MEDICC Review

International Journal of Cuban Health & Medicine

October 2018

Vol 20, No 4

Editors' Choice

Peer reviewed since 2007

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MEDICC Review

October 2018, Vol 20, No 4

Cover photo: *PAHO, from Mais Médicos photo exhibit September 26, 2016 (See interview with Cristian Morales this issue).*

Available online only

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Medical Education Cooperation with Cuba

MEDICC Review is published by MEDICC (Medical Education Cooperation with Cuba), a nonprofit organization founded in 1997 to enhance cooperation among the US, Cuban, and global health communities aimed at better health outcomes. The organization's mission is to support education and development of human resources in health committed to equitable access and quality care.

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MEDICC Review

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MEDICC Review (ISSN 1555-7960) is published quarterly by MEDICC (Medical Education Cooperation with Cuba) in January, April, July & October.

Submissions *MEDICC Review* publishes original peer-reviewed articles by Cuban and international authors. Send letters to editors@mediccreview.org. Guidelines for authors at www.mediccreview.org.

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Power, Politics and Population Health: The Strength of Collaboration

Rudolf Virchow, the father of public health, as far back as the late nineteenth century said, "Medicine is a social science, and politics nothing else but medicine on a large scale."

His point is well taken. Articles in this issue of ***MEDICC Review*** explore the often turbulent relationship between science and those with the power to use it. Eventually, it is they who can wield science for their benefit alone or to improve population and planetary health. They can pay attention to science, they can ignore science, or, in the worst cases, they can bend it to serve their own narrow interests.

As illustrated in several articles, breaking the decision-making monopoly of powerful forces requires that scientists and their allies come together to demand their place at the table. Of course, first must come the opportunity for scientists themselves to collaborate, to reach conclusions based on open, transparent and rigorous debate. This is driven home by Luis Velázquez-Pérez, President of the Cuban Academy of Sciences. Through his article, Cuban scientists call for a global scientific meeting to review the case of Havana-posted US diplomats' health complaints. Complementing his piece is an interview with Mitchell Valdés, director of the Cuban Neuroscience Center, who emphasizes the critical importance of information sharing as part of proper scientific process for investigating this or any other medical mystery.

In Blanco's paper on skyrocketing cancer drug prices worldwide, he and colleagues urge collaboration beyond the scientific community to establish a multinational force involving, first and foremost, the less-developed countries, to confront the major pharmaceutical manufacturers. Much is at stake in this people-vs.-profits struggle: unless drug prices for cancer patients come down, argues the paper, the world's health systems, even in richer countries, will simply go broke.

From Nigeria, comes Aregbeshola's Perspective proposing a path towards universal health care. It's a path that, once again, depends on those in power heeding the results of health and economic research to marshall the political will for change—in a country where less than 5% of the population has health coverage.

Collaboration in Latin America is brought into focus by two articles in this issue, the first an interview with outgoing PAHO/WHO representative in Cuba, Cristian Morales, who talks about PAHO's and Cuba's shared values of solidarity and health as a human right. In particular, he refers to the triangular collaboration among Brazil, Cuba and PAHO, responsible for over 11,000 Cuban physicians serving some 45 million people in Brazil's indigenous and otherwise underserved populations, as part of the Mais Médicos program.

The second is a research paper by Méndez-Rosado examining results of genetic testing in four Latin American countries, including Cuba. He and his colleagues detected hitherto unobserved genomic breakpoints related to de novo balanced structural chromosomal rearrangements. Far from being an esoteric exercise, the results will be useful in prenatal genetic counseling for pregnant women in Latin America and in long-term postnatal followup of patients with such abnormalities.

Perhaps there is no better illustration of the ability of collaboration in health and science to break through barriers than the joint venture between Roswell Park Comprehensive Cancer Center and Cuba's Molecular Immunology Center. This represents an important step to making Cuba's cancer-fighting biotech therapies available to US patients, given the encouraging early results (released in September) of early-phase clinical trials of CIMAvax-EGF in US lung cancer patients.[1]

> **Cuban scientists call for a global scientific meeting to review the case of Havana-posted US diplomats' health complaints**

CIMAvax's emergence on the global market is indicative of two major developments. First is the vindication of Cuba's persistence in researching cancer immunotherapy, a line of research at one point almost discounted, but this year the object of the Nobel Prize for Physiology and Medicine. Second, CIMAvax has benefited from the consolidation of Cuba's regulatory and clinical trials infrastructure. María Amparo Pascual, founding director of the National Clinical Trials Coordinating Center, is featured in the Interview section of this issue, as part of our series on outstanding Cuban women in science, technology and medicine (STEM).

Another outstanding woman scholar and practitioner who would certainly have been included in the women-in-STEM series, but for her untimely death a year ago September, was renowned educator Emelia Ycart Pereira, a longtime member of ***MEDICC Review***'s editorial board. Dr Ycart dedicated her five-decade career to improving the lives of people with disabilities, among her many activities, directing Havana's La Castellana Psychopedagogical Center. We dedicate this issue to her memory.

The Editors

1. Roswell Park Comprehensive Cancer Center. Media release: Roswell Park Lung Cancer Expert Shares Initial Findings From First North American Study of CIMAvax [Internet]. 2018 Sep 26 [cited 2018 Oct 18]. Available from: https://www.roswellpark.org/media/news/roswell-park-lung-cancer-expert-shares-initial-findings-first-north-american-study

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Strengthening National Health Priorities for Diabetes Prevention and Management

To the Editors:

In his report in the October 2017 issue of ***MEDICC Review***, Vega-Jiménez stressed that the design and integration of a prediabetes (intermediate hyperglycemia) registry would serve as an essential prevention strategy to improve population health outcomes in Cuba.[1] Cuba's universal health system, with its robust health workforce and established primary care services from family doctor-and-nurse offices, polyclinics and hospitals, is well prepared for this ambitious step forward in prevention and management of non-communicable diseases (NCD) like diabetes.

In the Dominican Republic (DR), a neighboring Caribbean nation of almost 11 million residents, the prevalence of type 2 diabetes is a growing concern, with 2016 prevalence estimated at 9.3% (7.8% in men; 10.6% in women), and all-age attributable mortality at 4%.[2] Reported risk factors were overweight (54.8%), obesity (23%) and physical inactivity (35%).[2] One recent study by the National Institute of Diabetes, Endocrinology, and Nutrition and Iberoamerican University, with a national sample of 10,500 adults, reported that 13.5% had type 2 diabetes, and 9.3% had prediabetes.[3] Although DR health leaders tout a national diabetes registry and strategic plan to combat diabetes and reduce sedentary behaviors, no national guidelines are available, and appropriate referral practices for diabetes management are inconsistently utilized.[2]

To combat rising diabetes prevalence, national health systems such as the DR's should consider a twofold approach that targets reduction of risk factors such as prediabetes and hypertension. First, a national advisory board can develop a consensus for evidence-based national guidelines that support clinical decision-making and best practices. Second, strengthened links between primary care and specialized health centers can facilitate patient management and referral measures for additional medical services. This approach would complement the Pan American Health Organization's 2012 Passport to Healthy Lifestyle initiative, which provided personal logbooks for patients diagnosed with diabetes and other NCDs.

Extensive connections between primary care and specialized health programs are essential to promote paradigm changes that integrate NCD prevention and management approaches for health service delivery. A prediabetes registry and appropriate patient referral measures would be a transcendental step to improving population health outcomes in vulnerable populations across Latin America and the Caribbean.

1. Vega-Jiménez J. Cuba needs a prediabetes registry now. MEDICC Rev. 2017 Oct;19(4):44.
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The Power of Persistence: María Amparo Pascual MD MS

Founding Director, National Clinical Trials Coordinating Center, Havana

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Throughout the 1980s, Cuban researchers at the country's biotech campus known as the Scientific Pole were making innovative discoveries and began developing unique therapies and vaccines unavailable elsewhere in the world. The pace and level of innovation meant prioritizing the establishment of a dedicated, internationally-certified institute for clinical trials. These and other accomplishments in science and related sectors, coupled with statistics revealing that 53% of all scientists in Cuba are women, prompted ***MEDICC Review*** to publish a series of interviews with outstanding Cuban women in science, technology and medicine.

In this, the second installment in the series, we spoke with Dr María Amparo Pascual, a biostatistician, researcher and professor, and the driving force behind the design and establishment of Cuba's Clinical Trials Coordinating Center (CENCEC). From 1991 to 2014, Dr Pascual served as founding director of CENCEC. During that time the center implemented internationally-recognized good clinical practices (GCP), launched the National Clinical Trials Coordinating Network to support trials overseen by CENCEC, began conferring master´s and doctoral degrees in clinical trials; initiated a quality management system for all trials (receiving ISO 9001 certification in 2008) and created the Cuban Public Registry of Clinical Trials—a bilingual, WHO–accredited Primary Registry, the first in the Americas. In 2013, the BBC recognized Dr Pascual as one of the most influential female scientists in Latin America for her achievements, including becoming Cuba's first biostatistician and her work at CENCEC.

In 2014, Dr Pascual stepped down as director of CENCEC but didn't slow down or leave the center she helped build, literally from the ground up: she currently serves as management consultant and researcher at CENCEC, as well as director of the Research Ethics Evaluation System Project there. She is also a professor in the Center's clinical trials graduate degree program and of bioethics at the Medical University of Havana. She is finishing her doctoral dissertation on clinical trials in Cuba and the founding and evolution of CENCEC.

MEDICC Review: **You're a biostatistician with a remarkable career in science and research. How did you get involved in clinical trials?**

María Amparo Pascual: I love biostatistics and I've dedicated my life to science. It's my passion. Early in my career, I worked at the Ministry of Public Health (MINSAP) evaluating how Cuban institutes conduct research and our research methodology: Is it relevant? Is it rigorous? Is it replicable? In the mid 1980s, I began my first foray into clinical trials as head of the Clinical Research Department at the National Institute of Oncology and Radiobiology (INOR) . I was a one-woman department, working completely alone; all my time was devoted to researching clinical trials methodology, international protocols and related topics. I was fascinated.

By that time, the research campus known as the Scientific Pole was developing innovative therapies and biopharmaceutical products, so it became imperative to establish a center for clinical trials [founded in 1981, the Scientific Pole received a billion dollars in investment from the Cuban government and within a decade included 52 institutions dedicated to scientific research and development—Eds.]. A group of Cuban scientists with related experience was convened and I was proposed to direct the new center.

I enjoyed my work immensely at INOR and was very good friends with my colleagues there, so it was difficult for me to decide to make the move. Agustín Lage, INOR's director at the time, convinced me that it was an exciting opportunity and that I was the right person for the job. But he also warned me that such a move wasn't without risk: I would be organizing a clinical trials center from scratch and in an uncertain context. This was during the 1990s, what's known as the Special Period, when resources were scarce and Cuba was in dire economic straits. But I'm a

Sagittarius! I love challenges and exploring beyond my comfort zone, so I decided to take the leap.

MEDICC Review: **That was almost 30 years ago. Were there many women scientists in leadership positions at that time in Cuba?**

María Amparo Pascual: Back then it was quite rare to have a woman directing a scientific institution. There were a handful, like Dr Concepción Campa, who led the Finlay Vaccine Institute team that developed the VA-MENGOC-BC vaccine for meningitis, and Lidia Tablada, who founded and directed the National Center for Animal Health, but the rest were men. For a man to be named director of an institution was unremarkable. But women have to work doubly hard to reach that level—they're usually responsible for resolving issues related to their children or home for instance, and there's also a certain level of gender bias women have to overcome—especially back then since it was so novel to have a women director. It wasn't easy. But where other people see limitations and difficulties, I see opportunity.

Today it's a different story. I've noticed younger men, in my own family, among my colleagues, assuming much more responsibility at home and for child care, for instance. They're present and actively participating in domestic life. It's also striking how many women are directing institutions or hospitals today. Likewise, we have many women in leadership positions in politics, ministries and the like. Nevertheless, while over 50% of scientists in Cuba are women, we don't represent half the directors of scientific institutes.

MEDICC Review: **In your experience, have you noticed a difference in leadership styles between men and women?**

María Amparo Pascual: In my opinion, men and women *do* have different styles of leading but I think generalizing can be dangerous, so this doesn't apply to all men or all women, but I've found that women are generally more demanding of themselves. And this translates into being more demanding in their work and with the team they're leading. We see a problem and we want to solve it—I think this is an attitude towards life that not all, but many women have. Like a dog with a bone, we don't let go until we've solved the problem and that leaves an impression on those working with us. Women leaders in my experience tend to be more human as well—when it comes to conflict resolution, for example, or when someone is having personal challenges, they are more likely to offer counsel when it's requested and appropriate.

I also think part of the reason Cuban women scientists make effective leaders is their persistence. Of course, they have to know the subject matter inside and out. In my case, I have to know about clinical trials. I have to know what has been achieved, I have to have experienced it firsthand. A director can't lead and delegate if they haven't done the work themselves. This provides an undeniable authority to their leadership, underscores their persistence and helps others believe in their vision.

Leaders and directors aren't perfect—we're human after all! But the best leaders—regardless of gender—are those who can marshal and allocate resources responsibly, who can walk in other people's shoes and empathize. It helps if they are humble and lead by example. When you lead by example, you will make mistakes and commit errors, we all do, but when workers see their leader walking the walk and not just talking the talk, as the saying goes, that has an impact. With good, effective leadership, workers feel confident and secure. We have seen cases of worthy, important institutions that are deteriorating due to poor leadership.

MEDICC Review: **You mention persistence as an important characteristic of leadership. Can you talk about some examples from your own experience establishing CENCEC?**

María Amparo Pascual: Persistence was key since we basically started from scratch. First we had to design a strategic program and learn what we didn't know. To achieve this we visited clinical trial centers and consulted with experts around the world: Italy, Belgium, France, Canada—from that experience, we learned how to design, conduct and evaluate clinical trials according to international protocols; we trained specialists, and organized and equipped the entire institution, from laboratories to work spaces. Although Cuba was in terrible economic shape at the time, the political will was there and resources were made available to establish the center based on standardized norms and best practices.

We began in a cramped office, only 120 square feet. My desk was right beside the bathroom, so anytime anyone used it, they had to squeeze by my desk! We worked in rotating shifts for lack of space. But as the Scientific Pole grew and more products required trials, we moved to an old convent. We had an entire floor, so it was bigger, but it didn't have the proper conditions for the work we were doing and the strategy we were developing. So we persisted, insisting that we needed a larger, more conducive space.

It took three years, but again, the political will was there and we received resources to further develop CENCEC as a center for conducting efficient, high quality clinical trials in accordance with standard protocols. It wasn't an easy task: we had a full staff by this time, and a very robust product pipeline with ongoing and pending trials. Moreover, the decision was made to erect a new building with all the proper conditions, to house both CENCEC and Cuba's regulatory agency, the State Center for Quality Control of Medicines, Equipment and Medical Devices. Luckily, we had the full support of MINSAP and then Minister Dr Roberto Morales, allowing us to inaugurate a new, high-tech and fully equipped center in 2014. By that time, we had a staff of 150 people—the majority women; and I'm proud to say that half of the people who were part of our desk-by-the-bathroom team are still working at CENCEC today.

MEDICC Review: **Given the day-to-day challenges in Cuba, including low salaries, how has CENCEC been able to retain scientists and researchers?**

María Amparo Pascual: Obviously, everyone needs a minimum salary to maintain themselves and their families and, while salaries are low, it's important to recognize that money isn't the only factor driving why and where people work. Motivations beyond money include having a passion for what you're doing and working in conditions favorable to pursuing that passion, enjoying opportunities for professional advancement, and receiving recognition for your achievements. This helps create a sense of support, appreciation, commitment and pride—especially, in our case, since producing safe, effective biotechnology and

pharmaceutical products is strategically and economically important for Cuba. Our workers receive recognition not only from their colleagues, but also from national leaders and international authorities.

When CENCEC started, it was obligatory for our researchers to pursue higher degrees—they were given one day a week to study, go to school. We instituted an academic development strategy early on and in 2008 initiated master's degrees and doctorates in clinical trials. We were also one of the first scientific centers to link pay to performance, which also helps retain talent. Together, these factors paved the way for one of CENCEC's major achievements: designing, implementing and maintaining a quality management system for all clinical trials. It was launched in 2006 and in 2008 received ISO 9001 certification from the Spanish Standardization and Certification Association.

If I leave any kind of legacy, it's having helped create a team of people who are engaged, who love what they do and come to work every day motivated to do more. Is this because I'm a woman and does it have to do with my managerial style? Could be . . . but retaining workers—in any sector—depends on the individual, on the recognition they receive, and on leadership.

MEDICC Review: **And the younger generations? Are they pursuing careers in science?**

María Amparo Pascual: Recently a young CENCEC colleague and I were talking about this same issue. I see a new generation of scientists hungry for knowledge, traveling to the provinces to work with our national network of clinical sites, visiting hospitals and colleagues within the network, and studying for higher degrees, all while earning a salary which doesn't resolve their day-to-day problems. And I began to analyze why . . . why do people choose to work here even though they don't earn that much? What motivates them to choose a career in science? As a graduate school professor, I ask young people thinking of going into the sciences: Do you like it? Are you passionate about it? Would you rather be doing science than anything else? When a student is lukewarm, waffling between science and another field, I tell them: Science is not for you. Sometimes here in Cuba we put a fine point on how many scientists we have, how many doctors we have. It's an accomplishment to have so many doctors, so many dentists and health professionals, but these statistics, while laudable, don't tell the whole story. We have to ensure that those who pursue science are passionate about it, that science is what keeps them engaged and excited. If that means we're training fewer scientists, let's train fewer scientists! It's a question of quality not quantity.

MEDICC Review: **And would having fewer scientists mean better paid scientists?**

María Amparo Pascual: Again, I don't want to generalize but I think many people are so hyper focused on money, they lose sight of other virtues and advantages implicit in a given profession. There are Cubans today who abandon their careers, opting for whatever job will make the most money the fastest. Others look overseas and emigrate. But others stick with science and research, despite low salaries. It's important that leaders transmit what makes a certain career or workplace attractive, highlighting favorable factors for different kinds of work and recognizing that everybody has other pursuits and passions in their lives that are important. It can't be all work, all the time—that doesn't make for a well-rounded or happy individual.

The salary issue is nothing new: it has been debated and analyzed at all levels and providing solutions and raising salaries is high on the agenda. But it's taking a long time. Meanwhile, day-to-day economic challenges persist for these workers; the situation can cause researchers to lose enthusiasm and motivation. Nevertheless, I don't think raising salaries is enough to resolve all these issues.

Unfortunately, sometimes the discourse here sounds like a parent lecturing a child: "You have to motivate workers to produce more! They have to work harder! Low production is why salaries are low!" That's not the way to motivate people—even if it comes from good intentions. It may have worked 30 years ago, but we're in a different historical moment and need different strategies so people feel committed to their work, so they feel appreciated and compensated for their contribution. This doesn't transcend the salary challenges, but it helps to some degree.

MEDICC Review: **The Cuban Public Registry of Clinical Trials lists more than 200 trials underway in different phases. Conducting these trials depends on a national network of clinical sites, specialists and participants. Can you talk about this piece of CENCEC's work?**

María Amparo Pascual: A Canadian colleague once told me, "CENCEC is the largest clinical trial center in the world!" I didn't understand what he meant until he said, "It covers the entire nation; few centers have such extensive reach." And it's true: Since the beginning, CENCEC's strategy has incorporated different elements to take advantage of our single, universal health system. In 1993, we established the National Clinical Trials Coordinating Network, including a certification program for clinical sites, ensuring they're applying GCPs; that same year, Cuba conducted its first controlled, randomized and concurrent multicenter clinical trial. More than a hundred independent ethics committees for scientific research were also established across the country; these operate in accordance with WHO guidelines. People working in these clinical trial sites are indispensable to the process. Yet, I see challenges here. What they do isn't directly linked to production, strategy or services and thus while their work is just as important, it's not as tangible or visible. So they generally receive less recognition for their contributions and lower salaries than colleagues working in other parts of the process. How can we raise their spirits and keep them motivated? Higher salaries of course, but we need to forge other solutions as well. This is an area warranting more study.

MEDICC Review: **Trials are underway with Cuban biotech products in countries around the world, including the USA. Can you comment on the recent collaboration between our two countries to test and produce one of Cuba's market leaders, CIMAvax-EGF?**

María Amparo Pascual: There has been tremendous enthusiasm for this non-small cell lung cancer therapy since Phase IV clinical trials concluded in Cuba in 2015 showing CIMAvax-EGF to be safe and effective. These were highly complex trials, including over 1000 participants across 61 clinical sites. That same year,

the Roswell Park Cancer Institute in Buffalo, New York signed an agreement to start clinical trials of CIMAvax-EGF and several other Cuban biotechnological products in the USA. Just recently, this collaboration has taken a step further, with Cuba's Molecular Immunology Center and Roswell Park joining to manufacture CIMAvax-EGF at the Mariel Special Development Zone west of Havana.

Collaboration like this doesn't happen overnight—the agreement is the result of years of hard work. Once again, persistence is paying off. Fortunately, there has always been interest in bilateral cooperation between US and Cuban scientists and health professionals. I hope that as a result of this persistence, US patients will one day have access to CIMAvax-EGF and other Cuban vaccines and therapies [the embargo and related travel restrictions prohibit US citizens and residents from traveling to Cuba for medical treatment—Eds.]

Remember, this is brand new territory for both countries, but perhaps it's the first step towards closer collaboration in other sectors. Bringing new biotech and pharmaceutical products to market involves high levels of risk and few are willing to be the first to take that risk—particularly in a climate of uncertainty and fear. And there has been a lot of fear manufactured about Cuba, especially recently in the USA. When other entities and companies see that Roswell Park can do it, that fear begins to wane and they begin to think: If they can do it, maybe we can too. I don't want to get ahead of myself, but this agreement could be a bellwether; already we're seeing stronger cooperation in the cultural and environmental sectors, so who knows what doors this might open? I have colleagues at the University of Miami, for instance, who are very interested in working with us on bioethics but restrictions for legal travel on the US end make it difficult; it would be great to be able to deepen this collaboration in the future.

***MEDICC Review*: You have so much energy . . . Any plans for retirement?**

María Amparo Pascual: When CENCEC's new center was inaugurated in 2014, I asked to be relieved of my position as director so I could finish my doctoral dissertation—or try to finish it! I'm in my 70s, I have a 92-year old aunt I take care of, plus I head CENCEC's ethics committee and am a graduate school professor. I'm no longer the director, but I still work at CENCEC, with a regular desk in the open office work space with the other researchers there. I like being around other scientists and most of them are young, which I like too.

I don't want to admit it, but I'm ancient! Many times I've asked myself: why am I still working?! But I love what I do and think it's in my blood. My mother was a teacher and my father was still working at age 92—he instilled in me the idea that work should be a passion, that you live to work, not the other way around. I'm convinced that our future success depends on passing the baton to younger generations who share this philosophy. We're seeing this right now in politics with our new President who embraces new technologies, employs new methods of communication, and explores different solutions. We have very talented young people, with the knowledge, experience and maturity to assume leadership roles to move Cuban science forward; we need to encourage and support them.

Translating the Shared Value of Solidarity
Cristian Morales PhD
PAHO/WHO Representative in Cuba

Gail A. Reed MS

Cristian Morales, an economist by training, has dedicated his career to improving health and health equity in the Americas through his work with PAHO/WHO. This has taken him from floods and earthquakes in Haiti to PAHO's Washington DC offices, where he was instrumental in achieving consensus on a resolution aiming for universal health—coverage plus access—approved by all governments in the Americas. Since 2015, he has served as PAHO/WHO Permanent Representative in Cuba and has recently been appointed to the analogous post in Mexico. At the end of his three years in Havana, ***MEDICC Review*** talked with Dr Morales about his experience, the Cuban health system, and the values it shares with the organization he represents. This is part one of the interview, the second part to be published in our January 2019 issue, in which we'll talk more about the health system in Cuba itself, its achievements and also its challenges.

MEDICC Review: **Since our last interview, soon after your arrival in Cuba, we've seen many changes in Cuba, in the region, and in several bilateral relations. Now Cubans are even debating a sweeping constitutional reform. One thing you said to me then was that Cuba shares PAHO's values in terms of health. Is that still true, and if so, in what sense?**

Cristian Morales: Yes, I think the first thing we have in common is recognizing the right to health, not simply as a legal construct, which is important of course, and is included in the proposed Cuban constitution as well as the current one. But I'm speaking of the right to health as a value essential to structuring the health system. A health system structured from a strictly economic vision, a market where health services are consumed, isn't the same as one structured based on health as a right, that guarantees universal access to quality health services of various kinds: health promotion, disease prevention and treatment, as well as rehabilitation. This kind of system is accompanied by access to comprehensive human development policies that address the social determinants of health. This seems fundamental to me.

The way Cuba's health system is structured around the right to health is expressed quite well in the breadth of primary health care, with two important characteristics that PAHO espouses. The first is that the system itself rests on a strong primary health care approach, and the second is that it is immersed in the community. The family doctor-and-nurse offices have a role that goes beyond protecting health, caring for Cubans in their geographic area. They also play an important part in how the community is structured, the ties that bind it internally, and finally in community development itself.

Another common value has to do with solidarity, which of course is promoted by PAHO, but is also in the genesis of the Cuban health system since the very beginning of the revolution. Perhaps the great inequalities existing prior to 1959 in the health sector also pushed forward this concept of a health system based on solidarity, one in which those who couldn't pay had the same rights and access to care as those with more means.

Those are the two main points of coincidence. But I could also mention a third value shared with PAHO, which is excellence. There are several expressions of excellence you can see in the Cuban health system: first, of course, is the excellence of its professionals, the excellence and quality medical sciences education they receive, and then the excellence of their professional practice. But you can also see this in innovation, in their capacity to generate innovations for world health, which is the foundation of Cuba's specialized institutes, its biotech industry (BioCubaFarma). So this is another very concrete example of how a value-based construction can

achieve results that benefit not only Cubans, but also all of humanity.

MEDICC Review: **From the point of view of the system, and also its results, what does Cuba have to share with the rest of the countries of the Americas? What can its experience contribute?**

Cristian Morales: The results aren't a product of fortune or chance, but rather above all, a product of political will, very clearly expressed from the start of building Cuba's health system. I think this is important to emphasize, and personally, I believe these results have been achieved mainly thanks to the strength of the primary level of care built into the system. By this I mean not only the doctor-and-nurse offices, but also the network of community polyclinics that act as a bridge, integrating primary health care with more complex levels.

That is, there is sufficient capacity nationally to learn the needs of the population, using the local health situation analyses that are intended, for example, to determine the various problems that affect Cuban families, and from there decide the resources needed to address these problems. But this also means the national capacity to refer patients [from the family doctor] to the polyclinic or to more complex levels of care when needed. This is what we would call care centered in people, families and communities. I think this is the key to their success and their results, which are many.

Starting towards the beginning: Cuba was the first country to eliminate polio, in 1962; (certified by WHO in 1994); and in the 1970s, malaria was eliminated. These are just two important examples of diseases that continue to affect countries in the Americas, in particular the Caribbean. In Cuba, measles was eliminated in the 1990s, and more recently in 2015, WHO certified elimination of mother-to-child HIV transmission and congenital syphilis.

These are impressive results. Infant mortality has been less than 5 per 1000 live births for the last 6 or 7 years, and in 2017, it was 4, with the goal of reducing it further. There are not many countries in the world where you can observe such an indicator. And you have to remember that we're talking about a country with few material resources, with few natural resources, but with a lot of determination and human resources that are ultimately responsible, through the dedication of their health professionals and scientists, for these contributions to the health of Cubans and people throughout the world.

I mentioned that one of the values underpinning the Cuban system is solidarity, and this is concretely expressed in its health outcomes. You can also see it expressed in results achieved beyond Cuba, across the world, through the contributions of Cuban medical teams in over 60 countries. Some 30 to 40 thousand health professionals are posted abroad in a given year. This extent of cooperation indicates an important level of solidarity, which has an impact in the health of other peoples. Not only are thousands of health professionals providing services daily, but the Henry Reeve Emergency Medical Contingent is also activated in cases of disaster or health emergencies. Thus, for example, many lives were saved by Cuban health professionals during the Ebola crisis in Western Africa in 2014.

We could also be talking about Haiti, Afghanistan or Algeria (the latter the first country with long-term Cuban health cooperation, in 1963), and also about my own country, Chile, to illustrate how rooted this concept of solidarity is in Cuba's health system. In 1960, we experienced the greatest earthquake recorded in history, and Cuba immediately organized a medical team that spent several months helping in our country, which was simply devastated. That is, over the decades, the lives of tens of thousands of people . . . the precise number difficult to say . . . have been saved thanks to the Cuban health system and its professionals.

MEDICC Review: **The experience in Chile was very personal for you . . . and that in Haiti, too, where you have been posted. More recently, the PAHO/WHO offices in Cuba and Brazil received an important PAHO award for their participation in the Mais Médicos program in Brazil, where Cubans have also played an important part. Can you tell me a bit more about this collaboration?**

Cristian Morales: We'd have to start by saying that Mais Médicos (More Doctors) is the largest project that PAHO has been involved in during the last few years, perhaps in its entire history. This is true not only because of the number of human and financial resources involved, but also because of its impact in the Brazilian population. In Mais Médicos, which began in 2013, PAHO has played a facilitating role between Cuba and Brazil, which decided to collaborate to provide health care for people who had never had access before. In its first phase, the program mobilized over 11,500 Cuban health professionals, who went to Brazil's most vulnerable municipalities. That is, they didn't go just anywhere but rather to places that had a level of vulnerability that was much worse, weaker than the rest of Brazil's municipalities. Thus, in this first stage, some 65 million people received health services, many who had never seen a doctor before. So you get a sense of the magnitude of the program. Of the 65 million, it's estimated that 40 to 45 million were cared for by Cuban doctors, because Mais Médicos also involves health professionals from Brazil and other countries.

The program revolves around three pivotal points: a main one is urgent care, which doesn't mean in a hospital emergency room, but rather the urgent need to get essential services to an entire underserved population. And this is where Mais Médicos–Cuba–Brazil–PAHO is inserted, in which we have the potential to recruit Brazilians in the first round, and then if vacancies still exist, appeal to doctors from other countries who are licensed to practice in Brazil. And when those vacancies aren't filled, we then have the possibility of recruiting Cuban physicians. So in addition to 11,500 Cubans, the first phase involved several thousand more from Brazil and other countries reaching a total of 18,000 doctors. This was the "emergency" or urgent care part of the program.

The second pivotal point of Mais Médicos has to do with expanding primary health care infrastructure in Brazil's universal health system. This means construction of more

health care centers. The third pivotal point, very important, is increasing the number of Brazilian physicians in these areas, because the intent of a program like Mais Médicos is that it be a transition, until the country has enough of its own doctors per inhabitant to provide the services required by all Brazilians. So the success of Mais Médicos will come when it ends with Brazil being able to provide quality health services to its entire population, services being provided now in part by Cuban doctors.


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This is a very complex program, whose success is reflected in very clear indicators. For example, patient surveys reveal satisfaction at over 85% in terms of the quality of care offered by Cuban physicians. And we're talking about physicians who, before going to Brazil, were not familiar with Brazilian culture, didn't know Portuguese or how the Brazilian health system worked. Nevertheless, they have managed to successfully integrate into that system.

There are zones in Brazil covered 100% by Cuban physicians, in particular indigenous areas. Every one of the indigenous districts today is served exclusively by Cuban doctors. This is important, because these are services provided in the most remote areas, where Cuban physicians sometimes have to travel several days to get to a village, or a place that connects to the rest of the world. This is a measure of tremendous dedication, of sacrifice, and represents part of the contribution they're making.

It's also important to mention that these Cuban doctors are literally inserted into Brazil's health system, into teams that work at the primary care level. This is enriching, because insertion in another system can result in new learning for professional and clinical practice back home and may also contribute to addressing some of the challenges in terms of efficiency that Cuba still faces. So for us, this sharing of experiences is part of the success of Mais Médicos.

The final element worth noting about Mais Médicos is that learning is formalized. That is, collaborators return to Cuba with postgraduate diplomas in primary health care. So they aren't only acquiring new learning and experience in daily practice, but also in a formal teaching environment to further consolidate their technical capacities.

Today, we're in the second phase of Mais Médicos, 2016 to 2019. In this period, there has been an important political shift in the Brazilian government. Nevertheless, the program not only continued but was consolidated, and goes forward despite political differences. And I think this is one of PAHO's merits, putting the health of people at everyone's center of attention, of concern, and one of the reasons why our office and Brazil's received PAHO's 2017 prize for outstanding team, awarded in 2018. We were able to fulfill, to concretize, PAHO's mission of facilitating collaboration among member states to achieve the highest levels of health for whole populations.

MEDICC Review: **Another element of Mais Médicos that seems interesting is that it provides financial support not only to the Cuban physicians involved, but also to Cuba's health system, since the salaries are divided between the two.**

Cristian Morales: Yes, correct. I think it's worth noting that Brazil's is considered one of the emerging economies, one of the 20 most important of the world's economies. It is part of the BRICS group, along with China, Russia, South Africa and India. Thus, in this context, they receive cooperation in a project like Mais Médicos, in which they also provide compensation for Cuba's technical assistance. And this is certainly interesting, because it allows Cuba to earn the hard currency so important to ensure the functioning of its own universal health care system.

MEDICC Review: **Now that we're speaking about solidarity in various directions, it seems we should talk a bit about Havana's Latin American School of Medicine, the training of international medical students by Cuba. What are your thoughts about this education and the possibilities for graduates to insert themselves in health systems, as the world aims for universal health?**

Cristian Morales: The Latin American School of Medicine (ELAM) is another of the Cuban health system's great expressions of solidarity. Nearly 30,000 doctors have graduated from the school. When you go to ELAM and talk with

students there, you begin to understand the real significance of what that means. I think very little is known about ELAM, which in 2019 will be 20 years old. It seems to me that we should further disseminate what ELAM has done, training so many thousands of physicians, first for Central America, then for Venezuela, all of South America, and finally we have ELAM graduates nearly the world over.

These 30,000 have been educated in the competencies needed to practice medicine, but also to practice a different kind of medicine, very different from that taught in most countries of the Americas. What do I mean? First and above all, they are taught to approach medicine from the perspective of primary health care, which means they are doctors concerned mainly with people, people in their environment, that is, their families, their communities. This is the foundation, not only of ELAM, but of all Cuban medical education. This is what distinguishes it and allows Cuba to prepare professionals beyond the technical aspects . . . and the technical aspects are important. But they're able to instill different values in their graduates, interacting with the community, potentially becoming the true agents of change needed by the countries of the Americas.

In 2014, PAHO's member states passed a transcendental resolution that today we call the resolution on universal health, including both universal access and universal coverage. And that resolution proposes four essential lines of strategic action to transform our health systems, to be able to reach the main objective, which is to resolve people's health problems, and above all, to preserve their health.

The first of these strategies is to change the model of care, from the hospital-centered one prevalent in most countries, which concentrates on diseases and privileges specialization and super-specialization. It's not that such specialization is bad, since specialists and subspecialists are needed in hospitals, but health systems need to be centered on people, their families and communities. And health services need to be organized starting there, not the other way around.

That is, when we say that this is a pivotal point, we're being very concrete: appropriate human resources—in number, kind and quality—constitute ELAM's contribution. And not only ELAM's, but the contribution of Cuban medical education in general: physicians and health professionals concerned with preserving health, with looking at people, at the environment where these people evolve, in order to understand better the characteristics of their families and have an impact on the social determinants of health.

MEDICC Review: **I know you prefer not to talk about yourself, but I'd like to ask you what you think is the most important contribution you made while in Cuba, and what do you take with you from Cuba, both professionally and personally?**

Cristian Morales: The contribution is perhaps easier for others to judge than for me. There have been important milestones that I've had the privilege to accompany in these three years, which give me great personal and professional satisfaction. And I hope that others see them that way, too. Undoubtedly important for me was being able to participate as PAHO in bringing the US and Cuba closer together in favor of better health, two countries that had not formally spoken for some time . . . although dialogue was always the practice in the spheres of health and science. At the beginning of my term, this was very important.

I recall the regional meeting on arboviruses held in Havana in October 2016, in midst of the Zika epidemic. Here, for the first time, a US secretary of health came to Cuba and we were able to work together on a series of actions—not only organizing the meeting itself with Cuba, but also at the highest level with our PAHO Director. Being able to establish a dialogue that, apart from any other consideration, concentrated on what was most important: people's health. I think that was a key moment.

The other was the Cuba Salud 2018 convention, organized by Cuba's Ministry of Public Health (MINSAP), which we supported every way we could. There, we had the privilege of participation by WHO's Director General and PAHO's Director, as well as thousands of attendees, including over 60 health ministers from the Americas, Africa, Asia and Europe, plus 90 high-level leaders, a once-in-a-lifetime experience. And this also represents a constant in our work, integrated as it was with MINSAP here. That's the greatest satisfaction I take with me from Cuba on the professional side: having achieved a high level of working relations with the ministry that corresponds with PAHO's mandate of collaboration, and thus being able to carry forward that mandate during these three years.

On the personal side, I've learned about everything. I've learned much, very much, every day and through the last day I'm here, I'll continue learning from each of the conversations I've had with the great figures of Cuban public health, with current leaders in Cuban public health, from the times I've been able to talk with students, workers, all kinds of people visiting the periodic health fairs. I've gained something from each one, and I think I leave with a very, very positive balance for myself personally, and with the satisfaction that I've given the job the very best I could. And I have had a great team here at the PAHO offices that has proven its mettle facing the challenges, willing to do what was needed even after hours, on weekends, and I think together we have learned much and achieved all this that gives me a great sense of tranquility. You always think you could have done more and better, and undoubtedly that's true, but I leave knowing I did my best, and I think we accomplished a great deal.

MEDICC Review: **Any special memory you'll take with you to Mexico and beyond?**

Cristian Morales: Yes, it was when, jointly with the MINSAP, we recognized those physicians who graduated before [the revolution of] 1959. They were the ones who laid the foundation for the health system we know today. The privilege of meeting them, getting to know those professionals, now quite elderly, was something extraordinary that will go with me wherever I am, the kind of thing that leaves an indelible impression and accompanies you always.

What Happened to the US Diplomats in Havana?
Mitchell Valdés MD PhD
Director, Cuban Neuroscience Center

Gail A. Reed MS

He was born in Chicago, Illinois, USA, but his family is Cuban. After 1959, they returned to the island, where Dr Mitchell Valdés received his medical degree at the University of Havana in 1972. He went on to study clinical neurophysiology, earning his PhD with a dissertation on the auditory system's sensory physiology. When the Neuroscience Center opened (as part of western Havana's Scientific Pole). he became its director, a post he holds today. Dr Valdés, a Distinguished Member of the Cuban Academy of Sciences, is widely published and has collaborated with colleagues in dozens of countries, including the USA, UK, Italy and Holland. He is a full professor of clinical neurophysiology, sits on Cuba's National Coordinating Group for Persons with Disabilities, and serves as an honorary professor at the University of Illinois at Chicago. What brought me to his office is the set of symptoms reported by some two dozen US diplomats in Cuba and more recently in China as well. And the controversy surrounding what might be the root cause—a topic that has crossed the line from medicine into politics. ***MEDICC Review***'s intent was to hear from Dr Valdés on the science pertinent to the controversy.

***MEDICC Review*: Can you walk me through the process in Cuba: when did you find out about these symptoms, what did Cuban authorities do, and how have you been involved in trying to get to the bottom of the problem?**

Mitchell Valdés: Cuba assembled a scientific team to study the problem from the first moment. At the beginning, it particularly involved specialists in hearing and ear, nose and throat (ENT) disorders, because some people reportedly complained of hearing strange sounds and feeling pain in the ear. So initially, it seemed to be a hearing problem. But, as the reports started coming in, apparently other disorders were involved. So immediately the Ministry of Foreign Relations asked the Cuban Ministry of Public Health and our Academy of Sciences to contribute with specialists in various fields to study the problem.

One of the handicaps we've had in this work is the very limited amount of hard data. There have been very few reports on the complaints: initially, a one-page summary of some of the cases, a general description of symptoms. But we've seen no lab tests, no images, no results of audiograms, for example. So the information has been quite limited.

In any case, what the Cuban team has done is first to examine possible explanations, looking at the scientific literature and at the little information provided; and second, to study persons in the environment, because if there was some sort of harmful event, some agent that was damaging people, then logically it could have spilled over and affected people working in the same environments around the US diplomats' homes or the hotels.

And to our surprise, there has been continuous speculation in the press about different hypotheses and theories, but very few facts. Actually, more information has been handled in the press than through the normal scientific channels. Usually scientific discussions are direct, person-to person—there's a possibility to see patients, to see the medical records. But all this was limited.

Then, suddenly early this year, a report appeared in the *Journal of the American Medical Association* (*JAMA*) that purported to describe a new syndrome. When this article was published, we carefully studied all the details it presented. Since publication, the report has been debated, criticized and not found acceptance in the international scientific community.

***MEDICC Review:* I know in the early days there were many people interviewed by the Cuban specialists. Can you give me a sense, first of all, what kind of specialists composed the Cuban team? How many people were interviewed? And then, later, what was the role of the FBI when its people came to Cuba?**

Mitchell Valdés: I've heard that the FBI has come to Cuba six times and that essentially they found no evidence of an "attack," no evidence of any kind of weapon, or any kind of intentional action directed to harm the diplomats.

But, I can speak more directly to the role of the Cuban medical team. First, as I said, our ENT specialists were involved. Then, we immediately involved neurologists, epidemiologists, people working in environmental health, specialists in acoustics (for example, measurements of harmful sound levels in the environment), physicists, neurophysiologists like myself, and internists. So, many medical fields were tapped, some 20 to 30 specialists.

Several hundred people were interviewed: all the neighbors around the diplomats' homes, all employees at the hotels where diplomats were lodged. And the essential result of the clinical examinations of Cuban controls was negative. That is, there was no increased prevalence of any of the symptoms described by the diplomats in the environment around the diplomats' living quarters. Some people with hearing loss were found, but they had hearing loss of long duration, so these were preexisting conditions. Nothing was found that would indicate a spillover of some noxious agent that was harming the diplomats. Nothing, in any of the several hundred interviewed.

Since sound was mentioned from the start, it is worth noting that it is common scientific knowledge that, for sound to produce damage, it has to be very high intensity; it has to be above 80 or 90 decibels. This would have produced a sound heard by many people. And, among all the people, the witnesses, the controls that were examined, nobody reported hearing such a sound. So, this precluded the possibility of loud sounds causing some sort of damage in the hearing or brains of the US diplomats.

***MEDICC Review:* What about the other symptoms reported by the diplomats? And the conclusion of brain injury?**

Mitchell Valdés: If you look carefully at what has been provided as evidence, which is the information in *JAMA*, the first obvious thing is that there is no real evidence for brain damage or injury. Yet, this is something that has been repeated continuously and you see in all the news reports, and so everybody builds on this. Even some people have started to do research on possible physical agents, based on the apparent "fact" that there is damage to the brain.

But this is a very flimsy construction, because it's all built on the idea that there has been brain damage in a large group, 21 of the US diplomats and their families included in the study. The evidence for brain injury just isn't there. In the *JAMA* paper, you first see that the neuroimaging studies were negative. Second, you find erroneous interpretation of neuropsychological tests. For example, according to their tests, there were claims of cognitive deficits, as well as memory, attention and concentration problems. But the thresholds selected by the authors were unusual, and so lenient that if you applied the same criteria to any sample of normal subjects, all oif them would be ill according to some of the tests.

This has been discussed thoroughly by scientists in the UK, the US and elsewhere. So we are now seeing publications severely criticizing the criteria used for these neuropsychological tests. If you discount the neuropsychological results, which are largely negative—perhaps one or two cases do have neuropsychological findings that indicate some sort of abnormality, yet with negative neuroimages—then you have no evidence that this group of 21 subjects has brain injury, which is what was asserted in the *JAMA* paper and has been repeated by the media, quite irresponsibly I think.

Now, we don't say that some of the patients, the diplomats, aren't ill. We're saying that there is no evidence for brain injury and there is very limited evidence for hearing loss. In fact, if you look carefully at the data provided, there are only three cases with audiograms (hearing tests) that show a loss that can be considered pathological. But the interesting thing is that each of the hearing-loss curves is different. Some showed loss at very high frequencies, something that commonly occurs with aging. Another case had loss concentrated at certain frequencies, typical of acoustic trauma. And another had hearing loss at very low frequencies, consistent with many conditions, such as Meniere's disease, for example—completely different pathologies. This also speaks against the idea that there's a common agent of harm, because it would be impossible with a common agent to produce such different profiles of hearing loss.

So, there is simply no evidence for the State Department's argument from day one that there has been an "attack," and that such an attack produced similar effects of brain injury and hearing loss in all the cases. There is no physical agent that could produce brain injury or hearing loss under the conditions in which it is alleged that the symptoms happened. Nor is there evidence of brain injury or hearing loss in the whole group, as has been repeatedly suggested.

***MEDICC Review:* What about the psychological component in the diplomats' symptoms? Should this be considered?**

Mitchell Valdés: Many of the complaints reported—such as dizziness, headaches and sleep disorders—are very frequent in the general population and could be due to functional disorders and stress. I imagine in any embassy in the world—let's say a Cuban embassy—if the government said to its employees, "You guys are under attack. Somebody's suffering brain damage because of a mysterious agent," the result would be severe stress. Everybody would be stressed, anxious and really freaked out. And this could lead to headaches, sleep disorders and many other symptoms. And if someone had a functional disorder or preexisting condition, this situation would amplify their symptoms. People would start searching and find these symptoms and amplify them in their minds.

It's interesting to see that for the first time, some researchers who have been studying these diplomats have publicly recognized that there could be a psychological ripple effect. It's not the University of Pennsylvania group that wrote the

JAMA article, but rather people from the University of Miami and the University of Pittsburgh, a second team. Here in Cuba, we've not said that this is all psychological, we're not saying that no one is sick. Certainly, some could be sick. But, we're saying that many of the people reporting symptoms could be also suffering from psychological amplification, because they were informed that they were under attack. And, of course, many other symptoms could be due to preexisting conditions, because we have no evidence from medical records of what their health status was before.

MEDICC Review: **Returning to the *JAMA* article, can you speak about reactions to it internationally?**

Mitchell Valdés: The first reaction was in *JAMA* itself: you'll see that the paper is accompanied by an editorial by two *JAMA* editors, Drs [Christopher] Muth and [Steven] Lewis. They make a long list of criticisms pointing out flaws, severe flaws, in the paper and urge readers to interpret the findings with caution, because the data do not support the paper's conclusions. This is very unusual, because normally, when you send an article to a high-impact journal and there are so many flaws, the journal doesn't publish it; they reject it. But in this case, they accepted it, along with this cautionary note that really invalidates the conclusions of the paper. Later, the paper was criticized in letters to the editors, and the authors' responses were quite unsatisfactory.

The letters criticizing the paper came from the world over. *JAMA* said that four out of a large group of letters were published, so I think they were flooded with letters finding flaws in the study. For example, Dr [Robert] Bartholomew of New Zealand, who has analyzed the psychological aspect, was critical of dismissing any psychological contribution, because in the *JAMA* article and in the responses, the authors initially stated that the diplomats were not malingering*;* that they were not faking. But, that's not what would be involved if there were psychological functional disorders involved. It's not people malingering; it's people who really feel ill. And in fact, if you look at the Handbook of Neurology, there's a whole volume dedicated to functional disorders. The people really feel ill, and, in fact, there's evidence of abnormalities in the brain's electrical activity. But the cause is not what they think. And the best way to cure them would not be to tell them that they have been attacked with something mysterious or that they have a mysterious new disease; it's to discuss with them the real science as part of treatment.

Criticism also came from a group that works in one of the US Veterans Administration hospitals, who considered that interpretation of some of the tests was flawed. They had problems with the balance disorder tests and eye movement tests, noting that functional explanations that are not neurological diseases were not considered. Other people criticized the thresholds for the neuropsychological tests, which I mentioned earlier.

Now we see more critiques, such as that of Professor Sergio Della Sala, head of the Cognitive Neuropsychology Department at the University of Edinburgh, in the *Journal of Neurology*. He also edits *Cortex,* whose editorial board in full was consulted, and as a result has published their dissatisfaction with the *JAMA* paper and the authors' responses to criticisms, saying they believe that either an erratum should be published or the article should be retracted.

MEDICC Review: **As you mentioned, the US State Department has continued to charge that the symptoms suffered by their diplomats in Havana were the result of some kind of attack. Is this a possibility, scientifically speaking?**

Mitchell Valdés: There is no evidence to support that charge. Take the idea of some acoustic weapon for example: it would be very difficult for audible sound to produce hearing loss, much less brain damage. There is no report in the literature, whatsoever, of any case of brain damage due to sound. Joe Pompei, a retired psychoacoustics expert from MIT, said that to produce brain damage with sound, you'd have to put someone's head in a swimming pool and fill it with powerful ultrasound transducers. It's just not plausible.

Then, consider the types of sound: ultrasound dissipates with distance. Yes, you can use ultrasound to damage tissues such as tumors in the brain; but, you have to place the transducers directly on the head. It's not possible to produce damage with ultrasound from a distance, because it dissipates rapidly with distance. In the case of infrasound, you can't focus it directionally because it has a very long wave length. So a weapon based on infrasound wouldn't explain why some people in the same room were affected by symptoms and others weren't. The reported cases of possible health effects from infrasound have been, for example, related to agricultural equipment, big harvesters. So, this again is also implausible.

Then, some people started floating the idea of microwaves, even reported in *The New York Times* like a very big thing. But, immediately experts from the US side, people such as Dr Ken Foster from the University of Pennsylvania (independent of the group that published in *JAMA*) rejected the notion. Dr Foster, who has studied the subject extensively for years, said it is impossible to use microwaves to injure the brain without first injuring skin, muscles and bones.

So, when you look at all the potential alleged weapons that could have been employed, none of them are possible according to the laws of physics and principles of engineering. And on the other hand, you have no evidence for brain injury and for hearing loss in a large group of subjects; so, the whole case collapses. It's simply a construction that I think has spiraled out of control, based on theories that have been accepted as facts and then these pseudo facts are used to construct other theories . . . none of which are scientifically sustainable or acceptable. Putting it all together, it's very difficult to accept the so-called "explanation" put forward by the US State Department that there's been an attack on their diplomats.

MEDICC Review: **Is there any progress in sight, in terms of finding a credible scientific explanation for what happened to these diplomats?**

Mitchell Valdés: Recently, a Cuban delegation visited the USA at the State Department's invitation. I think that was a very small step forward, a positive step because it was the first direct engagement. But unfortunately, the people directly

studying the patients, those from the University of Pennsylvania responsible for the clinical study and the *JAMA* paper, were not at the meeting that the Cuban delegation held with group of medical officials from the State Department. And what was discussed was essentially the *JAMA* paper, which we already had reviewed. So, we didn't come out of that meeting with any new information. Yet, it was positive in that we were able to state our concerns that the conclusions of the paper were not really validated by the data, and that there was no evidence for brain injury.

Of course, this was far from the normal scientific process—that is, aside from the *JAMA* article, we came to the table essentially informed of supposed medical results, alleged medical results, mainly through leaks to the media, which either came from the State Department or the University of Pennsylvania research group. The normal thing would have been to sit down with the researchers for a serious scientific discussion. And this is what we've been asking for from day one.

MEDICC Review: **So, the Cuban scientists have not had access to these patients or their clinical records?**

Mitchell Valdés: No. The only information we have is from the *JAMA* paper. The US side has given two explanations for why they are not giving us access to the clinical records, two arguments. One is that they're protecting patient privacy. This is something we respect, of course, but there are ways of carrying out scientific discussions where you protect subjects' identities. If this weren't so, it wouldn't be possible to carry out clinical trials, or collaborative research. As we speak, thousands of neuroimages are being exchanged around the world, where all identifying patient information has been erased. So, although privacy has been an argument, we do not think it is an insurmountable obstacle. The second argument, which I've seen in the press, is that sharing detailed data would give feedback to the perpetrators, the people who designed the alleged weapons. But this is absurd. Because if there's any power in the world so evil that it is capable of designing such an advanced weapon, they would already have had enough subjects on which to test it. They would not need feedback from what happened to these diplomats.

I think the information could be shared and that we could really get to the bottom of the problem. The first thing needed is a case definition. There is actually past experience where we did this with US researchers, when Cuba had an epidemic of peripheral and optic neuropathy. It wasn't kept secret. We asked for international collaboration and the USA contributed by sending the CDC. So we collaborated with people in the CDC; we shared information.

We could have gone paranoid and said: "Oh, this is some kind of poisoning or toxic event sent by the US to Cuba." But our ideas, our actions, were the opposite: we said, "let's collaborate," and we had a very positive experience working with CDC doctors. The first thing they told us was, "Let's make a case definition," because right away, there are people, because of psychological contagion, who feel symptoms and want to be included or feel they're included. This muddies the waters, because the first thing you have to do is separate real cases from cases that are not part of whatever you think is an outbreak.

We could have done the same thing here. And we should have worked together for a case definition. And maybe, as a result of careful study, we could have excluded some far simpler explanations than trying to find a mysterious weapon that, I think, no agency in the world, no defense agency, knows about. Because, there's no evidence in the literature of any kind of weapon that can do this kind of thing.

We also need the clinical histories to develop more evidence-based hypotheses. For example, did any of the diplomats have previous experience with blasts nearby, with explosions? If someone had military service, were they near explosives? Did any of them carry out shooting practice? Because it's well known that a shot from a gun or a rifle nearby can produce acoustic trauma, and this produces hearing loss, tinnitus, discomfort, pain. Did any of the diplomats practice any contact sports, like football, soccer or judo? I don't know. Any of these sports can produce mild brain injury or balance disorders. Did any of them have hypertension? Did any of them have any other conditions?

But I think that the fact of treating it as an attack, and the fact of informing officially that they had to be evacuated, as if from a war zone, is something that could have amplified any disorder they already had, any discomfort they had, any symptoms they had. And that completely confuses the whole issue. Many things can muddy the waters, even here in Cuba. Recently,

for example, we had a case that was investigated by police. A person was reporting strange sounds. Our police and the FBI went to the house, and the sound turned out to be coming from a water pump next door. And I have a personal experience with a US citizen who came to us and said that there were noises in their apartment.

This person was so afraid, they couldn't sleep in the apartment, had to go someplace else. We really appreciated that this person had the confidence to come to us and ask us to investigate. When we went to the apartment there was nothing abnormal, except a sound that came from some lights in the street; there was a buzzing. The environment is full of sounds you usually ignore by filtering them out. But, if you are informed by your government that you're under attack, you may start tuning in any strange sound you hear, because you're now anxious, afraid, worried. This could be part of the answer. Yet, to be certain, we need more information sharing.

MEDICC Review: **In the *JAMA* article it said that the mean time between symptom onset and examination by the University of Pennsylvania team was 203 days. Is it now too late to go back and do some of these case studies? Is it too late for real collaboration to get to the bottom of it?**

Mitchell Valdés: I don't think it's too late, because the claim is that there's permanent damage. That's what you read in the article and in the press, noting that some people will have to undergo a long period of rehabilitation. That means permanent, more or less permanent damage. So, I think that if that's true—which I doubt, and in fact, we were told by the State Department that many of the diplomats are already working again—but if that's true, then the evidence would still be there. But, it would be in the medical information.

At the same time, the fact that people were studied so long after the alleged events leads to all sorts of problems with recall, because the reports are not reliable, and they are necessarily influenced. Memory is an active process, you're continuously updating your memories and they are influenced by things that happen after or information that comes in after. In this case, there's been a bombardment of information in the press and discussions among the diplomats themselves that has to influence recollection.

Unfortunately, US diplomats would usually go to Cuban hospitals for many of their complaints. And the fact that they didn't go to any Cuban doctor makes it very difficult for us to assess firsthand information on what was happening. This was, let's say, a departure from what was normal up to that time, consulting Cuban doctors for their common health problems.

MEDICC Review: **So, speaking as a scientist then, would you say that you don't accept as a fact that there was an attack. And in any case, what would be your proposal on how to proceed?**

Mitchell Valdés: We have to start with the suggestion that these individuals are all suffering from a medical disorder that can be attributed to something that happened in a certain period of time in Havana. And from what has been stated in the *JAMA* article and in other sources, this would be brain injury and damage to the inner ear. Yet, once again, there is no evidence for that: we have not seen evidence that a large group of subjects suddenly suffered brain injury or injury to the inner ear in Havana in a specific period of time. I repeat, this is consistent with perhaps one or two people feeling ill for whatever reason, and then perhaps a process of psychological contagion; an amplification of functional disorders due to stress, anxiety; first, because they are in a foreign country, where the relations between their country and Cuba had not been cordial for many years, to say the least. And second, they were informed that in Cuba, where diplomatic relations had only recently been renewed, they were under attack. So, this would create all the conditions for psychological contagion, of anxiety, of functional disorders, of stress.

In any case, we don't accept that there is group of people that were injured because of an attack. There's no evidence for this. We don't accept it, because we have not been convinced. We have no preconception, but simply as a scientist, you sit down, look at the evidence, and it's not there.

The way forward would be to collaborate, and perhaps to ask other people to participate. We discussed this in Washington with the National Academies of Science and with the American Association for the Advancement of Science. They have the necessary specialists. I also think the NIH should be involved; they have very good scientists who could collaborate. And perhaps, people from other countries could participate in a scientific discussion, to hear many opinions. I would really like to see science proceed as it normally does: if somebody has findings, they usually discuss them in scientific meetings even before they publish, and certainly before they go to the media.

Yet here, we have seen the opposite. They go to the press first, because there have been leaks from the University of Pennsylvania group and from the State Department from the beginning. Then they published without any previous scientific discussion or debate. So it's been the complete opposite of the normal scientific process. I'm sure that we would have gotten to the bottom of this problem if we had followed the normal scientific process.

MEDICC Review: **Because even the hypothesis that you put forward that a few people may have been affected, we don't know by what, and then there was a psychological ripple effect. Even that's a hypothesis…**

Mitchell Valdés: It's a hypothesis, yes, and we say it can't be excluded. And in fact the arguments that have been made that there are no psychological effects are absurd. They're very flimsy. I mean, in science, sometimes you have something called a confirmation bias. You have a hypothesis and when you look at all the facts, you're not completely objective. And, the first things that fit with your preconceived theory are the ones you use more and the rest you sort of brush under the rug. Peer review and open scientific discussion are precisely designed to avoid confirmation bias. That's why any scientific result does not rest only on what's affirmed by the group that's proposing it. It has to be submitted to the scrutiny of the international community.

And in this case, there was a theory from the start: that there were attacks. And then everything that we've seen published and the leaks to the media, all are based on this unconfirmed idea. Other hypotheses are not considered. So, I think this whole thing is messed up due to the failure to apply the scientific process.

Of course, and this is my interpretation, if somebody has a political agenda and wants to take advantage of a situation, then that person doesn't wait for the facts. And I think some politicians involved in this obviously have their agenda and are not interested in whether what you're saying is scientifically sound. They don't care. They just use it. But in the long term, this is going to blow up in their faces. Because, if it is not sustained by scientific facts, it will fall apart, the whole construction, the whole theory, the whole thing will fall apart.

MEDICC Review: **Have you had personal experience with US scientists who say they're willing to collaborate with you?**

Mitchell Valdés: Yes, there are many who are interested. In fact, we've written a letter to *Nature*, signed by a large group of Americans and scientists from around the world. And then we wrote a letter to *The Guardian*, signed by a list of scientists who are concerned about the way this has been handled, both on the political side and on the medical side.

What's more, there are many scientists who have spoken to us, people from the NIH, who have told me that they're muzzled; they can't speak, because they've been told that it's not government policy to allow them to speak, but they have strong opinions on this problem. People from the universities and serious scientists all over the US are willing to collaborate. And as scientists, they have no preconceived hypothesis. And I say, I'm willing to accept any hypothesis if the evidence is good. But the problem is that everything that we've heard up to now is not sound from a scientific point of view: neither the medical claims nor the claims about the supposed weapons. They are simply not valid.

MEDICC Review: **So, collaboration is the only way forward?**

Mitchell Valdés: Yes, collaboration is necessary and we're very willing to do so, to collaborate. And we would expect the same thing we did when Cuba had a serious medical problem and opened up its medical records. People from the US came, from the CDC, and examined the patients. We examined them together and we were willing to clarify a problem, which was a very serious problem for Cuba. And it was international collaboration that opened the way to finding the solution. We think that kind of collaboration can and should be repeated in this case.

Estimating Normal Values of Rare T-Lymphocyte Populations in Peripheral Blood of Healthy Cuban Adults

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ABSTRACT

INTRODUCTION Flow cytometry allows immunophenotypic characterization of important lymphocyte subpopulations for diagnosis of diseases such as cancer, autoimmune diseases, immunodeficiencies and some infections. Normal values of rare lymphoid cells in blood, quantified by cytometry, vary among different populations; so it is indispensable to obtain normal national values that can be used in clinical practice.

OBJECTIVE Characterize distribution of rare T-lymphocyte populations in peripheral blood, specifically double-positive T, natural killer T and activated T lymphocytes, as well as their relationship to sex and age.

METHODS A cross-sectional study was carried out in 129 adults (68 women, 61 men) aged >18 years, without chronic diseases or unhealthy habits, who signed informed consent. Peripheral blood was collected for immunophenotyping of lymphocyte subpopulations with monoclonal antibodies specific for $CD4^+CD8^+$ double-positive T cells, $CD3^+CD56^+$ natural killer T cells, and $CD3^+CD25^+HLA\text{-}DR^+$ activated T cells. An eight-color flow cytometer (Beckman Coulter Gallios) was used. The analytic strategy was modified, associating variables of interest in a single graphic, using conventional monoclonal labeling antibodies. Medians and minimum and maximum percentiles (2.5 and 97.5, respectively) were used as descriptive statistics, stratified by sex, for cell counts and percentages. A linear regression model was applied to assess age effects and a two-tailed Mann-Whitney *U* test for independent samples was used to assess sex differences. The significance threshold was set as $p \leq 0.05$.

RESULTS Median percentages of total lymphocytes: natural killer T cells 6.3% (1.4%–23%) in men and 4.7% (0.8%–11.3%) in women ($p = 0.003$); activated T cells 1.0% (0.2%–2.2%) in men and 1.2% (0.4%–3.1%) in women, without statistical significance; and double positives 0.8% (0.1%–4.2%) in men and 0.9% (0.3–5.1) in women, also without statistical significance. Median cell counts (cells/mL) were: natural killer T cells, 126 (27–580) in men and 105 (20–279) in women ($p = 0.023$); activated T cells: 20 (4–46) in men and 25 (7–75) in women, ($p = 0.013$) and double-positive T cells: 17 (2–85) in men and 21 (7–154) in women, without statistical significance. Sex influenced natural killer T cells, but age did not.

CONCLUSIONS Age does not affect counts and percentages of rare T lymphocyte subpopulations in the blood of healthy Cuban adults. Sex differences found for some phenotypes suggest the need for different reference values for women and men.

KEYWORDS Normal values, T-lymphocyte subpopulations, flow cytometry, Cuba

INTRODUCTION

Flow cytometry (FC) is a useful technology for analytical and quantitative characterization of cells, because it offers rapid, simultaneous and complete information on distinctive cell aspects. Thus, it has become an essential tool in diagnosis, monitoring and management of numerous diseases, such as immunodeficiencies, infections, autoimmune diseases, cancer and others with immunopathogenic components. Because of its high sensitivity, it is the gold standard for assessing leukemias and natural killer (NK)/T-cell lymphoma, particularly in special biological samples such as cerebrospinal fluid. Its use has extended to other areas of medicine such as post-transplant care.[1–8]

FC has enabled discovery of different lymphocyte populations and shown their great heterogeneity. Previously, the classic lymphocyte populations reported were NK B and T lymphocytes. The latter have two types, TCD4+ and TCD4+8, which once were considered unique and mutually exclusive because of limitations in early cytometry technology that did not allow more than two or three markers on the same cell.[2] We now know that several cell subpopulations form each of these markers (CD4 and CD8). This shows the diversity of lymphocyte subpopulations, each with its own role in immunity; even if rare, they have important functions, so it is essential to consider them for correctly phenotyping peripheral lymphocytes.[6–15]

IMPORTANCE This study is an initial step towards the use of flow cytometry and conventional reagents to quantify rare but important lymphocytes in diagnosis and prognosis of adult Cuban patients with cancer, autoimmune diseases, HIV/AIDS and chronic infections.

The lymphoid cells of innate immunity are an example of recently discovered lymphocyte diversity.[9,10,16–24] These cells are rare in the blood (together <10% of circulating T lymphocytes) but are not necessarily in the minority in other compartments, in lymphoid tissues or elsewhere. They have important functions in maintenance of homeostasis and in immunopathogenesis of some diseases. They are quantified in counts and percentages of total lymphocytes by FC.

T lymphocytes are more heterogeneous than B lymphocytes and some subpopulations can be characterized with the same monoclonal antibodies used for conventional T, B and NK cell protocols, using a different reading window, which implies changing the analytic strategy and associating the variables of interest in a single graphic in the flow cytometer.

Thus far, normal values of these lymphocyte populations in healthy Cuban adults have not been established. Immunophenotyping of rare lymphocytes in peripheral blood extends analysis to more circulating variants, avoiding underreporting of cells with demonstrable clinical importance and not requiring other technologies or additional reagents for their characterization.

The study objective was to characterize the distribution of rare T-lymphocyte populations in peripheral blood and their relation to sex and age in healthy Cuban adults, with the purpose of eventually using them as reference values in diagnosis and prognosis of multiple diseases.

METHODS

Design, subjects and sample collection A cross-sectional study was carried out from January through April 2017 at the Hermanos Ameijeiras Clinical–Surgical Teaching Hospital. We included 129 apparently healthy adults, companions of patients who were seen at the immunology service of the Hermanos Ameijeiras Clinical–Surgical Teaching Hospital, and who provided written informed consent to participate. The sample consisted of 68 women and 61 men, aged 18–80 years (average 40 years). Exclusion criteria were habits, diseases and treatments that could structurally and functionally modify the immune system: toxic habits (smoking, alcohol consumption >40 g daily or its equivalent per week,[25] >4 cups of coffee a day); history of infections; use of antibiotics, immunosuppressants, immunostimulants, anti-inflammatories or anticoagulants in the previous 6 months; diabetes, immunodeficiencies, autoimmune or neoplastic diseases; (for women) pregnancy.

Blood was obtained by peripheral venipuncture; 4 mL were deposited in Vacutainer tubes using ethylene-deamine tetraacetic acid as anticoagulant. Samples were processed within six hours after extraction and the process followed good laboratory practice standards.

Flow cytometry A Beckman Coulter Gallios 8-color cytometer of (Beckman Coulter, France) was used; 100 µL of blood were dispensed for staining with fluorochrome-conjugated monoclonal antibodies from the same manufacturer (Beckman Coulter, France): anti-CD45 AA750 (Clone J33), anti-CD19 (Clone J3-119), anti-CD3 FITC (Clone UCHT1), anti-CD4 PC5.5 (Clone 13B8.2), anti-CD8 AA700 (Clone B9.11), anti-CD56 PE (Clone N901) (NKH-1), anti-HLA-DR PE (Clone Immu-357), anti-CD25 PC5 (Clone B1.49.9). Two panels were designed:

1. phenotype of double-positive mature subpopulations and NKT, and
2. phenotype of activated T cells.

To characterize the phenotype of T lymphocyte populations, immunophenotyping was done as follows:

1. mature double-positive alpha beta T cells ($CD45^+$, $CD3^+$, $CD4^+CD8^+$) and their subtypes—
   a. $CD45^+CD3^+CD4^{high}CD8^{low}$
   b. $CD45^+CD3^+CD4^{low}CD8^{high}$
2. NKT cells ($CD45^+CD3^+CD56^+$) and subtypes—
   a. $CD45^+CD3^+CD56^+CD4^+CD8^-$
   b. $CD45^+CD3^+CD56^+CD4^-CD8^+$
   c. $CD45^+CD3^+CD56^+CD4^-CD8^-$
3. activated T cells—
   a. $CD45^+CD3^+HLA\text{-}DR^+$
   b. $CD45^+CD3^+CD25^+$
   c. $CD45^+$ $CD3^+HLA\text{-}DR^+CD25^+$

Sample preparation was carried out according to manufacturer's specifications for cell surface immunophenotyping, using a protocol of unwashed red blood cells with Versalyse buffer (Beckman Coulter, France). Cytometer quality control was performed daily with Flow-Check fluorospheres (Beckman Coulter, France) to align lasers and check the water system. Fluorescence intensity was controlled with Flow-Set fluorospheres from the same company.

Acquisition data were processed using Kaluza Analysis Software V1.5A, with a minimum of 50,000 acquired events, which refers to the number of formed elements contained in a blood sample that pass through the cytometer's laser light beam and are counted and analyzed. In each case >4000 events were obtained in the lymphocyte region characterized by high expression of CD45 and low complexity as indicated by side scatter. Rare subpopulations were identified with modification of the analysis strategy to associate the variables of interest in a single graphic, permitting analysis of six monoclonal antibodies specific to six cell surface antigens at once, using a single 50-µL blood sample. This is illustrated in Figure 1, where each dot plot represents two parameters analyzed by the cytometer.

Bidimensional analyses were concatenated hierarchically to enable multiparametric analysis and characterization of several cell population phenotypes in a single tube. Thus, the first dot plot in Figure 1a reflects a window through which single cells passed through the laser beam for cell-by-cell analysis. Next a second dot plot was generated to relate cell size to complexity, distinguishing cells from artifacts or detritus. Once cell events were selected, a third dot plot was created that identified groups of leukocytes based on expression of a pan-leukocyte antigen, CD45. The lymphocyte population for analysis is circled at the bottom of the third dot plot. Then antigens representative of each strain were combined successively, from general to specific, each population displayed in a separate quadrant (Figure 1).

Absolute counts were obtained by double platform; results obtained were combined in an automatic hematological counter and by cytometry. The following formula was applied:

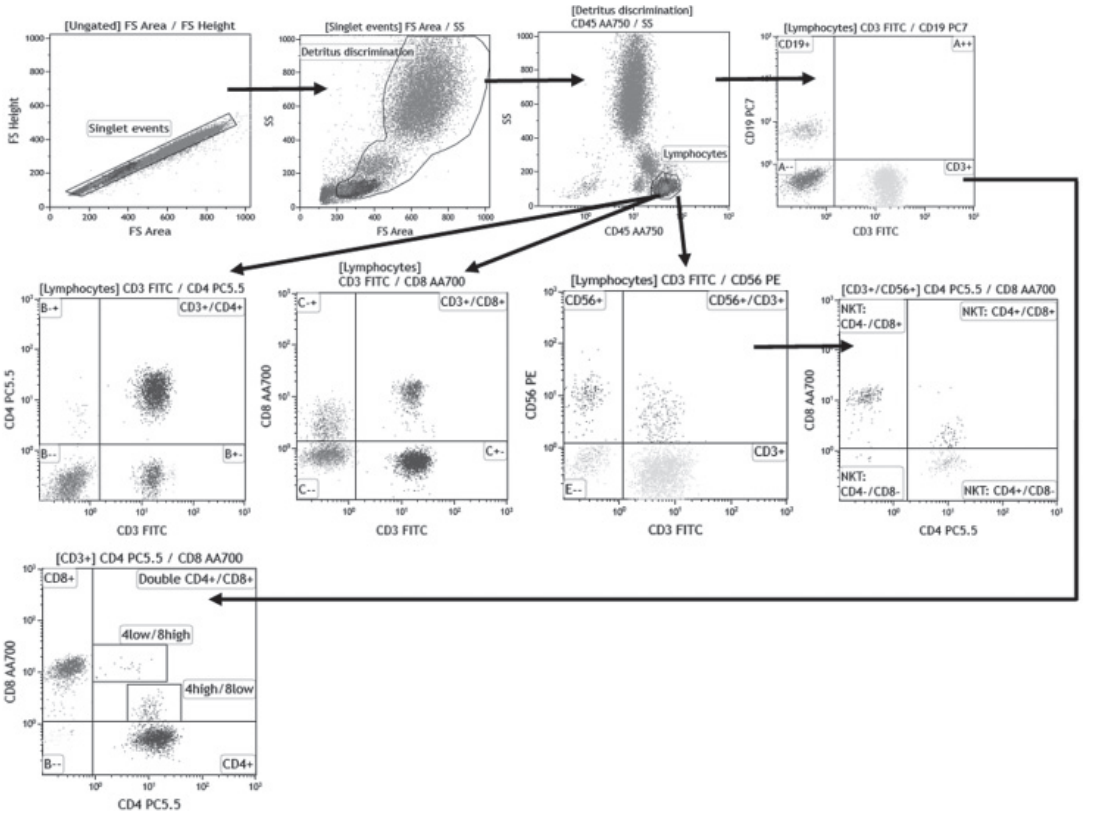
> Absolute count (cells/µL) = lymphocyte count (number of cells/µL in blood count) x % of the cellular subpopulation of interest ÷ 100.

Analysis Descriptive statistics were calculated: absolute and relative frequencies, means, medians and SD. The Kolmogorov–Smirnov test was used to assess normality of distribution of variable values. Because of variable asymmetry, percentiles 2.5 and 97.5 were specified as lower and upper limits, respectively, for reference intervals. A linear regression model was used to assess the effect of age and the two-tailed Mann–Whitney *U* test for independent samples to measure the effect of sex. The significance threshold was set at $p < 0.05$.

Ethics Study participants provided written informed consent per the Helsinki Declaration.[26] The consent document described the importance of participation and explained the study's characteristics and possible risks and benefits. All data were kept confidential and participant identity was delinked. The selection of diagnostic tools followed the ethical principles of maximum benefit and nonmaleficence.

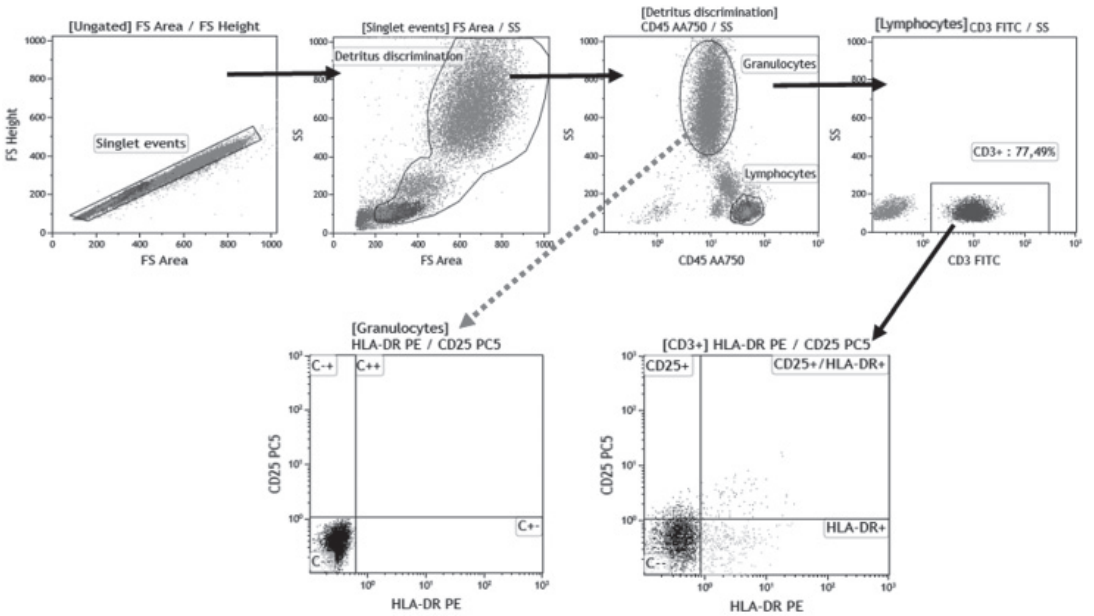
Figure 1: Reading window strategies

a. Design for panel 1 of T and NKT cell phenotypes[a]



[a]To identify double-positive populations, a CD4 vs. CD8 graphic from the $CD3^+$ region was added.

b. Design for activated T lymphocyte panel[b]



[b]The scatter plot marked with a dotted arrow (which comes from the granulocyte region) was used as a control to establish cutoff points for HLA-DR and CD25 tertiary antigens, since these cells do not express these antigens, which were cloned to obtain values for activated subpopulations in the $CD3^+$ region.

NKT: natural killer T

(available in color online at www.mediccreview.org/estimating-normal-values-of-rare-t-lymphocyte-populations-in-peripheral-blood-of-healthy-cuban-adults)

RESULTS

Age did not affect counts and percentages of the cell subpopulations studied; r^2 values were distant from unity and differences by age did not meet the specified significance level of $p < 0.05$. For some subpopulations, significant differences were found between men and women.

No significant sex differences were found in counts and percentages (of total lymphocytes) of double-positive alpha beta T lymphocytes subpopulations, except for the $CD3^+CD4^{low}CD8^{high}$ phenotype. Median percentages were less than one percent, but the median was one percentage point higher for women than for men. The upper range limit did not exceed 5.1% (Table 1).

Counts and percentages of NKT cells and their subtypes are summarized in Table 2. The $CD3^+CD56^+$ phenotype was influenced by sex, with both counts and percentages significantly higher in men ($p = 0.003$ and $p = 0.023$, respectively). Percentages of $CD3^+CD56^+CD8^+$ were also significantly higher in men. For all other subtypes, values were higher in men, but without statistical significance. Wider ranges in counts and percentages were found in this lymphocyte subpopulation than in other subpopulations studied, especially among men.

Counts and percentages of activated lymphocyte subpopulation phenotypes are summarized in Table 3. Modest sex differences were found in subtype medians, with statistical significance for $CD3^+$HLA DR^+CD25^+ ($p = 0.013$) counts, but not percentage of total lymphocytes (Table 3).

Table 1: Reference ranges for peripheral double-positive T lymphocytes in healthy Cuban adults (n = 129)

Phenotype	Men		Women		p Value[a]
	Median	Percentiles (2.5–97.5)	Median	Percentiles (2.5–97.5)	
$CD3^+CD4^+CD8^+$ (%)	0.8	0.1–4.2	0.9	0.3–5.1	0.173
$CD3^+CD4^+CD8^+$ (cells per µL)	17	2–85	21	7–154	0.080
$CD3^+CD4^{high}CD8^{low}$ (%)	0.3	0–3.8	0.4	0–4.7	0.312
$CD3^+CD4^{high}CD8^{low}$ (cells per µL)	7	0–77	10	0–142	0.218
$CD3^+CD4^{low}CD8^{high}$ (%)	0.2	0–1.9	0.3	0–2.5	0.044[b]
$CD3^+CD4^{low}CD8^{high}$ (cells per µL)	5	0–33	8	0–57	0.028[b]

[a] Mann–Whitney *U* test [b] significant

Table 2: Reference ranges for NKT lymphocytes and subtypes in healthy Cuban adults (n = 129)

Phenotype	Men		Women		p Value[a]
	Median	Percentiles (2.5–97.5)	Median	Percentiles (2.5–97.5)	
$CD3^+CD56^+$ (%)	6.3	1.4–23	4.7	0.8–11.3	0.003[b]
$CD3^+CD56^+$ (cells per µL)	126	27–580	105	20–279	0.023[b]
$CD3^+CD56^+CD4^+$ (%)	0.2	0–3.4	0.2	0–4	0.969
$CD3^+CD56^+CD4^+$ (cells per µL)	5	0–86	7	1–100	0.436
$CD3^+CD56^+CD8^+$ (%)	3.7	0.6–18	3.4	0.5–8.5	0.037[b]
$CD3^+CD56^+CD8^+$ (cells per µL)	81	9–395	66	11–212	0.082
$CD3^+CD56^+$CD4CD8 (%)	1.1	0.2–6.1	0.9	0.04–5.6	0.383
$CD3^+CD56^+$CD4CD8 (cells per µL)	22	3–125	20	1–141	0.579

[a] Mann–Whitney *U* test [b] significant NKT: natural killer T

Table 3: Reference ranges for activated lymphocytes and subtypes in healthy Cuban adults (n = 95)

Phenotype	Men		Women		p Value[a]
	Median	Percentiles (2.5–97.5)	Median	Percentiles (2.5–97.5)	
$CD3^+$HLA-DR^+ (%)	12.2	2.7–26.4	10.4	3.1–28.2	0.717
$CD3^+$HLA-DR^+ (cells per µL)	219	54–588	253	59–695	0.243
$CD3^+CD25^+$ (%)	3.7	1.4–12.0	3.6	1.0–10.3	0.612
$CD3^+CD25^+$ (cells per µL)	82	19–208	80	17–235	0.970
$CD3^+$HLA-DR^+CD25^+ (%)	1.0	0.2–2.2	1.2	0.4–3.1	0.550
$CD3^+$HLA-DR^+CD25^+ (cells per µL)	20	4–46	25	7–75	0.013[b]

[a] Mann–Whitney *U* test [b] significant

DISCUSSION

Normal blood percentages of rare lymphocytes are influenced by many factors, such as age, sex, viral infections, stress, medications, chronic diseases, lifestyles and even study methodology. Therefore, it is recommended that reference values be established through regional studies and even by countries and that they be updated periodically.[1,2,4,6] To obtain these values and their ranges, immunophenotyping of these subpopulations and their subtypes should be performed using flow cytometry in persons with immune systems unaffected by diseases, treatments or lifestyles. Once determined, they can be used in clinical practice to diagnose and predict the clinical course of immune system diseases and other diseases involving the immune system, such as cancer and infections.[2,4,6]

Values found for the double-positive (DP) alpha beta T population were similar to those published by authors in other countries and showed no differences between men and women.[27–32] However, some studies report an increase in persons aged >60 years.[32] Differences from similar studies are explained by the variety of factors influencing the composition of these populations, which cause great variability in the ranges, underscoring the need to establish country-specific reference values.[28,32]

The population of DP T lymphocytes in the peripheral blood of healthy individuals was described by Nascimbeni in 2004.[27] DP lymphocytes have been associated with antitetanus vaccination, good response to influenza vaccination in older adults, and lymphoproliferation (especially when the monoclonal phenotype is expanded after viral infections such as influenza A or Vaccinia).[28,32] Absence of lymphocytosis is more common in persistent viral infections, either latent or chronic, caused by cytomegalovirus, Epstein–Barr, herpes simplex, varicella zoster, HIV, or hepatitis B or C.[27,28] An increase in DP lymphocytes has been linked to autoimmune diseases (lupus, multiple sclerosis and rheumatoid

arthritis), cancer (melanoma and Hodgkin lymphoma) and beta thalassemia (especially after splenectomy). Thus quantification of DP cells is important for prognostic purposes. However, a decrease in or absence of these cells has not been associated with any disease and is considered normal.[6,11,29,30]

DP cell functions are varied due to their double phenotype, with production of cytokine patterns whose balance is related to the most strongly expressed coreceptor, as well as cooperative or cytotoxic functions. In addition, they maintain the ability to recognize antigens presented by both class I and II antigen-presenting molecules.[6,11,28–31] This functional capacity, which demonstrates the lymphoid cell plasticity, allows the immune system to adapt to antigenic challenges, especially long-lasting ones, as in chronic infections and cancer. In the case of HIV infection, their increase has been related to good viral control, even in the acute phase.[32]

DP cells have shown a mainly effector memory phenotype combined with markers of replicative senescence, suggesting that they have undergone continued stimulation over time.[28] This may explain the great variability observed, since they will be more or less abundant depending on the number of exposures to different antigens.

If this DP subpopulation in blood is not considered when performing cell counts, there will be incorrect duplication of their markers, biasing calculation of the CD4/CD8 index. This is a serious problem in followup of HIV patients, since this index requires precision to be useful for classifying patients for antiretroviral therapy, assessing antiviral resistance, monitoring treatment adherence and detecting possible evolution towards AIDS.

NKT populations are characterized mainly by a $CD3^+$, $CD56^+$ phenotype and in humans by absence or differential expression of CD4 or CD8. In 1995, the first articles were published about lymphocyte populations that not only had T cell receptor and NK markers, but also showed a unique type of receptor chain rearrangement and a frequency well above the expected for a specific rearrangement. These cells are called invariant NKT cells.[18–19]

The NKT ranges we found are similar to those reported by Rojas-Pandales in Colombia, who did not observe sex or age effects.[33] The great interindividual variability and wide ranges can be related to the biological characteristics of these lymphocytes, which accumulate in blood as they encounter their cognate antigens. Each person's NKT levels depend on the number of previous exposures to different agents capable of stimulating this cell population. However, the range for the normal (healthy) population in a given country has lower and upper limits, which allows detection of abnormal findings.[18,23,34,35]

Increased NKTs have been observed in chronic infections, allergies, cancer and autoimmune diseases. However, values can also be normal or decreased in allergies and cancer. Decreased values indicate poor cancer prognosis. NKTs comprise different subpopulations with functional diversity. NKT $CD8^+$ and NKT $CD4^-CD8^-$ phenotypes are a source of interferon alpha and tumor necrosis factor gamma, which possess obvious antitumor activity and are cytotoxic due to perforin secretion and binding of Fas/Fas-L (molecules that mediate apoptosis). NKT cells recognize nonprotein antigens coupled to CD1 molecules on cell surfaces, detecting malignant cells by recognizing antigens missed by their conventional T cell counterparts. This shows that NKTs complement cancer defense and immunosurveillance differently than classical T and NK cells.[24,36–39]

Among lymphocyte activation markers, HLA-DR and CD25 showed great variability among individuals, with an especially wide range in HLA-DR. This may be related to the presence of effector memory populations of T lymphocytes in blood, which also express these molecules, and to the fact that the size of this subpopulation is determined, among other factors, by the number of previous personal exposures to different immunogens.[40]

These molecules are classified as T lymphocyte late activation antigens and are synthesized after the cellular activation process has begun;[40] in flow cytometry interpretation, they are considered tertiary. Since expression cannot be predicted until cellular activation has occurred, they could influence range variability. Some authors report that age and sex do not greatly influence these expression levels, but do affect the process triggering such activation.[41] Considering that regulatory T cells characteristically express the CD25 marker, measuring lymphocyte activation by other markers, such as HLA-DR is more reliable.[40–43] One trait that can inform flow cytometry analysis is that regulatory T cells express CD25 more intensely, so that it does not behave like a typical tertiary marker.[44]

Expression of markers that reflect cell function are important to assess the activation state of the T cell compartment in patients with autoimmune diseases, because such markers discriminate between the active and compensated states of the disease.[45] There is evidence that the greater the number of activated T cells, the greater the severity of decompensation, through autoimmune pathogenesis. This has been observed in diseases such as multiple sclerosis, Wegener granulomatosis, Kawasaki disease, systemic lupus erythematosus and autoimmune aplastic anemias.[46,47] HLA-DR is used as a marker to predict therapeutic response in autoimmune diseases such as Kawasaki disease, in which patients with a higher percentage of $HLA\text{-}DR^+$ T cells fail to respond to therapy with intravenous immunoglobulins.[45–47]

In cancer, activation is analyzed by cell population. Activation of suppressor T cells indicates worsened clinical evolution and poorer prognosis. Evidence indicates correspondence between blood and tumor microenvironments, so that, if activated suppressor cells in the blood increase, so do tumor-infiltrating lymphocytes. But if the activated cells are cytotoxic, this increase can be beneficial, because they are known to help eliminate tumor cells.[43,44]

In the course of infections such as HIV, chronic immune activation is part of immunopathogenesis and is one of the most damaging phenomena in disease progression, since it increases viral load and decreases $CD4^+$ lymphocytes. Monitoring expression of CD25 and HLA-DR, as markers of the

degree of T cell activation in these patients, has been found to be very useful.[47]

It is important to consider activation markers to assess immune system function in many diseases, in some cases as an immunopathogenic factor and in others as pathological or physiological consequence of a previous illness or condition. For example, increased HLA-DR expression has even appeared in patients with chronic spinal cord injuries, possibly contributing to persistence of chronic inflammation or decreased resistance to infection.[44]

The levels of T-lymphocyte populations we observed are similar to those found in healthy controls in published research.[43–47]

CONCLUSIONS

Age does not affect counts and percentages of rare T lymphocyte subpopulations in the blood of healthy Cuban adults. Sex differences found for some phenotypes suggest the need for different reference values for women and men.

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Submitted: December 18, 2017
Approved for publication: October 6, 2018
Disclosures: None

Antenatal Diagnosis of De Novo Balanced Structural Chromosomal Aberrations in Latin America

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ABSTRACT

INTRODUCTION The consequences of de novo balanced structural chromosome aberrations diagnosed antenatally are unpredictable, and, as a result, they introduce uncertainty into genetic counseling decisions.

OBJECTIVE Describe de novo balanced structural aberrations present at antenatal diagnosis in samples from pregnant women in five Latin American countries and determine their effect on carrier individuals.

METHODS This was a retrospective observational study based on analysis of 109,011 antenatal tests conducted from January 1981 to December 2016 in Cuba, Uruguay, Costa Rica, Mexico, and Colombia. Thirteen cytogenetic laboratories provided information that included the cases analyzed during the study period; number of de novo balanced structural aberrations diagnosed antenatally; number of diagnoses with de novo balanced structural aberrations that resulted in termination of pregnancy; detailed descriptions of the karyotypes of de novo balanced structural aberration carriers, and descriptions of the form of diagnosis, including types of samples used (amniotic fluid, chorionic villus or fetal blood). Each laboratory also provided pathology reports and genetic counseling at time of diagnosis. Postnatal followup for pregnancies carried to term continued for at least two years.

RESULTS Of the 109,011 antenatal tests studied, 72 (0.07%) showed de novo balanced structural aberrations. These events primarily involved chromosomes 1, 2, 7, 14, 18, and 20. Of the 79 breakpoints identified, the most common were 5p15.3, 7q11.2, 7q22, and 14q24. We identified three breakpoints corresponding to 3.8% (3q13.1, 3q13.2, and 9p12) that were not reported in other studies of de novo balanced structural aberrations diagnosed antenatally in patients from other geographic regions or in studies of chromosomal fragile sites. Two of these breakpoints (3q13.1 and 3q13.2) were associated with high risk of phenotypic abnormalities. Information on antenatal or postnatal followup was available for 62 (86%) of de novo balanced structural aberration carriers; of the 44 carriers with postnatal followup, 10 had phenotypic abnormalities.

CONCLUSIONS Three new de novo breakpoints were identified, presumably related to genetic admixture characteristics in Latin America. Since some diseases associated with de novo balanced structural aberrations detected antenatally have a late onset, followup for at least two years is recommended for carriers of these aberrations. The information in this study is useful in genetic counseling for pregnant women in Latin America.

KEYWORDS Antenatal diagnosis, prenatal diagnosis, antenatal screening, chromosomal aberrations, chromosomal breakpoints, pregnancy, Colombia, Costa Rica, Cuba, Mexico, Uruguay, Latin America

INTRODUCTION

Structural chromosome aberrations usually involve changes in the linear sequence of genes due to loss, gain, or reallocation of segments, but the number of chromosomes generally remains constant. Structural changes may involve one or both chromatids. Chromatid-type aberrations differ from chromosome-type aberrations.[1] Structural chromosome rearrangements are considered balanced if disomy is maintained for all autosomes and a normal complement of sex chromatin is present, even if the positions of homologous segments on the chromosomes have been changed.

Structural chromosome rearrangements may arise at different stages of human development. When rearrangements occur in the postnatal period, they are referred to as acquired and may cause malignant diseases.[1]

IMPORTANCE These results will be useful in antenatal genetic counseling for pregnant women in Latin America and support long-term postnatal followup in patients with de novo balanced structural aberrations.

If a balanced chromosome rearrangement (BCR) is inherited, risk for physical or mental abnormalities in the newborn is low. However, if the aberration occurs de novo, i.e., if neither parent is a carrier, risk of a genetic disease or phenotypic abnormalities increases. The consequences of de novo BCRs diagnosed antenatally are especially difficult to predict. Microarray-based comparative genomic hybridization in antenatal diagnosis has shown that de novo BCRs can cause submicroscopic chromosomal imbalances—deletions, duplications and variations in the number of copies—that could alter gene dosage, inactivate genes susceptible to uniparental disomy, inactivate dominant genes (with the resulting expression of recessive deleterious genes in the homologous chromosome) and modulate gene expression around breakpoints.[2–4]

In 1991, Warburton studied 377,000 antenatal diagnoses in the USA and Canada to determine chromosomal distribution of breakpoints in de novo BCRs and to compare them with known breakpoints often associated with fragile sites in humans.[5] In this pioneer study, Warburton determined that de novo Robertsonian translocations carried a low risk for development of abnormalities (3%), while de novo inversions and translocations carried a higher risk, 9.4% and 6.1%, respectively.[5]

The demographic history of populations—with their fluctuating composition, size, and structure—may affect whole-genome patterns of variation. In addition, evolutionary processes—natural selection, mutation and genetic recombination—have affected variation in specific regions.[6]

In 2009, Giardino gathered data from 269,371 antenatal cytogenetic studies from 29 laboratories in Italy. Even though there was no pregnancy followup, she identified the chromosomes and breakpoints most involved in de novo BCR events.[7] Other studies have been conducted on prevalence of de novo BCRs in antenatal diagnosis in Asia and Australia, but the number of cases studied was smaller.[8–12]

In a study of individuals from 52 populations in Africa, Europe, the Americas, the Middle East, Asia, and Oceania, Rosenberg found that only 3%–5% of genetic variation was among populations, the majority of variation being within populations.[13]

In Latin America and the Caribbean, little has been published on de novo BCRs and their effects on neonatal phenotype by breakpoint involved.[14–17] Due to lack of information in the Region, genetic counseling for de novo BCRs in antenatal diagnosis is based on data compiled by other authors in populations that are geographically very distant and have some differences in their genome compared to our populations.[13] It is unknown what happens in relation to de novo BCRs in our Latin American populations, which are characterized primarily by a high admixture of Caucasian, African and Amerindian descent, with a lower percentage of Asian influence.[18–22] This study's objective was to identify de novo BCRs detected antenatally in samples from five Latin American countries, and to determine their effect on individual carriers.

Table 1: Study variables and description

Variable	Description
Type of invasive procedure	Chorionic villus biopsy (10–14 weeks) Amniocentesis (16–20 weeks) Fetal blood sampling (21–25 weeks)
Type of balanced structural aberration	Reciprocal translocation Robertsonian translocation Inversion Apparently balanced complex rearrangement
Chromosome involved	1–22 (autosomes) X and Y (sex chromosomes)
Breakpoint (international nomenclature)	p (short arm) q (long arm) Centromere (10) Chromosome region (1–3) Chromosome band (1–9) Sub-band (1–3)
Phenotype	Normal Altered
Followup	Antenatal Postnatal
Phenotypic findings	**Fetal ultrasound findings:** nuchal translucence >3 mm during 1st trimester or nuchal fold >6 mm during 2nd trimester of pregnancy, hypoplasia or absence of nasal bone, suspected cardiopathy, pyelocalyceal dilation, clubfoot, intrauterine growth retardation, oligoamnios, polyhydramnios, increased intestinal echogenicity, hydronephrosis, single umbilical artery, holoprosencephaly, malformations of the anterior wall and harelip **Postnatal findings:** dysmorphic features, intellectual disability, azoospermia, cryptorchidism, brachydactyly, epilepsy, coloboma of the iris, cardiopathy, inability to support the head, hypotonia, asymmetry of cerebral ventricles, hydrocephalus, growth retardation, psychomotor retardation
Parents' decision regarding continuation of pregnancy	Elective abortion Continued pregnancy

METHODS

Study type and population A retrospective observational study was conducted based on database information from eight cytogenetic laboratories in Cuba, one in Costa Rica, one in Colombia, two in Mexico, and one in Uruguay. Data were compiled from 109,011 antenatal tests done January 1981 through December 2016. An aberration was considered a de novo BCR if neither parent in the study had the chromosomal aberration detected in the fetus. There were 72 cases with de novo BCRs in the sample.

Data collection All participating laboratories were sent a questionnaire regarding the total number of cases tested, the cases in which a de novo BCR was detected, and the antenatal diagnostic method used (amniocentesis, chorionic villus biopsy or fetal blood sampling). Table 1 lists and describes the study variables. Laboratories submitted anatomic pathology reports of fetal testing in cases of pregnancy termination after detection of a de novo BCR.

Antenatal cytogenetic diagnosis Cultures and chromosome preparations were conducted according to the AGT Cytogenetics Laboratory Manual protocol[23] adapted to each laboratory's conditions. GTG or QFQ bands were used for chromosomal antenatal diagnosis. Chorionic villus (CV) biopsy, amniocentesis and fetal blood sampling (FBS) were carried out in essentially the same way in all participating laboratories, according to procedures reported.[23]

Followup In fetuses with de novo BCRs detected antenatally whose parents decided to continue the pregnancy (49/72, 68%), a minimum two-year postnatal followup was performed, even though this was not mandatory for inclusion in the study. This two-year followup period was selected because neurodevelopmental or motor disorders are more easily detected at age 2 years, and dysmorphic features become more evident. In addition, if there is any kind of metabolic disorder, symptoms will have already appeared in most cases. Followup was performed by clinical genetics specialists. Followup was not possible in some cases because the mother did not attend the genetic followup appointment, parents emigrated or other undetermined reasons. Phenotypic abnormalities were recorded in databases (municipal, provincial, or national) and/or in medical records.

Analysis Breakpoints were not determined by advanced molecular techniques (FISH and/or microarray) due to their

high cost or unavailability. The breakpoint was determined in translocations and inversions by studying the chromosome ideogram with 450 bands. Due to their low risk, Robertsonian translocations were not included in assessing risk associated with breakpoints. The International System for Human Cytogenetic Nomenclature 2005 was used to define chromosome formulae and specify sites within chromosomes: The centromere is assigned the number 10, with numbers assigned to regions on the short and long arms (p and q, respectively), getting larger in proportion to distance from the centromere. There are a limited number of bands in each region; if these bands are subdivided into sub-bands, a decimal point is added after the band designation, followed by the number of each sub-band. For example, 1p31.3 means: chromosome 1, short arm, region 3, band 1, and sub-band 3.[24] The resolution level of the analyzed metaphases is generally 450 bands.

Table 2: Cytogenetic diagnoses in participating laboratories

Laboratory	Period	Sample			
		Amniotic fluid	Chorionic villus	Umbilical cord blood	Total
Cuba[a]:					
Havana	1984–2015	25,692	1,785	562	28,039
Holguín	1990–2011	6,237	—	—	6,237
Villa Clara	1987–2012	10,194	2,199	—	12,393
Granma	2000–2014	4,111	—	—	4,111
Guantánamo	2004–2014	2,137	—	—	2,137
Sancti Spiritus	2004–2015	3,951	—	—	3,951
Camagüey	1990–2013	5,009	—	—	5,009
Santiago de Cuba	1986–2012	11,531	1,721	—	13,252
Mexico[b,c]	1983–2016	4,945	—	—	4,945
Colombia[d]	2010–2016	1,662	16	36	1,714
Costa Rica[e]	2013–2015	549	—	—	549
Uruguay[f]	1981–2016	21,772	4,902	—	26,674
Total (%)	1981–2016	97,790 (89.7)	10,623 (9.7)	598 (0.5)	109,011 (100)

[a]National Medical Genetics Center and provincial medical genetics centers
[b]Reproduction and Genetics, National Archives of Mexico
[c]20 de Noviembre National Medical Center, Institute for Social Security and Services for State Workers, Mexico City
[d]Biogenetic Diagnosis SAS, Bogota
[e]National Children's Hospital, San José
[f]Human Genetics Laboratory, Italian Hospital of Montevideo

While use of ultrasound findings can introduce bias in research on de novo BCRs, this study included cases in which some type of abnormality was detected antenatally by fetal ultrasound. Antenatal abnormalities detected by ultrasound have been shown to be important markers in detecting certain chromosome defects and, as a result, ultrasound findings are valuable in genetic counseling.[25]

The frequency distributions of detected aberrations (reciprocal translocations, Robertsonian translocations, inversions and complex rearrangements) were obtained for the different sample types.

Ethics Genetic counseling was provided to couples in all participating sites and written informed consent was obtained for specimen collection. Once antenatal diagnosis was made, all unused samples were discarded. Couples were informed of the results of cytogenetic diagnosis. In all laboratories, each patient was assigned a code in the database and anonymity was maintained during data processing. The study was approved by the ethics committees of all participating institutions.

RESULTS

Frequency of de novo BCR Table 2 summarizes data provided by the 13 participating laboratories, from 97,790 amniotic fluid samples, 10,623 chorionic villus samples and 598 umbilical cord blood samples for a total of 109,011 antenatal diagnoses. There were 72 de novo BCRs detected, for a frequency of 1/1535 (0.07%) diagnosed cases. The laboratories detected 36 reciprocal translocations (50%), 21 Robertsonian translocations (29.1%), 10 inversions (13.9%) and five complex rearrangements (6.9%).

Amniocentesis was used for diagnosis in 89.7% (97,790/109,011) of cases (Table 1); as a result, 95.8% (69/72) of de novo BCRs were detected using this form of antenatal diagnosis.

Three mosaics were detected in reciprocal translocations, two mosaics in Robertsonian translocations, and one mosaic in cases with inversion. Of five cases with complex rearrangements, three inherited structural aberrations and one de novo aberration were found. Two cases had rearrangements involving three or more chromosomes (Data available in Appendix 1 at www.mediccreview.org/antenatal-diagnosis-of-de-novo-balanced-structural-chromosome-aberrations-in-latin-america).

As seen in Table 3, the frequency of de novo BCRs in this study is slightly different than seen in other studies with a much greater number of cases.[5,7] In general, de novo BCRs appear less frequently in our study.

Table 3: Comparison of the frequency of de novo BCRs reported in three studies

Study	n	ACD Method	Frequency of de novo BCR (%)	Frequency of reciprocal translocations (%)	Frequency of Robertsonian translocations (%)	Frequency of inversions (%)
Warburton[5]	377,357	Amniocentesis	1/1129 (0.08)	1/2,000 (0.05)	1/9,000 (0.011)	1/10,000 (0.010)
Giardino[7]	269,371	Amniocentesis, chorionic villus sampling, fetal blood sampling	1/1095 (0.09)	1/1,500 (0.07)	1/6,000 (0.016)	1/16,000 (0.006)
This study	109,011	Amniocentesis, chorionic villus sampling, fetal blood sampling	1/1,514 (0.07)	1/3,028 (0.03)	1/5,191 (0.019)	1/10,901 (0.009)

ACD: antenatal cytogenetic diagnosis BCR: balanced chromosomal rearrangement

Distribution of de novo BCR in chromosomes Figure 1 shows the chromosomes most commonly involved in de novo BCRs (1, 2, 7, 14, 18, and 20). Chromosomes 10, 11, 15, 17, 19, and X were very rarely involved in rearrangements. Chromosome Y did not appear in any of the reported rearrangements.

Breakpoint distribution by chromosome for reciprocal translocations and inversions in the long and short arms of the chromosomes is also shown. Many of these breakpoints coincide with those reported by Warburton[5] and Giardino.[7] However, we found breakpoints not reported in these previous studies.

Distribution of de novo BCR frequency None of the reciprocal translocations were found more than once in this study. The common translocations reported by Warburton and Giardino, t(11;22)(q23;qll) and t(18;21)(pll;qll), also appeared in our study.

Ten cases were found with inversions in which chromosomes 2, 3, and 19 were involved twice. In this study, the chromosome inversion 2(p11q13) commonly appears, even though it has been primarily described as familial.[26,27]

Breakpoints most commonly found in our study that coincide with other studies[5,7] were: 5p15.3, 7q11.2, 7q22, and 14q24. Other breakpoints that appeared once in our study and that were commonly reported by Warburton and Giardino were: 1p13.2, 2p15, 2p21, 2q21.3, 3q21, 6q21, 7p15.3, 7p22, 8q24.1, 9p13.3, 11p15, 11q23, 11q13, 21q22.3, and 22q11 (Figure 1).

Breakpoints 1q25 and 7q22 were found three times in our study. Other recurring breakpoints were: 2q21.3, 4q35, 5p15.3, 7q11.2, 14q24, 20p12, and 20q13.1, each occurring twice (Figure 1).

Of the 79 breakpoints we found, 8 (10%) had not been published previously in studies on de novo BCR in antenatal diagnoses. These were: 2p22, 3p24, 3q13.1, 3q13.2, 5p11, 8q21.1, 9p12, and 12q23.[5,7,9–12,28–44] (Figure 1).

Followup Antenatal or postnatal followup was successfully conducted in 62 (86%) of the 72 de novo BCR carriers. In 44 carriers, followup lasted at least two years. Table 4 shows the cases of de novo BCR carriers who had some phenotypic abnormality.

Neurodevelopmental disorders were the predominant abnormal phenotypic traits in cases assessed postnatally. There were two individuals who exhibited no mental disability despite having complex rearrangements in more than three chromosomes.

DISCUSSION

Reciprocal translocations constitute the biggest difference between our results and those of Warburton[5] and Giardino.[7] This kind of defect was not detected antenatally by chorionic villus sampling, and our result could be explained by low quality of GTG bands from using acetic acid in sample processing, which sometimes made bands difficult to distinguish and could have contributed to failure to detect this kind of rearrangement.

Figure 1: Comparison of breakpoints by chromosome

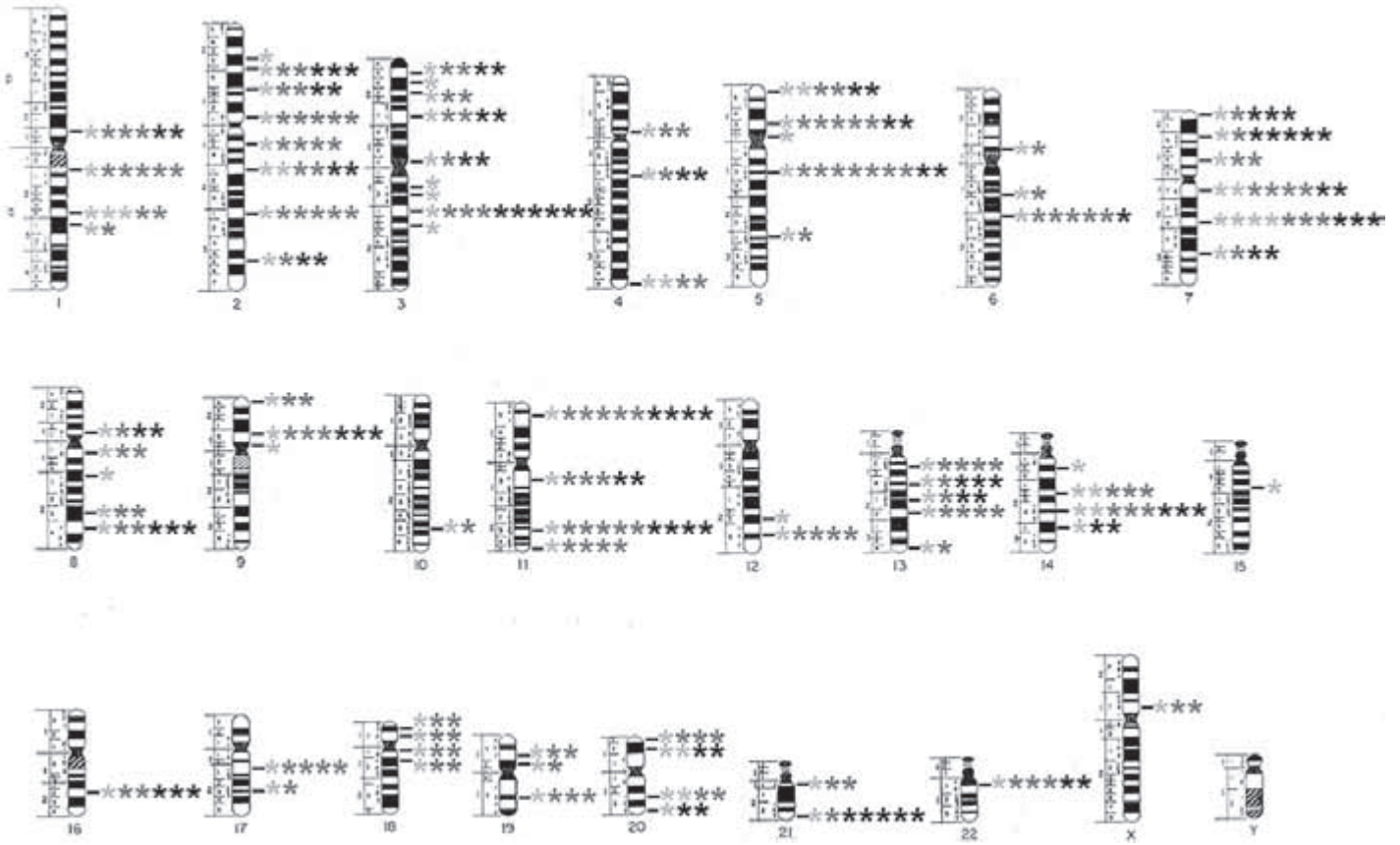

Table 4: Abnormal phenotypic findings in de novo BCR carriers

Karyotype	Pregnancy outcome	Laboratory	Abnormal finding (age of individual)
46,XX,t(7;14)(q11.2;q22)	Live birth	Havana, Cuba	Psychomotor retardation (6 years)
46,XX,inv(2)(p21q35)	Live birth	Mexico	Nuchal fold, dysmorphic features, hypotonia (newborn)
46,XX,inv(2)(p11q13)	Live birth	Mexico	Dysmorphic at birth
46,XX,inv(3)(q13.1q23)	Live birth	Havana, Cuba	Dysmorphic features, brachydactyly, growth and developmental retardation, epilepsy (25 years)
46,XY,t(1,3,20)(q21.1,p21,p12)	Live birth	Havana, Cuba	Azoospermia (26 years)
46,XY,t(3;7)(q13.2,q22)	Live birth	Villa Clara, Cuba	Dysmorphic features, asymmetry of cerebral ventricles, intellectual disabilities, coloboma of the iris (3 years)
46,XY,t(2,20)(q32;p13)	Live birth	Uruguay	Cardiopathy, convulsions, inability to support the head (4 years)
45,XX,t(18;21)(p11.2;q11)	Elective abortion	Granma, Cuba	Bilateral hydrocephaly, harelip
45,XX,t(5;17)(q31.3;q25)de novo, der(15;22)(q10;q10)mat	Live birth	Havana, Cuba	Moderate intellectual disability (5 years)
45,XY,der(13;14)(q10;q10)	Live birth	Holguín, Cuba	Moderate intellectual disability, bilateral cryptorchidism, mildly dysmorphic features (7 years)

Table 5: Comparison of breakpoints in this study with fragile sites and breakpoints in populations of different ethnic origins

This study	Coincidence with fragile sites reported by Mrasek[51] (%)	Coincidence with breakpoints reported by Liehr[52] (%)
Recurring breakpoints (n = 19)	17 (89.0)	13 (68.0)
New breakpoints (n = 8)	4 (50.0)	3 (37.5)

This method was applied in 9.7% of cases in our study and in 12% of cases in Giardino's, which detected 8% of reciprocal translocations through chorionic villus sampling.[7]

Breakpoint distributions and the chromosomes involved do not occur randomly in these de novo rearrangements.[5,7] Recurring breakpoints have been found in AT-rich palindromic regions. Genome architecture is also known to predispose certain chromosomes to structural rearrangements, due to their spatial disposition within the cell nucleus.[45,46]

We found many reported breakpoints in other antenatally diagnosed de novo BCR studies, but we also found eight breakpoints that previous studies did not identify.[5,7, 9–12, 30–44] All those studies primarily included Caucasian and Asian populations, and the majority, as in this study, used cytogenetic banding methods to analyze breakpoints.

In postnatal studies, many of these de novo BCRs were examined using more sophisticated molecular techniques, such as whole-genome high-resolution array, which includes matrix-based comparative genomic hybridization or single-nucleotide polymorphism.[3,4,47] These methods can reveal cryptic chromosome imbalances, but they are used in postnatal studies in individuals with an abnormal phenotype. This introduces a bias into breakpoint analysis because samples come from individuals with phenotypic traits (mental disability, dysmorphic features, delayed growth, etc.) that signal possible chromosome rearrangement. Therefore, such cases are not included in our comparisons.

Even though the origin of a chromosome aberration is much more complex than the simple association with fragile sites inside the genome,[48–50] it is indisputable that these fragile sites are prone to formation of chromosome aberrations, primarily de novo. Fragile sites are specific loci that are prone to forming gaps and breaks on metaphase chromosomes due to partial inhibition of DNA synthesis.[51] Table 5 shows a comparison of breakpoints we found and fragile sites reported in recent international studies.[51,52] Mrasek reported 230 fragile sites induced by aphidicolin, a substance that partially inhibits DNA synthesis, in human chromosomes.[51] Liehr demonstrated that approximately 71% of breakpoints found in Caucasian individuals, studied because they had balanced aberrations, are co-located in these fragile sites, and he determined that these regions prone to chromosome breaks play an important role in formation of structural chromosome rearrangements.[52] Of the eight new breakpoints identified in this study, three (3q13.1, 3q13.2, and 9p12) do not coincide with fragile sites previously reported by these other authors. Breakpoint 3q23 identified in this study has been reported in only three people.[51,52]

Mrasek's testing to identify fragile sites identified was performed in three Caucasian individuals (one of whom had a parent of Asian origin).[51] Liehr compared the 230 fragile sites detected by Mrasek with breakpoints found in 251 Caucasian patients with BCR and found greater than 70% overlap.[52]

In our study, we identified three breakpoints of de novo BCR not reported previously by these authors.[51,52] These three breakpoints (3q13.1, 3q13.2, and 9p12) were not reported in the large-scale studies by Warburton and Giardino,[5,7] in studies in Asia,[9–11] or in other studies with smaller sample sizes conducted in Europe, North America and Australia.[12,30–46] Rosenberg studied 1056 individuals from 52 populations and found that most genetic variation was within populations; only 3%– 5% of genetic variation was due to major group differences.[13] In 2006, Bastos-Rodrigues, using a more sophisticated methodology (a set of 40 biallelic slow-evolving short insertion-deletion polymorphisms) than the one used by Rosenberg, found greater genetic variation (12.1%) across the different population groups and confirmed Rosenberg's identification of five well-defined groups: the Americas, Africa, East Asia, Oceania, and a cluster comprising Europe, the Middle East and Central Asia.[53] Finding these previously unreported breakpoints could be due to the unique nature of our genome with its distinctive admixture of African, European and Asian populations.

Followup Problems were reported during the perinatal period in two of the children studied, but followup studies showed that their development was completely normal. For example, the carrier of the inv(2)(p11q13) rearrangement who showed dysmorphic features at birth had subsequent normal development. Identifying dysmorphic features in the perinatal period can be difficult because sometimes the process of labor can cause physical abnormalities in the newborn that are confused with dysmorphic features.

There are some de novo BCR carriers who, even though they are apparently normal at birth, can exhibit late-onset phenotypic traits during long-term followup, primarily due to neurodevelopmental disorders, which suggest gene mutations caused by de novo chromosome rearrangement. This commonly occurs when there are cryptic chromosome aberrations.[11,30] In this study, we present the example of a woman with inv(3)(q13.1q23) who was apparently normal at birth but at the time this manuscript was being prepared had a mental disability, epilepsy, autism-like behavior, and dysmorphic features.

Two breakpoints in the long arm of chromosome 3 (3q13.1 and 3q13.2) were detected very close together. These breakpoints are not reported in the literature consulted on de novo BCR in antenatal diagnosis in the USA and Canada, Europe, Asia and Australia.[5,7,9–12,30–44] Since these breakpoints were associated with severe phenotypic abnormalities and mental disability in carrier individuals, their presence should be carefully considered during antenatal diagnosis and genetic counseling for couples. In such cases, we recommend thorough ultrasound monitoring and molecular techniques, such as FISH and microarray, to supplement diagnosis in order to detect possible cryptic genome aberrations.

Two patients with complex chromosome rearrangements involving three or more chromosomes had no neurological abnormalities. In one case, the individual had undergone antenatal diagnosis in the 1990s and was encountered again because he exhibited azoospermia and attended an infertility service. In the other case, the carrier was a completely normal 12-year-old child, for whom microarray analysis found no gain or loss of inherited material.[54] This is unusual because when several chromosomes are involved in a de novo rearrangement, the possibility of phenotype disorders increases due to poor segregation of derivative chromosomes or generation of recombinant chromosomes.[55]

One study limitation is that the genome breakpoints were detected using conventional cytogenetics, not molecular techniques such as FISH and microarray. Molecular cytogenetic analysis could have provided more sensitivity in locating breakpoints at the chromosome band and sub-band level and would have helped in comparing our breakpoints with those of Mrasek[51] and Liehr.[52] These techniques were not available to the laboratories participating in our study, in part because of when the tests were conducted and in part due to their high cost. Nevertheless, the results are relevant because the study compiled data from five Latin American laboratories and was supplemented by long-term followup of carriers of these de novo rearrangements.

Another limitation is the relatively small number of cases analyzed, compared to the Warburton and Giardino studies,[5,7] and that not all participating countries provided a sufficient number of cases. It was not our objective to make comparisons among the Latin American countries that contributed samples, as these countries also have different genetic characteristics due to diverse ancestries. We must consider that data from Mexico, Costa Rica, and Colombia only make up 6.6% of the sample.

As did previous studies, our findings suggest a relationship between constitutional breakpoints during the antenatal period and fragile sites. However, confirmation of a relationship with biological implications requires molecular analyses of breakpoints. Molecular cytogenetic techniques provide greater precision in antenatal diagnosis,[49,50] but are still unavailable in most less-developed countries. Therefore, even with the study's limitations, it demonstrates the utility of conventional techniques for studying de novo BCRs with a focus on chromosome aberrations and their possible relationship with genome admixture in Latin American countries. Our results are useful in genetic counseling for pregnant women in Latin America.

CONCLUSIONS

Three new breakpoints were identified in the genome, related to de novo BCRs, which may be due to the typical genetic admixture in Latin America. Two of these breakpoints identified for the first time are considered high risk because they are associated with severe phenotypic abnormalities in carriers. In carriers of de novo BCRs, a minimum two-year followup period is recommended, as many phenotypic disorders have late onset.

ACKNOWLEDGMENTS

The authors thank Silvia Méndez, Luis Casaco, and Judith Pupo for their support during preparation of this manuscript. We thank all the medical geneticists and cytogenetic specialists for their considerable contribution to the study. Thank you to Joe Munch in MD Anderson's Department of Scientific Publications for editing the manuscript.

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Submitted: May 7, 2018
Approved for publication: October 7, 2018
Disclosures: None

Rising Cancer Drug Prices: What Can Low- and Middle-income Countries Do?

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ABSTRACT

Public health systems face the contradiction of skyrocketing cancer incidence and cancer drug prices, thus limiting patient access to more effective treatments. The situation is particularly dire in low- and middle-income countries. We urgently need consensus on the main determinants of this problem, as well as specific, effective and feasible solutions.

Analysis of available data reveals that the problem has reached its current magnitude only recently and is not related to the growing complexity of drug production technology, but rather to corporate profits and the failure of market mechanisms to allocate resources based on health needs.

Despite the obstacles, there is ample room for effective intervention: joint price negotiations, cost transparency, greater support for creation of manufacturing capacity, and regulatory measures that facilitate introduction of generic and biosimilar drugs and reduce intellectual property barriers to better use of flexibilities in the Agreement on Trade-Related Aspects of Intellectual Property Rights.

Such actions will not be effective if there is no consensus around them, or if low- and middle-income countries act in isolation. This is precisely where international organizations must intervene.

KEYWORDS Public health, price, cancer drugs, inequality, less-developed countries, developing countries, Cuba

INTRODUCTION

Cancer is one of the main causes of mortality worldwide, with 8.8 million deaths in 2015. In 2012, there were 14 million new cases, a number expected to grow by 70% in the coming two decades.[1]

The economic impact of cancer is substantial and growing, with US$1.16 trillion in treatment costs in 2010.[2] According to Prasad, at current prices, providing drugs to all patients with cancer, as the treatment paradigm for cancer indicates, would lead all nations, even the most prosperous, to bankruptcy.[3]

This paper reviews the possible causes and repercussions of rising prices for cancer drugs and offers suggestions for addressing these challenges in low- and middle-income countries.

DEVELOPMENT

Rising prices are a recent problem Surgery and radiotherapy were the main therapeutic options until antitumor drugs appeared in the mid-twentieth century. Availability of these drugs is now a determining factor in treatment quality. With implementation of the Agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPS) in 1995, cancer treatment costs increased. Prasad found that the average cost of these treatments surpassed average family income in 2004 and were twice average family income in 2014.[3]

The impact of unreasonably high prices for lifesaving drugs is similar to that of price gouging for necessities in isolated areas experiencing natural disasters.[4] The UN Sub-Commission on the Promotion and Protection of Human Rights recognized that, although States have the primary responsibility for promoting, respecting and protecting human rights, transnational corporations "are also responsible for promoting and securing . . . human rights."[5]

IMPORTANCE High prices for cancer drugs are a stumbling block to the goals of ensuring universal drug coverage and access to better treatments. This article reviews the possible reasons for and repercussions of rising cancer drug prices and offers suggestions for how developing countries can respond.

Annual treatment costs per patient for 12 of 13 cancer drugs approved in the USA in 2012 are over US$100,000.[6] Furthermore, according to an analysis of 71 cancer drugs approved from 2002 through 2012 for treatment of patients with solid tumors, median overall survival was 2.1 months, while progression-free survival was 2.3 months.[7] In another analysis of 47 pharmaceuticals approved by the US FDA in 2014–2016, only 9 (19%) met the significant clinical benefit standard with regard to overall survival established by the American Society of Clinical Oncology.[8] In addition, in an analysis of 226 randomized trials, only 70 (31%) met the threshold for significant clinical benefit proposed by the European Society for Medical Oncology.[9] These marginal results come from randomized controlled trials that supported regulatory approval of these drugs. These therapies' benefits are even lower in the general population (i.e., outside research settings), which is generally older with more comorbidities than patients recruited into clinical trials.

A systematic examination of cost–effectiveness of drugs for treatment of hematologic cancers found that only 9 of 24 drugs analyzed (37.5%) had incremental cost–effectiveness ratios below the benchmark of US$50,000 per quality-adjusted life year.[10]

Low- and middle-income countries have it worse Age is the strongest risk factor for cancer. The numbers of older adults worldwide are increasing; two thirds of the population aged ≥60 years live in low- and middle-income countries. In 2015, 47.2% of persons aged >80 years were living in developed countries and 52.8% in low- and middle-income countries. By 2050, the latter proportion is expected to increase to 70.6%.[11]

Although cancer is frequently identified as a problem of the industrialized world, 70% of cancer deaths occur in low- and middle-income countries. In 2017, only 26% of these countries reported having pathology services in the public health sector, where the greatest share of patients receive care, if they receive

care at all. Fewer than 30% of these public health systems had cancer treatment services, contrasting with over 90% in high-income countries. Only one in five low- and middle-income countries compiles data needed to support cancer policies. In low- and middle-income countries, even though cancer drug prices might be lower than in developed countries, treatments are less affordable due to citizens' lower average purchasing power.[12]

It could be argued that high drug costs are not a problem in countries with universal health coverage (of the 194 UN-member countries, only 36% guarantee the broad right to health in their constitutions),[13] but with current drug costs, budget constraints become inevitable, even in countries with the political will to assume the financial burden of citizens' treatment needs. Cancer drugs are simply unaffordable in many countries, and if current pricing trends continue, it is only a matter of time before they become unaffordable for all countries. Rising expenditures on cancer drugs can also siphon off funds from drugs to treat other types of illnesses, which risks endangering the health of the population as a whole.

Individual nations have very limited bargaining power to bring down prices, due to the modest size of their markets.[7] Therefore, capacity for collective bargaining with the pharmaceutical industry is becoming increasingly important.

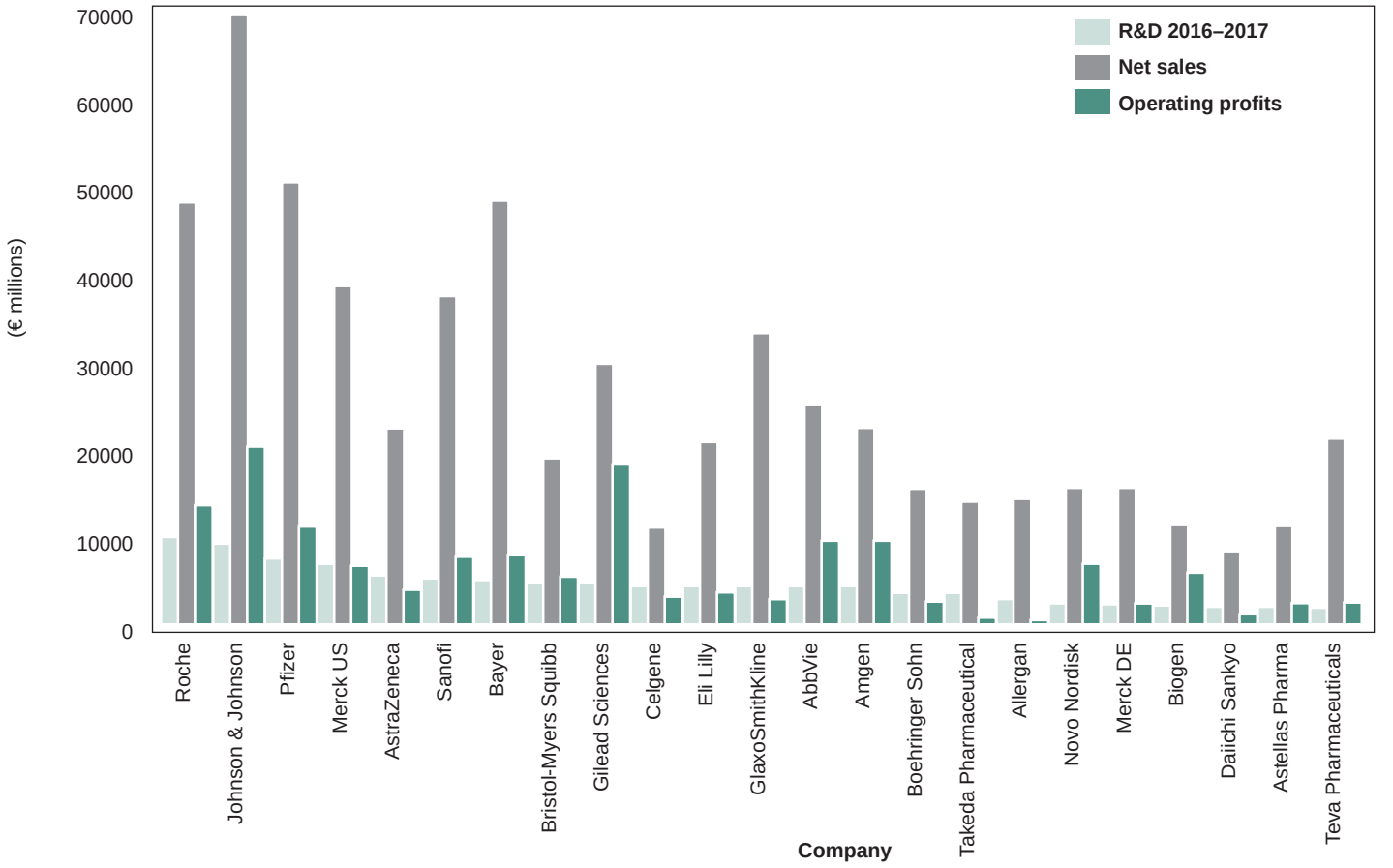
The problem is profit, not technological complexity Managing drug development, production and trade in a globalized world leads industry to evade the problem of accessibility, which it frames as a function of "market failure." A determining factor in price hikes is that pharmaceutical and biotechnology companies tend to be private, and they prioritize amassing wealth at the expense of the public interest in obtaining access to drugs at affordable prices.

The industry argues that unfettered price-setting is crucial to incentivizing innovation, since it allows for recovery of investments in product development. However, evidence suggests that research funded with public money has a direct role in innovation of 10%–40% of new drugs and that the indirect role of science funded with public funds is even more substantial.[14–16]

In the absence of corporate transparency and independent auditing of their accounting records, it is difficult to accurately calculate drug development costs and to what extent public or private funders cover these costs. Pharmaceutical corporations continue reporting annual profits of up to €20 billion,[17] suggesting that revenues substantially exceed expenditures. Evidence indicates that more money is spent on marketing than on research and development (R&D), generating high profit margins for the few companies that control the bulk of the market, most of which are located in only nine countries (Figure 1).[17]

Furthermore, pharmaceutical companies use various strategies to delay or prevent introduction of generic or biosimilar drugs. Patent holders often pay potential manufacturers to delay introduction

Figure 1: Profit margins for pharmaceutical/biotechnology companies among the world's 100 largest investors in R&D



Source: European Commission Project, Economics of Industrial Research & Innovation[17]

of generically equivalent drugs.[18] Another trend includes FDA Risk Evaluation and Mitigation Strategies (REMS) programs.[19] The FDA requires prescribers and patients to be informed of possible risks and benefits associated with a medication's use. Some drug manufacturers patent their own REMS program, and later deny access to generics manufacturers that wish to conduct bioequivalence studies. Major pharmaceutical corporations have responded to expiration of their patents by pressuring regulatory agencies to implement increasingly stringent controls, raising the entry bar for new competitors. These strategies have been successful for corporate interests, but not for the needs of public health systems or the patients they serve.

No discussion of generic and biosimilar drugs would be complete without considering intellectual property and its relationship to market exclusivity. Patents determine the exclusivity that businesses enjoy of exercising a monopoly under protection of law.[3] In an analysis of 437 top-selling drugs, market exclusivity lasted an average of 12.5 years; for drugs categorized by the FDA as hematology/oncology drugs, this period was 14.3 years.[20]

Elements that shore up drug prices include a dearth of competitors and an overall lack of a relationship among a given drug's price, sales volume and clinical performance. In fact, lack of competitors and bargaining power made cancer drug prices in the USA among the highest in the world, increasing by 10% annually between 1995 and 2013, far exceeding the USA's average inflation rate for the period.[21]

The pharmaceutical industry has defended these high prices by arguing high development costs due to the large clinical studies required to obtain regulatory approval. However, in this new era of personalized medicine, cancer-fighting drugs are often genotype-selective, which increases their success rate with more precise patient selection. Consequently, it is estimated that approval of these drugs will require less costly clinical trials. The European Medicines Agency has launched an adaptable licensing program that enables companies to obtain marketing authorization on the strength of well-designed trials based on biomarkers.[22] Other regulatory agencies might follow this example.

However, major challenges still need to be overcome regarding efficiency in the overall drug development process. For example, there are currently 803 clinical trials of therapeutic candidates with immune checkpoint inhibitors, which are expected to enroll more than 166,000 patients.[23] Enormous redundancy in these studies comes from many companies conducting similar trials with comparable drugs, but not sharing their data, all to protect commercial interests.

How is the international community dealing with these challenges? WHO has developed initiatives to resolve the conflict between market-driven approaches and public health interests. However, such efforts are still insufficient to sustainably address health priorities. As part of these initiatives, WHO publishes Model Lists of Essential Medicines (EML) aimed at meeting priority population health care needs and helping establish the principle that some drugs are more useful than others.[24] Several controversies have arisen around the list, due to its influence in determining reimbursements in drug procurement programs in many developing countries. For example, there was a time when HIV treatments, which save lives, were left off the EML because their purchase was not cost-effective. As a result, countries such as the BRICS (Brazil, Russia, India, China and South Africa) began to produce them even though their patents had not expired, which is how they came to be included in the EML.

However, the 20th edition of the EML (March 2017) still does not include products for cancer treatment on its core list. Cancer drugs registered several years ago remain on the complementary list, which includes drugs that require specialized facilities or care, or are very expensive. At present, the EML only includes three biological products.[24]

CONTROVERSIAL BUT POSSIBLE SOLUTIONS

Change the rules for price negotiations between governments and industry If nations had greater capacity to negotiate prices as a group, entering negotiations with consensus-based prices could have worldwide repercussions. To facilitate this process, WHO could establish an international open database of drug prices in all countries where they are commercially available. This should be a requirement for all drugs in the EML and should be complemented by introduction of a new category: medicines that would be classified essential if they were available at affordable prices.

An interesting example of this approach, although still controversial, is that of the UK National Institute for Health and Care Excellence, which has proposed provisional funding for use of certain drugs within the cost–effectiveness threshold and gathering data on their use to more accurately gauge their cost–effectiveness.[25]

Make drug development costs transparent The pharmaceutical industry argues that R&D expenses are a key component of the high cost of cancer drugs. However, R&D expenses associated with introduction of a new drug are for the most part unknown or speculative. In the USA there are initiatives to make composition of drug prices more transparent, such as Vermont's 2016 cost-transparency legislation.[26] This trend expresses the will to put an end to legal monopolies on products when prices are excessive. Such laws require manufacturers to provide details on R&D, manufacturing and marketing costs, as well as probable clinical benefits and prices set in other countries.[3]

Introduce rigorous programs to develop biomarkers Regulatory agencies should set standards for drug approval using validated and clinically useful biomarkers for patient selection. This would decrease costs by reducing the number of patients treated with drugs that do not improve survival or quality of life. In addition to discovering innovative pharmaceuticals, efforts should be aimed at repurposing drugs with expired patents, by looking for new indications linked to effective biomarkers that predict response.[3]

Speed up introduction of generic and biosimilar drugs Generic drugs are 80%–85% less expensive than their brand name equivalents. Hence, there is considerable public interest in ensuring their early and safe introduction, especially in oncology. It would be desirable to require patent holders to provide samples of drugs to manufacturers of generics and biosimilar drugs, to facilitate the studies needed to gain regulatory approval. This should also be extended to REMS programs and to knowhow, which are currently kept confidential

and are required for health registration of both generic and biosimilar drugs.

Expand and deepen use of TRIPS flexibility The Doha Declaration on the TRIPS Agreement affirmed that it "can and should be interpreted and implemented in a manner supportive of WTO members' right to protect public health and, in particular, to promote access to medicines for all."[27]

A recent study found that TRIPS flexibility mechanisms were used by 89 countries from 2001 through 2016, most (56.8%) in the form of compulsory licenses or public noncommercial use licenses.[28] Although the authors concluded that TRIPS flexibilities have been applied more often than commonly assumed, expanded and more thorough implementation is still needed.

In 2016, the report of the UN Secretary-General's High-Level Panel on Access to Medicines stressed: "Countries have the right to authorize and issue compulsory licenses. This right is explicitly safeguarded in leading intellectual property and trade treaties and in national laws." However, the same report also recommended better coordination among UN interagency working groups to "ensure greater coherence in the advice and support to governments and other stakeholders," clearly recognizing that the problem is complex and far from being resolved.[29]

WHO could advocate for mechanisms that maximize innovation, promote access and introduce creation of manufacturing capacity in developing countries through technology transfer incentives. Geographic concentration of manufacturing capacity in only a few countries does not contribute to price controls or affordability.

Increasingly, WHO has been leading stakeholder discussions aimed at designing solutions to the issue of high drug prices. To develop an effective policy framework, WHO must work with other UN system agencies, such as the WTO, the World Intellectual Property Organization and the UN High Commissioner for Human Rights.

CONCLUSIONS

Rising cancer incidence, which increasingly affects nations of the Global South, is incompatible with the global trend of increasing cancer drug prices. Solutions depend on collaboration to enhance the right to treatment for cancer patients, irrespective of their country of origin or socioeconomic situation.

The pharmaceutical industry' economic power makes the struggle to promote policies to reduce prices more difficult. Developing countries are particularly defenseless, unless they can work in concert to boost their bargaining power. UN institutions can play a decisive role by providing a more coherent policy framework that brings incentives for innovation and trade into line with efforts to ensure basic human rights, including access to medicines, wherever the market fails to do so.

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Submitted: August 10, 2018
Approved for publication: October 6, 2018
Disclosures: None

A Tax-based, Noncontributory, Health-Financing System Can Accelerate Progress toward Universal Health Coverage in Nigeria

Bolaji S. Aregbeshola MScPH

ABSTRACT

A major challenge to achieve health coverage in Nigeria is expansion of health access to the poor, vulnerable and informal sectors, which constitute over 70% of the population of more than 186 million. Evidence from other countries suggests that it is difficult for contributory insurance schemes to achieve universal health coverage in such conditions, especially with such a large informal sector. In fact, Nigeria's national social health insurance program has provided coverage to less than 5% of the population since its implementation in 2005, private voluntary health insurance has shown poor potential to extend coverage, and community-based health insurance has failed to expand access to poor, vulnerable and informal sector populations as well. Decentralization of health insurance to the states has limited potential to expand health insurance coverage for the poor, vulnerable and those in the informal sector. Furthermore, social health insurance in many developed countries has taken many years to achieve universal health coverage.

This paper suggests that policy makers should consider adopting a tax-based, noncontributory, universal health-financing system as the primary funding mechanism to accelerate progress toward universal health coverage. Social health insurance and its decentralization to states for formal sector workers should serve as a supplement, while private voluntary health insurance should cover better-off groups. Simultaneously, it is critical to tackle issues of poor governance structures, mismanagement of funds, corruption, and lack of transparency and accountability within regulatory and implementing agencies, to ensure that monies allocated for expanded health insurance coverage are well managed.

Although the proposed universal health coverage reform may take some years to achieve, it is more feasible to collect taxes, improve tax administration and expand the tax base than to enforce payment of contributions from nonsalaried workers and those who cannot afford to pay for health insurance or for services out of pocket.

KEYWORDS Health financing, access to health care, health services accessibility, tax-based universal system, financial risk sharing, Nigeria

INTRODUCTION

Nigeria—Demographic, administrative, socioeconomic and health context Nigeria has the largest population of any African country, an estimated 186 million in 2016.[1] It is rich in natural resources, particularly oil. Bordered by Benin to the west, Chad and Cameroon to the east and Niger to the north, Nigeria is a political federation with 36 states and a federal capital territory (in six geopolitical zones), and multiple ethnic groups. It is classified among the low- and middle-income countries; Table 1 displays selected demographic, socioeconomic, health and health-financing indicators.[1−8]

As can be observed, Nigeria's performance in health indicators is poor and has not improved as fast as might be expected, given its per capita GDP greater than $2000.[1,2,8,9] In 2016, the first ten causes of death were malaria, HIV/AIDS, diarrheal diseases; lower respiratory tract infections, neonatal encephalopathy, ischemic heart disease, preterm birth, congenital defects, meningitis and neonatal sepsis.[10] In addition, there are variations in health status and health outcomes between rural and urban areas and across geopolitical zones and socioeconomic groups, as well as inequities in access to health care services. Informal employment constitutes over 90% of total employment in Nigeria.[11] Furthermore, 53.5% of Nigerians live in poverty ($1.90 a day) with 70% of the poor residing in rural areas.[12] The majority of Nigerians who remain uninsured are poor, unemployed and nonsalaried workers.

IMPORTANCE The article highlights the need for governments in Nigeria to adopt a tax-based, noncontributory, universal health-financing system in order to significantly extend health coverage and access, given the country's large poor, vulnerable and informal sector population.

Nigeria's health system is structured in primary, secondary and tertiary levels,[13] with the three tiers of government (national, provincial and local) sharing responsibility for provision of health services. The private sector delivers approximately 60% of health care services and the public health sector 40%.[14] Nigerians should have reasonable geographic access to health care with about 32,000 public and private primary health care (PHC) facilities spread across the country.[15] Nonetheless, new PHC facilities are needed in rural areas and the Northern states, especially those affected by conflict, in order to address geographical barriers to access.

The fact that Nigeria has consistently had the largest economy in Africa provides fiscal space for financing to meet the health needs of the population, including all Nigerians, but there has been limited progress in addressing health-financing challenges to date.[9]

Universal health coverage (UHC) and Nigeria UHC aims to increase equity in access to quality health care services and reduce associated financial risk.[16] Evidence from other countries suggests that it is difficult to implement contributory insurance schemes for UHC where there is a large informal sector.[17−19] According to WHO, expanding health insurance coverage to the entire population requires substantial funding

Table 1: Nigeria's demographic, socioeconomic, health and health-financing indicators

Indicator	Value
Demographic	
Surface area (2017)	923,768 km^2[1,2]
Population (2016)	186 million[1]
Population growth rate (2017)	2.43%[2]
Population aged <15 years of age (2017)	43.0%[1,2]
Rural population (2016)	60.0%[3,4]
Birth rate (2017)	36.9 births/1000 population[2]
Socioeconomic	
Gross domestic product (GDP) (2016)	US$405.1 billion[1]
GDP per capita at purchasing power parity (2017)	US$5900[2]
GDP growth rate (2017)	0.8%[2]
Human development index (2016)	0.527[4]
Poverty rate at $2.50/day PPP terms (2017)	51%[5]
Poverty rate at $4/day PPP terms (2017)	75%[5]
Literacy rate (2017)	59.6%[2]
Unemployment rate (2017)	13.4%[2]
Health services and outcomes	
Life expectancy at birth (2016)	55.2 years[6]
Neonatal mortality (2016)	34.1/1000 live births[6]
Under-five mortality (2016)	104.3/1000 live births[6]
Maternal mortality ratio (2015)	814/100,000 live births[6]
Malaria incidence (2016)	381/1000 population at risk[6]
Tuberculosis incidence (2016)	322/100,000 population [6]
Probability of dying from non-communicable diseases (2017)	20.8%[7]
Contraceptive prevalence (2017)	13.4%[7]
Unmet contraception need (2017)	27.6%[7]
Total fertility rate (2017)	5.8%[7]
Births attended by skilled health personnel (2017)	35.0%[7]
Births in health facility (2017)	37.5%[7]
Postnatal health check for mother and newborn (2017)	37.1%[7]
Prevalence of stunting aged <5 years (2017)	43.6%[6]
Health-financing indicators	
Government health expenditure as share of GDP (2018)	0.59%[8]
Government health expenditure as share of total expenditure (2018)	5.3%[8]
Out-of-pocket expenditure as share of total health expenditure (2018)	72.3%[8]

PPP: purchasing power parity

from general tax revenues, these fully or partially subsidizing care for poor and vulnerable groups.[20] Countries like Thailand, Brazil and Costa Rica were able to achieve UHC by adopting tax-financed, noncontributory, universal coverage schemes to benefit particularly poor, vulnerable and informal sector (PVIS) populations.[21] General tax revenues have also been used to cover PVIS populations in Argentina, Colombia, Mexico and Vietnam, with tax financing predominant.[22]

Documentation from the 2014 Presidential Summit on UHC held in Nigeria,[23] the 2018 Health Policy Dialogue on UHC,[24] and the launch of the Basic Health Care Provision Fund under the National Health Act of 2014,[25] indicates that successive Nigerian governments have supported the objectives of UHC. Yet, health coverage is far from universal.

Nigeria's National Health Insurance Scheme (NHIS) and UHC

After several attempts to introduce health insurance beginning in the 1960s, legislation establishing the NHIS was passed in 1999 and the program launched in 2005 (after delays due to political instability).[26] NHIS is a contributory health insurance scheme (combining compulsory and voluntary contributions) targeted at formal sector workers as well as PVIS populations. It aims to ensure access to quality health care services, provide financial risk protection, reduce rising health care costs and ensure health care efficiency. NHIS has been implemented through programs such as the Formal Sector Social Health Insurance Programme, Mobile Health, Voluntary Contributors Social Health Insurance Programme, Tertiary Institution Social Health Insurance Programme, Community Based Social Health Insurance Programme, Public Primary Pupils Social Health Insurance Programme and Vulnerable Group Social Health Insurance Programme, which aim to provide health care services for children under 5 years, pregnant women, prison inmates, disabled persons, retirees and older adults.[27] However, NHIS has been performing poorly with very low coverage; since its implementation in 2005, evidence suggests that NHIS has provided health insurance coverage to less than 5% of the population.[21]

The Social Health Insurance Programme has entrenched inequities in access to health care as only federal government workers and their dependents are provided with coverage.[28] Community-based health insurance has also failed to expand coverage to PVIS populations,[29] and private voluntary health insurance has shown poor potential to extend health insurance coverage.[30] Voluntary membership, limited government support and poor management are some of the reasons why private voluntary and community-based health insurance do not work in Nigeria and result in high dropout rates, high administrative costs and low coverage of the poor.

Exemption schemes and waivers aimed at the poor and vulnerable groups have not been effective in increasing enrollment and addressing barriers to access for these groups due to problems associated with targeting and failure to enforce exemption systems. Thus, PVIS populations are disproportionately exposed to catastrophic and impoverishing effects of high out-of-pocket expenditures. Furthermore, the NHIS has been bedeviled with poor governance structures, mismanagement of funds, corruption, and lack of transparency and accountability.[31] Nigeria has to provide health insurance coverage for the >90% of its population who remain uninsured in order to achieve UHC by 2030.

A tax-financed noncontributory scheme has emerged as a viable option for financing UHC in countries at all income levels,

including those with large informal sector populations.[17,32−35] In addition, providing health insurance to PVIS populations represents a bottom-up approach to expanding coverage that is a feasible option for developing countries, including Nigeria.[22] Policy makers should consider a tax-based noncontributory universal health-financing system as the primary financing mechanism toward achieving UHC, as it is capable of addressing the factors responsible for poor enrollment in NHIS by PVIS populations.

Against this backdrop, this paper's objective is to propose the design and effective implementation of a noncontributory mechanism for health financing toward achieving UHC in Nigeria. This UHC reform proposal is in the spirit of those used in Thailand, Mongolia, Sri Lanka, Philippines, Brazil, Argentina and Mexico, aiming to expand health insurance coverage to PVIS populations.[22,32]

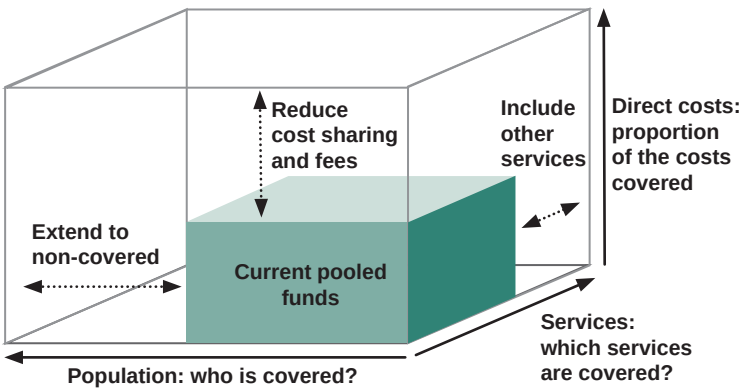
DESIGNING A UHC STRATEGY (UHCS)

Figure 1 illustrates the dimensions of UHC: depth (proportion of costs covered), scope (range of services covered), and breadth (proportion of the population covered).[20]

A comprehensive health benefit package should be designed for the whole population (including PVIS populations) in order to ensure equity and access to essential health services. This package should include a portfolio of health services, complemented by a negative list of services, products and interventions that would not be tax funded under any circumstances—for example, medications not considered cost effective by health technology assessment agencies.[36,37] The following health services should be made available under the UHCS for the whole population (including PVIS populations): maternity services, emergency services, hospital services, physician services, pharmaceuticals, public health services, outpatient primary care, inpatient specialist care, preventive care, curative care, mental health care services, health promotion services, palliative care services and interventions for diseases.[22] This should be modelled on experiences in countries such as Chile and Colombia,[38] Liberia pre-Ebola[39] and Thailand.[40]

The package should also be guided by equity, financial protection and empirical evidence on cost-effectiveness of health services and interventions.[18,36,37] It should be constantly reviewed and refined as new evidence, technologies and preferences emerge.

Figure 1: Three dimensions to consider when moving toward UHC (WHO)



Source: WHO 2010[20] used with permission

[36,37] Beneficiaries of a noncontributory UHCS would not be allowed to seek medical care at secondary and tertiary care levels except with a referral from PHC facilities or in emergency situations. Efforts should be made to make the gatekeeper policy effective by ensuring improved quality in PHC and smooth running of referral processes.[30] The bulk of additional public health spending should be allocated to pay for PHC services because these services are currently woefully inadequate in Nigeria, and are globally recognized as the foundation of good health care.

With respect to provider payment mechanisms, capitation and diagnosis-related group mechanisms should be attached to prioritized and nonprioritized health care services under the UHCS rather than fee for service and copayments, which undermine the objectives of UHC.[41]

Tax base and revenue collection In Nigeria, governments at national and subnational levels need to increase the tax base to finance such a comprehensive health benefit package, a noncontributory UHCS, in order to expand coverage to all. This will provide the large funding pool needed for provision of health services for PVIS populations, the majority of the Nigerian population. Efforts to expand taxation should include taxes on tobacco, alcohol and sugary soft drinks, without increasing relative tax burden on PVIS populations who pay proportionately more of their income in taxes than their wealthier compatriots. Other tax sources that should be explored include effective collection of corporate and business taxes, especially of natural resources profits—including oil. Taxing oil profits properly would bring in significantly more funds without adversely impacting the poor.

Funds for the UHCS should be collected by government tax agencies from all potential sources of taxes: in addition to those mentioned above, these might include mobile phone use, luxury goods (such as cars, yachts and private jets), unhealthy foods, tourism and imported goods (such as salt, plastics, cereals, machinery, frozen fish, vehicles, iron and steel). Other sources of funding should include special levies on large and profitable companies, currency exchanges, financial transaction flows, diaspora bonds and luxury air travel.

In general, it is important to ensure that a progressive taxation model is adopted for such prepaid health financing, in which richer population groups pay a higher percentage of their income than poorer groups. The UHCS should be financed through allocation of these tax funds and innovative financing mechanisms by government tax agencies. Expected revenue from the various types of "sin taxes" proposed and the financial implications of the UHCS would be calculated by a technical working group composed of technical experts and stakeholders—such as legislators, academics, policy makers and civil society groups.

Pooling UHCS funds should create a pool that guarantees coverage of PVIS population health care costs. In other words, under the UHCS, risk associated with illness would be shared, rather than borne individually. Needs-based resource allocation mechanisms should be used rather than historical budgeting. It is recommended that the NHIS, together with the state social health insurance schemes (SSHIS) should simply manage, coordinate and regulate the UHCS, social health insurance, and voluntary health insurance. In addition, a different national purchasing agency and fund manager—

preferably the National Primary Health Care Development Agency (NPHCDA) at the national level and subnationally, the State Primary Health Care Development Agencies (SPHCDA)—should manage this pool of funds in order to ensure inclusivity and facilitate resource allocation and redistribution.[42,43]

Nigeria has a decentralized health system; hence, funding for a UHCS should come from the national and subnational levels of government. Federal and state governments should work together to enroll PVIS populations in the UHCS. The Boards of the NPHCDA and SPHCDAs as the new purchasing agencies and fund managers should allocate annual per capita budget directly to public and private PHC providers in the states based on registered population, weighted by health needs.

Strategic purchasing Tax funds generated for the UHCS should be allocated to purchase health services from public and private PHC providers, guaranteeing a comprehensive health benefit package for PVIS populations, a responsibility assigned to both the NPHCDA and SPHCDAs as administrators/managers of the UHCS. These agencies should also coordinate registration and accreditation of public and private PHC facilities for participation in the UHCS scheme and negotiate prices for health services included in the comprehensive health benefit package. A contract should be drawn up between the new national purchasing agency and PHC providers, specifying the health benefit package, cost of health services, payment mechanisms and other performance requirements related to provision of health services for PVIS populations.[32] The proposed mode of payment such as capitation and diagnosis-related group rates should be fair and updated annually, as is done in Thailand,[20] in contrast to Nigeria's situation with failure to update capitation rates for years.[28]

The NPHCDA and SPHCDAs should guarantee monitoring and supervision of health services provided by enlisted public and private PHC facilities, to ensure quality of care and take necessary actions against poor practices. They should also ensure continuous availability of medicines, diagnostic equipment and constant improvements in health service quality. Programs should be developed to strengthen NPHCDA and SPHCDA purchasing skills, operational capacities and administrative capabilities.

BENEFITS OF A UHC STRATEGY

Affordability and financial accessibility The lack of direct out-of-pocket payment and user fees for public health care by PVIS populations would ensure financial accessibility for health services, thereby expanding coverage. Enlarging the tax base and achieving greater efficiency in managing health care resources would provide additional annual health spending needed to provide UHCS for PVIS populations. Public health spending of at least 3% of GDP is needed to kick-start this UHC reform, while governments work toward the goal of 5% of GDP in the long term. Government health expenditure as a percentage of total health expenditure would increase as a result of such a tax-based noncontributory universal health-financing system. Also, government health spending as a percentage of GDP would increase, since out-of-pocket payments for priority health services among poor and vulnerable groups would be virtually eliminated. However, the increase in government health spending as a percentage of total health expenditure and GDP would require significant political commitment and a serious attempt to increase the tax base and revenues, allocating a large share of these resources to health.

Equity and efficiency The UHCS would ensure equity in access to health care by allocating funds and purchasing health services based on health needs of PVIS populations. The design of a comprehensive health benefit package for the whole population, including PVIS groups, can be expected to ensure equity in health benefits in several ways. It would increase health services utilization among PVIS populations and prevent catastrophic health expenditures and resulting increased impoverishment. NPHCDA and SPHCDAs' purchasing power would help control prices and increase access to quality care for beneficiaries. UHCS would generate greater funding with lower administrative costs.[32] It would shift funding away from tertiary care to PHC services for the PVIS population and ensure resource allocation to cost–effective interventions and services that address the heaviest burden of ill health among PVIS populations.[32] Thus, UHCS would ensure provision of resources to a range of health services at lower cost, while improving health outcomes and financial risk protection.[32]

Political acceptability, implementation and sustainability Political support for UHCS can be obtained based on the following principles and arguments:

- Access to health care is a right of all Nigerian citizens and should not depend on individual income or wealth.
- The current targeting of specific programmes for the poor is ineffective.
- There is low coverage with voluntary health insurance.
- PVIS populations cannot afford premiums/contributions.
- The current basic minimum package does not provide financial risk protection.
- Middle- and high-income households will support this proposal because of the benefits accruing from UHCS: increased utilization of and access to health services, reduction in out-of-pocket health payments, improved financial risk protection and improved health service quality.

Beneficiaries of a tax-based noncontributory health-financing scheme would ensure the reform's continuity by demanding political and financial commitment from political parties during election campaigns. Effective and strong political leadership as well as succession planning within implementing and regulatory agencies—NHIS, SSHIS, NPHCDA and SPHCDAs—would enhance UHCS sustainability.

Payment mechanisms (capitation and diagnosis-related group rates) would be used along with a spending limit in order to enhance political acceptability of the UHCS by political actors at national and subnational levels. Development of operational capacity and administrative capability in purchasing organizations would promote effective UHCS implementation, also enhanced by effective communication and awareness among beneficiaries. The proposed UHCS is sustainable if governments can generate more tax revenue and effectively tackle corruption in the health sector by using an e-payment mechanism to block leakage of health care resources, as well as ensuring transparency and accountability. Funding UHCS through general tax revenue in the context of a strong tax collection mechanism would ensure its implementation over the long term.

As Nigeria has consistently had the largest economy in Africa, there is a potential for increasing the fiscal space for health financing to meet the health needs of the population and provide a comprehensive health benefit package. There is also fiscal space for increasing tax funding.

The creation of this health benefit package for PVIS populations would ensure increased utilization of and access to needed health care services without financial hardship. Poor service delivery for PVIS populations should be bridged by providing financial incentives for health workers in rural areas and increasing the number of health workers. Efforts should be made to address other factors affecting motivation and retention of health workers in rural areas such as low and unpaid salaries and incentives; housing difficulties and distance of housing from the workplace; travel costs and hardships incurred from commuting to health facilities as well as unmet career development priorities.[44]

DRAWBACKS OF CURRENT UHC REFORM EFFORTS

Social health insurance in many developed countries has taken many years to achieve UHC.[45] Although the proposed UHC reform for Nigeria may also take some years, it is easier to collect taxes, improve tax administration and expand the tax base than to enforce payment of contributions from nonsalaried workers and those who cannot afford to pay for health insurance premiums or out of pocket. Efforts to decentralize health insurance to the states have limited capacity to expand insurance coverage for PVIS populations, who constitute >70% of Nigerians.

The National Health Act (No. 8, 2014) established a Basic Health Care Provision Fund (BHCPF) with 50% to be used to provide a basic minimum package of health services to all citizens in eligible primary and secondary health facilities.[46] However, this is not an adequate health-financing mechanism toward achieving UHC in Nigeria, because a limited health benefit package will not reduce high out-of-pocket payments that result in a high incidence of catastrophic medical expenditures and impoverishment for poor and vulnerable households.[47] Furthermore, donor funding fluctuates from year to year and is dwindling, while the Federal Government annual grant of ≤1% of its Consolidated Revenue Fund (CRF) to the BHCPF is at the mercy of political uncertainty. In fact, the 1% of the CRF to BHCPF has yet to be allocated or disbursed; the federal government promised to begin disbursement in August 2018.[48]

While uncertainties remain about inclusion of 1% of the CRF in the 2018 appropriations bill almost four years after the National Health Act became law, there was presidential approval, without legislative approval, of withdrawal of $469.3 million for purchase of aircraft from the US government.[49] This alone indicates a lack of political will or commitment to address the issue of inadequate health spending for Nigeria's health system. Nor is it clear that state and local governments are committed to implementing the BHCPF, since their refusal to support similar policies over the decades has affected successful implementation.

In addition, the eventual approval of 57.15 billion naira (US$158.75 million) for BHCPF in the 2018 Appropriations Bill[50] may be affected by poor budget implementation and lack of clarity on how to manage the fund.

In any serious reform towards UHC, it will be critically important to tackle issues of inadequate governance, funding mismanagement, corruption, and lack of transparency and accountability within the NHIS, SSHIS, NPHCDA and SPHCDAs. This is essential to ensure that funds for expanding health insurance coverage to PVIS populations are well managed to provide equitable access to quality health care, financial risk protection and improved health outcomes. As indicated in this paper, there is urgent need for major tax policy reforms at national and subnational levels to increase general tax revenues from 6% of GDP to the international benchmark for developing countries of ≥15%,[51,52] and for these funds to be dedicated to provision of health insurance coverage, particularly for PVIS populations.

CONCLUSIONS

Expanding health coverage to PVIS populations is a major challenge for achieving UHC in Nigeria. Evidence from other countries suggests that it is difficult to achieve UHC through contributory insurance schemes when there is a large informal sector. Therefore, policy makers should ensure design and effective implementation of a tax-based, noncontributory, universal health-financing system. This will be critical if Nigeria is to achieve UHC by 2030.

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Submitted: May 9, 2018
Approved for publication: October 7, 2018
Disclosures: None

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UK Medical Students' Experience of Culture & Communication in Cuban Health Care

Jacob Levi MBBS and Isabelle Erbacher MBBS

Final-year medical students at UK universities are able to do elective placements elsewhere, to learn about other health care systems. We chose Cuba because of its excellent health outcomes despite relatively few resources. We were struck, for instance, when Cuba made global headlines in 2015 as the first country to eliminate mother-to-child transmission of HIV and syphilis. And, since the UK's underfunded National Health Service is experiencing high demand and increasing pressure, we sought to understand more about Cuba's socialist health system, where health care spending per capita is much lower than the UK's, but life expectancy is almost the same.

In spring 2018, we worked for several weeks at two large obstetric hospitals, one in Havana and another in Santiago de Cuba. While there, we noted differences in doctor–patient and doctor–doctor relationships, compared with the UK (realizing that these are of course subject to cultural norms). Since these were specialized secondary hospitals, we cannot comment on communication norms in primary care settings, where doctors and patients tend to know each other better and have more time to discuss concerns and expectations, and where most women get their antenatal care (with many questions probably answered there before labor even begins).

Communication is fundamental to quality health care. Much of our communication training in the UK focuses on providing patient-centered care, emphasizing provision of clear, understandable information about patients' examinations, diagnosis and treatment. The goal is to facilitate autonomy as well as listening to and answering patients' questions. Obstetrics is a particularly intimate specialty, and pregnancy and childbirth can induce anxiety. In the UK where the doctor–patient ratio is 1:357 (one of the poorest in Europe,[1]) doctors are often unable to dedicate enough time to individual patients because hospitals are dramatically understaffed.

We wondered if Cuba's 1:125 doctor–patient ratio[2] would give doctors more time for communication, but observed that patients often neither expected nor were given copious amounts of information, and instead trusted their doctors to act in their best interests. We witnessed situations where laboring or miscarrying patients were frightened and had questions. Often, they got the information they sought—evidence shows that patients benefit from being involved in treatment decisions and require sufficient information for informed consent—[3] but it was delivered in a very different style from that in the UK. For instance, when a young woman presented with severe abdominal pain in her ninth month and was diagnosed with placental abruption (a dangerous obstetric emergency) she was given only minimal explanation before being rushed to the OR for an emergency C-section; both mother and child were in good health following the birth. It was apparent that she trusted hospital staff to make the right decisions and do what was necessary to deliver her child safely; she did not demand more information from doctors and nurses.

Another contrast between the UK and Cuba is the notion of privacy, possibly due to differing space and resource availability, or to different cultural norms and expectations. The ob-gyn history involves intimate questions about the patient's sexual life, and we observed that, unlike in the UK, Cuban patients often chose to see doctors with a large support network of family members who remained present throughout the consultation, and did not seem to mind if others were present. In Cuban ob-gyn emergency departments in particular, multiple patients sometimes occupied the same room—even sometimes undergoing intimate examinations with relatives present, or even with other nonmedical hospital workers in the room. In contrast, breaching the UK's strict privacy and confidentiality rules can have serious consequences for doctors. UK patients would not tolerate this lack of privacy, but Cuban patients did not seem to feel uncomfortable about it. We also observed that there is less of a taboo around sexuality in Cuba, and people discuss their sexual histories more openly.

The communication among doctors was in some ways more open and honest than we were accustomed to in the UK, where a hierarchy among health care providers can make it difficult (and daunting) for juniors to speak up if they have questions, or importantly, if they think a senior is making a mistake. A hostile work environment can be dangerous, impedes junior doctors from taking responsibility and denies them valuable learning opportunities. The interactions we witnessed between Cuban doctors were respectful, and juniors' concerns were listened to and taken seriously. Such a supportive environment fosters open and honest communication, comradeship and ultimately, we feel, improves patient safety.

It took some time for us to learn, accept and become comfortable with these different cultural norms, before we were able to participate fully in Cuban hospitals. Once we did, however, we found that health care staff were exceptional teachers and keen to share their knowledge with juniors. We learned a great deal about these cultural differences, especially after discussing them with Cuban doctors who had had medical placements abroad.

In medicine, as in many aspects of life, learning to appreciate and understand cultural differences can lead to mutual benefits and new ideas. Our Cuban colleagues and patients must have had similarly bewildered thoughts about our culture and behavior. Our experiences in Cuba helped reinforce the importance of knowledge sharing and understanding the diversity of attitudes and styles in health care provision.

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Submitted July 6, 2018
Approved: October 10, 2018
Disclosures: None
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The Enigma of US Diplomats' Health Symptoms in Havana: Call For a Global Scientific Meeting

Luis Velázquez-Pérez MD PhD DrSc

In today's globalized world, scientific endeavor and its conclusions rest more than ever on dialogue and the ability to critically assess a full and transparent array of evidence, to maximize opportunities for accuracy and truth to prevail.

But such a normal, essential and prudent process has not been followed in the case of the health incidents reported by US diplomats in Havana in 2017. Instead, we see transparency and open scientific discussion hampered by lack of access to vital information, as well as publication of methodologically faulty research, analysis and unsubstantiated conclusions. For want of a thorough scientific approach, the essential questions remain unanswered, and those reporting symptoms still have no real explanation for their experience.

As President of the Cuban Academy of Sciences, I am surrounded by some of the best minds in Cuba. As in other nations, our mission is to advise government agencies and maintain links with counterparts and others in the global scientific community based on the premise that sharing and communicating knowledge contributes to the well-being of all our peoples.

For nearly 20 years, I directed the Center for Hereditary Ataxias Research and Rehabilitation in the eastern province of Holguín, an institution that accumulated notable successes nationally and internationally, in large part due to the priority accorded health by the Cuban government and its universal public health system. Research was the cornerstone of such advances, which depended on national and international scientific cooperation. Our scientists trained with others from the USA, Canada, Germany, France and other countries. Together, we organized events, published papers, examined and treated patients, and created networks of collaborators, mainly with researchers in the USA and Cuba, based on open exchange without ideological prejudice.

As a neuroscientist and as President of the Cuban Academy of Sciences, I address my comments here to the international scientific community, concerning the health symptoms reported by US diplomats in Havana and subsequent hypotheses. By and large, my observations are critical.

In particular, Swanson's 2018 article in *JAMA* describes a series of nonspecific neurological manifestations—including cognitive, vestibular and oculomotor alterations—in 21 US diplomats in Havana, initially associated with exposure to auditory and sensory phenomena and categorized by the US State Department as "sonic attacks."[1]

The article has been criticized by the Cuban and international scientific communities for its inconsistencies in research design, long time lapse between symptoms and interviews, and use of subjective tools to assess oculomotor deficits [2]. Moreover, cognitive alterations were diagnosed using test thresholds with low specificity.[3]

However, in a description also criticized internationally, the authors interpret their results as a new neurological syndrome involving brain damage without previous trauma. Brain damage is one of the conditions most studied by clinical neuroscientists, leading to knowledge of multiple aspects of its physiopathology and the main therapeutic options' primary mechanisms of action. These advances are based fundamentally on traumatic brain lesion research, since this represents the largest share of morbidity and mortality, and a serious health and socioeconomic problem. Nevertheless, the appearance of common neurological symptoms without evidence of trauma or exposure to toxins is an enigma. Their diagnosis is complex and requires exhaustive case studies and use of highly objective tools that lead to a differential diagnosis that rules out multiple neurological conditions exhibiting a similar clinical picture. The fact that all MRI results in the Swanson study were normal means that there is no objective clinical basis for assuming brain damage.

At the same time, Cuban specialists have not been permitted access to the US patients in order to contribute to a multidisciplinary, multinational examination. Nor, despite repeated requests to the US government, have we been able to establish personal contact with the physicians who assessed the diplomats, to develop a scientific exchange in which we could share observations and analysis.

On the basis of such a broad array of symptoms, without evidence of damage, and given the faulty methodology of the only US-government–authorized publication on the subject, it is impossible to conclude scientifically that any of these patients was targeted by an outside entity or person, as has been suggested.

It is essential to convene an international, broadly attended meeting to discuss the health symptoms described and review the evidence. I would propose such a meeting include the US scientists who directly participated in assessing the diplomats, scientists from Canada and Cuba, and other specialists who might contribute to clarifying this problem. With the sponsorship of our respective academies of sciences and on the basis of open exchange and full access to relevant information, we could discuss the date, format, venue and participation.

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Submitted: October 8, 2018
Approved for publication: October 15, 2018
Disclosures: None
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ISSN 1555-7960