Should FISH Test be Performed to All Patients With Breast Cancer?

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Abstract

CerbB2 receptor determination is very important for the prognosis and treatment of breast cancer. The most used two methods for CerbB2 receptor determination are immunohistochemistry and FISH methods. FISH method is recommended for immunohistochemically CerbB2 (++) patient group. Therefore, even though the immunohistochemically CerbB2 (++) patients are in the same group, FISH test divides the results into two separate groups. Our study compared these two groups in terms of relaps duration. Total of 85 patients from The Kayseri Research and Training Hospital in Kayseri, Kayseri Erciyes University Hospital and Mersin State Hospital were included in this study. The relaps duration of the patients were determined and then compared statistically. When the mean disease free survival was compared for FISH(+) and FISH(-) groups, the mean DFS was 46±5 and 73±8 months. However, the median DFS was 38±9 months for the FISH positive group. No median PFS was reached for FISH(-) group. Immunohistochemically CerbB2 (++) breast cancer patients are considered as in the same group, however actually they have different prognostic features. Similarly, when the immunohistochemical tests and FISH tests are compared for CerbB2 positivity, different results are obtained. As a consequence of that, the therapy alternatives will be changed. Therefore, we conclude that the FISH test should be performed to all patients.

Key Words: Breast Cancer, FISH, Cerb B2(++), HER 2

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Introduction

In the context of the genetic studies on breast cancer, the molecular classification became the main topic of conversation and the breast tumors are classified into molecular subtypes: Luminal A and B (recently a third group as C group is considered to be added), HER-2 Like, Basal Like and unclassified [1]. The amplification and overexpression of HER-2 oncogen was known to be an important prognostic factor for breast cancer [2]. It was shown in the studies that, HER2 Like and Basal Like groups have worse prognosis than other groups [3,4]. HER -2 oncogen, also known as CerbB2 is a member of epidermal growth factor receptor (EGFR) family [5-7]. It's located on chromosome 17q21 and plays a role in cell growth, differentiation, adhesion and motility, signal conduction [8]. The expression in breast cancer gives tumor aggressive characteristics. The importance of HER 2 oncogen is mentioned above and, HER 2 receptor determination in breast cancer patients is a very important milestone for the determination of the prognosis and treatment modalities. CerbB2 positive tumors had poor prognosis. However the introduction of the anti-tumor agents that target this receptor and pathway have changed the poor prognosis of this tumor group as adjuvant or metastatic context [9-12]. Several methods were tested for HER 2 receptor determination. However two of them are used more common worldwide: Immunohistochemical staining and fluorescence in situ hybridization (FISH) methods [13-14]. The score for immunohistochemical method and their meanings are provided at Table 1. Food and Drug Administration (FDA) approved several tests for these methods. Some of those are Hercep Test (Dako Cytomation, Carpinteria, CA) and Pathway (Ventana Medical System, Inc, Tucson, AZ) for immunohistochemical method, PathVysion (Vysis Inc, Downers Grove, IL), Inform (Ventana Medical Systems Inc) and Dako HER2 FISH pharmDx tests for FISH method. However, there is still no consensus on which method is the most predictive and useful [13]. FISH that accepted in the literature and practice was considered as the gold standard test for immunohistochemically CerbB2 (++) tumors [13-19]. Breast tumors are divided into 4 groups according to the scores of CerbB2 receptor: 0 (negative), 1(+), 2(+) and 3(+). Practically, the prognostic features are considered as similar for each group. CerbB2 (++) tumors are divided into 2 groups by FISH and therefore, prognosis needs to be re-evaluated for these subgroups (FISH(+) and FISH(-)).

The aim of our study is to compare FISH(+) and FISH(-) groups determined by the FISH test in terms of relaps duration in the CerbB2 (++) non-metastatic breast cancer patient group.

Material and Methods

Total of 85 patients from The Kayseri Research and Training Hospital in Kayseri, Kayseri Erciyes University Hospital and Mersin State Hospital were included in this study. These patients were in the adjuvant context during diagnosis, and their CerbB2 results were found to be immunohistochemically (++). The time to relaps were evaluated for the patients. They were entered into the SPSS 16.0 statistical software and Kaplan Meier was used.

Results

Total of 85 patients were evaluated. The number of FISH(+) cases were 37 and FISH(-) cases were 48. HER-2 score was shown in table 1. The prognostic factors of the patients were provided at Table 2. No statistically significant difference in the age, stage, menopausal status, ER and PR status, grade and adjuvant chemotherapy regimes was found between groups in the statistical evaluation. This evaluation is provided at Table 3. In the analysis of disease-free survival, the mean DFS was 46±5 and 73±8 months for FISH(+) and FISH(-) groups. However, the median DFS was 38±9 months for the FISH positive group. No median DFS was found for FISH(-) group. In this comparison, no significant difference was found between two groups in terms of PFS. However there was a trend in favor of FISH negative group. The DFS curve was provided in Figure 1.

Table 1 HER-2 score (Newcastle system) for immunohistochemical staining

Score to Report	HER-2 Protein Overexpression	Assessment Staining Pattern
0	Negative	No staining is observed or membrane staining is observed in <10% of the tumor cells
1+	Negative	A faint/barely perceptible membrane staining is detected in >10% of tumor cells. The cells exhibit incomplete membrane staining
2+	Weakly Positive	A weak to moderate complete membrane staining is observed in >10% of tumor cells
3+	Strongly Positive	A staining complete membrane staining is observed in >10% of tumor cells

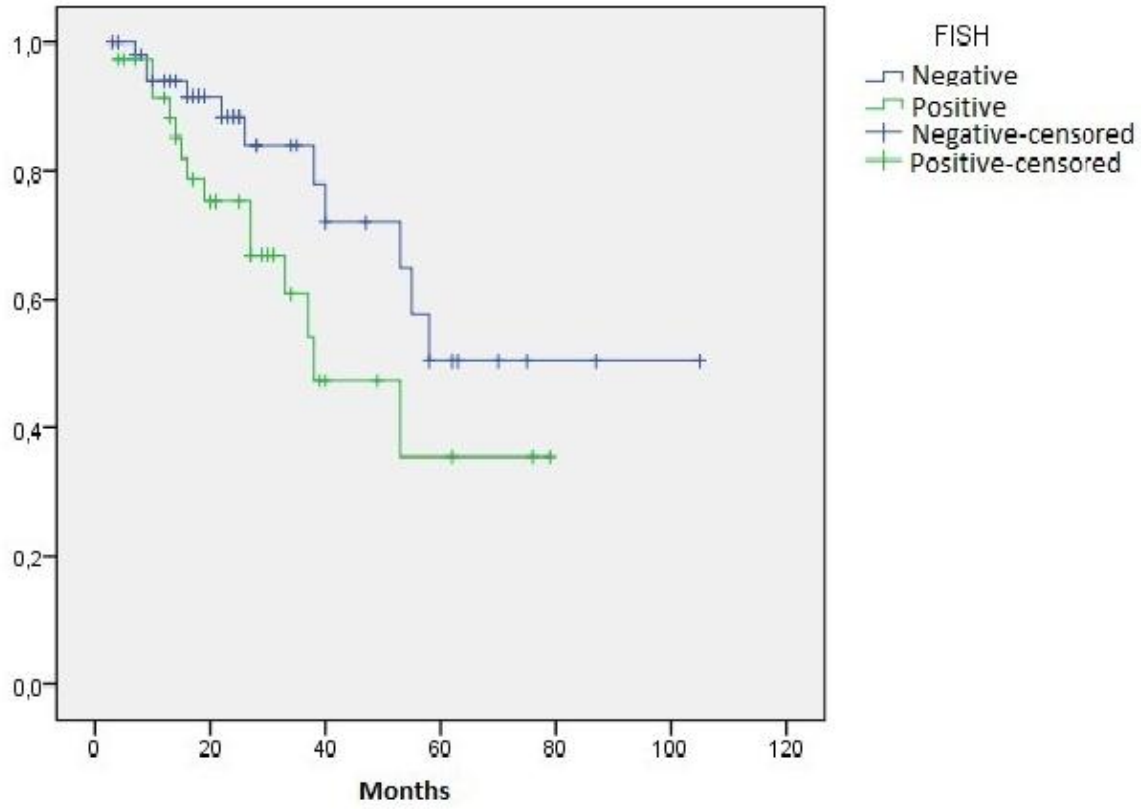
Table 2 The features of the groups.

Parameter	**FISH(+) (n:37)**	**FISH(-) (n:48)**
Age	48±13 (Mean)	47±10 (Mean)
	(n,%)	(n,%)
Stage		
Stage 1	1(2.7%)	7(14.6%)
Stage 2	11(29.7%)	18(37.5%)
Stage 3	25(67.6%)	23(47.9%)
Menopausal status		
Premenopausal	16(43.2%)	27(56.2%)
Perimenopausal	5(13.6%)	4(8.3%)
Postmenopausal	16(43.2%)	17(35.4%)
ER Status		
ER(+)	17(46%)	30(62.5%)
ER(-)	20(54%)	18(37.5%)
PR Status		
PR(+)	20(46%)	35(72.9%)
PR(-)	17(54%)	13(27.1%)
Grade		
Grade 1	2(5.4%)	2(4.2%)
Grade 2	18(48.6%)	26(54.2%)
Grade 3	12(32.4%)	15(31.2%)
Unknown	5(13.5%)	5(10.4%)
Adjuvant CT		
None	3(8.1%)	2(4.2%)
Anthracyclin based	6(16.2%)	19(39.6%)
Anthracyclin +Taxane based	28(75.7%)	27(56.2%)
Adjuvan Herceptin	20(54%)	0

Table 3 P values for parameters.

Parameter	FISH(+) vs FISH(-) p value
Age	0.651
Stage	0.085
Menopausal Status	0.459
ER	0.128
PR	0.071
Grade	0.915
Adjuvant CT	0.059

Figure 1. The DFS curve



Discussion

FISH positivity rate was calculated according to immunohistochemical score category in several studies [24-26]. The percentage of positive FISH results (median) was 3.5 for IHC(0), 5.9 for IHC (1+), 17.6 for IHC (2+), and 83.5 for IHC (3+) [26]. The percentage of positive

FISH results for IHC (2+) cases were found to be 16.9%, 6.7%, 92.3%, 25.7% and 16.9% in the studies [20-25]. In our study, percentage of positive FISH cases was 43.5%.

The most important reason to use FISH in Cerb B2 receptor IHC(2+) cases is to determine the prognosis and to modify the treatment modalities. As is known, Cerb B2 (3+) cases benefit from trastuzumab at the adjuvant or metastatic step, lapatinib-based regimes at the metastatic step. However Cerb B2 (2+) and Cerb B2 (3+) cases were identified as separate groups at IHC evaluation. So, should all Cerb B2 (2+) cases be considered as proper for "anti- HER2 receptor treatment"? Do CerB B2 IHC (2+) cases, even if they are FISH(+), give response to anti-receptor treatment as IHC(3+) cases.

At the end of our study, DFS was calculated as mean and median for FISH(+) and FISH(-) groups. In the analysis of progression-free survival, the mean DFS was 46±5 and 73±8 months for FISH(+) and FISH(-) groups. However, the median DFS was 38±9 months for the FISH positive group. No median PFS was found for FISH (-) group.

According to this analysis, even though the cases are IHC(2+), i.e. in the same group, they are divided into 2 groups prognostically, and these two groups are determined by FISH test. No significant difference in the age, stage, menopausal status, ER and PR receptor status, grade and adjuvant chemotherapy regimes was found between groups. This finding suggests that, the most important and the only factor in the formation of two separate groups is the FISH result.

In HERA study, CerbB2 (2+) FISH(+) cases were evaluated in two groups. Local CerbB2 (2+) FISH(+) cases benefited from adjuvant herceptin treatment significantly, however central CerbB2(2+) FISH(+) cases didn't. No significant difference was found in the combination of two groups [9]. Again, for CerbB2 (2+) FISH(+) cases in the adjuvant studies, significant difference was found for DFS in NSABP B31 study, and was not found in NCCTG N9831 study [10-11].

Conclusion

The percentage of FISH positivity ranged from 0% to 20% for Cerb B2 IHC(0+) cases and from 3.1% to 50. 0% for Cerb B2 IHC (1+) in the studies [20-25]. The percentage of FISH negativity ranged from 6.2% to 23.5% for Cerb B2 IHC(3+) cases [20-25]. Therefore we

consider that, direct FISH test is proper for all cases with breast cancer when evaluating Cerb B2 receptor status. It may be expensive however given that 6.3-23.5% of the CerbB2 IHC(3+) cases will not use anti-receptor. In conclusion, only IHC is not sufficient to show Cerb B2 receptor status. Even though these cases are IHC(2+) according to IHC scoring, i.e. in the same staining pattern, they have different prognosis.

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