Journal of Medical and Health Studies (JMHS)
ISSN: 2710-1452
DOI: 10.32996/jmhs
Journal Homepage: www.al-kindipublisher.com/index.php/jmhs

Immunohistochemical Profile of Molecular Markers of Mammary Carcinomas in Libreville

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ARTICLE INFORMATION

Received: August 08, 2021
Accepted: September 14, 2021
Volume: 2
Issue: 2
DOI: 10.32996/jmhs.2021.2.2.6

KEYWORDS

Immunohistochemical profile; Breast carcinomas; Molecular markers; Hormonal receptors; HER2/Neu; Ki67

ABSTRACT

To establish the immunohistochemical profile of the molecular markers of free-ranging breast carcinomas. Descriptive retrospective study over 3 years from July 2014 to September 2017. The tumour samples came from the Anatomical Pathology Laboratories of the Omar Bongo Ondimba Army Instruction Hospital and the University of Health Sciences. In total, the records of 60 patients with histologically proven breast carcinoma with the immunohistochemical study were included in the study. The following molecular markers, hormone receptors (estrogen receptors, progesterone receptors), the HER2/Neu oncogene, and the Ki67 cell proliferation marker, was identified using the Immunohistochemistry technique.The average age was 47.6 years, with extremes of 15 and 69 years. Depending on the location, the right breast was most commonly affected (50%). Histologically, there was a predominance of infiltrating ductal carcinomas (66.6%) and a majority SBR III grade (50%). This study revealed an immunohistochemical profile of positive hormone receptors: ERs + (13.3%); PRs + (8.3%) and a Ki67 profile positive in 10% of tumors. The molecular classification into 4 subtypes (Luminal A, Luminal B, HER2 and Triple Negative) places the Luminal A group in the first rank (33.3%) followed by the Luminal B (15%), Triple Negative (11.6%) and HER2 (8.3%). The present work is the first study reporting the immunohistochemical profile of molecular markers of mammary carcinomas in Gabon. It would be necessary to continue this study on a larger and wider cohort throughout Gabon because the knowledge of the immunohistochemical profile gives an indication of the origin of breast cancers and allows to consider better management of patients by a targeted therapy to avoid unnecessary toxic effects resulting from ineffective treatment.

1. Introduction

Breast cancer is the most common female cancer in the world, with more than 14 million new cases per year (Mbalawa-Gombe et al., 2012). In industrialized countries, it is the most common cancer in women with an incidence that has been steadily increasing for 20 years (Mbalawa-Gombe et al., 2012). In France, it is the leading cause of death from cancer in women between 30 and 50 years old with 53000 new annual cases in 2011 (Bossard et al., 2011). In Africa, the number of new annual cases is estimated at more than 847000 (Collège Français des Pathologistes, 2013) including more than 2500 cases in Senegal (Contesso et al., 1998) or 11,000 cases in Morocco (Belkacémi et al., 2010). In Gabon, breast cancer is the first female cancer, with 52% of cases in uterine cancers, or 36% of cases (Belembaogo, 2019). Epidemiological and experimental studies around the world have revealed genetic, environmental and nutritional factors involved in the etiology of this cancer (Nkondjock & Ghadirian, 2005). The treatment of this pathology, which was based for years on surgery and the work of the pathologist, was limited to confirming the malignancy and analyzing the axillary lymph nodes. The evolution of medical knowledge in the field of cancer biology and the development of diagnostic and treatment techniques (hormonal therapy, for example) have led to considerable

changes in the management of breast cancer. The multifactorial origin of breast cancer has led to the search for prognostic factors in order to improve patient management. In addition, there is no breast cancer but breast cancer, depending on the biological characteristics of the cells from which cancer has developed and depending on the stage of evolution at the diagnosis, and therefore it became important to '' establish prognostic factors to optimize patient management. Thus, in addition to conventional histopathological prognostic factors (tumor size, histological type, cell proliferation rate, possible lymph node invasion, histological grade), an evaluation of hormone-dependent receptors is increasingly required in breast cancers. The presence of hormone receptors is systematically sought to allow hormone treatment. This evaluation is carried out using immunohistochemical techniques whose impact on therapy is obvious. Immunohistochemistry is a technique for locating and detecting proteins in cells of a tissue section by the detection of antigens by means of antibodies. It exploits the fact that an antibody specifically binds to antigens in biological tissues. A common technique in developed countries, it was not until 2014 that it was applied in the study of the cancer process in Gabon.

2. Literature Review

Gabon is thus part of the limited circle of sub-Saharan African countries with the immunohistochemistry technique. There is partial data on breast cancer in Gabon but no known study on the immunohistochemical profile of molecular markers of breast carcinomas. Thus, in view of the increase in cancerous pathology, the present study aims to initiate the configuration of a profile of immunohistochemical markers of mammary carcinomas from histological and immunological data.

3. Methodology

3.1 Ethical considerations

This study was reviewed and approved by Gabon's National Research Ethics Committee (NRC). No name has been written on the pre-established datasheet to ensure confidentiality and all records have been kept secure.

3.2 Places, type and period of study

This study was carried out at the Pathological Anatomy Laboratory of the University of Health Sciences, the oldest laboratory in Libreville, Gabon. It is a retrospective preliminary study with descriptive aims, relating to diagnoses established over three years going from July 2014 to September 2017.

3.3 Study population, inclusion and exclusion criteria

The study was carried out on 60 patients from the Anatomy Pathology Laboratories of the Omar Bongo Ondimba Army Instruction Hospital (HIAOBO) and the University of Health Sciences (USS) for 3 years, from July 2014 to September 2017. These were patients for whom immunohistochemistry was performed after a diagnosis of breast carcinoma. The material included all diagnostic files or reports of mammary carcinoma. The elements retained for each patient were: age, sex, side of the breast, histological diagnosis, antibodies used and immunohistochemical results. All records of patients with histologically proven breast carcinoma with immunohistochemical study were included in the study. However, breast carcinoma records without immunohistochemistry and incomplete records were excluded from the study.

3.4 Histopathological study

The samples (biopsies and excision pieces) were sent to the pathological anatomy laboratory, where they underwent the first treatment by the different stages of the classic histology technique (Hermanek et al., 1998). Briefly, the samples were fixed in 10% formalin, the fixing time was variable and the amount of fixative used was 10 times greater than the volume of tissue to be fixed. The fragments were then incubated in cassettes which underwent dehydration and then inclusion in paraffin. The inclusion was carried out using an automaton, called histokinette. The 3-5 µm sections were made with a rotary microtome. These bowls were spread out by unrolling them on the slide by flotation on the surface of a hot bath. The slides were placed in an oven at 70°C for 1 hour, then dipped in toluene to remove paraffin from the tissue section so that the dyes could penetrate the tissue. The slides were then passed through a running water bath to replace the alcohol with water. Hematoxylin and eosin were used for histological staining to differentiate all the elements of a tissue. The aim is to highlight the nuclei, the cytoplasm of the cells and the collagen fibers, the hematoxylin colors the nucleus in purple, the eosin colors the cytoplasm in pink and the saffron colors the collagen fibers in yellow. After staining, the glass slides were fixed to the tissue section using a mounting medium (toluene) to provide mechanical and chemical protection of the sections. The slides were examined under a microscope.

3.5 Immunohistochemical study

Immunohistochemistry is a technique combining immunology and histochemistry. It aims to highlight certain cellular protein (antigens) on a histological section, whether cytoplasmic, membrane or nuclear specific for a type or a cell function using an antigen-antibody reaction. The formed complex being made visible, therefore localizable, thanks to a colored marker (Marck, 2010). In short, the fine paraffinized sections were spread on pretreated blades (positively charged blade type *SuperFrostPlus*) and fixed in the oven for 1 to 2 hours at 56°C, paraffin melting temperature, then at 37°C overnight. The rest of the paraffin was

removed by passing the section through two toluene baths of 5 min each. Dehydration was carried out by three baths of 3 min in a toluene solution of decreasing concentration (100%, 90% and 70%). The unmasking of the sites was carried out using a microwave at 98 ° C. for 12 minutes in a Tris-EDTA buffer buffer solution (pH 9). Then the slides were cooled to room temperature. The surface of the sample to be analyzed was circumscribed using a latex pencil (Dakopen). Rinsing was done in a buffer solution for 1 min and then for 5 min. The endogenous peroxidases (TA 060-H202Q) were blocked by incubation with hydrogen peroxide (3%) in methanol for 10 min. The slides were incubated with the primary antibody for 30 minutes to 1 hour. The tissue sections were then incubated with the Power Block TM reagent (BioGenex, San Ramon, California, United States), a universal protein blocking reagent, for 15 minutes at room temperature to avoid any non-specific fixation, then incubated with specific antibodies overnight at 4°C. After three washes, the sections were incubated with a secondary antibody conjugated to horseradish peroxidase for 30 minutes at room temperature, then detected using 3,3'-diaminobenzidine (Sigma, USA). The sections were then treated with hematoxylin, dehydrated with alcohol, air-dried, mounted on DPX and visualized under a microscope.

3.6 Statistical analysis

The data collected was entered on an Excel file (Microsoft Office 2007), then imported on the stat view software (version 5.0) and the Statistical Package for Social Science software (SPSS) (version 10.1) for statistical analysis. The data were divided into qualitative and quantitative variables: the results of the qualitative variables (nominal) are given in numbers (n) and percentages (%); Quantitative variables were averaged; Statistical significance thresholds were considered for $p < 0.05$.

4. Results and Discussion

4.1 Results

4.1.1 Epidemiological aspects

From 2014 to 2017, 60 cases of breast carcinoma were diagnosed in the Pathological Anatomy laboratory with a peak in 2014 (27 cases). The mean age of the patients at the time of diagnosis was 47.6 years with extremes of 15 years and 69 years and a peak between 41 years and 50 years (Figure 1A). Of the 60 cases, 30 patients had a tumor in the right breast (50%), 20 patients in the left breast (33.3%) and 3 cases had the tumor in both breasts (5%). For 7 other patients, the topographic location of the breast was not specified (Figure 1B).

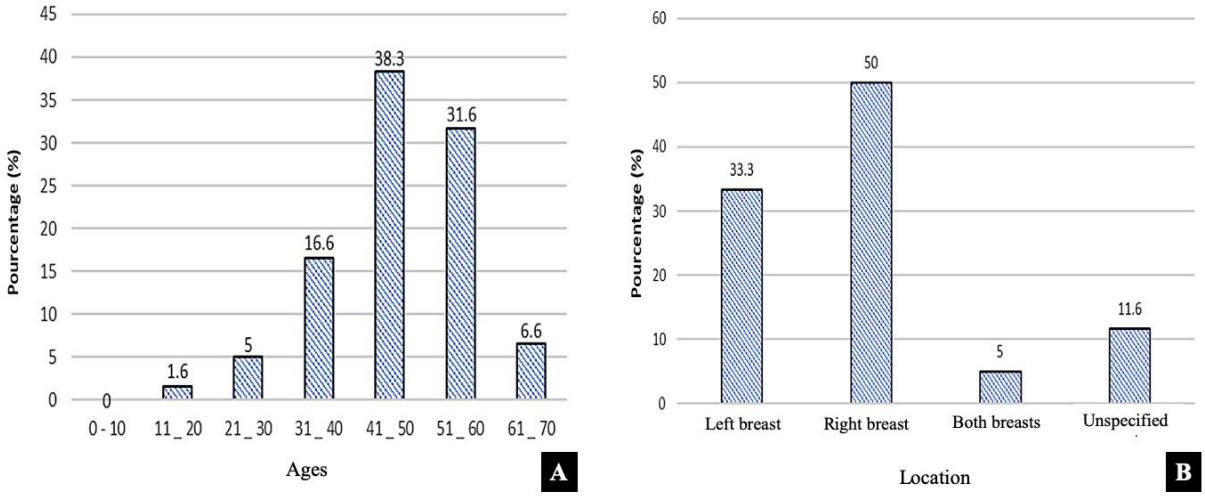

Figure 1: Epidemiological aspect

A: Distribution of breast carcinoma as a function of age, **B**: Location of the tumor

4.1.2 Histological aspects

Breast carcinomas have been identified and classified according to their locations by the classic histology method. Alone or in combination with another, invasive ductal carcinoma was the most frequent (66.6%) (Figure 2A). Of the 60 cases, 40 patients had an SBR grading recorded on the histological analysis results. SBR III tumors were in the majority (50%) followed by SBR II (10%) and SBR I (6.7%) (Figure 2B).

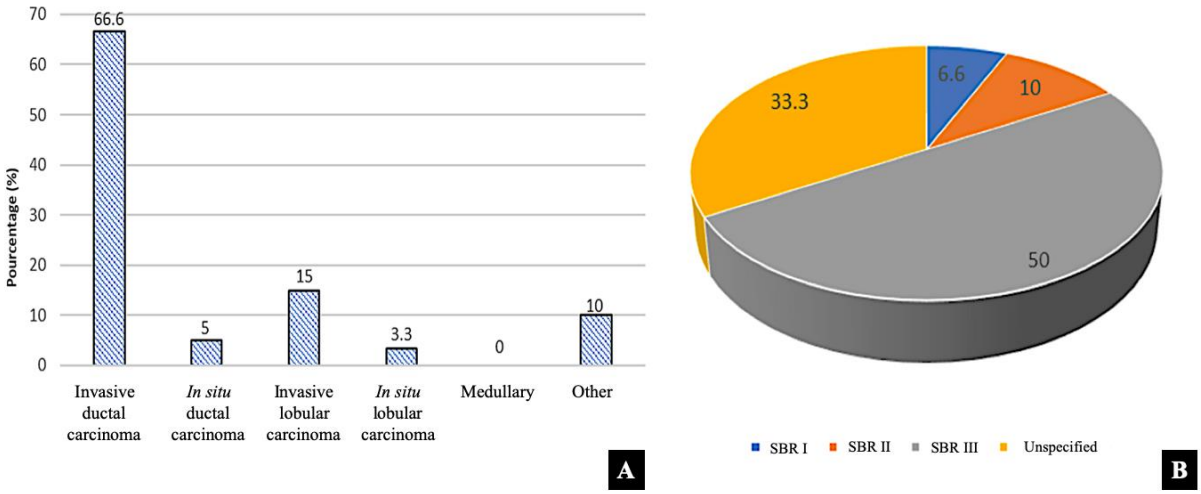

Figure 2: Histological aspects

A: Histological types of mammary carcinomas, **B**: SBR grade distribution of breast carcinomas expressed in percentages.

4.1.3 Immunohistochemical aspects

Antibodies to Estrogen Receptors (ERs), Progesterone Receptors (PRs), HER2/Neu Oncoprotein (HER2) and Ki67 were used. Only 50 out of 60 patients were tested for HER2 and Ki67. Tumors are predominantly Luminal A (33.3%), followed by Luminal B (15%). The most used hormone receptors are estrogen receptors (13.3%) compared to progesterone receptors (8.3%). Few tumors involving oncogenes, including HER2 Neu (8.3%)). Ki67 (10%) shows that the tumors were not proliferative (Table 1).

Table 1: Distribution of molecular markers

Molecular markers	Numbers	Percentages (%)
ERs+	8	13.3
PRs+	5	8.3
HER2+	5	8.3
Luminal A (ERs+ and/or PRs+/HER2-)	20	33.3
Luminal B (ERs+ and/or PRs+/HER2+)	9	15
Triple Negative (ERs-/PRs-/HER2-)	7	11.7
Ki67	6	10
Total	60	100

Ers : Estrogen Receptors; **PRs :** Progesterone Receptors

The primary anti-RP antibody is revealed by the secondary antibody conjugated to peroxidase on a tissue section of an invasive galactophoric carcinoma. The nuclear marking (brown) is obtained after the colored peroxidase / H_2O_2 reaction. The intensity of the labeling is amplified by means of the Avidin-Biotin-peroxidase complex (Magnification using a photon microscope x 400) (Figure 3A). Negative controls are reported in Figures 4A and 4B.

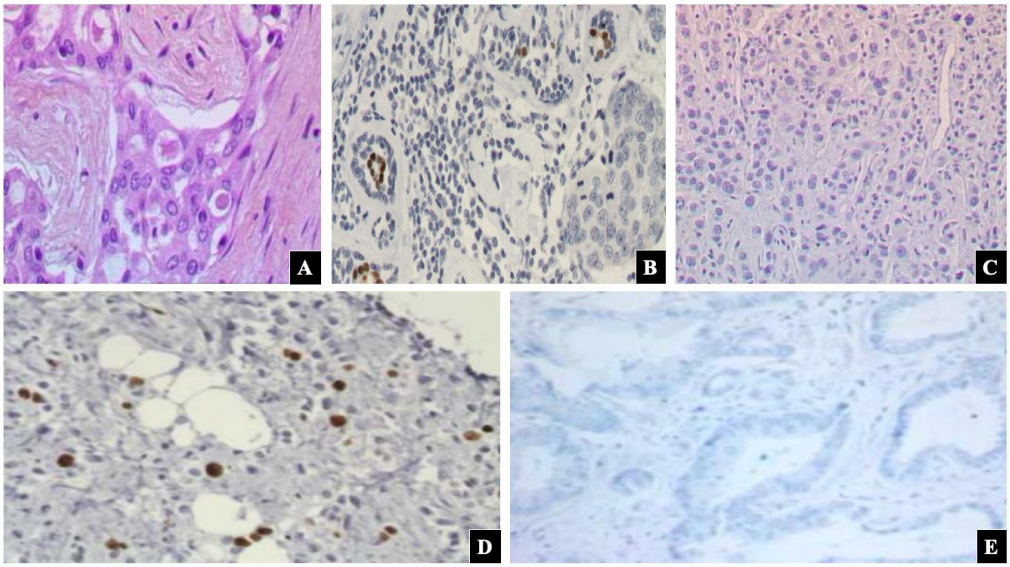

Figure 3: Tissue sections of an invasive galactophoric carcinoma

A: Detection of Progesterone Receptors; **B:** Detection of estrogen receptors; **C:** Demonstration of the Ki67 Protein; **D:** Demonstration of the HER2-Neu protein.

The secondary antibody conjugated to peroxidase on a tissue section of an invasive galactophoric carcinoma reveals the primary anti-RO antibody. The nuclear marking (brown) is obtained after the colored peroxidase / H_2O_2 reaction. The intensity of the labeling is amplified thanks to the Avidin-Biotin-peroxidase complex (Magnification using a photon microscope x 400) (Figure 3B). The negative controls are reported in Figures 4C and 4B.

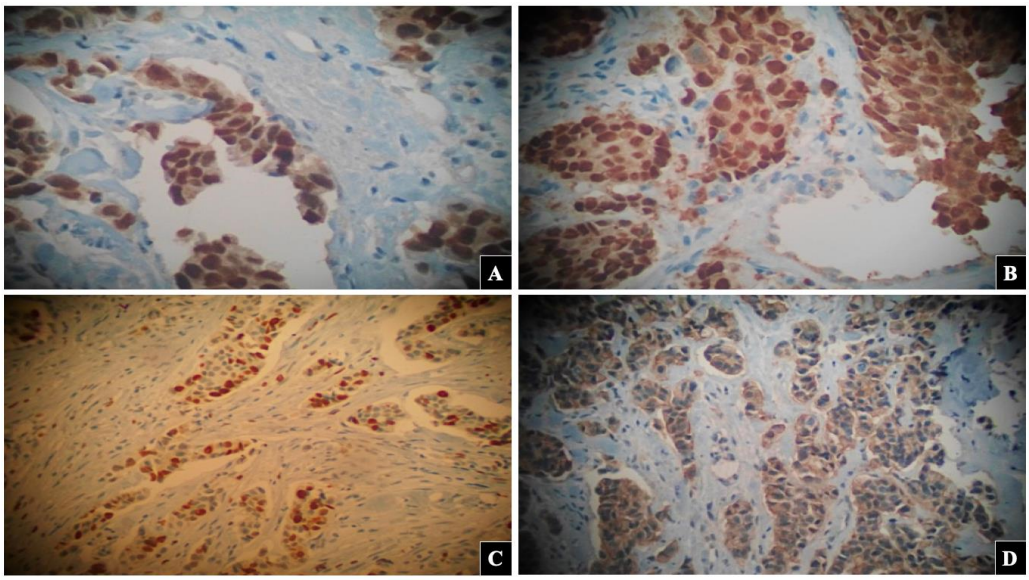

Figure 4 : Control tissue sections for the Immunohistochemistry study

A : Search for Progesterone Receptors (PRs) : The absence of brown coloring indicates an absence or a low level of Progesterone Receptors. **B :** Search for Hormonal Receptors (ERs and PRs) : The weak brown color in certain areas indicates the presence of a few Receptors (positive internal hormonal controls). **C :** Search for Estrogen Receptors (ERs) : The absence of brown coloring indicates an absence or a low level of Estrogen Receptors. **D :** Search for the non histone nuclear protein Ki67 : A weak brown marking which indicates a low rate of the Protein Ki67 and therefore a low proliferation coefficient. **E :** HER2-Neu protein search : The absence of brown coloring indicates an absence or a weak amplification of HER2.

The primary anti-Ki 67 antibody is revealed by the secondary antibody conjugated to peroxidase on a tissue section of a galactophoric carcinoma. Cytonuclear labeling of neoplastic cells (Brown) is obtained after the colored peroxidase / H_2O_2

reaction. The intensity of the labeling is amplified thanks to the Avidin-Biotin-peroxidase complex. (Magnification with photon microscope x 200) (Figure 3C). The negative control is reported in Figure 4D.
The primary anti-HER2-Neu antibody is revealed by the secondary antibody conjugated to peroxidase on a tissue section of a galactophoric carcinoma. Cytonuclear labeling of neoplastic cells (Brown) is obtained after the colored peroxidase / H_2O_2 reaction. The intensity of the labeling is amplified thanks to the Avidin-Biotin-peroxidase complex. (Magnification with photon microscope x 200) (Figure 3D). The negative control is reported in Figure 4E.
Hormonal receptors were detected by immunohistochemistry. The mammary tumors studied mainly express the molecular markers RO (estrogen receptors) compared to RP (progesterone receptors) (Table 2).

Table 2 : Profile of hormone receptors

Hormonal Receptors	Numbers	Percentages (%)
ERs + / PRs +	20	33.3
ERs + / PRs -	8	13.3
ERs - / PRs +	5	8.3
ERs - / PRs -	7	12

4.2 Discussion

In Gabon, breast cancer is a public health issue as it is the leading cause of death for women's cancers (Belembaogo, 2019). The average age of the study population is between 45 and 50 years, 47.6 years with extremes of 15 and 69 years. This result has also been found in other developing African countries such as Cameroon (Engbang et al., 2015), Algeria (Bekkouche et al., 2013) and Morocco (Fouad et al., 2012). However, this average age is higher (60 years) in developed countries (Bekkouche et al., 2013). Several hypotheses could justify this difference in particular: the demographic difference, the population being mostly young in developing countries, genetic factors and better monitoring for women in developed countries.

Clinically, the right breast is the most frequently affected in 50% of cases while other studies describe an injury in the same proportions of the left breast as in Morocco where we find a majority of breast carcinomas of 50% at the same level left breast (Mengue-M'Ella, 2008; Sahraoui et al., 2017). 5% of cases (3 cases out of 60 patients) of synchronous bilaterality were found for patients whose age varies from 37 to 49 years. It is possible that these cases of synchronous bilaterality are related to a family history of cancer (although not having information on the family history of these patients) as reported by previous work (Achaaban et al., 2013). The upper external quadrant was affected in 30 patients (50% of the cases) and only 10% of the lymph node dissection was observed.

Histologically, carcinomas are predominantly invasive (82%), including 67% ducts and 15% lobular cells. The same data were reported by most studies (Achaaban et al., 2013). The histopronotic classification reveals a predominance of grade III in 50% of patients followed by grade II in 10% of patients. These results confirm those already reported in a previous study conducted in Libreville (Meye et al., 2004). The invasive nature of the cancers in the present study could be explained by the mitogenic action of estrogens through their receptors on mutated mammary epithelial cells, thus stimulating tumor progression (Contesso et al., 1998; Pujol & Rochefort, 1995).

According to the classification in molecular sub-types defined according to the Saint Gallen consensus (Balic et al., 2019), immunohistochemical results in the search for molecular markers of mammary carcinomas predominance of luminal subtype A (tumors expressing at least one hormone receptor without expression of HER2/Neu) at 33.3%; followed by luminal subtype B (tumors expressing at least one hormone receptor with HER2/Neu expression) at 15%; and the Triple Negative subtype (tumors expressing neither hormone receptor nor HER2/Neu) at 11.7% and finally the subtype HER2 (tumors expressing only HER2/Neu without hormone receptor expression) at 8.3%. In addition, it is observed in the present study that 13 tumors in total (21.6%) express hormonal receptors with a slight predominance for estrogen receptors (8 tumors or 13.3%) compared with progesterone receptors (5 tumors or 8.3%). Estrogens have been shown to exert their mitogenic effects on the ductal epithelium (Contesso et al., 1998; Pujol & Rochefort, 1995). The carcinomas belonging to the Luminal A subtype express both the estrogen and progesterone receptors, and are therefore hormone-dependent and therefore sensitive to hormone therapy. Indeed, previous work has shown that the expression of estrogen receptors is correlated with other clinical and biological parameters, which made it possible to establish the prognostic value of these receptors (Contesso et al., 1998; McGuire, 1978). In addition, this work has demonstrated that the estrogen receptor status is an independent prognostic factor and is a predictor of the response to hormone therapy (McGuire, 1978). In contrast, the role of progesterone in the etiology of breast cancer is less well established than that of estrogen because its effects vary in the presence or absence of estrogen (Contesso et al., 1998; Pujol & Rochefort, 1995).

Indeed, depending on the case, progesterone may act on its receptor and on that of glucocorticoids, androgens, or estrogen (Contesso et al., 1998; Pujol & Rochefort, 1995). Progesterone receptors may be induced by estrogen receptors that may be reflected in them (Pujol & Rochefort, 1995). However, anti-progesterone molecules such as RU 486 inhibit the progression of breast cancer containing progesterone receptors, and could be used in the treatment of estrogen-resistant breast cancers. (Pujol & Rochefort, 1995; Rochefort et al., 2000).

The Luminal B group (tumors expressing at least one hormone receptor with Her/Neu expression) represents the 2nd class in our study, ie 15% of tumors. Tumors classified as Triple Negative (tumors that do not express either hormone receptors or HER2/Neu) represent 11.7%. The results obtained in our study are very close to those presented by other works on the continent. Thus, in a Tunisian study on the molecular classification of breast cancers, the authors report that 12.5% of tumors are associated with the Triple Negative subtype. (Belkacémi et al., 2010). In order to determine the origin of such cancers, extensive analysis is often carried out by looking for basal markers such as CK5/6 or p53. The technical resources made available during the present study did not make it possible to explore the origin of these Triple Negative cancers. Nevertheless, as a hypothesis, these tumors could be due to a mutation of the p53 gene. Indeed, the p53 gene is a tumor suppressor gene that plays an essential role in controlling the cell cycle and protecting the integrity of the genome. Its mutation is often the main cause of tumor pathology in almost all cancers (Soussi, 2000).

The HER2 molecular class (tumors expressing only the Her/Neu oncogene without expressing any hormone receptor) is poorly represented with 8.3% of cases and constitutes the fourth class of the present study. The study of the molecular markers of mammary carcinomas shows that the tumors analyzed are predominantly hormone-dependent and weakly related to an oncogene activation, in this case HER2, because the group corresponding to the Triple Negative subtype (ERs-, PRs-, HER2-) is weakly expressed (11.7%). Only 10% of tumors express the Ki67 protein, which indicates a high potential for cell proliferation in these cases. The results show that the systematic search, by immunohistochemistry, for tumor markers is essential for better patient management.

5. Conclusion

The present work is the first study reporting the immunohistochemical profile of molecular markers of mammary carcinomas from samples from the Anatomical Pathology Laboratories of the Omar Bongo Ondimba Army Instruction Hospital and of the University of Health Sciences from July 2014 to September 2017 in Gabon. On a sample of 60 patients, the majority profile consists of 33.3% of the luminal subgroup A (ERs + and/or PRs + / HER2-). The status in Hormonal Receivers is: Estrogen receptors (13.3% of tumors) and Progesterone receptors (8.3% of tumors). The protein Ki67, a marker of cell proliferation, was positive in 10% of the tumors. It would be necessary to continue this study on a larger and wider cohort throughout Gabon because the knowledge of the immunohistochemical profile gives an indication of the origin of breast cancers and allows to consider better management of patients by a targeted therapy to avoid unnecessary toxic effects resulting from ineffective treatment.

Funding: This work has not received any specific subsidy from the public, commercial or non-profit funding organizations.

Acknowledgements: We would like to thank the ***Service Edition Publication*** for correcting and proofreading this document.

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

[1] Achaaban, M., Maakoul, R., Fathi, K., Ragala, A., & Bargach, S. (2013). Cancer du sein bilatéral et synchrone à propos de deux cas. *Journal Marocain Des Sciences Médicales*, *18*(2), 1–5.

[2] Balic, M., Thomssen, C., Würstlein, R., Gnant, M., & Harbeck, N. (2019). St. Gallen/Vienna 2019: A brief summary of the consensus discussion on the optimal primary breast cancer treatment. *Breast Care*, *14*(2), 103–110. https://doi.org/10.1159/000499931

[3] Bekkouche, Z., Guedouar, Y., Ben Ali, F., & El Kebir, F. Z. (2013). Caractéristiques des carcinomes mammaires triple-négatifs dans l'Ouest-algérien. *Journal Africain Du Cancer / African Journal of Cancer*, *5*(3), 155–161.

[4] Belembaogo, E. (2019). *Le cancer du sein est le plus fréquent au Gabon*. Agence Gabonaise de Presse.

[5] Belkacémi, Y., Boussen, H., Hamdi-Cherif, M., Benider, A., Errihani, H., Mrabti, H., Bouzid, K., Bensalem, A., Fettouki, S., Ben Abdalah, M., L Abid, & Gligorov, J. (2010). Épidémiologie des cancers du sein de la femme jeune en Afrique du Nord. *32è Journées de La Société Française de Sénologie et de Pathologie Mammaire. Journées, Strasbourg, FRA, 2010-11-03: La Femme Jeune Face Au Cancer Du Sein (Strasbourg, 3-5 Novembre 2010)/Young Woman Facing Breast Cancer*, 56–68.

[6] Bossard, N., Remontet, L., Colonna, M., Grosclaude, P., Velten, M., Jougla, E., Rey, G., Belot, A., Danzon, A., A.Thuret, Uhry, Z., Bousquet, P. ., & Cerf, N. (2011). Projection de l'incidence et de la mortalité par cancer en France en 2011. *Veille Sanitaire (InVS) et l'Institut National de Cancer*, 1–78.

[7] Collège Français des Pathologistes. (2013). Item 309 (ex item 159) – Tumeurs du sein. *Université Médicale Virtuelle Francophone*, 1–11. http://campus.cerimes.fr/anatomie-pathologique/enseignement/anapath_32/site/html/cours.pdf

[8] Contesso, G., Mathieu, M.-C., & Guinebretiere, J.-M. (1998). Anatomie pathologique des cancers du sein: Cancer du sein. *La Revue Du Praticien*, *48*(1), 29–35.
[9] Engbang, J. P. N., Essome, H., Koh, V. M., Simo, G., Essam, J. D. S., Mouelle, A. S., & Essame, J. L. O. (2015). Breast cancer in Cameroon, histo-epidemiological profile: about 3044 cases. *The Pan African medical journal*, *21*, 242.
[10] Fouad, A., Yousra, A., Kaoutar, Z., Omar, E. M., Afaf, A., & Sanae, B. (2012). Molecular classification of breast cancer in Morocco TT - Classification moléculaire du cancer du sein au Maroc. *The Pan African medical journal*, *13*, 91.
[11] Hermanek, P., Hutter, R. V. P., Sobin, L. H., Wagner, G., & Wittekind, C. (1998). *Atlas TNM : Guide illustré de la Classification TNM / pTNM des tumeurs malignes* (Springer (ed.); 4th ed.). Broché.
[12] Marck, V. (2010). *Manuel de techniques d'anatomo-cytopathologie, théories et pratiques* (Masson (ed.)).
[13] Mbalawa-Gombe, C., Godet, J., & Gueye, S. (2012). *Les cancers en Afrique francophone, alliance de ligues francophones africaines et méditerranéennes contre le cancer*. 1-136.
[14] McGuire, W. L. (1978). Hormone receptors: their role in predicting prognosis and response to endocrine therapy. *Seminars in Oncology*, *5*(4), 428–433.
[15] Mengue-M'Ella, M. (2008). *Cancer du sein de la femme jeune âgée de moins de 40 ans*. Université des Sciences de la Sante.
[16] Meye, J. F., NGOMO, K. M. J., & Diallo, I. (2004). Le cancer du sein au centre hospitalier de Libreville. *Médecine d'Afrique Noire*, *51*(8–9), 479–482.
[17] Nkondjock, A., & Ghadirian, P. (2005). Facteurs de risque du cancer du sein. *Médecine/Sciences*, *21*(2), 175–180.
[18] Pujol, P., & Rochefort, H. (1995). Hormones stéroïdes et cancers du sein. *Médecine Thérapeutique*, *1*(4), 361–366.
[19] Rochefort, H., Garcia, M., Glondu, M., Laurent, V., Liaudet, E., Rey, J.-M., & Roger, P. (2000). Cathepsin D in breast cancer: mechanisms and clinical applications, a 1999 overview. *Clinica Chimica Acta*, *291*(2), 157–170.
[20] Sahraoui, G., Khanchel, F., & Chelbi, E. (2017). Anatomopathological profile of breast cancer in cape bon, Tunisia TT - Profil anatomopathologique du cancer du sein dans le cap bon tunisien. *The Pan African medical journal*, *26*, 11.
[21] Soussi, T. (2000). Cycle cellulaire et apoptose: le gène suppresseur de tumeur p53. *Médecine/Sciences*, *16*(4), 469–472.