Problems In Determining Her2 Status In Breast Carcinoma

Emel Ebru Pala, Ümit Bayol, Alp Özgüzer, Ülkü Küçük, Çağlar Yıldız Akdeniz, Özlem Sezer
Clinic of Pathology, İzmir Tepecik Training and Research Hospital, İzmir, Turkey

ABSTRACT

Objective: Human epidermal growth factor receptor 2 (HER2) oncoprotein is overexpressed in 15-25% of breast carcinomas and associated with poor outcome. Assessment of HER2 status accurately is important to select patients who will benefit from targeted therapy.

Materials and Methods: In this study immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) were used to determine the HER2 status in 308 breast carcinoma cases of which 129 were consultation. The major problems in determining HER2 status and the reasons of discordant results between methods were discussed.

Results: HER2 expression was (-) in 124, (+) in 29, (++) in 92, (+++) in 63 cases. 25 of 76 cases consulted as (++) were evaluated as (++) and 15 of 35 cases consulted as (+++) were evaluated as (+++). HER2 amplification was found in 88 (28.6%) of 308 cases by FISH. 3 of 124 (-), 1 of 29 (+), 22 of 92 (++) , 62 of 63 (+++) cases were amplified by FISH. The relation between HER2 expression and amplification was statistically significant ($p < 0.001$). Centromere 17 (CEN 17) region amplification was noted in 11 cases of which 2 were (+++), 9 were (++) . 6 of the 11 cases showed focal low level, 1 of them showed diffuse high level amplification.

Conclusion: The concordance rate between IHC (+++) cases and FISH was 95.4% for consultation cases, 100% for our cases. The final concordance rate for both case groups was 98.4%. The possible reasons of discrepancy were triple negativity, preanalytical and analytical procedures of consultation cases and trucut samples.

Keywords: Breast cancer, erbB genes, fluorescence in situ hybridization

Introduction

Human epidermal growth factor receptor 2 (HER2, *cerbB2*) gene that is located on the long arm of the 17th chromosome, encodes a trans-membrane surface receptor protein by intrinsic tyrosine kinase activity (1). HER2 protein shows structural homology to epidermal growth factor receptor (EGFR), and similar to EGFR is involved in cell proliferation (2). HER2 plays a role in the oncogenesis of different cancer types. Over-expression of HER2 (increase in HER2 receptors on cell membrane) is the result of gene amplification (an increase in HER2 gene copy number) by 95%, and is detected in approximately 15-25% of breast cancer cases (3, 4). For the first time in 1987, Slamon et al (5) concluded that HER2 amplification was together with decreased overall survival and disease-free survival in breast cancer patients with lymph node metastasis. HER2 status indicates response to chemotherapeutics, hormonal agents, recombinant human anti-HER2 antibody trastuzumab (Herceptin® Genentech, California, USA) and the dual HER1 / HER2 tyrosine kinase inhibitor lapatinib (Tykerb, GlaxoSmithKline, Philadelphia, USA) that positively influences clinical progress in advanced stage patients when combined with capecitabine (6). Trastuzumab is a human monoclonal antibody that is generated against the HER2 receptor. In tumors with HER2 overexpression, it binds to the extracellular part of the receptor, inhibits HER2 mediated signals, induces antibody-mediated cellular cytotoxicity and inhibits cell proliferation.

The most common routine methods for determining HER2 status are fluorescence in situ hybridization (FISH), silver in situ hybridization (SISH), chromogenic in situ hybridization (CISH) and immunohistochemistry (IHC) methods. The most important advantage of SISH and CISH methods is that they allow evaluation by light microscope. Although the issue on which method is the gold standard is still controversial, the compatibility between SISH /CISH and FISH is very high (7).

In this study, 308 breast cancer patients including consultations were evaluated for HER2 protein expression by IHC method and for HER2 / Chromosome17 (chr 17) gene region changes by FISH method, and we investigated the causes of discrepancy between these two methods. In addition, we evaluated reasons for discrepancy between our IHC results and results of the referring unit in consulted cases, and we examined

Address for Correspondence:

Emel Ebru Pala, Department of Pathology, İzmir Tepecik Training and Research Hospital, İzmir, Turkey
Phone: +90 232 469 69 69 e-mail: emelozkok@yahoo.com

Received: 12.06.2014

Accepted: 14.10.2014

pre-analytical, analytical and post-analytical processes that may influence HER2 IHC results.

Materials and Methods

A total 308 cases, including 179 cases who were consulted from other centers after evaluation of HER2 protein expression levels, 76 of which were (++), and 179 cases who were diagnosed at Tepecik Training and Research Hospital between 2008-2012, 57 of which were (++), were included into the study. According to the results of first evaluation 43% of 308 patients had (++) score. This study examined the compatibility between IHC and FISH as well as factors that influence IHC/FISH results, rather than reflecting the incidence of HER2 over-expression/amplification in our department.

In order to evaluate 308 cases by IHC and FISH, two 4 microns thick sections were obtained from paraffin blocks on lysine slides. In all cases, HER2 protein expression percentage and intensity were detected by using polyclonal rabbit anti-human HER2 antibody (Clone A0485, Dako®, Glostrup, Denmark, dilution 1/300) with the Auto-stainer Link 48 (Dako®, Glostrup, Denmark) fully automated IHC staining device. The FISH method was applied to all 308 cases by HER2 DNA and Chr 17 centromeric PNA probe mix (DAKO, Glostrup, Denmark). The slides were left in the incubator at 58°C for an hour, and passed through xylene-alcohol series for deparaffinization. The slides were incubated in pretreatment solution in 95°C water bath for 15 minutes, and were treated with pepsin after washing solution at 37°C for 4 minutes. After washing and dehydration process, 10µl probmix was dropped and slides were covered with 24x24 mm slide and coverslip sealent. Denaturation was performed with hybridiser apparatus (Dako, Glostrup, Denmark) at 82°C for 5 min, and hybridizations were performed at 45°C for 12 hours. In a water bath at 65°C, following 10 minutes of posthybridisation washing and dehydration, 15 µl DAPI was dropped and kept at +4°C for 30 min. The HER2 gene region was represented in red, and the chr 17 centromeric gene region was represented in green. The evaluations were performed with Olympus BX51 fluorescence microscope equipped with Texas Red, FITC and DAPI filter under x100 immersion objective. The American Society of Clinical Oncology and the College of American Pathologists (ASCO / CAP) 2007 criteria were used for interpretation of IHC and FISH results (Table 1, 2) (8). HER2/chr 17 centromere rate ≥ 5 was accepted as high-level amplification, and $2 < \text{HER2/chr 17} < 5$ was accepted as low-level amplification. The IHC and FISH results, both internal and external evaluation results, as well as histological subtype, tumor grade, estrogen and progesterone receptor (ER/PR) status have been documented. The reasons for discrepancy between IHC and FISH results were also discussed.

Statistical Analysis

Statistical analysis was performed with SPSS version 15.0 (SPSS Inc, Chicago, Illinois, USA). $p < 0.05$ was considered significant.

Results

The mean age was 53.7 (24-91), and the mean tumor diameter 2.84 cm (0.3-13cm). A statistically significant relationship was detected between increase in age and HER2 amplification ($p = 0.02$) (Table 3). 279 patients (90.7%) had invasive ductal carcinoma histology (Table 4), 125 (40.6%) were grade 2, and 178 (57.8%) were assessed as grade 3. The specimens were obtained by tru-cut / incisional biopsy in 14.5%, by excision in 51.9%, and by mastectomy in 33.6% of patients. IHC evaluation for ER and PR status could not be performed in eight patients due to inability to obtain sufficient lysine slides containing tumor tissue. 84 patients were ER (-), 109 were PR (-) while

Table 1. ASCO/CAP immunohistochemical HER2 evaluation protocol (2007)

Negative (0/+)	No staining or weak interrupted membranous staining
Equivocal (++)	Weak-moderate complete membranous staining (>10 tumor cells) or intense complete membranous staining in less than 30%
Positive (+++)	Uniform intense complete membranous staining in more than 30% tumor cell
ASCO/CAP; American Society of Clinical Oncology and the College of American Pathologists HER2; Human epidermal growth factor receptor 2 oncoprotein	

Table 2. ASCO/CAP(2007); Evaluation criteria for HER2 gene amplification

	Amplification (-)	Equivocal Amplification	Amplification (+)
HER2 gene copy number	<4	4-6	>6
HER2/CEP17 ratio	<1.8	1.8-2.2	>2.2
ASCO/CAP; American Society of Clinical Oncology and the College of American Pathologists HER2; Human epidermal growth factor receptor 2 oncoprotein CEP17; Chromosome 17 centromere			

216 were ER (+), and 191 PR (+). There was a statistically significant correlation between decreased ER / PR expression and HER2 over-expression ($p = 0.00$). In addition, as the percentage of PR decreased the incidence of HER2 amplification increased ($p = 0.00$). IHC evaluation for HER2 expression revealed 124 (40.3%) (-) cases, 29 (9.4%) (+), 92 (29.9%) (++), and 63 (20.5%) (+++). Twenty-five out of 76 patients who were previously identified as (++) in other centers were evaluated as (++) in our center, and 15 out of 35 cases who were previously identified as (+++) in other centers were evaluated as (+++) in our center. IHC results of patients consulted from external centers and our department are compared in Table 5. Amplification by FISH analysis was detected in only 17 of 35 patients who were previously evaluated as (+++) in other centers (Table 6). Focal low-level amplification was detected by FISH analysis in 3 patients out of 124 IHC (-) patients, one consultation and 2 of our cases (Figure 1), and diffuse amplification was detected in one case, our patient, out of 29 (+) cases (Figure 2). Amplification was observed in 22 of 92 (++) patients and 62 of 63 (+++) patients (Table 7). Amplification by FISH was not detected in one patient whose tru-cut biopsy was interpreted as IHC (+++) (Figure 3). The correlation between immunohistochemical HER2 expression levels and HER2 amplification was statistically significant ($p < 0.001$). Amplification was diffuse high-level in 67 of 88 patients, and focal low level in the remaining 21. Amplification was observed in centromeric gene region of chromosome 17 by FISH in 11 cases (Figure 4). Immunohistochemically 9 of these 11 were (++), and 2 were (+++). Diffuse HER2 amplification was noticed in one of these 11 patients, and focal amplification was detected in six patients.

Discussion and Conclusions

The ASCO / CAP guideline defined features of samples that cannot be evaluated by IHC method as specimens fixated with materials other than

Table 3. Age - HER2 amplification correlation (p=0.02)

	HER2 amplification	N	Mean	Std. Deviation	Std. Error Mean
age	positive	220	52.68	12.525	.844
	negative	88	56.51	13.108	1.397

HER2; Human epidermal growth factor receptor 2

Table 4. Histologic subtypes of tumors

Histologic subtype	Case number and percentage
Invasive ductal carcinoma	279 (90.7%)
Invasive lobular carcinoma	7 (2.4%)
Mixed carcinoma	6 (1.9%)
Invasive papillary carcinoma	5 (1.6%)
Micropapillary carcinoma	5 (1.6%)
Metaplastic carcinoma	2 (0.6%)
Apocrine carcinoma	2 (0.6%)
Mucinous carcinoma	2 (0.6%)

Table 5. Comparison of HER2 IHC results of other centers with results of our center

	Tepecik HER2 result				
Consult HER2 result	-	+	++	+++	Total
-	5	1	0	0	6 (4.6%)
+	7	3	2	0	12 (9.3%)
++	36	8	25	7	76 (59%)
+++	10	2	8	15	35 (27.1%)
Total	58 (45%)	14 (10.9%)	35 (27.1%)	22 (17%)	129 (100%)

HER2; Human epidermal growth factor receptor 2 oncoprotein
IHC; Immunohistochemistry

Table 6. Comparison of HER2 IHC results from other centers with FISH results from our center

Consult IHC result	HER2 amplification by FISH		
	positive	negative	Total
-	6	0	6 (4.6%)
+	11	1	12 (9.3%)
++	65	11	76 (59%)
+++	18	17	35 (27.1%)
Total	100 (77.5%)	29 (22.5%)	129 (100%)

FISH; Fluorescent in situ hybridization
HER2; Human epidermal growth factor receptor 2 oncoprotein
IHC; Immunohistochemistry

buffered neutral formalin, excisional biopsy materials that were fixated in formalin for less than 6 hours or more than 48 hours, tru-cut biopsy materials with retraction and compression artifact, strong membrane staining in normal ductus and lobules, and control cases with unexpected results

(8). The reasons for discrepancy between HER2 gene and protein product are expressed as chromosome 17 polysomy, low specificity and sensitivity of the primary antibody used in IHC, aggressive antigen retrieval methods and problems in tissue fixation-processing procedures (8).

When applying IHC, it is necessary to know the differences of HER2 antigene from the other antigens. HER2 is a thermo-labile antigen. When the lysine slides are kept in the incubator ($\geq 60^{\circ}\text{C}$) overnight, drying and loss of specific staining is observed in tissues. It is recommended that the slides that will undergo HER2 IHC should be kept in an incubator overnight at 37°C or at 60°C for one hour.

Standardization of tissue processing steps and preservation of cell morphology is very important since IHC evaluates HER2 staining on cell membrane. Assessment of membranous staining becomes very difficult in case of retraction artifact in cells. In this context, if there is a discrepancy between HER2 score and histopathological parameters at centers where standardization of the pre-analytical, analytical and post-analytical processes could not be provided, confirmation of IHC and ISH results at another center with standardization will be suitable.

Although international committees issue guidelines in order to determine HER2 accurately, there is a variety of inter-observer or inter-laboratory variables in both FISH and IHC, the compliance is low (9). In our study, IHC results from other centers and our unit were compared. The slides of patients from other centers were evaluated if their IHC slides could be obtained. HER2 score was based on pathology reports in consultation cases. Out of the 76 cases that were reported as (++) in other centers, 25 were interpreted as (++), and 44 were interpreted as (- / +) in our center. Out of the 35 (+++) cases, 15 were evaluated as (+++) and 12 as (- / +). In other centers (+++) considered amplification was observed in 18 of 35 cases. In particular, given the discrepancy between (++) / (+++) scores and FISH results, it was concluded that cytoplasmic and incomplete membranous staining was reported as complete membranous staining in other centers. Although compatibility was low in IHC (++) / (+++) scores, compatibility was high between (- / +) scores from other centers and IHC and FISH results from our unit. Based on this finding, the fundamental problem appears to be linked to non-standardized pre-analytical and post-analytical processes rather than the antibodies and methods used.

Amplification was detected by FISH in one patient who had (+) IHC in our unit. On re-evaluation of hematoxylin-eosin stained tumor sections of this case, it was noticed that tumor morphology was not optimal due to pre-analytical process problems. The IHC result of the slide with fixation problems was evaluated as (+). In this case, amplification was detected by FISH technique. FISH is the least affected method by pre-analytical processes and results in the least damage to the tissue. FISH to determine HER2 status is considered the gold standard (12). Disadvantages of FISH analysis are the long technical procedures, signals fading over time, and failure to store

Table 7. Correlation of HER2 expression and amplification in consult and non-consult cases

Immunohistochemistry			HER2 Amplification		Total
HER2			negative	positive	
Non-consult	HER2 expression	(-)	64	2	66
		(+)	14	1	15
		(++)	42	15	57
		(+++)	0	41	41
Consult	HER2 expression	(-)	57	1	58
		(+)	14	0	14
		(++)	28	7	35
		(+++)	1	21	22
		Total	220	88	308
HER2; Human epidermal growth factor receptor 2 oncoprotein

Figure 1. a, b. a) High-grade solid tumor islands containing lymphocytic infiltrate (HE, 20x), b) fluorescence in situ hybridization (FISH) amplified cells scattered between non-amplified cells (100x)

slides for a long-term, requirement of fluorescence microscopy and expertise for evaluation. It is very difficult to assess histomorphology under fluorescent microscope. Therefore, the SISH and CISH methods have been developed that enable assessment by light microscope. With these methods, it is possible to evaluate morphology and archive slides for long-term.

In the literature, the incidence of amplification in IHC (++) cases have been reported as 6-25% (10, 11). ASCO / CAP stated this rate as 23.9% (8). In our study, amplification was observed in 22 out of 92 IHC (++) (23.9%), the compatibility between IHC and FISH was calculated as 98.4%.

The prospective subgroup analyses of adjuvant randomized trastuzumab studies have shown the misinterpretation rate of HER2 protein expression level as 20% (13, 14). In this study, we also re-evaluated cases with incompatible IHC and FISH results. It was found that one case with (+++) IHC and no amplification had a tru-cut biopsy. Nowadays, tru-cut breast biopsies are commonly used for both diagnostic and therapeutic purposes. Thus, hormone receptor status and HER2 expression level can be determined in patients who will receive neoadjuvant chemotherapy. In the literature, concordance between tru-cut biopsy and excisional biopsy in terms of HER2 IHC evaluation was reported to be 87-98.8% (15, 16).

Chivukula et al. (17) even stated that tru-cut biopsies are more reliable in IHC and ISH since they do not have any fixation problems. However, tru-cut needle biopsies only sample a small area of the tumor and can have false (-) results especially in heterogeneous cases. On re-evaluation of our tru-cut biopsies, it was seen that they were extremely thin and contained severe compression artifacts. Therefore, non-specific cytoplasmic and granular staining was interpreted as complete membranous positivity. Although tru-cut biopsies harbor less fixation problems interpretation of IHC score is difficult if compression artifact is present. In these cases, confirmation of IHC by any ISH method will be appropriate.

Mixed probes including the centromeric region of the chromosome are recommended for assessment of HER2 amplification. When both gene regions are amplified, the HER2 / CEP17 rate may be below the limit of amplification. How this condition affects response to treatment is still controversial. Hofmann et al. (18) reported that in 2 IHC (++) cases with amplification in Chr centromere, FISH was negative and these patients were positive responders to trastuzumab therapy. Ultimately, it was suggested that, HER2 gene copy number might be more important than the ratio in determining the response to trastuzumab. One of the handicaps of ASCO / CAP criteria is situations when both gene regions are

Figure 2. a-c. a) Tumor cells with pre-analytical problems (HE, 10x), b) HER2 (+) tumor cells by immunohistochemistry (DAB, 10x), c) diffuse amplification with FISH (100x)

amplified. Patients who would be considered as amplified based on the number of HER2 copies, remain below the amplification limit if rate is considered. Therefore, modification of ASCO / CAP criteria is proposed.

Previously centromeric gene region amplification was considered as Chr 17 polysomy. Recently many genes on Chr 17 were examined simultaneously by comparative genomic hybridization (CGH) method and it was detected that real polysomy is extremely rare (19). However, amplification

Figure 3. a-c. a) Thin tru-cut biopsy with compression artefact (HE, 10x), b) Tumor cells assessed as (+++) HER2 by IHC due to artefacts (DAB, 20x), c) Tumor cells with no HER2 amplification by FISH (100x)

is frequent in pericentromeric gene regions of HER2 (-) and (+) cases. In Chr 17 aneusomy with pericentromeric rearrangements, the aberrant patterns (clusters) observed in the centromeric region leads to misinterpretation of HER2 / Chr 17 rate (20). In this situation, the individual number of monitored signals in HER2 and centromere region should be specified. We also observed Chr 17 centromere region amplification in 11 cases. Nine cases were evaluated as (++), and two as (+++). In six of these cases focal low-level, and in one case diffuse high-level amplification was observed. In these cases, when assessing amplification individual signal number of these regions were taken into consideration as well as HER2/ Chr 17 rate.

In this study, one noteworthy aspect was detection of focal heterogeneous low-level amplification by FISH technique in three cases that

Figure 4. a-c. a) IHC HER2 (++) tumor (DAB, 20x), b) Amplification of Chromosome 17 centromere region (green filter) and c) HER2 gene region (red filter) by FISH (100x)

were triple (-) (ER, PR, cerbB2 (-)) by IHC. Amplification was observed as small amplified clones (amplification in $\leq 5\%$ neoplastic cells) in all cases. Bernasconi et al. (21) reported small amplified clones in 27 out of 291 cases. Two of these 27 cases were IHC negative and one was triple negative. The existence and meaning of focal low level of HER2 amplification in triple negative cases should be examined in larger series.

Recent studies indicate the presence of genetic heterogeneity in breast tumor (21). IHC may be insufficient due to compression artifacts and genetic heterogeneity in tru-cut biopsies that are increasingly being used in routine clinic practice. In these cases, the addition of an ISH method to IHC will contribute to the accurate determination of HER2 status. In addition, when reporting ISH method in any type of material distribution and number of amplified cells, the type of amplification (low-high / diffuse-focal), and whether amplification of chromosome 17 centromere region accompanies or not should be indicated. In service training on standardization of pre-analytical, analytical, and post-analytical processes should be more comprehensive.

Ethics Committee Approval: Due to the retrospective design of the study, ethics committee approval was not taken.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - E.E.P., Ü.B.; Design - E.E.P.; Supervision - Ü.B.; Data Collection and/or Processing - E.E.P., Ç.Y.A., Ö.S., Ü.K.; Analysis and/or Interpretation - E.E.P., A.Ö.; Literature Review - E.E.P.; Writer - E.E.P.; Critical Review - Ü.B.

Financial Disclosure: The authors declared that this study has received no financial support.

Conflict of Interest: No conflict of interest was declared by the authors.

References

1. Popescu NC, King CR, Kraus MH. Localization of the human erbB-2 gene on normal and rearranged chromosomes 17 to bands q12-21.32. *Genomics* 1989; 4:362-366. (PMID: 2565881) [\[CrossRef\]](#)
2. Bargmann CI, Hung MC, Weinberg RA: The neu oncogene encodes an epidermal growth factor receptor-related protein. *Nature* 1986; 319:226-230. (PMID: 3945311) [\[CrossRef\]](#)
3. Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, Levin WJ, Stuart SG, Udove J, Ullrich A. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science* 1989; 244:707-712. (PMID: 2470152) [\[CrossRef\]](#)
4. Pauletti G, Godolphin W, Press MF, Slamon DJ. Detection and quantitation of HER-2/neu gene amplification in human breast cancer archival material using fluorescence in situ hybridization. *Oncogene* 1996; 13:63-72. (PMID: 8700555)
5. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* 1987; 235:177-182. (PMID: 3798106) [\[CrossRef\]](#)
6. Geyer CE, Forster J, Lindquist D, Chan S, Romieu CG, Pienkowski T, Jagiello-Gruszfeld A, Crown J, Chan A, Kaufman B, Skarlos D, Campone D, Davidson N, Berger M, Oliva C, Rubin SD, Stein S, Cameron D. Lapatinib plus capecitabine for HER2-positive advanced breast cancer. *N Engl J Med* 2006; 355:2733-2743. (PMID: 17192538) [\[CrossRef\]](#)
7. Dietel M, Ellis IO, Höfler H, Kreipe H, Moch H, Dankof A, Kölblle K, Kristiansen G. Comparison of automated silver enhanced in situ hybridisation (SISH) and fluorescence ISH (FISH) for the validation of HER2 gene status in breast carcinoma according to the guidelines 11 of the American Society of Clinical Oncology and the College of American Pathologists. *Virchows Arch* 2007; 451:19-25. (PMID: 17562074) [\[CrossRef\]](#)
8. Wolff AC, Hammond ME, Schwartz JN, Hagerty KL, Allred DC, Core RJ, Dowsett M, Fitzgibbons PL, Hanna WM, Langer A, McShane LM,

- Paik S, Pegram MD, Perez EA, Press MF, Rhodes A, Sturgeon C, Taube SE, Tubbs R, Vance GH, van de Vijver M, Wheeler TM, Hayes DF. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *J Clin Oncol* 2007; 25:118-145. (PMID: 17159189) [\[CrossRef\]](#)
9. Cell Markers and Cytogenetics Committees College Of American Pathologists. Clinical laboratory assays for HER-2/neu amplification and overexpression: quality assurance, standardization, and proficiency testing. *Arch Pathol Lab Med* 2002; 126:803-808. (PMID: 2088449)
10. Tsuda H, Akiyama F, Terasaki H, Hasegawa T, Kurosumi M, Shimadzu M, Yamamori S, Sakamoto G. Detection of HER-2/neu (c-erbB-2) DNA amplification in primary breast carcinoma: interobserver reproducibility and correlation with immunohistochemical HER-2 overexpression. *Cancer* 2001; 92:2965-2974. (PMID:11753973) [\[CrossRef\]](#)
11. Zhang H, Ren G, Wang X, Zhao J, Yao H, Bai Y, Bo W. HER-2 gene amplification by fluorescence in situ hybridization (FISH) compared with immunohistochemistry (IHC) in breast cancer: a study of 528 equivocal cases. *Breast Cancer Res Treat* 2012; 134:743-749. (PMID: 22678158) [\[CrossRef\]](#)
12. Schiavon BN, Jasani B, de Brot L, Vassallo J, Damascena A, Cirullo-Neto J, Ivanildo Neves J, Augusto Soares F, Gobbi H, Malagoli Rocha R. Evaluation of reliability of FISH versus brightfield dual-probe in situ hybridization (BDISH) for frontline assessment of HER2 status in breast cancer samples in a community setting: influence of poor tissue preservation. *Am J Surg Pathol* 2012; 36:1489-1496. (PMID: 22982892) [\[CrossRef\]](#)
13. Paik S, Bryant J, Tan-Chiu E, Romond E, Hiller W, Park K, Brown A, Yothers G, Anderson S, Smith R, Wickerham DL, Wolmark N. Real-world performance of HER2 testing--National Surgical Adjuvant Breast and Bowel Project experience. *J Natl Cancer Inst* 2002; 94:852-854. (PMID: 12048273) [\[CrossRef\]](#)
14. Roche PC, Suman VJ, Jenkins RB, Davidson NE, Martino S, Kaufman PA, Addo FK, Murphy B, Ingle JN, Perez EA. Concordance between local and central laboratory HER2 testing in the breast intergroup trial N9831. *J Natl Cancer Inst* 2002; 94:855-7. (PMID: 12048274) [\[CrossRef\]](#)
15. Tsuda H, Kurosumi M, Umemura S, Yamamoto S, Kobayashi T, Osamura RY. HER2 testing on core needle biopsy specimens from primary breast cancers: interobserver reproducibility and concordance with surgically resected specimens. *BMC Cancer* 2010; 10:534. (PMID: 20925963) [\[CrossRef\]](#)
16. Usami S, Moriya T, Amari M, Suzuki A, Ishida T, Sasano H, Ohuchi N. Reliability of prognostic factors in breast carcinoma determined by core needle biopsy. *Jpn J Clin Oncol* 2007; 37:250-255. (PMID: 17485439) [\[CrossRef\]](#)
17. Chivukula M, Bhargava R, Brufsky A, Surti U, Dabbs DJ. Clinical importance of HER2 immunohistologic heterogeneous expression in core-needle biopsies vs resection specimens for equivocal (immunohistochemical score 2+) cases. *Mod Pathol* 2008; 21:363-368. (PMID: 18246053) [\[CrossRef\]](#)
18. Hofmann M, Stoss O, Gaiser T, Kneitz H, Heinmöller P, Gutjahr T, Kaufmann M, Henkel T, Rüschoff J. Central HER2 IHC and FISH analysis in a trastuzumab (Herceptin) phase II monotherapy study: assessment of test sensitivity and impact of chromosome 17 polysomy. *J Clin Pathol* 2008; 61:89-94. (PMID: 17412870) [\[CrossRef\]](#)
19. Yeh IT, Martin MA, Robetorye RS, Bolla AR, McCaskill C, Shah RK, Gorre ME, Mohammed MS, Gunn SR. Clinical validation of an array CGH test for HER2 status in breast cancer reveals that polysomy 17 is a rare event. *Mod Pathol* 2009; 22:1169-1175. (PMID: 19448591) [\[CrossRef\]](#)
20. Gunn S, Yeh IT, Lytvak I, Tirtorahardjo B, Dzidic N, Zadeh S, Kim J, McCaskill C, Lim L, Gorre M, Mohammed M. Clinical array-based karyotyping of breast cancer with equivocal HER2 status resolves gene copy number and reveals chromosome 17 complexity. *BMC Cancer* 2010; 10:396. (PMID: 20667129) [\[CrossRef\]](#)
21. Bernasconi B, Chiaravalli AM, Finzi G, Milani K, Tibiletti MG. Genetic heterogeneity in HER2 testing may influence therapy eligibility. *Breast Cancer Res Treat* 2012; 133: 161-168. (PMID: 21901388) [\[CrossRef\]](#)