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Research article

Correlation of Histological type, Grade and Results of IHC and SISH regarding Cerb2-HER2/neu Amplification

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SUMMARY. Breast cancer is one of the most common causes of death worldwide. The increasing incidence of breast cancer and its associated mortality highlights the need for new diagnostic procedures and immunohistochemical techniques improving the therapeutic management, especially the targeted treatment. To highlight and assess the expression of oncogene HER2, regarding to the results of Immunohistochemistry (IHC) scoring 2+ and Silver DNA in Situ Hybridization (SISH) technique and identify breast cancer type, special histological types, grade, and the correlation regarding to histological type, grade, and other biomarkers such as estrogen and progesterone receptors, and Ki-67.

Study design: Immunohistochemical detection of HER2 amplification in 151 formalin-fixed paraffin embedded tissue blocks of female patients of Pathological Anatomy Laboratory of Hippokration General Hospital with breast carcinoma and median age 60.3 years. 78 cases with equivocal score 2+ HER2 of IHC underwent in SISH technique to determine the HER2 gene amplification status; amplified or not.

The correlation of HER2 gene status by the SISH technique was made with different parameters including histological type, Grade, ER, PR, and Ki-67 immunoreactivity.

Results: 16 histological types of breast cancer were recognized with the most common histological type of Invasive Ductal Carcinoma Non Special Type (NST) which was found in 98 cases (64.9%), followed by Pleomorphic Invasive Lobular Carcinoma (6.6%), Ductal Invasive Carcinoma with Medullary Features (6%), Classic Invasive Lobular Carcinoma (4%), Ductal in situ carcinoma with microinvasion (4%) and Mucinous Invasive Carcinoma (3.3%), while other histological types were found in one or two breast cancer cases (0.7% to 1.3 %).

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The most common Grade was Grade III (64.4%), followed by Grade II (3.5%), and Grade I (2.1%).

Regarding the association of breast histological type and Grade, the IDC NST carcinoma which consists the 64.9% of our breast cancer cases, showed a significant association with Grade III (39.7%) and Grade II (23.2%), while the association with Grade I, was very low (1.3%). Other histological types were Invasive Lobular Carcinoma (ILC) pleomorphic type (6% Grade III), IDC with medullary features (5.3% Grade III), Ductal Carcinoma in Situ (DCIS) with microinvasion (4% Grade III), ILC classic type (4% Grade II), and Mucinous Invasive Carcinoma (2% Grade II). Also, there was an association between IDC NST and positive >2% ER and PR receptors (57.6%) followed by ILC pleomorphic type (6.6%), and classic type ILC (4%). Regarding histological type and the proliferative marker Ki-67, there was also a significant association between IDC NST in >20% percentage of the marker (39.7%), followed by IDC with medullary features (6%) and ILC pleomorphic type (4%).

As it concerns the correlation of histological type of Breast Cancer and HER2 with the IHC technique IDC NST, is the only type that shows the larger number of HER2 amplification (IHC 2+ and 3+ scores) resulting in a 41.8% percentage, followed by ILC pleomorphic type and IDC with medullary features (9.2%) while the rest histological types showed an overall amplification 12.6%, an overall 21.8% including ILC pleomorphic type and IDC with medullary features. Also, IDC NST is the only histological type of the79 IHC 2+score cases which gave positive results with SISH in 8 breast cancer cases (10.25%).

Conclusions: Our study showed that from the 16 studied histological types of the 151 cases of breast cancer, IDC NST, is mainly associated with a higher grade of malignancy (Grade III), positive ER and PR receptors >2%, higher levels of biomarker of cellular proliferation Ki-67 >20%, higher amplification of HER2 41.8%, and finally, is the only histological type giving positive results of equivocal HER2 2+ IHC score with the SISH technique (10.25%).

Thus, the further study of grading, molecular mechanism of oncogene HER2 combined with the hormone receptors and biomarker Ki-67 of the histological type IDC NST may lead to the improvement of breast cancer therapy.

INTRODUCTION

Cancer is a major public health problem worldwide and is the second leading cause of death in the United States. Breast cancer is also one of the most common causes of death, worldwide. The increasing incidence of breast cancer and its associated mortality highlights the need for new therapeutic development, especially targeted treatment [1]. Breast cancer represents a significant global health challenge, most diagnosed cancer in the world with an estimated 2.26 million cases recorded in 2020. It is the leading cause of cancer mortality among females and historically considered to be a disease of developed countries, since over half of breast cancer diagnoses and two-thirds of breast cancer related deaths occurred in the less developed regions of the world in 2022 [2].

Breast cancer subtypes are biologically distinct and may have distinct etiologies [3, 4]. For the morphological study of breast cancer, is essential to specify whether the tumor is limited to the epithelial component of the breast or has invaded the surrounding stroma, and whether this tumor appeared in the mammary ducts or lobes [5]. However, in histopathological practice, cell type characteristics, number of cells, type, and location of secretion, immunohistochemical profile and architectural characteristics determine if the tumor is ductal or lobular, in addition to its sub-classifications, rather than its precise location in the mammary tissue [6, 7]. About 50% to 80% of newly diagnosed breast cancer cases are called invasive ductal carcinoma (IDC); the rest of the cases are classified as invasive lobular carcinoma (ILC) [8, 9].

Non invasive breast cancer, is a cancer that has not extended away from the lobule or ducts where is located [5, 10]. An example of a kind of non-invasive breast cancer is ductal carcinoma in situ. Ductal carcinoma in situ (DCIS), appears when atypical cells develop within the milk ducts, are in proximity to breast tissue and not extended outside. On the other hand, lobular carcinoma in situ (LCIS), is a less common type of breast cancer which develops into breast lobules without invasion of the basal cellular membrane and has not extended into the breast tissue [10, 11]. Lobular carcinoma in situ is usually identified as non-invasive breast cancer [8, 12].

Ductal carcinoma in situ, it is the most general type of non-invasive breast cancer limited to breast duct. An example of ductal carcinoma in situ is ductal comedo carcinoma [9, 13].

Infiltrating lobular carcinoma (ILC), is also recognized as invasive lobular carcinoma, originates from the breast lobules, and frequently extends to other areas of the body [14, 15]. Infiltrating ductal carcinoma (IDC) is also recognized as invasive ductal carcinoma. IDC originates from the milk ducts of the breast and infiltrates the duct wall, invading the breast fatty tissue and probably other parts of the body [8, 16].

The prevalence of breast cancer enhances quickly with increasing age [17]. Invasive breast cancer that extends to different organs of the body is also recognized as metastatic breast cancer [18]. Most common organs to which cancer cells are spread – metastasize are brain, bones, lungs, and liver.

IDCs can be classified as "no specific type" because these tumors do not present sufficient morphological characteristics to be determined as a characteristic histological type. On the other hand, IDCs can also be recognized as a "special type", if they present sufficient distinctive characteristics, and cellular and molecular behavior [9, 19].

The most common special histological types of breast cancer include medullary carcinoma, metaplastic carcinoma, apocrine carcinoma, mucinous carcinoma, cribriform carcinoma, tubular carcinoma, neuroendocrine carcinoma, classic lobular carcinoma, and pleomorphic lobular carcinoma, in addition to the non specific type of invasive ductal carcinoma, which constitutes most newly diagnosed cases [9].

Medullary carcinoma is an invasive breast cancer with medullary features [20]. Mucinous carcinoma recognized as colloid carcinoma, is an uncommon breast cancer developed by mucus-forming cancer cells. Females with mucinous carcinoma usually have a better prognosis than females with additional general histological types of invasive carcinoma [21]. Tubular carcinomas are a particular histological type of invasive breast carcinoma. Females with tubular carcinoma usually have also a better prognosis than women with additional general histological types of invasive carcinoma [22].

Inflammatory breast cancer occurs when cancer cells block the lymphatic vessels in skin covering the breast, causing the characteristic red, swollen appearance of the breast [23]. Paget's disease of the breast is also an uncommon type of breast cancer that usually shows visible changes to the nipple of the breast [24]. Phyllodes tumors are either benign or malignant uncommon breast tumors which develop in the connective tissue of the breast and may be treated by surgical removal [25, 26].

Breast cancer is at the present extensively reported that is a heterogeneous lesion with special subtypes distinguished by their different clinico-pathological characteristics, prognosis, and responses to therapy. Triple-negative breast cancer (TNBC) is a distinct breast cancer with characteristic clinical and histopathological features, described by the lack of progesterone (PR) and estrogen (ER) receptors, and human epidermal growth factor receptor 2 not overexpressed (ER-negative, PR-negative, HER2-negative) [4, 27]. Women with positive estrogen or progesterone receptors and those that overexpress the tyrosine kinase human epidermal growth factor receptor-2 (HER2), due to amplification of its encoding oncogene Cerb2 (HER2), can receive hormone therapy or targeted HER2 drugs, since the amplification of HER2 gene is associated with rapid progression of the disease, increased metastatic potential and resistance to tamoxifen. On the other hand, TNBC is a clinical problem because of its relatively poor prognosis, aggressive behavior, and lack of targeted therapies, leaving chemotherapy as the main treatment [27].

The discovery of targeted therapy against the HER2 gene has brought an effective treatment modality for breast cancer patients [28]. Immunohistochemistry (IHC) is the most frequently used, convenient and cost-effective initial test for HER2 protein expression. HER2 IHC results are generally divided to four scale scores (range, 0 to 3+), depending on the percentage of positive tumor cells and staining intensity. The US Food and Drug Administration (FDA) recommends that HER2 IHC scores of 0 and 1+ must be considered as HER2 negative and those with HER2 (3+) scores should be regarded as HER2 positive, while HER2 (2+) score invasive breast cancer is considered as equivocal. This must be further assessed by Fluorescent in Situ Hybridization (FISH), which is more accurate and reliable than IHC. However, the need for a skilled operator, long procedure, special equipment, and difficult preservation of slides for later review are the disadvantages [29].

A fully automated method, Silver In Situ Hybridization (SISH) may overcome the disadvantages of FISH as it has the same accuracy of FISH. It is performed by counting black signals, which are dot-like on the

conventional bright field light microscopy [30]. Advantages of SISH include high sensitivity for detection of single gene copies, quantifying DNA targets with high resolution, and tissue staining with high contrast for viewing the signal separately and determining tissue morphology [31].

Thus, the main scope of this research is to assess the expression of oncogene HER2, regarding to the results of IHC scoring 2+ and SISH technique and identify breast cancer type, special histological type, Grade, and the correlation regarding to histological type, Grade, and other biomarkers such as estrogen and progesterone receptors, and Ki-67.

MATERIALS AND METHODS

151 formalin-fixed paraffin embedded tissue blocks of female patients with breast carcinoma and median age 60.3 years (32 years the youngest and 95 years the oldest, Table 1a), were collected during the period from May 2020 to February 2022. All cases were studied by IHC. The ones with equivocal score 2+ HER2 underwent SISH technique in Pathological laboratory of Hippokration General Hospital of Athens, Greece to determine the HER2 gene amplification status; amplified or non-amplified.

All cases were studied by the IHC technique in the same laboratory for ER, PR HER2 and Ki-67 status, for positive or negative reactivity. The correlation of HER2 gene status by the SISH technique was made with different parameters including histological type, grade of carcinoma, ER, PR and Ki-67 immunoreactivity.

IHC for all 151 cases for HER2, ER, PR and Ki-67 was studied by the VENTANA-BenchMark-XT computerized automated system, using the ultraview Universal DAB Detection Kit. Three-micrometer-thickness tissue sections were used. The used antibodies were:

1. HER2 rabbit monoclonal antibody (clone Cerb2).
2. Estrogen receptor (ER) rabbit monoclonal antibody (clone SP1).
3. Progesterone receptor (PR) rabbit monoclonal antibody (clone 1E2).
4. Ki-67 cellular marker of proliferation (clone Mib-1).

The ultraview Universal DAB Detection Kit detects specific mouse and rabbit primary antibodies bound to an antigen for paraffin-embedded tissue sections. The specific antibody is located by a cocktail of enzyme labeled secondary antibodies (HRP Multimer). The complex is then visualized with hydrogen peroxide substrate and, 3'-diaminobenzidine tetrahydrochloride (DAB) chromogen, which produces a brown precipitate that is readily observed by light microscopy. The staining protocols followed for the four immunostains (HER2, ER, PR and Ki-67) were in accordance with standard staining protocols of VENTANA-BenchMark-XT computerized automated system for each antibody.

The IHC results and scores were recorded independently by two pathologists, followed by a common review for agreement.

- 0+ negative score
- 1+ negative score
- 2+ equivocal score
- 3+ positive score

Those of cases, scored with 2+, equivocal score, where put through SISH, so the final expression of HER2 could be evaluated.

HER2 amplification using SISH procedure was studied by the VENTANA-BenchMark-XT computerized automated system. 3 μm thickness tissue sections were used. In brief, the procedure combined two basic detection kits, HER2 & chromosome 17 DNA probes cocktail, and other supplementary bulk solutions and reagents:

1. INFORM HER2 Dual ISH DNA Probe Cocktail of HER2 DNA probe and chromosome 17 DNA probe.
2. ultraView SISH DNP (dinitrophenyl) Detection Kit for the HER2 DNA probe sequence.
3. ultraView Red ISH DIG (digoxigenin) Detection Kit for chromosome 17 DNA probe sequence.

Each detection kit used: a chemical compound that labels the DNA probes, primary antibody, secondary antibody (HRP Multimer), enzymes, and a series of chromogen reagents. DNP and DIG labeled probes are co-hybridized to their respective specific target DNA sequences (HER2 and chromosome 17, respectively) within the cell nuclei. Then detection of HER2 signals occurred first by ultraView SISH DNP Detection Kit, as a black intranuclear signal, followed by detection of

chromosome 17 signals by ultraView Red ISH DIG Detection Kit, as a red intranuclear signal.

The staining protocol was in accordance with standard dual ISH staining protocol of VENTANA-BenchMark-XT computerized automated system, a procedure roughly required eight and a half hours to complete.

The slides where independently reviewed by two pathologists. The HER2 black signals and chromosome 17 red signals were enumerated in tumor invasive cells nuclei, according to VENTANA HER2 SISH interpretation algorithm, and results were reported as a function of (HER2/chromosome 17) signals ratio to determine HER2 amplification status: HER2/chr17 ratio of ≥2.0 was considered amplified, while a ratio of <2.0 was considered as non-amplified.

DATABASE DESIGN-DATABASE CREATION-STATISTICAL ANALYSIS

The original database was created with the help of Microsoft Office Excel, then the data was encoded, and a new database was produced in SPSS [32] with the data of 151 breast cancer patients in whom the detection of the amplification of Her2/neu HPV was investigated by SISH method in Her2 2 + IHC score [31].

The research process included the descriptive and inductive analysis of 8 variables from the patients' medical record: 1) age, 2) histological type, 3) degree of malignancy, 4) estrogen receptors, 5) progesterone receptors, 6) Ki-67 cellular proliferation marker, 7) detection of HER2 amplification by IHC technique and, 8) detection of HER2 2 + IHC equivocal score by SISH. The analysis of the results resulted from the use of the distribution of percentage frequencies through Frequencies and Descriptive Statistics, the cross-referencing of variables to receive combined results using the Crosstabs method [32].

RESULTS

16 histological types of breast cancer were recognized according to Table 1b.The most common histological type was Invasive Ductal Carcinoma Non Special Type (NST) which was found in 98 cases (64.9%) followed by Pleomorphic Invasive Lobular Carcinoma (6.6%), Ductal Invasive Carcinoma with medullary features (6%), Invasive Lobular Carcinoma classic type (4%), Ductal in Situ Carcinoma with microinvasion (4%), and Mucinous Invasive Carcinoma (3.3%), while other histological types were found in percentages from 0.7% to2%.

The most common Grade of the 151 breast cancer cases was Grade III (64.4%), as a result of a tumor which looks different to normal breast cells and is usually fast growing likely to spread, followed by Grade II (33.5%), moderate Grade between Grade III and Grade I, and Grade I (2.1%), referring to slowly growing tumor and less likely to spread (Table 2).

Regarding the association of breast histological type and Grade, the IDC breast carcinoma NST, which consists the 64.9% of our breast cancer cases, according to Table 3, showed a significant association with Grade III 39.7% and Grade II 23.2 %, while the association with Grade I, was very low (1.3%). Other histological types regarding Grade II and III, are Pleomorphic ILC (Grade III, 6%), IDC with medullary features (Grade III, 5.3%), DCIS with microinvasion (Grade III, 4%), ILC classic type (Grade III, 4%), and mucinous Invasive Breast Carcinoma (Grade II, 2%). Also, as it is observed in Tables 4 and 5, an association between IDC NST and positive >2% ER and PR receptors was found in 87 breast cancer cases (57.6%), followed by ILCs pleomorphic (6.6%) and classic type (4%), accordingly.

On the other hand, the same histological type, didn't show ER and PR receptors in 11 breast cancers cases (7.3%).

Our statistics showed that most cases (88.8%) of the 98 IDC NST were finally ER positive (+), similar with the 70-80% ER positive IDC NST breast cancer cases of recent bibliography [33]. Regarding histological type and the proliferative marker Ki-67, there was also an association between IDC NST in all percentages of the marker (1-9%, 10-19%, >20%), followed by pleomorphic and classic type ILCs carcinomas and IDC with medullary features (Table 6).

As it concerns the correlation of histological type of breast cancer and HER2 with the IHC technique (Table 7), IDC NST, is the only type that shows the larger number (63) of HER2 amplification (figures 1, 2, 5, 6, 7), resulting in a 41.8% percentage, followed by 14 cases of ILC pleomorphic type and IDC with medullary features (9.2%) while the rest (13) histological types showed an overall amplification 12.6 %.

Those 79 cases, scored with 2+, an equivocal IHC score, were put through SISH, so the final

overexpression of HER2 could be evaluated positive or negative.

According to Table 8, the only histological type of IHC 2+ score which gave positive results with SISH (figures 3, 4, 8, 9) was the IDC NST, in 8 breast cancer cases (10.25%).

Our final correlation between histological type and the final expression of HER 2 with SISH method confirmed that is present in finally 25 breast cancer cases (16.5 %), according to the approximately 15 - 20% of breast tumors mentioned by the recent bibliography [34] but the most interesting result of this study is that all the valid HER2 positive cases discovered with SISH method, were from the subtype IDC NST.

DISCUSSION

The role of Grading, hormone receptors ER and PR, biomarker Ki-67 and oncogene HER2 plays a crucial role for patients with breast cancer. Good Hematoxylin counter stain slide sections and the classic IHC are the first weapons to determine with great accuracy the type and subtype of breast cancer. IHC is a good and low cost method applied to determine all the above biomarkers.

New technologies for the determination of the equivocal expression (IHC score 2+) of oncogene HER2 such like SISH, give a perfect and clear result which is important, because patients can take targeted therapy with trastuzumamb.

In our study all the results are close to the worldwide bibliography. Most of our cases were IDC NST (98/151). Cases with high Grade of malignancy, Grade III (62.3%), according to Table 3, had high cell proliferation, high Ki-67>20% (59.6%), according to Table 6. IDC NST cases had overexpressed ER and PR receptors (88.8%), according to Tables 4 and 5. From 151 breast cancer cases 79 (52.3%) had the equivocal HER2 score 2+ result in IHC and most of them were also from the same histological type, IDC NST (35.8%), according to Table 7. We performed SISH technique to those 79 cases and we found that finally only 8 from the whole 79 cases had the oncogene HER2 amplified. The interesting finding of this research is that those 8 amplified HER2 cases where from the same subtype of IDC NST (10.25% of all equivocal 2+ IHC HER 2 cases and 5.3% of all breast cancer cases), according to Table 8. These results are also following the recent bibliography. Hormone PR receptor is playing a significant role in breast cancer survival. Based on the hormone receptor, the overall survival rate with positive hormone PR/ER receptor has a better chance of improved survival [34, 35].

Biomarker Ki-67 is high in high grade carcinomas. Ki-67 expression is higher in hyperplastic enlarged lobular units than in adjacent normal terminal duct lobular units [36] and is related to the subsequent risk of breast cancer [37]. The exclusive Ki-67 expression pattern with ER is disrupted during breast carcinogenesis [38, 39]. The statistical analysis of correlation between histological type and oncogene HER2 showed a result that is synchronized with the latest bibliography, 10-20% of IDCs NST had the ERBB2 (HER2) gene amplified [34, 40].

CONCLUSIONS

From all studied breast cancer cases with IHC 2+ score most of them were finally HER2 non amplified by SISH method (89.7%), while only eight cases of the IDC NST histological type (10,25%) were positive. This result is important to be taken into consideration by oncologists during their management planning of patients with breast cancer. Amplified oncogene HER2 is associated with negative ER and PR status that affects patients' management therapy protocols [41].

Our study showed that from the 16 studied histological types of the 151 cases of breast cancer, IDC NST, is mainly associated with a higher grade of malignancy (Grade III), positive ER and PR receptors >2%, higher levels of biomarker of cellular proliferation Ki-67 >20%, higher amplification of HER2 especially in IHC score 2+, and finally, is the only histological type giving positive results of equivocal IHC HER2 score 2+ with the SISH technique, helping the targeted breast cancer therapy.

The further study of grading, the molecular mechanism of oncogene HER2 combined with the hormone receptors and biomarker Ki-67 of the histological type IDC NST may lead to a major therapy of breast cancer.

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TABLES

Table 1a: Age incidence of Breast Cancer

	Female age	Frequency	Percent	Valid Percent	Cumulative Percent
Valid	30-40	6	4.0	4.0	4.0
	41-50	38	25.2	25.2	29.1
	51-60	41	27.2	27.2	56.3
	61-70	26	17.2	17.2	73.5
	71-80	27	17.9	17.9	91.4
	81-90	12	7.9	7.9	99.3
	91-100	1	0.7	0.7	100.0
	Total	151	100.0	100.0	

Table 1b: Prevalence of Breast Cancer Histological Types

	Frequency	Percent	Valid Percent	Cumulative Percent
Missing Data	2	1.3	1.3	1.3
ILC classic	6	4.0	4.0	5.3
DCIS with microinvasion	6	4.0	4.0	9.3
IDC with medullary features	9	6.0	6.0	15.2
IDC with squamous differentiation	2	1.3	1.3	16.6
IDC Non Special Type (NST)	98	64.9	64.9	81.5
IDC with apocrine differentiation	1	0.7	0.7	82.1
LPBC (Lymphocytic Predominant Breast Cancer)	2	1.3	1.3	83.4
IBC Micopapillary type	2	1.3	1.3	84.8
IBC Mucinous type	5	3.3	3.3	88.1
IBC Multifocal classic type	1	0.7	0.7	88.7
Multifocal ILC lassic type and IDC Non Special Type (NST)	1	0.5	0.7	89.4
DCIS multifocal with apocrine features	1	0.5	0.7	90.1
Multifocal ILC pleomorphic type	1	0.5	0.7	90.7
IBC Papillary type	1	0.5	0.7	91.4
ILC pleomorphic type	10	4.9	6.6	98.0
Residual IBC	3	1.5	2.0	100.0
Total	151	74.4	100,0	

Table 2. Prevalence of Breast Cancer Grade

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Missing Data	5	3.3	0.0	0.0
	Grade I	3	2.0	2.1	2.1
	Grade II	49	32.5	33.5	35.6
	Grade III	94	62.3	64.4	100.0
	Total	151	100.0	100.0	

Table 3. Correlation of Histological type of Breast Cancer and Grade

Histological Type		Grade of malignancy				
		Missing Data	Grade I	Grade II	Grade III	Total
Missing Data*	Count	1	0	1	0	2
	% of Total	0,7%	0,0%	0,7%	0,0%	1,3%
ILC classic	Count	0	0	6	0	6
	% of Total	0,0%	0,0%	4,0%	0,0%	4,0%
DCIS with microinvasion	Count	0	0	0	6	6
	% of Total	0,0%	0,0%	0,0%	4,0%	4,0%
IDC with medullary features	Count	0	1	0	8	9
	% of Total	0,0%	0,7%	0,0%	5,3%	6,0%
IDC with squamous differentiation	Count	0	0	0	2	2
	% of Total	0,0%	0,0%	0,0%	1,3%	1,3%
IDC Non Special Type (NST)*	Count	1	2	35	60	98
	% of Total	0,7%	1,3%	23,2%	39,7%	64,9%
IDC with apocrine differentiation	Count	0	0	0	1	1
	% of Total	0,0%	0,0%	0,0%	0,7%	0,7%
LPBC (Lymphocytic Predominant Breast Cancer)*	Count	2	0	0	0	2
	% of Total	1,3%	0,0%	0,0%	0,0%	1,3%
IBC Micopapillary type	Count	0	0	0	2	2
	% of Total	0,0%	0,0%	0,0%	1,3%	1,3%
IBC Mucinous type	Count	0	0	3	2	5
	% of Total	0,0%	0,0%	2,0%	1,3%	3,3%

IBC Multifocal classic type	Count	0	0	1	0	1
	% of Total	0,0%	0,0%	0,7%	0,0%	0,7%
Multifocal ILC classic type and IDC Non Special Type (NST)	Count	0	0	1	0	1
	% of Total	0,0%	0,0%	0,7%	0,0%	0,7%
DCIS multifocal with apocrine features	Count	0	0	0	1	1
	% of Total	0,0%	0,0%	0,0%	0,7%	0,7%
Multifocal ILC pleomorphic type	Count	0	0	0	1	1
	% of Total	0,0%	0,0%	0,0%	0,7%	0,7%
IBC Papillary type	Count	0	0	1	0	1
	% of Total	0,0%	0,0%	0,7%	0,0%	0,7%
ILC pleomorphic type	Count	0	0	1	9	10
	% of Total	0,0%	0,0%	0,7%	6,0%	6,6%
Residual IBC*	Count	1	0	0	2	3
	% of Total	0,7%	0,0%	0,0%	1,3%	2,0%
Total	Count	5	3	49	94	151
	% of Total	3,3%	2,0%	32,5%	62,3%	100,0%

The pathology wasn't available for review
ILC: Invasive Lobular Carcinoma IDC: Invasive Ductal Carcinoma
DCIS: Ductal Carcinoma in Situ
IBC: Invasive Breast Carcinoma

Table 4. Correlation of Histological type of Breast Cancer and ER

Histologic Type		Estrogen Receptors			
		Missing Data	Negative (-) < 2%	Positive (+) > 2%	Total
Missing Data*	Count	0	1	1	2
	% of Total	0,0%	0,7%	0,7%	1,3%
ILC classic	Count	0	0	6	6
	% of Total	0,0%	0,0%	4,0%	4,0%
DCIS with microinvasion	Count	0	1	5	6
	% of Total	0,0%	0,7%	3,3%	4,0%
IDC with medullary features	Count	0	5	4	9
	% of Total	0,0%	1,3%	0,0%	1,3%
IDC with squamous differentiation	Count	0	2	0	2
	% of Total	0,0%	1,3%	0,0%	1,3%
IDC Non Special Type (NST)	Count	0	11	87	98

	% of Total	0,0%	7,3%	57,6%	64,9%
IDC with apocrine differentiation	Count	0	0	1	1
	% of Total	0,0%	0,0%	0,7%	0,7%
LPBC (Lymphocytic Predominant Breast Cancer)*	Count	2	0	0	2
	% of Total	1,3%	0,0%	0,0%	1,3%
IBC Micopapillary type	Count	0	0	2	2
	% of Total	0,0%	0,0%	1,3%	1,3%
IBC Mucinous type	Count	0	0	3	5
	% of Total	0,0%	2,0%	1,3%	3,3%
IBC Multifocal classic type	Count	0	0	1	1
	% of Total	0,0%	0,0%	0,7%	0,7%
Multifocal ILC classic type and IDC Non Special Type (NST)	Count	0	0	1	1
	% of Total	0,0%	0,0%	0,7%	0,7%
DCIS multifocal with apocrine features	Count	0	1	0	1
	% of Total	0,0%	0,7%	0,0%	0,7%
Multifocal ILC pleomorphic type	Count	0	0	1	1
	% of Total	0,0%	0,0%	0,7%	0,7%
IBC Papillary type	Count	0	0	1	1
	% of Total	0,0%	0,0%	0,7%	0,7%
ILC pleomorphic type	Count	0	0	10	10
	% of Total	0,0%	0,0%	6,6%	6,6%
Residual IBC	Count	0	2	1	3
	% of Total	0,0%	1,3%	0,7%	2,0%
Total	Count	2	26	123	151
	% of Total	1,3%	17,2%	81,5%	100,0%

**The pathology wasn't available for review*
IDC NST: Invasive Ductal Carcinoma Non Special Type
LBPC: Lymphocytic Predominant Breast Cancer

Table 5. Correlation of Histological type of Breast Cancer and PR

Histologic Type		Progesterone Receptors			
		Missing Data	Negative (-) < 2%	Positive (+) > 2%	Total
Missing Data*	Count	0	1	1	2
	% of Total	0,0%	0,7%	0,7%	1,3%
ILC classic	Count	0	0	6	6

	% of Total	0,0%	0,0%	4,0%	4,0%
DCIS with microinvasion2	Count	0	1	5	6
	% of Total	0,0%	0,7%	3,3%	4,0%
IDC with medullary features	Count	0	3	6	9
	% of Total	0,0%	2,0%	4,0%	6,0%
IDC with squamous differentiation	Count	0	2	0	2
	% of Total	0,0%	1,3%	0,0%	1,3%
IDC Non Special Type (NST)	Count	0	11	87	98
	% of Total	0,0%	7,3%	57,6%	64,9%
IDC with apocrine differentiation	Count	0	0	1	1
	% of Total	0,0%	0,0%	0,7%	0,7%
LPBC (Lymphocytic Predominant Breast Cancer)*	Count	2	0	0	2
	% of Total	1,3%	0,0%	0,0%	1,3%
IBC Micopapillary type	Count	0	0	2	2
	% of Total	0,0%	0,0%	1,3%	1,3%
IBC Mucinous type	Count	0	1	4	5
	% of Total	0,0%	2,0%	1,3%	3,3%
IBC Multifocal classic type	Count	0	0	1	1
	% of Total	0,0%	0,0%	0,7%	0,7%
Multifocal ILC classic type and IDC Non Special Type (NST)	Count	0	0	1	1
	% of Total	0,0%	0,0%	0,7%	0,7%
DCIS multifocal with apocrine features	Count	0	1	0	1
	% of Total	0,0%	0,7%	0,0%	0,7%
Multifocal ILC pleomorphic type	Count	0	0	1	1
	% of Total	0,0%	0,0%	0,7%	0,7%
IBC Papillary type	Count	0	0	1	1
	% of Total	0,0%	0,0%	0,7%	0,7%
ILC pleomorphic type	Count	0	0	10	10
	% of Total	0,0%	0,0%	6,6%	6,6%
Residual IBC	Count	0	2	1	3
	% of Total	0,0%	1,3%	0,7%	2,0%
Total	Count	2	22	127	151
	% of Total	1,3%	14,6%	84,1%	100,0%

**The pathology wasn't available for review*

Table 6. Correlation of Histological type of Breast Cancer and Ki-67

Histological Type		Ki-67 proliferation marker				
		Missing Data	1-9%	10-19%	>20%	Total
Missing Data	Count	0	0	1	1	2
	% of Total	0,0%	0,0%	2,4%	1,1%	1,3%
ILC classic	Count	0	3	2	1	6
	% of Total	0,0%	2,0%	1,3%	0,7%	4,0%
DCIS with microinvasion	Count	0	2	2	2	6
	% of Total	0,0%	1,3%	1,3%	1,3%	4,0%
IDC with medullary features	Count	0	0	0	9	9
	% of Total	0,0%	0,0%	0,0%	6,0%	6,0%
IDC with squamous differentiation	Count	0	0	0	2	2
	% of Total	0,0%	0,0%	0,0%	1,3%	1,3%
IDC Non Special Type (NST)	Count	0	11	27	60	98
	% of Total	0,0%	7,3%	17,9%	39,7%	64,9%
IDC with apocrine differentiation	Count	0	0	1	0	1
	% of Total	0,0%	0,0%	0,7%	0,0%	0,7%
LPBC (Lymphocytic Predominant Breast Cancer)*	Count	2	0	0	0	2
	% of Total	1,3%	0,0%	0,0%	0,0%	1,3%
IBC Micopapillary type	Count	0	0	1	1	2
	% of Total	0,0%	0,0%	0,7%	0,7%	1,3%
IBC Mucinous type	Count	0	1	1	3	5
	% of Total	0,0%	0,7%	0,7%	2,0%	3,3%
IBC Multifocal classic type	Count	0	0	1	0	1
	% of Total	0,0%	0,0%	0,7%	0,0%	0,7%
Multifocal ILC classic type and IDC Non Special Type (NST)	Count	0	0	0	1	1
	% of Total	0,0%	0,0%	0,0%	0,7%	0,7%
DCIS multifocal with apocrine features	Count	0	0	0	1	1
	% of Total	0,0%	0,0%	0,0%	0,7%	0,7%
Multifocal ILC pleomorphic type	Count	0	0	1	0	1
	% of Total	0,0%	0,0%	0,7%	0,0%	0,7%
IBC Papillary type	Count	0	0	0	1	1
	% of Total	0,0%	0,0%	0,0%	0,7%	0,7%
ILC pleomorphic type	Count	0	1	3	6	10

Residual IBC	% of Total	0,0%	0,7%	2,0%	4,0%	6,6%
	Count	0	0	1	2	3
Total	% of Total	0,0%	0,0%	0,7%	1,3%	2,0%
	Count	2	18	41	90	151
	% of Total	1,3%	11,9%	27,2%	59,6%	100,0%

**The pathology wasn't available for review*

Table 7. Correlation of Histological type of Breast Cancer and HER2 with the IHC technique

Histologic Type		Immunohistochemistry					
		Missing Data	HER2 0+	HER2 1+	HER2 2+	HER2 3+	Total
Missing Data	Count	0	0	0	1	1	2
	% of Total	0,0%	0,0%	0,0%	0,7%	0,7%	1,3%
ILC classic	Count	0	2	3	1	0	6
	% of Total	0,0%	1,3%	2,0%	0,7%	0,0%	4,0%
DCIS with microinvasion	Count	0	1	1	3	1	6
	% of Total	0,0%	0,7%	0,7%	2,0%	0,7%	4,0%
IDC with medullary features	Count	0	0	2	7	0	9
	% of Total	0,0%	0,0%	1,3%	4,6%	0,0%	6,0%
IDC with squamous differentiation	Count	0	0	0	0	2	2
	% of Total	0,0%	0,0%	0,0%	0,0%	1,3%	1,3%
IDC Non Special Type (NST)	Count	0	13	22	54	9	98
	% of Total	0,0%	8,6%	14,6%	35,8%	6,0%	64,9%
IDC with apocrine differentiation	Count	0	0	0	1	0	1
	% of Total	0,0%	0,0%	0,0%	0,7%	0,0%	0,7%
LPBC (Lymphocytic Predominant Breast Cancer)*	Count	2	0	0	0	0	2
	% of Total	1,3%	0,0%	0,0%	0,0%	0,0%	1,3%
IBC Micopapillary type	Count	0	0	0	1	1	2
	% of Total	0,0%	0,0%	0,0%	0,7%	0,7%	1,3%
IBC Mucinous type	Count	0	2	1	2	0	5
	% of Total	0,0%	1,3%	0,7%	1,3%	0,0%	3,3%
IBC Multifocal classic type	Count	0	1	0	0	0	1
	% of Total	0,0%	0,7%	0,0%	0,0%	0,0%	0,7%
Multifocal ILC classic type and IDC Non Special Type (NST)	Count	0	1	0	0	0	1
	% of Total	0,0%	0,7%	0,0%	0,0%	0,0%	0,7%

DCIS multifocal with apocrine features	Count	0	0	0	1	0	1
	% of Total	0,0%	0,0%	0,0%	0,7%	0,0%	0,7%
Multifocal ILC pleomorphic type	Count	0	0	1	0	0	1
	% of Total	0,0%	0,0%	0,7%	0,0%	0,0%	0,7%
IBC Papillary type	Count	0	0	0	1	0	1
	% of Total	0,0%	0,0%	0,0%	0,7%	0,0%	0,7%
ILC pleomorphic type	Count	0	0	3	6	1	10
	% of Total	0,0%	0,0%	2,0%	4,0%	0,7%	6,6%
Residual IBC	Count	0	0	0	1	2	3
	% of Total	0,0%	0,0%	0,0%	0,7%	1,3%	2,0%
Total	Count	2	20	33	79	17	151
	% of Total	1,3%	13,2%	21,9%	52,3%	11,3%	100,0%

*The pathology wasn't available for review.
IHC: Immunohistochemistry

Table 8. Correlation of Histological type of Breast Cancer and Silver in Situ Hybridization results

Histological Type		SISH				
		Missing Data	Negative (-)	Positive (+)	Not evaluated	Total
Missing Data	Count	0	1	0	1	2
	% of Total	0,0%	0,7%	0,0%	0,7%	1,3%
ILC classic	Count	0	1	0	5	6
	% of Total	0,0%	0,7%	0,0%	3,3%	4,0%
DCIS with microinvasion	Count	0	3	0	3	6
	% of Total	0,0%	2,0%	0,0%	2,0%	4,0%
IDC with medullary features	Count	0	7	0	2	9
	% of Total	0,0%	4,6%	0,0%	1,3%	6,0%
IDC with squamous differentiation	Count	0	0	0	2	2
	% of Total	0,0%	0,0%	0,0%	1,3%	1,3%
IDC Non Special Type (NST)*	Count	1	45	8	44	98
	% of Total	0,7%	29,8%	5,3%	29,1%	64,9%
IDC with apocrine differentiation	Count	0	1	0	0	1
	% of Total	0,0%	0,7%	0,0%	0,0%	0,7%
LPBC (Lymphocytic Predominant Breast Cancer*	Count	2	0	0	0	2
	% of Total	1,3%	0,0%	0,0%	0,0%	1,3%

IBC Micopapillary type	Count	0	1	0	1	2
	% of Total	0,0%	0,7%	0,0%	0,7%	1,3%
IBC Mucinous type	Count	0	2	0	3	5
	% of Total	0,0%	1,3%	0,0%	2,0%	3,3%
IBC Multifocal classic type	Count	0	0	0	1	1
	% of Total	0,0%	0,0%	0,0%	0,7%	0,7%
Multifocal ILC classic type and IDC Non Special Type (NST)	Count	0	0	0	1	1
	% of Total	0,0%	0,0%	0,0%	0,7%	0,7%
DCIS multifocal with apocrine features	Count	0	1	0	0	1
	% of Total	0,0%	0,7%	0,0%	0,0%	0,7%
Multifocal ILC pleomorphic type	Count	0	0	0	1	1
	% of Total	0,0%	0,0%	0,0%	0,7%	0,7%
IBC Papillary type	Count	0	1	0	0	1
	% of Total	0,0%	0,7%	0,0%	0,0%	0,7%
ILC pleomorphic type	Count	0	6	0	4	10
	% of Total	0,0%	4,0%	0,0%	2,6%	6,6%
Residual IBC	Count	0	1	0	2	3
	% of Total	0,0%	0,7%	0,0%	1,3%	2,0%
Total	Count	3	70	8	70	151
	% of Total	2,0%	46,4%	5,3%	46,4%	100,0%

*The pathology wasn't available for review
SISH: Silver in Situ Hybridization

FIGURES

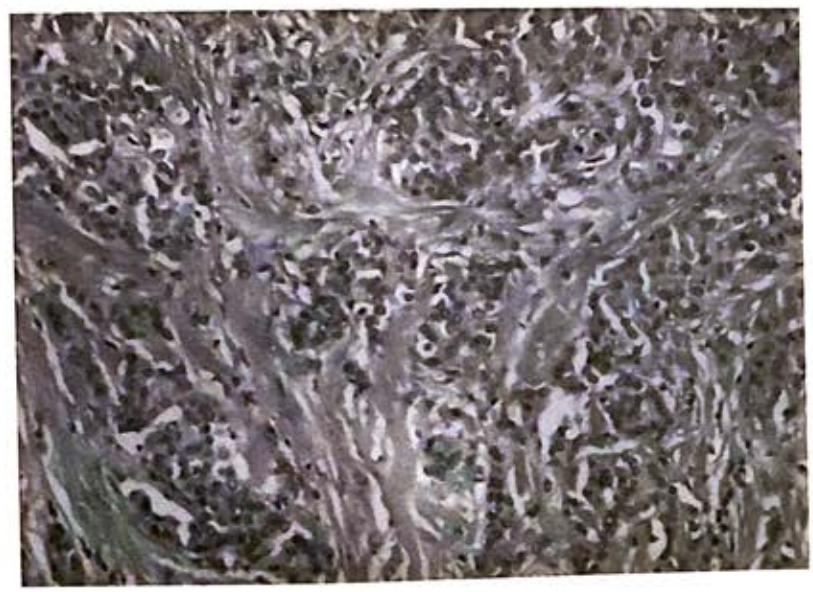

Figure 1. Invasive Ductal Carcinoma Non Special Type (NST), hematoxylin/eosin X20

Figure 2. Invasive Ductal Carcinoma Non Special Type (NST), IHC HER2 score 1+ X20

Figure 3. Invasive Ductal Carcinoma Non Special Type (NST), SISH (40X). Negative HER2 expression, intranuclear signals typically appear smaller than chromosome 17 red ISH intranuclear signals with an overall ratio of (black SISH signals/red ISH signals) <2.

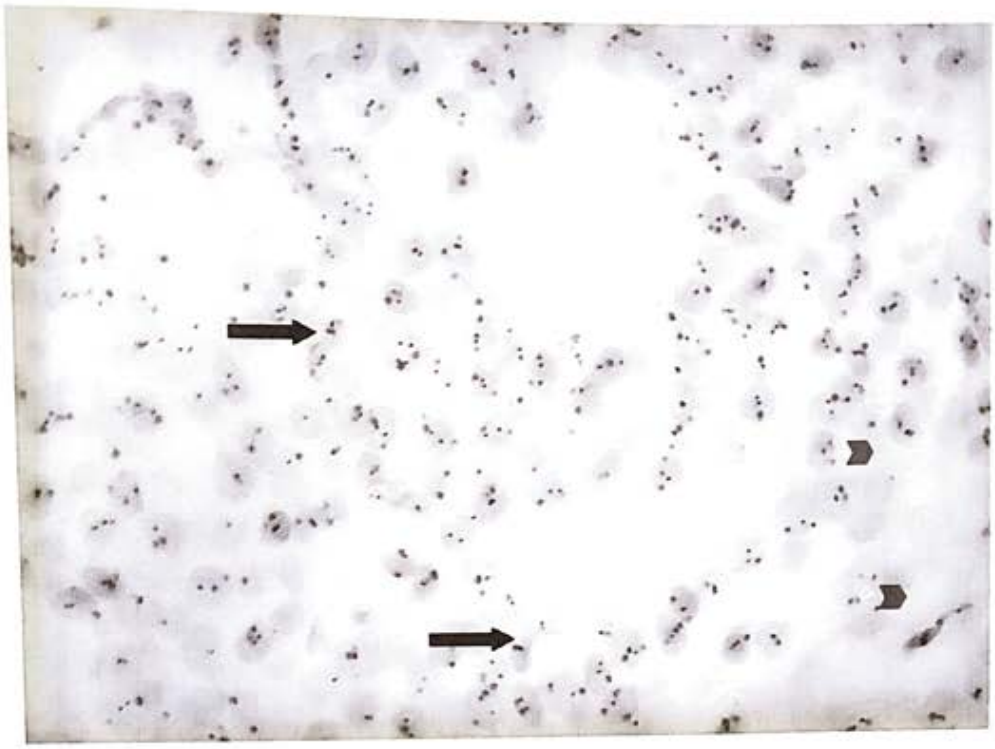

Figure 4. Invasive Ductal Carcinoma Non Special Type (NST), SISH (60X) Negative HER2 expression, intranuclear signals (arrows) typically appear smaller than chromosome 17 red ISH intranuclear signals (arrow heads) with an overall ratio of (black SISH signals/red ISH signals) <2.

Figure 5. Invasive Ductal Carcinoma Non Special Type (NST), hematoxylin/eosin (10X).

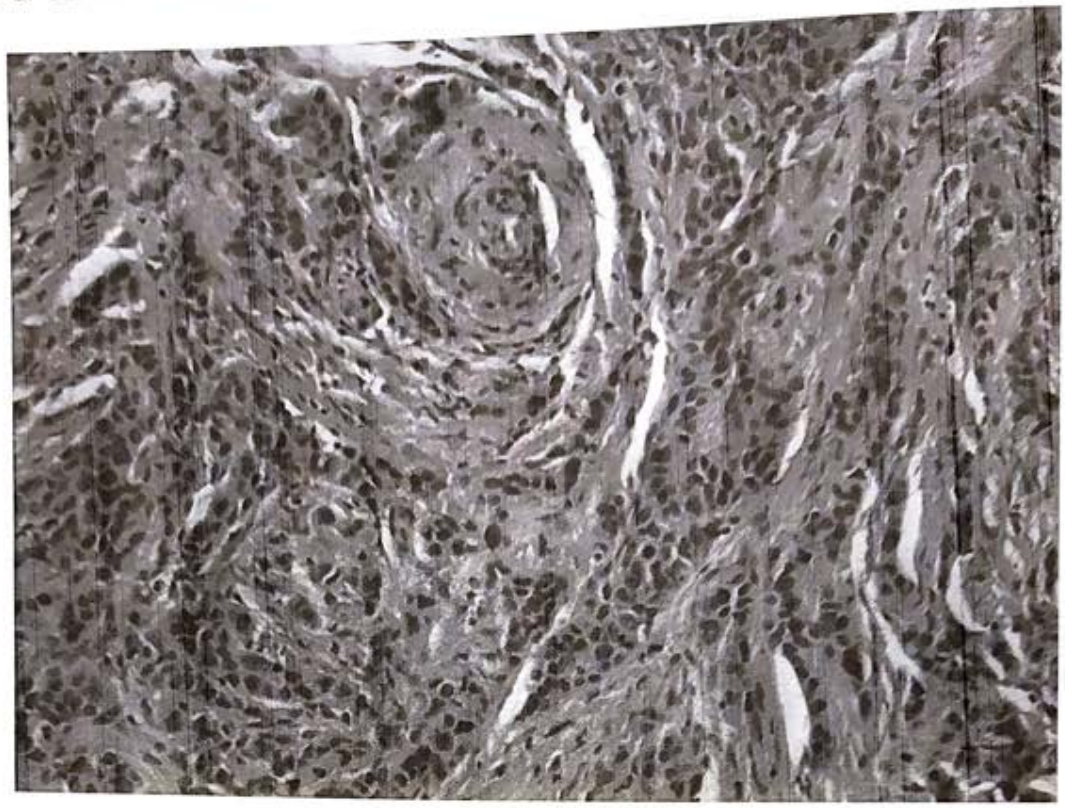

Figure 6. Invasive Ductal Carcinoma Non Special Type (NST), hematoxylin/eosin (20X).

Figure 7. Invasive Ductal Carcinoma Non Special Type (NST), IHC HER2 score 2+ (20X).

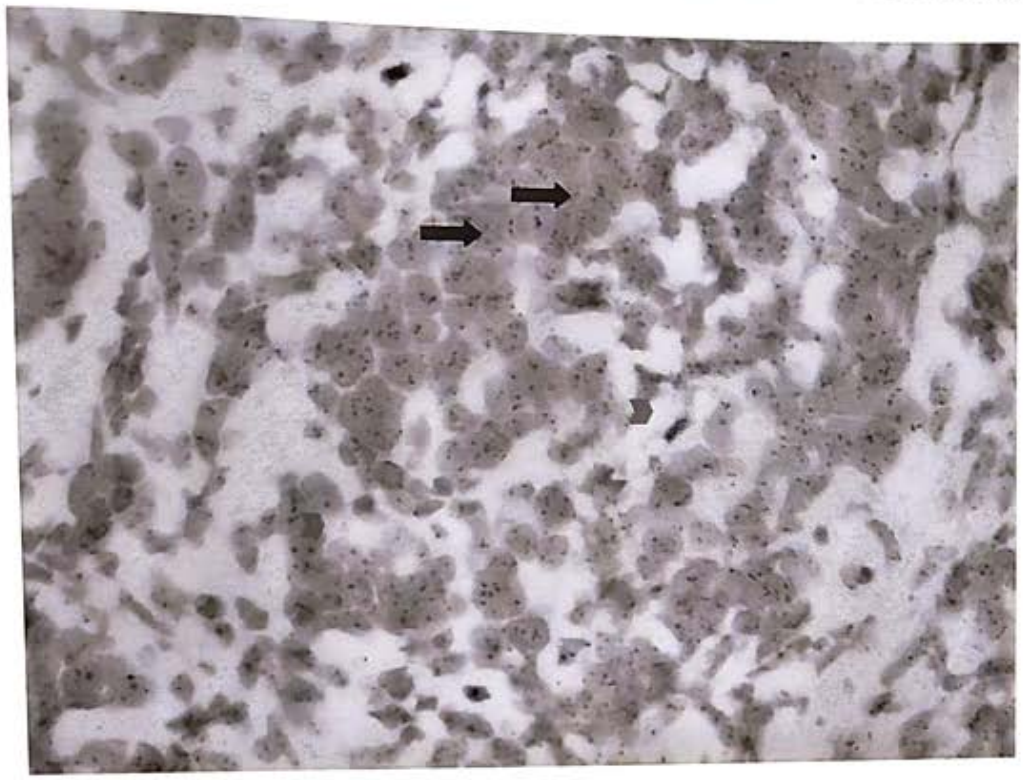

Figure 8. Invasive Ductal Carcinoma Non Special Type (NST), SISH (40X). Positive HER2 expression, HER2 gene black SISH intranuclear signals (arrows) typically appear smaller than chromosome 17 red SISH intranuclear signals (arrow heads) with an overall ratio of (black SISH signals/red ISH signals) >2.

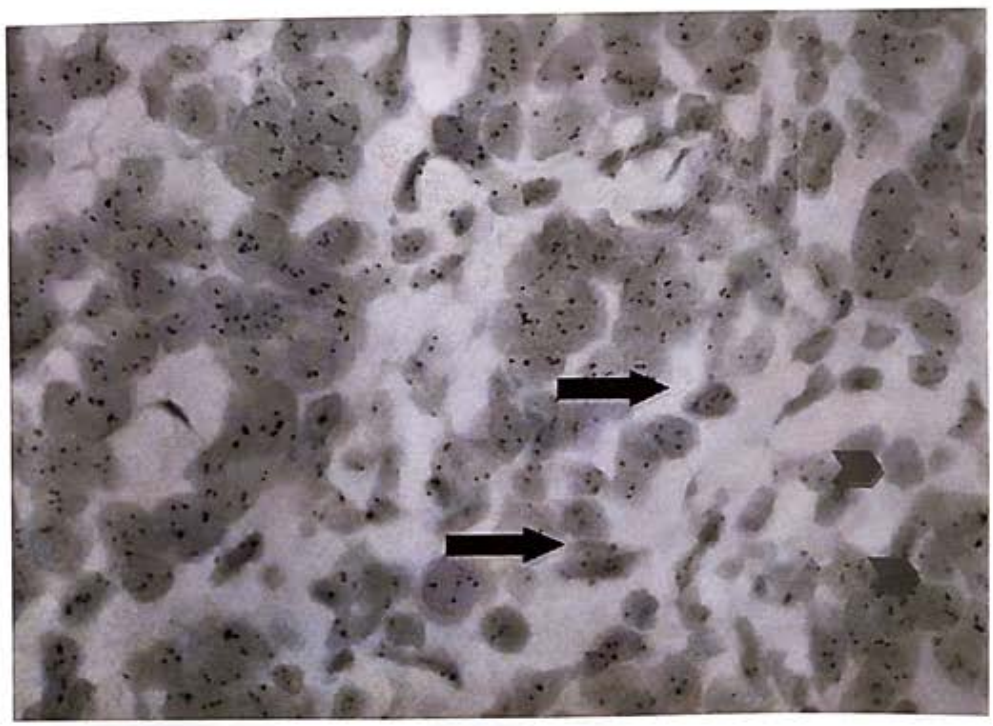

Figure 9. Invasive Ductal Carcinoma Non Special Type (NST), SISH (60X), Positive HER2 expression, HER2 gene black SISH intranuclear signals (arrows) typically appear smaller than chromosome 17 red SISH intranuclear signals (arrow heads) with an overall ratio of (black SISH signals/red ISH signals) >2.