

ORIGINAL ARTICLE

Evaluation of and quality assurance in HER2 analysis in breast carcinomas from patients registered in Danish Breast Cancer Group (DBCG) in the period of 2002–2006. A nationwide study including correlation between HER-2 status and other prognostic variables

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Abstract

Introduction. In Denmark, analysis for HER2 is situated in the pathology laboratories dealing with breast pathology. The analysis was introduced during the late 1990's, and was gradually intensified in the following years up to now. The present study deals with the experience with the analysis during the last 5 years, from 2002 – 2006. **Patients and methods.** All patients, registered in DBCG (Danish Breast Cancer Group) and with a HER2-test were included. The analysis followed international recommendations, with an initial immunohistochemical (IHC) analysis with a semiquantitative grading of the reaction in four grades, 0 and 1+, defined as HER2-negative, 2+, equivocal and 3+, HER2-positive. In the 2+ group, a FISH-test was applied to identify the presence of gene amplification, defined as a ratio ≥ 2 . We investigated the number of analyses performed, the number of positive cases and the relation between the result of IHC and the result of FISH. Furthermore we looked at the relation to other prognostic factors. **Results.** The number of analyses gradually increased during the years of investigation, from 30% of patients in 2002 to 71% in 2006. The increase was seen in all laboratories resulting in all laboratories but one having a substantial number of analyses. Sixty-two percent of all cases were HER2-negative, 18% were equivocal and 21% positive in the IHC-analysis. Of the 2+, equivocal cases, 23% had gene-amplification. Thus, 23% of patients were defined as HER2-positive and eligible for treatment with trastuzumab. There was a significant correlation to other prognostic factors. The results are in accordance with what is found elsewhere. The quality of the test is further assured by all laboratories participating in external quality assurance schemes.

Since the registration of trastuzumab (Herceptin®) as treatment for both metastatic breast cancer and in the adjuvant setting, the identification of the target, HER2 has been a major issue. HER2, a transmembrane receptor tyrosine kinase is a member of the epidermal growth factor family [1]. HER2 is coded for by a gene situated on chromosome 17 [2]. Overexpression of HER2 can be assessed by immunohistochemistry (IHC) and amplification on the gene level by in situ hybridisation, with fluorescence (FISH) or chromofob (CISH) detection systems [3–5]. The immunohistochemical reaction is semi quantitatively divided into four grades, 0, 1+, 2+ and 3+, whereas the FISH test provides the ratio between the number of genes and the number of

chromosomes. Eligibility for treatment with trastuzumab in accordance with recent clinical practice includes patients having a tumour that is defined as HER2 positive, either as an IHC-level of 3+ or a FISH ratio ≥ 2.0 [6]. Internationally, the algorithm for testing for HER2 will be an IHC, in case of a 2+ reaction followed by FISH [7]. In Denmark, testing for HER2 is performed in the Departments of Pathology dealing with breast pathology. The test was gradually introduced during the late 1990's and early 2000, being fully implemented for selected high risk patients during accrument to the HERA-trial 2002–2004. In the St. Gallen criteria from 2005 [8], the definition of high-risk patients includes HER2-status, and today all patients newly diagnosed with

breast cancer, will have a HER2-test. The present work represents the experience obtained with HER2 testing in Denmark in the period of 2002–2006.

Patients and methods

All patients, registered in the DBCG-database with a HER2-test in the period of 2002–2006 are included. In the period, 18 401 patients were registered. Of these 6 984 (38%) had a HER2-test.

Methods

All HER2-tests on the IHC-level were performed in the 18 pathological laboratories dealing with breast pathology. The FISH-test was confined to seven laboratories in the beginning of the period, nine laboratories toward the end of the period. To ensure the quality of the analysis, it was recommended to use the HERCEPTEST® for the IHC-analyses and for the FISH-test either the FDA-approved kit from Vysis or the kit from DAKO. Furthermore, all laboratories participated in external quality assurance schemes, either UK Neqas, NordicQ or both. The principles in these schemes are identical. Laboratories are provided with unstained slides containing four pieces of tissue or cell lines, with a confirmed HER2-status of 0, 1+, 2+ and 3+. The participating laboratories are asked to stain the slides and grade the reaction, returning the slides with their evaluation. A group of independent assessors will evaluate the slides, both with regard to the quality of the immunohistochemical reaction and the decentral grading of the slides. The result is returned to the participating laboratories, in case of a less optimal result, with recommendations for what to change in the used method in order to improve the reaction.

Tumours were defined as negative in IHC 0 or 1+, 2+ was defined as equivocal to be followed by FISH, while 3+ was deemed positive on the IHC alone. The FISH-ratio had to be ≥ 2.0 for a tumour to be amplified. All results were reported to the database with a note on whether the analysis was performed on the primary tumour or on a metastatic focus.

Statistical analysis

Descriptive statistics were presented by frequencies. Tests for independence between the HER2 IHC score and HER2 status defined by IHC score and FISH ratio and prognostic factors (size, nodal status, grade, receptor status and age) were done using the χ^2 test. Calculations were performed using SAS (v9.1, SAS Institute, Cary, N.C.). P-values less than 5% were considered significant.

Results

Table IA and B show the number of patients analyzed in the period under investigation, stratified by year of analysis, and in Table IB by pathology laboratory. It is seen that the number of analyses increased from approximately 30% in 2003 to 71% in 2006 where the St. Gallen criteria for definition of high risk patients was implemented. Today, ideally all patients should have a HER2-test. Table IB shows the distribution of the number of analyses performed in the participating pathology laboratories, for each year from 2002 to 2006. The frequency of HER2 analysis increased clearly in this period. Four laboratories stopped the analysis during the period, all of them because the corresponding department of breast surgery was centralized, thus leaving the laboratories without breast pathology. Today, the analysis is performed in 14 laboratories, with total number of analyses during the period under investigation ranging from 43 to 1 085. In 2006, four laboratories had less than 100 analyses. The laboratory with just two analyses has since stopped performing HER2-analysis, leaving just one laboratory with very few analyses.

Table IA. Number of analyses in the period of investigation.

Year	No. of patients	HER2 test done (%)
2002	3 720	877 (23)
2003	3 578	1 194 (33)
2004	3 788	1 139 (30)
2005	3 549	1 173 (33)
2006	3 588	2 601 (71)

Table IB. Number of analyses in the participating laboratories and over the years of investigation.

Laboratory\YEAR	2002	2003	2004	2005	2006	Total
1	156	194	138	154	443	1 085
2	109	84	110	39	299	641
3	57	92	66	91	173	479
4	47	67	56	26	88	284
5	51	73	41	157	273	595
6	16	66	71	0	0	153
7	7	30	31	19	0	87
8	12	21	3	5	2	43
9	86	176	229	171	224	886
10	45	14	13	136	162	370
11	0	0	1	16	94	111
12	91	119	101	145	224	680
13	36	28	14	0	0	78
14	21	19	11	30	147	228
15	10	6	3	4	127	150
16	42	70	136	118	142	508
17	29	70	19	10	116	244
18	27	38	10	0	7	82

Table IIA shows the results of the IHC in the four groups of 0, 1+, 2+ and 3+. Seventy-one percent of the tumours were negative, with a grade 0 or a grade 1+, 21% were positive with at 3+ reaction, whereas 18% were equivocal with a 2+ reaction. Table IIB shows the relation of HER2, IHC-score and FISH-ratio. Since the recommendation of when to perform a FISH-test was an IHC-score of 2+, the majority of FISH-analyses are in this group. In the 2+ group, 142/477 (23%) were amplified. In total 23% of patients were HER2-positive, either with a 2+ and gene-amplification or an IHC-score of 3+. There were a few cases in the group of IHC-score 0 or 1+ with a FISH-analysis. Not unexpectedly, only 7% were amplified. On the contrary, only 56% in the 3+ in the group of IHC 3+ were amplified. This figure is lower than expected, but the number of analyses is very low (16 cases), giving wide confidence limits. ((31–82%) exact confidence limits)

Table IIIA and B shows the relation between HER2 positivity in IHC (Table IIIA) and in the combination of IHC and FISH (Table IIIB) and other prognostic factors. There is a strong significant relation between the presence of HER2 and other prognostic factors ($p < 0.0001$).

As mentioned, laboratories performing the analysis participated in external quality schemes, the UK Neqas scheme and the NordicQ quality assurance scheme, taking place every 3 months. In both schemes, laboratories generally score satisfactorily. The scoring in both systems depended on the quality of the immunohistochemical reaction. Whether it was too strong or too weak, whether the reaction was in the cell membrane as expected and not elsewhere in the cell or the surroundings, or whether there was unspecific background staining present. In the UK Neqas scheme, participating laboratories can be classified as “passed”, “passed but improvement

needed” and “failed”. Only one of 12 laboratories participating in this scheme failed, whereas the remaining 11 laboratories all passed. Likewise, in the NordicQ, you could score as “optimal”, “good”, “borderline” or “poor”. Of the 12 laboratories participating in this scheme, none scored as poor, whereas 10 scored as optimal or good.

Discussion

The immunohistochemical detection of overexpression of the HER2 protein and the FISH detection of amplification of the coding HER2 gene has gradually over the past decade become part of the diagnostic options in pathological laboratories. The analysis is like the analysis for hormone receptors, unique since it is detecting a predictive factor, and the IHC result is independent of morphology. This is in contrast to other IHC procedures meant to support a morphologic diagnosis. It is thus of outmost importance that the quality of the analysis is optimal, since the treatment offered to the patient depends on the result of the analysis.

The present investigation shows that a decentral analysis, performed in 18 pathological laboratories seem to yield results that are consistent with what is found in the literature. We found a strong correlation to other prognostic factors, a finding that is also well documented [1]. In the IHC analyses, 39% of patients had either a 2+ or a 3+ reaction, whereof 21% were 3+, consistent with what is reported in the literature [9]. In the FISH-analyses in the 2+ tumours, 23% were amplified. Only approximately half of the patients with a 2+ tumour had a FISH-test. Most of these were from the beginning of the period since the FISH analysis was introduced somewhat later than the IHC-analysis. Patients participating in the HERA-trial had a central FISH-test but unfortunately the result is not registered in the DBCG database. Nevertheless, the 23% amplified cases are on the level of what is usually reported [9]. Combining the IHC-result with the FISH-result, the number of HER2-positive cases comes to 23%. However, we did find some inter laboratory variation. The number of negative cases (0 or 1+) ranged from 35% up to 73% (data not shown). However, the majority of laboratories (13 of 17) gave results only deviating 10% from the median of 62% negative.

Several publications recommend a certain amount of analyses per year for a laboratory to maintain the expertise to perform the analysis [7,9]. As can be seen in Table II, several of the laboratories in the present investigation that only had few analyses gave up analyzing for HER2 in the period so that toward the end, in 2006, only two laboratories have very few

Table IIA. IHC-scores, all laboratories and all years.

HER2-score	N	%
0	1 688	24.40
1+	2 528	36.55
2+	1 224	17.70
3+	1 439	20.80

Table IIB. The relation between IHC-score and gene-amplification.

HER2 IHC	Fish ratio <2 (%)	Fish ratio ≥2 (%)
0	5	0
1+	22 (92)	2 (8)
2+	477 (77)	142 (23)
3+	7 (44)	9 (56)

Table IIIA and IIIB. The relation between HER2 (IHC-score Table IIIA) and positivity (Table IIIB) and other prognostic factors.

Her2 IHC Prognostic variable	0 (%)	1+ (%)	2+ (%)	3+ (%)	p-value
Age					
<36	56 (24)	66 (28)	34 (14)	79 (34)	<0.0001
36-45	254 (25)	355 (34)	180 (17)	247 (24)	
46-55	516 (26)	704 (36)	362 (18)	380 (19)	
56-65	450 (24)	677 (36)	320 (17)	425 (23)	
66-75	252 (22)	468 (41)	210 (19)	204 (18)	
>75	160 (25)	258 (40)	118 (18)	105 (16)	
Size					
0-20	885 (25)	1 408 (40)	581 (17)	642 (18)	<0.0001
21-50	681 (25)	907 (33)	530 (19)	606 (22)	
50+	60 (20)	95 (32)	54 (18)	86 (29)	
Nodal					
N -	755 (27)	1 114 (39)	485 (17)	482 (17)	<0.0001
status					
N+	847 (24)	1 231 (34)	665 (19)	837 (23)	
Grade 1	395 (29)	663 (48)	238 (17)	74 (5)	
Grade 2	636 (23)	1 072 (38)	580 (21)	533 (19)	<0.0001
Grade 3	418 (23)	484 (26)	287 (16)	653 (35)	
Receptor					
-	467 (26)	413 (23)	220 (12)	661 (37)	<0.0001
Status					
+	1 174 (24)	2 011 (41)	950 (20)	695 (14)	

Table IIIB.

Her2 status Prognostic variable	Negative (%)	Positive (%)	p-value
Age			
<36	137 (62)	84 (38)	<0.0001
36-45	672 (71)	272 (29)	
46-55	1 356 (76)	420 (24)	
56-65	1 260 (73)	458 (27)	
66-75	805 (78)	229 (22)	
>75	463 (80)	118 (20)	
Size			
0-20	2 511 (78)	708 (22)	<0.0001
21-50	1 795 (73)	664 (27)	
50+	178 (65)	94 (35)	
Nodal			
N -	2 043 (79)	535 (21)	<0.0001
status			
N+	2 349 (72)	915 (28)	
Grade 1	1 158 (93)	93 (7)	
Grade 2	1 921 (76)	600 (24)	<0.0001
Grade 3	1 017 (59)	694 (41)	
Receptor			
-	977 (59)	681 (41)	<0.0001
Status			
+	3 535 (81)	809 (19)	

analyses, and these two have later renounced from analyzing for HER2. Thus, today all laboratories have a minimum of close to or more than a hundred analyses per year.

Recently, ASCO has published new guidelines for HER2-analyses, shifting the cut-off points for positivity to more than 30% cells with a strong, full membrane reaction in the IHC instead of the present definition of more than 10% positive cells [6]. Likewise, it is recommended that FISH-ratio should be ≥ 2.2 instead of as today ≥ 2.0 . Applying this cut-point to the current dataset results in a reduction of amplified samples to 17% in 2+ group, a reduction from 23% using the current cut-point. The guide-lines are set up in concern for the quality of the analyses, especially in decentral laboratories, with a discrepancy of 20% in decentral vs. central, high volume laboratories. However the guidelines have no obvious argument for changing cut-off levels, and we consider it of concern to compensate bad quality by changing cut-off levels although it may reduce the number of false positive cases. Rather, the quality as recommended should be assured in external quality assurance schemes, and regular check that the HER2-positive rate is within reasonable limits [10,11].

The percentage of amplified cases in the group of 3+ tumours is somewhat lower than what is expected [9]. However, the number of analyses is very low, only 16, yielding wide confidence limits (31-82%). On the other hand, the percentage of amplified cases in the 2+ group is 23% which is exactly as otherwise published [9]. Probably the low number in the 3+ group can be explained by the statistical variation, although a genuine number of false positive cases cannot be ruled out [12]. It could

be considered to routinely perform a FISH-test on 3+ tumours, to ensure that no false positive patients are offered a treatment that is ineffective.

Ultimately the clinical correlations, that is the responsiveness to treatment with trastuzumab, will be the final answer. Hopefully, reanalyses of the recent large adjuvant trastuzumab studies of the relation between disease free survival and the result of HER2-testing (IHC score and FISH ratio) will elucidate this topic.

We find it very significant and recommend that laboratories practising IHC and FISH participate in quality schemes to ensure and maintain the demanded high quality of the analyses. Furthermore they should be prepared to accept centralization of the analysis if the quality does not fulfil the demands.

In conclusion, our investigation has shown that HER2-analyses performed in pathology laboratories throughout the country yield acceptable results with respect to the relation to other prognostic factors, the number of positive cases and the results of evaluation in external quality assurance schemes. We did find an inter-laboratory variation that could be of some concern. It is in that context of importance that each laboratory constantly is aware of the results of their analysis as mentioned above.

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