Predominio del subtipo molecular Luminal B en un grupo de mujeres con cáncer de mama infiltrante del Eje Cafetero de Colombia: análisis por técnica de inmunohistoquímica

Predominance of the Luminal B molecular subtype in a group of women with infiltrating breast cancer from the Coffee-growing Region of Colombia: Analysis by immunohistochemistry technique

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Abstract

Breast cancer is the most common cancer in women and constitutes a public health problem due to its high morbidity and mortality rates. Diagnosis is made through routine histopathology studies that are complemented by expression studies of hormone receptors (Estrogen and Progesterone Receptors), human epidermal growth factor 2 (HER2) and cell proliferation index (Ki67) for their subtyping into molecular groups (Luminal A, Luminal B, Her2

and triple negative), which have different prognostic and therapeutic implications. Globally, a high prevalence of the Luminal A subtype has been reported, predominantly in North America, Europe, and some Latin American countries; however, the reports in the Colombian population are heterogeneous. The objective of this research is to establish an incidence profile of these molecular subtypes in a population of the coffee-growing region in Colombia. In retrospect, samples of 377 patients with a diagnosis of infiltrating breast cancer were analyzed, between the years 2015 and 2018. The histological diagnoses included: NOS infiltrating ductal carcinomas (339 cases; 89.9 %), infiltrating lobular (23 cases; 6.1%), infiltrating mucinous (6 cases; 1.5%), infiltrating papillary (1 case; 0.2%) and mixed patterns: ductal - lobular (3 cases; 0.7%) and ductal with mucinous component (5 cases; 1.3%), of which 56.2% (212 cases) correspond to luminal B, 22.2% (84 cases) to Luminal A, 14.8% (56 cases) to triple negative and 6.6% (25 cases) to HER2. These findings contrast with the prevalence reported worldwide. Therefore, in the population of the Colombian coffee-growing region, this predominance of the luminal pattern B should be considered when establishing prognosis and treatment from medical staff.

Keywords: Breast Cancer, Immunohistochemistry, Prognosis.

Resumen

El cáncer de mama es el cáncer más frecuente en las mujeres y constituye un problema de salud pública debido a sus altas tasas de morbimortalidad. El diagnóstico se hace a través de estudios rutinarios de histopatología que se complementan con estudios de expresión de receptores hormonales (Receptores de Estrógenos y Progesterona), del factor de crecimiento epidérmico humano 2 (HER2) e índice de proliferación celular (Ki67) para su subtipificación en grupos moleculares (Luminal A, Luminal B, Her2 y triple negativo), los cuales tienen implicaciones pronósticas y terapéuticas diferentes. Globalmente, se ha reportado una alta prevalencia del subtipo Luminal A, predominantemente en Norteamérica, Europa y algunos países latinoamericanos, sin embargo, los reportes en la población colombiana son heterogéneos. El objetivo de esta investigación es establecer un perfil de incidencia de dichos subtipos moleculares en una población del eje cafetero en Colombia. Retrospectivamente se analizaron muestras de 377 pacientes con diagnóstico de cáncer de mama infiltrante, entre los años 2015 a 2018. Los diagnósticos histológicos incluyeron: Carcinomas Ductal infiltrante NOS (339 casos; 89.9 %), lobulillar infiltrante (23 casos; 6.1 %), mucinoso infiltrante (6 casos; 1.5

%), papilar infiltrante (1 caso; 0.2 %) y patrones mixtos: ductal-lobulillar (3 casos; 0.7 %) y ductal con componente mucinoso (5 casos; 1.3 %), de los cuales 56.2 % (212 casos) corresponden a luminal B, 22.2 % (84 casos) a Luminal A, 14.8 % (56 casos) a triple negativo y 6.6 % (25 casos) a HER2. Estos hallazgos contrastan con la prevalencia reportada a nivel mundial. Por lo tanto, en la población del eje cafetero colombiano, se debe considerar este predominio del patrón luminal B al momento de establecer pronóstico y tratamiento por parte del personal médico tratante.

Palabras clave: Cáncer de mama, inmunohistoquímica, pronóstico.

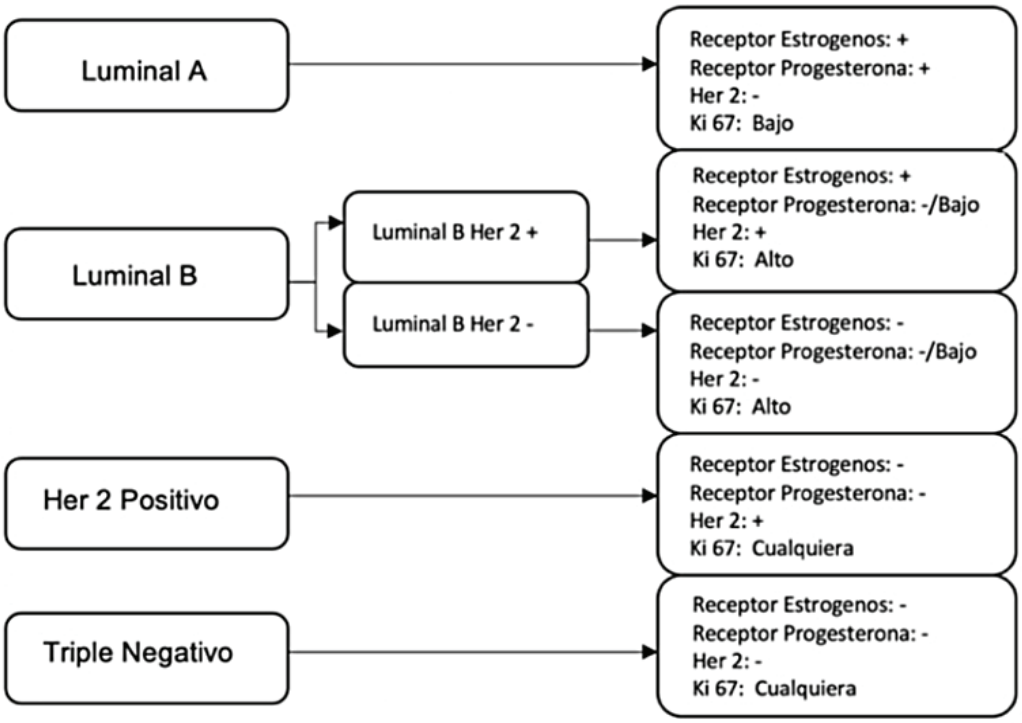
Introduction

Breast carcinoma is the most frequent cancer in the female sex, regardless of race or ethnic group (1) and leads cancer deaths in Latin America (2). Annually, about 1.2 million new cases and approximately 500,000 deaths due to this entity are reported globally. The latest analysis of the Department of Noncommunicable Diseases (NCD) of the Ministry of Health and Social Protection of Colombia reports an increase in the number of cases of breast cancer in the country and more than 2,500 deaths due to this cause (3). Therefore, this pathology is currently considered a public health problem.

At the molecular level, breast carcinoma is heterogeneous, with variable immunophenotypic patterns that in turn lead to differences in tumor behavior and prognosis. Regarding infiltrating breast carcinoma (IBC), the most frequently used molecular sub-classification, due to its easy implementation and worldwide accessibility, is based on the tumor cell expression pattern of estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor 2 (HER2) and the cell proliferation marker Ki67 markers, all of them are analyzed by immunohistochemistry and/or in situ hybridization in the case of HER2, the latter in case of an equivocal pattern in the immunohistochemistry. Figure 1 shows this classification.

«Breast carcinoma is the most frequent cancer in the female sex, regardless of race or ethnic group and leads cancer deaths in Latin America.

Figure 1. Molecular classification based on immunohistochemistry studies.



Regarding the prognosis of these molecular groups, the luminal ones present better rates of disease-free survival, total survival, and distant metastasis-free survival. However, the luminal B subtype performs worse than luminal A with statistically significant lower survival rates (4, 5). The triple negative subtype has been characterized as having the worst performance of all, with high risk of recurrence and early death due to disease, as well as poor response to treatment. The non-luminal HER-2 positive subtype, despite having a bad biological behavior, has targeted pharmacological treatment, which provides patients with relatively optimal management options that translate into better survival as well as a higher percentage of complete pathological response, in comparison with the triple negative subtype (Glück et al. 2013).

Most literature reports from North America and Europe consistently indicate a higher prevalence of the Luminal A subtype with approximate ranges between 40 and 60% (6,7). The distribution of the rest of the subtypes is heterogeneous among these populations. Latin American studies in Mexico, Peru, Argentina, Venezuela, and Cuba show a similar trend (8, 9, 10, 11, 12) and studies in the Colombian population show a variable distribution among the different groups; although the luminal subtypes continue to predominate,

some studies are consistent with world reports (prevalence of Luminal A), while others indicate a predominance of luminal B subtype.

The National Institute of Cancerology, a national reference center, along with the Hospital Universitario del Caribe, conducted a study led by Serrano and collaborators (13), in which 301 patients with a diagnosis of IBC were included, of which 37.2 % corresponded to subtype Luminal B, while 26.2 % corresponded to Luminal A. On the other hand, in Antioquia, Bonilla-Sepúlveda et al (14) analyzed a cohort of 114 patients, finding a predominance of Luminal A subtype with 38.5% followed by Luminal B patients with 32.4% of the population studied; in parallel, Gómez et al (15) analyzed a group of 328 patients, also from Antioquia, of which more than half corresponded to the Luminal B group. More recently, Jaramillo (16) analyzed a Magdalena population of 162 patients, finding an equal distribution between Luminal A and B groups (34.5% each).

Given the importance of molecular subtyping due to the prognostic and therapeutic impact it has on individuals affected by IBC, together with the heterogeneity in the different reports at the national level, it was decided to conduct a retrospective study in a Colombian population of the coffee-growing region, in order to establish a prevalence profile of the different molecular subtypes of IBC, determined through immunohistochemistry and in situ hybridization techniques (for HER2, when required).

1. Materials and Methods

The present work corresponds to a cross-sectional descriptive retrospective study, in which patients with histological diagnosis of IBC between the years 2015 to 2018 were included. The diagnosis was performed in a pathology laboratory that processes specimens from the Colombian coffee-growing region and the Caldense Institute of Pathology, located in the city of Manizales, Colombia. The inclusion criteria of the patients were:

- **i.** Availability of personal data (year of diagnosis and age) Infiltrating breast carcinoma
- **ii.** Diagnosis between 2015 and 2018
- **iii.** Complete immunohistochemistry and/or in situ hybridization (ISH) markers study.

The patient selection flowchart is shown in **Figure 2.**

Figure 2. Flow chart of inclusion and exclusion of patients in the study

INCLUDED PATIENTS: 377			CASES PER YEAR				GENDER	
			2015	2016	2017	2018	FEMALE	MALE
			176 (46,68%)	115 30,50%)	38 (10,07%)	48 (12,73%)	377 (100%)	0 (0%)
AGE GROUP								
10-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100
0 (0%)	7 (1,85%)	23 (6,10%)	67 (17,77%)	112 (29,70%)	103 (27,32%)	48 (12,73%)	15 (3,9%)	2 (0,53%)

Routine histopathological diagnosis of the samples evaluated was performed on biopsies or surgical specimens from the laboratory archive and took into account ASCO/CAP (American Society of Clinical Oncologists/ College of American Pathologists) guidelines and World Health Organization diagnostic criteria. Histopathologic slides with Hematoxylin and Eosin staining were again reviewed by two expert pathologists for the purpose of this work. ER, PR, HER2 and cell proliferation studies with KI67 were performed using immunohistochemistry technique. Hormone receptor (ER/ PR) positivity was determined taking into account the criteria established by the ASCO/CAP guidelines according to which a reactivity of 1% or more is considered positive. To establish the presence or not of HER2 overexpression, the clinical practice guidelines of the CAP and the American Association of Clinical Oncology (17) were considered, where a strong and complete membranous positivity in 10% or more of tumor cells is considered positive; in case of obtaining an equivocal overexpression, a fluorescence in situ hybridization (FISH) study was performed. The cell proliferation index, studied with the KI67 marker, was determined taking into account the guidelines of the International Working Group on Ki67 in breast cancer (18), which establishes that proliferation less than or equal to 14% corresponds to a low index, and greater than 14% to a high proliferation index. All immunohistochemistry studies were performed by automated technique, using the BenchMark GX equipment of the Roche Diagnostics laboratory with the marker's estrogen receptors (clone SP1), progesterone receptors (clone 1E2), Ki67 (clone MIB-1) and HER 2 (clone PATHWAY HER2 4B5). Finally, each patient was assigned a molecular group by immunohistochemistry technique.

The variables studied for each individual were:

1. Age at diagnosis,
2. Gender,
3. Histological diagnosis,
4. Year of diagnosis, and
5. Molecular classification by immunohistochemistry.

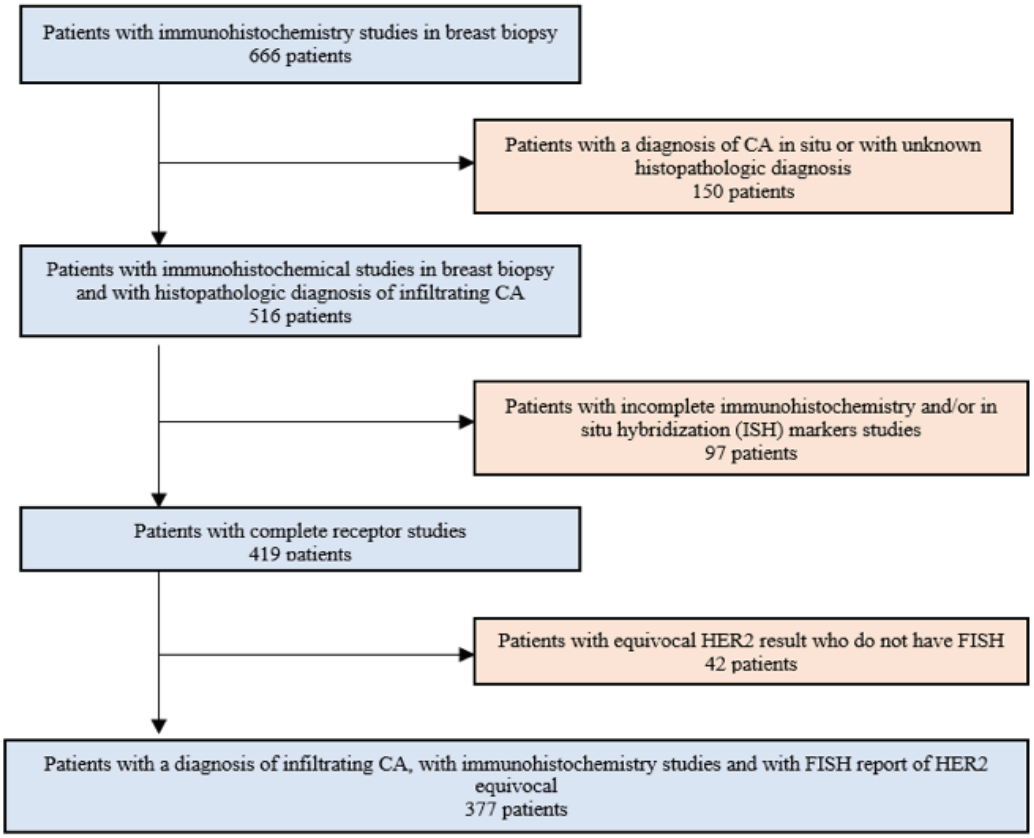
After collection, the information was filtered and entered into a protected database for subsequent storage and analysis. For each parameter, the average of each subgroup was identified.

Names, hospital records or any information that violates the privacy of the patients are not published in this work. The research was presented to the bioethics committee of the Universidad de Manizales, which endorsed the work. The histopathological studies performed, as well as the immunohistochemistry and FISH technique, correspond to routine procedures.

Results

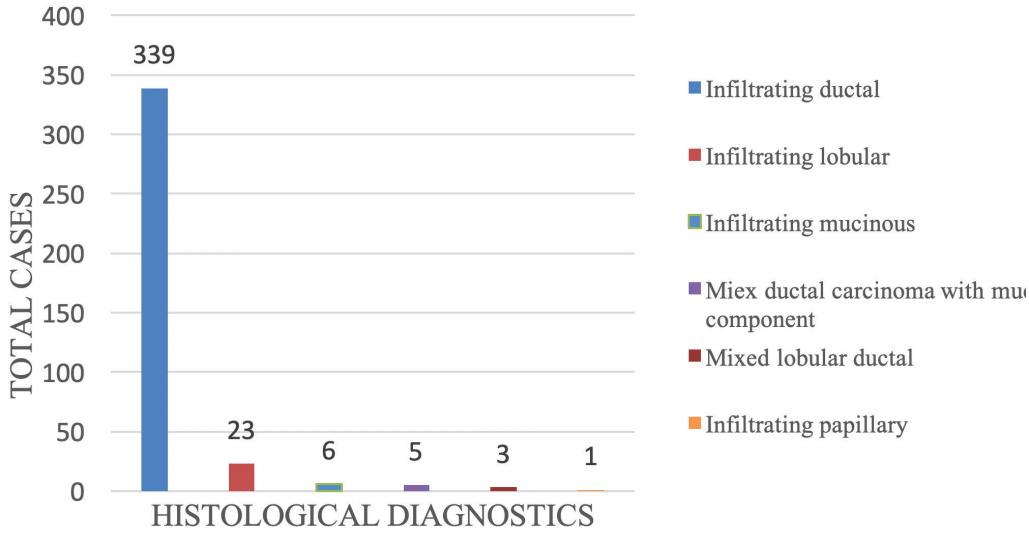
The data related to age, cases diagnosed by year, gender and age group are shown in **Figure 3**.

Figure 3. Distribution of cases according to year of diagnosis, a ge range and gender.



The diagnoses found were the following: Infiltrating ductal carcinoma, NOS (339 cases; 89.9 %), infiltrating lobular carcinoma (23 cases; 6.1 %), infiltrating mucinous carcinoma (6 cases; 1.5 %), infiltrating papillary carcinoma (1 case; 0.2 %) and mixed patterns: lobular ductal (3 cases; 0.7 %) and ductal carcinoma with mucinous component (5 cases; 1.3 %) as shown in Fig 4.

Figure 4. Total cases by histological diagnosis.



The percentage of receptors obtained in the samples were as follows: Estrogen receptor positive 296 cases (78.5 %), Estrogen receptor negative 81 cases (21.5 %), Progesterone receptor positive 244 cases (64.7 %), Progesterone receptor negative 133 cases (35.3 %). The percentage of HER2 receptor obtained in the samples were as follows: 0 (138 cases; 36.6 %), 1+ (157 cases; 41.6 %), 2+ (18 cases; 4.7 %), 3+ (63 cases; 16.9 %), and 3+ (63 cases; 16.9 %)).

Of the cases that initially presented HER-2 error, which were subsequently analyzed by FISH, 16 cases were negative (86.8%), and 2 cases were positive (13.2%). This brings the total number of cases that are HER2 positive to a total of 65 cases (17.5 %) and includes both estrogen receptor positive (40 cases) and estrogen receptor negative (25 cases). HER2 negative in total are 311 cases (82.5 %), including all 0, 1+ and 2+ that were negative for FISH. Ki67 proliferation index showed the following results: Ki67 equal to or less than 14 % are 107 cases (28.3 %). Ki67 greater than 14 % is 270 cases (71.7 %). This statistic also includes all HER2 positive and triple negative cases.

The molecular subtypes defined by immunohistochemistry technique were distributed as follows **(see figure 5)**:

Luminal B (212 cases; 56.2 %).

Luminal A (84 cases; 22.3 %)

HER2+ (25 cases; 6.6 %).

Triple negative (56 cases; 14.9 %)

Figure 5: Example of a case of Luminal B molecular subtype by immunohistochemistry. immunohistochemistry. HE= Hematoxylin and Eosin, at 200x. KI67= cell proliferation index. ER= Estrogen receptors. HER2= epidermal epithelial growth factor 2.

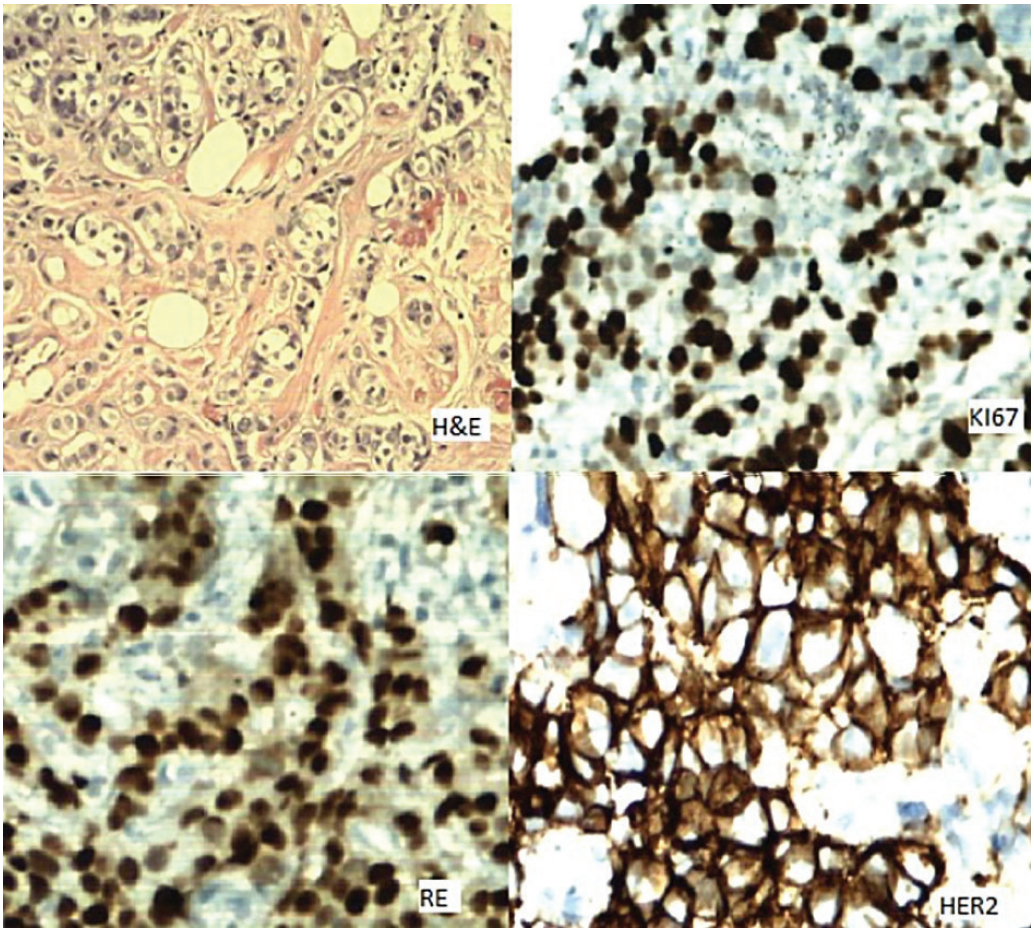
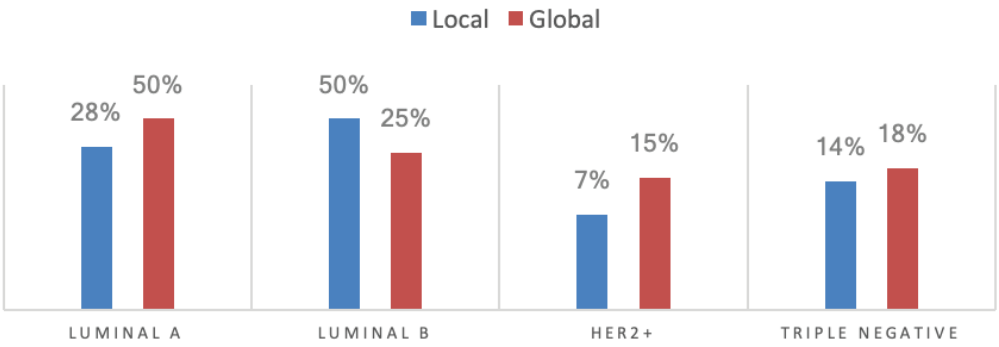


Figure 6. shows the distribution of molecular subgroups by immunohistochemistry technique in the studied population compared to the worldwide distribution.

Figure 6. Local Vs global incidence of breast cancer subtypes.



Discussion

Breast cancer is the most common cancer in women, regardless of race or ethnicity. It is one of the leading causes of cancer death worldwide, predominantly among Latinas and women of African descent. In order to determine which patients are at high risk of dying from the disease, as well as of recurrence, relapse and/or distant metastasis, some histopathology and molecular analysis tools have become available that allow tumor subtyping at the molecular level.

The most widely studied and applied molecular classification worldwide, thanks to its ease of implementation and availability, especially in developing countries, is molecular subclassification by immunohistochemistry. This classification uses the expression or not of estrogen and progesterone hormone receptors by tumor cells, as well as the overexpression or not of epidermal growth factor (HER2) and the cell proliferation index with the ki67 marker. All these parameters are routinely analyzed in anatomic pathology laboratories using immunohistochemistry and, in case of an erroneous result for HER2, further analysis is required using in situ hybridization techniques (FISH/CISH). Finally, patients will be assigned to a molecular group depending on the expression profile found (see Figure 1). However, this classification by immunohistochemistry has some limitations such as the inter-observer variability of some markers such as ki67 expression.

This molecular subtyping, together with other parameters such as tumor size and lymph node involvement (pathological T and N), allows the treating physicians to know the prognosis, including the probability of recurrence, overall survival time and disease-free survival. Thus, a personalized and targeted therapy can be planned for each individual patient.

In the general population, as shown in Figure 6, the predominant molecu-

lar subtype is Luminal A, characterized by a better prognosis and response to treatment in general. In Colombia, reports are heterogeneous and, although they favor predominance of luminal subgroups, some studies indicate a majority of luminal A subtype and others of Luminal B subtype. The luminal B subtype has the worst prognosis among luminal subtypes, not only with a worse biological behavior, but also a poor response to treatment compared to luminal A subtype.

Our study is the first reported in the Colombian coffee-growing region population. After applying the previously mentioned inclusion criteria, a cohort of 377 patients diagnosed with IBC between the years 2015 to 2018 in an anatomic pathology referral center located in the Colombian coffee-growing region was analyzed. Demographic parameters such as age and gender were identified, as well as histopathological characteristics such as tumor histological subtype and molecular sub-classification. One hundred percent of the patients who met the inclusion criteria were women, more than a quarter of the patients were between 51 and 60 years of age, approximately 90 % obtained a histologic diagnosis of non-special type infiltrating ductal IBC (NOS) and half were classified as Luminal B. This implies that, compared to most of patients reported in world and national literature reporting higher prevalence of Luminal A subtype patients (6,7), our patients will probably have worse prognosis at the expense of statistically lower survival (overall and disease-free) (4,5) and, therefore, should be subjected to a strict and personalized follow-up and treatment regimen.

Future studies should include the identification of molecular expression patterns according to race and ancestry, perform studies only in patients who have the molecular studies in the initial biopsy, as well as study separately patients who underwent neoadjuvant vs. those who did not undergo neoadjuvant treatment, in order to identify or rule out clonal selection by treatment, analyze patients diagnosed in other pathology diagnostic centers and complement molecular studies based on immunohistochemistry with complementary studies based on genomic and transcriptomic data.

«Our study is the first reported in the Colombian coffee-growing region population. »

In conclusion, our study is the first reported in the literature in patients from the Colombian coffee-growing region, in which we identified a prevalence of luminal subtype B by immunohistochemistry among patients affected by IBC. These findings contrast with those reported worldwide, which have consistently reported a prevalence of luminal subtype A. This should be considered by the treating medical staff, not only to carry out appropriate and personalized treatment plans, but also to implement stronger preventive measures, according to the population.

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