therapies is contradictory and thus more evidence is needed. If prospective studies show these effects, patients at risk for developing tubular damage would be identified early. Use of pharmacogenetic markers together with quantification of TDF can alert physicians to the advisability of monitoring renal function.[23]

*Lamivudine (3TC) and zidovudine (*AZT*): polymorphisms associated with increased plasma levels* 3TC has high oral bioavailability, wide biodistribution and is generally well tolerated. Common adverse reactions include nausea, vomiting, diarrhea, stomach ache, hair loss, fever, insomnia, rash, nasal congestion and joint ache. AZT can cause serious adverse reactions such as hematological toxicity, including neutropenia and severe anemia, lactic acidosis and severe hepatomegalia with steatosis. Cases of hepatic decompensation have been reported for AZT and prolonged use has been associated with redistribution of body fat and lipodystrophy syndrome.[31]

A pilot study on the pharmacogenetic aspects of therapy with indinavir, AZT and 3TC in 33 adults living with HIV reported that the average AZT level was 49% higher in carriers of variant ABCC4 3724G>A than in wild genotype (GG) carriers.[32] Carriers of variant 4131T>G of gene ABCC4 also showed a 20% increase in intracellular 3TC levels.

Few studies have evaluated pharmacogenetics with respect to AZT and 3TC, but the aforementioned results indicate that variations in gene coding for drug metabolism can influence ART efficacy and toxicity. Large-cohort studies are needed for more in-depth analysis and to validate the role of pharmacogenetic factors in AZT pharmacokinetics and pharmacodynamics.[33]

Non-nucleoside reverse transcriptase inhibitors (NNRTIs)

Efavirenz (EFV): polymorphisms in metabolizing enzymes and transport proteins that affect plasma levels EFV presents few clinically significant side effects, but frequently produces psychological and neurological symptoms, especially during the first three to four weeks of therapy. Not uncommonly, these symptoms cause discontinuation. This ARV drug has a narrow therapeutic window, since EFV plasma levels over 4 mg/mL have been associated with high toxicity in the central nervous system, and at levels below 1 mg/mL incidence of virologic failure appears to increase.[34]

EFV is metabolized by isoenzyme 2B6 of cytochrome P450 (CYP2B6) through primary oxidative hydroxylation.[34] Several polymorphisms have been associated with variations in this protein's expression. The gene's allelic variation that appears to have the biggest effect on hepatic expression of CYP2B6 and metabolism of EFV is the change of G to T in codon 516.[35-38] The effect of this change is decreased activity of the encoded protein; so carriers of this allele (especially homozygous persons) eliminate EFV more slowly, have higher EFV plasma levels[38] and higher incidence of especially intense and prolonged neuropsychological toxicity. The marked increase in EFV's half-life increases the patient's risk of developing resistance to the drug if it is suspended at the same time as all other ART components. In most of one study population, the polymorphism is associated with varying EFV plasma levels and central nervous system toxicities, suggesting that in patients presenting T/T genotype, prescribing lower EFV dosage could ensure reduced side effects without compromising drug efficacy,[39] but another study found no correlation between neurotoxicity and EFV plasma levels.[40] In any case, a combination of genotyping CYP2B6 and drug monitoring has been proposed to minimize toxicity and viral resistance.[41] Besides neurotoxicity, elevated EFV plasma levels have been associated with prolonged QTc interval in subjects with genotype CYP2B6 *6/*6, since they presented higher EFV levels in comparison with genotype *1/*1,[42] although the full potential of EFV to prolong the QTc interval may not be completely verified until conclusion of the study.[43]

Genetic polymorphisms of P glycoprotein (P-gp) also called multidrug resistance protein 1 (MDR1) have been widely studied. Among the most important are 3435 C>T and 2677 G>T/A, associated with decreased expression of the protein.[43] Several studies suggest that these polymorphisms could be related to low EFV levels. However, results are not conclusive.[41] One study notes that although these MDR1 variations have been significantly associated with resistance to EFV, exposure to these plasma levels is not the only pharmacological determinant of resistance, indicating the need for further studies to determine the mechanism by which an MDR1 polymorphism can affect virologic response.[40] According to Alonso-Villaverde[44] genotype C/C in codon 3435 of the MDR1 gene is associated with increased high-density lipoprotein (HDL) cholesterol levels in patients who receive EFV. This observation is supported by the fact that P-gp is involved in lipoprotein metabolism.

The fact that certain genetic polymorphisms may influence and condition significant differences in EFV pharmacokinetics between individuals could have important implications for effective ART. EFV is currently administered at a fixed dose of 600 mg daily. The possibility that a lower dose could reduce ADRs while sustaining efficacy in patients with allelic variations of CYP2B6 associated with higher exposure to the drug is attractive and has already been successfully applied in isolated cases.[41]

Nevirapine (NVP): hypersensitivity reaction NVP is metabolized mainly by enzymes CYP3A4 and CYP2B6, with a smaller contribution from CYP3A5. Several studies have reported that polymorphisms 516G>T[38] and 983T>C[45] in the CYP2B6 gene are associated with variations in NVP pharmacokinetics in different ethnic populations. Patients who are homozygous for this allelic variation presented NVP plasma levels 1.7 times higher than patients homozygous for the common allele.[37] Clinical implications of this observation are unclear, but since higher NVP plasma levels have been associated with greater risk of hepatic toxicity, patients with polymorphic allele 516G>T may present elevated hepatic enzymes during NVP therapy.[39]

P-gp could be involved in transporting NVP outside cells. Reduced P-gp expression could cause NVP accumulation and higher plasma drug levels. Polymorphism 3435*C>T* in the MDR1 gene has been associated with low P-gp expression. In a study on patients in South Africa, this polymorphism was associated with lower risk of hepatotoxicity after starting NVP therapy.[46] Another report described the same observation, in which the T allele in MDR1 was associated with a lower risk of hepatotoxicity in patients receiving EFV or NVP.[47] A lower risk of hepatotoxicity in T/T carriers is paradoxical, since higher intracellular concentrations of NVP would be expected.[39]

Approximately 5% of patients treated with NVP experience an HSR consisting of rash and fever; hepatitis and other systemic symptoms also may occur occasionally, and in some cases can be fatal. Most side effects appear one to six weeks after start of therapy. The mechanism involved in appearance of NVP-related ADRs is not well understood. Skin reactions are most likely mediated by the major histocompatibility complex (MHC-I) and influenced by the CYP2B6 metabolism of NVP, while hepatic toxicity is most likely mediated by MHC-II and not affected by its metabolism. The simultaneous presence of allele HLA-DRB1*01:01 and more than 25% of LT-CD4+ significantly increases risk of HSRs and hepatotoxicity from NVP.[48]

Patients in Sardinia, Italy, who presented unusually high levels of HSRs to NVP compared with other ethnic groups were studied to see if there was a relationship with specific HLA antigens.[49] The study examined 49 HIV patients who were treated with NVP and 82 patients who were not. Approximately 26% of the patients receiving NVP (13/49) developed HSRs; of these, 46% (6/13) presented haplotypes HLA–Cw*8 and HLA–B*14, compared with only 5% (2/36) in the group who received NVP and did not develop HSR. Although the study was not able to determine which of the two haplotypes was more related to HSR, in a later study on a Japanese population where haplotype HLA–B*14 was not present, association between haplotype HLA–Cw*8 and risk of developing HSR due to NVP was confirmed.[50]

Protease inhibitors (PIs)

Atazanavir (ATV): hyperbilirubinemia ATV is included in first-line ART, both for patients initiating ART and those who have interrupted therapy due to intolerance of other ARVs. Atazanavir is metabolized primarily by isoenzyme CYP3A4 of cytochrome P450 and acts as an inhibitor of this isoenzyme and of others, such as uridine-glucuronosyltransferase 1, polypeptide A1 (UGT1A1).

One disadvantage associated with ATV use is that 20%–50% of patients treated with this drug develop hyperbilirubinemia, and about 6% present jaundice. Prevalence of the UGT1A1*28 variation (the most common polymorphism in the UGT1A1 superfamily) was found in 9%–60% of patients, with the highest prevalence in Africa.[51] This alteration does not have clinical consequences, but treatment is suspended in some patients, usually due to jaundice intensity. This ADR is attributed to competitive inhibition by ATV and the UGT1A1 enzyme responsible for conjugation and clearance of bilirubin and occurs more often (5%–10%) in the population with Gilbert syndrome (benign congenital disorder characterized by spontaneous increase in bilirubin plasma values). This syndrome is associated with the UGT1A1*28 allele, defined by the presence of seven repetitions of the dinucleotide TA (TA7) in the gene's promoting region that codes the enzyme, which translates into reduced enzyme activity and asymptomatic hyperbilirubinemia.[52]

Another study confirmed the association between this allele and risk of hyperbilirubinemia; 67% of the individuals homozygous for the UGT1A1*28 allele who received ATV or IDV had at least two episodes of hyperbilirubinemia, compared with 7% in the group not treated with either of these drugs. The UGT1A1*28 allele is the cause of Gilbert syndrome. Prevalence of the UGT1A1*28 allele varies significantly between populations. Hyperbilirubinemia associated with atazanavir is more predictable if considered in conjunction with UGT1A1 genotypes, basal bilirubin and the initial hemoglobin values.[53]

A study in Spain on 118 patients treated with ATV enhanced with ritonavir (RTV) showed a correlation between bilirubin values and ATV plasma levels, and a relationship between ATV plasma levels and polymorphism 3435C>T in the MDR1 gene that codes P-gp.[54] This is the same profile observed in Caucasian patients with genotype CT/TT, who have lower ATV levels even when ATV is enhanced with RTV. Although ATV plasma levels are directly related to bilirubin levels, risk of severe hyperbilirubinemia increases even more in the presence of the UGT1A1*28 (TA7) allele. In the study, C/C carriers had higher ATV plasma levels than patients with C/T or T/T genotype, which suggests that P-gp plays an important role in the bioavailability of ATV. The change in nucleotide C for T in position 3435 and in the MDR1 gene is silent (does not generate phenotypic changes) so it does not change the coded amino acid.[55] Consequently, this may be a marker for another P-gp gene polymorphism with functional effects.

Differences in ATV plasma levels in patients with different P-gp genotypes could be particularly relevant and some patients may need higher ATV plasma levels to inhibit replication of the virus. In such cases, patients with genotype MDR1 CC could have a pharmacokinetic advantage over patients with other genotypes. These studies also show the clinical value of the UGT1A1 test before prescribing ATV, at least in Caucasian patients.[54]

Indinavir (IDV): hyperbilirubinemia IDV is another PI that is not currently widely used due to its difficult dose prescription and its narrow therapeutic index. From 5% to 25% of patients who receive it develop hyperbilirubinemia through the same mechanism as atazanavir.[17] Patients with the additional dinucleotide (TA) present in allele *28 have increased bilirubin levels compared with those who do not have the polymorphic allele.[56] A study in Thailand of patients treated with IDV suggests that a different allele, UGT1A1*6, can predispose a patient to hyperbilirubinemia more than the UGT1A1*28 allele.[57]

IDV is metabolized mainly in the liver by isoenzyme CYP3A5, which shows wide genetic variation. The *3 allele is one of the best known in CYP3A5; its prevalence varies with ethnicity .[58] A guanine in position 6986 in this allele creates a cryptic splicing site in intron 3, which leads to an aberrant splicing in the majority of transcriptions and reduced expression of CYP3A5 protein.[59] Presence of this variation has been associated with low or null expression of CYP3A5 protein, and thus, low IDV clearance. As a result, individuals with this polymorphism tend to present higher IDV plasma levels and more commonly suffer from kidney stones (nephrolithiasis) in IDV-containing therapy. Although these data can appear clinically insignificant due to the benign nature of increased bilirubin, such findings illustrate an important point: genetic variability in pharmacological targets affects drug response.[32]

Nelfinavir (NFV): polymorphisms that affect its plasma levels Metabolism of NFV is mainly hepatic due to the CYP450 pathway. Enzymes CYP3A and CYP2C19 appear to be predominant in humans. An association has been noted between 681G>A (CYP2 C19*2) and NFV plasma concentrations.[40] Individuals who are heterozygous or homozygous for the rare allele present higher concentrations of this PI than individuals homozygous for the common allele. The same study also showed that heterozygous individuals have lower NFV levels.

NFV plasma levels are influenced by polymorphisms in the MDR1 gene. A correlation between NFV plasma levels and genotypes in position 3435 has been described.[43] A study described lower levels in patients carrying the T/T variation, although carriers of the T/T allele have reportedly shown higher levels of T CD4 cells when receiving NFV therapy, despite its unfavorable pharmacokinetic profile. However, this observation has not been confirmed by other authors.[39] Another study showed that heterozygous (C/T) children who are carriers of polymorphism 3435 had higher NFV plasma levels than children with C/C or T/T genotypes.[60] The researchers measured intracellular NFV levels in lymphoblastoid cell lines and examined genotypes of the 3435 position, finding significantly higher NFV levels in the T/T genotype than in the C/C or C/T genotypes. These data support the prior observation of better immunologic recovery in NFV therapy for T/T carriers. It is not clear whether presence of the T allele is associated with higher or lower NFV plasma levels. Differences between the two studies [39,60] e.g., age of population studied and concomitant drugs that may influence P-gp activity, could explain differing results.

Lopinavir (LPV): polymorphisms associated with increased plasma levels One study showed significant association of polymorphism SLCO1B1 T521C with higher LPV plasma levels in patients who are homozygous for the mutant allele,[61] which would suggest that LPV's entry into the liver via transporter SLCO1A2 is an important determinant of exposure to LPV. However, no significant associations were observed between LPV levels and polymorphisms of genes SLCO1A2 and SLO1B3.[62]

Another study examined the influence of several genetic variations of gene SLCO1B, showing higher LPV plasma levels in carriers of mutant allele (521C) than in patients who were homozygous for the wild type allele (521TT). Reduced LPV capture by hepatocytes in carriers of allele 521C could explain these results. However, additional studies are needed to confirm clinical importance of SLCO1B1 polymorphisms in LPV pharmacokinetics.[63]

Ritonavir (RTV): dyslipidemia RTV is widely used at low doses as an enhancer of other PIs, increasing their plasma levels due to its inhibitor effect on the cytochrome P450 enzymatic system. Serious adverse reactions to RTV include possible metabolic toxicity and its effect on adipose tissue due to possible long-term consequences for cardiovascular risk.

A longitudinal cohort study of 329 patients followed for 3 years in Switzerland confirmed an association between SNPs of C-III (APOC3) apolipoprotein and hyperlipidemia. A group of patients carrying both variations of APOC3 and APOE (5.8%) presented higher risk of severe hypertriglyceridemia from treatment with RTV.[64]

A 4-year longitudinal study of 438 HIV-1 patients examining the effect of 20 SNPs on 13 genes associated with dyslipidemia in the general population suggests participation of other genes in lipidic ART response.[65] The objective was to help predict which patients were at risk for developing secondary dyslipidemia from ART. The study concluded that variations in 5 genes (ABCA1, APOA5, APOC3, APOE and CETP) helped explain triglyceride and cholesterol plasma levels, especially in the ART context. However, these findings lack current clinical applicability because of the partially unknown polygenic nature of the mechanism involved.

Variations in the APOC3 allele and its genes appear to contribute significantly to increased triglyceride plasma levels, more or less equal in measure to factors such as age and RTV use. Polymorphisms in gene APOC3 associated with higher risk of hyperlipidemia include -482C>T, -455T>C and 3238C>G.[64] Foulkeset al. found that race/ethnicity was a predictor of plasma lipids in HIV-1 patients on ART and a significant difference in the influence of apoC-III genotypes on PI use across ethnic groups in the association with triglycerides.[66]

Integrase inhibitors (INIs)

Dolutegravir (DTG): polymorphisms that can affect DTG plasma levels Dolutegravir is a potent and well-tolerated INI drug. It is metabolized in the liver via the uridine diphosphate glucuronosyltransferase 1 (UGT1A1) family and cytochrome P450 (CYP3A4) family.

Genetic polymorphisms of ABCG2 were associated with maximum DTG plasma levels.[67] The genetic polymorphism of ABCG2 altered the protein's expression level in experiments on plasmid transfection. Substitution of nucleotide C for A in position 421 significantly reduced the protein's expression and activity. This genotype's low expression level can help explain the high DTG levels, because absorption of DTG from intestinal lumen to capillaries increases in the presence of low expression of ABCG2 in the intestines. The authors propose that it is possible to predict high DTG plasma levels by identifying genotype before starting DTG-containing therapy. In ABCG2 patients, DTG dose can be lowered to minimize possible toxicity and cost without compromising its potency.[67]

Summary Analysis

In summary, a literature review identified 12 ARV drugs with therapeutic responses affected by genetic polymorphisms. To facilitate application by health professionals working with HIV patients, Table 1 summarizes data obtained on these 12 drugs and the associated polymorphisms and effects.

In the drug safety studies consulted,[5,8,68] an HSR to abacavir (ABC) was not among the most frequently reported ADRs in Cuba; this could be due as much to underreporting of ADRs to ARV drugs as to the infrequent prescription of ABC.[5] In treatment-naive patients, therapy preferably begins with 3TC, AZT and NVP. In the Pedro Kourí Tropical Medicine Institute, various cases of rashes and Stevens Johnson syndrome associated with NVP

use have been reported.[68] The authors suggest that this could be because HIV patients have higher exposure to medications and pronounced immunosuppression, as well as coinfection with other viral agents that could stimulate the immune system and predispose them to ADRs. However, appearance of these ADRs could also result in patients carrying the alleles associated with them—an association yet to be demonstrated.

The presence of genetic polymorphisms in metabolizing enzymes or transport/receptor proteins in certain individuals affects the PK/PD processes of some ARV drugs.[39,62,69] Increased ARV plasma levels can have various side effects, including rash,[13] anemia,[32,33] and hepatotoxicity.[38,70] When concentrations drop to suboptimal levels, viral suppression is incomplete and selective pressure triggers viral resistance to that drug. For this reason, we recommend monitoring blood levels in Cuban HIV patients who present ADRs or viral resistance to determine if ARV levels are within the therapeutic window and to adjust the dosage accordingly.

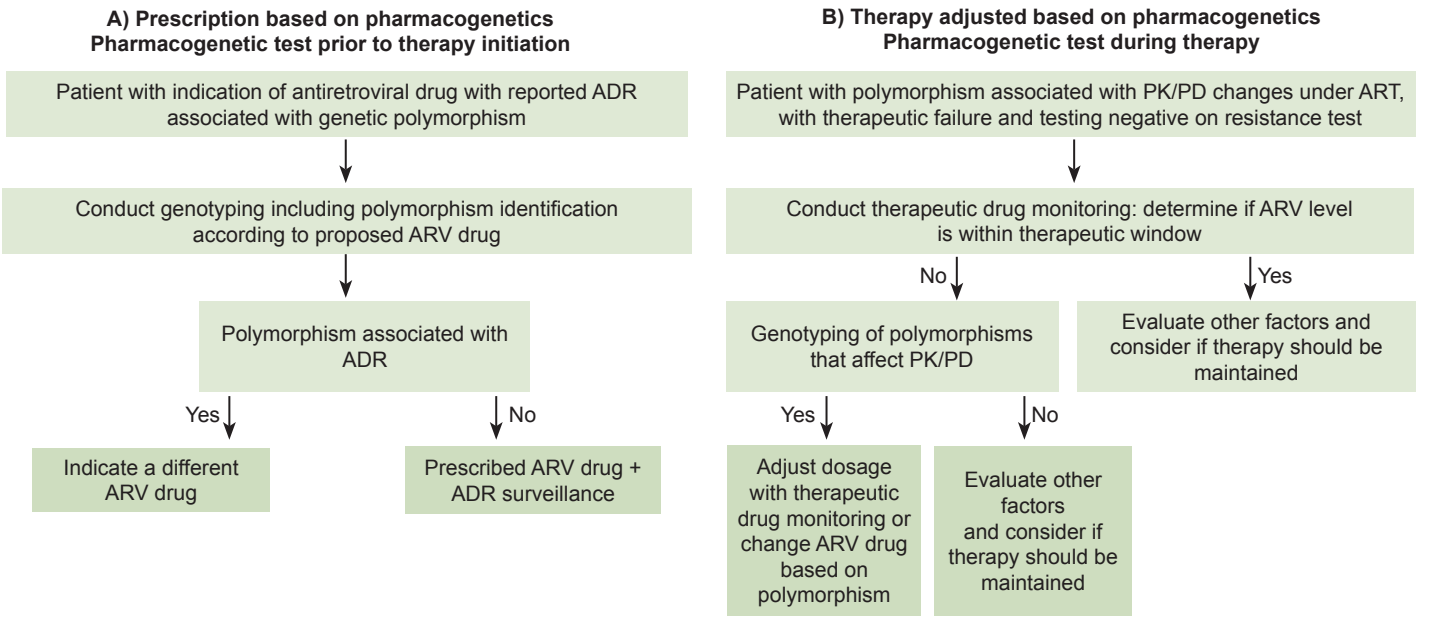
Most ARV drugs belong to the PI family. This group of drugs is one of the most widely used clinically. PIs are generally enhanced with RTV due partly to that drug's high genetic barrier for developing resistance (meaning that accumulation of multiple mutations is needed to induce resistance). Patients with HIV receiving PIs as part of their ART are at high risk of suffering dyslipidemias and lipodystrophy syndrome. This association is relevant because these ADRs are among those often associated with treatment interruption and they occur frequently in Cuba.[68]

In their drug labelling, the US Food and Drug Administration (FDA), European Medicines Agency (EMA), and Japan's Pharmaceuticals and Medical Devices Agency (PMDA) now include information concerning the following pharmacogenetic biomarkers:
—HLA-B for ABC, due to its proven association with appearance of an HSR. It is one of the most cited examples in the literature consulted. Before starting treatment with ABC, all patients should be tested to detect the HLA-B * 5701 allele as this drug is contraindicated in positive cases. Even if the association is negative or cannot be determined, suspension of the drug is clearly indicated after any hypersensitivity reaction to ABC appears, since its continuation could lead to more severe symptoms or even death;[71]
—CYP2B6 for EFV due to association in carriers of CYP2B6 *6/*6 genotype with high EFV plasma levels and prolonged QTc interval, according to the FDA;
—CYP2B6 516 G>T (rs3745274) genotype for EFV due to recognized association with increased EFV plasma levels, according to the PMDA and EMA; and
—UGT1A1 for DTG since carriers of this polymorphism tend to have higher plasma DTG levels, according to the FDA. In the literature consulted, however, Chen[72] concluded that the association is not clinically significant.

In current clinical practice abroad, specifically in management of HIV patients, most drugs are submitted to dosage adjustments *a priori* based on each patient's genetic information and *a posteriori* based on the patient's response. However, in light of therapeutic failure rates, new strategies are needed to optimize ART. Figure 1 shows two proposed interventions combining plasma level monitoring and pharmacogenetic analysis. The purpose of the first intervention (A) is to determine the most appropriate prescription according to a patient's genetic information and is a useful guide for preventing the severe ADRs reported in therapeutic guidelines and drug safety studies. Based on the scientific evidence, we recommend that this strategy be implemented in patients prescribed ART containing NVP or ABC. The second recommended action (B)—applicable to patients receiving ART who present therapeutic failure and whose resistance test was negative—is to conduct therapeutic drug monitoring and identify polymorphisms to determine if a dosage should be adjusted or therapy changed. The triggering ARV drugs in this case are EFV and TDF, due to the numerous dosing regimens proposed, associated toxicities and potential interactions, together with the patient's noncompliance with the therapeutic regimen. Incorporating these recommendations into treatment implementation protocols requires a laboratory with molecular biology capacity, drug quantification and specialized personnel to conduct the proposed diagnostics.

In Cuba, these biomarkers could be introduced in clinical practice according with the government's newest set of guidelines for

Figure 1: Intervention proposals based on pharmacogenetic tests for Cuban HIV patients



ARV: Antiretroviral ART: Antiretroviral therapy ADR: Adverse drug reaction PK: Pharmacokinetics PD: Pharmacodynamics

pharmacogenomics studies enacted in 2018.[73] The first recommendation is to study the incidence of adverse events and therapeutic failures associated with ARV drugs, since current statistics are inaccurate due to underreporting. Also, findings from this review have led to recommendations for clinical practice innovation to combine plasma level monitoring and pharmacogenetic analysis. We recommend that ARV plasma levels be monitored to help estimate the extent of influence of polymorphisms in ARV efficacy and that operational and cost-benefit studies be conducted to measure the impact and feasibility of this type of diagnosis. Incorporating these measures into clinical practice could help extend the therapeutic index of ARV treatment regimens, minimize the likelihood of developing drug resistance and ADRs, and result in a sustained response that improves patient quality of life and helps attain viral suppression in 90% of patients treated, thus reducing HIV transmission.

CONCLUSIONS

Twelve ARV drugs were identified that have been studied for their possible association with genetic variations (SNPs) in several genes, although in most cases there was no statistical support for a causal association.

To the extent that statistical analysis supports associations between these SNPs, clinical response and other pharmacological factors, combining drug monitoring with the identification of polymorphic variations before or during therapy is a medium- to long-term strategy to optimize ART.

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Cuban Public Health History: The 19th Century Board of Health in the City of Holguín

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ABSTRACT

INTRODUCTION In 19th century colonial Cuba, Boards of Health (*Juntas de Sanidad)* were created to administer public health, in tandem with and later replacing the older Royal Protomedicato Court (*Real Tribunal de Protomedicato*). Development of the Board of Health in the northeastern city of Holguín reflected local historical processes, as well as class relations and social issues characteristic of this period.

Among the highlights of the Board's activities were epidemic control during cholera and smallpox outbreaks, monitoring the city's sanitary conditions, and support for charitable work. Studying the history of such epidemiological surveillance activities may benefit design and implementation of related current research and prevention/control campaigns.

OBJECTIVE Describe the development of the 19th century Board of Health in the city of Holguín.

EVIDENCE ACQUISITION The research was conducted through a critical analysis of primary sources contained in the Historical Archives of (today's) Holguín Province, specifically relevant documents from the regional and city government (*Fondo Tenencia de Gobierno y Ayuntamiento*) and town council (*Cabildo*). Cuban and international scientific publications were also consulted.

DEVELOPMENT The Board of Health was the main institution conducting health and hygiene control and charitable activities in the city of Holguín during the 19th century. It was created mainly to take preventive measures against diseases affecting the population, an effort it undertook with support from the Urban Health Police. Its efforts to confront smallpox and cholera epidemics greatly helped to reduce the toll of these diseases on the population, albeit not sufficiently to prevent their reccurrence. Beginning in the 1870s, weakened government support eroded the Board's position, and health-related measures were implemented mainly by the Board of Charity, which focused on matters concerning the city's Civil Hospital.

CONCLUSIONS Although established in 1820, Holguín's Board of Health carried out preventive actions most actively from 1850 to 1865, with support from the Urban Health Police. Its gradual disappearance from the health policy arena beginning in the 1870s reflects its failure as an institution, in large part due to weak government support.

KEYWORDS Board of Health, prevention, epidemics, Cuba

INTRODUCTION

Boards of Health (*Juntas de Sanidad*) were institutions created in Spain in the early 18th century to organize measures to combat the epidemics that plagued the country at the time. The Supreme Board, a judicial administrative body established in 1720, centralized Spanish health policy. Although it collaborated with other institutions such as the Royal Protomedicato Court (*Real Tribunal del Protomedicato*), the Supreme Board relied on provincial and local boards to ensure strict compliance with its policies. In addition to ensuring passage of laws and measures to protect population health, the Board's influence in the 18th century extended to other areas all the way from shelters for the poor, sick and aged to medical publications.[1]

Early 19th century Cuba was shaped by important economic transformations such as development of the sugar industry, but also by political and social changes. An increase in the demand for slaves on the island and Spain's neglect of the education and health sectors had major social impacts.[2] In this context, the colony's public health measures fell short in the preventive control of diseases and public education in hygiene. Each of these developments manifested differently in different regions of the country, depending on their economic activities, local government policies and the general conditions in each location.

IMPORTANCE The history of health and hygiene surveillance in Cuban public health adds meaningful knowledge relevant to current health promotion campaigns and research on more recent disease trends and outbreaks, including those of dengue and cholera.

Boards of Health were created in Cuba in the early 19th century to carry out preventive actions. Initially, they performed this function jointly with the Royal Protomedicato Court, until the latter was dissolved in 1833 during the island's first cholera epidemic. The work of the Boards of Health was essential in the fight against cholera, smallpox and yellow fever epidemics and facilitated health surveillance on the island during the colonial period. Its Superior Board was located in the Cuban capital of Havana, with subordinate boards in provincial and municipal capitals. This topic has been researched by several health historians in Cuba, including Gregorio García Delgado,[3] Enrique Beldarrain Chaple[4] and Gabriel José Toledo Curbelo.[5] Holguín city is located in northeastern Cuba. Its main 19th century health-related achievement was the creation in 1820 of the city's Board of Health.

New outbreaks of dengue in Cuba since 1997,[6] as well as cholera in 2012 and 2013[7] demonstrate the ongoing importance of surveillance of infectious disease threats, particularly when effective vaccines may still be lacking. Studying the history of such epidemiological surveillance activities may benefit design and implementation of related current research and prevention/control campaigns.

This study is one of the first to research the development of the Board of Health's prevention and control work in 19th century Holguín. (NOTE: unless otherwise indicated, Holguín hereafter refers to the city, not the province of the same name, established in 1976). It aims to describe the institution's antecedents and its evolution during that period. The Board's development reflects local historical processes, class relations and social problems of the era. Local historiography includes several works from this period

partially related to this topic, such as those by José García Castañeda[8] and Adisney Campos,[9] although they do not detail the Board of Health's work.

EVIDENCE ACQUISITION

To interpret the available sources, the authors applied critical, analytical and hermeneutic approaches to primary sources from the regional and city government (*Fondo Tenencia de Gobierno y Ayuntamiento*) and town council (*Cabildo*) historical archives of (today's) Holguín Province. These contain the minutes of Board of Health and town council meetings. A literature review was also conducted of publications, both Cuban and non-Cuban authors.

DEVELOPMENT

Historical Antecedents of the Boards of Health Early civilizations suffered greatly from ignorance of the causes of diseases and were thus ill-equipped to protect communities from them, leaving populations exposed to infectious disease epidemics in particular. With the development of science, more policies were devised to successfully combat problems of health and hygiene. In 18th century Europe, studies were conducted using a social approach to medicine in order to improve the structure and function of existing health institutions.[10,11]

The Royal Protomedicato Court, founded in the 15th century, was the first institution created to organize public health in Spain, although it was not integrated into the country's health surveillance system until 1752.[10] In 1780, it was subdivided into three new boards—Medicine, Surgery and Pharmacy—which would continue to operate under the Protomedicato until its final dissolution in 1833.[12,13]

The Urban Health Police, an institution established in Europe during the 18th century and operating throughout the 19th century, had its greatest impact in Western Europe. Its mission consisted of health surveillance and control, as set forth in the work of German physicist and hygienist Johann Peter Frank,[14] who proposed a set of actions aimed at protecting pregnant women, controlling epidemics and organizing hospitals and shelters for the poor, sick and aged. In Spain, the works of Valentín de Foronda, Vicente Mitjavila and Tomás Valeriola were noteworthy. They contained regulations aimed at maintaining sanitary control in cities and towns but did not take into account the social causes linked to epidemics.[15–17]

From 1832 to 1833, the Iberian peninsula and its colonies suffered a cholera epidemic that in Spain alone affected 449,264 people, of whom 102,511 died. This led to the creation of another important institution, the Central Board of Charity, to facilitate cooperation between government and religious authorities to confront the epidemic and supervise handling of donations, alms, budgets and charitable facilities such as hospitals and shelters.[17]

The Supreme Board of Health, created in Spain in 1720 in response to an outbreak of plague in Marseille, France, remained in operation until 1847. It was the country's main institution dedicated to managing health policy, and established protocols to follow during epidemics such as plague and yellow fever. It was also charged with control and surveillance of ports, pharmacies, hygiene in prisons and shelters, and management and supervision of scientific research. Concerning the latter, the Board provided guidance to physicians studying contagious diseases and the best practices known at the time to treat and manage them.[1,11]

In Latin America, Boards of Health were established early in the 19th century and functioned similarly to those in the metropole. However, Spain's centralized governance of its American colonies—which triggered the region's independence movements in the early 1820s—also hindered the functioning of the Boards of Health. This dysfunction was exacerbated by local colonial governments' disregard for sanitation, as evidenced in the Viceroyalty of New Spain (today Mexico), where problems such as poverty and precarious sanitation facilitated the spread of epidemics in its growing cities.[18] Cuban independence leader José Martí identified such problems as the main cause of disease and death in the American colonies, and referred to the importance of applying proper sanitation policies, noting: *The true medicine is not that which cures, but that which prevents: hygiene is the true medicine.* [19]

With the culmination in 1826 of the Latin American struggles for independence in South America and the final recognition by the Spanish government of the status of independent republics in 1836, Boards of Health ceased to exist as such and were replaced by similar institutions responding to the interests of the new republics.[20,21] However, Spain maintained its colonial rule over Cuba until 1898, and thus the Boards of Health continued to operate with more or less effectiveness during the greater part of the 19th century.

Genesis of Boards of Health in Cuba The period of Spain's conquest and colonization of the island began in 1492 with the arrival of Christopher Columbus on Cuban coasts. The indigenous population was decimated by the conquerors in a process that began in 1502. Nicolás de Ovando, the first Spanish governor of the island, was charged with promoting colonial expansion by allocating lands that would lead to formation of towns. Diego Velázquez, Ovando's capitan and later governor, became a main player in this process when he founded five of the first seven towns—the last of them, Santiago de Cuba, in 1515. The towns, organized Spanish style, had as their main form of government the *cabildo,* a council led by the most powerful residents.[2,15]

In the 16th and 17th centuries, Cuba's population was small compared to other American colonies, as the indigenous population had been all but wiped out and many Spaniards abandoned the island when they discovered there was no gold. Cattle ranching became the main economic activity, making for autonomous population centers, where trade in contraband goods also flourished. However, by the end of the 18th century, tobacco and sugar cultivation had gained more prominence.[2,15]

In colonial Cuba, the first public health institution established was the Royal Protomedicato Court of Havana in 1634, which Spain previously had instituted only in the Viceroyalties of Mexico and Peru.

In 1804, the Economic Society of Friends of the Land (Sociedad Económica de Amigos del País) promoted the founding of the Board of Vaccines, whose main goal was to immunize the island's population. One of the most illustrious members of this Society was Dr Tomás Romay who was familiar with Edward Jenner's

work. Dr Romay's efforts were essential in the fight against smallpox. He played an important role in introducing the vaccine:[22] in cooperation with the Spanish mission that brought it to Cuba in 1804, he immediately vaccinated his own children as a first step. On July 30, 1804 the Central Board of Vaccines was established and Dr Romay appointed as secretary. He directed the Board for more than 30 years.[15,22]

Boards of Health were established in Cuba in 1807 to help the Royal Protomedicato Court carry out its functions. The Superior Board was located in Havana, several subordinate boards in the provincial capitals and local boards in the rest of the country's municipalities. Their functions were similar to those of their counterparts in Spain; they were tasked with confronting diseases (specifically epidemics) afflicting the population, inspecting ships arriving at Cuban ports and monitoring sanitary conditions where food was sold. In 1848, following the closing of the Board of Vaccines, shortly after Dr Romay's death, and due to organizational reasons, they took on implementation of vaccination programs.[15,22] Yet, constant political changes in early 19th century Spain made for instability in the organization's functioning.[21]

The Holguín Board of Health: Creation and early years The city of Holguín is located in northeastern Cuba, about 774 km from Havana. On January 18, 1752, the Governor of Santiago de Cuba, Alonso de Arcos y Moreno, in compliance with a Royal Decree issued by Spain's King Ferdinand VII, awarded the title of city to the settlement of Holguín and handed over a large portion of land, previously belonging to the territory of Bayamo. The municipality of Holguín thus gained 5084 useful hectares for agriculture and a 2.4 km^2 area to be used for the city's subsequent growth.[8] From an initial population of 1426, the jurisdiction grew nearly ten fold in the early years of the 19th century, to 12,695 inhabitants by 1820. In 1822, the construction of a major port facility in the village of Gibara, located 32 km north of the city on the Atlantic Ocean, provided an important stimulus for the region's agriculture and livestock industry and led to extensive economic growth for Holguín.[23]

The 1820s were a period of economic prosperity, at least for some classes, under the administration of Lieutenant Governor Francisco de Zayas, who was responsible for major building projects, development of agriculture and commerce, and revitalization of Holguín's Board of Health.[8] This was indeed the main health-related achievement in this period.

After some irregularities and changes, Holguín's Board of Health was definitively installed on May 24, 1820. Presided over by Dr Juan Buch, the Board's main function was monitoring and control of the city's health and hygiene. Table 1 summarizes some of the main events associated with development of this institution in Holguín.[24]

Illustrative of the Board's importance is a document dated May 17, 1815, detailed a meeting of Holguín's town council members, who called on the city's doctors to maintain strict control over diseases most affecting the jurisdiction, especially leprosy. This provision was in addition to the existing obligation for doctors to report illnesses in their populations by order of the Royal Protomedicato Court which, in turn, sent monthly reports to Spain.[25,26]

Table 1. Key events in the history of the Holguín Board of Health in the 18th and 19th centuries

Years	Events
1720	Supreme Board of Health created in Spain.
1804	Central Board of Vaccines established in Havana.
1807	Superior Board of Health created in Havana.
1820	Board of Health installed in Holguín.
1825	Holguín's Board of Vaccines created to fight the smallpox epidemic.
1832	Cholera pandemic affects Holguín's population.
1851	Urban Health Police established in Holguín to support the Board of Health and fight the new cholera epidemic.
1865	Smallpox epidemic affects Holguín inhabitants.
1879	Cuban health sector reorganized according to new provincial and municipal Boards of Health (coastal and territorial), operating under the Superior Board of Health.

The Holguín Board of Health carried out activities similar to those of other municipal boards across Cuba, such as control of ports, hospitals, asylums and pharmacies. Another important function was sanitary control of food markets, especially meat sales, due to various cases of dysentery that appeared from consuming spoiled beef. The first agreement was *(...) making the Constabulary and the slaughterhouse manager understand the need to ensure that spoiled meat...must not be admitted.*[27]

Repeated smallpox and cholera epidemics severely affected Holguín's population in the 19th century, hindering the city's economic and social development. Holguín implemented vaccination against smallpox with Dr Buch leading this effort. On April 6, 1825, Lieutenant Governor De Zayas presided over the establishment of the Board of Vaccines.[27]

However, Holguín Board of Health actions during the 1820s were few and limited to small-scale inspections in the city's public establishments and sanitation. These are only recorded in town council minutes, since separate documentation of Board sessions did not begin until 1832. Francisco Javier Martínez poses one possible reason why the Board of Health actions are not discussed in other documents from the period:[28]

> *We are ignorant of the difficulties faced by [Cuba's] Superior Board of Health between the Wars of Independence [in the Americas] and the restoration of absolutism in 1824 [in Spain], during which there were frequent and often contradictory changes in public health in Spain. The Superior Board of Health may have temporarily disappeared because, as a result of the dengue epidemic in the Antilles, Mexico, Colombia, Peru and the United States that affected Cuba in 1828, Captain General Francisco Vives decided to establish a new board that functioned as the higher body of health on the island until 1832.*

Nevertheless, an 1832 document alludes to a report sent by the first heads of the Royal Treasury of Santiago de Cuba and Puerto Príncipe (charged with the colony's fiscal records and tax collection),[29] expressing concern for strict control of ports. Specifically, the port of Gibara, a small village lacking a local government of its own, was deemed to require strict surveillance and control by the Holguín town council, lest it pose a danger to the city's population. [30] Also noteworthy in the same report is a donation by the Count of Villanueva of 1636 pesos and 2 reales for the

first month and 579 pesos and 7 reales for the remaining months during the epidemic duration to provide care for the infected poor, a fact underscoring the scarce resources available to the Board, which often needed to seek funds from private citizens.[31]

Martínez's conjecture[28] may explain why, in the documents consulted, the first one referring to a Board of Health meeting is dated September 22, 1832 and relates to the cholera epidemic that struck several countries and eastern Cuba. This epidemic, which lasted until 1833, forced Spain to review its health policies and reorganize the structure of its main institutions. One of its actions was the creation of the Central Board of Charity, giving the colonial government a leading role in functions previously undertaken by the Catholic Church.

The Board of Charity arrived late in Holguín. During the epidemic of 1833, no documents were found drawing a connection between it and the Board of Health's actions to fight the outbreak. The first document mentioning the Board of Charity's work is dated 1861.[32,33] Unlike the Board of Vaccines, the Board of Charity did not have a close relationship with the Board of Health at the time. When its work became more systematic and organized beginning in the 1860s, the Board of Charity focused solely on managing the city's Civilian Hospital and on collecting donations.[33]

Prevention and control efforts by Holguín's Board of Health (1848–1865) The main topics discussed at Board of Health meetings reflected the priority of attention to diseases most affecting the population, especially those that reached epidemic proportions: thus, cholera and smallpox were the most frequent subjects in the first half of the 19th century.

The measures mandated by the territorial and Urban Health Police, approved on December 12, 1848 by the Superior Board of Health, were aimed at preventing the spread of cholera. These were endorsed in the Royal Order of December 26. In the same year, a regulation was approved establishing the economic and administrative structure of Cuba's health sector.

Another example of the Board's actions during epidemics is found in a document dated March 15, 1850, in response to a rabies outbreak in Holguín. Since a main cause was determined to be the many stray dogs roaming the streets, an order was issued to eliminate them. In addition, local leaders were required to ensure that only dogs needed for collecting or herding farm cattle would circulate, but these would also be eliminated if they showed any rabies symptoms.[34]

The creation of the Urban Health Police in Holguín in 1851 supported the Board of Health work by putting into practice many measures that, though officially adopted, had not been implemented with the priority required. It also bolstered the Board's role by adding an executive branch to carry out its decisions. Although still dependent on the city's town council, the new surveillance body gave the health institution greater freedom of action.[34]

Some of the first tasks entrusted to Holguín's Urban Health Police were related to the appearance of a new cholera epidemic and were mentioned in the minutes of the Board of Health meeting of January 19, 1851. Police commissioners were ordered to abide by the following guidelines: defer cadaver burial for 16 hours after certification of death by an attending physician; publicly burn clothes and other belongings of deceased; provide 14 cots for the Charity Hospital and two stretchers, one for patient transfers to the hospital and the other for cadavers.[34]

Repeated outbreaks tested the Board's level of organization and effectiveness. Cholera, in its variant known as Asiatic morbus, was a major public health problem in the Americas during the 19th century.[35] In Holguín, four periods were recorded in which, according to minutes of the town council and of the Board of Health, the disease appeared with greater strength: the first in 1832–1833, the second in 1851–1852 and the third and fourth in 1870 and 1883.

Local government and Board actions during the 1851–1852 epidemic were insufficient to stop one of the century's most dangerous cholera outbreaks. This prompted a communication in January 1851, from the Santiago de Cuba Provincial Board of Health to the president of the Holguín Board. It argued for stepped-up efforts to address the epidemic, given 29 cholera deaths in Holguín, continued infection among civilian and military populations, and the ineffectiveness of control measures thus far. This included the requirement that every doctor submit a daily report to the Board of Health detailing cholera cases and their evolution, as well as a general report to the Provincial Board.[36]

Other tasks undertaken by Holguín's Board of Health in 1851 related to management of garbage dumps in empty lots to control their harmful effects. The Urban Health Police was in charge of controlling improper disposal practices.[34] This problem was not unique to Holguín. In Havana, poor sanitary conditions spread alongside the growth and development of the urban nucleus. Enrique Beldarrain also mentions contamination of Havana's port from waste accumulation, causing diseases that spread throughout the population.[4]

In Holguín, the shortage of doctors to care for the affected population was another main challenge in fighting the epidemics. In July, 1853 local authorities asked the Board of Health to transfer Dr Manuel Castellanos to a rural area to care for cholera patients. Despite the absence of doctors offering services to this area, the Board denied the request arguing there were too few doctors in Holguín for the urban population. The city had been divided into five zones, with one doctor taking the lead and another physician in charge in each of the other four zones, plus one in the Military Hospital, another in the Charity Hospital and two in the Board of Health. This made a total of nine doctors, not enough to address the epidemic. The Board also argued that if Dr Castellanos attended to this rural population, he would have to leave another doctor in charge of his work.[34] This shows how difficult it must have been for doctors to care for the sick in epidemic periods. The lack of medical services in other towns highlights an overall problem in the colony, albeit more serious in the eastern region.[4]

Board of Health members continued to conduct routine inspections of food and liquor points of sale. In 1857, these establishments were inspected and spoiled food and adulterated liquor were found. The Urban Health Police ordered that they be collected and burned.[34]

In April 1862, Holguín suffered a new smallpox outbreak which, like the others, disproportionately affected the poor. The public health sector had few resources to tackle the epidemic, as dem-

onstrated by the terrible conditions patients faced at the Charity Hospital. The Administrator of this facility wrote to the Board of Health, that the hospital was incapable of caring for the infected *(...) due to the risk of spreading the epidemic to other sick people.* Thus it was decided *(...) to rent a house with six beds (...) for the poor, indolent and outsiders with no family.*[34]

Smallpox outbreaks continued unabated over the next several years. In January 1865, in response to numerous smallpox outbreaks in several communities since the beginning of the decade, the Cuban Superior Board of Health published a circular attributing the main causes of smallpox propagation to absence of early diagnosis and treatment, insufficient monitoring of reported cases and health authorities' abandonment of vaccination efforts. The Board of Health decided to inform Holguín's Governor of the circular's contents, to promote joint efforts to prevent further spread of the disease.[36]

Holguín's Board of Health during Cuba's independence wars: 1868–1878 and 1895–1898 During the Ten Years' War (1868–1878), the public health system operated under the Spanish military health system. Documentation on Boards' of Health work is sparse for this period. However, smallpox, yellow fever, cholera and other diseases returned with force to population centers in Holguín and throughout the island, afflicting both the Cuban population and Spanish troops.[38]

Despite Boards' of Health insistence on smallpox vaccination, this was not effectively accomplished in Holguín during the Ten Years' War, since public health was all but completely neglected. There is only one document available from this period, dated 1875, regarding a meeting of the Board of Health which, practically bankrupt, was forced to address a new outbreak of this dreadful disease. Lacking the necessary resources, the Board requested help from all the city's administrative, judicial and religious entities to support the vaccination campaign and promote prompt reporting of new cases. This call for help reveals the institution's inability to address public health problems and reflects the scant level of support by colonial governing authorities. [39]

Yellow fever, one of the main pandemics of the 19th century, did not severely affect the population of Holguín until the beginning of the independence war of 1868. Board of Health minutes make no mention of yellow fever in the city during this period. However, Toledo, in his study of yellow fever in Cuba, records its occurrence there in 1857, although without data on the number of people infected.[40]

Once the first war was over in 1878, the colonial government approved a new territorial division into six provinces: Pinar del Río, Havana, Matanzas, Santa Clara, Puerto Principe and Santiago de Cuba. The new divisions led to a reorganization of the health sector that appears in the plans of March 12, 1879 approved by the Governor General of Cuba, establishing Provincial and Municipal Health Boards, which would report to the then named Superior Health Board. The Provincial Boards corresponded to each of the six new provinces, while the Municipal Boards were divided into coastal and territorial. The plan confirmed the new Governor General of Cuba and the new Civilian Governors and Mayors as *higher directors of civilian health* in their respective administrative demarcations, leaving the Boards as mere advisory bodies. [28]

From 1878 to 1895, Cuba made great strides in the field of health: in 1881, Dr Carlos J. Finlay presented his discovery of the vector transmitting yellow fever and his recommendations of measures for the disease's eradication; cholera was eliminated in 1882, and the administration of rabies vaccine began.[15] However, information on the work of the Holguín Board of Health is virtually nil for this period.

With the independence war of 1895, public health was put to the test throughout the country. The return of diseases such as cholera, smallpox and yellow fever severely taxed the national and provincial health systems. According to research on the mortality rate of Spanish troops in Holguín, 1073 soldiers died during the war, 550 due to yellow fever. In 1896, the forced relocation of entire rural populations into the cities under the governorship of Valeriano Weyler caused an increase in infectious diseases. The Boards of Health across Cuba, deprived of funds and authority and completely disorganized, were weak in the face of the many diseases that affected Spanish troops and the general population (dysentery, smallpox, malaria and typhoid fever).[38,41]

CONCLUSIONS

Cuba's Boards of Health were created in the 19th century by the Spanish colonial authorities to organize measures for public health and hygiene, and to address the frequent epidemic outbreaks that plagued the island. To do so, they directed inspections of ports, asylums, hospitals, food markets and pharmacies. To carry out their responsibilities, they collaborated with other organizations such as the Royal Protomedicato Court, the Board of Vaccines, the Central Board of Charity and the Urban Health Police.

In Holguín during this period, the Board of Health took measures to prevent diseases such as cholera and smallpox. No records are available on its work to counteract another great pandemic of the century: yellow fever. The Board tackled health problems by mitigating damage. It was more active from 1850 to 1865. Its decline, beginning in the 1870s, was due to the colonial government's inability to support the actions of such an important institution created to safeguard the population's health and sanitary conditions.

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Increasing Research Productivity across Africa

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Empowering scientific innovation, building knowledge management and strengthening research capacity across Africa are fundamental for improving high-level policy decision-making. Such contributions can distinguish the continent as a reputable source of scientific expertise, prepared to manage local and global challenges towards achieving the Sustainable Development Goals (SDGs).

However, lack of robust higher education policies and political commitment in African nations has affected scientific research training and overall knowledge productivity. This factor helps explain Africa's abysmal research productivity, with the continent contributing just 1% of published global research findings.[1] Thus, we observe that while a research project is a minimum criterion for most undergraduate and graduate degrees, results are not reflected in the literature. One key to addressing this is to implement education policies that foster studies leading to dissertations that meet standards worthy of peer-reviewed journals. Even so, this cannot be the only goal of such policies: they must also prioritize those academic studies that address pressing real-world issues at local and national levels.

Concerning health, several factors highlight challenges for the African research community that may hinder scientific advancement and its role in achieving optimal population health in the continent's 54 nations. Indigenous research output as well as consistent national monitoring and evaluation programs are essential for data-driven decisions, which would promote better population health outcomes.[2] Yet, in most African countries, promoting quality higher education programs in science, technology, engineering, and math (STEM), and engaging the academic community, have ranked low on political agendas. Ironically, this community is perhaps the best prepared to propose solutions to the crises topping these very agendas: infectious disease outbreaks, economic disparities, social and health inequities, to name but a few.

The result is a paucity of research scientists and research-trained clinicians, and a depletion in the research workforce. Brain drain is an exacerbating factor in some academic programs, further limiting the pool of local research mentors.

To address these factors, we propose a three-pronged approach to facilitate collective dialogue across STEM disciplines and support sustainable African research initiatives to contribute to population health and other relevant social policy decision-making.

First, strengthened national data systems Timely, accurate statistics should be gathered from health and development interventions already under way, to help identify health priorities and improve decision-making. This would contribute to health policy formulation that prioritizes use of empirical data and scientific recommendations, while discarding that based mainly on political inclinations. Government and other stakeholders should be pressed to eliminate bottlenecks and bureaucracy so that researchers can easily access secondary data from international, federal, state, and local government sources.

Second, investment in research and related training Scientists across disciplines should be encouraged to participate and present at national and international professional conferences. Mastering scientific communication skills is essential for promoting quality scientific dialogue. Formal mechanisms should be set up for senior-level researchers to mentor junior-level researchers. Efficient use of current budgets for research, coupled with international collaboration and grants, can mitigate the financial burden implied in this process of change necessary to improve research productivity across the continent. Research grants should be accessible to faculty and graduate students to encourage positive scholarly pursuits and provide funding for coordinated research activities.

African leadership should actively engage scientists and other stakeholders from public and private sectors to fund high-quality, high-impact research projects. If their findings show significant influence on health outcomes, they can be scaled up and later translated into practical strategies to enhance population health, growth, and development. African scientists should also be encouraged to seek leadership positions at national and international health and research institutions to build research capacity and strengthen human capital.

Third, prioritize research training in higher education Faculty reviews of curricula and identification of areas for enriching pedagogical methods in classroom and community settings can strengthen their quality and scope. Strict mentorship mandated for undergraduate and graduate research projects can provide students with direct supervision and immediate feedback. Faculty mentors can serve as role models, guiding students to explore basic and applied science disciplines. Visiting professors can be linked with local faculty in related scientific fields, stressing the value of transdisciplinary engagement in One Health research.[2] These networking opportunities can enhance critical analysis and innovation through academic partnerships and co-authored publications.

Against a backdrop of fast-growing populations, African governments must pledge sustainable political commitment and policy development to enhance higher education reforms across all disciplines. Strengthening education systems at all levels and prioritizing support for the academic community towards research for data-driven decisions and policy should be key elements on each national agenda. This will empower citizens to pursue additional academic training, engender research mentorship and transdisciplinary collaborations, and support national and international initiatives to achieve SDG targets.

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Physiology without Borders: US and Cuban Scientists Meet in Space

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Over 400 delegates from 28 countries gathered recently in Havana for the 2nd Pan-American Congress of Physiological Sciences (May, 2019). It was quite an event, the level of science attesting to the quality of participants from the world over. The conference attracted more US scientists to Cuba than any other in the field of medicine over the last several years: 228. I find this number particularly noteworthy amidst the pressures they faced to keep them from coming.

However, such robust US participation was no accident: the event's International Scientific Committee encouraged it, and the US delegates themselves were clearly determined to attend despite the rumor-mongering, the new restrictions announced by the White House and the added bureaucracy of traveling to Cuba—even legally. One by one the obstacles to their participation were overcome and so they joined other prestigious scientists from Latin America and the world, including Cubans. Unfortunately, several NASA specialists were prohibited from attending the Satellite Symposium on Space Physiology—which proved to be one of the most fascinating sessions.

The Symposium was a high-level meeting, with participation by both European and US organizations and institutions. The themes were quite original, many of them unfamiliar to many attendees, such as the medical-biological experiments carried out by Cuban Arnaldo Tamayo Méndez and Soviet Yuri Romanenko during their joint space flight in September 1980, almost 40 years ago.

Some of these experiments were purely Cuban[1,2] and were conducted on this and subsequent flights of the Intercosmos program, and later referenced by virtually all astronauts. This is the case of the so-called "Cuban boots", a life-support experiment carried out for the first time by Tamayo and Romanenko. It addressed the problem of spacial disorientation, specifically the sense of verticality during prolonged weightlessness.

The Cuban boots were designed to "apply pressure to the bottoms of the feet to simulate standing on solid ground" in 1 g conditions, and thus were also one of the features to help distinguish "floor" from "ceiling."[1–3] A Soyuz 38 cosmonaut noted that use of the boots "reduced the severity of spatial illusions and motor disturbances, phenomena thought to be produced by conditions which also produce motion sickness."[3]

A number of other experiments were presented related to astronauts' health during and after long space voyages, including prevention of such problems in preparation for any travel to Mars—a 34 million-mile trip that would take 6 months. The adverse health effects addressed included effects on body and bone mass, vision issues, shortening of telomeres and other DNA alterations that have afflicted astronauts in previous flights.

Ecologists learned of the germs that astronauts would take to Mars and that might contaminate that planet, because of course these human space explorers are also microbial vectors. Presenters also referred to high-resistance tests and psychological analyses, such as those involving the six women who crossed Antarctica, assessing problems they encountered along their trek when exposed to extreme natural conditions while reaching the limits of their endurance, followed by psychological analyses. Another study compared molecular adaptation between a set of identical twins, both astronauts, associated with the different trips made by each.

For scientists, walls are simply meant to be scaled, preferably together

Training needs for future astronauts were also projected, including recommended exercises during prolonged space voyages, based on specially equipped pre-flight simulators. Yet another area of research looked into the consequences of inactivity during long space flights, which can reduce insulin resistance, among other effects on vascular health.

In summary, this event illustrated that such scientific research can build bridges of useful exchange and cooperation, in particular between professionals in the USA and Cuba. The congress, whose theme was "physiology without borders," proves once again that we can work jointly in the interests of health. It offered further evidence that for scientists, walls are simply meant to be scaled, preferably together.

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