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EDITORIAL

Organ donation and transplantation in Mexico, is everything solved?

Donación de órganos y trasplantes en México, ¿todo está resuelto?

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In 1954, in Boston, United States, was the first successful kidney transplant performed in humans. In 1963, in that same country, Hardy carried out the first lung transplant, and Starzl, the first liver transplant. In 1967, in South Africa, Barnard performed the first heart transplant.

In Mexico, in 1963, doctors Manuel Quijano, Regino Ronces, Federico Ortiz Quezada and Francisco Gómez Mont performed the first kidney transplant from a living donor at the National Medical Center (CMN – *Centro Médico Nacional*) of the Mexican Institute of Social Security (IMSS – *Instituto Mexicano del Seguro Social*), today CMN *Siglo XXI*.[1] In 1976, at the National Institute of Nutrition (currently National Institute of Medical Sciences and Nutrition "Salvador Zubirán" [INCMNSZ - *Instituto Nacional de Ciencias Médicas y Nutrición "Salvador Zubirán"*]), Dr. Héctor Orozco carried out the first auxiliary liver transplant, with a pediatric donor graft, in an adult female patient; in 1985, Dr. Orozco performed the first orthotopic liver transplant, also at INCMNSZ.[2] In 1988, Dr. Rubén Argüero performed the first heart transplant at the Specialty Hospital of IMSS *La Raza* Medical Center.[3] In 1989, doctors Jaime Villalba Caloca and Patricio Santillán performed the first lung transplant at the National Institute of Respiratory Diseases.[4] All these "first transplants" in Mexico took place in the capital of the country.

Obtaining a heart with effective beats for transplantation purposes transformed the culture related to brain death: it favored the development of transplant programs, not only of the heart but of other organs and tissues, since from then on, using biological material started being allowed. The procurement of organs and tissues from people with brain death was promoted, which in Mexico was accompanied by successive reforms and adaptations to the General Statute of Health with regard to organ and tissue donation and transplantation. This way, an opportunity was opened to the benefit of numerous patients.

Thirty-one years after the first heart transplant, the scenario in Mexico is characterized by a shortage of donations and transplants. There is an unmet demand that grows year after year, even when, in 2017, there were 255 authorized centers for kidney transplantation, which makes our nation one of the countries with the largest number of them in the world; regarding other organs such as liver, heart, lung and pancreas, it is the country with the highest number of authorized transplantation centers in Latin America.[5] Nevertheless, the processes related to donation and transplantation suffer from a lack of standardization, comparable quality, supervision, analysis and strict adherence to protocols that enable assessing the results in authorized centers in order to grant or revoke licenses when the opinion of an *ad hoc* committee thus recommends.

In addition to recurrent financial problems, there is uncertainty regarding the continuity of, and support to, transplantation programs by federal authorities. There is a documented lack of inter-institutional

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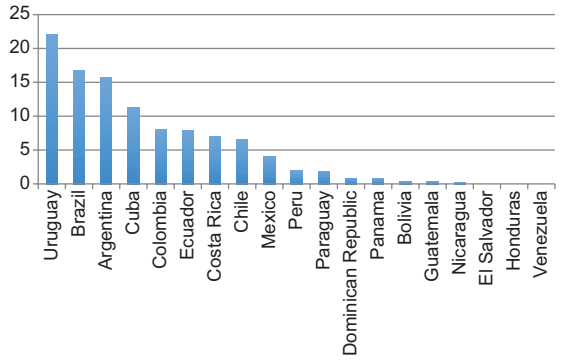

Figure 1. *Rates of organ donation from brain-dead donors per million population in different countries of Latin America, according to Global Observatory on Donation and Transplantation data (http://www.transplant-observatory.org/) corresponding to 2018.*

collaboration in agreements and information on inter-institutional productivity that includes private hospitals. In Mexico, reflecting on the distributive inequality of donations and transplants is necessary, which is a problem the fractionation of the National Health System, among other factors, has contributed to.

An important measure to correct this trend would be the establishment of fair inter-institutional collaboration agreements that are open to public knowledge and that contribute to minimize the distrust and the privileged position of some sectors with regard to others. Said agreements must create mechanisms that in practice overcome the division of the National Health System and drive to a universal health system, that make of the right to receive a transplant a reality without prejudice for being affiliated to one health institution or another.

An exercise that reveals the productivity of Mexico's National Transplantation System, currently known as the National Donation and Transplantation Subsystem (SNDT – *Subsistema Nacional de Donación y Trasplante*), is the comparison of the rates of donated organs obtained from people with brain death per million population in various Latin American countries in 2018, an information that stems from the Global Observatory on Donation and Transplantation (http://www.transplant-observatory.org/). The rates are higher than those of Mexico in most analyzed Latin American countries, which shows that, although organ transplantation absolute numbers in Mexico have increased, when the figures are expressed as rates per million population, cadaveric organ donation in Mexico is observed to have not had a significant increase over the last 40 years,[6] which explains the increasing number of patients waiting for an organ in our country (Fig 1).

Together, these elements of analysis characterize SNDT as a system that lacks a common and comprehensive program that groups both health institutions and private hospitals, as well as a definition of objectives, goals, indicators and growth strategies; in addition, it lacks inter-institutional collaboration mechanisms or possible remuneration to the personnel that enable the efficiency of SNDT members. In a few words, SNDT lacks planning, leadership, programs, and requires deep reengineering.

An omnipresent characteristic of donation-transplantation programs in Mexico has been an orientation towards creating a "donation culture", under the assumption that the main obstacle to the performance of transplantations is family refusal; little attention is given to measuring and evaluating the efficiency of medical processes and sub-processes of hospitals that are authorized for these activities. It is necessary for the flaws or limitations in the donation process to be identified beyond family refusal.

Organ donation is a process that involves various stages, each one with sub-processes where services, departments and multiple health professionals interact; in the course of these sub-processes, there are setbacks, inefficiency and shortages. Although the family interview is important, it constitutes only one of those sub-processes and there is a tendency to forget that the other ones –of medical, technical, logistical or resource management-type– can hinder or obstruct the willingness to donate.

Changes in hospital dynamics are required in order to improve donation processes, and not only attributing the low number of donations and transplants to the refusal of potential donor relatives. We must strive for programs that guarantee quality and facilitate internal and external auditing in hospitals and in the various medical services involved, in order to continuously improve them and eliminate obstacles that prevent reaching figures similar to those of countries that are successful in organ donation and transplantation. It is clear that there is a need to make a stop along the way and carry out a critical analysis in order to improve the results and rearrange whatever is required to reach international standards.

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ORIGINAL ARTICLE

Prevalence of overweight and obesity in school-age children

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Abstract

Introduction: *Childhood obesity is a public health challenge. Between 1999 and 2012, the prevalence in Mexico of overweight and obesity in schoolchildren went from 25.5 to 32 %.* **Objective:** *To report current prevalence of overweight and obesity in schoolchildren from the municipality of Durango, Mexico.* **Method:** *Cross-sectional survey conducted between January 2017 and December 2018. A total of 24,600 children aged between six and 11 years from 138 schools of the municipality of Durango were included. The body mass index reference values established by the World Health Organization were used to determine the presence of overweight and obesity.* **Results:** *The prevalence of overweight was 19.7 %, of obesity, 16 %, and of overweight and obesity combined, 35.7 %. In the six-year-old group, a prevalence of overweight-obesity of 25.4 % was found, and in the 11-year-old group, 41.1 %.* **Conclusions:** *The prevalence of overweight-obesity in children aged from 6 to 11 years in the municipality of Durango is higher than those reported in the national survey by states in 2012 and in the 2016 national survey; a trend towards an increase with age was observed.*

KEY WORDS: *Obesity. Overweight. School-age children.*

Prevalencia de sobrepeso y obesidad en niños escolares

Resumen

Introducción: *La obesidad infantil es un reto de salud pública. Entre 1999 y 2012, en México la prevalencia de sobrepeso y obesidad (SO) en niños escolares pasó de 25.5 a 32 %.* **Objetivo:** *Reportar la prevalencia actual de SO en niños escolares del municipio de Durango, México.* **Método:** *Encuesta transversal realizada entre enero de 2017 y diciembre de 2018. Se incluyeron 24 600 niños de seis a 11 años, de 138 escuelas del municipio de Durango. Se utilizaron los valores de referencia del índice de masa corporal establecidos por la Organización Mundial de la Salud para determinar la presencia de SO.* **Resultados:** *La prevalencia de sobrepeso fue de 19.7 %, la de obesidad de 16 % y la de SO de 35.7 %. En el grupo de seis años se encontró una prevalencia de SO de 25.4 % y en el de 11 años, de 41.1 %.* **Conclusiones:** *La prevalencia de SO en niños de seis a 11 años del municipio de Durango es más elevada que la reportada en la encuesta nacional por entidad federativa en 2012 y la nacional en 2016; se observó tendencia al incremento en la prevalencia conforme aumenta la edad.*

PALABRAS CLAVE: *Obesidad. Sobrepeso. Escolares.*

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Introduction

Although the prevalence of overweight and obesity in children has increased over the past 50 years, both in developing and developed countries, the childhood obesity increase rate is up to 30 % higher in countries with low or moderate income than in those with a high income.[1] This has made for childhood obesity to be considered as an emerging global public health problem, with not-yet-determined social and economic consequences.

Children with overweight and obesity generally continue to be obese throughout their lives and are more likely to develop non-communicable diseases such as diabetes, cardiovascular disorders, dyslipidemia, and orthopedic, neurological, lung and liver problems.[2,3]

In Mexico, the prevalence of overweight and obesity in school-age children showed a significant increase between 1999 and 2012: it went from 25.5 to 32 %.[4]

The results of the 2012 National Health and Nutrition Survey (ENSANUT – *Encuesta Nacional de Salud y Nutrición*)[5] showed that, in the state of Durango, the prevalence of overweight and obesity in children aged from five to 11 years was 33.8 %, and it was higher in boys (37.5 %) than in girls (29.8 %). "Half-Way" ENSANUT in 2016[6] reported a national prevalence of overweight-obesity of 32.8 % in girls and 33.7 % in boys; in northern Mexico, the prevalence of overweight-obesity in children aged from 5 to 11 years was 29.5 %.

The purpose of this study was to determine the prevalence of overweight-obesity in children who attend primary schools in the municipality of Durango.

Method

After approval of the study by the Research Ethics Committee of the Mexican Institute of Social Security (2016-785-103), and after informed consent was granted by the parents of all participants, a descriptive, cross-sectional study was carried out in collaboration with the National System for Comprehensive Development of the Family of Durango, through the Comprehensive Health program that the state government has implemented in primary schools, which among its actions includes nutritional assessment of the children enrolled in primary schools of the public system.

Between January 2017 and December 2018, all children aged between six and 11 years included in the Comprehensive Health program for that period and who were enrolled in 138 (68.3 %) schools out of a total of 202 located in the municipality of Durango were included.

Previously trained nutrition professionals determined weight and height using scales (Beurer, model MS 50) and portable stadiometers (Seca, model 213).

The overweight-obesity diagnosis was established according to the body mass index (BMI) based on the World Health Organization reference tables (BMI Z-values-for-age from five to 19 years).[7] According to the number of inhabitants, the localities were classified as urban (more than 2,500 inhabitants) and rural (less than 2,500 inhabitants).

The information was collected in Excel spreadsheets and analyzed with the SPSS statistical program, version 15.

Results

A total of 24,600 children were included in the study, out of which 77.1 % came from urban localities and 22.9 % from rural localities. The prevalence of overweight-obesity was 35.7 % (19.7 % overweight and 16.0 % obesity); in urban schools, the prevalence was 36.7 %, while in rural schools it was 31.7 %.

In urban localities, the prevalence of overweight was higher in girls (21.2 %) than in boys (19.1 %), while the prevalence of obesity was higher in boys (19.2 %) than in girls (14.3 %). A similar pattern was observed in rural areas, with more overweight in girls (18.9 %) than in boys (17.7 %) and higher prevalence of obesity in males (15.0 %) than in females (11.7 %) (Table 1).

When the results were analyzed by age, the prevalence of overweight-obesity was 25.4 % in six-year-old children and 41.1 % in those aged 11 years (Table 2).

Discussion

The prevalence of overweight-obesity in children aged from 6 to 11 years in the municipality of Durango was 35.7 %, which is 1.9 % higher (1.2 % overweight and 0.7 % obesity) than that reported in ENSANUT 2012[5] (Table 3).

According to Half-Way ENSANUT 2016 data, the national prevalence of overweight-obesity was 33. 2%.[6] Given that the survey did not provide results by state, the prevalences recorded in our study were compared with those reported in ENSANUT 2012.[5] By gender, the prevalence of overweight-obesity had an increase of 4.5 % in girls, while in boys it decreased by 0.5 %.

Table 1. Prevalence of overweight and obesity by type of locality and gender

Locality	Girls									Boys								
	Normal weight		Overweight		Obesity		Overweight + obesity		Total	Normal weight		Overweight		Obesity		Overweight + obesity		Total
	n	%	n	%	n	%	n	%	n	n	%	n	%	n	%	n	%	n
Urban	6147	64.6	2014	21.2	1358	14.3	3372	35.4	9519	5829	61.7	1803	19.1	1813	19.2	3616	38.3	9445
Rural	1977	69.4	537	18.9	334	11.7	871	30.6	2848	1876	67.3	493	17.7	419	15	912	32.7	2 788
Total	8124	65.7	2551	20.6	1692	13.7	4243	34.3	12367	7705	63	2296	18.8	2232	18.2	4528	37	12233

Table 2. Prevalence of overweight and obesity by age

Age (years)	Girls									Boys								
	Normal weight		Overweight		Obesity		Overweight + obesity		Total	Normal weight		Overweight		Obesity		Overweight + obesity		Total
	n	%	n	%	n	%	n	%	n	n	%	n	%	n	%	n	%	n
6	1640	76.1	334	15.5	180	8.4	514	23.9	2154	1606	73.1	353	16.1	237	10.8	590	26.9	2196
7	1417	69.0	372	18.1	266	12.9	638	31.0	2055	1437	66.7	376	17.4	344	15.9	720	33.3	2157
8	1349	62.6	475	22.1	330	15.3	805	37.4	2154	1258	63.6	346	17.5	374	18.9	720	36.4	1978
9	1338	63.9	468	22.3	290	13.8	758	36.1	2096	1194	59.4	379	18.9	435	21.7	814	40.6	2008
10	1270	61.8	463	22.5	323	15.7	786	38.2	2056	1153	55.9	444	21.5	467	22.6	911	44.1	2064
11	1110	59.9	439	23.7	303	16.4	742	40.1	1852	1057	57.8	398	21.7	375	20.5	773	42.2	1830

Table 3. Prevalence of overweight and obesity in Durango according to ENSANUT 2012 and to the present study

	Girls			Boys		
	Overweight	Obesity	Overweight + obesity	Overweight	Obesity	Overweight + obesity
2012*	15.9 (10-2 23.9)	13.9 (9.2-20.3)	29.8 (21.9-39.0)	20.8 (15.7-27.0)	16.7 (11.0-24.4)	37.5 (30.2-45.4)
2018**	20.6 (19.8-21.3)	13.7 (13.0-14.3)	34.3 (33.4-35.1)	18.8 (18.1-19.4)	18.2 (17.5-18.8)	37.0 (36.1-37.8)

The values represent the prevalence and 95 % confidence intervals.
*ENSANUT 2012 data[5]; **Data from this study.

The increase in the prevalence of overweight-obesity in girls was determined by the significant increase in the prevalence of overweight, which went from 15.9 % to 20.6 % in six years. In boys, overweight decreased by 2 %: from 20.8 %, it went to 18.8 %. The prevalence of obesity in girls decreased by 0.2 %, while in boys, it increased by 1.5 %.

Regarding the type of locality, the prevalence of overweight-obesity increased by 1.2 % (0.6 % overweight and 0.6 % obesity) in urban localities, while in rural areas the increase was 1.7 % (1.9 % overweight and 0.2 % obesity).

When the data were analyzed by age, the prevalence of overweight-obesity was lower at six than at 11 years of age (25.4 and 41.1 %). When the information was broken down by gender, the same trend was observed. This result suggests that, at the time of admission to primary school, one fourth part of the children have overweight or obesity problems, probably related to lifestyle in the family environment and genetic factors (genome and gut microbiome) and to the fact that in schools there are adverse environments that favor the development of overweight and obesity.

In comparison with ENSANUT 2006,[8] which, like our study, provided results by age groups, the fact that the prevalence of overweight-obesity in 11-year-old children (32.5 %) was higher than in those aged five (17.7 %) stood out. Similarly, the prevalence of overweight-obesity in 11-year-old girls was higher (29.5 %) than that of five-year-old girls (21.2 %).

In comparison with ENSANUT 2012,[5] ENSANUT 2006[7] reported a slight decrease in the prevalence of overweight in girls (16.5 % versus 15.9 %), and thus the prevalence of 20.6 % documented in the present study is the highest observed in the last 12 years in the state.

The increase in the prevalence of overweight in girls should be considered as a warning, since the risk of developing obesity and the comorbidities associated with it increase if this condition continues until adolescence, a stage in which there are changes in the body composition associated with puberty (characterized by a decrease in insulin sensitivity, which promotes an increase in body fat), togetheer with a decrease in regular physical activity and increased intake of foods with higher caloric content.[9]

Even when the problem represented by the high prevalence of overweight-obesity is well documented in schoolchildren, and various nutritional education interventions and physical activity recommendations have been established, these have not been effective for stopping it, which suggests that public policies in this area require substantial modifications.[10,11]

Access to the entire sample conferred robustness and reliability to the results, as demonstrated by the reduced confidence intervals, which are considerably lower than those obtained in ENSANUT 2006 and 2012.[7,5] One weakness of our study is that the recorded prevalence might not represent that of other rural populations of the state, due to their proximity to the city of Durango.

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Conflict of interests

None

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Ethical disclosures

The authors declare that no experiments were performed on humans or animals for this investigation.

Confidentiality of data. The authors declare that they followed the protocols of their workplace regarding the publication of third-party data.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

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ORIGINAL ARTICLE

Natural killer cell reconstitution after hematopoietic stem-cell transplantation in children

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Abstract

Introduction: *After hematopoietic stem cell transplantation (HSCT), natural killer (NK) cells reconstitution is the main barrier against viral infections.* **Objective:** *To determine that the knowledge on the kinetics of NK cell reconstitution after HSCT contributes to transplant efficient monitoring, which increases the possibility of its success.* **Method:** *Twenty-one patients undergoing HSCT were included, as well as a control group of clinically healthy individuals. At different time points after transplantation (range of 21 to 670 days), CD3- CD16+ CD56+ NK cells were quantified by flow cytometry in peripheral blood samples.* **Results:** *NK cell recovery occurs at three to six months and 10 to 12 months post-transplantation; their number was significantly lower (in comparison with the control group) in the rest of the monitoring time.* **Conclusions:** *The first period of NK cell recovery occurs between three and six months after transplantation. Reconstitution is transient and the number of NK cells varies in the first years.*

KEY WORDS: *Natural killer cells. Transplantation. Hematopoietic cells. Cell reconstitution.*

Reconstitución de células natural killer después del trasplante de células progenitoras hematopoyéticas en niños

Resumen

Introducción: *Después de un trasplante de células progenitoras hematopoyéticas (TCPH), la reconstitución de las células natural killer (NK) es la principal barrera contra las infecciones virales.* **Objetivo:** *Determinar que el conocimiento sobre la cinética de la reconstitución de las células NK posterior al TCPH contribuye a un eficiente monitoreo del trasplante, lo que incrementa la posibilidad de éxito de este.* **Método:** *Se incluyeron 21 pacientes sometidos a TCPH, así como un grupo control de individuos clínicamente sanos. En diferentes momentos después del trasplante (intervalo de 21 a 670 días), mediante citometría de flujo se cuantificaron las células NK CD3– CD16+ CD56+ en muestras de sangre periférica.* **Resultados:** *La recuperación de las células NK ocurre a los tres a seis meses y a los 10 a 12 meses postrasplante; su número fue significativamente menor (en comparación con el grupo control) en el tiempo restante del monitoreo.* **Conclusiones:** *El primer periodo de recuperación de las células NK ocurre entre los tres y seis meses posteriores al trasplante. La reconstitución es transitoria y el número de células NK varía en los primeros años.*

PALABRAS CLAVE: *Células natural killer. Trasplante. Células hematopoyéticas. Reconstitución celular.*

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Introduction

Natural killer (NK) cells are generated from a lymphoid precursor originating in bone marrow progenitor cells.[1,2] Their morphology is similar to that of T and B lymphocytes, but they are profusely granulated and are identified by the absence of CD3 (a molecule that is exclusive to T lymphocytes) and CD16 and CD56 expression (more than 90 % of NK cells in peripheral blood express both), although there are subpopulations with the CD16- CD56+ and CD16+ CD56- phenotypes, which constitute between 5 and 10 % of the total numbers of this type of cells.[3] NKs are the first defense barrier against pathogens (mainly viruses) and tumor cells. Their activation and inhibition occurs after the recognition of human leukocyte antigen (HLA) alleles through immunoglobulin-like receptors that inhibit killer cells, which can interact with a single HLA allele or with several.[4]

Regardless of the graft source (bone marrow, mobilized peripheral blood, or umbilical cord cells), reconstitution of the NK cell population is achieved one to two months after allogeneic hematopoietic stem cell transplantation (HSCT). In general, NK cells that are generated during this period do not derive from the expansion of mature NK cells of the graft, but mainly from differentiation and maturation of progenitor cells.[5-8]

Furthermore, in allogeneic transplants, alloreactivity of graft cells[9-11] (mature cells) or of those generated in the recipient after HSCT can occur, which is an event that can have a cytotoxic effect on the tumor cells that resisted or reappeared after HSCT.[12]

Reconstitution of innate immunity cells is critical in immunosuppressed patients, as well as in allogeneic or autologous HSCT recipients, owing to their role in infection control. In the latter case, since the reconstitution of T and B cells requires at least six months, prompt recovery of cell lines associated with the control of pathogens, such as NK cells, is relevant.

NK cell reconstitution after HSCT requires time to achieve homeostasis of this cell line in the first years after allogeneic transplantation. Monitoring of this process will contribute to a comprehensive assessment of the transplant recipient, by including laboratory tests and clinical follow-up, in order to increase the possibility for the procedure to be successful.

Method

An observational, prospective and descriptive study of children undergoing allogeneic HSCT between January 2017 and January 2018 was carried out at the Stem Cell Transplantation Unit and the Clinical Laboratory of the Children's Hospital of Mexico "Federico Gómez". The protocol was approved by the Research, Ethics and Biosafety Committees of the hospital. Parents, legal guardians or caregivers granted written consent after being informed on the purposes of the research.

The patients received a myeloablative conditioning or reduced-intensity regimen (according to the underlying pathology), and subsequently were infused at least one dose of 2 x 10^6 CD34+ cells/kg of body weight. A control group (n = 20), made up of clinically healthy individuals, with no fever, or infection diagnosis or on treatment with antibiotics or antivirals in the previous five days, was included.

From day 21 to day 670 of the post-transplantation stage, approximately 1 mL of peripheral blood was collected from each individual. Blood count was carried out on a Coulter LH 780® equipment (Beckman Coulter, Brea, CA, USA) in order to determine leukocyte total count, as well as the percentage of lymphocytes of the individual. NK cell phenotyping and quantification (CD3- CD16+ CD56+) were performed by flow cytometry; at least 50,000 cells were analyzed. NK cell determinations were made and absolute values (number of cells per liter) were calculated. In addition, HLA genotyping data, which was previously obtained at the Histocompatibility Laboratory, were collected.

Results

During the period from January 2017 to January 2018, 21 patients undergoing HSCT were included. The diagnoses they were referred to the Hematopoietic Stem Cell Transplantation Unit for were the following: acute lymphoblastic leukemia, 38.09 % (n = 8); medullary aplasia, 28.57 % (n = 6); acute myeloid leukemia, 19.04 % (n = 4); other solid tumors, 14.28 % (n = 3) (Fig. 1); nine patients (43 %) were males and 12 (57 %) were females; age ranged from one to 17 years (Table 1).

Some patients received a myeloablative regimen, while others received a reduced-intensity regimen (depending on the underlying pathology) prior to allogeneic peripheral blood hematopoietic stem cell transplantation, after mobilization with granulocyte-colony stimulating factor. Compatibility between donor and recipient was defined based on HLA-A, HLA-B, HLA-C and HLA-DP alleles genotyping in two patients, and in the remaining 19, HLA-DQ and HLA-DR were also

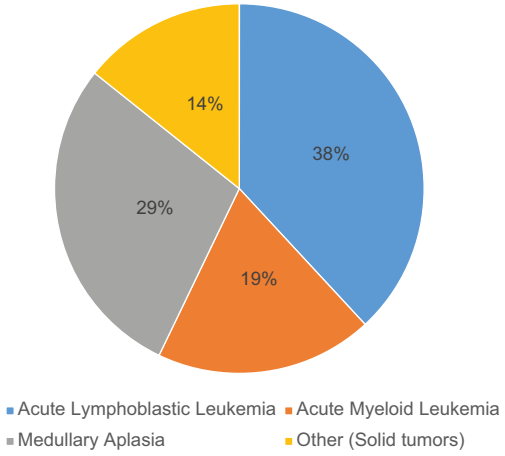

Figure 1. *Diagnoses hematopoietic stem cell transplantation was carried out for.*

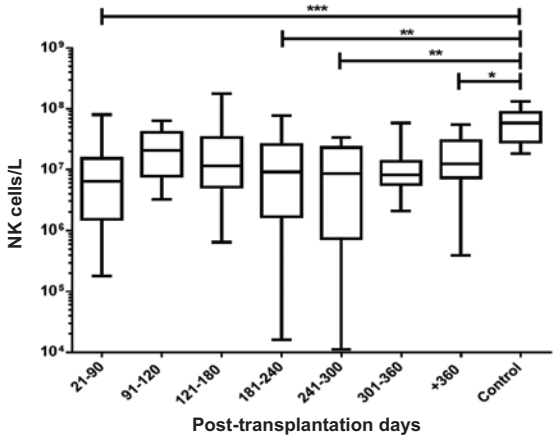

Figure 2. *NK cell reconstitution kinetics after hematopoietic stem cell transplantation. Values were transformed to logarithms, and the p-values according to Kruskal-Wallis test ($p < 0.001$) and the multiple comparison are presented. *p = 0.043, **p = 0.0059, ***p < 0.0001.*

analyzed. Table 2 shows the shared alleles and if it was a haploidentical transplantation when the donor was one of the parents.

To analyze NK cell reconstitution kinetics in all 21 patients, 105 determinations were made, with an average of five per patient (minimum two and maximum 11). The obtained values were grouped by periods: from 21 to 90 days, from 91 to 120 days, from 121 to 180 days, from 181 to 240 days, from 241 to 300 days, from 301 to 360 days and more than 360. All categories were compared with the values obtained in the clinically healthy individuals (Table 3 and Figure 2). The origin of the cells identified during the first weeks could be due to expansion of the cells included in the graft (given that no CD34+ cell enrichment is made), while those subsequently detected would be generated by the recipient from cell precursors, mainly from the donor, as demonstrated by 95 % of chimerism detected in peripheral blood cells (Fig. 3).

Table 1. Demographic characteristics of patients undergoing HSCT and clinically healthy subjects of the control group

Demographic data	Patients with HSCT	Control subjects
Gender		
Males	9 (43 %)	17 (85 %)
Females	12 (57 %)	3 (15 %)
Age		
Range	1 to 17 years	8 to 17 years
Mean	7 years 6 months	10 years 1 month
Median	8 years 5 months	9 years

HSCT = hematopoietic stem cell transplantation.

Significant differences were observed with regard to NK cell absolute numbers in patients undergoing HSCT: during the entire study period, the numbers were lower than those in the control group (Table 3 and Figure 3).

Discussion

According to our observations in the analyzed patients, NK cell early recovery occurs in the period of three to six months post-transplantation; however, there are periods when the absolute number is observed to be lower, and various events such as infections and graft-versus-host disease (GVHD) could therefore negatively affect NK cell reconstitution.

No significant difference was found when the number of NK cells was analyzed in 36 samples from patients with GVHD versus 69 samples from patients without GVHD (data not shown), or when quantifications of samples with or without viral agents were compared: BK polyomavirus, cytomegalovirus , adenovirus, Epstein-Barr virus (data not shown); therefore, NK cell reconstitution in this group of patients was considered not to be significantly affected by these clinical entities.

This could be due to the fact that, in the four patients in whom BK polyomavirus viral DNA was detected, immunosuppressive treatment was reduced in order to allow efficient cell function (expansion and effector capacity, such as the production of interferon-gamma and cytotoxicity). When the detected viral DNA was from Epstein-Barr virus, cytomegalovirus or adenovirus (viral load lower than 1000 copies/mL of plasma),

Table 2. **Transplantation cell dose, conditioning regimen and HLA compatibility**

Patient	CD34+/kg cell dose	Conditioning regimen	HLA allele compatibility*
1	1.71×10^7	Fludarabine/cyclophosphamide	Haploidentical
2	7.4×10^6	Busulfan/cyclophosphamide	Allogeneic (12/12)
3	6.7×10^6	Cyclophosphamide/etoposide/radiotherapy	Allogeneic (8/8)
4	3.19×10^6	Busulfan/cyclophosphamide	Allogeneic (12/12)
5	5×10^6	Busulfan/cyclophosphamide	Allogeneic (12/12)
6	1.03×10^7	Busulfan/fludarabine	Haploidentical
7	4×10^6	Busulfan/cyclophosphamide	Allogeneic (12/12)
8	8×10^6	Busulfan/cyclophosphamide/radiotherapy	Haploidentical
9	7.09×10^6	Fludarabine/cyclophosphamide	Haploidentical
10	1.47×10^6	Busulfan/cyclophosphamide/radiotherapy	Allogeneic (8/8)
11	5×10^6	Busulfan/cyclophosphamide	Allogeneic (12/12)
12	1.11×10^6	Busulfan/cyclophosphamide	Allogeneic (12/12)
13	1.73×10^6	Busulfan/cyclophosphamide	Allogeneic (12/12)
14	2.52×10^6	Busulfan/cyclophosphamide	Allogeneic (12/12)
15	1.54×10^5	Busulfan/cyclophosphamide	Allogeneic (11/12)
16	6×10^6	Fludarabine/cyclophosphamide	Haploidentical
17	6×10^6	Busulfan/cyclophosphamide/radiotherapy	Allogeneic (12/12)
18	1.23×10^6	Busulfan/cyclophosphamide	Allogeneic (12/12)
19	1×10^6	Busulfan/cyclophosphamide	Allogeneic (12/12)
20	9.92×10^6	Fludarabine/cyclophosphamide	Haploidentical
21	4.8×10^6	Busulfan/cyclophosphamide	Allogeneic (11/12)

HLA = human leukocyte antigen.
*Shared alleles/determined alleles.

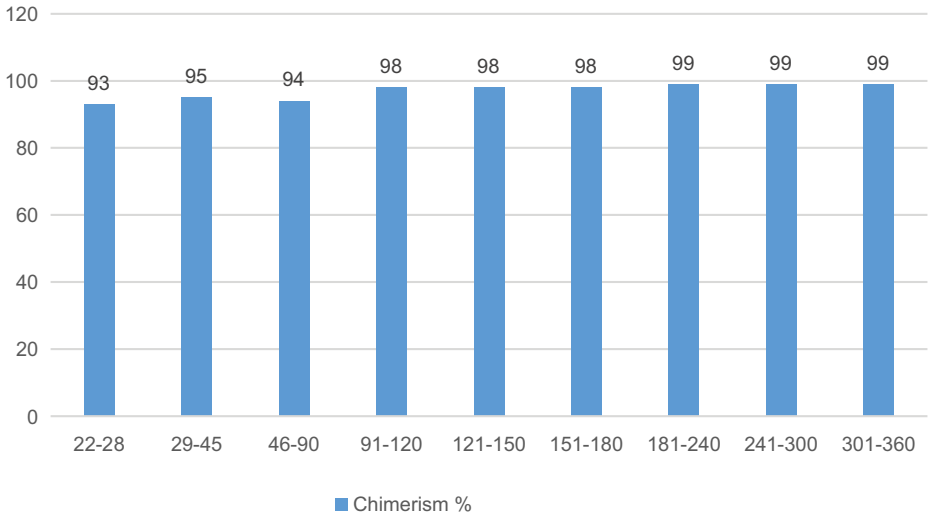

Figure 3. *Cell chimerism kinetics after hematopoietic stem cell transplantation. Means obtained at different post-transplantation time-points.*

Table 3. NK cell/L measured values at different performed determinations

Statistical value	Post-transplantation days							Control group
	21-90	91-120	121-180	180-240	241-300	301-360	+360	
Lowest value	0.018×10^8	0.0321×10^8	0.0065×10^8	0.00016×10^8	0.000011×10^8	0.0208×10^8	0.00040×10^8	0.183×10^8
25th percentile	0.161×10^8	0.0778×10^8	0.051×10^8	0.0167×10^8	0.00743×10^8	0.0563×10^8	0.0730×10^8	0.283×10^8
Median	0.635×10^8	0.205×10^8	0.0115×10^8	0.0909×10^8	0.0854×10^8	0.0819×10^8	0.124×10^8	0.582×10^8
75th percentile	0.144×10^8	0.406×10^8	0.0335×10^8	0.258×10^8	0.231×10^8	0.135×10^8	0.295×10^8	0.874×10^8
Highest value	0.794×10^8	0.633×10^8	1.76×10^8	0.765×10^8	0.335×10^8	$0.5.82 \times 10^8$	0.548×10^8	1.31×10^8
Mean	0.114×10^8	0.252×10^8	0.289×10^8	0.165×10^8	0.126×10^8	$0.1.47 \times 10^8$	0.183×10^8	0.605×10^8
Standard deviation	0.160×10^8	0.203×10^8	0.442×10^8	0.232×10^8	0.126×10^8	$0.1.95 \times 10^8$	0.148×10^8	0.334×10^8
Standard error	0.0296×10^8	0.0642×10^8	0.111×10^8	0.067×10^8	0.0419×10^8	0.074×10^8	0.0316×10^8	0.0927×10^8
95 % CI lower limit	0.0530×10^8	0.107×10^8	0.0531×10^8	0.0172×10^8	0.0292×10^8	0.034×10^8	0.118×10^8	0.403×10^8
95 % CI upper limit	0.174×10^8	0.398×10^8	0.525×10^8	0.312×10^8	0.223×10^8	0.327×10^8	0.249×10^8	0.807×10^8
Comparison vs. control group, difference (p)	Yes ($p < 0.0001$)	No	No	Yes ($p = 0.0059$)	Yes ($p = 0.0059$)	No	Yes ($p = 0.043$)	—

standard treatment and constant viral load monitoring were continued, since having control of the infection by the recipient him/herself was intended. In order to reduce GVHD severity, immunosuppressive treatment was intensified, which is a measure that is also taken when the number of NK cells is dramatically decreased in order to favor their proliferation.

NK cell recovery in patients undergoing HSCT has been described to occur in the first two months,[8,13-15] but it can be delayed for up to six months in patients with infections (mainly fungal) within the first 100 post-transplantation days.[15] This phenomenon was observed in the analyzed patients, in whom various adverse events (infections and GVHD) were documented during the first 100 days (Epstein-Barr virus, cytomegalovirus and adenovirus were identified in four patients and GVHD in two, who throughout the first year did not have an efficient NK cell reconstitution).

According to the literature, during the first month of the post-transplantation stage, the number of NK cells should be > 0.75×10^8/L when the source of CD34+ cells is the bone marrow; when CD34+ cells are collected from peripheral blood, reconstitution is obtained from the fourth month on in pediatric patients.[16] In a series of patients, it was possible to identify that reconstitution reached a median of 305 NK cells/μL (range: 30 ± 1200) by day 130 on average.[7] These values are higher than those obtained in patients and in the control group of the research herein presented, and numerical comparison between the different series is therefore not pertinent given the different characteristics of each group, in which various external factors have an influence:

- The disease HSCT is carried out for.
- The conditioning regimen (which together with the administration of 50 mg/kg/day of cyclophosphamide on days 3 and 4 post-transplantation as a prophylactic measure for the prevention of acute GVHD generated a decrease in lymphopoiesis in our patients and, therefore, NK cell recovery was affected during the first 90 days of the post-transplantation stage).

- Type of transplantation.
- Type of donor and HLA compatibility (13 HSCTs of the series herein described [62 %] were allogeneic with a 100 % compatible donor, two HSCTs [9.5 %] were allogeneic with a 90 % compatible donor and six [28.5 %] were haploidentical).

When the number of NK cells was compared in patients who underwent HSCT versus that of control individuals, a significant difference was observed in some periods (Fig. 2), which shows that the reconstitution was transient and deficient in some cases, probably due to NK cells sequestration at the infection target site (i.e., urinary tract in the patient in whom BK virus was identified) or to cell migration to specific tissues in the GVHD cases (skin and liver tissue). The highest number of NK cells in the post-transplantation stage was observed in the quantifications performed at between 91 and 120 days, with a homogeneous distribution, from which it is inferred that there was delayed NK cell reconstitution in our patients, considering data reported in the literature. Another factor that influences NK cell quantification is immunomodulation at post-transplantation stage, which is performed as part of transplantation maintenance follow-up.

There are two factors that directly influence on the delay of immune reconstitution after HSCT:

- Cell damage in the microenvironment generated by the conditioning regimen.
- Slow de novo generation of donor-derived immune system cells,[7,17] which is why one of the strategies to promote an efficient immune reconstitution is the protection of the recipient's microenvironment.[18]

In the present research, only NK cell absolute numbers and percentages were analyzed; however, immune system homeostasis also depends on their functional capacity, since NK cell effector capacities can be efficient in the production of cytokines, antibodies or cytotoxic capacity even without the values observed in healthy subjects being reached.

In HSCT recipients, who are immunosuppressed, antigenic stimulation events occur due to opportunistic viral or bacterial infections; therefore, NK cells participation is essential since they are the first line of immune defense, and their reconstitution is important for transplantation long-term success.[19-21]

Assessing the role of different NK cell populations in hematopoietic stem cell receivers may assist the design of new therapeutic interventions aimed at enhancing HSCT effects.[19,22]

Conflict of interests

None.

Funding Source

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Ethical disclosure

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors have obtained written informed consent of the patients or subjects mentioned in the article. The corresponding author is in possession of this document.

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ORIGINAL ARTICLE

Flow-cytometry as an auxiliary in the diagnosis of primary humoral immunodeficiencies

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Abstract

Background: *Antibody deficiencies encompass a wide spectrum of pathologies and constitute approximately 50 % of primary immunodeficiencies; with cytometry, it is possible to evaluate the immune status rapidly, effectively and at low cost.* **Objective:** *To assess, by means of flow cytometry, the cells of patients with three types of primary humoral immunodeficiencies.* **Method:** *Using flow cytometry, blood samples from patients and healthy subjects were analyzed with different monoclonal antibodies.* **Results:** *Using various stains, a severe decrease in B lymphocytes was shown in patients with X-linked agammaglobulinemia, as well as a lack of CD154 expression in patients with hyper-immunoglobulin M syndrome, and heterogeneity of B lymphocyte subpopulations in patients with common variable immunodeficiency.* **Conclusion:** *Flow cytometry enables early diagnosis of primary immunodeficiencies with a high level of confidence and, in many cases, identification of the genes involved.*

KEY WORDS: *Agammaglobulinemia. Hyper-immunoglobulin M. Common variable immunodeficiency.*

La citometría de flujo como auxiliar en el diagnóstico de las inmunodeficiencias primarias humorales

Resumen

Antecedentes: *Las deficiencias de anticuerpos abarcan un amplio espectro de patologías y constituyen aproximadamente 50 % de las inmunodeficiencias primarias; con la citometría es posible evaluar el estado inmunológico de forma rápida, efectiva y a bajo costo.* **Objetivo:** *Evaluar mediante citometría de flujo, las células de pacientes con tres tipos de inmunodeficiencias primarias humorales.* **Método:** *Mediante citometría de flujo se analizaron muestras de sangre de pacientes y sujetos sanos con distintos anticuerpos monoclonales.* **Resultados:** *Mediante diversas tinciones se demostró disminución severa de linfocitos B en pacientes con agammaglobulinemia ligada al cromosoma X, la falta de expresión de CD154 en pacientes con síndrome de hiperinmunoglobulina M y heterogeneidad de subpoblaciones de linfocitos B en pacientes con inmunodeficiencia común variable.* **Conclusión:** *Con la citometría de flujo es posible realizar el diagnóstico temprano de inmunodeficiencias primarias con un nivel de confianza elevado y, en muchos casos, identificar los genes implicados.*

PALABRAS CLAVE: *Agammaglobulinemia. Hiperinmunoglobulina M. Inmunodeficiencia común variable.*

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Table 1. Classification of predominantly antibody immunodeficiencies*

Disease	Serum Ig	Characteristics	Type of inheritance	Genetic defects
X chromosome-linked agammaglobulinemia	All isotypes decreased	Serious bacterial infections. Absence or highly significant decrease of mature B cells	X chromosome-linked	Mutations in BTK
Autosomal recessive agammaglobulinemia	All isotypes decreased	Serious bacterial infections. Absence or highly significant decrease of mature B cells	Autosomal recessive	Mutations in μ heavy chain, in l5, Igα, Igβ and BLNK
Common variable immunodeficiency	Decreased IgG, IgA and/or IgM	Recurrent bacterial infections, some patients experience autoimmunity, lymphoproliferation or granulomatous disease	Variable	Mostly unknown, in some cases, mutations in ICOS, CD19, TACI, BAFF-R
Hyper-IgM syndrome	Normal or elevated IgM with decreased IgA and IgG	Opportunistic infections, neutropenia, autoimmune diseases	X chromosome-linked (CD154) or autosomal recessive	Mutations in CD154, CD40, AICDA, UNG
Isotype deficiencies with B cell normal numbers	One or more IgG and/or IgA and IgE subclasses decreased	Asymptomatic in some cases, or subjects can experience recurrent bacterial and viral infections	Variable	Mutations or deletions in chromosome 14q32 or in λ light chain
Antibody-specific deficiency with normal concentrations of Ig and B cells	Normal	Inability to produce antibodies against some specific antigens	Variable	Unknown
Transient hypogammaglobulinemia of infancy	Decreased IgG and IgA	Moderate to recurrent bacterial infections	Variable	Unknown

*Classification modified from International Union of Immunological Societies Expert Committee on Primary Immunodeficiencies. Notarangelo LD, Fischer A, Geha RS, Casanova JL, Chapel H, et al. Primary immunodeficiencies: 2009 update. J Allergy Clin Immunol. 2009;124(6):1161-1178.
BTK = Bruton's tyrosine kinase.

Introduction

Primary immunodeficiencies (PID) are genetic conditions that lead to susceptibility to infections by germs that generally are poorly virulent. PIDs can be inherited or acquired by de novo mutations during embryonic development.[1] In Mexico, no reliable statistics regarding the magnitude of the problem are available. Due to the lack of appropriate methods, PIDs are not diagnosed in most cases. More than 180 conditions have been identified in the world thanks to the development of high-impact analysis tools, such as flow cytometry, which is combined with molecular diagnosis.[2] In the PID classification accepted by the International Union of Immunology Societies, various conditions are recognized, among which defects in antibody production are the most common.[2] In the European Society of Immunodeficiencies statistics[3] and in various publications of the United States, defects in the production of antibodies comprise about half the diagnosed cases. Mexico ranks third in Latin America in recorded PID cases; according to data from the Latin American Society of Immunodeficiencies, at least one in 200 to 500 children has an antibody PID.[4] Notwithstanding the foregoing, the region is still far from having real statistics available.[4]

Antibody deficiencies encompass a wide spectrum of pathologies, from severe insufficiency in the production of all immunoglobulin isotypes and total absence of mature B lymphocytes, to selective deficiency of one isotype (Table 1).[5] Patients with these deficiencies show no signs of disease during the first months of life, due to the antibodies they receive from the mother; however, the number and severity of infections increases when those antibodies decrease. Regular administration of human gamma-globulin can prevent progressive deterioration and allows patients to lead almost normal lives, as long as therapeutic interventions are carried out at an early age, which is why early diagnosis is essential.[6] Flow cytometry enables making early diagnoses with a high level of reliability and, in many cases, investigating genes with possible defects.[7]

In this work, we show the assessment of patients with defects in the production of antibodies by using simple and rapid stains; the comparison was established with healthy individuals (control group).

Method

Cross-sectional, prospective, descriptive study. Children previously diagnosed at the National Institute of Pediatrics or at *La Raza* National Medical Center of the Mexican Institute of Social Security, Mexico City, with X-linked agammaglobulinemia (XLA), hyperimmunoglobulinemia M syndrome (hyper-IgM syndrome) or common variable immunodeficiency (CVID), according to the criteria established for PIDs, were included.[8] Parents or legal guardians granted their consent after being informed on the purposes and procedures of the investigation (in accordance with the ethical principles established in the Declaration of Helsinki). Patients with secondary causes of hypo- or agammaglobulinemia were excluded[8], and patients with insufficient samples or who decided to withdraw from the investigation were censored.

Three to 10 mL of peripheral blood were obtained, with heparinized Vacutainer® tubes (Becton Dickinson, San Jose, CA, USA); 1 mL was used to determine leukocyte populations and the rest was used to obtain mononuclear cells by centrifugation in Histopaque® (Sigma, Chemical Co., St Louis, MO, USA).

By means of staining, the percentages of the different cell populations were determined. 30 μL of non-activated peripheral blood were used and incubated for 20 minutes with 5 μL of the following monoclonal antibody mixtures:

- Anti-CD45-FITC/anti-CD14-PE.
- Anti-CD3 FITC/anti-CD19 PE/anti-CD45 PerCP.
- Anti-CD4 FITC/anti-CD8 PE/anti-CD3 PerCP.
- Anti-CD3 FITC/anti-CD16+56 PE/anti-CD45 PerCP.

Anti-CD45 PerCP/γ1 FITC/γ1 PE was used as isotype control antibodies. Most antibodies used were obtained from Becton Dickinson; otherwise, the laboratory of origin is indicated. The samples were incubated 20 minutes at room temperature, in the dark. After incubation, the erythrocytes were lysed with 500 μL of FACS® lysis solution (Becton, Dickinson, San Jose, CA, USA), incubated for an additional 10 minutes, and then washed with phosphate buffered saline (PBS) and 1 % human serum albumin. The cells were fixed with 1% formaldehyde in PBS. The acquisition and analysis will be described later on.

B cell subpopulations were determined from mononuclear cells, which were stained with the anti-CD27 PE/anti-CD19 APC/anti-IgD FITC and anti-CD24 PE/anti-CD38 APC/anti-CD19 FITC mixtures; incubation, washing and fixation were carried out as described and erythrocyte lysis was omitted.

Bruton's tyrosine kinase expression determination

One million mononuclear cells were fixed with 1% formaldehyde in PBS for 10 minutes. The mononuclear cells were washed with PBS with 10 % of human serum albumin at 300 × g for five minutes and permeabilized for 10 minutes with 0.1% saponin in PBS (PBS-S). Labeling was carried out with 30 μL of the anti-Btk monoclonal antibody (Bruton's tyrosine kinase, Pharmingen, San Diego, CA, USA), followed by 30 minutes of incubation and two washes with PBS-S. Staining was carried out with 30 μL of anti-IgG2a PE (Upstate Biotechnology, Lake Placid, NY, USA) and the cells were then incubated for 30 minutes in the dark. Subsequently, the cells were washed with PBS-S, stained with 5 μL of anti-CD14 FITC and incubated for 15 minutes in the dark; finally they were washed and fixed with 300 μL of 1% formaldehyde in PBS.

CD40 and TACI expression determination

The mononuclear cells were incubated with anti-CD40 PE and anti-CD19 APC to identify their expression in B cells, and with anti-CD40 PE and anti-CD14 PerCP to identify their expression in monocytes. In another tube, the mononuclear cells were incubated with anti-TACI PE and anti-CD22 PECy5. Incubation, washing and fixation were carried out according to the described procedures.

CD154 and ICOS expression determination

For the determination of CD154, two million mononuclear cells were cultivated for 12 hours at 37 ºC, under 5 % CO_2 conditions, in RPMI 1640 medium (Gibco-BRL®, Gaithersburg, MO, USA) supplemented with 10 % fetal bovine serum (PharmAust, Bentley, Western Australia), 1 mM L-glutamine, 100 units/mL penicillin, and 10 μg/mL streptomycin (Gibco). They were activated with 100 ng/mL of phorbol 12-myristrate 13-acetate (Gibco) and 1 μg/mL ionomycin (Sigma). The mononuclear cells were stained with anti-CD3 PerCP (anti-CD154 PE or anti-ICOS PE) and

Table 2. Clinical and laboratory data of patients with X chromosome-linked agammaglobulinemia

Patient	Hospital admissions (n)	Pneumonias (n)	Sinusitis (n)	Otitis media (n)	Diarrhea episodes (n)	IgG (486-1211 mg/dL)	IgM (45-211 mg/dL)	IgA (30-182 mg/dL)	B cell % (19 ± 8)
P1	6	6	2	10	0	6.2	14.2	0.5	0
P2	4	0	0	0	0	28	6	11	0.12
P3	6	0	20	1	0	27	12	0.3	0
P4	2	0	0	0	Several	6.6	16.8	22.1	0.1
P5	1	1	0	0	0	6.7	17	23.4	0
P6	1	0	0	0	0	33.3	4.1	6.6	0
P7	1	6	0	0	0	6.6	17	1.5	0
P8	2	1	4	1	1	0	6	0	0
P9	1	1	0	0	0	41	8.6	6.7	0.3
P10	2	1	0	0	1	47.7	24.1	11.5	0

anti-CD69 FITC. Incubation, washing and fixation were carried out as described.

Non-activated cells were used as negative controls and CD69 expression as positive control. The analysis is described later on.

Acquisition and analysis

For the analysis of peripheral blood leukocyte populations, 10,000 cells were observed and 100,000 were examined for mononuclear cell staining. The samples were read on the FACScalibur® platform. Data were examined using the FlowJo 10.0.07 software.

Statistical analysis

Demographic, clinical and laboratory data are indicated as medians and ranges or means and standard deviations, depending on the type of distribution; categorical variables, with frequencies and percentages. Patient phenotype is described with frequencies and percentages. The comparisons between both groups were made using Mann-Whitney's test. The results are expressed as the mean; values with $p < 0.05$ were considered significant. Statistical analyses were performed using GraphPad Prism version 5.0.

Results

Table 2 summarizes the clinical and laboratory findings of 10 patients diagnosed with XLA; serum immunoglobulin residual values and clinical data, both of the number of hospitalizations and infections, can be appreciated. Serum immunoglobulin values were correlated with B lymphocytes reduction.

Figure 1 shows representative results of one patient with XLA who showed a severe decrease in CD19+ B lymphocytes. To ensure that this cell population was absent, labeling with anti-CD20 was used, which confirmed the result. To corroborate the defect, BTK expression was analyzed; in no patient could this protein be identified (data not shown).

Figure 2 shows the results of CD19+ B lymphocyte staining, supplemented with IgD and CD27 staining. Both markers allow to define three subpopulations: naïve B lymphocytes (IgD+ CD27-), memory B cells without isotype change (IgD+ CD27+) and B cells with isotype change (IgD- CD27+). In patients with hyper-IgM syndrome, although B lymphocyte values were normal, an important decrease was observed in memory B lymphocytes (with and without isotype change); the diagnosis can be corroborated by the absence of CD154 in activated T lymphocytes and by efficient activation in T cells through CD69 expression. The graphs on the right side show the overall results of seven patients with hyper-IgM syndrome, in comparison with those obtained in the control subjects.

Figure 3 shows the staining of the B lymphocyte subpopulations, which allowed classifying patients with CVID. No patient exhibited agammaglobulinemia and CD40 and CD154 expression was normal. For the

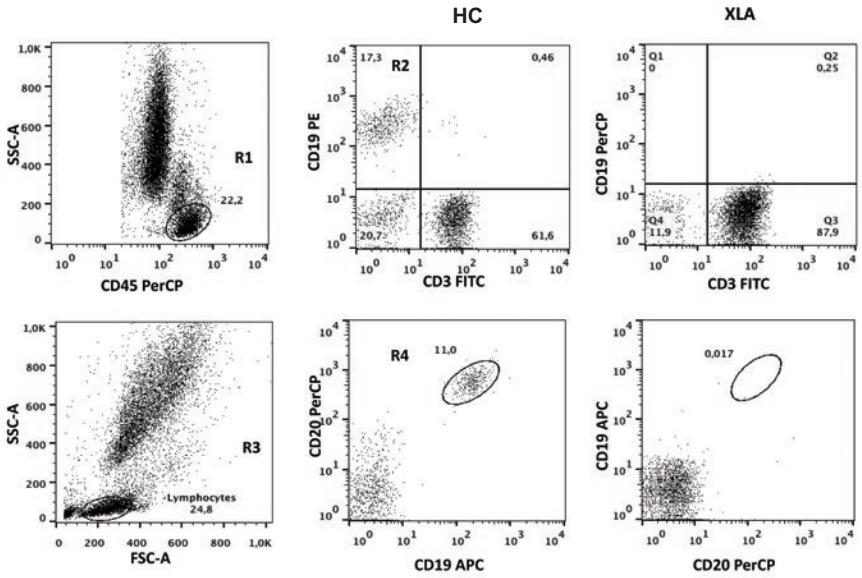

Figure 1. *Flow cytometry results in patients with X-linked agammaglobulinemia (XLA). B cells were identified with CD45+ (R1) and CD19+ (R2). CD20+ (R3) and CD19+ (R4) expression is observed in the total lymphocyte region. HC = healthy control.*

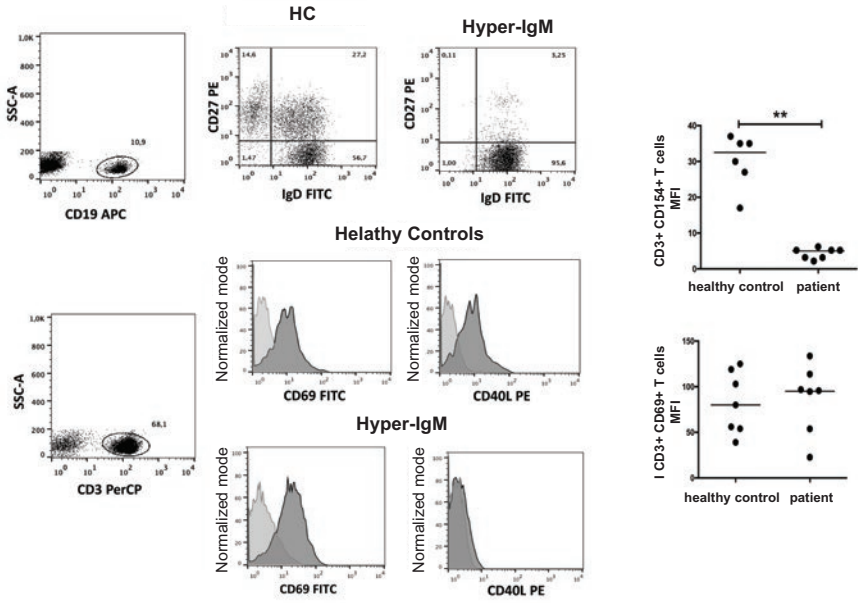

Figure 2. *Analysis of patients with hyper-IgM syndrome. B cell subpopulations were identified with antibodies against CD19, CD27 and IgD, and CD154 and CD69 expression in the CD3+ T-cell region. The light gray histogram represents non-stimulated T cells and the dark gray histogram represents cells stimulated for 12 hours with ionomycin and phorbol 12-myristrate 13-acetate. CD154 (CD40L) and CD69 (activation control) expression can be observed in seven healthy controls and seven patients with hyper-IgM syndrome, as well as their respective values in mean fluorescence intensity (MFI). The horizontal bar shows the median, and the asterisks, significant differences using Mann-Whitney's U statistical test. ***Highly significant, p < 0.01.*

naïve B cells, with and without isotype change, IgD+ CD27 staining was used. Subpopulations with low CD21 expression (CD19+ CD21- CD38-) were also identified.

The patients were stratified according to the Freiburg classification:

– Group I, patients with reduced values (< 0.4 %) of memory B lymphocytes with isotype change;

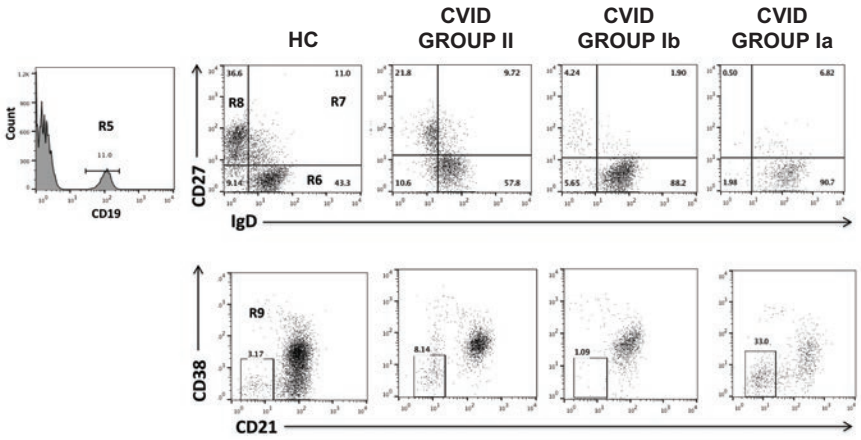

Figure 3. *Analysis of patients with common variable immunodeficiency (CVID). B cells are identified with CD19+ (R5) and then subdivided as follows: Naïve B cells are identified with CD19+ CD27- IgD+ (R6); B cells without isotype change or marginal zone, with CD19+ CD27+ IgD+ (R7); B cells with isotype change, with CD19+ CD27+ IgD- (R8); CD21^{low} B cells, with CD19+ CD21- CD38- (R9). Patients were stratified in groups Ia, Ib and II according to the Freiburg classification.*

Table 3. Clinical and laboratory data of patients with common variable immunodeficiency, grouped according to the Freiburg classification

Characteristic	Group Ia (n = 10)		Group Ib (n = 10)		Group II (n = 4)	
Female/male gender	7/3		7/3		3/1	
	Mean		Mean		Mean	
Age (years)	14.3		15.2		10.2	
IgG (mg/dL)	210		213		243	
IgA (mg/dL)	27		18		27	
IgM (mg/dL)	52		23		71	
B cells (%)	9		11		17	
	n	%	n	%	n	%
Pneumonia	5	50	6	60	2	50
Sinusitis	6	60	5	50	1	25
Otitis media	5	50	5	50	1	25
Chronic diarrhea	2	20	3	30	0	0
Acute infectious gastroenteritis	5	50	5	50	0	0
Autoimmune diseases	5	50	2	20	0	0
Bronchiectasis	5	50	4	40	1	25
Splenomegaly	2	20	1	10	0	0
Lymphadenopathies	4	40	1	10	0	0

this group is subdivided in patients with an increased percentage (> 20 %) of CD21^{low} B cells (group 1a) and patients with CD21^{low} cells normal expression (group 1b).

– Group II, patients with normal values of memory B lymphocytes with isotype change.

As detailed in Table 3, patients in group II showed moderate characteristics of the disease. In group I, a clear separation was observed when the presence of autoimmune diseases and lymphadenopathies were analyzed in patients with CD21 reduced expression.

Discussion

XLA is the result of mutations in the BTK gene, which is essential in the development of B cells.[9] BTK is also expressed in peripheral blood monocytes; their search by cytometry yielded negative results in all patients with XLA (data not shown). Patients with XLA appear to have exacerbated inflammatory responses. Our group has proposed that BTK regulates the inflammatory response of monocytes, macrophages and polymorphonuclear cells.[10]

Patients with hyper-IgM syndrome are susceptible to opportunistic germs, they experience neutropenia, and some may develop autoimmune diseases and cancer.[11] B cells of these patients are unable to form germ centers and change isotype. The patients exhibit a decrease or frank absence of memory B cells,[11] a condition that was verified in all the patients in this cohort. The most common defect was the absence of CD154 in activated T cells,[12] a result that was obtained in all seven patients with hyper-IgM syndrome of this cohort.

Since the description of CVID in 1953,[13] its complexity became relevant. Patients have highly diverse

clinical manifestations, the severity of which is varied. The mutations that cause CVID phenotypes are observed in less than 20 % of patients. Several research groups have stratified patients with CVID.[14-16] In this work, we used the Freiburg classification. Group Ia patients are those with the lowest total B cell expression in peripheral blood, in addition to experiencing the most serious clinical manifestations.

Through the use of flow cytometry, it was possible to diagnose XLA, hyper-IgM syndrome and CVID, which are conditions that involve a decrease or absence of antibody production. The diagnosis was obtained with a high level of reliability and will be able to guide the search for candidate genes with possible defects.

Conflict of interests

None.

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Ethical disclosures

Protection of people and animals. The authors declare that the procedures followed adhered to the ethical standards of the responsible human experimentation committee and were in accordance with the World Medical Association and the Declaration of Helsinki.

Confidentiality of data. The authors declare that they have followed the protocols of their work center regarding the publication of patient data.

Right to privacy and informed consent. The authors have obtained the informed consent of the patients and/or subjects referred to in the article. This document is in the possession of the corresponding author.

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ORIGINAL ARTICLE

Diagnostic accuracy of the Edinburgh Postnatal Depression Scale: consequences of screening in Mexican women

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Abstract

Introduction: *Postpartum depression is a non-psychotic depressive episode with serious repercussions on the bond between the mother and her child, hence the importance of detecting it in a timely manner.* **Objective:** *To determine the accuracy of the Edinburgh Postnatal Depression Scale as a diagnostic test and to analyze the consequences of screening and the probability of depression after applying the test.* **Method:** *Screening of 411 women with the Edinburgh Postnatal Depression Scale during the postpartum period; Beck's Depression Inventory was used as reference.* **Results:** *At a cutoff point of 12, a sensitivity of 70.4 %, specificity of 72.2 %, positive predictive value of 36.9 % and negative predictive value of 91.4 % were obtained with Edinburgh Postnatal Depression Scale, as well as an area under the curve of 0.729 and a p-value of 0.0003. Out of 49 women without treatment for postpartum depression, five were identified to require it.* **Conclusions:** *The Edinburgh Postnatal Depression Scale has moderate accuracy; its application is simple, accessible and should be routine. It is necessary for strategies to detect and treat postpartum depression to be implemented in Mexico.*

KEY WORDS: *Postpartum depression. Edinburgh Postnatal Depression Scale. Sensitivity. Specificity. Screening. Diagnostic accuracy.*

Exactitud diagnóstica de la Escala de Depresión Posnatal de Edimburgo: consecuencias del tamizaje en mujeres mexicanas

Resumen

Introducción: *La depresión posparto es un episodio depresivo no psicótico con repercusiones graves en el vínculo de la madre con su hijo, de ahí la importancia de detectarla oportunamente.* **Objetivo:** *Determinar la exactitud de la Escala de Depresión Posnatal de Edimburgo como prueba diagnóstica y analizar las consecuencias del tamizaje y la probabilidad de depresión después de aplicar la prueba.* **Método:** *Tamizaje con la Escala de Depresión Posnatal de Edimburgo a 411 mujeres durante el posparto; se utilizó el Inventario de Depresión de Beck como referencia.* **Resultados:** *En un punto de corte de 12, con la Escala de Depresión Posnatal de Edimburgo se obtuvo sensibilidad de 70.4 %, especificidad de 72.2 %, valor predictivo positivo de 36.9 % y valor predictivo negativo de 91.4 %, así como un valor del área bajo la curva de 0.729 y p = 0.0003. De 49 mujeres sin atención para depresión posparto, en cinco se identificó que la necesitaban.* **Conclusiones:** *La Escala de Depresión Posnatal de Edimburgo tiene una exactitud moderada; su aplicación es sencilla, accesible y debería ser rutinaria. Es necesario que en México se implementen estrategias para detectar y tratar la depresión posparto.*

PALABRAS CLAVE: *Depresión posparto. Escala de Depresión Posnatal de Edimburgo. Sensibilidad. Especificidad. Tamizaje. Exactitud diagnóstica.*

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Introduction

Postpartum depression (PPD) is a mild to moderate non-psychotic depressive episode that begins after delivery and extends throughout the first postpartum year.[1] Its global prevalence is 10 to 20 %,[2-4] and in Mexico, from 13 to 24 %.[5] It usually resolves spontaneously in three to six months, although in 25 % of cases it can last more than one year.[6] It is important for PPD to be diagnosed and treated due to the deep emotional impact on the mother-child bond; children of depressed mothers have been observed to have three times more risk to develop emotional and behavioral problems.[7-10]

It is essential for health personnel to become aware of the importance of identifying PPD with rapid, inexpensive and simple tests. In 2016, Place et al. stated that there are few publications on the prevention, detection and treatment of PPD in Mexico. PPD is mentioned in various health policies, but protocols to routinely detect and treat it in obstetric units have not been implemented.[11] Furthermore, even when screening tests are applied, the results are not always used for follow-up and treatment; only 23 to 60 % of women with a positive screening test follow an adequate treatment.[12-14]

The most widely used, recommended and internationally validated test for PPD screening is the Edinburgh Postnatal Depression Scale (EPDS); Beck's Depression Inventory (BDI) is an internationally validated scale to determine the severity of depression.[15,16] Studies on the validity of EPDS as a diagnostic test show differences in sensitivity, specificity and predictive values, in the type of population, sample size, cutoff points and diagnostic instruments used as reference.[17] In Mexico, there are only few reports on screening with EPDS. Alvarado et al. recommend a cutoff point of 11/12 (sensitivity of 75 % and specificity of 93 %) at four weeks postpartum.[18] However, no reports have been published on the consequences of these results in clinical practice, and neither has an analysis of EPDS performance and diagnostic accuracy.

The main purpose of this investigation was to determine the accuracy of EPDS as a diagnostic test, with BDI being used as the reference method; sensitivity, specificity, predictive values, ROC curve and Youden index were calculated at different cutoff points, and the consequences of screening and the probability of PPD after applying the test were analyzed.

Method

Observational, cross-sectional, descriptive study, carried out in the outpatient clinic of *Hospital Juárez de México*, Ministry of Health, from February to November 2009, registered with number HJM1411/07.10.09.

Women aged between 15 and 45 years, with a minimum education of fifth year of primary school, whose delivery had occurred at between 15 days and eight weeks before the application of the EPDS, and who verbally granted their consent after being informed on the purposes and procedures of the research, were included. Women with known thyroid disease, and those whose children had congenital malformations or disease requiring medical attention were excluded. Women who met the selection criteria answered the EPDS questionnaire, which explores the mood of the woman in the previous seven days with 10 questions, each question with a score from 0 to 3 (highest total score of 30 points); a score ≥ 10 was considered to be indicative of PPD. Subsequently, those who obtained an EPDS score ≥ 10 were asked to answer the BDI questions which is a self-applied 21-question questionnaire whose 0-to-63 score indicates the severity of depression: from 5 to 9 points, minimal depression; 10 to 18 points, mild to moderate depression; from 19 to 29 points, moderate to severe depression; from 30 to 63 points, severe depression.

Frequencies and percentages of demographic characteristics and and central tendency and dispersion measures of the scale scores were obtained. Prevalence, sensitivity, specificity, predictive values, and likelihood ratios (LR or post-test probabilities) were calculated with 95 % confidence intervals (CI), whereby the MedCalc program, version 19.0.3, was used. For the BDI, a score of 10 was used as reference.

Sensitivity was defined as the percentage of individuals with the disease who have a positive test, and specificity, as the percentage of individuals without the disease who have a negative test. The positive predictive value (PPV) is the probability that a patient with a positive result (abnormal) has the disease; the negative predictive value (NPV) is the probability of not having the disease when the test result is negative (normal).[19] According to reports in the literature, for the calculation of predictive values, a PPD prevalence (prior probability) of 18.7 % was considered.

LRs indicate how much the result of a diagnostic test will increase or decrease pretest probability. The following criteria were considered:[20]

- higher than 10 or lower than 0.1, significant changes.
- 5-10 or 0.1-0.2, moderate changes.

Table 1. Demographic characteristics of women with suspected postpartum depression who obtained ≥ 10 points on the Edinburgh Postnatal Depression Scale

Marital status	Level of education	Occupation	Deliveries	Addictions
Married 44.8 % (n = 37)	Primary school 12.7 % (n = 10)	Homemaking 89.9 % (n = 71)	One 44.3 % (n = 35)	No 91.1 % (n = 72)
Cohabitating 40 % (n = 32)	Secondary school 60.8 % (n = 48)	Work away from home 7.6 % (n = 6)	Two 30.4 % (n = 24)	Yes, tobacco 3.8 % (n = 3)
Single 12.7 % (n = 10)	High school 5.1 % (n =4)	Students 2.5 % (n = 2)	Three 15.2 % (n = 12)	Yes, alcohol 3.8% (n = 3)
Widow/divorced 0 % (n = 0)	College degree 0 % (n = 0)		Four 3.8 % (3)	Yes, drugs 3.8 % (3)

Table 2. Postpartum depression severity in women with ≥ 10 points on Edinburgh Postpartum Depression Scale

BDI score	Frequency (n = 79)		PPD severity
	%	n	
0-9	22.8	18	Minimal
10-18	39.2	31	Mild to moderate
19-28	24	19	Moderate to severe
29-63	13.9	11	Severe

Beck's Depression Inventory was used as reference method.

- 2-5 or 0.5-0.2, small changes.
- >10 or <0.1, significant changes.
- 5-10 or 0.1-0.2, moderate changes.
- 2-5 or 0.5-0.2, small changes.
- 1-2 and 0.5-1, very small and rarely important changes.

To determine the probability of correctly classifying a pair of randomly selected women, one healthy and the other with the disorder, a ROC curve was plotted with the results of their tests. Women with a BDI score ≥ 10 were regarded as having PPD. The area under the curve was reported with 95 % CI and a value of 0.5-0.7 was considered to indicate low accuracy; 0.7-0.9, moderate accuracy; > 0.9, high accuracy.[21] Finally, the Youden index was calculated to assess EPDS performance as a diagnostic test (a value of 1 indicates a perfect test, and a value of 0, useless test).

Results

The EPDS was applied to 441 women; 17.9 % (n = 79) obtained a score ≥ 10. Table 1 describes the sociodemographic characteristics. Average age was 26 ± 7 years, and most were married women (44.8 %), primigravidas (44.3 %), had secondary education (60.8 %) and were exclusively dedicated to household chores (89.9 %). Average number of days after delivery in which the test was positive was 28 ± 13.

Average EPDS score was 5.7 ± 5; 82.1 % (n = 362) obtained less than 10 points. The 79 women with a score ≥ 10 had an average of 14.58 ± 4.08 points; in that group, BDI average score was 17 ± 11.1. Despite having an EPDS score of ≥ 10, 22.8 % (18/79) had no data consistent with PPD. Mild to moderate PPD was the most common (39.2 %), followed by moderate to severe (24 %), and only 13.9 % had data consistent with severe PPD (Table 2).

Table 3 shows how EPDS validity and safety were modified according to different cutoff points:

- Sensitivity: from 93.4% at a cutoff point > 10, it decreased to 70.4 % at a cutoff point > 12 (95 % CI = 84.1-98.2 and 57.4-81.5, respectively).
- Specificity: from 16.6 % with a cutoff point of 10, it increased to 94.4 % when the cutoff point was 17; specificity was 72.2 % when the cutoff point was > 12 (95 % CI = 3.6-41.4, 72.7-99.9 and 46.5-90.3, respectively).
- PPV: from 20.5 % when the score was> 10, it increased to 36.9 % with a cutoff point > 12 (95 % CI = 17.2-24.3 and 21.5–55.6, respectively).
- NPV: from 91.7 % when the score was < 10, it went to 91.4 % when it was < 12 (95% CI = 73.1-97.8 and 86.8-94.5, respectively).

Considering a cutoff point of 12 for EPDS and 10 for BDI, positive LR was 2.5 and negative LR 0.4 (95 % CI = 1.2-5.4 and 0.3-0.7, respectively), which represent a small, but significant change.

Figure 1 shows the consequences of conducting the screening with EPDS, and figure 2, the ROC curve: area

Table 3. Validity and safety values and likelihood ratios according to the cutoff point on Edinburgh Postnatal Depression Scale and Beck's Depression Inventory as reference method

EPDS cutoff point	Sensitivity % (95 % CI)	Specificity % (95 % CI)	Positive likelihood ratio (95 % CI)	Negative likelihood ratio (95 % CI)	PPV % (95 % CI)	NPV % (95 % CI)
≥ 10	100 (94.1-100)	0.00 (0.0-18.5)	1.0 (1.0-1.0)	—	18.7 (18.7-18.7)	—
> 10	93.4 (84.1-98.2)	16.6 (3.6-41.4)	1.1 (0.9-1.4)	0.3 (0.1-1.6)	20.5 (17.2-24.3)	91.7 (73.1-97.8)
> 11	80.3 (68.2-89.4)	38.8 (17.3-64.3)	1.3 (0.9-1.9)	0.5 (0.2-1.1)	23.2 (17.0-30.8)	89.6 (79.9-94.9)
> 12	**70.4 (57.4-81.5)**	**72.2 (46.5-90.3)**	**2.5 (1.2-5.4)**	**0.4 (0.3-0.7)**	**36.9 (21.4-55.6)**	**91.4 (86.8-94.5)**
> 13	57.3 (44.1-70)	83.3 (58.6-96.4)	3.4 (1.2-9.9)	0.5 (0.4-0.7)	44.2 (21.6-69.5)	89.5 (85.6-92.4)
> 14	47.5 (34.6-60.7)	83.3 (58.6-96.4)	2.8 (1.0-8.3)	0.6 (0.5-0.9)	39.6 (18.4-65.6)	87.4 (83.4-90.5)
> 15	37.7 (25.6-51)	88.8 (65.3-98.6)	3.3 (0.9-13.0)	0.7 (0.5-0.9)	43.8 (16.9-75.0)	86.1 (82.8-88.9)
> 16	31.1 (19.9-44.3)	88.8 (65.3-98.6)	2.8 (0.7-10.9)	0.7 (0.6-1.0)	39.2 (14.2-71.5)	84.9 (81.6-87.7)
> 17	24.5 (14.5-37.3)	94.4 (72.7-99.9)	4.4 (0.6-31.3)	0.8 (0.7-1.0)	50.4 (12.6-87.8)	84.5 (81.9-86.7)
> 18	21.3 (11.9-33.7)	100 (81.5-100)	—	0.7 (0.7-0.9)	100	84.7 (82.9-86.3)
> 19	13.1 (5.8-24.2)	100 (81.5-100)	—	0.8 (0.8-1.0)	100	83.3 (81.9-84.7)
> 20	11.4 (4.7-22.2)	100 (81.5-100)	—	0.8 (0.8-1.0)	100	83.1 (81.8-84.3)
> 21	9.8 (3.7-20.2)	100 (81.5-100)	—	0.9 (0.8-1.0)	100	82.8 (81.6-84.0)
> 22	6.5 (1.8-15.9)	100 (81.5-100)	—	0.9 (0.9-1.0)	100	82.3 (81.3-83.3)
> 23	4.9 (1.0-13.7)	100 (81.5-100)	—	0.9 (0.9-1.0)	100	82.1 (81.2-82.9)
> 26	1.6 (0.04-8.8)	100 (81.5-100)	—	0.9 (1.0-1.0)	100	81.5 (81.1-82.0)
> 29	0.0 (0.0-5.9)	100 (81.5-100)	—	1.0 (1.0-1.0)	—	81.3 (81.3-81.3)

CI = confidence interval, NPV = negative predictive value, PPV = positive predictive value.

under the curve = 0.729 (95 % CI = 0.617-0.823), p = 0.0003, with a standard error of 0.0634. Youden index was 0.4271 and a cutoff point at 12 was suggested.

Discussion

The present study is the first to assess EPDS performance as a diagnostic test in Mexican women whose screening result was positive for PPD. The frequency of positivity in EPDS (17.9 %) agrees with previously published results[2-4] with different cutoff points, ranging from 9 to 12 depending on the study.

EPDS positivity was found at 28 ± 13.2 days postpartum, suggesting the convenience of routinely screening from the second week after delivery and thereafter, when the women attend for examination of

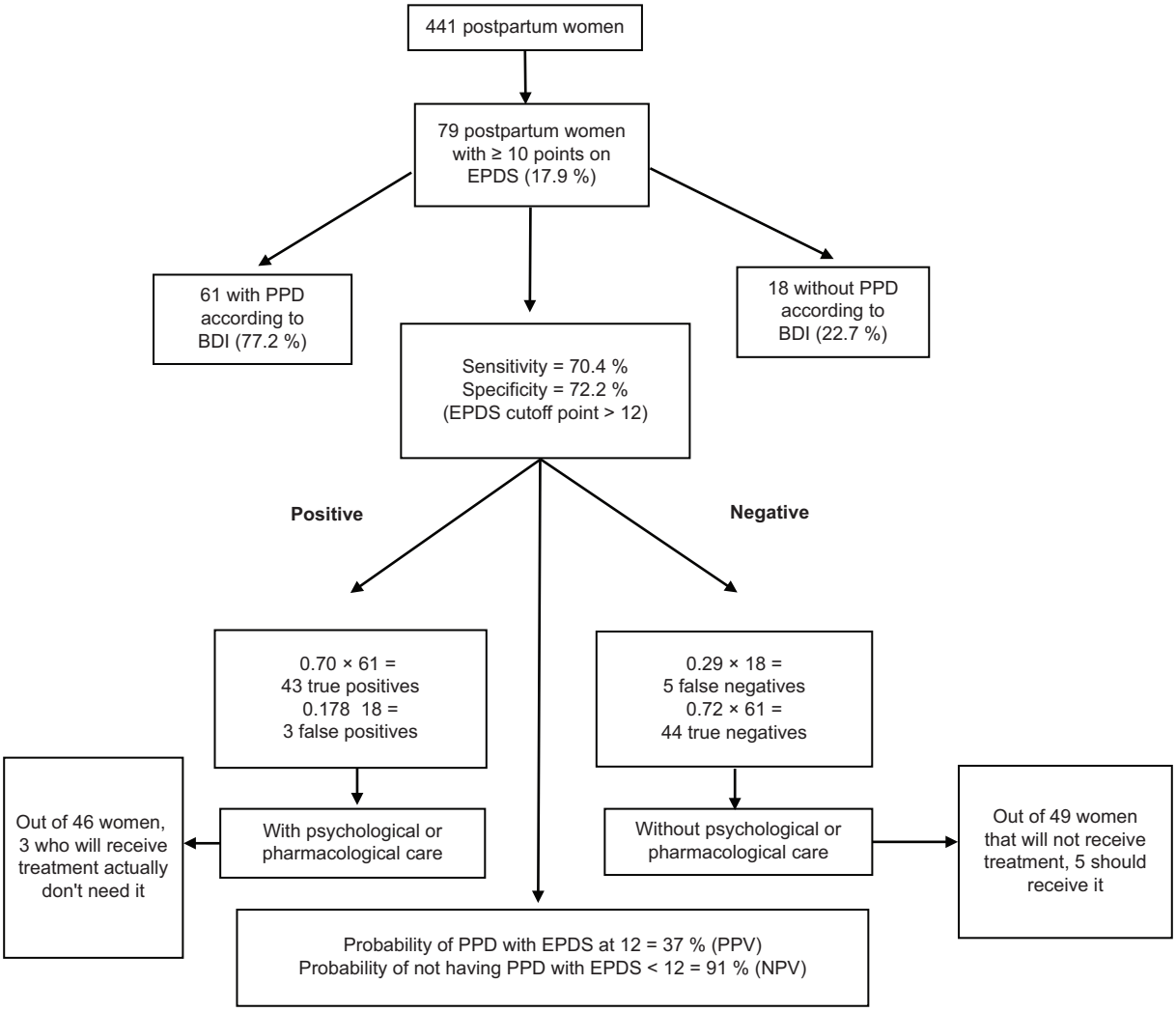

Figure 1. *Consequences of performing the screening with Edinburgh Postnatal Depression Scale in woman during the postpartum period. EPDS = Edinburgh Postnatal Depression Scale, PPD = postpartum depression, BDI = Beck's Depression Inventory, PPV = positive predictive value, NPV = negative predictive value. Adapted from Leeflang MMG. Systematic reviews and meta-analyses of diagnostic test accuracy. Clin Microbiol Infect. 2014;20:105-113.*

the newborn. Although our objective was not to study risk factors for PPD, it is important to highlight that their detection would provide useful information to implement prevention and treatment strategies.

Hospital Juárez de México serves low-income populations without social security, which probably have risk factors such as domestic violence; according to the National Institute of Statistics and Geography, 43.9 % of Mexican women have had at least one episode of violence generated by their partners.[22] Mexico needs to implement national health policies to look after the emotional development of families; specifically, timely care of PPD should be a priority.

It is important to note that, of those women with positive EPDS, 22.7 % had no PPD according to BDI criteria, i.e., the result was false positive; in addition, 22.8 % of the women who had an EPDS score ≥ 10 had no depression. The highest percentage, 39.2 %, had mild to moderate depression, which is relevant because depression identification at this stage will allow early intervention in order to avoid an increase in severity and chronicity of the disorder.

Diagnosis is a dynamic process the purpose of which is to support correct decision making. Although having absolute diagnostic certainty is not necessary to adopt the correct therapeutic decision,[23,24] diagnostic tests seek to reduce uncertainty. In this study, post-test probability (predictive value, PV) decreased uncertainty, since the probability of PPD increased with positive results or decreased with negative

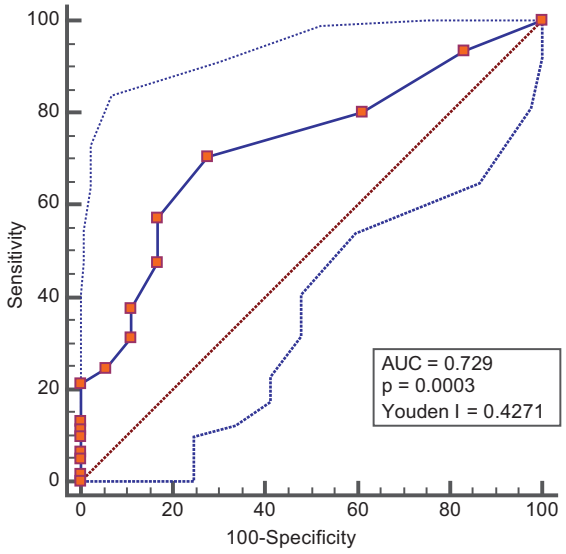

Figure 2. *Edinburgh Postnatal Depression Scale ROC curve of postpartum depression detection in Mexican women. AUC = area under the curve; Youden I = Youden Index.*

results when EPDS was applied. Given that PPD pretest probability (prevalence) (18.7 %) is not so high or close to 100 % to cross the therapeutic threshold and start treatment, or so low (0 %) as to cross the diagnostic threshold and rule out PPD, applying a simple, accessible and accurate test is required. Hence the importance of screening with EPDS, since these thresholds depend on each clinical scenario, on PPD severity and on the cost of false positive and false negative cases, both in clinical (risk of delaying or not establishing the diagnosis, with consequences on the mother, the family and the development of the child, due to underdiagnosed depression) and economic terms (if not diagnosed, inability of the mother to work or expenses due to neglect of the child owing to maternal depression).

The present study contributes to know EPDS validity and accuracy, since it allowed knowing the agreement it had with BDI to correctly classify the presence or absence of PPD. The higher the sensitivity and specificity values, the more valid the test is known to be. With a cutoff point of 12, the best sensitivity (70.4 %) and specificity (72.2 %) values were obtained for EPDS, with these data reflecting the intrinsic value of the test before knowing the result.

Little has been published about EPDS in Mexico. Alvarado et al. reported similar sensitivity (75 %), but higher specificity (93 %) with a cutoff point of 11/12:[25] PPV (36.9 %) in our study was lower than that reported by Alvarado et al. (50 %), whereas NPV was similar (91.4 %). It is important to remember that predictive values depend on the prevalence of PPD (18.7 %) and, therefore, are not directly applicable to scenarios with different pretest probabilities; regarding PPD, the reports differ depending on the type of study, place of study or instrument used as reference method. Therefore, to determine the probability of PPD (post-test) in scenarios with different pre-test probabilities, LRs were calculated, which constitute sensitivity and specificity summary indices that are independent of pretest probability. Thus, in our study, the probability of finding a positive result was 2.5 times higher, which constituted a small change, although it was important due to the psychological, economic and social repercussions PPD has. There was also a small change (0.4) in the probability of finding a negative result in the absence of PPD after applying the EPDS.

The present study has the strength that the analysis goes beyond the knowledge of EPDS sensitivity, specificity and predictive values. To the best of our knowledge, and taking into account that the diagnostic process does not end with the calculation of post-test probability, in no previous study in Mexico have the consequences of screening with EPDS been discussed. Furthermore, the profitability of a diagnostic test does not only depend on its validity, but also on its performance in diagnostic-therapeutic decision-making and its cost.[23] EPDS result does change initial clinical attitude and influences on the management of women with PPD; in addition, EPDS application is simple, inexpensive and accessible in routine clinical practice, which does not compromise its profitability. In the performance of a diagnostic test, the benefits women will experience with its application should also be considered: better bond with the child, better physical and emotional development of the children, more family harmony and reincorporation of the woman to work, among other.

A woman with an EPDS score > 12 should be evaluated and, if necessary, opportunely treated. According to the results of this study, out of 49 women who were considered healthy (and therefore did not receive treatment), five should have been referred for PPD care, which means that almost 10 % were without treatment despite the screening test.

Finally, the ROC curve and the area under the curve (0.729, 95% CI = 0.617-0.823) show that, although statistically significant, EPDS has a moderate accuracy; therefore, it will be better complementing it with another instrument or psychiatric assessment of suspected cases, in order to have a more accurate diagnosis.

Conclusion

It is important to raise awareness among health personnel and the general population about the relevance of PPD and of routinely screening mothers for PPD with EPDS during newborn assessment visits. When any woman has a score > 12 points, an additional evaluation will be needed with some other instrument and, sometimes, referring her to the specialist, who will determine the treatment. EPDS results are positive in 70 % of women with PPD and negative in 72 % of those without PPD. A woman with an EPDS positive result has a 37 % probability of having PPD; if her result is negative, she has 91 % probability of having PPD. Therefore, we can claim that EPDS is a diagnostic test with moderate accuracy.

Conflict of interests

None.

Ethical disclosure

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author is in possession of this document.

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ORIGINAL ARTICLE

The new coronavirus that came from the East: analysis of the initial epidemic in Mexico

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Abstract

Introduction: *As of March 23, 2020, suspension of non-essential activities was declared in Mexico throughout the country in order to mitigate the spread of the COVID-19 pandemic.* **Objective:** *To analyze data on the first 1,510 laboratory-confirmed cases of COVID-19 in Mexico, and to describe the geographical distribution of the disease and its transmission dynamics.* **Method:** *Description of the first COVID-19 cases with real-time RT-PCR-positive test, as well as evaluation of epidemiological measures, cumulative incidence, rate of transmission, and mortality and lethality rates during the first month of the epidemic.* **Results:** *Average age was 43 years, and 58 % were males; 44 % of initial cases were imported. Lethality in the population during the first month went from 1.08 to 3.97 per 100 cases; however, the trend is linear and similar to that observed in Europe.* **Conclusions:** *In Mexico, social distancing is being applied, but studies are still required on the dynamics of the epidemic, person-to-person transmission, incidence of subclinical infections, and patient survival.*

KEY WORDS: *Human coronavirus. SARS-CoV-2. Respiratory tract infections. Respiratory viruses.*

El nuevo coronavirus que llegó de Oriente: análisis de la epidemia inicial en México

Resumen

Introducción: *A partir del 23 de marzo de 2020, en México se declaró la suspensión de actividades no esenciales en todo el país para mitigar la diseminación de la pandemia de COVID-19.* **Objetivo:** *Analizar los datos sobre los primeros 1510 casos de COVID-19 confirmados por laboratorio en México, describir la distribución geográfica de la enfermedad y su dinámica de transmisión.* **Método:** *Descripción de los primeros casos de COVID-19 con prueba positiva de RT-PCR en tiempo real, así como evaluación de las medidas epidemiológicas, incidencia acumulada, razón de contagios y tasas de mortalidad y letalidad durante el primer mes de la epidemia.* **Resultados:** *La edad promedio fue de 43 años y 58 % fue del sexo masculino; 44 % de los casos iniciales fue importado. La letalidad en la población durante el primer mes pasó de 1.08 a 3.97 por 100 casos; sin embargo, la tendencia es lineal y similar a la observada en Europa.* **Conclusiones:** *En México se está aplicando el distanciamiento social, pero aún se requieren estudios sobre la dinámica de la epidemia, la transmisión de persona a persona, la incidencia de infecciones subclínicas y la supervivencia de los enfermos.*

PALABRAS CLAVE: *Coronavirus humano. SARS-CoV-2. Infecciones del tracto respiratorio. Virus respiratorios.*

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Introduction

A new virus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was identified in January 2020 as the cause of a series of pneumonia cases initially detected in the city of Wuhan, Hubei Province, China.[1] Shortly, SARS-CoV-2 disease has spread all over the world.[2] It was not until March that the spread of COVID-19 was recognized by the World Health Organization as a pandemic.[3] At the time of this report, more than 130,000 people have become infected in more than 100 countries, and the mortality rate went from 2 to 4 % in a short time.

Coronaviruses are members of the *Coronavirinae* subfamily of the *Coronaviridae* family, and of the *Nidovirales* order (according to the International Committee on Taxonomy of Viruses classification). This subfamily consists of four genera: alphacoronavirus, betacoronavirus, gammacoronavirus, and deltacoronavirus, depending on their phylogenetic relationships and genomic structures. The two highly pathogenic viruses, SARS-CoV and MERS-CoV, cause severe respiratory syndrome in humans, and the other four human coronaviruses (HCoV-NL63, HCoV-229E, HCoV-OC43, and HKU1) only induce mild upper respiratory diseases in immunocompetent hosts, although they can cause serious infections in infants, young children, and older people.[4,5]

SARS-CoV was the causative agent of severe acute respiratory syndrome outbreaks in 2002 and 2003 in the Guangdong province, China, with a lethality of 10 %.[6] Ten years later, MERS-Cov was the cause of Middle East respiratory syndrome (MERS-CoV), which has become a global health problem since 2012.[7] On that occasion, it affected more than 2,000 people in 27 countries of four continents, with an average lethality of 34 %. In the Middle East, MERS-CoV epicenter was Saudi Arabia,[8] a country to which millions of Muslims from all over the world travel annually to make the pilgrimage to Mecca (*hajj*), a tradition that contributed to global distribution of the virus. MERS-CoV infection is transmitted from animals to humans and from humans to humans.[9] Evidence shows that bats helped the spread of SARS-CoV[10] and MERS-CoV[11,12] as the original host species. The main cause of the occurrence of MERS-CoV infections is exposure to animals, mainly to bats and camels, which can act as an intermediate host.[4,13]

We currently know that SARS-CoV uses angiotensin II converting enzyme receptor as a binding site to infect ciliated bronchial epithelial cells and type II pneumocytes,[14] while MERS-CoV uses the dipeptidyl peptidase receptor 4 to infect non-ciliated bronchial epithelial cells and type II pneumocytes.[15]

In numerous countries, a desperate response has been given to get health systems ready and confront this unprecedented challenge. Unfortunately, China, Iran and Italy did not have the opportunity to prepare and resist the rush of sick people seeking medical attention, which caused more than 100,000 confirmed infections and 4,000 deaths from COVID-19.[16] Containment measures implemented in China have reduced the occurrence of new cases by 90 %.

With the identification of the first cases in Mexico, as of March 20, the federal government declared the suspension of educational activities in public and private systems and requested to postpone massive events involving more than 5,000 people. On March 23, the "Healthy Distance" (*"Sana Distancia"*) campaign was launched, which seeks to reduce contact between people by temporarily suspending non-essential activities in the public, social and private sectors. The campaign includes the following strategies:

- Promotion of frequent hand washing.
- Distant greeting.
- Diffusion of the habit of sneezing or coughing into the elbow.
- Domiciliary isolation of sick people.
- Protection, isolation and care of older adults.

The purpose of this document is to analyze data on the first 1,510 laboratory-confirmed COVID-19 cases in Mexico, describe the geographical and age distribution, and the dynamics of transmission.

Method

Based on information published by the World Health Organization (www.who.int), the Center for Science and Systems Engineering at Johns Hopkins University in Maryland, United States (coronavirus.jhu.edu) and the Unit of Epidemiological and Sanitary Intelligence of the Ministry of Health of Mexico (www.coronavirus.gob.mx), an analytical, cross-sectional study was carried out. Cases are reported as positive for SARS-CoV-2 infection when they meet the following criteria: clinical symptoms (fever, cough, malaise) and positive test by real-time RT-PCR for COVID-19 at any of the national laboratories authorized for this purpose. The following variables were recorded for this study:

- Date of the case report.
- Date of symptom onset.

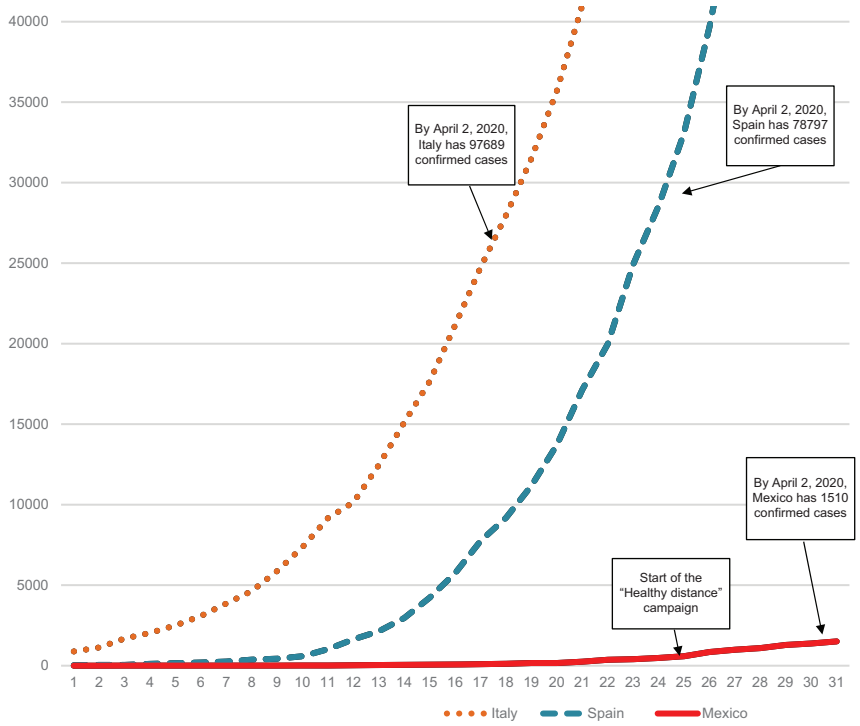

Figure 1. *COVID-19 new cases in Italy and Spain in comparison with Mexico in the first month after the first cases were recorded in the latter country.*

- Date of diagnosis.
- State of the republic where the case report was generated.
- Gender.
- Age.
- Origin of the cases that got infected abroad.

With the statistical program SPSS version 21, a database was constructed for descriptive analysis, with measures of central tendency and dispersion, of the number of patients, origin of the "imported" cases and distribution in the country. Epidemiological measures such as cumulative incidence, rate of infection, mortality and lethality rates were used to determine the evolution of the epidemic during the first month of evolution in the country. For the variables described in frequency, 95 % confidence intervals were calculated. Scatter plots were generated.

Results

Between February 29 and April 2, 2020, 1,510 COVID-19-positive cases were reported in Mexico, out of which 872 cases were of the male gender (58 %, 95 % CI = 55-61) and 638 were females (42%, 95 % CI = 38-46). Figure 1 describes the comparison of new COVID-19 cases in Italy, Spain and Mexico for the referred period.

Age of the cases ranged from 0 to 89 years, with an average of 43 ± 16 years; the percentile calculation showed that 25 % of the cases (P25) were younger than 31 years, 50 % (P50), younger than 42 years, and 75 % (P75) were younger than 54 years. The results show a high prevalence of the disease in an economically active young population, at least 10 years younger than the Chinese population, 5 % of the cases were even younger than 20 years, while only 10 % of cases were older than 65 years (highest-risk population). Figure 2 describes COVID-19-positive cases age distribution during the first month of the epidemic in Mexico according to their frequency.

Forty-four percent of the cases that started the epidemic in Mexico came from Europe and the United States: 296 cases from the United States (45%, 95 % CI = 39-51); 272 cases from Spain (41 %, 95% CI = 35-47). The rest of the countries and their impact on total onset of the epidemic are described in table 1 and figure 3.

When analyzing the incidence of cases by states of the country, most were identified in Mexico City (n = 346,

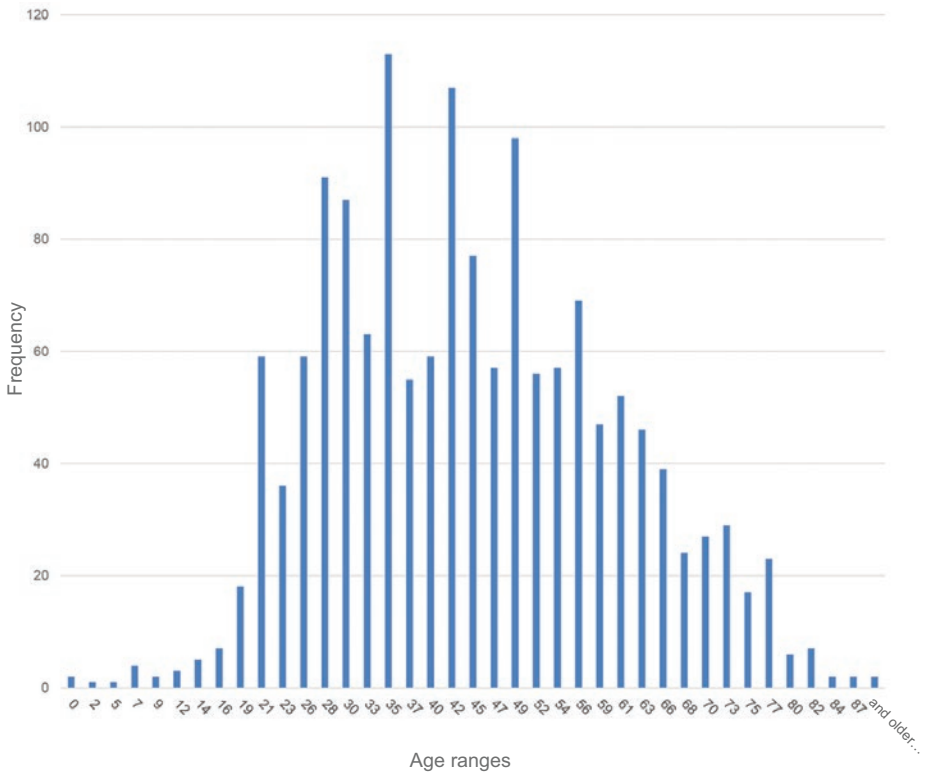

Figure 2. *COVID-19-positive cases by age ranges during the first month of the pandemic in Mexico.*

Table 1. Origin of the 661 cases that started the spread of COVID-19 during the first month of the pandemic in Mexico

	n	%	95 % CI
United States	296	45	39 to 51
Spain	272	41	35 a 47
France	54	8	1 a 15
Italy	24	3.6	−4 a 11
Germany	13	1.9	−6 a 9
Singapore	2	0.5	−9 a 10

95 % CI = 95 % confidence interval. Source: *Unidad de Inteligencia Epidemiológica y Sanitaria de la Secretaría de Salud de México* (www.coronavirus.gob.mx).

22.9 %), State of Mexico (n = 175, 11.6 %), Puebla (n = 102, 6.7 %), Jalisco (n = 113, 7.4 %) and Nuevo León (n = 79, 5.2 %). The remaining states had approximately 50 cases or less. An important aspect is the rate of infection directly related to the spread of the virus in the local population by cases infected abroad. Among the states with an accelerated infection rate, Michoacán (5.7), Sinaloa (4.8), Tabasco (4.1) and Coahuila (3.0) were identified. The incidence and rate of transmission of COVID-19-positive cases during the first month of the pandemic in Mexico are presented in table 2.

As for COVID-19-associated mortality, unlike Italy, which in the analyzed period went from 2.36 to 11.04 per 100 cases, and Spain, which went from 0.51 to 8.28 per 100 cases, Mexico was observed to have a slower evolution: it went from 1.08 to 3.97 per 100 cases, although the trend was linear and similar to that recorded in European countries, which indicates that if the required epidemiological actions are not carried out, the result could be similar to that of Spain or the United States.

The incidence of mortality has been higher in Mexico City (15 cases), with a cumulative incidence of 42.6 and a lethality of 3.9 per 100 cases. However, the states of the country that had the highest lethality were Morelos (25.0), Zacatecas (14.3), Durango (12.5), Nayarit (12.5), Hidalgo (10.0), Sinaloa (9.8), Baja California (9.6) and Baja California Sur (9.5), higher than global disease lethality: 4.7 per 100 cases. Deaths, cumulative incidence, mortality rate, and lethality of COVID-19-positive cases during the first month of the pandemic in Mexico in comparison with Italy and Spain are described in table 3 and figure 4.

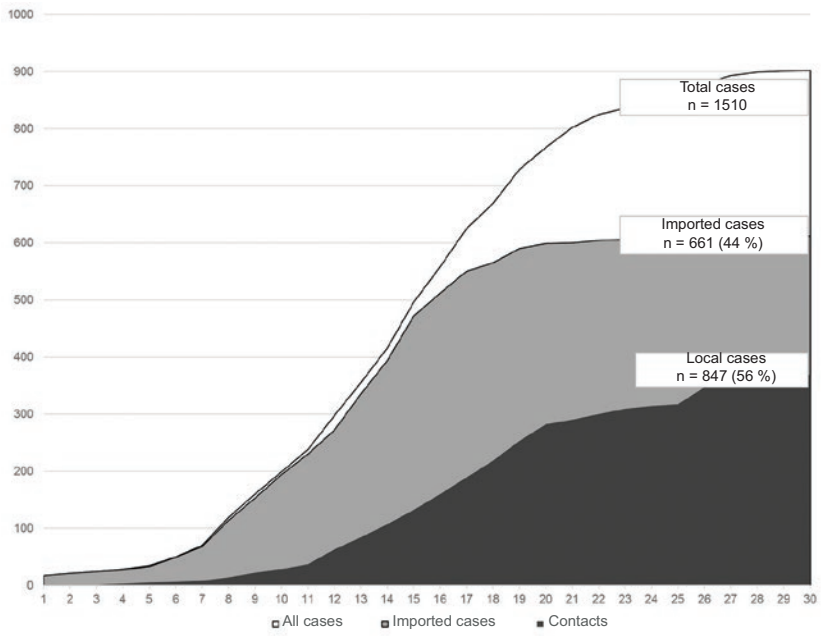

Figure 3. *Cumulative number of COVID-19-positive cases during the first month of the pandemic in Mexico according to their origin.*

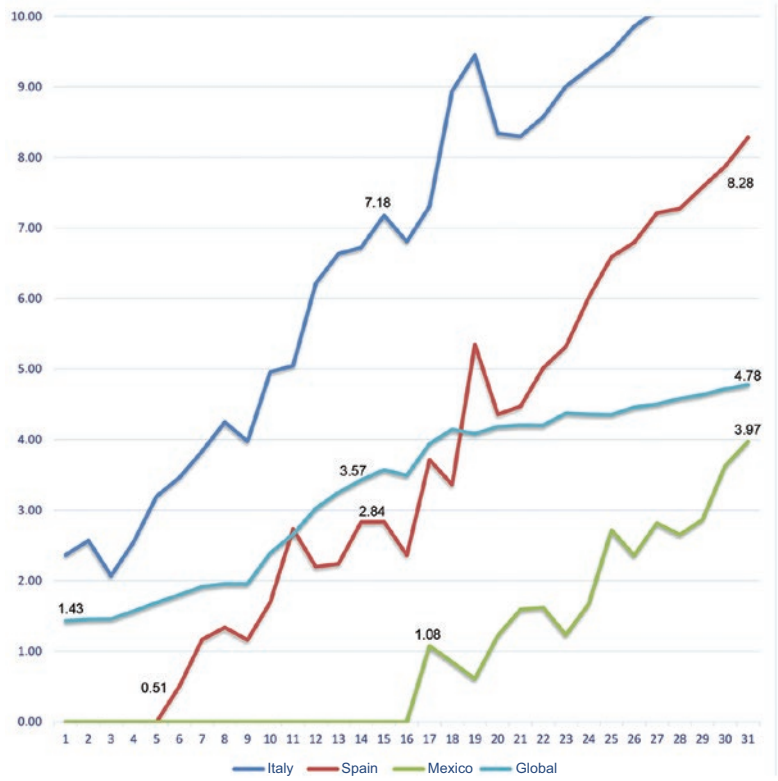

Figure 4. *COVID-19 lethality in Italy and Spain in comparison with Mexico in the first month after the pandemic started in the latter country.*

Discussion

This study presents a summary of epidemiological information during the first month of the COVID-19 epidemic in Mexico. This disease arrived in a very short time at two main fronts, the United States (which at the time of this report is the first focus of infection worldwide) and Spain, the health system of which has collapsed.

Table 2. COVID-19-positive cases incidence and rate of transmission during the first month of the pandemic in Mexico

State	Imported cases		Local cases (contacts)		Total		Rate of transmission
	n	%	n	%	n	%	
Mexico City	162	24.5	184	21.7	346	22.9	1.12
Jalisco	65	9.8	48	5.7	113	7.4	0.73
Nuevo León	56	8.5	23	2.7	79	5.2	0.41
State of Mexico	54	8.2	121	14.3	175	11.6	2.24
Puebla	40	6.1	62	7.3	102	6.7	1.55
Guanajuato	35	5.3	24	2.8	59	3.9	0.68
Quintana Roo	26	3.9	30	3.5	56	3.7	1.1
Yucatán	24	3.6	30	3.5	54	3.5	1.2
San Luis Potosí	18	2.7	12	1.4	30	1.9	0.6
Veracruz	17	2.6	20	2.4	37	2.4	1.1
Querétaro	17	2.6	16	1.9	33	2.1	0.9
Baja California	16	2.4	22	2.6	38	2.5	1.3
Aguascalientes	14	2.1	28	3.3	42	2.7	2.0
Sonora	13	2.0	7	0.8	20	1.3	0.5
Coahuila	13	2.0	40	4.7	53	3.5	3.0
Tabasco	11	1.7	46	5.4	57	3.7	4.1
Guerrero	8	1.2	11	1.3	19	1.2	1.3
Hidalgo	8	1.2	13	1.5	21	1.3	1.6
Tamaulipas	7	1.1	2	0.2	9	0.5	0.2
Oaxaca	7	1.1	15	1.8	22	1.4	2.1
Chiapas	7	1.1	6	0.7	13	0.8	0.8
Chihuahua	6	0.9	4	0.5	10	0.6	0.6
Sinaloa	6	0.9	29	3.4	35	2.3	4.8
Baja California Sur	5	0.8	13	1.5	18	1.1	2.6
Durango	5	0.8	2	0.2	7	0.4	0.4
Campeche	4	0.6	2	0.2	6	0.3	0.5
Michoacán	4	0.6	23	2.7	27	1.7	5.7
Morelos	4	0.6	5	0.6	9	0.5	1.2
Nayarit	3	0.5	4	0.5	7	0.4	1.3
Colima	2	0.3	0	--	2	0.1	--
Tlaxcala	2	0.3	3	0.4	5	0.3	1.5
Zacatecas	2	0.3	4	0.5	6	0.4	2.0

Source: *Unidad de Inteligencia Epidemiológica y Sanitaria de la Secretaría de Salud de México* (www.coronavirus.gob.mx). Downloaded on April 2, 2020.

According to Mexico's geographical distribution, the highest incidence of cases was observed in the country's central states, where there is a higher flow of international travelers and higher population density, and the disease is gradually spreading throughout the country. The debate on the advisability of early closure

Table 3. Deaths, cumulative incidence, mortality rate and lethality of COVID-19-positive cases during the first month of the pandemic in Mexico

State	Non-fatal cases	Deaths	Cumulative incidence	Mortality rate	Lethality
Mexico City	369	15	42.6	1.7	3.9
Quintana Roo	64	1	37.7	0.6	1.5
Aguascalientes	48	0	33.5	0.0	0.0
Tabasco	70	3	28.4	1.2	4.1
Yucatán	59	0	26.1	0.0	0.0
Baja California Sur	19	2	26.1	2.5	**9.5**
Coahuila	67	3	21.7	0.9	4.3
Puebla	108	1	16.5	0.2	0.9
Sinaloa	46	5	16.2	1.6	**9.8**
Querétaro	33	2	15.4	0.9	5.7
Nuevo León	85	0	15.2	0.0	0.0
Baja California	47	5	14.3	1.4	**9.6**
Jalisco	110	6	13.8	0.7	5.2
San Luis Potosí	33	2	12.2	0.7	5.7
State of Mexico	176	1	10.2	0.1	0.6
Hidalgo	27	3	9.7	1.0	**10.0**
Sonora	28	0	9.1	0.0	0.0
Guanajuato	52	0	8.3	0.0	0.0
Guerrero	23	1	6.6	0.3	4.2
Nayarit	7	1	6.2	0.8	**12.5**
Campeche	6	0	6.0	0.0	0.0
Morelos	9	3	5.9	1.5	**25.0**
Tlaxcala	8	0	5.8	0.0	0.0
Oaxaca	21	1	5.3	0.2	4.5
Colima	4	0	5.1	0.0	0.0
Michoacán	23	1	5.0	0.2	4.2
Tamaulipas	16	0	4.4	0.0	0.0
Durango	7	1	4.3	0.5	**12.5**
Zacatecas	6	1	4.2	0.6	**14.3**
Veracruz	33	2	4.1	0.2	5.7
Chihuahua	12	0	3.2	0.0	0.0
Chiapas	18	0	3.1	0.0	0.0
Total national	1634	60	13.3	0.47	3.5

95 % CI = 95 % confidence interval. Lethality higher than world average indicated in bold numbers (approximately 5.0). Source: *Unidad de Inteligencia Epidemiológica y Sanitaria de la Secretaría de Salud de México* (www.coronavirus.gob.mx). Downloaded on April 4, 2020.

of borders and civil aviation interruption remains open; these measures were carried out by other nations where currently there are few cases of the disease.

According to a report published by Young et al., 14 % of COVID-19 cases will be severe[17,18] and will require hospital care. Should the disease affect 70 % of the

120 million inhabitants of Mexico, there would be a scenario of approximately 18 million patients seeking medical attention in gradual waves. That same study showed that 5 % of cases were admitted to an intensive care unit, 2.3 % required assisted mechanical ventilation and 1.4 % died.

This leads to the projection that approximately 900,000 beds at intensive care units and 20,700 ventilators will be required in Mexico and that 12,600 deaths from COVID-19 might occur. Although these figures sound unlikely at this moment, the figures published by the World Health Organization (www.who.int) indicate that 14,681 patients have died in Italy and 10,935 in Spain. An advantage in Mexico is the younger age of the cases in comparison with China, where the average is 56 years (range 46 to 67);[19] moreover, 5 % of cases in Mexico are younger than 20 years and only 10 % are older than 65 years. However, it cannot be ignored that comorbidities (high blood pressure, obesity, diabetes mellitus and immunosuppression, among others) increase mortality, and that Mexico has a clear disadvantage in this regard.[20]

Early establishment of social distancing in Mexico was an adequate measure. According to studies carried out in China, the disease has a propagation speed among the population (R0) of 2.2 (95 % CI = 1.4-3.9),[1] which is much higher than that of influenza (R0 = 1.3),[21] and is a value that can be decreased with the isolation of positive cases.

With regard to lethality, analytical curves show a similar trend to those of Spain or Italy, but with a slower daily change. This means that the epidemic in Mexico will probably be slower and last longer, and the recommended measures should therefore be continued in the states of the country where the spread of the disease is more accelerated (infection rate). No country could face a disease that is transmitted so quickly without the effort of the population to stop the spread.

As weaknesses of this investigation, the type of study and the lack of follow-up of COVID-19 cases can be mentioned, which is information we did not have access to. Despite the above, the results provide an initial approach to the way the disease is distributed in Mexico.

Conclusions

In the worst case scenario, the evolution in Mexico of the COVID-19 pandemic could be similar to that of Spain, which is a situation that can only be modified with efforts of the population, continuation of the social distancing strategies implemented by the Ministry of Health and with the identification of more effective control measures to reduce transmission in the community. In the future, studies could be carried out on the dynamics of the epidemic, person-to-person transmission in households and other settings, serological tests results to determine the incidence of subclinical infections (carriers of the disease) and patient survival in different types of population. It is difficult to define whether the hospital infrastructure in our country will be sufficient to contain the pandemic. In Mexico, the coin is still in the air.

Conflict of interests

The author declares that he has no conflicts of interest.

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Ethical disclosures

Protection of people and animals. The author declares that no experiments were performed on humans or animals for this study.

Confidentiality of data. The author declares that no patient data appear in this article.

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ORIGINAL ARTICLE

Predictors of anthracycline-induced cardiotoxicity in a retro-prolective cohort of children surviving cancer

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Abstract

Introduction: *Cardiotoxicity is an adverse reaction associated with the use of anthracyclines.* **Objective:** *To estimate the factors associated with the development of anthracycline cardiotoxicity in pediatric patients surviving cancer.* **Method:** *Retro-prolective cohort of children diagnosed with cancer and treated with anthracyclines. Baseline echocardiographic determination of ejection fraction (LVEF0) was carried out before the start of treatment and again at 12 months (LVEF1). Demographic characteristics and treatment were obtained from the medical record. A multiple logistic regression (MLR) model was constructed; LVEF1 < 50 % was the dependent variable, which was adjusted for the main confounding variables.* **Results:** *Sixty-five patients were included, out of which 36.9 % were females and 56.8 % had a solid tumor. LVEF0 was 74.79 ± 7.3 % and LVEF1, 67.96 ± 6.7 % (p = 0.001); 60 % developed cardiotoxicity. In the MLR, only a cumulative dose > 430 mg was associated with cardiotoxicity (p = 0.001).* **Conclusions:** *In Mexican children, an anthracycline cumulative dose > 430 mg should be avoided in order to prevent cardiotoxicity.*

KEY WORDS: *Anthracyclines. Cardiotoxicity. Risk factors.*

Factores predictores de cardiotoxicidad inducida por antraciclinas en una cohorte retroprolectiva de niños supervivientes de cáncer

Resumen

Introducción: *La cardiotoxicidad es una reacción adversa asociada al uso de antraciclinas.* **Objetivo:** *Estimar los factores asociados al desarrollo de cardiotoxicidad por antraciclinas en pacientes pediátricos supervivientes de cáncer.* **Método:** *Cohorte retroprolectiva de niños con diagnóstico de cáncer tratados con antraciclinas. Se realizó determinación ecocardiográfica basal de la fracción de expulsión (FEVi0) antes del inicio del tratamiento y a los 12 meses (FEVi1). Del expediente se obtuvieron las características demográficas y el tratamiento. Se realizó un modelo de regresión logística múltiple (RLM); la FEVi1 < 50 % fue la variable dependiente, que se ajustó por las principales variables confusoras.* **Resultados:** *Se incluyeron 65 pacientes, 36.9 % fue del sexo femenino y 56.8 % presentó un tumor sólido. La FEVi0 fue de 74.79 ± 7.3 % y la FEVi1, de 67.96 ± 6.7 % (p = 0.001); 60 % desarrolló cardiotoxicidad. En la RLM solo la dosis acumulada > 430 mg se asoció a cardiotoxicidad (p = 0.001).* **Conclusiones:** *En los niños mexicanos se debe evitar una dosis acumulada > 430 mg de antraciclinas para evitar la cardiotoxicidad.*

PALABRAS CLAVE: *Antraciclinas. Cardiotoxicidad. Factores de riesgo.*

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Introduction

The incidence of cancer in Mexico has been estimated at 126 cases/million/year.[1,2] Reports made by the World Health Organization show that the incidence of cancer in children younger than 15 years has increased by more than 200 % in developing countries, with a predominance of leukemias and lymphomas.[3-5] In addition to the increase in cancer global incidence, treatment improvements have allowed to increase survival, but treatment-associated adverse reactions have also grown.[6] In the case of anthracyclines,[7] adverse events have been reported both during therapy administration and in the long term.

Pediatric patients who survive cancer are eight times more likely to die from heart disease and 15 times more likely to suffer from heart failure than their siblings who did not have cancer.[8,9] Cardiotoxicity (CTX) is the leading non-cancerous cause of serious complications, and the risk persists for up to 45 years after treatment conclusion, in addition to constituting the second leading cause of death, after secondary cancer.[10]

Anthracycline-treated childhood cancer survivors experience subclinical cardiac dysfunction for up to six years after treatment completion.[1] Furthermore, CTX can develop in the long-term and occur at between four to 20 years after treatment conclusion.[11] Some studies report that the prevalence of this complication is as high as 57 %.[12] In Mexico, the incidence of CTX in children surviving cancer is not known.

Acute CTX can cause from mild electrocardiographic changes to life-threatening arrhythmias.[13] Long-term cardiotoxic effects have been described to depend on drug doses[14] and to be clinically more important than the acute ones.

Although some factors associated with acute CTX have been described, few studies address the factors associated with the development of long-term CTX. The purpose of the present study was to assess the factors associated with the development of long-term CTX in anthracycline-treated children who survived cancer.

Method

Retro-prolective cohort study of cancer surviving patients attended to at the Oncology Department of the Pediatric Hospital "Dr. Silvestre Frenk Freund" between 2013 and 2018. Patients aged < 16 years who were cancer survivors (disease-free period ≥ 5 years), who received treatment with anthracyclines, who had no evidence of previously-existing heart disease, corroborated by echocardiography, were included. All participants had to have a baseline echocardiogram, obtained within the first month after starting anthracycline treatment at the Cardiology Department by attending physicians. Patients with clinical anemia, corroborated by blood count (hemoglobin < 7 mg/dL) or with hemodynamic repercussion were excluded. Ejection fraction determination was performed before treatment (LVEF0) and 12 months after treatment initiation (LVEF1) at the Pediatric Cardiology Department of the hospital. Information on demographic characteristics and treatment was obtained from the medical record. CTX was defined as a 10 % decrease in LVEF1 in relation to the baseline value, in accordance with the guidelines for cancer treatments and cardiovascular toxicity of the European Society of Cardiology.[15]

The sample size was calculated with the difference between proportions formula, assuming an alpha = 0.05 and 1-beta = 0.20. The Epi Info program was used. Eight percent of subjects with CTX prior to chemotherapy and 40 % one year after treatment were estimated. The sample was defined with at least 60 patients, a size that ensured stability of the multiple logistic regression (MLR) models adjusted for six variables.

The following quantitative variables were summarized in the descriptive statistics: age, weight, height, LVEF0, LVEF1. Inferential methods were used to corroborate the type of distribution; if the variables did not show normal distribution with this procedure, Kolmogorov-Smirnoff normality tests were carried out. Variables with normal distribution were expressed as means and standard deviations, and freely distributed variables, as medians and 25-75 interquartile ranges (IQR). Nominal variables (gender, dichotomous age, tumor type) were recorded using frequencies and percentages.

To determine the risk factors, nominal variables were analyzed using Pearson's chi-square test. To assess clinical relevance, relative risks (RR) were calculated with 95 % confidence interval (CI). Quantitative variables with normal distribution were analyzed with Student's t-test or Mann-Whitney's U-test. Variables that were statistically significant in the bivariate model were selected to be included in the MLR model, which was carried out by descending steps, in order to find the independent variables with the highest association. To assess the dose with the highest risk for

CTX, a ROC curve was plotted in order to find the point with the highest positive likelihood ratio (PLR).

The SPSS program, version 25, was used for statistical analysis. A p-value < 0.05 was determined to be statistically significant in all cases.

This study was carried out following the ethical guidelines for research in human subjects[16] set forth in the Declaration of Helsinki, the Nuremberg Code, the Belmont Report, the CIOMS Guidelines and the Good Clinical Practice Guidelines for the Americas, as well as in accordance with the Regulations of the General Statute of Health in Matters of Health Research, in particular with article 13 regarding the respect, dignity and protection of the rights of the pediatric patient; article 17, with regard to which the research was considered to be of minimum risk; and 20 and 21, which regulate informed consent and assent. The research was also limited to the indications of Mexican Official Standard NOM 012-SS3-2012 for the execution of research projects for health in human subjects. All patients who received anthracyclines and who were in the cohort of cancer survivors (equitable subject selection) were invited to participate. All parents of patients who agreed to participate in the protocol signed a consent letter after being informed about the purposes of the research. Children older than eight years signed informed assent letters.

As part of the follow-up protocol, all patients received dexrazoxane as a cardioprotective agent. Those in whom CTX was detected were referred to the Cardiology Department to receive an angiotensin converting enzyme inhibitor treatment and were followed-up (risk-benefit balance).

The study has social value because cancer survivors are currently not systematically evaluated in pediatric practice. The parents of all participants will be contacted to inform them on the results of the research.

The research was reviewed and accepted by the National Commission for Scientific Research of the Mexican Institute of Social Security with number R-2013-785-040.

Results

Sixty-five patients who met the inclusion criteria were recruited. Baseline characteristics of the population are summarized in Table 1. Among the participants, 36.9 % were females. As for age, 60 % of participants were older than five years at diagnosis. Solid neoplasms accounted for 56.8 % of tumors.

Table 1. Baseline characteristics of 65 children surviving cancer

Variable	n	%
Gender		
Female	24	36.9
Age at diagnosis		
≤ 5 years	26	40.0
> 5 years	39	52.3
Type of tumor		
Hematolymphoid	26	40.0
Solid	39	60.0
Baseline weight in kg (IQR)	15.5 (11.5-24.7)	
Cumulative dose in mg (IQR)*	320 (165-485)*	
Baseline height in cm	107 ± 27	
LVEF0 % (mean)	74.79 ± 7.3	
LVEF % (mean)	67.96 ± 6.7	

*Cumulative dose of all three anthracycline groups.
IQR = interquartile range, LVEF0 = left ventricular ejection fraction prior to chemotherapy, LVEF1 = left ventricular ejection fraction after chemotherapy.

Baseline weight of the participants was 15.5 kg (IQR = 11.5-24.7 kg), baseline height was 107 ± 26.6 cm. Anthracycline cumulative dose (epirubicin, doxorubicin, or daunorubicin) had a median of 385 mg/m^2 body surface area (IQR = 184-325). Participants' mean LVEF0 was 74.8 ± 7.3 %.

Thirty-nine patients (60 %) developed early CTX. When the risk factors were analyzed, the male gender was found to have a RR = 2.09 (95 % CI = 0.7-6.13) for the development of cardiotoxicity. Among the three types of anthracyclines used in the study, epirubicin had a RR = 2.35 (95 % CI = 0.7-7.61). Hematolymphoid-type neoplasms had a RR = 1.75 (95 % CI = 0.96-3.15). The rest of RRs are summarized in Table 2. RRs are represented in Figure 1.

Mean LVEF0 and LVEF1 were compared with Student's t-test, with a mean difference of 10 % being found (p = 0.001), which can be observed in Figure 2.

The prevalence of CTX in our population was 60 %. The rest of anthracycline-associated complications were neutropenia and fever (18 %), mucositis (6.2 %), thrombocytopenia, and sepsis (3.1 %).

A logistic regression model was calculated, where the dependent variable was CTX, adjusted for age and cumulative dose, which were the main confounding variables (Table 3).

The stepwise descending logistic regression method was used in order for the main variables associated with the development of cardiotoxicity to remain within the model. In step 4 of the model, the epirubicin

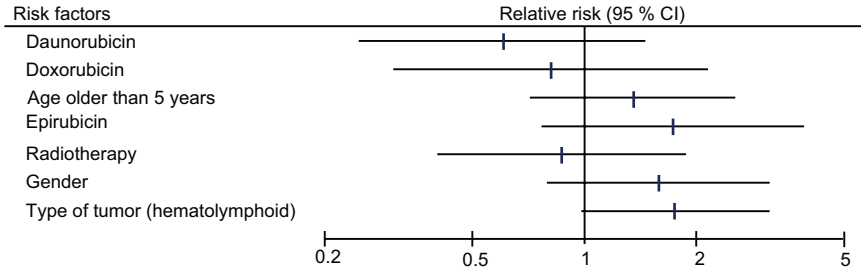


Figure 1. *Main risk factors associated with the development of cardiotoxicity.*

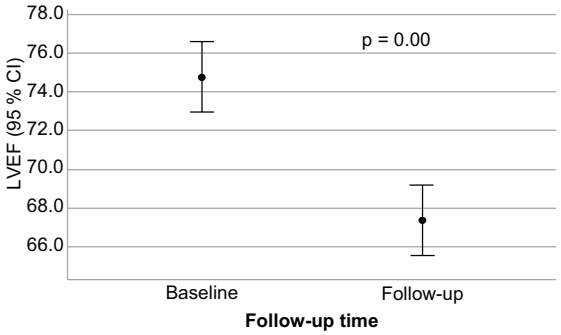


Figure 2. *Comparison of ejection fraction means at baseline versus at 12 months. LVEF = left ventricular ejection fraction.*

variable had an odds ratio (OR) = 4.69 (95 % CI = 0.75-29.03), cumulative dose had an OR = 1.004 (95 % CI = 1.001-1.007).

Since the cumulative dose was the only variable associated with CTX, a ROC curve was plotted (Fig. 3), where an area under the curve of 0.716 was found (95 % CI = 0.55-0.882). The 260 mg dose had a PLR of 1.84; the 380 mg dose, of 2.15; and the 430 mg dose, of 3.51 (Fig. 4).

Discussion

Some risk factors associated with anthracycline CTX have been identified, which are potentially modifiable, such as cumulative dose, rate of administration, concomitant treatments, or physical activity. However, there are few studies with long-term follow-up where these possible risk factors are adjusted according to possible confounders. To the best of our knowledge, this is the first study in Mexico where risk factors for the development of CTX are assessed with multivariate models.

The present study focused on cancer surviving patients, i.e., those who developed CTX after treatment, which rules out patients who had acute toxicity, as we described in other investigations.[17]

Table 2. Risk factors associated with the development of cardiotoxicity

Variable	Cardiotoxicity		Relative risk	95 % CI
	Yes (n = 26)	No (n = 39)		
Males	19	22	1.6	0.78-3.21
Doxorubicin versus	3	6	Reference	
Epirubicin	21	25	1.4	0.51-3.63
Daunorubicin	4	11	0.81	0.22-2.78
Hematolymphoid neoplasm	15	12	1.8	1.01-3.22
Solid tumor	12	27		
Age > 5 years	16	18	1.4	0.72-2.57
Radiotherapy	5	9	0.9	0.39-1.88

RR = relative risk, CI = confidence interval.

International reports indicate that the prevalence of CTX in patients receiving anthracyclines is approximately 40 %; however, in our analysis it was 60 %, which can be attributed to the fact that in our population the incidence of hematolymphoid neoplasms is higher than in other populations.

In the bivariate model, hematolymphoid neoplasms had a RR = 1.75, which was not significant, probably due to an insufficient sample size, since an interval lower limit of 0.97 indicates a trend towards risk. A similar situation was observed with the male gender and epirubicin; these factors may be significant if the sample size is increased, as indicated in other studies.[15]

Although cardiac evaluation has evolved, ejection fraction reduction and symptomatic congestive heart failure (current CTX outcomes)[18] generally occur weeks or years after treatment conclusion.[19] Ejection fraction measurement continues to be used in clinical practice because it is readily accessible and highly

Table 3. Logistic regression model for the development of cardiotoxicity associated with the use of anthracyclines

Variables		B	OR	95 % CI for OR	
				Lower	Upper
Step 1 (p = 0.116)					
r^2 = 0.215	Gender	0.04	1.04	0.17	6.46
	Epirubicin	1.93	6.88	0.81	58.4
	Diagnosis*	0.379	1.461	0.264	8.077
	Age**	–0.726	0.484	0.056	4.202
	Cumulative dose	0.003	1.003	1	1.007
Step 2 (p = 0.062)					
r^2 = 0.215	Epirubicin	1.926	6.859	0.815	57.709
	Diagnosis*	0.38	1.462	0.265	8.083
	Age**	–0.704	0.494	0.071	3.427
	Cumulative dose	0.003	1.003	1	1.007
Step 3 (p = 0.029)					
r^2 = 0.213	Epirubicin	1.784	5.955	0.795	44.586
	Age**	–0.752	0.471	0.069	3.237
	Cumulative dose	0.003	1.003	1	1.007
Step 4 (p =0.013)					
r^2 = 0.203	Epirubicin	1.546	4.692	0.758	29.035
	Cumulative dose	0.004	1.004	1.001	1.007

*Hematolymphoid versus solid diagnosis. **Age > 5 years.

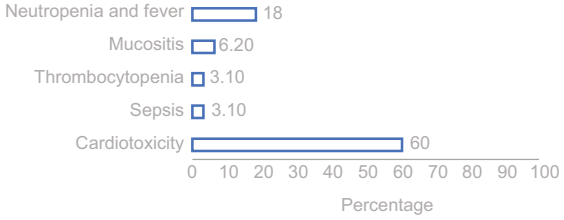


Figure 3. *Prevalence of anthracycline-associated complications.*

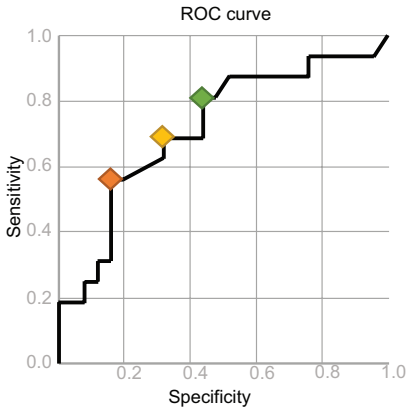
useful to determine the initiation of heart protection treatment.[20]

The main mechanism of anthracycline-induced CTX appears to involve the generation of highly reactive oxygen radicals, which promote lipid peroxidation, which in turn damages the cell membrane.[21] Anthracycline toxic effects on myocardiocytes lead to left ventricular wall thinning, which increases afterload and decreases contractility, thus resulting in congestive heart failure.[22] The European guidelines recommend surveillance of patients with high clinical risk, even though risk factors in pediatric patients are not well described.[23]

In clinical practice, follow-up after cancer complete remission is for one year after chemotherapy is concluded, whereby the incidence of this phenomenon could be underestimated, especially in children, with whom contact may be lost.

In the multivariate model, adjustment was made according to the main confounding variables: age, gender, type of tumor and cumulative dose. The sample size limited the number of variables in the model; however, an OR = 1,004 was observed for the cumulative dose, which was statistically significant. This has important implications: it appears that it is total dose and not the type of anthracycline that is associated with cardiac complications in children, unlike to what occurs in adults.

We can relate this finding to the fact that hematolymphoid neoplasms were the main risk factor in the bivariate model, since patients with this type of tumors



Area	SD	Sig.	95 % CI	
			Lower	Upper
0.716	0.085	0.021	0.55	0.882

Dose	Sensitivity	Specificity	PLR
260 mg	0.813	0.44	1.84
380 mg	0.68	0.32	2.15
430 mg	0.56	0.16	3.51

Figure 4. *ROC curve for the association of cumulative dose and higher likelihood of cardiotoxicity. PLR = positive likelihood ratio. SD = standard deviation. Sig. = significance.*

receive the highest doses of chemotherapy and for a longer period.

The importance of performing these analyses at each country lies in the genetic variants that modify the pharmacology of chemotherapy.[24]

The study herein presented is part of the follow-up of a cohort of pediatric cancer survivors, which has allowed the detection of other adverse events such as hearing loss,[25] nephrotoxicity,[26,27] neutropenia and fever;[28,29] hence the relevance of follow-up studies[30] and clinical trials that allow adequately addressing the consequences of chemotherapy-associated toxicity.[31] The cure is not enough, it is necessary to continue with efforts to limit reversible and non-fatal and irreversible adverse events.[32,33]

In this research, we propose that high doses of anthracyclines (regardless of the type) constitute the main risk factor. Consequently, clinicians will be able to offer protective treatments to children who require high doses of anthracyclines, which shall, in theory, lessen one of the most catastrophic complications.

Conclusion

The main risk factor associated with early cardiotoxicity in Mexican children surviving cancer is a cumulative dose higher than 430 mg.

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Conflict of interests

None

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Ethical disclosures

Protection of people and animals. The authors declare that the procedures followed adhered to the ethical standards of the responsible human experimentation committee and were in accordance with the World Medical Association and the Declaration of Helsinki.

Confidentiality of data. The authors declare that they have followed the protocols of their workplace regarding the publication of patient data.

Right to privacy and informed consent. The authors have obtained the informed consent of the patients referred to in the article. This document is in the possession of the corresponding author.

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EDITORIAL

Double or triple combination therapy in systemic arterial hypertension: to whom, when and with what?

Terapia dual o triple en hipertensión arterial sistémica, ¿a quiénes, cuándo y con qué?

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Systemic arterial hypertension (SAH) is the main risk factor for premature death in the world and in Mexico.[1] Around 15.2 million Mexicans have been estimated to be diagnosed with SAH, out of which 7.48 million are affiliated to IMSS (evaluation of the financial risks considered in the Institutional Risk Management Program, ENSANUT, 2018).[2] SAH is a complex, chronic disease that requires continuous medical attention with multifactorial risk reduction strategies, which go beyond numerical control in mm Hg of blood pressure and have been shown to be effective in reducing the vascular, cardiac and renal complications of the disease, as well as its impact on premature death. Continuous education and support in SAH self-management are essential to overcome the main challenges: treatment adherence and self-monitoring by the patient for the rest of his/her life.[3,4]

In the American Heart Association and American College of Cardiology (AHA/ACC) 2017 guidelines, which update the Joint National Committee seventh report, reducing the cutoff point for the diagnosis of SAH to ≥ 130/80 mm Hg was proposed, as well as starting pharmacological treatment in patients at stage 1 (130-140/80-90 mm Hg) and with high risk.[5] On the other hand, the new recommendations of the European Society of Cardiology and European Society of Hypertension (ESC/ESH) 2018 guidelines indicate that, in patients with borderline or elevated blood pressure (130-139/80-89 mm Hg), only lifestyle changes, diet and exercise should be indicated. These apparent divergences have generated uncertainty, especially among experts.[6] Another innovative aspect is the recommendation to use double or triple combination therapy, either separately or in a single pill, as first-line treatment in patients with SAH, especially with blood pressure levels ≥ 160/100 mm Hg or ≥ 140/90 mm Hg if the subject has high-risk factors.[6]

In Mexico, as in most Latin American countries, there are serious lags in SAH timely detection, as well as in risk stratification, timely treatment initiation, optimal control, control follow-up and treatment adherence.

Whom to give double or triple combination therapy?

The most recent guidelines (AHA/ACC and ESC/ESH)[4,5] recommend starting treatment with two drugs, preferably in a single pill, in patients with blood pressure ≥ 160/100 mm Hg or ≥ 140/90 mm Hg when they are classified as being at high risk or have target organ damage (retinopathy, left ventricular hypertrophy, kidney disease, diabetes). Furthermore, approximately 25 % of patients will require three antihypertensive agents to achieve treatment goals, which have become stricter (<130/80 mm Hg but not <120/70 mm Hg).[5,6]

We do not fully agree with some ACC/AHA criteria established in the 2017 guidelines:

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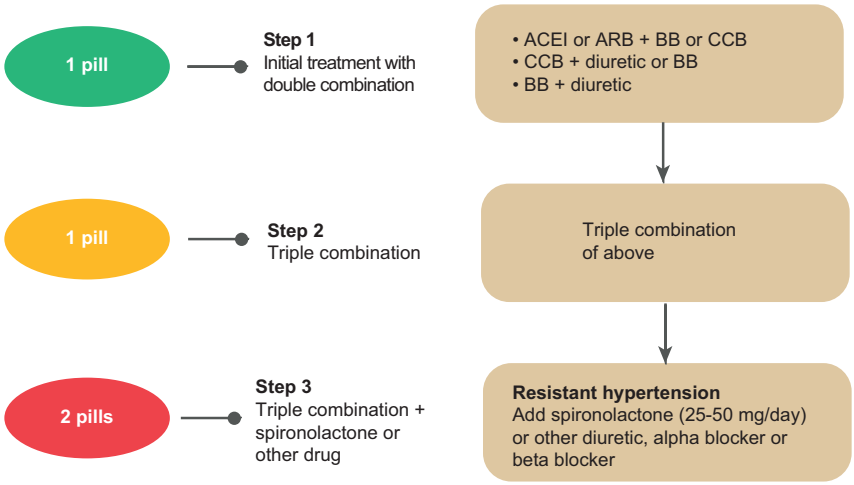

Figure 1. *Stepwise approach to the treatment of high-risk patients with stage 1, 2 and 3 systemic arterial hypertension. ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; BB, beta blocker; CCB, dihydropirine calcium channel blocker. Adapted from the European guidelines (ESC/ESH).*[6]

- The 130/80 mm Hg threshold to determine the presence of hypertension, under the premise that most patients at stage 1 (130-139/80-89 mm Hg) can be controlled with non-pharmacological strategies and that only patients with high cardiovascular risk require pharmacological treatment and only with one drug.
- A blood pressure goal < 130/80 mm Hg, given that the context in Mexico is very different and that the goal with the highest net clinical benefit (effectiveness/side effects) is the level accepted by the 2018 European guidelines, i.e., < 140/90 mm Hg and only if the patient tolerates it, < 130/80 mm Hg but not less than 120/70 mm Hg.

We agree with the tendency in both guidelines to start with a double pharmacological combination, preferably in a single pill, given that most patients are of medium-high risk, except for older, frail and high-risk younger patients with blood pressure > 130/80 mm Hg, in whom monotherapy would be indicated.

With what? Double and triple combination therapy

The simultaneous effect of the renin-angiotensin-aldosterone system, autonomic nervous system, vascular reactivity and endothelial function, among others, shows that the blockade of a single mechanism may not be sufficient and monotherapy is therefore insufficient, whereas the combination of two antihypertensive agents with a different mechanism of action can reduce coronary events by up to approximately 35 % and cerebrovascular events by 54 %.[7]

If there is sufficient evidence of multiple pathophysiological aspects, why does the prescription of monotherapy predominate? The reason most commonly expressed by the general practitioner is related to the fear of suddenly and extremely lowering blood pressure. However, there is increasing evidence that with the combination of drugs, control figures with higher consistency and without side effects are achieved. A calcium channel blocker (CCB) with a diuretic or an angiotensin II receptor blocker (ARB) or an angiotensin converting enzyme inhibitor (ACEI) with a diuretic, or an ACEI or ARB with CCB are the combinations popularized as “double combination antihypertensive therapies”, which already even exist in a single pill, which in turn facilitates their prescription and treatment adherence by the patient.[8]

The use of a beta-blocker with a diuretic is also a double combination therapy; however, it is only recommended in certain patients (with ischemia, tachyarrhythmia, or heart failure). There are combinations that should not be used, such as two agents that inhibit the renin-angiotensin axis (on April 20, 2012, the Food and Drug Administration issued a warning on the subject), specifically an ACEI with an ARB.[9] Combinations of diuretics with beta-blockers have been observed to have the tendency to increase the risk for developing type 2 diabetes, as recorded in the ASCOT trial,[10] and thus they should be used with caution, especially in

diabetogenic populations such as the Mexican population. The combination of beta-adrenergic blocking agents with non-dihydropyridine calcium channel blockers is also not recommended, due to the increased risk of bradycardia or atrioventricular block.[11] The treatment steps for patients with blood pressure > 150/100 mm Hg according to the ESC/ESH 2018 guidelines are shown in Figure 1.

Monotherapy should only be tried in subjects with mild SAH (140-149/90-99 mm Hg) and low cardiovascular risk, or in special cases, such as in frail patients or in case of gestational hypertension.[12-16]

When to prefer double combination therapy with a diuretic and when with a calcium channel blocker?

In patients with obesity or predisposition to metabolic problems, the combination of ACEI or ARB with dihydropyridine CCB (amlodipine, nicardipine, felodipine) is preferable; if there is no obesity or metabolic problems but there is evidence of fluid retention, the combination of an ACEI or ARB with a thiazide diuretic is preferable if the renal function is preserved (glomerular filtration rate > 40 mL/minute) or a loop diuretic if renal function is impaired (< 30 mL/minute).[11-16]

The ACCOMPLISH trial included 11,462 patients older than 50 years with high cardiovascular risk (60.4 % with diabetes mellitus). They were divided into two groups: one group received the benazepril plus amlodipine combination, and the other group received benazepril plus hydrochlorothiazide. Trial duration was designed for five years; however, the study was suspended at month 39 because the calcium channel blocker plus ACEI combination was found to be superior to the ACEI plus hydrochlorothiazide combination in the reduction of cardiovascular, cerebrovascular and renal events.[16]

Conclusion

By blocking several pathways of blood pressure increase, combination therapy has higher antihypertensive power than high-dose monotherapy, in addition to providing greater protection to target organs and having less potential for side effects.

The most commonly used combinations include an ACEI or ARB associated with a calcium channel blocker or a natriuretic agent. Combinations that include a diuretic have yielded better results in patients with heart failure or impaired kidney function.

A significant percentage of patients will require triple therapy: an ACEI or ARB, a CCB and a natriuretic agent, which should be administered to patients who fail to respond to double combination treatment at six to eight weeks, since the benefit is beyond the shadow of a doubt. The use of these combinations in the form of a single tablet has a highly favorable impact on treatment adherence by the patient.

Conflict of interests

None

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Ethical disclosures

The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data

The authors declare that no patient data appear in this article.

Right to privacy and informed consent

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REVIEW ARTICLE

Antibody-drug conjugates: the new generation of biotechnological therapies against cancer

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Abstract

Therapeutic antibodies are recombinant proteins used in the treatment of cancer. There is a new generation of monoclonal antibodies with activity against cancer cells, known as antibody-drug conjugates. These molecules are made up of three elements: a monoclonal antibody, a highly potent cytotoxic drug, and a chemical linker that binds them together. The antibody recognizes tumor antigens, thereby allowing targeted delivery of the cytotoxic agent to cancer cells. After recognizing its antigen, the antibody-drug conjugate is endocytosed by the target cells, where the protein fraction is degraded into lysosomes, releasing the cytotoxic drug. This article reviews antibody-drug conjugates general characteristics and describes the clinical evidence of efficacy and safety of the first four approved by regulatory agencies in the United States and Europe.

KEY WORDS: *Antibody-drug conjugates. Gemtuzumab ozogamicin. Brentuximab vedotin. Trastuzumab emtansine. Inotuzumab ozogamicin.*

Anticuerpos conjugados a fármaco: la nueva generación de terapias biotecnológicas contra el cáncer

Resumen

Los anticuerpos terapéuticos son proteínas recombinantes empleadas en el tratamiento del cáncer. Existe una nueva generación de anticuerpos monoclonales con actividad contra las células cancerosas, conocidos como anticuerpos conjugados a fármacos. Estas moléculas están integradas por tres elementos: un anticuerpo monoclonal, un fármaco citotóxico con alta potencia y un enlazador químico que los une. El anticuerpo reconoce antígenos tumorales, por lo que permite la entrega dirigida del agente citotóxico hacia las células cancerosas. Tras el reconocimiento de su antígeno, el anticuerpo conjugado a fármaco es endocitado por las células blanco, donde se induce la degradación lisosomal de la fracción proteica y se libera el fármaco citotóxico. En el presente artículo se revisan las características generales de los anticuerpos conjugados a fármacos y se describe la evidencia clínica de la eficacia y seguridad de los primeros cuatro aprobados por las agencias reguladoras de Estados Unidos y Europa.

PALABRAS CLAVE: *Anticuerpos conjugados a fármacos. Gemtuzumab ozogamicina. Brentuximab vedotina. Trastuzumab emtansina. Inotuzumab ozogamicina.*

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Therapeutic antibodies overview

Antibodies are excellent agents that with high specificity inhibit specific molecules. Clinical use of an antibody was first carried out in 1986, after the development of the technology to produce monoclonal antibodies. It was an anti-CD3 murine antibody (muromonab), the use of which, although effective in reducing transplant rejection, was limited by its rapid elimination, poor capability to induce effector functions in the immune system and and due to the development of antibodies against the drug.[1]

To avoid these problems, chimeric, humanized or totally human antibodies have been generated and, by means of protein engineering, the solubility, aggregation and stability profiles have been improved in order to scale up their production. Currently, there are recombinant antibodies that can be therapeutically used. These products, generically known as therapeutic antibodies (TA), are used in the treatment of cancer and other chronic-degenerative or infectious diseases. Owing to the incidence of diseases that can be treated with TAs, as well as to TAs effectiveness and positioning in the market, these agents constitute the fastest growing segment among biotech products, with sales that annually grow by 5.3 %.[2] Only in 2018, the Food and Drug Adninistration (FDA) approved 11 new TAs.[3]

TAs are immunoglobulins G

All TAs on the market are immunoglobulins G (IgG) that have stability in the circulation, good effector capacity and adequate physicochemical properties for their formulation and administration.[4] IgGs are glycoproteins composed of two light chains and two heavy chains (Fig. 1A). The light chain has two immunoglobulin domains: one variable (V_L) and one constant (C_L) at N-terminus. The heavy chain has four immunoglobulin domains, one variable (V_H) and three constant (C_H1, C_H2, and C_H3) at N-terminus. The structure of the antibody is stabilized by disulfide bonds that link light to heavy chains and heavy chains to each other, which confers flexibility and a "Y" shape with two identical antigen-binding fragments (Fab). Each Fab is made up of the variable domains of a heavy and a light chain (Fig. 1B). Hypervariability of the Fab sequences can generate an almost infinite diversity of antibodies with different specificity.[5]

The fragment crystallizable region (Fc region) is made up only of heavy chains and plays an effector role by binding to specific receptors (FcR) expressed on cells

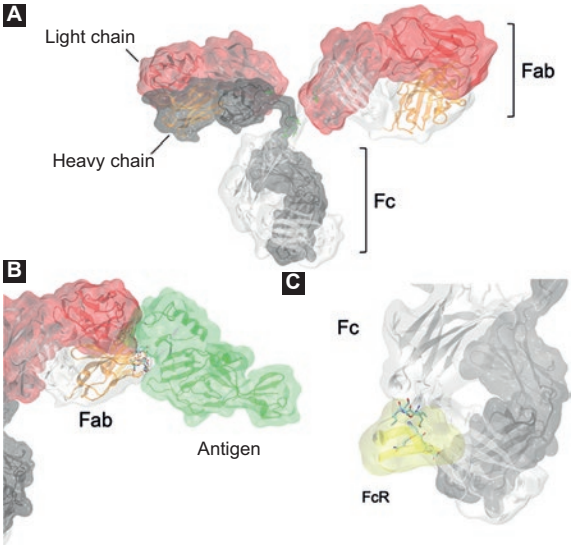

Figure 1. **A:** *Representation of the structure of an IgG: in red, the light chains; in gray, the heavy chains; in green, the disulfide bridges that bind them. The image was generated with Protein Data Bank information (PDB ID: 1IGT).* **B:** *Interaction of an antigen-binding fragment (Fab) with an antigen (green). In this region is where the specificity of the antibody lies. Image generated based on PDB information (ID: 5KEL).* **C:** *Interaction of the Fc region with a FcR fragment (yellow). Other sites within the Fc region can mediate the interaction with Cq1 or FcR. Image generated based on PDB information (ID: 1DN2).*

of the immune system, the kidney or placenta (Fig. 1C), or by activating the complement. In humans, there are four IgG subclasses with different heavy chains (γ1, γ2, γ3, and γ4), which despite being highly similar (90 % identical), each one has a unique profile with regard to:

- Responsiveness to the antigen.
- Antibody-dependent cytotoxicity activation.
- Complement-dependent cytotoxicity activation.
- Antibody-dependent phagocytosis induction.
- Half-life time.
- Placental transfer.[6]

Fc region glycosylation induces changes in the quaternary structure, which affect its functions; therefore, it is crucial knowing the glycosylation patterns of the different subclasses and their relationship with the antibody functional properties.[7] Most TAs used in neoplasms are of the IgG1 isotype, due to its higher capability to induce antibody-dependent cytotoxicity or complement-dependent cytotoxicity in cells that express the target antigen.[4]

Conjugated antibodies, a new generation of antineoplastic drugs

Antibody-drug conjugates (ADCs) are monoclonal antibodies targeted against tumor antigens, which deliver

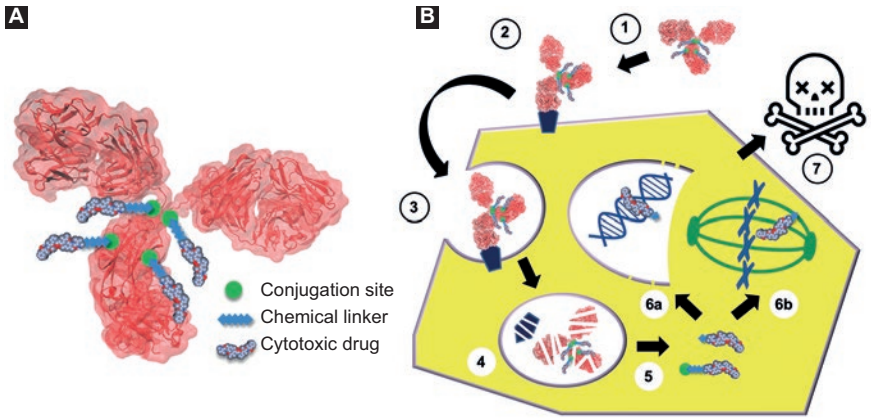

Figure 2. A: *ADC consists of three essential elements for its functioning: an antibody (red), a cytotoxic drug (gray) and a linker that binds both (blue). As the conjugation site (green), lysines, cysteines or modified amino acids can be used.* **B:** *ADCs pharmacokinetics and pharmacodynamics are complex and involve bringing the conjugate to the tumor (1), binding to the tumor antigen (2), incorporation of the complex by endocytosis (3), lysosomal hydrolysis of proteins (4), release of the cytotoxic drug (5), interaction of the drug with its intracellular target, either DNA (6a) or tubulin (6b), and cell death induction (7).*

cytotoxic payloads to cancer cells; hence, ADCs are considered prodrugs.[8] ADCs have three central elements: the monoclonal antibody carrier, the cytotoxic drug and the chemical linker that allows the conjugation of both (Fig. 2A). The antibody allows antigen-specific recognition in cancer cells. After binding, the ADC-antigen complex is incorporated into the cell by endocytosis and its lysosomal degradation is induced. Consequently, the cytotoxic payload is released inside the cell and triggers cell death by interacting with its target (Fig. 2B). This mechanism takes advantage of the specificity of the antibody to deposit the cytotoxic payload on tumor tissue,[8] thus reducing systemic toxicity and possible resistance.

Antigen and antibody characteristics

The target tumor antigen directs ADC biodistribution, and it must therefore be overexpressed in the cells or in the tumor microenvironment and be present at very low levels or absent in healthy tissues.[9] Cell surface proteins are the most widely used targets owing to their accessibility. For example, the efficacy of anti-HER2 TAs is associated with high receptor expression, since up to two million molecules have been found on cancer cells surface.[10] However, in B-cell lymphomas, it is enough for 30,000 molecules per CD19 antigen cell to be expressed for an ADC to exert its effect.[11]

Cells that lack the antigen of interest on their surface may be exposed to the cytotoxic drug if they are close to those that are recognized by the ADC. This mechanism, known as the "innocent bystander effect", is relevant when the antibody identifies tumor vasculature or stromal antigens or when antigen expression is not homogeneous in solid tumors.[12]

After selecting the antigen, identifying the antibody's optimal isotype is required, which influences on ADC efficacy, pharmacokinetics and therapeutic index. Most ADCs that have been clinically tested are of the IgG1 isotype.[9] As previously mentioned, IgG1s induce antibody-dependent or complement-mediated cytotoxicity; therefore, whether or not maintaining these functions is desired should be evaluated. In general, the antibodies used to generate ADCs should not trigger effector functions.[9] However, when the antibody to be conjugated has therapeutic activity by itself, it may be relevant to retain these functions. The IgG2 and IgG4 isotypes are inefficient to stimulate secondary immune responses.[13] IgG2s allow conjugating a larger number of cytotoxic molecules, since they have four disulfide bridges in the hinge region that can be reduced for conjugation, whereas IgG1 and IgG4 only have two.[14] Although comparative studies have shown that ADCs made up of IgG1 or IgG2 antibodies have similar tolerability and toxicity profiles,[15] IgG2 hinge regions remain more difficult to reduce. Therefore, the design of IgG1 mutated in the Fc region has been chosen in order to attenuate its effector function,[15] while preserving its ability to bind to FcR in order to optimize its half-life.

Chemical linker characteristics

The linker is a fundamental part of an ADC since it must allow the release of the drug exclusively at the

site of action. If the drug is released into the circulation, systemic toxicity increases and ADC efficacy decreases. Ideally, the linker should not interfere with the chemotherapeutic agent cytotoxicity, since the cytotoxic agent-linker by-product is what generates the innocent bystander effect.[16] In addition, the linker may serve to counteract resistance to the cytotoxic drug, since hydrophilic linkers generate metabolites that are not substrates for efflux or drug-expulsion active pumps, such as P-glycoprotein (P-gp).[17]

Two main types of linkers are currently used in the development of ADCs: cleavable and non-cleavable.[17] Cleavable linkers are stable for as long as they are circulating and efficiently release the drug after the ADC is endocytosed by the tumor cell. Non-cleavable linkers remain bound to an amino acid of the antibody after its lysosomal degradation. This type of linker is unsuitable for inducing the innocent bystander effect, since the amino acid-linker-cytotoxic agent complex does not spread outside the tumor cell.[16]

Both types of linkers take advantage of lysines or cysteines reactivity in the antibody to form covalent bonds. Conjugation to lysines is efficient, but it generates multiple antibody species with differences in the number of cytotoxic drug molecules and their localization.[18] In addition, since many reactive lysines are found in the C_H2 domain, conjugation increases ADC aggregation.[19] Conjugation to cysteines requires for them to be in their reduced form and, thus, if those that form interchain disulfide bridges are employed, linkers with two reactive groups must be used in order to allow the bridge to regenerate. This strategy allows better control of the number of molecules of the conjugated cytotoxic drug, but still, more than 100 ADC different species can be generated.[18]

The diversity of species affects ADC's stability, pharmacokinetics and pharmacodynamics.[20] For this reason, technologies have been developed that seek to increase the proportion of a particular species, such as site-specific conjugation, incorporation of new cysteine residues or non-natural amino acid residues or enzymatic conjugation.[18]

Cytotoxic drugs characteristics

The drug conjugated to the antibody is responsible for exerting the cytotoxic effect on tumor cells; therefore, it must meet several characteristics (recently, Yaghoubi et al. carried out a review[21]):

- It must have a mean inhibitory concentration below the nanomolar range, since only 1 to 2 % of the cytotoxic agent reaches its intracellular target.
- Covalent binding to the chemical linker must not interfere with its activity.
- It must be poorly sensitive to P-gp-mediated transport in order to avoid the generation of resistance.
- It must have physicochemical properties that allow its formulation for intravenous administration.
- It must be stable within the pH range existing in the lysosome.

It is difficult to find molecules that meet all these characteristics, which is why most ADCs assessed in humans use agents from three families: calicheamicins, auristatins or maytansinoids.

Calicheamicin is an antitumor agent isolated from the actinomycete *Micromonospora echinospora*. Calicheamicin γ 1l is approximately 1000 times more potent than doxorubicin for inducing cytotoxicity. N-acetyl-γ-calicheamicin, a modified analog, is used in ADCs. Calicheamicins induce cell death by binding to DNA minor groove, preferably to the TCCT/AGGA sequence,[22] forming diradical species that cause DNA strand scission.[23] These compounds are hydrophobic and, therefore, few molecules can be conjugated to the antibody without causing aggregation.[9]

Auristatins are synthetic analogues of dolastatin 10, an antimitotic isolated from the sea hare *Dolabella auricularia* and subsequently from cyanobacteria. Auristatins generate microtubule continuous and excessive growth by binding to the β subunit of tubulin dimers and preventing guanosine triphosphate (GTP) hydrolysis; consequently, sister chromatid separation and mitosis are blocked.[24] While mean inhibitory concentrations of other drugs that inhibit tubulin polymerization, such as vincristine or vinblastine, are in the range of 10^{-8} to 10^{-9} M, auristatins have average inhibitory concentrations ranging from 10^{-10} to 10^{-12} M.[25] Monomethyl auristatin E is a synthetic molecule with optimized physicochemical properties, which is why it has been used in the development of multiple ADCs.[26-29] When monomethyl auristatin E is conjugated to the antibody by cleavable linkers, the product is hydrophobic enough to induce the innocent bystander effect on neighboring cells.[30]

Maytansinoids are cytotoxic substances derived from maytansine, a macrolide antibiotic isolated from the *Maytenus ovatus* shrub. They bind to the tubulin located at the ends of microtubules, favoring their depolymerization and leading the cell to apoptosis.[31] Maytansinoids have been shown to be 100 to

1000-fold more potent in vitro than other cytotoxic agents;[32] emtansine and mertansine are characterized by a substituent that contains a thiol group, which facilitates their conjugation to linkers.[8] These compounds have good solubility and stability in aqueous solution, but can promote ADC aggregation or limit antigen binding, especially at high conjugation ratios.[21]

Other cytotoxic agents in ADCs used in clinical trials include doxorubicin,[33] pyrrolobenzodiazepines,[34] indolinobenzodiazepines,[35] camptothecin derivatives,[36] duocarmycins[37], and tubulisins.[38]

Available ADCs

Up to May 2019, four ADCs had received marketing approval by the FDA and the European Medicines Agency (EMA); each one is described below.

Gemtuzumab ozogamicin

Gemtuzumab ozogamicin is a humanized anti-CD33 IgG4 conjugated to N-acetyl-γ-calicheamicin by a bifunctional linker.[39,40] CD33 is a cell-adhesion molecule that belongs to the sialic acid-binding lectin superfamily,[41] which is expressed in myeloid cells and in approximately 85 to 90 % of patients with acute myeloid leukemia.

In 2000, gemtuzumab ozogamicin was the first ADC to be approved by the FDA for the treatment of patients with relapsed acute CD33+ myeloid leukemia, aged 60 years or older and who were not candidates to other chemotherapies. However, in the post-marketing period, its lack of efficacy and its association with serious side effects and premature death were demonstrated: gemtuzumab ozogamicin addition to standard induction chemotherapy (daunorubicin + cytarabine) did not improve the complete response rate or relapse-free survival,[42] increased mortality from 1.4 to 5.5 %,[42] and the incidence of hepatic veno-occlusive disease was approximately 10 %.[43] For these reasons, the drug was withdrawn from the market in 2010.

Various hypotheses have been proposed to explain the failure of the product: poor stability of the chemical linker, heterogeneity in the amount of drug molecules bound to the antibody, and susceptibility of the cytotoxic agent to be carried by transporters.[9,17] Given that it is poorly immunogenic, the relationship of anti-ADC antibodies with gemtuzumab ozogamicin efficacy and safety was not identified.[44]

Subsequent trials demonstrated that lower and more frequent doses increased gemtuzumab ozogamicin efficacy and safety. In adults with newly diagnosed acute myeloid leukemia, the addition of ADC to chemotherapy increased 2-year event-free survival, as well as overall survival.[40] As monotherapy, it favors obtaining complete responses in children with refractory or recurrent acute myeloid leukemia.[39] These results contributed to gemtuzumab approval in 2017 by the FDA for the treatment of patients with CD33+ acute myeloid leukemia during adulthood and in children who relapse or who do not respond to primary treatment.[45] Months later, it was approved by the EMA.[46] Side effects, although less serious, are still reported.[40,45]

Brentuximab vedotin

Brentuximab vedotin is a chimeric anti-CD30 IgG1 conjugated to monomethyl auristatin E by a cathepsin B-sensitive valine-citrulline cleavable linker.[47-49] CD30 belongs to the tumor necrosis factor receptor family and normally it is expressed in lymphoid tissues and overexpressed in neoplastic cells of patients with Hodgkin's lymphoma, anaplastic large cell lymphoma or cutaneous T-cell lymphoma.[50]

In patients with Hodgkin's lymphoma who relapse and who fail to respond to autologous stem cell transplantation, brentuximab vedotin monotherapy improves complete response rates, as well as 5-year progression-free survival and overall survival.[47-49] In patients with previously untreated Hodgkin's lymphoma, the combination of brentuximab vedotin with doxorubicin, vinblastine, and dacarbazine is equally efficacious and less toxic than adding a fourth chemotherapeutic agent to the therapeutic regimen.[51,52] Brentuximab vedotin has demonstrated benefits when used as monotherapy in patients with anaplastic large cell lymphoma[53] or cutaneous T-cell lymphoma,[54] as it significantly increases overall response rate and progression-free survival.

Brentuximab vedotin induces adverse reactions, especially in patients with impaired liver or kidney function,[55] or who take drugs that inhibit CYP3A4, since monomethyl auristatin E is metabolized by this cytochrome P450 isoform.[56]

Trastuzumab emtansine

Trastuzumab emtansine is a humanized anti-HER2 IgG1 conjugated to emtansine via a non-cleavable thioether linker bound to lysine residues.[57,58] HER2 is overexpressed in a subgroup of breast tumors and,

for this reason, trastuzumab was developed as a TA.[59] Trastazumab pharmacokinetics, pharmacodynamics, and adverse reactions are well known.[59] Trastuzumab emtansine retains the mechanisms of action of the unconjugated antibody, in addition to inducing emtansine cytotoxicity.[60]

In patients with previously-treated locally advanced, unresectable or metastatic HER2+ breast cancer, trastuzumab emtansine is more effective than the combination of capecitabine + lapatinib[57] or a third-line treatment,[58] since it increases progression-free survival and median overall survival. In addition, adverse reactions were better tolerated in the groups treated with the ADC.[57,58] In contrast, in patients with treatment naïve HER2+ advanced breast cancer, trastuzumab emtansine is not more efficacious than trastuzumab combined with a taxane,[61] and the approval for its use is therefore limited to previously-treated patients.

There are reports of development of resistance to this ADC. This may be due to the following:[62]

- Mechanisms of resistance to trastuzumab.
- ADC inefficient internalization.
- Reduced lysosomal degradation.
- Action of recycling endosomes that allow the HER2-ADC complex to return to the membrane.

Although 5 % of patients receiving trastuzumab emtansine develop antibodies against this agent,[44] this does not impact on its efficacy.

Trastuzumab emtansine has been observed to induce multiple adverse responses; thrombocytopenia is the most serious and the one that motivates dose limitations.[63] Like trastuzumab, it can induce left ventricular dysfunction or liver failure.[63] These effects are increased by the consumption of drugs that interfere with emtansine metabolism.[62]

Inotuzumab ozogamicin

Inotuzumab ozogamicin is a humanized anti-CD22 IgG4 conjugated to N-acetyl-γ-calicheamicin via the same bifunctional linker used in gemtuzumab ozogamicin.[64-66] CD22 is a transmembrane glycoprotein of the sialic acid-binding lectins superfamily[41] that is overexpressed in more than 90 % of patients with acute lymphoblastic leukemia.[67] Inotuzumab ozogamicin is used as monotherapy for the treatment of adults with relapsing or refractory CD22+ acute lymphoblastic leukemia. In these patients, the use of the ADC significantly increases the rate of patients with complete remission, 2-year overall survival, and the quality of life.[64-66]

The linker in inotuzumab ozogamicin is stable in systemic circulation,[68] even though it is attributed the toxicity of other ADCs. Inotuzumab ozogamicin induces adverse effects that can be mitigated with preventive measures and signs and symptoms constant monitoring.[69] Although 3 % of patients receiving inotuzumab ozogamicin generate antibodies against the product, the impact of this reaction on the agent's efficacy and safety has not yet been determined.[44]

Conclusions and perspectives

Although ADCs represent a breakthrough in oncology, they have various limitations; for example, even when an ADC is indicated for one type of tumor, its usefulness is limited to specific patient subgroups. Therefore, analyzing the effect of ADCs in different populations and types of tumors that express the target antigen is required. An example of this type of analysis is the one related to trastuzumab emtansine efficacy in lung cancer.[70] In addition, the mechanisms by means of which resistance to ADCs is developed should be studied in order to propose strategies to reverse it. Citotoxic drugs efflux is an important factor in resistance and, therefore, combining the ADC with inhibitors of the transporters that allow this mechanism has been proposed,[71] or generating new agents that are not transported.[21] More research on the frequency whereby anti-ADC antibodies are developed and their role in the development of resistance is also necessary.[44]

ADC safety still needs to be improved. Adverse effects are generally caused by induction of healthy cell death. This cytotoxicity might be due to target antigen expression on the cells, to uptake of the conjugate by the cells independently of the antigen or to the cytotoxic drug being released to the circulation by target cells that processed the ADC.

It is to be expected that new ADCs that are more effective and safer will be developed in the near future, since there is sufficient information regarding what the nature of the selected antigen should be, what properties the antibody should have, which are the essential pharmacological and physicochemical properties of the cytotoxic agent and how the linker should work. ADCs will increase survival opportunities and quality of life for cancer patients.

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Conflict of interests

None

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Ethical disclosures

The authors declare that no experiments were performed on humans or animals for this research.

Confidentiality of data. The authors declare that no patient data appear in this article.

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REVIEW ARTICLE

From ISET to InDRE. V. Institute of Epidemiological Diagnosis and Reference. Global strategic position, 2012-2019

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Abstract

This document describes the changes at the Institute of Epidemiological Diagnosis and Reference (InDRE) from 2012 to 2019, the administrative and equipment modifications, the new headquarters and the National System of Epidemiological Surveillance legal modifications. The process of relocation is mentioned, especially the careful transfer of the biological material protected by the Institute, and the new way of studying epidemic outbreaks, endemic diseases and the negative network is analyzed. At the international level, the promotion of links with global networks of the Pan American Health Organization, the World Health Organization (WHO) and other international organizations is described. The assignation to InDRE of four WHO collaborating centres is also mentioned. The Global Health Security Initiative Laboratory Network acknowledged InDRE's leadership, which co-chaired the working group during the study period.

KEY WORDS: *Institute of Epidemiological Diagnosis and Reference. History of medicine. Public health.*

Del ISET al InDRE. V. Instituto de Diagnóstico y Referencia Epidemiológicos. Posición estratégica global, 2012-2019

Resumen

En este documento se describen los cambios en el Instituto de Diagnóstico y Referencia Epidemiológicos (InDRE) de 2012 a 2019, las modificaciones administrativas y de equipamiento, la nueva sede y las modificaciones jurídicas al Sistema Nacional de Vigilancia Epidemiológica. Se menciona el proceso de mudanza, en especial el cuidadoso traslado del material biológico que resguarda el Instituto y se analiza la nueva forma de estudiar los brotes epidémicos, los padecimientos endémicos y la red negativa. Respecto al ámbito internacional, se describe el fomento de la vinculación con redes globales de la Organización Panamericana de la Salud, la Organización Mundial de la Salud (OMS) y otros organismos internacionales. También se menciona la designación en el InDRE de cuatro centros colaboradores de la OMS. La Red de Laboratorios de la Iniciativa Global para la Seguridad en Salud reconoció el liderazgo del InDRE, cuyo director ocupó la copresidencia del grupo de trabajo en el periodo de estudio.

PALABRAS CLAVE: *Instituto de Diagnóstico y Referencia Epidemiológicos. Historia de la medicina. Salud pública.*

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… that which on the tree is flowering,
lives from that which it has buried in the ground.
Francisco Luis Bernárdez

Introduction

This article gives continuity to number IV of the "From ISET to InDRE" series,[1] where the complex operation of the National Network of Public Health Laboratories (RNLSP – *Red Nacional de Laboratorios de Salud Pública*) was described.

The changes at InDRE in the 2012-2019 period had repercussions on the entire structure of the National System of Epidemiological Surveillance (SINAVE – *Sistema Nacional de Vigilancia Epidemiológica*), which was organized according to the Official Mexican Standard NOM-017-SSA2-2012, for epidemiological surveillance (Fig. 1). On one hand, there are the National Committee of Epidemiological Surveillance, the General Directorate of Epidemiology, InDRE and the epidemiological surveillance units; and, on the other, the flows of information and samples, whose entrance door are the epidemiological surveillance units of all institutions of the health sector.[2] The entire process is regulated by the National Committee of Epidemiological Surveillance and the General Directorate of Epidemiology. When SINAVE requires confirmation of cases, RNLSP and InDRE are incorporated.[1]

In 2013, InDRE changed its headquarters, although preparations had started since the previous year. It took three months for the institution's teams and working groups stepwise mobilization. On April 7, 2014, the new facilities were formally opened.

Regarding the change of institutional headquarters

Administrative and work modifications

As part of the strategy for the management of the change towards the modernization and systematization of InDRE's attributions, the Quality Management System was implemented in 2012, with a focus on processes and a risk-based approach. The incorporation of the biological risk management standard for laboratories, the CWA15793:2011 standard, consolidated the Institute's comprehensive management model, so far the only one in the world for national reference laboratories.

The imminence of the change of institutional headquarters generated more commitment and a sense of belonging among the personnel and, at the same time, uncertainty and concern. By means of tripartite dialogue tables, with the participation of workers, union and institutional authorities, adapting the profiles of the positions in the job profile diagram was agreed, the staff members who were under contract were workwise institutionalized and the change of assignment of 59 workers (who represented around 10 % of the workforce) was allowed.[3] At the same time, 67 newly-created positions were obtained, which triggered more than a hundred hierarchical movements.

Legal modifications

In 2013, the Official Mexican Standard NOM-017-SSA2-2012, for epidemiological surveillance, was updated,[2] with InDRE acquiring legal support as a national reference laboratory. The previous year, the Institute had joined the General Directorate of Epidemiology, in accordance with the new Internal Regulations of the Ministry of Health.[4] The NOM-017-SSA2-2012 standard established the protocols to update InDRE's operational criteria and technical guidelines. These documents have incorporated new methodological developments in the diagnostic algorithms and express RNLSP technological innovation.[5]

Also in 2013, owing to its "high national importance", the new property was designated as a triple A strategic facility, in terms of the General Statute of the National System of Public Security, and as a National Security Agency, in accordance with the collaboration bases signed between the Ministry of Health and the Ministry of the Interior.[6]

Relocation

During the relocation process, InDRE had to retain the capacity to carry out "critical" diagnostic algorithms and continue operating should any epidemiological contingency occur in the country. Activities were maintained at the *Santo Tomás* facilities and the RNLSP members supported while all the diagnostic phases were transferred to the new headquarters. Technical and administrative processes were carefully planned and executed, including the chain of custody and biosafety.

The most important challenge in the relocation process was the transfer of the biological material stored in the Institute's facilities. It was a national security event in terms of biological risk management.[7]

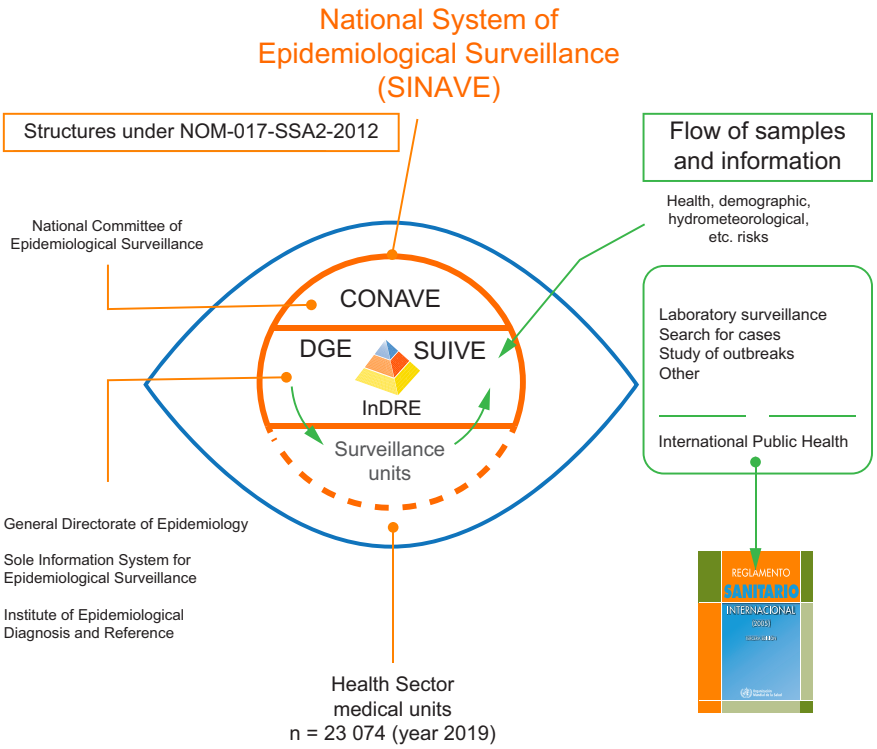

Figure 1. *Diagram of the National System of Epidemiological Surveillance, where the role of the national reference laboratory (InDRE) is shown. Mexico, 2019.*

After performing an exhaustive inventory of the biological material that had to be transported from the old facilities to the new ones, the Pan American Health Organization/World Health Organization (PAHO/WHO) regional office was informed. Hours before the transfer, InDRE staff packed the material, in accordance with international criteria. The transfer of all the material was carried out on Saturday, November 16, 2013, around midnight, in a single trip, with a caravan of trucks escorted by members of the Federal Police.

Due to its relevance, the operation was planned and coordinated by CISEN officials (Center for Investigation and National Security, now the National Intelligence Center) and the InDRE, with participation of members of the legal cabinet staff: Ministry of the Interior, Ministry of National Defense, Ministry of Communications and Transportation, in addition to the Federal Police. The High Level Specialized Committee on Disarmament, Terrorism and International Security Matters coordinated the compliance with international agreements on the subject. The WHO shipping pass was signed at the former facilities, and the mobilization process was notified to PAHO/WHO, the United Nations Office for Disarmament Affairs and the Biological Weapons Convention.

Work continued until dawn for InDRE staff. As the areas were vacated and the laboratories, laboratory animal facilities, insectariums, cold rooms, etc. were dismantled, bio-decontamination and disinfection (environmental biosafety) was carried out to ensure the safety of the furniture, materials, equipment and buildings at the time the old property was delivered.

After having been settled for 78 years in the *Santo Tomás* neighborhood, InDRE moved to the new property that had belonged to the grounds of an old hacienda, then to *La Castañeda* General Insane Asylum (1910-1968) and to the National Institute of Human Communication (1968-2002).[8]

Opening

Finally, on April 7, 2014, the World Health Day, that year dedicated to vector-borne diseases, the official opening ceremony of the new facilities was celebrated in presence of the President of the Republic, Enrique Peña Nieto; the Secretary of Health, Mercedes Juan López; and the representative of the PAHO/WHO

Table 1. **National Network of Public Health Laboratories. Integration of specific laboratory networks. 2019**[5]

Network		Public health state laboratories	Laboratories of epidemiological surveillance support (LAVE)*	Jurisdictional/local laboratories
1	Malaria	32	—	116
2	Influenza and other respiratory viruses	32	8	—
3	Tuberculosis	31	-	737
4	Dengue and other arboviroses	31	1	3
5	Acute bacterial diarrheic disease	31	1	—
6	Febrile exanthematous illness	31	1	—
7	Brucellosis	31	—	—
8	Sexually-transmitted infections	31	—	—
9	Hepatitis	30	—	—
10	HV-AIDS	30	—	—
11	Chagas disease	29	—	—
12	Acute bacterial respiratory infections	28	—	—
13	Pertussis	28	—	—
14	Leishmaniasis	28	—	116
15	Rotavirus and other enteroviruses	27	—	—
16	Entomology	27	—	1
17	Rabies	25	—	—
18	Cervical cytology	21	—	74
19	Leptospira	21	—	—
20	Rickettsiosis	4	—	—

*Participating institutions: Mexican Institute of Social Security (with four LAVEs), Institute of Social Security and Services of State Workers, National Institute of Respiratory Diseases, National Institute of Medical Sciences and Nutrition, General Hospital of Mexico.

office in Mexico, Maureen Birmingham. This event was reported in all national circulation newspapers, on eight front pages (*El Universal*, *Reforma*, *Excélsior*, *El Sol de México*, *Impacto Diario*, *DiarioImagen*, *Ovaciones*, *Unomásuno*) and on inside pages in the rest of the written press. The Secretary of referred to InDRE as follows:[9]

> ... main point of support for the national epidemiological surveillance system... (it) is part of the national security strategy to respond to highly dangerous biological emergencies.

Similar to the note "Finally, it will be founded", published in 1936 by *El Universal* on the occasion of the foundation of the Sanitary and Tropical Diseases Institute (ISET – *Instituto de Salubridad y Enfermedades Tropicales*), the newspaper *Reforma*, 78 years later, titled its note "Finally, InDRE's headquarters are opened".

A new way to approach epidemiological surveillance

Currently, the country has 20 specific diagnostic networks under the stewardship of InDRE as National Reference Laboratory (Table 1). In addition to the new infrastructure, practically all laboratory, computing and communication equipment was replaced. The laboratory equipment changed from monocular microscopes with external lamps to optical microscopes with LED illumination and digitized documentation; from classical bacteriology to gene sequencing; from agglutination tests, to automated chemiluminescence, to real-time quantitative PCR, to next-generation sequencing, and to bioinformatics. Together, these transformations constitute the most important technological renewal of the Institute since its foundation.

As a result, there were modifications in the Institute's participation in epidemiological surveillance. Below, some aspects are described.

Epidemic outbreaks

In the reviewed period, the Institute participated by characterizing epidemic outbreaks using a more expedite strategy with higher predictive value, based on the identification and analysis of nucleic acids by real-time PCR and whole-genome sequencing. A clear example of InDRE's contribution was the characterization of a highly pathogenic avian A influenza strain (H7N3) in poultry farm workers from Jalisco.[10] The transmission of the chikungunya[11], Zika[12], and dengue[13] viruses in our country was also studied, the circulation of non-polio D68 enterovirus was identified in respiratory conditions and the diagnosis of influenza in children was ruled out at the Institute.[14]

Detection of the *Aedes* mosquito in Mexico City[15] triggered focused monitoring and surveillance activities.[16] As a consequence, all state public health laboratories (LESP – *Laboratorios Estatales de Salud Pública*) have had, since then, molecular methodologies available for these diagnoses, which facilitated national response.

During the cholera outbreak in Mexico in the 2013-2014 period, diagnosis was obtained with conventional methods, and with molecular methods, the origin of the strain (Haiti) that caused the re-emergence of this disease was established. With a mobile field laboratory, the time of response for the care of affected individuals was reduced, which facilitated decision-making. The outbreak was controlled by SINAVE and inter-sectoral participation in only 13 weeks.[17]

Given the possible introduction in Mexico of highly infectious unknown pathogens such as Ebola, and the reintroduction of others, such as the yellow fever virus, InDRE strengthened the capacity to receive them at its new facilities and to contain them in level 3 biological safety laboratories; it also reinforced the communication networks with computer systems.

Endemics

The Mexican experience in malaria eradication facilitated WHO certification of all microscopists at InDRE Malaria Laboratory, which allowed the creation of the WHO Collaborating Center for Malaria. This certified personnel maintains its high level throughout the country and also carries out parasitological diagnoses for leishmaniasis and Chagas disease (Juan Manuel Serna-Velázquez, personal communication).

Chagas disease serological diagnosis is essential for strategies to control the transmission of this parasite. To that end, InDRE developed a national reference algorithm and a solid performance evaluation program where RNLSP members and the National Center for Blood Transfusion participate, to ensure diagnostic reliability.

The Tuberculosis Network started operating since the decade of 1970; currently, it has 768 laboratories of different levels of complexity throughout the country. Bacteriological tests are the basis of this network.[7]

The establishment of the Rickettsiosis Epidemiological Surveillance program allowed the creation of the Institute's most recent diagnostic network.

In November 2019, the WHO granted Mexico the first certification in the world as a country free of dog-transmitted human rabies, based on scientific evidence provided by InDRE and RNLSP.

Surveillance of eliminated or eradicated diseases (negative network)

The negative network includes diseases without autochthonous cases in Mexico[2] (Table 2). As a relevant example, InDRE has participated in the polio eradication process, with the diagnosis of probable cases in outbreak studies and through environmental surveillance; in addition, it is the only agency that generates laboratory information for epidemiological surveillance of acute flaccid paralysis in Mexico.

Recognitions, academic activity and scientific production

The Institute received the ISO 15189:2012 accreditation, ISO 9001:2015 certification and the 2015 National Health Quality Award. That year, InDRE was granted the Funsalud-GSK Award in the category of epidemiological research; in 2017, a recognition with the CANIFARMA Award and was a finalist in the Carlos Slim Award to the Exceptional Institution. In 2019, it was finalist for the National Quality Award granted by the Ministry of Economy.

In 2014, the National Council of Science and Technology distinguished Dr. Clara Gorodezky as emeritus researcher, and she was also named "Person of the Year 2019" at Forbes Health Forum, due to her contributions to the benefit of health.[18]

In the 2012-2019 period, 179 articles were published in peer-reviewed indexed journals, 2972

Table 2. InDRE participation in the surveillance of conditions with no autochthonous cases recorded in the national territory

Condition	Year the last cases were recorded	Origin
Polio	1990	Jalisco
Diphtheria	1991	Michoacán
Measles*	1996	Federal District
Onchocerciasis	1998	Oaxaca
Dog or cat-transmitted human rabies	2004	Sinaloa
Blindness caused by trachoma	2007	Chiapas
Congenital rubella syndrome*	2008	Nuevo León
Malaria caused by *Plasmodium falciparum**	2009	Sonora
Rubella*	2010	Distrito Federal and Nuevo León
Rhodnius prolixus-transmitted Chagas disease	2012	Chiapas
Cholera*	2018	Sinaloa

*The diagnosis is made by the entire RNLSP.

genetic sequences and complete genomes were deposited in public or restricted databases, and the number of members at the National System of Researchers increased by 143 %, due to the training and incorporation of young professionals with postgraduate degrees.

All these achievements illustrate the expertise, commitment and human talent that InDRE has developed and offered to the field of public health.

InDRE international participation

According to the WHO, among the top 10 health problems in 2019, six are related to infectious diseases: influenza as a pandemic event, antimicrobial resistance, the threat of spread of the Ebola virus and other pathogens (including X disease, which represents the need for preparedness for an unknown pathogen that might cause a serious epidemic), vaccine hesitancy, dengue and human immunodeficiency virus.

In addition to intensive technical meetings, InDRE has participated in many other on national laboratory policies, planning, regulation, biological risk management, and financing. It has also maintained collaboration with global networks and has joined other new ones (Table 3). The InDRE National Influenza Center has been maintained since 1951[19] and was re-designated in 2016. The InDRE Tuberculosis Laboratory has been a supranational laboratory for Central America since 2005; in 2016, it was accredited as member of the Network of Supranational Reference Laboratories for Tuberculosis by the WHO Global Tuberculosis Program.[20,21]

Through the United States Northern Command, in 2017, InDRE received a next-generation sequencer to decrease response times in epidemic events, such as Zika, influenza, and foodborne diseases.[22]

InDRE is part of the Global Health Security Initiative Laboratory Network (Table 3), where only the seven most industrialized countries and Mexico participate;[23] the InDRE director was unanimously elected co-chair for the 2012-2019 period. Mexico hosted the meetings of that body in 2014 and 2017.

WHO collaborating centres at InDRE

Between 2012 and 2019, the Institute received important designations from PAHO/WHO (Table 4). A WHO Collaborating Centre (WHOCC) is an institution designated to carry out activities in support of WHO's public health programs. InDRE received four designations as WHOCC:

- WHO Collaborating Centre for Training on Malaria Microscopy Diagnosis.[24]
- WHO Collaborating Centre on Laboratory Biosafety.[25]
- WHO Collaborating Centre for Arboviruses.[26]
- WHO Collaborating Centre on Laboratory Quality Management.[27]

With these designations, 28 % of the WHOCCs established in the country are under the care of InDRE.[28]

Table 3. InDRE participation in global and multinational diagnostic networks

Program	Coordinating body	Objective
PAHO/WHO global networks		
The Global Polio Laboratory Network	WHO	To differentiate polioviruses from other causes of acute flaccid paralysis
Global Influenza Surveillance and Response System (GISRS)	WHO	Global network for influenza epidemiology and virology surveillance
WHO External Quality Assessment Project for the detection of influenza viruses	WHO/Centre for Health Protection, (Hong Kong)	To assure quality and improve the capacity of laboratories at the global level for the detection and sub-typing of the influenza virus (support to GISRS)
Global Measles and Rubella Laboratory Network	PAHO	To monitor and verify viral transmission and monitoring the susceptibility profile of a population
System of Networks for the Surveillance of Agents Responsible for Bacterial Pneumonias and Meningitis	PAHO	Epidemiological surveillance of bacterial pneumonias and meningitis
Latin American Network for Antimicrobial Resistance Surveillance	PAHO	Surveillance of resistance in nosocomial and community-acquired pathogens
Pan American Cytology Network	PAHO	To improve the quality of the Papanicolaou test
Global Salm-Surv	WHO	To strengthen surveillance of foodborne diseases. Alert and response in case of outbreaks
Laboratory Network for Arbovirus Diagnosis	PAHO	To strengthen diagnosis in order to ensure a timely response to outbreaks and epidemics
Genomic Surveillance of Dengue in the Americas	WHO/PAHO	To promote genomic surveillance studies of dengue and other arboviruses in the Region of the Americas
External Evaluation of Performance in the Diagnosis of Emerging and Reemerging Infectious Diseases	PAHO/WHO/National Center of Tropical Diseases (Bolivia)	To assess the capacity of national reference laboratories of the Region of the Americas
Program for Quality Control in Bacteriology and Antimicrobial Resistance	PAHO/National Institute of Infectious Diseases (Argentina)	Quality Control Program to support ReLAVRA
Program for External Evaluation of Performance in Malaria Microscopy Diagnosis for the Countries of Mesoamerica and the Caribbean	PAHO	To establish the technical procedure for the organization, design and evaluation of national reference laboratories in the countries of the sub-region.
TB Supranational Reference Laboratory Network	WHO	Surveillance of *Mycobacterium tuberculosis* drug resistance
Mycobacterium tuberculosis Drug Susceptibility Quality Control Program	PAHO/WHO	To strengthen the capacity of laboratories in the countries to identify the global magnitude of *Mycobacterium tuberculosis* resistance
Network for Evaluation of Vaccine Effectiveness in Latin America and the Caribbean. Influenza	PAHO/CDC (United States)	To provide information on the effectiveness of the seasonal influenza vaccine
The External Quality Assurance System of the WHO Global Foodborne Infections Network	WHO/Technical University of Denmark (Denmark)	To assess the ability of Member States to detect and respond to outbreaks of foodborne diseases and to the emergence of antimicrobial resistance
Multinational networks		
Global Health Security Action Group-Laboratory Network	Global Health Security Initiative (Group of Seven + México)	Partnership between laboratories in the countries of the Group of Seven and Mexico in order to strengthen global public health preparedness and response to chemical, biological, radiological and nuclear terrorism threats

(Continued)

Table 3. InDRE participation in global and multinational diagnostic networks (*Continued*)

Program	Coordinating body	Objective
Multinational networks		
International Proficiency Testing Scheme for the Leptospirosis MAT	International Leptospirosis Society (National Serology Reference Laboratory, Australia)	To improve the diagnosis of leptospirosis in the world
Performance Evaluation Program	Division of Laboratory Systems, CDC	To improve surveillance and public health, clinical laboratory quality and safety, data science, biorepositories, and working competence
PulseNet and PulseNet-NGS	CDC	To compare patterns of bacterial DNA obtained from individuals involved in outbreaks related to food safety
Laboratory Response Network	CDC	Network of laboratories that can respond to biological and chemical threats, as well as to other public health emergencies
Global Microbe Identifier-Proficiency Test	Technical University of Denmark (Denmark)	To harmonize and standardize whole-genome sequencing of emerging pathogens, data analysis, and bioinformatics
United Nations Secretary-General's Mechanism-Proficiency Test	United Nations Office for Disarmament Affairs/Technical University of Denmark	To strengthen the ability to detect a biological threat, based on genomic and bioinformatics analysis

PAHO = Pan American Health Organization; WHO = World Health Organization; GISRS = Global Influenza Surveillance and Response System; ReLAVRA (*Red Latinoamericana de Vigilancia de la Resistencia a los Antimicrobianos*) = Latin American Network for Antimicrobial Resistance Surveillance; NGS = next-generation sequencing, CDC = Centers for Disease Control and Prevention.

Discussion

The opening of new and modern strategic facilities, the reengineering of processes, technological renewal, the creation of new positions and the associated hierarchical movements generated a favorable environment for the projection of InDRE in the 2012-2019 period. Together, these events constitute the deepest renovation of the Institute since its foundation in 1938.

In the review herein presented, three elements were considered: the lessons of ISET-InDRE historical recovery, Mexico's epidemiological challenges and the institution's forward planning.

The historical analysis showed the character traits present in the Institute since its birth:

- Multidimensional approach to health problems, considering the basic, entomological, clinical, epidemiological and humanistic aspects, to support priority health campaigns and programs of each period.
- The seriousness and robustness of scientific research carried out at the Institute, which offers answers to the most important health problems, although the complicated internal situation of the country, as well as in the borders, has posed challenges to national public health, which is inseparable of global public health.

From this significant institutional biography, we can conclude that InDRE must maintain its strong sense of identity and promote continuous and intense scientific, technological and humanistic development. With this, it will be able to help facing both infectious and chronic and non-communicable diseases.

In December 2019, in the province of Wuhan, China, an epidemic outbreak of the SARS-CoV2 coronavirus, the cause of Covid-19, was initiated, with disease quickly turning into a pandemic. In the face of this new global challenge, InDRE was the first institution in Latin America to carry out the confirmation of cases by molecular methods. The methodology would be transferred in a matter of few days to the RNLSP.[29]

The diagnostic task in charge of InDRE must go beyond the laboratory and include economic, social and ecological aspects in order to explain the complexity behind the morbidity and mortality figures. In the near future, InDRE should maintain the momentum in high-level research and link with clinical areas of national health institutes as well as with reference and high specialty hospitals.

Building upon its great past, InDRE should continue in the 21st century as a national security institution, leader in global public health.

Table 4. World Health Organization designations for InDRE, 2012 to 2019

Year of designation	WHOCC name	National program it supports	International program it supports
2016*	National Influenza Center	Prevention and Control of Respiratory Diseases and INFLUENZA	Global Influenza Surveillance and Response System
2016**	TB Supranational Laboratory	Prevention and Control of Tuberculosis	End TB Strategy
2017	WHO Collaborating Center for Training on Malaria Microscopy Diagnosis	Prevention and Control of Malaria	Global Technical Strategy for Malaria 2016-2030
2017	WHO Collaborating Center on Laboratory Biosafety	National Network of Public Health Laboratories	One World, One Health Agenda
2017	WHO Collaborating Centre for Arboviruses	Epidemiological Surveillance of Vector-Transmitted Viral Diseases	Arbovirus Diagnostic Laboratory Network in the Americas
2018	WHO Collaborating Centre on Laboratory Quality Management	National Network of Public Health Laboratories	Conference on Laboratory Quality Systems
2018***	Poliovirus-Essential Facilities	Polio and acute flaccid paralysis surveillance	Global Polio Eradication Initiative

*First appointment in 1951; re-designation in 2016.
**First appointment in 2005; re-designation in 2016.
***At the request of the health authority in 2018.

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Conflict of interests

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Confidentiality of data. The authors declare that no patient data appear in this article.

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BRIEF COMMUNICATION

Recommendations for the management of critically ill adult patients with COVID-19

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Abstract

Except for pregnant women, the management of critically ill patients with COVID-19 during the pandemic includes the standard procedures that are used for any patient that requires to be attended to at the intensive care unit, as well as limited administration of crystalloid solutions, orotracheal intubation, invasive mechanical ventilation in the event of patient clinical deterioration, and muscle relaxants continuous infusion only if necessary. Non-invasive mechanical ventilation and high-flow oxygen therapy are not recommended due to the generation of aerosol (associated with risk of viral spread among health personnel), and neither is extracorporeal membrane oxygenation or the use of steroids. So far, there is no specific antiviral treatment for patients with COVID-19, and neither are there results of controlled trials supporting the use of any.

KEY WORDS: *Coronavirus. COVID-19. Intensive care. Critical care.*

Recomendaciones de tratamiento para pacientes adultos graves con COVID-19

Resumen

Con excepción de las mujeres embarazadas, el manejo de los pacientes adultos graves con COVID-19 durante la pandemia incluye los procedimientos estándar que se llevan a cabo en cualquier paciente que requiere atención en la unidad de cuidados intensivos, así como la administración limitada de las soluciones cristaloides, la intubación orotraqueal, la ventilación mecánica invasiva ante deterioro clínico del paciente y la relajación muscular en infusión continua sólo cuando sea necesaria. No se recomienda la ventilación mecánica no invasiva, la oxigenoterapia de alto flujo debido a la generación de aerosol (asociado con riesgo de propagación del virus entre el personal de salud), la oxigenación por membrana extracorpórea ni el empleo de esteroides. Hasta el momento no hay tratamiento antiviral específico para pacientes con COVID-19 ni resultados de estudios controlados que avalen su uso.

PALABRAS CLAVE: *Coronavirus. COVID-19. Cuidados intensivos. Terapia intensiva.*

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Introduction

By March 19, 2020, 209,839 cases of coronavirus disease (COVID-19) had been confirmed worldwide, with global mortality associated with this cause being less than 5 % (8778/209,839).[1] Approximately 4.7 % (2087/44,672) of patients with COVID-19 had developed a severe form of the disease, and mortality in seriously ill patients was around 49 % (1023/2087).[2] At that time, total number of confirmed cases in Mexico was 164, with a mortality rate lower than 1 % (1/164), and there were 448 suspected cases under investigation in different states of the Mexican Republic. In the ensuing weeks, the number of confirmed cases has increased.[3] The purpose of this work is to present the main recommendations for the treatment of seriously ill adult patients with COVID-19, with the exception of pregnant women.

Main recommendations

- The management of critically ill patients with COVID-19 during the pandemic is the same that is provided to any critically ill patient that requires attention at the intensive care unit (ICU), i.e., standard treatment.[4]
- The definitions of sepsis, septic shock[5] and acute respiratory failure syndrome[6] do not change. The same operational definitions that apply to seriously ill patients without COVID-19 admitted to the ICU should be used.
- Administration of supplemental oxygen to patients with severe acute respiratory infection, respiratory distress, hypoxemia or shock is intended to achieve an oxygen saturation > 94 %.[4]
- Hospitalized patients with COVID-19 require close surveillance and monitoring. Using the National Early Warning Score (NEWS 2) is recommended in order to early identify those patients with high risk of in-hospital complications.[4]
- Performing a complete blood count, blood chemistry, venous or arterial blood gas testing, serum electrolyte testing and electrocardiogram when the patient is admitted to the ICU is suggested. If the tests were carried out in the emergency department and the patient clinical condition has not changed when transferred to the ICU, there is no need to repeat them.[4]
- If imaging studies such as chest X-ray or tomography were performed in the emergency department, there is no need to repeat them. Once the patient is in the ICU, follow-up can be carried out with lung ultrasound.
- If the patient shows clinical deterioration and there is any evidence of organ failure during his/her stay at the ICU, the necessary biomarkers should be assessed according to the affected organ or system.[4]
- Restrictively administering crystalloid solutions is recommended.[4,5]
- If the patient has risk factors for bacterial infections, intravenous antibiotic treatment should be started according to the epidemiological profile of the hospital during the first hour of assessment of the seriously ill patient with operational criteria consistent with sepsis.[5]
- For the time the influenza season continues, considering the use of oseltamivir at the usual dose and interval is suggested, according to the result of the rapid test performed at the time of patient assessment.[4]
- In the emergency department or in hospitalization, patients with type I acute respiratory failure can receive 10 to 15 L/minute of supplemental oxygen through a mask with a reservoir. If the patient clinical condition deteriorates, all necessary equipment should be prepared to perform orotracheal intubation and initiate invasive mechanical ventilation, which should be adjusted according to lung protective strategies.[4]
- Health personnel should carry out orotracheal intubation after putting on all the protective equipment for high-risk procedures (orotracheal intubation, orotracheal tube change, cardiopulmonary resuscitation). Using a videolaryngoscope is recommended.[6]
- Invasive mechanical ventilation should be started with a tidal volume of 4 to 6 mL/kg of predicted weight, following the recommendations for lung protection.[7]
- Deep sedation should be used if necessary, in order to avoid invasive mechanical ventilation-associated damage, always following the recommendations for sedation and analgesia for seriously ill patients.[5,6]
- Early changing the patient to the prone position should be considered if he/she continues with hypoxemia despite all adjustments to the ventilator.[8]

- Muscular relaxant continuous infusion should not be routinely used, but according to each patient's clinical conditions.[4,9]
- It is important to avoid ventilator disconnection, which results in loss of positive end-expiratory pressure, atelectasis and aerosol generation.[4]
- Non-invasive mechanical ventilation and high-flow oxygen therapy are not recommended due to the generation of aerosol and the associated risk of viral spread among health personnel.[6,10,11] The only scenario where these measures might be used is when a seriously ill patient requires invasive ventilatory support and the necessary equipment is not available; in those cases, the patient should preferably be moved to a cubicle with negative pressure.
- Extracorporeal membrane oxygenation is not recommended given that recent reports suggest high mortality in patients with COVID-19 treated with this form of organ support, which should not be used in hospitals that are not reference or high-volume centers and whose personnel has no experience with this procedure.[12,13]
- Using steroids is not recommended.[14]
- So far, there is no specific antiviral treatment for patients with COVID-19 or results of controlled trials supporting the use of any; the antivirals that are being proposed are under experimentation and are not part of the standard treatment for patients with COVID-19.
- The use of vasopressor drugs to maintain mean blood pressure ≥ 65 mm Hg is only indicated if hypovolemia has been excluded and mean blood pressure has not improved despite resuscitation with crystalloid solutions. Norepinephrine is the first-line vasopressor.[4,5]
- If data consistent with tissue hypoperfusion are identified despite adequate resuscitation with crystalloids or vasopressor use, using dobutamine should be considered.[4,5]

All the described recommendations are subject to change according to updates published in national or international journals.

Conflict of interests

The authors declare that they have no conflicts of interest.

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Ethical disclosures

Protection of people and animals. The authors declare that no experiments were performed on humans or animals for this study.

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BRIEF COMMUNICATION

Simultaneous mechanical ventilation of several patients with a single ventilator

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Abstract

Introduction: *Simultaneous mechanical ventilation of several patients with a single ventilator might reduce the deficit of these devices for the care of patients with acute respiratory failure due to Covid-19.* **Objective:** *To communicate the results of a mechanical ventilation exercise with a ventilator in a lung simulator, and simultaneously in two and four.* **Results:** *No statistically significant differences were observed between programmed, recorded and measured positive end-expiratory pressure, mean airway pressure and peak pressure, except when simultaneously ventilating four lung simulators.* **Conclusions:** *Simultaneous mechanical ventilation should be implemented by medical personnel with experience in the procedure, be restricted to two patients and carried out in the intensive care unit.*

KEY WORDS: *Controlled mechanical ventilation. Acute respiratory insufficiency. COVID-19. Lung simulators.*

Ventilación mecánica simultánea con un solo ventilador a varios pacientes

Resumen

Introducción: *La ventilación mecánica simultánea a varios pacientes con un solo ventilador podría disminuir el déficit de esos dispositivos para atender a los enfermos con insuficiencia respiratoria aguda por Covid-19.* **Objetivo:** *Comunicar los resultados de un ejercicio de ventilación mecánica con un ventilador en un simulador de pulmón, y simultáneamente en dos y cuatro.* **Resultados**: *No se observaron diferencias estadísticamente significativas entre la presión positiva al final de la espiración, presión media de la vía aérea y presión pico programadas, registradas y medidas, excepto al ventilar simultáneamente cuatro simuladores de pulmón.* **Conclusiones:** *La ventilación mecánica simultánea debe ser instaurada por personal médico con experiencia en el procedimiento, restringirse a dos pacientes y ser realizada en la unidad de cuidados intensivos.*

PALABRAS CLAVE: *Ventilación mecánica controlada. Insuficiencia respiratoria aguda. COVID-19. Simuladores de pulmón.*

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Introduction

The use of mechanical ventilation in clinical practice and its immediate acceptance by the medical community revolutionized the treatment of the seriously ill and critical patient. The possibility of keeping alive and helping patients with acute respiratory failure became a reality and a common practice in hospital medicine.[1] This unprecedented therapeutic intervention was the scenario that allowed the description of acute respiratory distress syndrome (ARDS), the therapeutic benefit of the use of positive end-expiratory pressure (PEEP), alveolar recruitment and prone ventilation.[1-7]

Since respiratory failure is always the first organic failure in the patient with multiple organ dysfunction syndrome, having enough ventilators is of paramount importance, particularly in ill-fated times such as those we are living in. Given the possibility that numerous patients experience acute respiratory failure in Mexico due to the Covid-19 pandemic, and the need to apply a protocol for massive mechanical ventilation to "victims" due to ventilator shortages, simultaneous ventilation of several patients with a single ventilator, especially those who share similar pathophysiological characteristics, can be a viable alternative.[8,9] Although this procedure was proposed more than 20 years ago, it is currently feasible with pressure-controlled ventilation, by means of which peak pressure (Ppeak) and conduction pressure can be controlled, which allows ventilation with lung protection measures. The purpose of this paper is to communicate the results of a single-ventilator multiple mechanical ventilation exercise in lung simulators.

Method

The ventilator and circuits were connected to latex anesthesia breathing bags (lung simulator); dual limb adult breathing circuits were used (RT200 series, Fisher & Paykel Healthcare, Auckland, New Zealand); two pieces, assembled and connected to the inspiratory and expiratory valve, were used to connect one, two or four lung simulators (Fig. 1) to a single mechanical ventilator (AVEA®, CareFusion, San Diego, CA, USA) programmed in the pressure-control mode. To corroborate the measurements, a pressure and volume calibrator was available (VT305®, Fluke Biomedical, Cleveland, OH, USA).

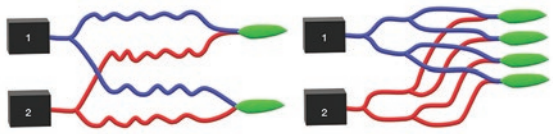

Figure 1. *Diagram of two and four lung simulators; in blue, inspiratory limbs of the circuit connected to the inspiratory valve;[1] in red, expiratory limbs of the circuit connected to the expiratory valve;[2] in green, the lung simulators.*

The programmed values, those recorded by the ventilator and those measured by the calibrator were consecutively recorded in one, two and four lung simulators. The ventilator was then programmed in the pressure-control mode with PEEP rising from 0 cm H_2O, fraction of inspired oxygen at 40%, respiratory rate at 20 breaths per minute, inspiration:expiration ratio at 2:1. Each PEEP level was maintained for 10 minutes, in order to favor stability of the readings; the process was repeated until reaching a PEEP of 11 cm H_2O due to the limitations of the lung simulator.

Statistical analysis was carried out with measures of central tendency and dispersion for quantitative variables, frequency and percentage were recorded for categorical variables, and the Kolmogorov-Smirnov test was used for normality of the curve. Two-tailed Student's t-test and Pearson's correlation test were applied for normally distributed data; abnormally distributed data were analyzed with Mann-Whitney's U-test and the Spearman correlation test. Statistical significance was established with a p-value < 0.05. The statistical programs used were Social Science Statistics (http://socscistatistics.com) and STATA (StataCorp LLC, http://stata.com/products/mac/).

Results

No statistically significant differences were observed between programmed, recorded and measured PEEP, mean airway pressure and Ppeak, except when ventilating four lung simulators, a situation in which Ppeak was significantly lower in the lung simulators than in the ventilator (Table 1 and Figure 2).

Discussion

The described results demonstrate that programmed and obtained pressures were statistically equivalent when one or two lung simulators were ventilated; therefore, the system reliably transmitted the projected

Table 1. Comparison of airway pressure and volume

	One lung Mean (SD)	Two lungs Mean (SD)	Four lungs Mean (SD)
Pressure (cm H_2O)			
Programmed PEEP *versus* ventilator PEEP	6.9 (3.21) versus 5.2 (3.21) p = 0.9203	5.2 (3.32) versus 5.7 (3.30) p = 0.4894	5.5 (3.50) versus 5.9 (3.23) p = 0.6584
Programmed PEEP *versus* measured PEEP	6.9 (3.21) versus 5.0 (3.19) p = 0.9326	5.2 (3.32) versus 4.7 (3.23) p = 0.5887	5.5 (3.50) versus 4.2 (3.14) p = 0.1134
Ventilator PEEP *versus* measured PEEP	5.2 (3.21) versus 5.0 (3.19) p = 0.8520	5.7 (3.30) versus 4.7 (3.23) p = 0.2159	5.9 (3.23) versus 4.2 (3.14) p = 0.0358
Ventilator Pmaw *versus* measured Pmaw	9.4 (3.43) versus 9.6 (3.42) p = 0.8711	9.7 (3.27) versus 9.0 (3.19) p = 0.3492	10.1 (3.41) versus 8.9 (3.30) p = 0.1193
Ventilator Ppeak *versus* measured Ppeak	21.2 (3.14) versus 20.8 (3.08) p = 0.8054	22.7 (3.22) versus 21.5 (3.14) p = 0.1328	26.3 (3.68) versus 24.4 (3.58) p = 0.0309
Volume (mm)			
Ventilator volume *versus* measured volume	163.1 (18.14) versus 146.6 (17.10) p < 0.0001	174.9 (20.64) versus 160.0 (18.54) p < 0.0001	221.3 (3.88) versus 205.3 (4.67) p < 0.0001

PEEP = positive end-expiratory pressure, Pmaw = mean airway pressure, Ppeak = peak pressure, SD = standard deviation.

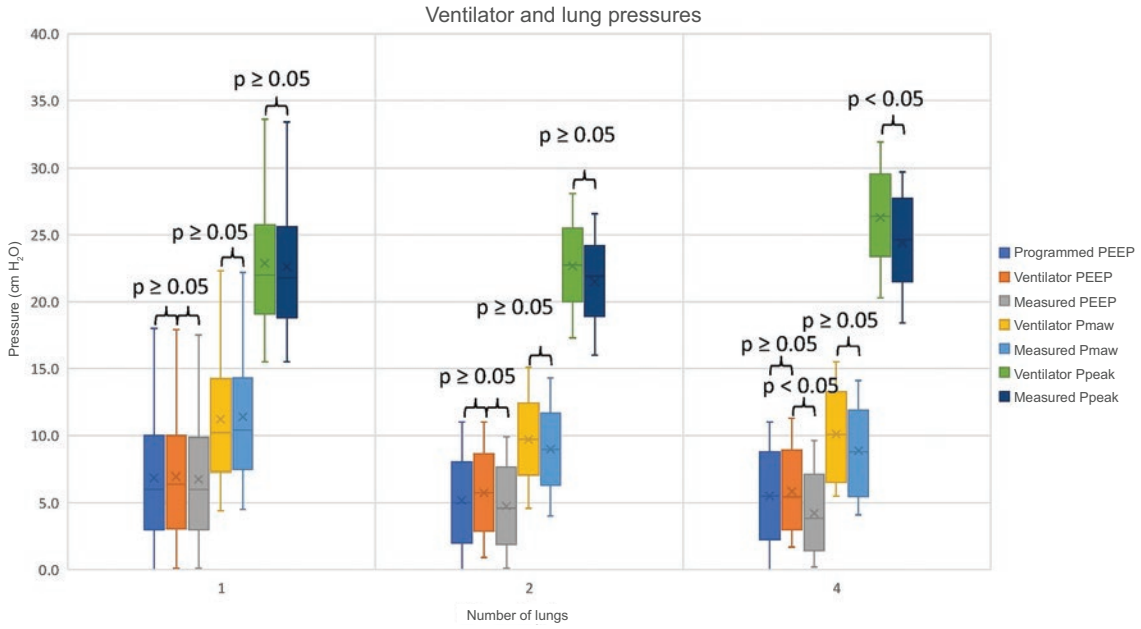

Figure 2. *Ventilator and lung pressures distribution. PEEP = positive end-expiration pressure, Pmaw = mean airway pressure, Ppeak = peak pressure.*

values. Simultaneous mechanical ventilation of multiple patients with a single device has the potential to double the access to mechanical ventilation until more supplies (ventilators) are received or the number of patients requiring them decreases.

Conclusions

Owing to the complexity of the connections and monitoring, the modality of simultaneous ventilation with a single ventilator should be applied by medical personnel with experience in the procedure, be restricted to two patients and carried out in the intensive care unit, where continuous monitoring is available, in addition to requiring ethical analysis and approval by health authorities.

Conflicts of interest

Dr. Gorordo Delsol reports receiving fees from Pfizer México and Merck, which are unrelated to the presented investigation. The rest of the authors declare that they have no conflicts of interest.

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Ethical disclosures

Protection of people and animals. The authors declare that no experiments were performed on humans or animals for this research.

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LETTER TO THE EDITOR

Challenges for medical education in Mexico in the time of COVID-19

Retos para la educación médica en México en los tiempos del COVID-19

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Introduction

By April 2, 2020, only 116 days after the description of the first case of SARS-CoV2 virus infection, which causes the COVID-19 disease, 1,014,673 cases and 50,030 deaths have been recorded in 181 countries.[1] In the United States, there have been 244,678 cases and 5,911 deaths recorded (in New York State alone, there are 93,053 cases and 2,538 deaths), while in Mexico, 1,378 cases and 37 deaths have been recorded. This number is expected to keep on increasing in both countries. The health system in Mexico, as well as in the rest of the world, will face an enormous problem in the months to come.

Mexico's medical education system has to adapt to the healthcare requirements generated by the pandemic. The undergraduate internship, the social service and the national system of medical residency are essential cycles in the training of doctors in Mexico. Undergraduate medical interns, house officers and residents represent an essential part of the physicians who carry out healthcare tasks and who will be exposed to patients with COVID-19. Between 2017, 2018 and 2019, 26,972 physicians (8480, 8821 and 9671, respectively) were accepted in the National System of Medical Residency in Mexico.[2] Furthermore, according to the 2018-2019 higher education statistical yearbook of the National Association of Universities and Institutions of Higher Education,[3] there were 139,272 undergraduate medical students, with 16,070 graduate physicians on training. Assuming that the number of graduates on training is similar to that of social service medical interns and undergraduate medical interns, there are approximately 32,000 undergraduate medical interns and house officers in Mexico. The addition of the above yields an approximate of 58,972 physicians in the medical education system clinical phase. However, health care burden and responsibilities at each stage are different, which requires defining the healthcare roles and recruitment strategies, and clarifying the level of risk that physicians on training will be exposed to.

The authors of this letter are doctors educated in the Mexican medical education system, and at this moment we are at the center of the pandemic caused by the SARS-CoV2 virus, New York City metropolitan area. We have experienced the lifestyle changes in the academic and healthcare areas, and we have observed the protection measures taken by institutions in the face of this emergency. We believe that some of our observations may be useful for the medical education system in Mexico.

Guaranteeing personal protection supplies availability

Regardless of the level of training, availability of supplies must be guaranteed in order to comply with personal protection measures. To assist COVID-19 patients, a regular mask or surgical mask is required (or N95 for aerosol-generating procedures such as endotracheal intubation), as well as eye protection

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(goggles or face shields), gloves, and isolation gowns. The correct use of personal protective equipment decreases the probability of nosocomial infection, reduces the number of doctors who must be hospitalized or quarantined and the probability of transmission by them to the population. This way, the health system has more doctors available to treat patients, while reducing the number of infected individuals. However, in the early stages of the health emergency, lack of supplies and protocols has already been reported in places such as the National Institute of Respiratory Diseases;[4] fellow residents from all over the country have expressed concerns for not having the supplies to comply with the personal protective measures at their hospitals, in addition to other resources. Mexico is not the only nation with this problem, which is also occurring in the United States and Italy,[5] the two countries with more recorded cases of the disease to date,[1] where several hospitals have been forced to depend on donations from external institutions or individuals.

Defining the acceptable exposure risk according to the level of training

The acceptable exposure risk of physicians on training should be defined. We celebrate the initiative taken by the General Directorate for Quality and Health Education (DGCES – *Dirección General de Calidad y Educación en Salud*) to protect trainee personnel.[6] As of March 24, all undergraduate medical interns should not be present in areas of risk for COVID-19. Historically, undergraduate medical interns have the double condition of students[7] and worker-employees.[8,9] This DGCES measure denotes that the well-being of undergraduate medical interns trumps over their healthcare-related activities and highlights the role of the undergraduate medical intern as a student rather than as an employee. However, if health services are overwhelmed by the number of patients during the pandemic, consideration could be given to assigning undergraduate interns where they are needed.

The DCGES will not interrupt the healthcare-related activities of medical interns on social service and residents; in addition, it establishes that these doctors must receive the necessary equipment for their protection in the care of suspected and confirmed COVID-19 patients. The legal framework that defines the positions of medical interns on social service makes it difficult to determine the level of healthcare obligations they should be assigned during the pandemic.[10,11] However, the work of these physicians is essential to the healthcare system at primary care level, since nearly one third of the primary care units of the public system are covered by them.[12] The DGCES establishes that resident physicians will continue with their clinical practice activities according to their academic program.

In addition, it is crucial to define the level of exposure of medical students who are on clinical rotations: which is the role of the student in the clinical setting during this pandemic? Unfortunately, unlike other situations that require an increase in medical attention, such as natural disasters in which the student can learn, in this pandemic, students can act as asymptomatic transmission agents, in addition to decreasing available personal protection supplies and COVID-19 tests in case they get sick.

This drove multiple medical schools in the United States to adopt immediate measures of social distancing and to restrict medical students access to healthcare areas,[13] which has generated new challenges for medical education. Although prior to the pandemic some subjects of the curriculum were already taught online at United States medical schools, social distancing measures required for all basic science courses to migrate to this format. Social distancing significantly affected teaching in clinical fields by limiting patient-student interactions. Alternative strategies are still being developed, including the use of virtual cases and the use of telehealth, among others.[13]

In a timely manner, the DCGES suspended academic activities in clinical fields for students in health areas from March 23 on. Furthermore, the education of medical students in the first years of medical school will be affected by the interruption of face-to-face classes in educational institutions. The first steps required to mitigate COVID-19 transmission have affected the paradigm of health education in Mexico, and it will therefore be important to assess the consequences of these actions. Medical undergraduate education system may need to adjust to an online education system in the immediate future, which will require the effort of all members of the medical academic community to solve the new challenges.

Adjusting the clinical activities of residents of different specialties according to health care burden

Multiple healthcare systems in New York City implemented actions to prevent nosocomial transmission,

including the cancellation of all elective surgical procedures, and numerous operating rooms have been transformed into intensive care units in order to increase mechanical ventilation devices availability. This led to a decrease in health care burden for surgical specialty medical residents, who remained in a "reserve status" and joined the pandemic response teams. Since surgical specialty residents sometimes lack sufficient experience in the management of patients with complex, non-surgical diseases, it was essential to create multidisciplinary teams, in which personnel with more experience in the management of medical diseases serve as group leaders. It is possible that his strategy may be necessary in Mexico. On the other hand, most patients with COVID-19 are admitted to internal medicine or intensive care departments, where health care burden is overwhelming.

Furthermore, various hospitals have created protocols or clinical practice guidelines for the management of patients with COVID-19, which are based on evidence and on available supplies and personnel at the institution. These protocols are continuously updated according to new findings on the disease. Following a consistent, evidence-based treatment allows health personnel on training to learn to manage COVID-19. The creation of these protocols by hospitals in Mexico will standardize the treatment of patients with COVID-19 at each institution.

Establishing measures to preserve mental health

Healthcare personnel participating in the pandemic response team will be exposed to high levels of stress. The sources of stress include witnessing scenes of human suffering, the health risk the personnel is exposed to, decision-making in matters of life and death, intense health care burdens, lack of resources and being separated from the family. The institutions those who wrote this letter work for have established different strategies for the management of stress in health workers; it is important for these strategies to be planned for trainee physicians who participate in the pandemic.

Adapting the recruitment of new residents to social distancing measures

Finally, although the recruitment of graduate and undergraduate medical interns in their health care positions may not be affected, this is not the case with resident physicians. The National Exam for Medical Residency Applicants (ENARM – *Examen Nacional para Aspirantes a Residencias Médicas*) has historically required physicians to travel to the sites where the exam is applied, which generates large congregations. The 44th ENARM will be held from September 25 to 30, 2020;[14] however, it has been estimated that social distancing will have to be active until vaccines or pharmacological strategies are created to treat the disease, i.e., perhaps more than 18 months.[15] What are the measures to be taken to adapt the selection of resident physicians?

Conclusions

Without a doubt, Mexican society will have to make difficult decisions when facing the pandemic generated by the SARS-CoV2 virus. The health system must be prepared to provide care to patients who require medical attention when contracting COVID-19. The medical education system in Mexico will have to be adapted to overcome as best as possible the challenges generated by the pandemic, as well as to define the risk of acceptable exposure according to the level of training, ensure that the supplies for personal protection are available, adjust the health care burden according to patient flow, protect the mental health of trainee physicians, and plan the recruitment and education of the new physicians who will join the medical education system. Undoubtedly, this is not an exhaustive list and there will be many other situations that should be considered in the future, but we hope that these reflections serve as a catalyst for future discussions for the planning of medical education in Mexico in times of COVID-19.

Ethical disclosure

Protection of people and animals. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

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LETTER TO THE EDITOR

Neurologic manifestations of COVID-19

Manifestaciones neurológicas por COVID-19

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The COVID-19 pandemic, which started in China, has spread rapidly to affect the entire world in a matter of months. Main manifestations of the disease include a febrile syndrome accompanied by respiratory symptoms; however, cases of systemic involvement are increasingly being reported, including cardiac and central nervous system compromise. In the series by Ling M. et al., 214 patients with COVID-19 were studied; 78 (36.4 %) had neurologic manifestations, which were classified into four main groups: acute cerebrovascular disease, impaired consciousness, peripheral nervous system involvement and muscular manifestations.[1] Another report published by Li et al. describes that, out of 221 patients with COVID-19, 13 developed acute cerebrovascular disease with cerebral infarction, venous thrombosis and intracerebral hemorrhage.[2]

Acute respiratory distress syndrome is the main cause of death in COVID-19 patients, which is explained by an intense pulmonary inflammatory reaction.[3] There is evidence that the virus might be able to invade the central nervous system through the brain stem and affect the regulation of respiratory centers, thus contributing to refractory respiratory failure, as well as to the development of some manifestations such as hyposmia and dysgeusia.[4]

In Mexico, COVID-19 cases are increasingly being identified, and thus we must bear in mind infectious and non-infectious complications that affect the nervous system such as encephalitis, seizures, Guillain-Barré syndrome, disseminated encephalomyelitis and hemorrhagic leukoencephalitis, which are disorders that can occur during or after viral infections. A neurological symptom could be the first manifestation of COVID-19.

Conflict of interests

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LETTER TO THE EDITOR

High-flow cannulas will be required with current COVID-19 crisis, not only mechanical ventilators

La crisis por COVID-19 hará necesaria cánulas de alto flujo, además de ventiladores mecánicos

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COVID-19 is characterized by acute respiratory distress syndrome progression,[1] which ranges from mild to severe.[2] A percentage of critically ill patients will require endotracheal intubation and mechanical ventilation; therefore, Mexican engineers from different places have had the initiative of creating mechanical ventilators.[3-5]

Although artificial ventilator is one of the last resources for the most severely ill patients, there are patients who are on the verge of not meeting the criteria for intubation; if they are directly intubated, the opportunity for them to overcome the severe phase with high concentration supplemental oxygen would be be missed. Intubation per se induces lung damage, increases the risk of superinfection, and the number of days of ICU stay.[6,7] Furthermore, non-selective use of artificial ventilators decreases the opportunity for patients who require a ventilator to survive and increases institutional care costs of both human and material resources.

High-flow oxygen cannulas function is to provide high concentrations of oxygen to the patient, which are not reached with normal nasal prongs or masks.[8-10] At some point, supplemental oxygen with nasal prongs is not tolerated by the patient and produces dryness, loss of heat in the airway, bleeding, nasal mucosa lesions and pain. High-flow oxygen cannulas are much simpler than an artificial ventilator, since their only function is to mix oxygen with warm water to humidify, bring the gas mixture to body temperature and decrease the discomfort of having oxygen at high concentrations in the airways (Fig. 1). In addition to the benefit of not requiring sedation, they reduce stress, improve breathing and promote deep breaths, thus increasing alveolar ventilation. Additionally, the patient requires less nursing care, he/she can even eat, drink and sleep under normal conditions. Owing to the nature of the patients with COVID-19, whose main problem is hypoxia, high-flow nasal cannula oxygenation appears more convenient than non-invasive intermittent ventilation, since respiratory needs can exceed 50 L/hour and a normal mask can only provide approximately 10 L/hour of oxygen, in addition to mixing air with oxygen; with high-flow cannulas, up to 50 liters of oxygen can be provided, without the need to dilute with air.[8]

In addition to the above advantages, it is important to make two observations:

- The use of high-flow oxygen cannulas can produce aerosol, and the procedure should therefore be carried out in rooms with negative pressure. When implementing negative pressure is not possible, devices can be used to isolate patient aerosols, for example, covers of different materials, helmets, etc.
- This material resource is not available in the secondary care public institutions that have been designated as COVID reconversion centers, and those existing in tertiary care hospitals will probably be insufficient.

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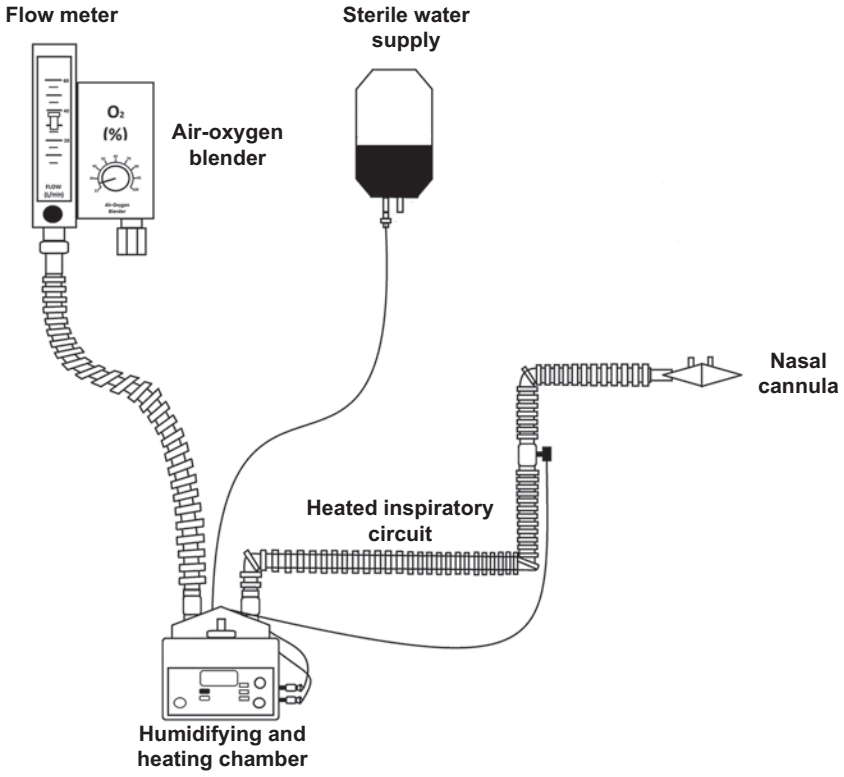

Figure 1. *General diagram of a high-flow cannula.*

The high-flow oxygen system would reduce the need for intubation in acute patients[11,12] and has the potential to save lives due to the easiness of its use in clinical practice, the reduction of costs, the limitation of biological risk for the medical team during the protection of the airway and the release of ventilators for other patients who do require them. In addition, it can decrease intensive care unit days of stay, which will be especially important when ventilators and beds in intensive care units become scarce or unavailable due to the number of patients.

We suggest to the government authorities to urgently purchase these devices, and to the engineers, companies and research centers, to also focus on the production of high-flow cannulas.

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