ORIGINAL ARTICLE
DOI: 10.29289/2594539420190000415

IMMUNOHISTOCHEMICAL PROFILE OF BREAST CANCER SUBTYPES IN PATIENTS SEEN AT NAPOLEÃO LAUREANO HOSPITAL, PARAÍBA, BRAZIL

Perfil dos subtipos de câncer de mama baseado no estudo imuno-histoquímico em pacientes do Hospital Napoleão Laureano – Paraíba

Jader Bruno Formiga Pinheiro[1*], Adriana de Freitas Torres[2], Alexandre Rolim da Paz[3]

ABSTRACT

Objective: To determine the profile of molecular subtypes of invasive breast carcinomas among women who underwent immunohistochemical study from May 2013 to December 2014, at Hospital Napoleon Laureano, Paraiba, Brazil, to characterize the mean age at diagnosis and describe the percentage of the following variables: estrogen and progesterone receptors, human epidermal growth factor 2 and proliferation index (Ki-67). **Method:** retrospective ecological study using the secondary databases at Hospital Napoleon Laureano Pathology Laboratory. The population consisted of 683 cases of invasive breast carcinoma with immunohistochemical study in this institution between May 2013 and December 2014. **Results:** Of the 683 patients, 46 were excluded because they presented inconclusive results for human epidermal growth factor 2 (++), totaling 637 recorded cases. Five hundred and fifty-six (87.28%) were 40 or older, and 81 (12.72%) under 40 years old. As for estrogen and progesterone receptor, 452 patients (70.96%) were positive for both receptors, while 185 (29.04%) showed no positivity. Four hundred and sixty-eight women (73.47%) did not show overexpressed human epidermal growth factor 2, while 169 (26.53%) did. The percentage of Ki-67 showed 474 individuals (74.41%) with a high proliferation index and 163 (25.59%) with a low index. The molecular subtypes showed the following prevalence: luminal A (143 cases; 22.45%), luminal B (250 cases; 39.25%), luminal B-enriched (113 cases; 17.84%), HER2 (57 cases; 8.95%) and triple-negative (74 cases; 11.62%). **Conclusion:** This study supported the notion of regional differences in the profile of breast tumors, since it showed a greater prevalence of triple-positive carcinomas and lower frequency of triple-negative tumors compared to studies of other Brazilian regions.

KEYWORDS: breast cancer; carcinoma, ductal; immunohistochemistry.

RESUMO

Objetivo: Determinar o perfil dos subtipos moleculares dos carcinomas invasivos de mama entre mulheres que realizaram o estudo imuno-histoquímico de maio 2013 a dezembro de 2014, no Hospital Napoleão Laureano, Paraíba, além de caracterizar a idade média ao diagnóstico e descrever os percentuais das seguintes variáveis: receptor de estrogênio e de progesterona, fator de crescimento epidérmico humano do tipo 2 e índice mitótico (Ki-67). **Método:** Estudo retrospectivo, ecológico, a partir da base de dados secundários do Laboratório de Anatomia Patológica do Hospital Napoleão Laureano. A população foi composta de 683 casos de carcinoma invasivo da mama, com estudo imuno-histoquímico realizado nessa instituição entre maio de 2013 e dezembro de 2014. **Resultados:** Dos 683 pacientes, foram excluídos 46 por apresentarem positividade para fator de crescimento epidérmico humano do tipo 2 inconclusiva (++), totalizando 637 casos contabilizados. Quinhentas e cinqüenta e seis pacientes (87,28%) eram

Study conducted in Hospital Napoleão Laureano – João Pessoa (PB), Brazil.
[1]Universidade Federal da Paraíba – João Pessoa (PB), Brazil.
[2]Department of Obstetrics and Gynecology, Universidade Federal da Paraíba – João Pessoa (PB), Brazil.
[3]Department of Surgery, Universidade Federal da Paraíba – João Pessoa (PB), Brazil.
***Corresponding author:** jadermdpb@gmail.com
Conflicts of interest: none to declare.
Received on: 07/15/2018. **Accepted on:** 08/20/2018

≥40 anos e 81 (12,72%), <40 anos. Quanto ao estrogênio e à progesterona, 452 pacientes (70,96%) possuíam receptores positivos para ambos, enquanto 185 (29,04%) não apresentaram positividade. Quatrocentas e sessenta e oito mulheres (73,47%) não superexpressaram fator de crescimento epidérmico humano do tipo 2, em contrapartida 169 (26,53%) o fizeram. A porcentagem do Ki-67 evidenciou 474 indivíduos (74,41%) com alto índice mitótico e 163 (25,59%) com baixo índice. Os subtipos moleculares apresentaram as prevalências: luminal A (143 casos; 22,45%), luminal B (250 casos; 39,25%), luminal B amplificado (113 casos; 17,84%), fator de crescimento epidérmico humano 2 (57 casos; 8,95%) e triplo negativo (74 casos; 11,62%). **Conclusões:** Este artigo ratificou a existência de diferenças regionais quanto ao perfil dos subtipos de tumores mamários, demonstrando maior prevalência de carcinomas triplo-positivos e menor frequência de tumores triplo-negativos quando comparado a outros estudos.

PALAVRAS-CHAVE: câncer de mama; carcinoma ductal; imuno-histoquímica.

INTRODUCTION

Breast cancer is the second most common type of cancer among women and has high morbidity and mortality. About 1.67 million new cases of this neoplasm were expected in the year 2012, worldwide[1]. For Brazil, in 2016, 57,960 new cases of breast cancer were estimated, with a risk of 56.20 cases per 100,000 women[2]. Classically, the prognosis and treatment of breast cancer are determined by clinical and pathological variables, such as tumor size, histological grade, nuclear grade and lymph node status, together with immunohistochemical markers[3]; however, tumors showing the same pathological characteristics may have different behaviors depending on its molecular biology[4].

Molecular evaluation by DNA microarray technique has allowed the classification of tumors into subgroups[5]. Subgroups show similarities and differences in gene expression, growth, cell composition, prognosis and therapeutic sensitivity. These tests are highly costly and complex, making it difficult to use regularly. A classification based on immunohistochemical markers, with similar but not identical criteria, is however feasible[6].

The expression of estrogen (ER) and progesterone (PR) receptors and increased human epidermal growth factor receptor 2 (HER2) and proliferation index (Ki-67) are the immunohistochemical parameters used in this alternative classification[7]. Analogous to molecular classification, tumors are divided into luminal A, luminal B HER2-negative, luminal B HER2-positive, HER2-overexpressed and triple-negative[8].

Luminal tumors have been associated with a more favorable prognosis, whereas triple-negative and HER2-overexpressed have a more guarded prognosis[9].

Overexpressed HER2 is associated with increased cell proliferation, angiogenesis, tumor invasion, high nuclear grade, and a greater likelihood of multifocal and multicentric involvement[10]. Triple-negative tumors also show greater aggression and are generally found in premenopausal women with histological grade II or III, in addition to having a greater tropism for solid organs[11].

The genomic atlas of the disease has emphasized its heterogeneity and has suggested that genetic studies can be potentially informative in treatment decisions[12], such as the use of aromatase inhibitors (in the subtypes with positive hormone receptors), reducing the need for axillary emptying, and optimal duration of the use of transtuzumab in the HER2-overexpressed subtype[13]. These data show the individualized character of the treatment based on immunohistochemical profile.

Carvalho et al., in a retrospective study, determined that the distribution of molecular subtypes of breast cancer differs between regions of Brazil. These authors point out that knowledge of the possible differences, regarding the immunohistochemical profile and its frequencies in certain geographic locations, in a large and ethnically complex country like Brazil, is beneficial for the understanding of the mechanisms involved in different molecular subtypes, besides the development of strategies for the treatment and prevention of breast cancer[14].

Such arguments strengthen the need for greater knowledge of the molecular profile of breast cancer by state or microregion. In Paraíba, there are still no studies focused on this aspect of breast oncology. The present study aimed to determine the profile of the subtypes of invasive breast carcinoma among women who underwent an immunohistochemical study, from May 2013 to December 2014, at the Laboratory of Pathological Anatomy of Hospital Napoleão Laureano (HNL), João Pessoa, Paraíba.

METHOD

We conducted a cross-sectional study based on secondary databases of HNL. Convenience sampling was used, where we included all cases of invasive breast carcinoma, with an immunohistochemical study performed at the Laboratory of Pathological Anatomy of HNL, during the period from May 2013 to December 2014. Exclusion criteria were incomplete immunohistochemical panel and immunohistochemical analysis performed on a secondary tumor.

The variables studied were age at diagnosis, percentage of positivity for ER (clone SP1), PR (clone 1E2), HER2 oncoprotein (clone 4B5) and Ki-67 proliferation index (clone 30-9).

The paraffin blocks of the patients studied were submitted to histological sectioning at 3.0-µm thickness for automated immunohistochemical study (Ventana Benchmark GX, Roche Diagnostics) and detection by means of the multimers system (Ventana ultraView Universal DAB Detection Kit, Roche Diagnostics). Positive and negative controls confirmed the method's reliability.

ER and PR were considered positive with more than 1% staining of tumor cells[15]. HER2 was recorded as positive with a 3+ score and negative if 0+ or 1+ [16]. Ki-67 index was determined to be low when less than 14 and high when greater than that value[8].

Tumors were classified according to the consensus recommendation of St. Gallen 2011[8], as luminal A (ER- and/or PR-positive, HER2-negative, Ki-67 low); luminal B HER2-negative (ER- and/or PR-positive, HER2-negative and Ki-67 high); luminal B HER2-positive or luminal B-enriched (ER- and/or PR-positive, HER2-positive and any Ki-67); and HER2-overexpressed (HER2-positive, ER- and PR-negative) and triple-negative (ER--and PR-negative, HER2-negative).

The data were tabulated and analyzed in the program EpiInfo™ version 7. The distribution of the absolute and relative frequencies was used to analyze the data. Measurements of association between the variables studied were determined using the $\chi 2$ test or Fisher's exact test, at a 5% significance level.

We observed and complied with the norms of Resolution No. 466/2012 of the National Health Council (CNS) at all stages of the study, which was approved by the Ethics Committee of the Federal University of Paraíba (UFPB) through process No. 1,376,053. The present study was funded by the researchers themselves, thus not presenting a conflict of interest.

RESULTS

A total of 683 immunohistochemical tests for breast cancer were analyzed, where 42 panels were excluded because they showed inconclusive positivity for HER2 (++), leaving 637 cases to be counted in the statistical calculations.

The age of the patients at diagnosis ranged from 24 to 97 years, with a mean of 53.3 years: 556 (87.3%) aged ≥40 years and 81 (12.7%) aged <40 years (Table 1).

Regarding ER, 452 tumors (71%) had positive receptors, while 185 (29%) displayed no positivity. In addition, 468 tumors (73.5%) were not HER2-overexpressed, while 169 (26.5%) were (Table 1).

According to the immunohistochemical markers, the tumors were classified as: luminal A, 143 cases (22.5%); luminal B; 250 cases (39.3%); luminal B-enriched, 113 cases (17.7%); HER2, 57 cases (8.9%); and triple-negative, 74 cases (11.6%). The percentage of Ki-67, on the other hand, showed that 474 tumors (74.4%) had a high proliferation index and 163 (25.6%), low proliferation index (Table 1).

Regarding the correlations with age, we found that that in patients younger than 40 years, tumors were ER-positive in 64.2% of cases, for HER2-positive in 34.6% of cases and high Ki-67 high in 90.1% of cases (Table 2). In the group of patients 40 and older, tumors were ER-positive in 71.9% of cases, HER2-positive in 25.4% of cases and Ki-67 high in 72.1% of cases ($p < 0.05$).

The distribution of molecular subtypes differed between the age groups. In the patients under 40 years of age, at diagnosis,

Table 1. General characteristics of the sample (n=637).

Variable	n	%
Age range		
Less than 40 years	81	12.7
40 years or older	556	87.3
Estrogen receptor		
Positive	452	71
Negative	185	29
HER2		
Positive	169	26.5
Negative	468	73.5
Ki-67		
Low	163	25.6
High	474	74.4
Subtype		
Luminal A	143	22.5
Luminal B	250	39.3
Luminal B-enriched	113	17.7
HER2	57	8.9
Triple-negative	74	11.6

HER2: human epidermal growth factor receptor 2; Ki-67: proliferation index.

Table 2. Distribution according to age group.

Variable	Age <40 (n=81) n (%)	Age ≥40 (n=556) n (%)	p
Estrogen receptor			
Positive	52(64.2)	400(71.9)	0.15
Negative	29(35.8)	156(28.1)	
HER2			
Positive	28(34.6)	141(25.4)	0.08
Negative	53(65.4)	415(74.6)	
Ki-67			
Low	8(9.9)	155(27.9)	0.0003
High	73(90.1)	401(72.1)	
Subtype			
Luminal A	7(8.6)	136(24.5)	0.0009
Luminal B	35(43.2)	215(38.7)	0.46
Luminal B-enriched	20(24.7)	93(16.7)	0.08
HER2	8(9.9)	49(8.8)	0.6
Triple-negative	11(13.6)	63(11.3)	0.5

HER2: human epidermal growth factor receptor 2; Ki-67: proliferation index.

the luminal B (43.2%) and luminal B-enriched (24.7%) molecular subtypes predominated. On the other hand, in patients 40 and older, at diagnosis, the highest percentages were for the luminal B subtypes (38.7%) and luminal A (24.5%), with statistical significance in the latter (Table 2).

DISCUSSION

The literature indicates a higher incidence of breast cancer among women older than 50 years, with only 5 to 7% of breast cancer cases in female patients younger than 40 years of age[17]. Our study supported this notion, as it demonstrated a much higher percentage of breast cancer in women aged 40 years or older (87.3%) when compared to the lower age group (12.7%), but we could see a particularity, a percentage of cases among those under 40 years of age that was significantly higher than the average.

ER positivity was more prevalent in women 40 and older (71.9%) compared to the younger age group (64.2%). Clagnan et al. also concluded that patients older than 40 showed higher ER positivity compared to those younger. The proportions found by these authors were: 72.3% (older than 50 years), 64.9% (between 40 and 50 years) and 58.7% (under 40 years)[18].

There was also a higher prevalence of HER2 positivity in younger women. Of the patients younger than 40 years, 28 (34.6%) were positive for this variable, while among those aged 40 and over, 141 (25.4%) overexpressed HER2. Dutra et al. also reached this conclusion from a study in which 236 patients were selected among pre- and postmenopausal women, which showed higher positivity for the HER2 protein in the premenopausal women (28.7 versus 16.9%; p = 0.03)[19].

These data confirmed a well-established finding in the literature that younger women have more advanced, higher-grade tumors with negative hormone receptor status, greater HER2 overexpression and lymphovascular invasion[20].

Carvalho et al. demonstrated the presence of regional differences in the profile of molecular subtypes of breast tumors. The Southeast and South regions of Brazil, with the highest proportion of inhabitants of European descent, showed the highest rates of luminal tumors. In the Central-West, there were higher triple-positive rates, while in the North, greater triple-negative and HER2-overexpressed rates. The Northeast, on the other hand, showed an intermediate frequency of the molecular subtypes[14].

Cintra et al. and Carvalho et al. revealed a predominance of the luminal B subtype with respect to luminal A, in line with the present study, which showed a prevalence of 39.3% for this subtype in contrast to luminal A (22.5%)[9,14]. Sarturi et al., however, found the opposite, a predominance of luminal subtype A over B[21] (Table 3).

We also saw a considerably larger percentage of luminal B-enriched in the present survey compared to the data referring to the Northeast region reported by Carvalho et al. and those presented by Cintra et al.[9,14]. The same could be observed for HER2 overexpression that we found (26.5%) compared to the latter study (16.8%) and that of Sarturi et al. (17.64%)[21]. The distribution of HER2 subtypes according to races and ethnicities is more difficult to analyze because many authors include luminal B-enriched in the HER2 group[22] (Table 3).

It is known, however, that the triple-negative subtype is more prevalent in black women[23], and that these patients have a higher proportion of aggressive tumors compared to Caucasian women. A study carried out in Nigeria and Senegal with 507 patients diagnosed with breast cancer showed a proportion of 27% for this subtype[24].

It would be expected, therefore, that the present study have a higher percentage of triple-negative tumors, since the northeastern state of Paraíba has a black population of 66.8%, notably higher compared to the Southeast (43.8%) and South (22.8%)[25], in which studies by the groups of Cintra and Sarturi[9,21] were performed (Table 3).

Regarding Ki-67, we found a higher percentage of this variable in patients under 40 years old (90.1%), contrasting with the 72.1% of patients above this age. This shows a greater aggressiveness of breast tumors in younger women, resulting in a worse prognosis[26].

CONCLUSION

We conclude, therefore, that there is regional difference regarding the profile of the subtypes of breast tumors. HNL is the state's referral center for cancer treatment. Thus, a sample taken from this place reliably expresses the profile of the Paraíba cancer patient.

The present study determined a greater prevalence of tumors of the luminal B-enriched subtype and a lower triple-negative frequency compared to similar studies performed in other

Table 3. Comparison of studies.

Variable	Authors			
	Carvalho et al.[14]	Sarturi et al.[21]	Cintra et al.[12]	Present study
Mean age	55.5	53.7	57.4	53.3
ER positivity (%)	80	71.4	–	71
HER2 positivity (%)	21.6	17.6	16.8	26.5
Subtype distribution (%)				
Luminal A	24.1	62.1	17.1	22.5
Luminal B	37.1	9.2	41.8	39.3
Luminal B-enriched	10.7	–	10.8	17.7
HER2	10.5	8.4	6	8.9
Triple-negative	17.4	20.1	24.2	11.6

ER: estrogen receptor; HER2: human epidermal growth factor receptor 2.

Brazilian regions. With a better understanding of this profile and greater epidemiological knowledge of breast cancer, more effective treatment strategies in confronting this disease can be developed in Paraíba.

ACKNOWLEDGMENTS

We thank the entire team of the Laboratory of Pathological Anatomy of Hospital Napoleão Laureano, for opening the doors of their service to allow us to conduct this study.

REFERENCES

1. Ferlay J, SoerjomataramI, 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:E359-86.
2. Instituto Nacional de Câncer. Coordenação de Prevenção e Vigilância. Programa Nacional de Controle do Câncer de Mama. Estimativa 2016: incidência de câncer no Brasil. Rio de Janeiro: INCA; 2015. 122p..
3. Dai X, Li T, Bai Z, Yang Y, Liu X, Zhan J, et al. Breast cancer intrinsic subtype classification, clinical use and future trends. Am J Cancer Res. 2015;5(10):2929-43.
4. Serra KP, Ramalho S, Torresan R, Vassallo J, Sarian LOZ, Silva GRP, et al. Nova classificação dos carcinomas da mama: procurando o luminal A. Rev Bras Ginecol Obstet. 2014; 36(12):575-80. http://dx.doi.org/10.1590/SO100-720320140005158
5. Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, et al. Molecular portraits of human breast tumours. Nature. 2000;406(6797):747-52. https://doi.org/10.1038/35021093
6. Barros ACSD, Leite KRM. Classificação molecular dos carcinomas de mama. Uma visão contemporânea. Rev Bras Mastologia. 2015;25(4):146-55. http://dx.doi.org/10.1590/S0104-42302012000200013
7. Goldhirsch A, Winer EP, Coates AS, Gelber RD, Piccart-Gebhart M, Thürlimann B, et al. Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. Ann Oncol. 2013; 24(9):2206-23. https://doi.org/10.1093/annonc/mdt303
8. Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thürlimann 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. http://dx.doi.org/10.1590/S0104-42302012000200013
9. Cintra JRD, Teixeira MTB, Diniz RW, Gonçalves Junior H, Florentino TM, Freitas GF, et al. Perfil imuno-histoquímico e variáveis clinicopatológicas no câncer de mama. Rev Assoc Med Bras. 2012; 58(2):178-87. http://dx.doi.org/10.1590/S0104-42302012000200013
10. Morrow M. Personalizing extent of breast cancer surgery according to molecular subtypes. Breast. 2013; 22(Supl. 2):S106-9. https://doi.org/10.1016/j.breast.2013.07.020
11. Chikarmane SA, Tirumani SH, Howard SA, Jagannathan JP, DiPiro PJ. Metastatic patterns of breast cancer subtypes: What radiologists should know in the era of personalized cancer medicine. Clin Radiol. 2015;70(1):1-10. https://doi.org/10.1016/j.crad.2014.08.015
12. Dunbier AK, Anderson H, Ghazoui Z, Salter J, Parker JS, Perou CM, et al. Association between breast cancer subtypes and response to neoadjuvant anastrozole. Steroids. 2011; 76(8):736-40. https://doi.org/10.1016/j.steroids.2011.02.025
13. Martelli G, Boracchi P, Ardoino I, Lozza L, Bohm S, Vetrella G, et al. Axillarydissection versus no axillary dissection in older patients with T1N0 breast cancer: 15-year results of a randomized controlled trial. Ann Surg. 2012;256(6):920-4. https://doi.org/10.1097/SLA.0b013e31827660a8
14. Carvalho FM, Bacchi LM, Pincerato KM, Van de Rijn M, Bacchi CE. Geographic differences in the distribution of molecular subtypes of breast cancer in Brazil. BMC Women's Health. 2014;14:102. https://doi.org/10.1186/1472-6874-14-102
15. Hammond ME, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. J Clin Oncol. 2010;28(16):2784-95. https://doi.org/10.1200/JCO.2009.25.6529
16. Wolff AC, Hammond ME, Hicks DG, Dowsett M, McShane LM, Allison KH, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. J Clin Oncol. 2013;31(31):3997-4013. https://doi.org/10.1200/JCO.2013.50.9984
17. Gabriel CA, Domchek SM. Breast cancer in young women. Breast Cancer Res. 2010;12:212. https://doi.org/10.1186/bcr2647
18. Clagnan WS, Andrade JM, Carrara HHA, Tiezzi DG, Reis FJC, Marana HRC, et al. Idade como fator independente de prognóstico no câncer de mama. Age as an independent prognostic factor in breast cancer. Rev Bras Ginecol Obstet. 2008;30(2):67-74. http://dx.doi.org/10.1590/S01007203200800020004
19. Dutra MC, Rezende MA, Andrade VP, Soares FA, Ribeiro MV, Paula EC, et al. Imunofenótipo e evolução de câncer de mama: comparação entre mulheres muito jovens e mulheres na pós-menopausa. Immunophenotype and evolution of breast carcinomas: a comparison between very young and postmenopausal women. Rev Bras Ginecol Obstet. 2009;31(2):54-60. http://dx.doi.org/10.1590/S0100-72032009000200002
20. Assi HA, Khoury KE, Dbouk H, Khalil LE, Mouhieddine TH, El Saghir NS. Epidemiology and prognosis of breast cancer in young women. J Thorac Dis. 2013;5:(Supl.1):S2-8. https://dx.doi.org/10.3978%2Fj.issn.2072-1439.2013.05
21. Sarturi PR, Cunha Júnior AD, Morais CF. Perfil imuno-histoquímico do câncer de mama de pacientes atendidas no Hospital do Câncer de Cascavel – Paraná. Rev Bras Oncol Clin. 2012;8(29):121-4.

22. Chuang E, Christos P, Flam A, McCarville K, Forst M, Shin S, et al. Breast cancer subtypes in Asian-Americans differ according to Asian ethnic group. J Immigr Minor Health. 2012;14(5):754-8. https://dx.doi.org/10.1007%2Fs10903-012-9577-7

23. Kurian AW, Fish K, Shema SJ, Clarke CA. Lifetime risks of specific breast cancer subtypes among women in four racial/ethnic groups. Breast Cancer Res. 2010;12(6):R99. https://dx.doi.org/10.1186%2Fbcr2780

24. Huo D, Ikpatt F, Khramtsov A, Dangou JM, Nanda R, Dignam J, et al. Population Differences in Breast Cancer: Survey in Indigenous African Women Reveals Over-Representation of Triple-Negative Breast Cancer. Clin Oncol. 27(27):4515-21. https://doi.org/10.1200/JCO.2008.19.6873

25. Instituto Brasileiro de Geografia e Estatística. Censo Demográfico 2010, [Internet]. Rio de Janeiro: Instituto Brasileiro de Geografia e Estatística; 2012 [acessado em jun. 2016]. Disponível em: http://www.ibge.gov.br/home/estatistica/populacao/censo2010

26. Keegan THM, DeRouen MC, Press DJ, Kurian AW, Clarke CA. Occurrence of breast cancer subtypes in adolescente and young adult women. Breast Cancer Res. 2012;14(2): R55. https://dx.doi.org/10.1186%2Fbcr3156