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Immunohistochemical and Molecular Evaluation of Oncoprotein HER-2 in Women's Breast Cancer in The Republic of Congo

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ABSTRACT

Introduction: *Breast cancer is a heterogeneous disease with a variety of morphological and molecular characteristics impacting treatment response. This study aimed to evaluate by immunohistochemistry and RT-PCR the overexpression of HER2 in breast cancer in women in the Republic of Congo.*

Materials and Methods: *We conducted an 8-month cross-sectional descriptive study. 25 paraffin biopsies of breast cancer cases in patients diagnosed at the University Hospital of Brazzaville were collected. Epidemiological, clinical, histological, immunohistochemical and molecular aspects were studied.*

Results: *The mean age of the patients was 49.64 ± 13.20 years (31-80 years). 60% of the patients had a right localization of the tumor. 76% of the patients had an invasive nonspecific type carcinoma. The T4b N1a M0 stage was predominant, representing 56% of the study population. SBR histopronostic grade 1 was represented by 60% of patients. Estrogen and progesterone receptors were positive in the range of 45% and 60%, respectively. The HER2 oncoprotein was positive in 12% (3/25) of 25 cases for IHC. The luminal group was in the majority with 32%. Molecular analysis of the HER2 gene by RT-PCR revealed over expression in 60% (15/25) of cases, 3 of which were already positive for IHC. With the "AmoyDx® HER2 Mutation Detection Kit", 12 mutations were identified, 10 of which involved exon 20, ie 83.33% and 2 mutations with exon 19, ie 16.67% of cases. The correlation of the over expression of the HER2 gene showed a statistically significant difference between the two techniques, p <0.00003.*

Conclusion: *HER2 is known as a prognostic and predictive marker in breast cancer, making this receptor a valuable therapeutic target. However, its highlighting by IHC remains cumbersome and subject to false negatives. Hence molecular analysis could play a crucial role in decision-making when implementing targeted breast cancer therapies in Congo.*

Keywords
HER2, immunohistochemistry, qRT-PCR, Breast cancer, Republic of Congo.

Introduction

Female breast cancer has now surpassed lung cancer as the leading cause of global cancer incidence in 2020, with around 2.3 million new cases, or 11.7% of all cancer cases. It is the fifth leading cause of cancer death in the world, with 685,000 deaths (1). In Congo, it is the first of all cancers according to the latest estimates of GLOBOCAN 2020 [1]. It is a real public health problem affecting both sexes with a large predominance of women in the order of 99% of cases [2]. Breast cancer is a heterogeneous disease with a variety of morphological and molecular characteristics and response to treatment and clinical outcome. It is divided into five molecular subtypes: luminal A, luminal B, basal type and normal type, human epidermal growth factor (HER2) positive receptor 2. The latter, is localized in chromosome 17 and encodes for a protein of 185 kPa that functions as a transmembran growth factor receptor. it is a cell proliferation marker with high metastatic potential [3,4]. Tumours that overexpress this gene have a poor prognosis. Its evaluation involves immunohistochemistry and currently molecular biology techniques to better study its profile and thus institute the new therapeutic approach which is none other than the indicated targeted therapies (Trastuzumab) [5,6].

The relationship between the overexpression of HER2 and the evolution of breast cancers on the one hand, and the new therapeutic possibilities by anti-HER2 antibodies on the other hand have made the HER2 status a commonly sought-after element currently in breast cancers. Knowledge of HER2 status is essential for the selection of breast cancer patients for treatment with trastuzumab (Herceptin®). According to clinical data, her2-targeted therapy significantly improves the survival of breast cancer patients with overexpression of HER2 [7-9]. However, recent data suggest the presence of oncogenic mutations in HER2 affects the technical and clinical outcomes of the IHC in patients with HER2-positive breast cancer [10]. Some authors attributed somatic mutations to HER2, a role in resistance to anti-HER2 treatment because differential regulation of HER2 was observed in patients [10-13].

A reliable assessment of HER2 status is essential for a selection of all patients to benefit from targeted therapy. Many tests are available to assess HER2 status. The two most commonly used tests are immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) [8,10,14]. The determination of the HER-2 status can be carried out either by the search for amplification of the gene or by that of the overexpression of the protein encoded by this gene. Several methods can be used, only morphological methods are currently the subject of recommendations by international bodies [9,10,15]. In addition, PCR-based methods have become important in clinical analyses, especially quantitative PCR methods such as real-time polymerase chain reaction (PCR), which is based on the detection of DNA amplification. Real-time PCR is cost-effective and many samples can be analyzed at the same time. The overexpression of HER2 is currently defined according to the scoring system of the ASCO/CAP 2014 guidelines to achieve reproducible dosing performance [10,16-19]. Several studies have been carried out around the world and in Africa studying different aspects including epidemiological, diagnostic and therapeutic. However, almost no studies have looked at immunohistochemical and molecular approaches to HER2 oncoprotein in breast cancer in Congo. The objective of this work was to evaluate the overexpression of oncoprotein HER-2 by IHC and molecular biology in order to contribute to a better therapeutic management of breast cancer patients in Congo.

Materials and Methods

It was a cross-sectional descriptive study with retrospective data collection, which took place from April 1 to November 1, 2020, a period of 8 months.

The study population involved 25 breast biopsies and operating parts included in paraffin blocks with available and interpretable clinical data, and with a confirmed diagnosis of breast cancer after triple reading. The paraffin blocks were identified on the basis of the selection criteria in the Department of Pathological Cytological Anatomy, Brazzaville University Hospital Center from January 2019 to March 2020.

This study was conducted successively in Brazzaville (Brazzaville University Hospital) for the selection of samples, in Oyo in the department of the basin (Edith Lucie BONGO ONDIMBA General Hospital) for the immunohistochemistry analysis (IHC) and in Pointe Noire (Marie Madeleine GOMBES Laboratory) for molecular analysis. This work has been approved by the Committee on Ethical Research in Health Science (CESRB) according to Opinion No. 061.

Technical support of the samples

Histopathology

All breast tissues were fixed in 10% neutral buffered formalin and included in paraffin under standard conditions. Sections of 4 µm were performed at the microtome for each of the 25 blocks of paraffin breast to examine microscopically the presence of more than 80% of breast cancer cells. The tissue ribbons were spread out on the door blades objects for hematxyline and eosin (H&E) staining. The resulting slides were deparaffin in three toluene baths for 5 minutes each. Then they were rehydrated with a series of alcohol baths to decreasing degrees (100%, 95% and 80%), each for 3 minutes. The cuts were then stained with hematxyline for 5 minutes, rinsed gently in tap water for 10 min, then colored with eosin for about 1 minute. The blades were subsequently dehydrated with an increasing series of alcohol (80%, 95% and 100% ethanol). After immersion for 5 min in a toluene bath, they were mounted with slats covering objects using an assembly medium (Eukkit). This phase was carried out in the Laboratory of Pathological Cytology Anatomy at the Brazzaville University Hospital.

The paraffin blocks diagnosed with breast cancer were all sent to the Morphological and Molecular Analysis Laboratory of the

Edith Lucie BONGO ONDIMBA General Hospital for the manual immunostaining technique. Clinical data including age, stage, histological grade and histological type were collected on the survey sheets. The histological classification was carried out using the Nottingham classification system and staging according to the 8th edition of the 2017 AJCC classification [11,20].

Immunohistochemistry

Immunohistochemistry procedures examining hormone receptor labeling (RO for estrogen receptor and RP for progesterone receptor) and her-2 expression were performed manually.

The technology used was the "Ultra vision quanto system" using horseradish peroxidase (HRP) and Diaminobenzydine (DAB). The following standard practices were performed: To analyze the labeling profile of hormone receptors and the expression of Her-2 with super Frost plus blades, each was deparaffin in xylene, rehydrated in alcohol baths to decreasing degrees (100°, 95°,70°) and boiled for 45 minutes (in a water bath set at 96°) in 10 mM citrate buffer (pH 6.0) for the restoration of antigenic sites.

Then the slides were incubated for 35 minutes at 37°C with primary monoclonal antibodies (See Table 1 below) using manual mode. Thermo Scientist's manual Ultra vision detection system uses an indirect biotin-avidine system with a universal biotinylated immunoglobulin secondary antibody. The slides were incubated for 5 minutes with 3,3'-diaminobenzidine (DAB) until appropriate brown staining.

Then the blades were counter-stained with hematxyline before assembly. The staining procedures were carried out in accordance with the manufacturer's recommendations. Negative and positive control slides were included in each test. The samples were interpreted according to the guidelines of the American Society of Clinical Oncology / College of American Pathology (ASCO / CAP): negative (0, 1+), weakly positive (2+) and strongly positive (3+), with a threshold of more than 10% of tumor cells that must have a homogeneous and dark circumferential pattern (mesh) to call the results 3+, HER2 positive [17]. The results of estrogen receptors (ER) and progesterone (PR) were interpreted in accordance with the guidelines recommended by asco/CAP [9,14,18,19].

DNA extraction

Sections of 5 µM were made using the Leica microtome from the paraffin blocks and put in the eppendorf tubes.

Dewaxing was achieved by adding 1 ml of xylene to each eppendorfs tube. After vortexing for 30 seconds, the tubes were incubated at room temperature for 15 min and then centrifuged at 6000 rpm at 15°C for 5 minutes. After removal of the supernatant, 1mL of ethanol 70% was added, voratexed, incubated for 15 min at room temperature and then centrifuged for 5 min at 6000 rpm at 15°C.

The operation was repeated 2 times. Finally the pellet was dried at room temperature and then washed with PBS (saline phosphate buffer) two (2) times. The samples were re-suspended in 180 .mu.l of lysis buffer to which 20 .mu.l of proteinase K was added. After incubation at 56°C for one hour, the extraction continued with the kit "ReliaPrepTM gDNA Tissue Miniprep System (Promega)" following the manufacturer's instructions. The elution was done with 50 .mu.l of elution solution and the collected DNA was immediately stored at -20°C before use. The concentration of the extract was determined using Qubit® 3.0 (life technologies, Invitrogen).

Molecular analysis by real-time PCR of the HER-2 gene

Her2/neu gene expression was analyzed using the "AmoyDx® HER2 Mutation Detection Kit (Amoy Diagnostics Co., Ltd. Xiamen, China)". The kit uses refractory mutation system amplification technology (ARMS) that includes specific primers and fluorescent probes to detect gene mutations in the HER-2 gene by real-time PCR. During nucleic acid amplification, the targeted mutant DNA is matched with the bases at the 3 ' end of the primer, amplified selectively, then the mutant amplicon is detected by fluorescent probes labeled with FAM. This kit is composed of 6 reaction mixtures and positive control. Table I shows the primer and probe concentrations used. The master mix and primers / probes were combined with 0.3 .3 µL of MATRIX DNA in a final volume of 35.3 .mu.l (Table 2). The amplification conditions were reported in Table 3.

Triple reactions were performed and the mean cycle cut-off value (Ct) was used to determine the relative amount of PCR product. The hybridization probes used for quantification were based on primers and probes described in Table 4.

Table 1: Contents of the kit according to the Master Mix.

Tube No	Contents	Main ingredient	Quantity	Fluorescent Ssignal
1	*HER2* Reaction Mix (1)	Primers, probes, Mg2+, dNTPS	550 µL/tube × 1	FAM, HEX/VIC
2	*HER2* Reaction Mix (2)	Primers, probes, Mg2+, dNTPS	550 µL/tube × 1	FAM, HEX/VIC
3	*HER2* Reaction Mix (3)	Primers, probes, Mg2+, dNTPS	550 µL/tube × 1	FAM, HEX/VIC
4	*HER2* Reaction Mix (4)	Amorces, sondes, Mg2+, dNTPS	550 µL/tube × 1	FAM, HEX/VIC
5	*HER2* Reaction Mix (5)	Primers, probes, Mg2+, dNTPS	550 µL/tube × 1	FAM, HEX/VIC
6	External control reaction mixture HER2	Primers, probes, Mg2+, dNTPS	550 µL/tube × 1	FAM
/	Enzyme mixture HER2	Taq DNA Polymerase, Uracil-N-Glycosylase	30 µL/tube × 1	/
/	Positive control HER2	plasmid DNA	250 µL/tube × 1	/

Table 2: HER2 Master Mix.

Content	Volume per test
Reaction mixture	35 µL
HER2 enzyme mixture	0.3 µL
Total volume	35.3 µL

Table 3: Represents cyclic parameters.

Cycles	Temperature	Time	Data collection
31	93°C	25s	/
	60°C	35s	FAM et HEX/VIC
	72°C	20s	/

Table 4: HER2 Mutations detected by the kit.

Reagents	Exon	Mutation	Base change
HER2 Reaction mixture (1)		A775_G776insYVMA	2325_2326 ins12 (TACGTGATGGCT)
	20	A775_G776insYVMA	2324_2325 ins12 (ATACGTGATGGC)
		M774_A775insAYVM	2322_2323 ins12 (GCATACGTGATG)
HER2 Reaction mixture (2)	20	G776> VC	2326_2327 ins3 (TGT)
		G776> VC	2326_2327 ins3 (TTT)
		G776R	2326G> C
		G776C	2326G> T
HER2 Reaction mixture (3)		P780_Y781insGSP	2339_2340 ins9 (TGGCTCCCC)
		P780_Y781insGSP	2339_2340 ins9 (GGGCTCCCC)
	20		2340_2341 ins9 (GGCTCCCCA)
		P780_Y781insGSP	
		P780_Y781insGSP	2339_2340 ins9 (CGGCTCCCC)
HER2 Reaction mixture (4)	20	V777L	2329G> T
HER2 Reaction mixture (5)	19	L755P	2263_2264 TT> CC

Statistical analysis

Qualitative variables were expressed as a percentage. The qualitative data was compared with McNemar's Fisher Exact test, at the risk $\alpha = 5\%$.

Results

Clinico-pathological features

All the clinico-pathological characteristics are reported in Table 5.

The average age of the study population was 49.64 ± 13.20 years with extremes ranging from 31 to 80 years. The majority age group of the study population was between 36 and 45 years of age, or 32%.

The right tumor localization was the most represented in our study with 15 patients or 60%. Invasive carcinoma was the most frequent with 76% for 19 patients. The T4b N1a M0 stage was in the majority representing 56% of the study population. Histo-prognostic grade 1 of SBR was represented by 60% of the patients. Estrogen receptors were negative in the order of 56% and positive in the order of 44%. Progesterone receptors were positive in 40% of cases. HER2 oncoprotein was positive with 12%. The luminal group was in the majority with 32%.

Immunohistochemical profile (IHC) of oncoprotein HER-2

A total of 25 samples were immunohistochemically analyzed for her-2 oncoprotein. Three (03) of the 25 expressed oncoprotein HER-2 or 12% positivity.

Molecular profile of the HER-2 gene

All 25 samples were analyzed. The expression of the HER2 gene was observed in 15 of the 25 samples or 60%. Of the 15 samples detected positive, 3 were also tested by the IHC technique. Analysis of the 12 samples exclusively positive for RT-PCR showed mutations that were described in Table 6.

Molecular and immunohistochemical correlations of HER-2

Since the expression of the HER-2 gene is crucial in the management of cases of breast cancer, its expression gives the possibility of prescribing Herceptin as an important molecular in the target therapy of this cancer. Table VII reports the bivariate analysis between the results of IHC and RT-PCR in the detection of oncoprotein HER-2. A statistically significant difference was observed for the detection of her-2 oncoprotein expression between the IHC technique and RT-PCR, $p<0.00003$.

Discussion

Oncoprotein HER-2 is recognized as one of the biomarkers of cell proliferation with high metastatic potential and very poor prognosis. His systematic research in breast cancer offers new therapeutic possibilities thanks to the discovery of new molecules such as Trastuzumab. Thanks to the IHC, the overexpression of HER-2 makes it possible to define the HER-2 status in breast cancers.

Table 5: Clinico-pathological characteristics.

Variables	Effectif (n)	Percentage (%)
Age groups		
Average age ± SD	49,64 ± 13,20	
Extremes of age	31 - 80 years	
≤ 35 years	5	20
36-45	8	32
46-55	7	28
56-65	2	8
≥ 66 yeans	3	12
Tumor localization		
Right breast	15	60
Left breast	10	40
Histological types		
Nonspecific infiltrating ductal carcinoma	19	76
Invasive lobular carcinoma	3	12
Medullary carcinoma	1	4
Mixed invasive carcinoma	1	4
Mucinous carcinoma	1	4
TNM Classification		
T0N0M0	1	4
T3N0M0	4	16
T3N1aM0	2	8
T4bN0M0	4	16
T4bN1aM0	14	56
Histo-prognostic grade of SBR		
Grade 1	15	60
Grade 2	8	32
Grade 3	2	8
Immunohistochemical aspect		
RO+	11	44
RO-	14	56
RP+	10	40
RP-	15	60
HER2+ (2+ ; 3+)	3	12
HER2-	22	88
Molecular profile		
RT-PCR of the HER2 gene		
HER2+ Gene	15	60
HER2- Gene	10	40
Molecular classification		
Luminal A	8	32
Luminal B	8	32
HER2 positive	3	12
Triple négative	6	24

Table 6: Distribution of the 12 MUTATIONS of the HER2 gene.

Reaction mixture	Exon	Mutations	Genes mutations	Number of Samples
HER2 Mix (1)	20	A775_G776insYVMA	2325_2326ins12 (TACGTGATGGCT)	3
HER2 Mix (2)	20	G776 > VC	2326_2327 ins3 (TGT)	2
		G776 > VC	2326_2327 ins3 (TTT)	
HER2 Mix (3)	20	P780_Y781insGSP	2339_2340 ins9 (TGGCTCCCC)	2
			2339_2340 ins9 (GGGCTCCCC)	
		P780_Y781insGSP		
HER2 Mix (4)	20	V777L	2329G > T	3
HER2 Mix (5)	19	L755P	2263_2264 TT > CC	2

Table 7: expression of oncoprotein HER-2 by IHC and RT-PCR.

IHC HER-2	RT-PCR Gene HER-2		Total	*P-value*
	Positive n (%)	Négative n (%)		
Positive	3 (20)	0 (00)	3	0,00003 (χ^2=12.25)
Négative	12 (80)	10 (100)	22	
Total	15 (100)	10 (100)	25	

Kappa coefficient = 0.16, poor agreement between the two tests.

However, this technique is still used empirically in resource-constrained countries such as Congo, leading to high proportions of false negatives. Molecular biology thus gives another alternative in the analysis of the expression of this oncoprotein, especially with the appearance of mutations at the level of the epitopes essential for the IHC technique [7-10]. It is with this in mind that this work has made it possible to evaluate the expression of the HER-2 oncoprotein by RT-PCR in a population of Congolese women with breast cancer.

In connection with the expression of oncoprotein HER-2, the IHC detected 3 positive cases out of 25 or 12% of the samples analyzed. These data are lower than those reported by Moudiongui et al. [20] in another study in Brazzaville which found a positivity of the oncoprotein HER2 at 37.5%. Bel Charlyne in France in 2019 [21] reported the presence of HER2 in 68% cases.

The low percentage of HER-2 positivity in our study population suggests two possible reasons : (i) either they are true negatives, the conditions have been well met from the sampling to the realization of the IHC technique; ii) either they are false negatives because of the IHC technique which is still widely practiced empirically in our country or, biopsies once taken, are often put in unbuffered formalin and spend more than 72 hours without being analyzed, which can strongly impact the epitopes resulting in these false negatives , or somatic mutations widely described at some of the exons, leading to a change in conformation of the HER-2 protein and during the antigen-antibody reactions of the IHC inducing false negatives [10].

However, HER-2 positive patients are eligible for her-2 antibody targeted therapy for example, although the presence of overexpression of HER2 in breast cancers is a sign of poor prognosis because the tumors are more aggressive, have greater metastatic potential and are less sensitive to hormonal treatments and/or chemotherapy [22].

Molecular amplification of the HER2 gene was observed in 15 cases out of 25 or 60% of the samples analyzed. These results made it possible to highlight a great divergence from those observed at the IHC which reported on the same population as 12% of positivity. In addition, 12 or 80% of PCR-positive cases were negative for HCI. With a kappa at 0.16 reflecting a poor concordance between these two techniques in the overexpression of HER-2, the low positivity observed at the IHC would reflect false negatives due to several causes mentioned above. The use of a mutation detection kit identified a high proportion of mutations in our study population. Indeed, 60% detection of HER2 by RT-PCR was obtained against 12% by the IHC. These observations confirm the results obtained at the IHC in particular the low positivity and show the importance of molecular biology in the expression of her-2 gene in breast cancer in Congo.

Moreover, this divergence of the overexpression of HER-2 observed can also be explained by the tumor heterogeneity of breast cancer and the HER2 gene described in the literature which is defined by the coexistence, within the same carcinomatous lesion, of several subpopulations of tumor cells with a different status of the HER2 gene. According to studies, it is found in 11 to 40% of breast cancers, [23,50]. There are currently 2 types, a form called "cluster", characterized by the presence of a population of contiguous cells with amplification of HER2 grouped in a specific geographical area within the tumor, well separated from tumor cells without amplification of HER2, and a form, called "mosaic", characterized by the presence of cells with amplification of HER2 scattered within a majority population of cells without amplification of HER2 [23]. Intra tumor heterogeneity may explain the discrepancies observed regarding the status of HER2 between the biopsy and the operating room or between 2 different blocks of the same operating room. It can also explain the inter-observer discrepancies in HIS, in the establishment of the average count of HER2 signals per nucleus or the RATIO HER2 / CEP17, depending on the part of the tumor analyzed. Little is known about the prognostic significance of this phenomenon and the sensitivity of different clones of the same tumor to anti-HER2 therapies. Intra-tumor heterogeneity is believed to be associated with a high histological grade and polysomy of chromosome 17. A study also showed that if less than 30% of tumor cells were amplified in a tumor, this had no prognostic impact [24]. Our results are discordant from those of Lehmann et al. [25] in England who rather demonstrated in 2011 a strong convergence in the order of 97% between the IHC and the PCR in real time. Bel Charlyne in France in 2019 [21] demonstrated a passage of 68% of the oncoprotein HER2 to the IHC to 92% of the HER2 gene to molecular biology, an average of 24% more positivity, this result is close to ours. Two other studies were carried out by Rosa et al. [26] and Olsson et al. [27] the concordance between IHC and q RT-PCR was observed in 59 cases out of 75 (78.7%). In the second, there was an 86% match rate between real-time PCR and IHC. Although there are several techniques to determine the expression of the HER2/neu gene; q RT-PCR is more convenient, easier and faster than IHC, FISH and CISH.

As for the kit used, it made it possible to describe the mutations of this gene (HER2) in other types of cancer including lung cancer. Indeed, in several studies conducted in China using the same kit, conducted respectively by Li X and al in 2016 [28]; Wang et al. in 2017 [29] and Ma Y et al. in 2020 [30] studied the 13 mutations in the gene during lung cancer.

According to existing data, the probability of HER-2 mutations is 1.67% in breast cancer, 1 - 4% in lung cancer and 2.9% in colorectal cancer [31-33]. The gene encoding HER-2 is located in chromosome 17 and encodes an 185 kPa protein that functions

as a transmembran growth factor receptor [34]. The intracellular domain of HER-2 contains about 500 residues and is composed of three parts: a cytoplasmic membrane juxta linker, a tyrosine kinase domain (TK) and a carboxyl-terminal tail [35,36].

In our study all mutations were observed in the Tyrosine Kinase (TK) domain: 83.3% of the mutations concerned exon 20 and 16.7% concerned exon 19. These results are in line with the data of the literature related to mutations of the HER2 gene which are mainly localized in the three exons [18,19,37] of the TK domain [3,10,38] and are encoded by dna sequences in exons 18 - 23 [39]. Mutations in the HER-2 kinase domain can be classified as follows: missense point mutations, small insertions in the frame or duplications that occur almost in exon 20 and in frame releases. Among these mutations, inframe insertions or duplications in exon 20 are the most frequently encountered types of mutations [40,41]. The publication of next-generation sequencing data indicated that somatic mutations in HER2 are present in about 2-5% of primary breast cancers [42-47]. Most HER2 somatic mutations have been reported in her2 breast cancers with negative amplification [48,49]. These mutations are proportionally more frequent in lobular cancers than ductal cancers. They mainly concern the extracellular domain of the HER2 gene (exon 8, residues 309-310) in 20% of cases and the tyrosine kinase domain (exons 19-20, between residues 755-781) in 68% of cases. Most mutations in the HER2 gene have been detected in exons 19 and 20 of the tyrosine kinase domain (TK), at the level of the C- α position of the protein helix [49]. Several authors propose that mutations in this area could be an alternative mechanism to her2 activation and effective sensitivity to anti-HER2 therapy, as a mechanism of acquired resistance to this form of therapy. The TK mutations described to date in HER2+ breast cancer promote the activation of protein functionality and increase the oncogenicity of HER2, in addition to inducing the phosphorylation of other cell signaling proteins [49,27,50]. Some of these mutations are activators (HER2 G309A, D769H, D769Y, V777L, P780-Y781insGSP, V842I, R896C, G309E and S310F), others would confer resistance to lapatinib (HER2 L755S), still others would be of unknown significance. These findings may explain the clinical response to anti-Her2 therapies in some patients, whose tumours are commonly considered Her2-negative by the IHC and HIS [9].

In addition, some authors have attributed somatic mutations to HER2, a role in resistance to anti-HER2 treatment because differential regulation of HER2 was observed in patients [3,51-55]. Recently, data from preclinical and clinical studies have attributed somatic mutations in HER2, a role in constitutive expression [54,55] or differential regulation of HER2 that leads to resistance (primary or acquired) to anti-HER2 therapy and endocrine therapy [51,53]. Such mutations therefore compromise the clinical benefits of HER2 targeted therapy in patients with HER2-positive breast cancer [10,56]. If these mutations prove clinically significant in future therapeutic trials, it will imply a change in the way we approach HER2 tests in breast cancer.

Limitations of the study: In addition to the small size of our study population, the correlation of these two variables made it possible to note that 12 samples previously negative to the IHC, after realization of RT-PCR by the kit "AmoyDx® HER2 Mutation Detection Kit" became positive with a statistically significant difference p =0.00003. The discrepancy between these methodologies could include inter-observer errors, due to the subjectivity of IHC interpretation and RT-PCR analysis, which can cause deviations in particular in the initial cycles, which depend not only on the melting temperature of the amplicon, but also on the behavior of the amplicon [57]. Using PCR-based methods, the expression of a specific tumor gene or tissue and the presence of genetic abnormalities can be detected in a clinical sample with higher sensitivity (one malignant cell out of 106-107 normal cells) than that of other techniques such as optical microscopy (one malignant cell out of 102-103 normal cells). Using RT-PCR, nucleic acid molecules can be amplified 1010 times [58].

Nistor et al. [59] conclude from their results, obtained from HER2 gene amplification, that real-time PCR combined with the IHC approach for determining HER2 status in breast cancer patients may be an effective and efficient strategy, but HER2 detection using qPCR was more accurate and reproducible compared to HCI.

Conclusion

This study focused on the great biological heterogeneity of HER2 in breast cancer and the need to implement reproducible and sensitive tests such as RT-PCR to measure the low expression of HER2 observed with the IHC technique.

Amplification of the HER2 gene, is still considered a major mechanism of HER2-induced tumorigenesis and is used as the main predictive biomarker to identify patients who could benefit from treatment with anti-HER2 agents.

Thus, the IHC should be considered as an initial screening strategy to distinguish between positive and negative cases of HER2 amplification; on the other hand, molecular biology through RT-PCR must remain by far the examination of choice for determining the profile of the HER2 gene, in order to effectively improve the therapeutic and personalized management of breast cancer patients.

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