Project profile: a multicenter study on breast cancer in young women in Latin America (PRECAMA study)

Isabelle Romieu, MD, PhD,[1] Carine Biessy, MSc,[2] Gabriela Torres-Mejía, MD, PhD,[1] Angélica Ángeles-Llerenas, MD, PhD,[1] Gloria Inés Sánchez, PhD,[3] Mauricio Borrero, MD,[4] Carlos Andrés Ossa, MD,[5] Carolina Porras, Msc, PhD,[6] Ana Cecilia Rodríguez, PhD,[6] Rebecca Ocampo MD,[6] María Luisa Garmendia, MD, PhD,[7] Eva Bustamante, PhD,[8] Magali Olivier, PhD,[9] Peggy Porter, MD,[10] Sabina Rinaldi, PhD.[*,2]

Romieu I, Biessy C, Torres-Mejía G, Ángeles Llerenas A, Sánchez GI, Borrero M, Ossa CA, Porras C, Rodríguez AC, Ocampo R, Garmendia ML, Bustamante E, Olivier M, Porter P, Rinaldi S. Project profile: a multicenter study on breast cancer in young women in Latin America (PRECAMA study). Salud Publica Mex. 2019;61:601-608.
https://doi.org/10.21149/10466

Abstract

Objective. To describe the rationale and the methodology of a multicenter project to study the etiology of breast cancer in young Latin American women. **Materials and methods.** The International Agency for Research on Cancer has established an international collaborative population-based case-control study in four countries in Latin America: Chile, Colombia, Costa Rica, and Mexico (the PRECAMA study). Standardized methodologies were developed to collect information on reproductive variables, lifestyle, anthropometry, diet, clinical and pathological data, and biological specimens. The study will be extended to other countries in the region. **Conclusion.** PRECAMA is unique in its multidisciplinary approach that combines genetics, genomics, and metabolomics with lifestyle factors. The data generated through this project will be instrumental to

Romieu I, Biessy C, Torres-Mejía G, Ángeles Llerenas A, Sánchez GI, Borrero M, Ossa CA, Porras C, Rodríguez AC, Ocampo R, Garmendia ML, Bustamante E, Olivier M, Porter P, Rinaldi S. Perfil de Proyecto: Un estudio multicéntrico sobre el cáncer de mama en mujeres jóvenes en América Latina (estudio PRECAMA). Salud Publica Mex. 2019;61:601-608.
https://doi.org/10.21149/10466

Resumen

Objetivo. Describir la justificación y la metodología para el establecimiento de un proyecto multicéntrico sobre el cáncer de mama en mujeres jóvenes de América Latina. **Material y métodos.** La Agencia Internacional para la Investigación del Cáncer (IARC) ha establecido un estudio colaborativo internacional de casos y controles con base poblacional en cuatro países de América Latina: Chile, Colombia, Costa Rica y México (el estudio PRECAMA). Se han desarrollado metodologías estandarizadas para recolectar información sobre variables reproductivas, estilos de vida, antropometría y dieta, datos clínicos y patológicos y muestras biológicas. **Conclusión.** PRECAMA es único en su enfoque multidisciplinario. Los datos generados a través de este proyecto serán fundamentales para identificar los principales factores de riesgo del cáncer de mama en mujeres jóvenes. Los ha-

(1) Centro de Investigación en Salud Poblacional, Instituto Nacional de Salud Pública, Cuernavaca, Mexico.
(2) Section of Nutrition and Metabolism, International Agency for Research on Cancer. Lyon, France.
(3) Group Infection and Cancer, School of Medicine, University of Antioquia. Medellín, Colombia
(4) Clínica Vida. Medellín, Colombia.
(5) Instituto de Cancerología Las Américas. Medellín, Colombia.
(6) Agencia Costarricense de Investigaciones Biomédicas (ACIB)-Fundación INCIENSA. Costa Rica.
(7) Instituto de Nutrición y de Tecnología de los Alimentos, Universidad de Chile. Santiago, Chile.
(8) Instituto Oncológico Fundación Arturo López Pérez, Providencia. Santiago, Chile.
(9) Molecular Mechanisms and Biomarkers Group, Section of Mechanisms of Carcinogenesis, International Agency for Research on Cancer. Lyon, France.
(10) Division of Human Biology, Fred Hutchinson Cancer Research Center. Seattle, USA.
* On behalf of the PRECAMA Team

Received on: March 26, 2019 • **Accepted on:** July 8, 2019
Corresponding author: Sabina Rinaldi. International Agency for Research on Cancer.
150 Cours Albert Thomas 69372. 69008 Lyon, France.
E-mail: rinaldis@iarc.fr

identify major risk factors for molecular subtypes of breast cancer in young women, which will be important for prevention and targeted screening programs in Latin America.

Keywords: breast cancer; women; premenopause; Latin America; risk factors

llazgos serán relevantes para la prevención y los programas de detección oportuna en América Latina, con beneficios clínicos inmediatos.

Palabras clave: cáncer de mama; mujeres; premenopausia; América Latina; factores de riesgo

Breast cancer (BC) is the second most common cancer in the world, and by far the most frequently occurring cancer among women, with an estimated 1.67 million new cases diagnosed in 2012 (25% of all cancers).[1,2] It is estimated that one-third of these cancers occur in women younger than 50 years.[3] However, this proportion varies according to geographical region and socioeconomic status. In Latin America, the proportion of incident BC cases among women younger than 45 years is about 20%, whereas in North America and the European Union the proportions are about 12%.[3] Similarly, mortality data show an important difference in the proportion between Latin America (14%) and Western countries (7%).[3] Although the age distribution differs substantially between Western and Latin American countries, the large number of incident BC cases currently observed among young women in Latin America can only be partly explained by the population age structure.[4] The Latin American population is culturally and genetically heterogeneous, with varying ancestral backgrounds, and the proportion of BC that is due to hereditary factors appears to vary with ethnicity.[5] In addition, the association of BC with risk factors in Latin American women could be confounded by the extensive diversity in genetic ancestry, which may differentially influence the risk of breast cancer.[6] This Latin American population is currently undergoing significant lifestyle and nutritional transitions that result in unique contrasts in exposures, providing opportunities to identify endogenous (genetics and genomics) and exogenous (metabolic modification, behavioral, and cultural) factors associated with BC subtypes.

BC is a heterogeneous disease, and the presence or absence of estrogen receptor (ER) and/or progesterone receptor (PR) expression, as well as the molecular pathological characteristics, are key determinants of the tumor clinical behavior and response to treatment.[3,7] BC in young women has aggressive characteristics, in particular a high proportion of triple-negative and human epidermal growth factor receptor 2 (HER2)-positive (HER2+) cancers,[8] high risks of local recurrence and of contralateral BC,[9] and high mortality associated with advanced stage at diagnosis.[8,9] Genetic background and/or different environmental exposures (including lifestyle, dietary habits, and sedentary behaviors) are likely to shape BC tumor characteristics and cancer progression.[10,11] However, so far, only a few studies have evaluated the impact of lifestyle on the incidence in BC subtypes in young women, and most of the studies have focused on Western populations.[12-14] In Caucasian premenopausal women, alcohol intake is associated with increased risk of BC,[15] and a reduction of risk is observed with breastfeeding, but there is still limited evidence on the role of diet and body fatness.[16] Data from Latin America are sparse, although previous studies suggest a potential effect of dietary factors and physical activity.[17-20]

To increase the knowledge of clinical and pathological profiles associated with specific genetic and lifestyle risk factors in Latin American women, the International Agency for Research on Cancer (IARC) in collaboration with Latin American partners is conducting a multicenter case-control study on BC in young women in four Latin American countries (Chile, Colombia, Costa Rica, and Mexico) to determine BC subtypes and associated risk factors for BC incidence and survival. The results of this investigation will have immediate benefits for specific preventive actions focusing on modifiable behavioral risk factors and for patient-tailored selection among the existing treatment options.

Materials and methods

This project is coordinated by IARC and involves scientific teams in four Latin American countries, with one coordinating center in each country and one or more hospitals collaborating with the coordinating center. Through IARC and a central pathology laboratory (the Porter Lab) at the Fred Hutchinson Cancer Research Center (FHCRC) in Seattle, the research teams have harmonized and standardized protocols for recruitment of cases and controls, collection of questionnaire data, collection and storage of blood and urine samples, tumor block fixation and handling, and pathology review. All questionnaires and forms are administered in the local language (Spanish). A central web-based database has been developed.

Ethics approval and consent to participate

All participants gave written informed consent before enrolment, and the study protocols were approved by the institutional review boards in Chile (Oncologic Institute Foundation Arturo López Pérez and National Cancer

Institute), Colombia (Cancer Institute Las Americas and University of Antioquia), Costa Rica (Costa Rican Institute of Clinical Research [ICIC] and Center for Strategic Development and Information in Health and Social Security [CENDEISSS] of the Costa Rican Social Security Fund [CCSS]), and Mexico (National Institute of Public Health [*Instituto Nacional de Salud Pública*, INSP] and the Mexican Social Security Institute [*Instituto Mexicano del Seguro Social*, IMSS]), and by the Ethics Committee of the International Agency for Research on Cancer (IARC). The pilot phase of the study started in 2012.

Recruitment of subjects

Selection of cases

Incident cases of primary invasive BC are recruited from general or cancer-specific hospitals or private oncology institutes. The inclusion criteria are: (1) age 20-45 years; (2) resident for ≥3 years in the same city district; (3) having an incident primary invasive BC with positive biopsy and clinical staging (according to tumor-node-metastasis standards). The exclusion criteria for eligible subjects are: (1) severe chronic disease (e.g. renal insufficiency); (2) limited ability to communicate; (3) pregnant or nursing; (4) previous diagnosis and treatment of cancer (except non-melanoma skin cancer).

Cases are recruited before any treatment and after signing an informed consent to participate. All of the recruiting health institutions provide services to populations with a wide range of socioeconomic status. Recruitment and sample collection are performed at each of the study clinics. Cases referred to the PRECAMA hospitals from other cities or regions are not included in the study, because of the difficulty of selecting adequate controls. Women with a recent biopsy diagnosis of BC (incident case) or a referral to the breast surgeon because of suspicious mammography that was confirmed as BC are approached by a nurse and/or a breast surgeon and invited to participate in the study. After the study is presented, women who are willing to participate provide their telephone number and contact information. Through telephone calls, nursing assistants check the eligibility criteria and invite the women to attend the recruitment clinical center. During the visit, trained nurses or physicians obtain informed consent, conduct the interviews, and collect samples.

Selection of controls

Controls are randomly selected from the underlying population residing in the same city district as the case for ≥3 years, using a multilevel sampling frame. In Chile, because of logistic constraints, some controls are referred by the cases (acquaintances but not relatives). Controls are matched to cases on age (± 3 years), city district of residence, and health insurance institution. The exclusion criteria are the same as for the cases. After the study is presented, women who are willing to participate sign an informed consent. In Colombia and Mexico, controls are interviewed at home; in Chile and Costa Rica, the interview is conducted at the study clinic.

Data collection

Lifestyle assessment

The questionnaire includes information on socioeconomic status during infancy and early childhood (based on parental education and occupation, place of residence, and type of housing during childhood), health and reproductive history (number of births, age at each birth, age at menarche, and duration of breastfeeding for each birth), history of benign breast disease, use of hormones (e.g. oral contraceptives), smoking habits, alcohol intake, maximum attained body weight, body silhouette at different ages, physical activity (household and recreational) and hours per day spent sedentary (e.g. watching television), diet, occupation, environmental exposures, ethnicity, and family history of cancer.

Dietary intake assessment

Dietary intake is assessed through a semi-quantitative food frequency questionnaire specific to each country that includes food items with a standard portion size.[21] These questionnaires have been validated in women residing in Mexico City [22,23] and in Costa Rica.[24] In each country, the list of food items was carefully reviewed with local nutritionists, and additional foods were added if necessary according to local nutrition surveys, in order to capture the local diet. The number of food and drink items in each food frequency questionnaire therefore varies across countries, and include 195, 217, 168, and 191 items, respectively, for Chile, Colombia, Costa Rica, and Mexico.

For each food item, a commonly used unit or portion size is indicated (specified serving size: a slice, a glass, or a natural unit such as one apple), and participants are asked how often, on average, over the previous year, they consumed the specified amount of each food, choosing among 10 frequencies of consumption: >6 times/day, 4-5 times/day, 2-3 times/day, 1 time/day, 5-6 times/week, 2-4 times/week, 1 time/week, 2-3 times/month, <1 time/month, or never. In addition, information is collected on intake of alcoholic and non-alcoholic drinks (sugar-sweetened beverages, tea, coffee, and water), as well as on added sugar, added salt, fat used for cooking, and intake of fat from meat. Women

are also asked to provide information on consumption of multivitamins and specific vitamin supplements.

Physical activity

Women are asked to report a representative week in terms of the number of hours of sleep and the level of physical activity: mild (1.0-3.4 metabolic equivalents [METs]), moderate (3.5-5.9 METs), and vigorous (≥6 METs) activity during work time, work at home, or recreational time on each day of the week and over the weekend. The women are provided with specific definitions and examples of these different types of activities, for a better characterization of the physical activity level. Hours per day are summed to calculate the number of hours per week of each type of activity to calculate MET-hours/week, using the following coefficients: mild (2), moderate (4), and vigorous (6-8). In addition, some information on vigorous physical activity during adolescence and early adulthood is obtained.

Anthropometric measurements

Anthropometric measurements are performed by trained personnel according to Lohman's recommendations.[25] The measurements include weight, height, sitting height, waist and hip circumferences. In addition, women are asked to identify their body silhouette from among six options (from very thin to very fat) at six different ages: childhood (6-11 years), adolescence (12-18 years), early adulthood (19-25 years), before pregnancy, adulthood (age 26 years until one year before diagnosis), and current. The validity of responses to these pictograms has been tested in different settings and has been proven to be reliable.[26]

Collection of biological specimens

Each center applies standardized protocols for specimen collection. The protocols have been previously developed and validated.[27,28] *Blood samples* are obtained at recruitment by venipuncture using vacutainers, aliquoted into serum (20 0.2-ml aliquots), plasma (20 0.2-ml aliquots), red blood cell (6 0.2-ml aliquots), and buffy coat (6 0.2-ml aliquots), and stored at -80 °C. *Spot urinary samples* are collected in 50-ml flasks, aliquoted (101-ml aliquots), and stored at -80 °C. *Tumor samples* are formalin-fixed and paraffin-embedded (FFPE) according to standardized protocols within one hour after excision. Paraffin blocks are stored at the local pathology service facilities. Each study site prepares serial thin (4 μM) sections of fixed tumor tissues mounted on glass slides in batches that are shipped immediately on cold packs to the Porter Lab at FHCRC for immunohistochemistry (IHC) analyses and centralized pathology review. Additional thicker (6 μM) sections from each tumor are cut into microfuge tubes and shipped to the Porter Lab for DNA extraction. Because of logistic issues, tumor samples from Mexico are shipped directly to the Porter Lab, where all procedures are conducted.

Pathology review and IHC analyses

Histology sections from tumor biopsies are reviewed for diagnosis, tumor grade, lymphovascular invasion, and stromal and lymphocyte response. IHC is performed for ER (SP1, Lab Vision, Fremont, CA), PR (PgR 636, Dako, Denmark), HER2 (AO485, Dako, Denmark), epidermal growth factor receptor (EGFR) (31G7, Invitrogen, Camarillo, CA), CK5/6 (D5/16 B4, Dako, Denmark), p53 (Pab 1801, Calbiochem, La Jolla, CA), and *nuclear protein Ki-67 (*Ki-67**,** MIB-1, Dako, Denmark) according to standardized and optimized protocols that include antigen retrieval when required. Tumor samples with ≥1% of immunostained tumor cell nuclei are considered positive for ER and PR expression (ER+, PR+). For HER2, samples are considered positive if there is strong membrane immunostaining (3+), and negative otherwise. In addition, the expression percentages of tumor protein p53 (p53, classified as positive if >10%)[29] and the proliferation marker Ki-67 (classified as high if >14%)[30] are determined. Triple-negative (TN) tumors are defined as ER-, PR-, and HER2-, and among the TN tumors, basal-like cancers are defined as ER-, PR-, HER2-, and *EGFR*+ and/or CK5/6+.

Storage, management, and quality control of data and biological samples

To facilitate data entry and validation, a computerized version of the questionnaire, based on previous BC studies in Mexico,[17,18] has been developed.

Questionnaire data are entered locally using a web-based application and centralized at IARC at regular intervals. IARC conducts consistency checks and quality controls; any queries are resolved directly with the center before files are merged for data harmonization and establishment of the central database. Mirror halves of the blood fractions and urinary samples are stored in local biobanks in each center, and half is stored and centralized at the IARC biobank, in order to minimize the risk of sample loss in case of freezer breakdown or other problems affecting samples in one of the locations, and to facilitate retrieval of samples for PRECAMA-wide projects. Tumor DNA is also centralized and stored at IARC.

The shipment of all biological samples will follow the International Air Transport Association (IATA) international regulations (http://www.iata.org/ps/publications/dgr/Pages/manuals.aspx).

Blood fractions and urine samples are shipped in dry ice, while tumor DNA is shipped in frozen gel packs.

Discussion

Major research questions and objectives

The primary aim of the PRECAMA study is to identify molecular subtypes of BC and their risk factors in young Latin American women in order to support targeted strategies for prevention and control. In addition, this project aims to support development of human resources and infrastructure for research on BC in Latin America. The availability of extensive and validated epidemiological data on current and past lifestyle exposures, as well as access to tumor blocks and biological samples collected through high-quality standard operating procedures, makes PRECAMA a unique resource for cutting-edge molecular epidemiology projects.

PRECAMA will be a very important platform to provide evidence to address still-unanswered questions about BC etiology in young LA women. The key questions that will be addressed are the following:

1) *What are the molecular phenotypes of breast cancer in premenopausal Latin American women, and do they differ from those in other populations?* Because of the molecular heterogeneity of the disease, one objective of the study is to subtype tumors according to IHC markers (ER, PR, HER2, EGFR, CK5/6, Ki-67, p53),[31] and to study their genomic features, such as somatic mutations in cancer genes and genome-wide mutational signatures.[32] This will enable better characterization of the tumor biology and assessment of the contribution of mutational processes (endogenous and exogenous) to tumor development. These genomic features have not been analyzed in detail in young women, and data on Latin American women are lacking. Associations between exposure/lifestyle factors and specific molecular features will provide important mechanistic insights into the etiology of different BC subtypes.

   Pilot data in PRECAMA[32] indicated that the majority of cases were positive for ER or PR (72%), and 21% were triple-negative. In 126 sequenced cases, *TP53, PIK3CA* and *AKT1* were the most frequently mutated genes. *TP53* mutations were more frequent in HER2-enriched and triple negative subtypes, while *PIK3CA*/*AKT1* mutations were more frequent in ER-positive tumors. Interestingly, in this population, a higher proportion of G:C>T:A mutations was observed in *TP53* compared with data from TCGA and METABRIC BC series. Overall, these pilot results show that although the overall mutation burden was as expected from data in other populations, mutational patterns observed in *TP53* suggested possible differences in mutagenic processes giving rise to these tumors compared with other populations.

2) *Which reproductive and lifestyle risk factors are specifically associated with major subtypes of breast cancer in young LA women?* Of particular interest are:
   (i) the role of earlier age at menarche and lactation[33] - reproductive factors are the group of factors with the strongest and most consistent associations with BC risk.[34] Known risk factors associated with BC include age at menarche, age at first birth, and parity, but only few studies have focused on risk factors for BC in young women, particularly by subtypes. Preliminary results on 288 cases and controls from the PRECAMA study indicated that older age at first full-term pregnancy, longer time between menarche and first full-term pregnancy, and an older age at last pregnancy were associated with an increased risk of ER+ tumors, while pregnancy, number of childbirths, time since last birth, and history of breastfeeding were inversely associated with ER+ tumors.[33] In addition, older age at menarche and longer duration of breastfeeding were inversely associated with ER- tumors. Overall, these first results indicate that reproductive factors may be differentially associated with BC subtypes in young Latin American women.
   (ii) the role of body fatness, fat distribution, and change in body shape over time. Although body mass index (BMI), a valid parameter of general adiposity, has been consistently related to a decreased risk of premenopausal BC, recent data suggest that waist circumference and waist-to-hip ratio, a better proxy of abdominal adipose tissue, which is more metabolically active than peripheral adipose tissue,[35,36] are related to an increased risk of premenopausal BC in specific subgroup.[37-39] Detailed anthropometric data, biological markers (inflammatory markers, hormones, and adipokines), and metabolomic analyses will improve our understanding of potential mechanisms.
   (iii) the role of dietary factors and dietary patterns, for which results are still inconclusive (particularly fatty acid components, diets with high glycemic load, processed foods, low vitamin D, and low folate);[16] and
   (iv) the role of physical activity level and sedentary behaviors.[16,40,41] Regular physical activity reduces the risk of premenopausal breast can-

cer.[16] However, data from Latin America and developing regions are still sparse.

3) *What is the role of genetic ancestry in breast cancer risk in young Latin American women, and the role of genetic variants involved in metabolic disorders?*

Population admixture studies have demonstrated that genetic ancestry is associated with BC risk in Latin women from the United States,[5] and these results have been replicated in a sample of Mexican women.[6] However, little is known about the role of admixture on genetic mapping in Latin American women in association with BC subtypes[41] and associated risk factors. In PRECAMA, ancestry will be estimated using validated Ancestry Informative Markers (AIMs) that allow distinguishing with a high probability the three ancestral populations.

Epidemiological studies have linked diabetes with BC risk in Caucasian populations, and recent meta-analyses have shown an increase of >20% in BC risk among women with type 2 diabetes compared with women without the disease.[42,43] This association could be due to shared lifestyle risk factors or intrinsic etiology, such as genetic susceptibility. Several susceptibility genes for type 2 diabetes and metabolic disorders have been identified in the Latin American population.[44,45] However, there are no data on shared genetic susceptibility to these diseases and BC in young women. The results will provide a better understanding of the potential mechanisms involved and will help to identify susceptible individuals.

4) *What are the factors that may play a role in breast cancer progression, recurrence, and survival according to specific subtypes*? Breast tumors in young women are often aggressive and have poor prognosis, leading to early death. Given the limited understanding of the basic biology, epidemiology, and optimal therapeutic strategies for tumors of young Latin women, it is essential to identify lifestyle factors that could have a beneficial impact on survival.[12]

Future directions

The PRECAMA study currently includes four Latin American countries, and the study will be extended to other countries in the region (Brazil and Guatemala). With a target sample size of 1 500 cases and 1 500 controls, this project will be the largest ongoing effort to characterize the relationship between different BC subtypes and risk factors in young, premenopausal women. Importantly, this project strongly supports the development of research infrastructure and capacity building to strengthen breast cancer research in Latin America. The Latin American countries involved in PRECAMA (Chile, Colombia, Costa Rica and Mexico, with Brazil and Guatemala joining) cover about 65% of the entire population in Latin America.

The ongoing development of a BC survival cohort in order to evaluate prognostic factors, treatment response, and recurrence patterns in this population will be extremely valuable for the treatment of the disease and improvement of survival. Family history and high-penetrance germline mutations will be explored in relation to the risk of BC in young Latin American women. The establishment of genetic counselling for women with inherited mutations and for their families would also be a priority in infrastructure development. Young women are more likely to have genetic susceptibility to BC[9] with specific germline mutations that could be related to ancestry. Some mutations with high penetrance, such as those in the *BRCA1* and *BRCA2* genes, have been widely described (80% risk of BC), and other mutations with lower penetrance are known in the Caucasian population,[46,47] however, there are limited data among Latin American women. Therefore, it will be important to assess the hereditary component in this population.

Conclusion

PRECAMA is unique in its multidisciplinary approach that combines genetics, genomics, and metabolomics approaches with extended documentation of lifestyle factors. The data generated through this project will identify new markers and pathways that influence BC incidence and survival in young, premenopausal women and can support the development of subtype-specific risk prediction models for BC in young women. Targeted screening of highly susceptible individuals will enable early diagnosis and timely treatment, with favorable consequences for survival. In Latin America, this will have immediate clinical benefits. Knowledge of genomic tumor characteristics, treatment response, and prognostic factors will enable clinicians to better select the most effective treatment options and to provide recommendations for behavior changes and referral to relevant community services to improve survival.

List of recruitment centers

In Chile, cases are recruited in Santiago through the Oncologic Institute Foundation Arturo López Pérez, the Hospital Santiago Oriente Dr. Luis Tisné Brousse, and the National Cancer Institute. In Colombia, cases are recruited in three cities: Medellín, from the Cancer Institute Las Americas, the Oncologic Center of Antioquia, and Clínica Vida; Barranquilla, from the Ultrasonographic

Diagnostic Center (CEDIUL) and the Bonnadona Prevenir clinic; and Cali, from the Hemato Oncologists Center. In Costa Rica, cases are identified in women attending large general hospitals and private practice physicians who are resident in districts located in the Greater Metropolitan Area, Pérez Zeledón, or the provinces of Guanacaste and Puntarenas. In Mexico, cases are recruited through the *Hospital de Oncología del Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social*.

Funding

The study is funded by the International Agency for Research on Cancer (IARC), the Union for International Cancer Control (UICC), the Pan American Health Organization (PAHO), the Ibero-American Programme for the Development of Science and Technology (CYTED), the Colombian Administrative Department of Science, Technology and Innovation (COLCIENCIAS) (grant no. 1115-569-348899), and CODI-Universidad de Antioquia (grant CPT-1229).

Disclaimer

Where authors are identified as personnel of the International Agency for Research on Cancer / World Health Organization, the authors alone are responsible for the views expressed in this article and they do not necessarily represent the decisions, policy or views of the International Agency for Research on Cancer / World Health Organization. Ana Cecilia Rodríguez was part of the *Proyecto Guanacaste* when this work was iniciated.

Acknowledgments

The PRECAMA team includes International Agency for Research on Cancer (Coordinating Center) Investigators and Staff: Carine Biessy, Magali Olivier, Sabina Rinaldi, Isabelle Romieu; Investigators and Staff in Chile: Maria Luisa Garmendia, Jose Luis Soto, Eva Bustamante, Leonor Moyano; Investigators and Staff in Colombia: Alberto Angel, Carlos Andres Ossa, William H. Arias, Gabriel Bedoya, Mauricio Borrero, Alicia Cock-Rada, Israel Díaz-Yúnez, Carolina Echeverri, Fernando Herazo, Ángel Hernández, Roberto Jaramillo, Edgar Navarro, Yorlany Rodas Cortés, Gloria I. Sánchez; Investigators and Staff in Costa Rica: Bernal Cortés, Paula González, Diego Guillén, Viviana Loría, Rebecca Ocampo, Carolina Porras, Ana Cecilia Rodríguez; Investigators and Staff in Mexico: Gabriela Torres-Mejía, Isabel Alvarado-Cabrero, Angélica Ángeles-Llerenas, Jenny Tejeda; Investigators and Staff in Seattle: Peggy Porter.

The authors wish to acknowledge the substantial support provided by the research nurses and health workers as well as Tracy Lignini, Karen Muller, Mathilde His, Dacia Christin, Cecile Le Duc, Jordi de Battle, Talita Duarte-Salles, and Ana Cristina Ocampo.

The authors also wish to thank the women participating in the project, for their time and commitment.

Declaration of conflict of interests. The authors declare that they have no conflict of interests.

References

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, *et al.* Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136(5):E359-86. https://doi.org/10.1002/ijc.29210
2. Curado MP. Breast cancer in the world: incidence and mortality. Salud Publica Mex. 2011;53(5):372-84 [cited march 25, 2019]. Available from: https://www.ncbi.nlm.nih.gov/pubmed/22218791
3. Villarreal-Garza C, Bargallo-Rocha JE, Soto-Perez-de-Celis E, Lasa-Gonsebatt F, Arce-Salinas C, Lara-Medina F, *et al.* Real-world outcomes in young women with breast cancer treated with neoadjuvant chemotherapy. Breast Cancer Res Treat. 2016;157(2):385-94. https://doi.org/10.1007/s10549-016-3811-2
4. Franco-Marina F, López-Carrillo L, Keating NL, Arreola-Ornelas H, Marie Knaul F. Breast cancer age at diagnosis patterns in four Latin American Populations: A comparison with North American countries. Cancer Epidemiol. 2015;39(6):831-7. https://doi.org/10.1016/j.canep.2015.10.004
5. Fejerman L, John EM, Huntsman S, Beckman K, Choudhry S, Perez-Stable E, *et al.* Genetic ancestry and risk of breast cancer among U.S. Latinas. Cancer Res. 2008;68(23):9723-8. https://doi.org/10.1158/0008-5472.CAN-08-2039
6. Fejerman L, Romieu I, John EM, Lazcano-Ponce E, Huntsman S, Beckman KB, *et al.* European ancestry is positively associated with breast cancer risk in Mexican women. Cancer Epidemiol Biomarkers Prev. 2010;19(4):1074-82. https://doi.org/10.1158/1055-9965.EPI-09-1193
7. Porter PL. Global trends in breast cancer incidence and mortality. Salud Publica Mex. 2009;51 Suppl 2:s141-6 [cited march 25, 2019]. Available from: http://saludpublica.mx/index.php/spm/article/view/4881
8. Lee HB, Han W. Unique features of young age breast cancer and its management. J Breast Cancer. 2014;17(4):301-7. https://doi.org/10.4048/jbc.2014.17.4.301
9. Narod SA. Breast cancer in young women. Nat Rev Clin Oncol. 2012;9(8):460-70. https://doi.org/10.1038/nrclinonc.2012.102
10. Mahoney MC, Bevers T, Linos E, Willett WC. Opportunities and strategies for breast cancer prevention through risk reduction. CA Cancer J Clin. 2008;58(6):347-71. https://doi.org/10.3322/CA.2008.0016
11. Colditz GA, Bohlke K. Priorities for the primary prevention of breast cancer. CA Cancer J Clin. 2014;64(3):186-94. https://doi.org/10.3322/caac.21225
12. Brenner DR, Brockton NT, Kotsopoulos J, Cotterchio M, Boucher BA, Courneya KS, *et al.* Breast cancer survival among young women: a review of the role of modifiable lifestyle factors. Cancer Causes Control. 2016;27(4):459-72. https://doi.org/10.1007/s10552-016-0726-5
13. Chajes V, Romieu I. Nutrition and breast cancer. Maturitas. 2014;77(1):7-11. https://doi.org/10.1016/j.maturitas.2013.10.004
14. Romieu I. Diet and breast cancer. Salud Publica Mex. 2011;53(5):430-9 [cited march 25, 2019]. Available from: http://saludpublica.mx/index.php/spm/article/view/7089

15. Coronado GD, Beasley J, Livaudais J. Alcohol consumption and the risk of breast cancer. Salud Publica Mex. 2011;53(5):440-7 [cited march 25, 2019]. Available from: http://saludpublica.mx/index.php/spm/article/view/7090
16. World Cancer Research Fund, American Institue for Cancer Research. Third Expert report: Diet, Nutrition, Physical Activity and Cancer: a Global Perspective [internet]. WCRF. 2018 [cited march 25, 2019]. Available from: https://www.wcrf.org/dietandcancer/about
17. Amadou A, Degoul J, Hainaut P, Chajes V, Biessy C, Torres-Mejía G, *et al*. Dietary carbohydrate, glycemic index, glycemic load, and breast cancer risk among mexican women. Epidemiology. 2015;26(6):917-24. https://doi.org/10.1097/EDE.0000000000000374
18. Ángeles-Llerenas A, Ortega-Olvera C, Pérez-Rodríguez E, Esparza-Cano JP, Lazcano-Ponce E, Romieu I, *et al*. Moderate physical activity and breast cancer risk: the effect of menopausal status. Cancer Causes Control. 2010;21(4):577-86. https://doi.org/10.1007/s10552-009-9487-8
19. Lajous M, Willett W, Lazcano-Ponce E, Sanchez-Zamorano LM, Hernandez-Avila M, Romieu I. Glycemic load, glycemic index, and the risk of breast cancer among Mexican women. Cancer Causes Control. 2005;16(10):1165-9. https://doi.org/10.1007/s10552-005-0355-x
20. Chajes V, Torres-Mejia G, Biessy C, Ortega-Olvera C, Ángeles-Llerenas A, Ferrari P, *et al*. omega-3 and omega-6 Polyunsaturated fatty acid intakes and the risk of breast cancer in Mexican women: impact of obesity status. Cancer Epidemiol Biomarkers Prev. 2012;21(2):319-26. https://doi.org/10.1158/1055-9965.EPI-11-0896
21. Willett WC. Nutritional Epidemiology, 2nd ed. ed. n, editor. New-York: Oxford University Press, 1998.
22. Hernandez-Avila M, Romieu I, Parra S, Hernandez-Avila J, Madrigal H, Willett W. Validity and reproducibility of a food frequency questionnaire to assess dietary intake of women living in Mexico City. Salud Publica Mex. 1998;40(2):133-40 [cited march 25, 2019]. Available from: http://saludpublica.mx/index.php/spm/article/view/6068
23. Romieu I, Parra S, Hernández JF, Madrigal H, Willett W, Hernández M. Questionnaire assessment of antioxidants and retinol intakes in Mexican women. Arch Med Res. 1999;30(3):224-39 [cited march 25, 2019]. Available from: https://www.ncbi.nlm.nih.gov/pubmed/10427874
24. Kabagambe EK, Baylin A, Allan DA, Siles X, Spiegelman D, Campos H. Application of the method of triads to evaluate the performance of food frequency questionnaires and biomarkers as indicators of long-term dietary intake. Am J Epidemiol. 2001;154(12):1126-35. https://doi.org/10.1093/aje/154.12.1126
25. Lohman TG RA, Martorell R. Anthropometric standardization reference manual. Champaign IL: Human Kinetics Books, 1988.
26. Tehard B, van Liere MJ, Com Nougue C, Clavel-Chapelon F. Anthropometric measurements and body silhouette of women: validity and perception. J Am Diet Assoc. 2002;102(12):1779-84. https://doi.org/10.1016/s0002-8223(02)90381-0
27. Vaught JB, Caboux E, Hainaut P. International efforts to develop biospecimen best practices. Cancer Epidemiol Biomarkers Prev. 2010;19(4):912-5. https://doi.org/10.1158/1055-9965.EPI-10-0058
28. Mendy M, Caboux E, Lawlor RT, Wright J, Wild CP. Common minimum technical standards and protocols for biobanks dedicated to cancer research. Lyon, France: IARC, 2017.
29. Lee SK, Bae SY, Lee JH, Lee HC, Yi H, Kil WH, *et al*. Distinguishing low-risk luminal a breast cancer subtypes with Ki-67 and p53 Is More predictive of long-term survival. PLoS One. 2015;10(8):e0124658. https://doi.org/10.1371/journal.pone.0124658
30. Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thurlimann B, Senn HJ, *et al*. Strategies for subtypes--dealing with the diversity of breast cancer: highlights of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. Ann Oncol. 2011;22(8):1736-47. https://doi.org/10.1093/annonc/mdr304
31. Jackisch C, Harbeck N, Huober J, von Minckwitz G, Gerber B, Kreipe HH, *et al*. 14th St. Gallen International Breast Cancer Conference 2015: Evidence, Controversies, Consensus - Primary Therapy of Early Breast Cancer: Opinions Expressed by German Experts. Breast Care (Basel). 2015;10(3):211-9. https://doi.org/10.1159/000433590
32. Olivier M, Bouaoun L, Villar S, Robitaille A, Cahais V, Heguy A, *et al*. Molecular features of premenopausal breast cancers in Latin American women: Pilot results from the PRECAMA study. PLoS One. 2019;14(1):e0210372. https://doi.org/10.1371/journal.pone.0210372. eCollection2019
33. Romieu I, Biessy C, Carayol M, His M, Torres-Mejía G, Sanchez GI, *et al*. Reproductive factors and molecular subtypes of breast cancer among young women in Latin America: the PRECAMA study. Sci Rep. 2018;8(1):13109. https://doi.org/10.1038/s41598-018-31393-7
34. Rosner B, Colditz GA, Willett WC. Reproductive risk factors in a prospective study of breast cancer: the Nurses' Health Study. Am J Epidemiol. 1994;139(8):819-3. https://doi.org/10.1093/oxfordjournals.aje.a117079
35. Fortner RT, Katzke V, Kuhn T, Kaaks R. Obesity and Breast Cancer. Recent Results Cancer Res. 2016;208:43-65. https://doi.org/10.1007/978-3-319-42542-9_3
36. Harvie M, Hooper L, Howell AH. Central obesity and breast cancer risk: a systematic review. Obes Rev. 2003;4(3):157-73. https://doi.org/10.1046/j.1467-789X.2003.00108.x
37. Amadou A, Ferrari P, Muwonge R, Moskal A, Biessy C, Romieu I, *et al*. Overweight, obesity and risk of premenopausal breast cancer according to ethnicity: a systematic review and dose-response meta-analysis. Obes Rev. 2013;14(8):665-78. https://doi.org/10.1111/obr.12028
38. Harris HR, Willett WC, Terry KL, Michels KB. Body fat distribution and risk of premenopausal breast cancer in the Nurses' Health Study II. J Natl Cancer Inst. 2011;103(3):273-8. https://doi.org/10.1093/jnci/djq500
39. John EM, Sangaramoorthy M, Hines LM, Stern MC, Baumgartner KB, Giuliano AR, *et al*. Overall and abdominal adiposity and premenopausal breast cancer risk among hispanic women: the breast cancer health disparities study. Cancer Epidemiol Biomarkers Prev. 2015;24(1):138-47. https://doi.org/10.1158/1055-9965.EPI-13-1007-T
40. Romieu I, Amadou A, Chajes V. The role of diet, physical activity, body fatness, and breastfeeding in breast cancer in young women: epidemiological evidence. Rev Invest Clin. 2017;69(4):193-203 [cited march 25, 2019]. Available from: https://www.ncbi.nlm.nih.gov/pubmed/28776604
41. Slattery ML, John EM, Torres-Mejia G, Lundgreen A, Herrick JS, Baumgartner KB, *et al*. Genetic variation in genes involved in hormones, inflammation and energetic factors and breast cancer risk in an admixed population. Carcinogenesis. 2012;33(8):1512-21. https://doi.org/10.1093/carcin/bgs163
42. Boyle P, Boniol M, Koechlin A, Robertson C, Valentini F, Coppens K, *et al*. Diabetes and breast cancer risk: a meta-analysis. Br J Cancer. 2012;107(9):1608-17. https://doi.org/10.1038/bjc.2012.414
43. Hardefeldt PJ, Edirimanne S, Eslick GD. Diabetes increases the risk of breast cancer: a meta-analysis. Endocr Relat Cancer. 2012;19(6):793-803. https://doi.org/10.1530/ERC-12-0242
44. Gamboa-Meléndez MA, Huerta-Chagoya A, Moreno-Macías H, Vázquez-Cardenas P, Ordónez-Sánchez ML, Rodríguez-Guillén R, *et al*. Contribution of common genetic variation to the risk of type 2 diabetes in the Mexican Mestizo population. Diabetes. 2012;61(12):3314-21. https://doi.org/10.2337/db11-0550
45. Weissglas-Volkov D, Aguilar-Salinas CA, Nikkola E, Deere KA, Cruz-Bautista I, Arellano-Campos O, *et al*. Genomic study in Mexicans identifies a new locus for triglycerides and refines European lipid loci. J Med Genet. 2013;50(5):298-308. https://doi.org/10.1136/jmedgenet-2012-101461
46. Villalobos-Comparan M, Flores-Dorantes M, Villarreal-Molina M, Rodríguez-Cruz M, García-Ulloa AC, Robles L, *et al*. The FTO gene is associated with adulthood obesity in the Mexican population. Obesity (Silver Spring). 2008;16(10):2296-301. https://doi.org/10.1038/oby.2008.367
47. Rudolph A, Chang-Claude J, Schmidt MK. Gene-environment interaction and risk of breast cancer. Br J Cancer. 2016;114(2):125-33. https://doi.org/10.1038/bjc.2015.439