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Effect of fixation time and sample type on tissue biomarker preservation in breast cancer in Cameroon

Effet de la durée de fixation et du type d'échantillon sur la préservation des biomarqueurs tissulaires dans le cancer du sein au Cameroun

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Article original

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ABSTRACT

Background: Reliable molecular profiling is essential for the effective management of invasive breast cancer. In the Cameroonian context, there is uncertainty about the factors that influence the preservation of tissue biomarkers for this profiling. The aim of this study was to determine the impact of fixation time and sample type on sample quality for molecular analysis and on the profile of four tissue biomarkers in invasive breast cancer.
Methods: A retrospective analytical study was conducted at the pathology laboratory of Douala Gyneco-Obstetric and Pediatric Hospital. After obtaining research authorization, data from archived histopathology and immunohistochemistry reports of patients with invasive breast cancer from 2017 to 2020 were collected. Statistical analysis was performed using SPSS 25 software, and Chi-square and Fisher's exact tests were conducted. Cramer's V was used to determine the strength of association between categorical variables.
Results: In univariate analysis, sample type and fixation time were statistically significantly associated with sample quality for molecular analysis, with a stronger effect observed for sample type than for fixation time. P-values were adjusted after multivariate analysis, revealing a bias in the association: fixation time. There was no significant association between fixation time and hormone receptor or Her2 profile, but a correlation was found with the Ki67 proliferation index.
Conclusion: To ensure good molecular profiling, emphasis should be placed on sample type rather than fixation time.

RESUME

Introduction : La prise en charge efficace du cancer du sein invasif, est tributaire d'une caractérisation moléculaire fiable. Le but de l'étude était de déterminer l'impact de la durée de fixation et du type d'échantillon sur la qualité de l'échantillon pour l'analyse moléculaire.
Méthodes :Une étude analytique rétrospective a été menée au laboratoire d'anatomie pathologique de l'Hôpital Gynéco-Obstétrique et Pédiatrique de Douala. Après avoir obtenu l' autorisation de recherche , les données des rapports d'histopathologie et d'immunohistochimie archivés des patients atteints d'un cancer du sein invasif de 2017 à 2020 ont été collectées. L'analyse statistique a été réalisée à l'aide du logiciel SPSS 25, et les tests exacts de Fisher et Khi-Deux ont été effectués. Le V de Cramer a été utilisé pour déterminer la force de l'association entre les variables catégorielles.
Résultats : En analyse univariée, le type d'échantillon et la durée de fixation étaient associés de manière significative à la qualité de l'échantillon pour l'analyse moléculaire, avec un effet plus important pour le type d'échantillon que pour le temps de fixation. Lors de l'analyse multivariée, il n'y avait pas d'association significative entre la durée de fixation et les récepteurs hormonaux ou le profil Her2, mais une corrélation a été trouvée avec l'indice de prolifération Ki67.
Conclusion : L'accent doit être mis sur le type d'échantillon plutôt que sur la durée de fixation dans le cancer du sein invasif, pour une meilleure caractérisation moléculaire.
Implications : Cette étude fournit des orientations au personnel des laboratoires d'anatomie pathologique pour améliorer l'efficacité du traitement des tissus.

Introduction

Efficient management of invasive breast cancer relies on a dependable pathological diagnosis. All stages of the analysis, particularly the pre-analytical phase, require special attention to ensure accuracy. In contrast to the flexible procedures traditionally used to prepare tissues for standard histopathological analyses, those used to preserve tumor tissue biomarkers for prognostic and predictive purposes are more stringent, taking into consideration fixation conditions, especially fixation time (1). We did not find any studies in the literature that considered the type of sample as a factor potentially associated with the preservation of tissue biomarkers, except for one that only looked at the Ki67 proliferation index (2).

To date, two tissue biomarkers, hormone receptors, and human epidermal growth factor receptor 2 (Her2), have shown the highest level of evidence for prognostic and predictive clinical utility in invasive breast cancer. Other markers, such as Ki67, which indicates tumor proliferation, are also utilized but with lower levels of evidence (1). The utilization of these biomarkers has increased in recent years as they enable targeted treatments (3). Unfortunately, in the Cameroonian context, the use of these biomarkers is suboptimal due to inadequate tissue preparation that compromises the integrity of tissue biomarkers. The aim of our study was to investigate the impact of fixation time and sample type on their quality for molecular analysis and on the status of four tissue biomarkers in invasive breast cancer.

Methodology

This study was a retrospective, descriptive and analytical analysis conducted in the pathology laboratory of Douala Gynaeco-Obstetric and Pediatric Hospital (DGOPH). The study population consisted of archived pathology and immunohistochemistry (IHC) reports from patients diagnosed with invasive breast cancer between 2017 and 2020. We excluded breast cytopathological analysis reports, cases of in situ carcinoma, and reports of samples from health institutions other than DGOPH due to the inability to determine the fixation time of samples before macroscopic examination. We obtained ethical clearance from the institutional ethics committee of Douala Gynaeco-Obstetric and Pediatric Hospital (DGOPH). Data were collected anonymously and solely for the purposes of the study. The nature of the histological samples (biopsy, nodulectomy, or mastectomy) was determined from the histopathological analysis reports. Biopsy samples included cores and/or tru-cut biopsies that were entirely placed in a cassette. Nodulectomy samples consisted of quadrantectomies and zonectomies, which were generally only partially included. Mastectomy involved the complete surgical removal of the breast. Fixation time was determined by considering the date and time of fixation, as well as the date and time of macroscopic examination indicated on the examination form and the laboratory register. Fixation time was deemed insufficient if it was less than 6 hours for biopsies, 12 hours for nodulectomies, and 24 hours for mastectomies. Optimal fixation time fell between 6 and 12 hours for biopsies, 12 and 24 hours for nodulectomies, and 24 and 72 hours for mastectomies. Fixation time was considered prolonged if it exceeded 12 hours for biopsies, 24 hours for nodulectomies, and 72 hours for mastectomies, taking into account the estimated penetration speed of 10% formalin at around 1 mm per hour (4). The clinical characteristics of age, gender and tumor location were extracted from the histological analysis reports. Histological characteristics such as tumor type, Scarff Bloom Richardson (SBR) grade and the presence of Paget's disease of the nipple were also extracted from the analysis reports. The quality of the samples was indicated on the IHC report. Samples were classified into two groups: satisfactory for interpretation and unsatisfactory for interpretation (due to poor fixation, presence of artefactual pigments, cytolysis, etc). The HR status of samples considered satisfactory for interpretation was expressed using the Allred score and extracted from the IHC reports along the Her2 status express according to the ASCO/CAP recommendations (5).

They were then grouped according to the classification of Sorlie and Perou (6) : Luminal A (ER+ and/or PR+; Her2-), Luminal B (ER+ and/or PR+; Her2+), HER2 (ER- and/or PR-; Her2+), Triple negative (Basal like: ER-; PR-; Her2- and CK5/6+ or CK14+ or EGFR2+), and unclassifiable (ER-; PR-; Her2- and CK5/6- and CK14- and EGFR2-). Since the IHC reports did not provide information on the status of cytokeratins (CK) 5/6 and 14, or that of Epidermal Growth Factor Receptor 2, it was not possible to distinguish between basal-like and unclassifiable tumors. The two phenotypes were therefore grouped together in the triple-negative category. Ki67 was expressed in accordance with the updated recommendations of the International

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Ki67 in Breast Cancer Working Group (7). The tumor was classified as slightly proliferative if Ki67 was less than 10%, highly proliferative if Ki67 was greater than 30%. We also identified an intermediate class for tumors with Ki67 levels between 10% and 30%.

In this study, statistical analyses were conducted using Statistical Package for Social Sciences (SPSS) 25 software. Quantitative variables were presented in terms of central tendency (mean and median), along with dispersion parameters (standard deviation, standard error, interquartile range), and extremes. For univariate analysis, Chi 2 and Fisher's exact test were employed to test hypotheses, while Cramer's V was utilized to assess the strength of the association between categorical variables. The independent-sample Student's t-test was used to compare means and explore the relationship between fixation time, hormone receptor status, and Ki67.

In multivariate analysis, adjustments were made using logistic regression. Throughout the study, any p-value less than 0.05 was considered statistically significant.

Results

During our study period, we identified 64 reports of patients with invasive breast cancer who underwent IHC analysis in the pathology laboratory of DGOPH. Our population consisted of patient files aged between 24 and 69 years, with a mean age of 44.38 ± 9.94 years. 54.69% of the population had invasive carcinoma of the left breast. There was one 54-year-old man included in our population. The most common type of specimen was biopsies (48.44%), and the vast majority of specimens (85.94%) underwent prolonged fixation. The majority of the population had ductal carcinoma (84.40%) of grade II (53.10%) without associated Paget's disease (90.63%). A significant number of specimens (31.25%) were unsatisfactory for IHC analysis (**Table 1**).

The Allred scores means (less than 3), as well as the medians (0) indicate that the majority of tumors were negative for HR (**Table 2a**).

Table 2b shows that the majority of patients in the study population (52.27%) had triple-negative invasive cancer with a highly proliferative tumor (45.46%). All samples, regardless of type, had a prolonged average fixation time. None of the biopsies had an optimal fixation time (**Table 3**).

Table 1: *study population characteristics (N=64)*

Variables	Numbers	Frequencies (%)
Age (year)	**Mean ± standard deviation**	**44.38 ± 9.94**
	Min-Max (24-69)	
< 30	2	3.10
[30-40[	21	32.80
[40-50[	19	29.70
≥ 50	22	34.40
Gender	**Ratio (M /F)**	**0,02**
Female	63	98.44
Male	1	1.56
Breast affected		
Left	35	54.69
Right	29	45.31
Sample type		
Biopsy	31	48.44
Mastectomy	23	35.94
Nodulectomy	10	15.62
Fixation time		
Prolonged	55	85.94
Optimal	9	14.06
Histological type		
Ductal carcinoma	54	84.40
Lobular carcinoma	7	10.90
Mucinous carcinoma	3	4.70
SBR Grade		
I	11	17.20
II	34	53.10
III	19	29.70
Paget's disease		
Present	6	9.37
Not present	58	90.63
Sample quality		
Satisfactory	44	68.75
Unsatisfactory	20	31.25
Total	**64**	**100.00**

The nature of the sample was found to be statistically significantly associated with fixation time, as indicated by the mean magnitude (in **table 4**). In univariate analysis, the type of sample and the fixation time were found to be statistically significantly associated with the sample quality for molecular analysis. However, the impact was greater for the sample type than for the fixation time. It is important to note that no sample had an insufficient fixation time (see **table 5**).

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Table 2a : *IHC characteristics of the study population (N =44)*

Variables	Mean ± standard error	Median (Interquartile range)	Min-Max
ER Allred score	2.66 ± 0.51	0 (0-7)	(0 – 8)
PR Allred score	1.77 ± 0.41	0 (0-5)	(0 – 8)
Ki67	34.27 ± 4.80%	25% (5%-56%)	(1% - 98%)

Table 2b : *IHC characteristics of the study population (N =44)*

Variables	Numbers	Frequencies (en %)
ER		
Positive	17	38.64
Negative	27	61.36
PR		
Positive	14	31.82
Negative	30	68.18
Her2		
Positive	5	11.36
Negative	39	88.64
Ki67		
Low proliferative	10	22.72
Intermediate	14	31.82
Highly proliferative	20	45.46
Molecular class		
Luminal A	16	36.36
Luminal B	3	6.82
Her2 +	2	4.55
Triple negative	23	52,27
Total	**44**	**100**

Table 3: *fixation time for different type of samples (N= 64)*

Variables	Min - Max	Mean ± Standard deviation
Fixation time in hour		
Biopsy	18 – 83	38.90 ± 2.45
Mastectomy	43 – 147	88.30 ± 5.40
Nodulectomy	19 – 82	43.50 ± 5.53

Table 4: *association between sample type and fixation time (N=64)*

Variables	Fixation time		p Value	Cramer V
	Optimal	Prolonged		
Sample type			**0.001**	**0.457**
Biopsy	0(0%)	31(100%)		
Mastectomy	8(34.78%)	15(65.22%)		
Nodulectomy	1(10%)	9(90%)		

Table 5: *factors associated with the quality of sample (N=64)*

Variables	Sample quality		p Value	Cramer V	Adjusted P Value
	Satisfactory	Unsatisfactory			
Sample type			**0.018**	**0.353**	**0,001**
Biopsy	26(83.87%)	5(16.13%)			
Mastectomy	11(47.82%)	12(52.18%)			
Nodulectomy	7(70%)	3(30%)			
Fixation time			**0.029**	**0.273**	**0,999**
Prolonged	35(63.63%)	20(36.37)			
Optimal	9(100%)	0(0%)			
Insufficient	0(0%)	0(0%)			

The p values adjusted after multivariate analysis (**Table 6**) reveal a bias in the association: fixation time, as also shown in **Figure 1** below. Biopsies had the highest rate of prolonged fixation (100%), yet they had the lowest rate of samples unsatisfactory for analysis (16.13%).

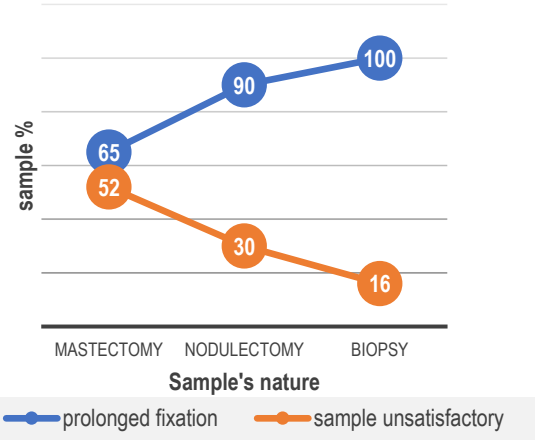

Figure 1: *variations in the percentage of samples with prolonged fixation and the percentage of unsatisfactory samples vary depending on the nature of the sample.*

Table 6 : *multivariate analysis (N=64)*

Variables	Sample Quality		Gross p Value	Adjusted p Value
	Satisfactory	Unsatisfactory		
	Sample nature		0.018	0.001
Biopsy	26(83.87%)	5(16.13%)		
Mastectomy	11(47.82%)	12(52.18%)		
Nodulectomy	7(70%)	3(30%)		
Fixation time			0.029	0.999
Prolonged	35(63.63%)	20(36.37)		
Optimal	9(100%)	0(0%)		
Unsatisfactory	0(0%)	0(0%)		

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Table 7 shows that fixation time is not significantly associated with hormone receptor status, but rather with the Ki67 proliferation index. We were unable to compare the means of the Allred score for biopsies and nodulectomies due to their small numbers (0 and 1): no biopsy had an optimal fixation time, and only one nodulectomy had an optimal fixation time.

Table 7: *effect of fixation time on molecular profile for mastectomies (N=23)*

Variable		HR Allred score and Ki67 for mastectomies				
Fixation time		Mean ± Standard Error	Mean difference	95 % confidence interval		p Value
				Lower	Upper	
Prolonged	ER	1.07 ± 0.72	Ref			
Optimal		3.13 ± 1.22	-2.06	-4.89	0.78	0.19
Prolonged	PR	0.33 ± 0.33	Ref			
Optimal		2.88 ± 1.20	-2.54	-5.41	0.33	0.07
Prolonged	Ki67	3.47 ± 2.15%	Ref			
Optimal		33.38 ± 10.49%	-29.91%	-0.55	-0.05	0.02

Table 8 shows a non-statistically significant association between fixation time and Her2 status.

Table 8: *effect of fixation time on Her2 status (N=64)*

Variable	Her2		p Value
	Positive	Negative	
Fixation time			**0.67**
Prolonged	6(10.9%)	49(89.1%)	
Optimal	1(11.1%)	8(88.9%)	

Discussion

The aim of this study was to determine the effect of fixation time and sample nature on the quality of tissue samples for molecular analysis as well as on the status of certain tissue biomarkers (ER, PR, Her2, Ki67) in invasive breast cancer. We identified 64 cases of invasive breast cancer that underwent IHC study at the pathology department of DGOPH from 2017 to 2020. The majority of the population (98.44%) was female (sex-ratio M/F 0.02), with an invasive malignant lesion of the left breast (54.69%); and whose age varied between 24 and 69 years with an average of 44.38 ± 9.94. The most represented age group was patients aged over 50 years (34.40%). The predominant histological type was ductal carcinoma (84.40%) Grade SBR II (53.10%), with no associated Paget's disease (90.63%). These results are similar to those of Engbang *et al.* (2015), both in terms of sex ratio, mean age, affected breast, and histological characteristics (8). Atangana and others found that the 30 to 39 age group was the most represented in Douala and Yaoundé in 2011, out of 208 cases and in 2015 out 404 cases (9,10). Other histological parameters, such as the nature of the surgical margins, TNM stage, presence of vascular emboli, perineural engorgement, microcalcification, necrosis, inflammatory infiltration, and in situ ductal component, were not taken into consideration because they were not mentioned in a sufficient number of analysis reports.

We did not find any studies in the literature that examined factors associated with sample quality (satisfactory or unsatisfactory) for molecular analysis. Most studies focused on factors associated with tissue biomarker expression, as these are the final objectives of IHC examinations and are of clinical interest (11,12). Although it may seem logical that the expression of these biomarkers would be influenced by sample quality, there are significant nuances to consider. The results of our study showed that fixation time had a statistically significant impact ($P<0.05$) on the quality of tissue samples for molecular analysis, but only in univariate analysis. Adjustment by multivariate analysis revealed that fixation time is a bias factor, falsely associated. It would be expected that samples with prolonged fixation would have the highest proportion of samples unsatisfactory for molecular analysis, but this was not the case. 100% of biopsies had prolonged fixation, yet these biopsies had the highest proportion of samples satisfactory for analysis. In fact, the nature of the sample significantly influences its final quality, much more than the fixation time.

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Biopsies are more likely to undergo prolonged fixation, which is easily explained. A biopsy collected by a gynecologist between 10am and 4pm and fixed immediately must be processed in the pathology laboratory between 4pm and 4am to ensure optimal fixation time. However, this time interval does not align with the working hours in pathology laboratories. This is not the case for mastectomies, for which the time interval corresponding to optimal fixation time is easily achievable by pathology laboratory staff (13). The large proportion of unsatisfactory samples from mastectomies can be explained by an intrinsic characteristic of this type of sample: the thickness of the surgical specimen. This characteristic is the cause of the slowdown in the speed of penetration of the fixative liquid into the tissue. This is known as a layer fixation artifact: fixation is adequate at the periphery of the tissue while the center of the specimen is poorly fixed or not fixed at all; between these two areas, the tissue is more or less well fixed. It is important to note that this type of artifact is impossible to correct technically (14).

Whether it be the proportion score, intensity score, or Allred score for HR and Her2 status, they were not significantly associated with fixation time as long as the sample quality was satisfactory for analysis. Most series in the literature support this fact, with the exception of a few studies that do highlight a decrease in HR immunoreactivity after 72 hours of fixation.(11,15).

This can be explained by the fact that cytolysis resulting from prolonged fixation affects cell membranes very little, but has minimal impact on tumor cell nuclei. This can leave the nuclei in a state of naked nuclei with intact hormone receptors. Additionally, the decrease in immunoreactivity is not always directly correlated with a decrease in the Allred score. For instance, the percentage of marked nuclei may decrease from 100% to 68%, while the proportion score remains constant at '5'. The parameter most likely to alter the Allred score is the intensity score, as it is a more subjective and operator-dependent measurement. We did not encounter any cases of insufficient fixation. In the literature, opinions are divided regarding its effect on tissue biomarkers (16,17).

The most represented tumor phenotype was triple negative (52.27%), followed by luminal A and B (36.36% and 6.82% respectively), with the least represented being Her2. Atangana *et al.* in 2011 also found triple negative and luminal A tumor phenotype to be the most represented in Douala and Yaoundé. However, in this study including 208 cases, tumors overexpressing the Her2 oncoprotein were not the least represented as in our study (9).

The results of this study revealed that the majority of participants had a highly proliferative tumor (45.46%), and the proliferation index was significantly associated with fixation time. Prolonged fixation time resulted in a decrease, leading to an underestimation of this index. Nobuyuki *et al.*, found that both insufficient fixation and prolonged fixation negatively affected the proliferation index. Additionally, their study had the particularity of considering the nature of the sample, which was significantly associated with the evaluation of the proliferation index. They found that a mastectomy specimen sectioned before fixation had a proliferation index strongly correlated with that of a biopsy of the same tumor (2).

Instead of allowing the mastectomy specimens to be fixed for 24 to 72 hours before macroscopic examination, we suggest that pathologists either cut sections a few centimeters thick as soon as the mastectomy specimen arrives in the laboratory and let them fix for 24 hours, or better yet, proceed directly to macroscopic examination and collect systematic samples a few millimeters thick (ideally 2 mm) that are representative of the surgical specimen. These samples should be included in cassettes and left to fix for 24 hours. Additionally, we recommend that the fixation time be documented on the examination report accompanying the tissue sample, not only for the standard histopathological examination but also for the IHC examination.

Limitations

We were unable to determine the effect of the sample type on the HR and Her2 oncoprotein status because the number of cases in certain categories of the independent variable was less than 2. This made it impossible to compare the means or variances of the Allred scores. Since it had been previously established that the sample type influences its quality for IHC analysis, we concluded that the sample type also influences the HR and Her2 status.

Conclusion

The type of sample and the fixation time significantly impact the proliferation index, while hormone receptor status and Her2 oncoprotein overexpression are only affected by the sample type.

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Smaller tissue samples preserve and assess tissue biomarkers better through IHC under our current fixation conditions.

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Author's contribution:
P.J.A.A. and C.E.E.M. were the project leaders, C.P.N. and L.B.T.K. were responsible for experimental and project design. C.P.N. performed most of the experiments. S.H.M. and E.T.M. made conceptual contributions. Calculations were performed by C.P.N.; C.P.N. and G.R.A. co-wrote the manuscrip**t.**

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Références

1. Bellocq JP, Egele C, De Cremoux P, Ruck L, Mathelin C, Luporsi E, *et al*. Prélèvements tissulaires tumoraux pour analyses moléculaires: comment les gérer au mieux? 32° Journ Société Fr Sénologie Pathol Mammaire Journ Strasbg FRA 2010-11-03 Femme Jeune Face Au Cancer Sein Strasbg 3-5 Novembre 2010Young Woman Facing Breast Cancer. 2010; pp. 153-162
2. Arima N, Nishimura R, Osako T, Nishiyama Y, Fujisue M, Okumura Y, *et al*. The importance of tissue handling of surgically removed breast cancer for an accurate assessment of the Ki-67 index. J Clin Pathol. mars 2016;69(3):255-9.
3. Boyle P. Breast cancer control: Signs of progress, but more work required. The Breast. 1 déc 2005;14(6):429-38.
4. MacGrogan G, Mathieu MC, Poulet B, Penault-Llorca F, Vincent-Salomon A, Roger P, *et al.* Recommandations du GEFPICS concernant la phase pré-analytique pour l'évaluation de HER2 et des récepteurs hormonaux dans le cancer du sein: mise à jour 2014. In: Annales de pathologie. Elsevier; 2014. p. 366-72.
5. Rakha EA, Starczynski J, Lee AHS, Ellis IO. The updated ASCO/CAP guideline recommendations for HER2 testing in the management of invasive breast cancer: a critical review of their implications for routine practice. Histopathology. avr 2014;64(5):609-15.
6. Sorlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, *et al.* Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci. 11 sept 2001;98(19):10869-74.
7. Nielsen TO, Leung SCY, Rimm DL, Dodson A, Acs B, Badve S, *et al*. Assessment of Ki67 in Breast Cancer: Updated Recommendations From the International Ki67 in Breast Cancer Working Group. JNCI J Natl Cancer Inst. 1 juill 2021;113(7):808-19.
8. Engbang JPN, Essome H, Koh VM, Simo G, Essam JDS, Mouelle AS, et al. Cancer du sein au Cameroun, profil histo-épidémiologique: à propos de 3044 cas. Pan Afr Med J. 4 août 2015;21:242.
9. Atangana PJA, Tchente Nguefack C, Kabeyene Okono A, Dina Bell E. Aspects immunohistochimiques des cancers du sein à Douala et Yaounde. Health Sci Dis. 2017, Volume 18 (3). 14-20p.
10. Ngowa JDK, Kasia JM, Yomi J, Nana AN, Ngassam A, Domkam I, *et al*. Breast Cancer Survival in Cameroon: Analysis of a Cohort of 404 Patients at the Yaounde General Hospital. Adv Breast Cancer Res. 2015;04(02):44-52.
11. Rugwizangoga B, Ndayisaba MC, Izimukwiye IA, de Dieu Baryabagaya J, Bosco J. Accuracy of the duration of tissue fixation and the receptor status profile of primary breast cancers in a tertiary hospital of Rwanda. Imp J Interdiscip Res. 2016;2(11):756-62.
12. Arnould L, Denoux Y, MacGrogan G, Penault-Llorca F, Fiche M, Treilleux I, *et al.* Agreement between chromogenic in situ hybridisation (CISH) and FISH in the determination of HER2 status in breast cancer. Br J Cancer. 2003;88(10):1587-91.
13. Hofman V, Ilie M, Gavric-Tanga V, Lespinet V, Mari M, Lassalle S, et al. Rôle du laboratoire d'anatomie pathologique dans l'approche pré-analytique des examens de biologie moléculaire réalisés en pathologie tumorale. In: Annales de pathologie. Elsevier; 2010. p. 85-93.
14. Fortier F, Hould R. Histotechnologie: théorie et procédés. 2ème. Montreal: CCDMD; 2013. 722 p.
15. Yildiz-Aktas IZ, Dabbs DJ, Cooper KL, Chivukula M, McManus K, Bhargava R. The Effect of 96-Hour Formalin Fixation on the Immunohistochemical Evaluation of Estrogen Receptor, Progesterone Receptor, and HER2 Expression in Invasive Breast Carcinoma. Am J Clin Pathol. 1 mai 2012;137(5):691-8.
16. Halilovic A, Bulte J, Jacobs Y, Braam H, van Cleef P, Schlooz-Vries M, et al. Brief fixation enables same-day breast cancer diagnosis with reliable assessment of hormone receptors, E-cadherin and HER2/Neu. J Clin Pathol 2017, 6p.
17. Kalkman S, Barentsz MW, Witkamp AJ, Van Der Wall E, Verkooijen HM, Van Diest PJ. Brief fixation does not affect assessment of hormone receptor expression in invasive breast carcinoma biopsies: paving the road for same-day tissue diagnostics. Am J Surg Pathol. 2014;38(8):1071-8.