Chromogenic in situ hybridization compared with other approaches to evaluate HER2/neu status in breast carcinomas

F.E. Rosa¹, R.M. Santos¹, S.R. Rogatto² and M.A.C. Domingues³

¹Departamento de Patologia, Hospital das Clínicas, Faculdade de Medicina, Universidade Estadual Paulista, Botucatu, SP, Brasil
²Departamento de Urologia, Faculdade de Medicina, Universidade Estadual Paulista, Botucatu, SP, Brasil and CIPE, Hospital A.C. Camargo, São Paulo, SP, Brasil
³Departamento de Patologia, Faculdade de Medicina, Universidade Estadual Paulista, Botucatu, SP, Brasil

Abstract

Human epidermal growth factor receptor 2 (HER2) has been evaluated in breast cancer patients to identify those most likely to benefit from herceptin-targeted therapy. HER2 amplification, detected in 20-30% of invasive breast tumors, is associated with reduced survival and metastasis. The most frequently used technique for evaluating HER2 protein status as a routine procedure is immunohistochemistry (IHC). HER2 copy number alterations have also been evaluated by fluorescence in situ hybridization (FISH) in moderate immunoexpression (IHC 2+) cases. An alternative procedure to evaluate gene amplification is chromogenic in situ hybridization (CISH), which has some advantages over FISH, including the correlation between HER2 status and morphological features. Other methodologies have also been used, such as silver-enhanced in situ hybridization (SISH) and quantitative real-time RT-PCR, to determine the number of HER2 gene copies and expression, respectively. Here we will present a short and comprehensive review of the current advances concerning HER2 evaluation in human breast cancer.

Key words: CISH; Breast cancer; HER2; Immunohistochemistry; FISH

Introduction

The v-erb-b2 erythroblast leukemia viral oncogene homolog 2, neuro/glioblastoma-derived oncogene homolog (avian), ERBB2/HER2 (other aliases: CD340, HER-2, HER-2/neu, MLN 19, NEU, NGL, TKR1) plays a role in the pathogenesis of a significant number of human cancers. This membrane receptor protein of the growth factor receptor gene family presents tyrosine kinase activity and is associated with cell growth, survival and differentiation. In human breast cancer, HER2 overexpression is reported in 20-30% of breast carcinomas (1-5), mostly due to HER2 gene gains or amplification. HER2 overexpression is associated with constitutive activation of different pathways, in particular the PI3K and ERK pathways, leading to a significant increase in cell proliferation (6).

For breast cancer management, discrimination of HER2 status is crucial for determining therapy and prognosis, since HER2 alterations are associated with a shorter disease-free period, overall survival and resistance to tamoxifen antiestrogen therapy and other chemotherapy regimens, regardless of the nodal or hormone receptor status (4,5,7,8). Moreover, patients with HER2-positive breast cancers can benefit from Trastuzumab (Herceptin™, Genentech, Inc., USA), while patients presenting HER2 amplification without Ch17CEP duplication have apparently limited benefit from the addition of the anthracycline (9,10).

As a result of the importance of HER2 assessment in clinical practice, several methods have been described to evaluate its status. Currently, three types of assays, already approved by the FDA (U.S. Food and Drug Administration), have been described for HER2 evaluation in formalin-fixed paraffin-embedded samples. HER2 protein expression can be determined by immunohistochemistry (IHC), while copy number alterations can be determined by fluorescence in situ hybridization (FISH) or chromogenic in situ hybridization (CISH).

IHC has been the most commonly used assay for determining HER2 status. It is easy to perform and of relatively low cost. However, wide variation in sensitivity and specificity has been reported among commercially available antibodies (11). Its scoring is highly applicable to

Correspondence: F.E. Rosa, Departamento de Patologia, Hospital das Clínicas, UNESP, 18618-000 Botucatu, SP, Brasil. Fax: +55-14-3811-6238. E-mail: fabiolarosa7@yahoo.com.br or fabiolarosa@fmb.unesp.br

Received July 9, 2012. Accepted December 3, 2012. First published online March 20, 2013.
In situ hybridization techniques are able to determine gene copy number using labeled DNA probes complementary to the target genomic sequences. For FISH, archival paraffin-embedded samples are pretreated to remove cytoplasmic and nuclear proteins, which can be a barrier to probe penetration, and the target DNA is denatured. Fluorescent-labeled probes are added to the tissue section to hybridize to HER2 gene sequences, whose signals are viewed with a fluorescence microscope. Tissue morphology and gene amplification are primarily disconnected, and although the nuclei can be identified by fluorescent DNA counterstain, such as DAPI (4′,6-diamidino-2-phenylindole), this does not always allow sufficient histopathological evaluation. Hematoxylin-and-eosin-stained sections from the same block are viewed in conjunction to enable morphologic analysis. The advantage of FISH testing is that the quantitative interpretation of the results is relatively straightforward and concordance rates among observers are higher than for IHC in some studies (for reviews, see Refs. 13,14).

More recently, CISH has emerged as a potential alternative to FISH for confirming ambiguous IHC results (15). CISH is a combination of in situ hybridization with antibodies or avidin conjugated with enzymes, such as alkaline phosphatase and peroxidase, to develop a chromogenic reaction similar to IHC staining. The principle of FISH is the hybridization of a fluorochrome-labeled DNA (probe) with a complementary target DNA sequence. A fluorescent counterstain is applied and the use of a fluorescent microscope with appropriate filters is necessary. Compared to FISH, CISH is much easier for pathologists to use for the analysis of gene amplification simultaneously with detailed morphologic features of tumors. Moreover, CISH signals do not diminish over time and can provide useful archives in the laboratories (9,16,17). This method has several advantages compared to FISH analysis, such as cost, the use of a light microscope, permanent staining, and available tissue morphology. Moreover, pathologists are more familiar with IHC labeling than with the FISH signal (18).

In addition to the CISH methodology, another bright-field in situ hybridization (BRISH) technique is the automated silver-enhanced in situ hybridization (SISH) developed by Ventana Medical System (Tucson, USA). SISH offers the advantages of a bright-field FISH test coupled with automation for HER2 amplification (17). It improves the efficiency and consistency of BRISH, reducing the risk of error. Probes for the HER2 gene and chromosome 17 are labeled with dinitrophenol. After DNA denaturation with enzyme digestion, goat anti-rabbit antibody conjugated to horseradish peroxidase is used as a chromogenic enzyme. Sequential addition of silver acetate as the source of ionic silver, hydroquinone, and hydrogen peroxide is used to yield a metallic silver precipitate at the probe site, which is visualized as a black dot. The slides are counterstained with hematoxylin for examination by light microscopy (17,19,20). A stable and discrete chromogenic reaction product is achieved allowing quantification of centromeric chromosome 17 and HER2 probe signals on the same slide by conventional bright-field light microscopy (19,21).

Methods based on the polymerase chain reaction (PCR) are also being increasingly applied to evaluate gene expression, in particular, quantitative real-time reverse transcription PCR (qRT-PCR), based on TaqMan methodology (11). This technique has successfully evaluated mRNAs expressed in mixed cell populations and specific mRNAs, especially those present in low copy numbers in a small number of cells or in small quantities of tissue. However, qRT-PCR suffers from the same drawback as other PCR-based methods. Besides isolating the tumor cell population within the tissue under evaluation, other technical aspects must be considered, including template quality, operator variability, and subjectivity in data analysis and reporting (14,22). Limitations related to tumor heterogeneity can be eliminated by the use of laser microdissection, although this seems to be impractical for routine diagnosis (14).

Due to the potential and application of these techniques, the aim of the current review is to summarize and compare available HER2 clinical routine findings by CISH methodology with IHC, FISH, SISH, and qRT-PCR results in breast cancer tissues.

Chromogenic in situ hybridization

HER2 amplification can be assessed by CISH in archival paraffin-embedded samples. This method is based on peroxidase- or alkaline phosphatase-labeled reporter antibodies that are detected using an enzymatic reaction (18). CISH was first used for HER2 screening by Tanner et al. (18), who demonstrated amplification by enzymatic detection as an additional method to be combined with IHC in breast cancer tumors. The main advantage of CISH is the use of chromogens instead of fluorochromes for signal identification, which can be viewed with a standard bright-field microscope.

The commercial system for CISH detection, known as the SPoT-Light HER2 CISH kit (Zymed Laboratories Inc., USA), recommends the following classification for HER2 status based on gene copy number in the nuclei: a) non-amplified, tumor cells with 2 to 5 brown intranuclear spots per nucleus; b) low-level amplification, when 6 to 10 signals per nucleus were detected in more than 50% of tumor cells or when a small coalescing signal cluster was identified; c) high-level amplification, defined as more than 10 copies per nucleus or when copy clusters were
observed in more than 50% of cancer cells. Non-amplified tumors can be grouped as disomy, when 1 or 2 copies of the gene are present, or polysomy, when 3 to 5 copies were detected per nucleus in more than 50% of cancer cells.

**CISH in comparison with other methodologies**

Using the PubMed database (http://www.ncbi.nlm.nih.gov/pubmed, accessed on August 24, 2011), 112 studies were found using the key words: “breast cancer”, “HER2” and “CISH”. Of these, 67 presented adequate data to enable comparisons between CISH and other methods, comprising more than 7000 cases of breast cancer. The concordance rate between CISH and IHC was achieved when: 1) cases scored as negative (0 or 1+) or positive (3+) by IHC were non-amplified and amplified, respectively, by CISH; 2) cases scored as 2+ by IHC were considered to be amplified by CISH. CISH and FISH technique agreement was achieved when: 1) cases were considered to be amplified by both methods independent of the status of low and high amplification level; 2) cases were not amplified by either methodology. Table 1 shows the comparison between CISH and IHC and between CISH and FISH. The concordance varied from 52 to 100% and 82 to 100% when CISH results were compared to IHC and to FISH, respectively.

**CISH compared to IHC.** Forty-seven studies compared CISH to IHC (Table 1) and 41% showed more than 90% of agreement (Figure 1A). Studies presenting lower concordance rates can be explained by the high number of 2+ cases evaluated by IHC, in which amplification by CISH was not detected. A subset of these cases could involve chromosome 17 polysomy, which is frequently associated with IHC 2+ tumors (37,80).

While evaluating IHC 2+ tumors at seven different centers, Di Palma et al. (31) observed a variation concerning amplified or non-amplified cases. Three of 12 IHC 2+ cases presented discrepant results by CISH among the centers. For IHC 0-1+ or 3+ cases, 100% agreement was achieved by CISH, indicating that it is a highly reproducible method.

**IHC 2+ cases.** Cases scored as 2+ by IHC showed amplification using CISH in 34% of 1397 tumors, ranging from 0 to 100% (Figure 1B). Sixteen studies detected amplification in more than 60% of IHC 2+ tumors. Six different antibodies were used for HER2 immunostaining evaluation, two of them polyclonal (Herceptest and A0485) and four monoclonal (CB11, 4B5, SP3, and TAB250). Herceptest was provided by Dako, while the other antibodies were supplied by different manufacturers. The studies were grouped according to the antibody used to discriminate IHC 2+ status. The percentage of amplification by CISH ranged from 21 to 69% of tumors (Table 2). A higher amplification rate by CISH was detected in studies that used monoclonal antibodies (CB11, SP3 and TAB250), compared to studies that used polyclonal antibodies (A0485 and Herceptest). These results agree with two studies that had used different antibodies for the same sample (15,49). Nunes et al. (49) observed that amplification was detected in 8 and 38% of cases in the IHC 2+ subgroup by polyclonal antibodies (A0485 and Herceptest, respectively) and in 80, 91 and 92% by monoclonal antibodies (NCL-CB11, CM-CB11 and 4D5, respectively). Zhao et al. (15) also reported lower agreement between IHC 2+ and amplified tumors using polyclonal antibodies (A0485, 11%) than monoclonal antibodies (57 and 80% for CB11 and TAB250, respectively).

According to Kostopoulou et al. (37), IHC 2+ tumors without gene amplification could indicate that at least some of these cases were either “overstained” or “over-read” in IHC analysis. The authors performed an immunohistochemical reevaluation of IHC 2+ cases and observed a decrease in, but not elimination of, this particular subgroup. Tumor heterogeneity is another plausible explanation for these findings. Kostopoulou et al. (37) reported that approximately 3% of their cases showed heterogeneous immunostaining and hybridization signals. Small areas from the same tumor were observed showing IHC 3+ and amplification next to areas showing IHC 2+ or 0 and non-amplification. The same intratumoral heterogeneity was described by Rosa et al. (55) using CISH, but not in IHC 2+ cases. The authors observed a pool of mixed cells presenting no amplification (2 or 3-5 copies) and high-level amplification in the same area. Hybridization heterogeneity was also observed by FISH in IHC 2+ tumors; the tumors presented low-level amplification or a mosaic mixture of high-level amplified and non-amplified cells (81). The major question is whether cells showing different levels of amplification make any difference or whether a threshold (or its value) percentage of amplified tumor cells is required to define non-amplified and amplified tumors (55).

**IHC 0-1+ and 3+ cases.** The distribution into subgroups according to IHC status is shown in Figure 1B. Of the 4594 cases scored as 0/1+ by IHC, 96% were confirmed as non-amplified, while 90% of 1612 cases classified as 3+ by IHC showed amplification by CISH (Figure 1B).

The concordance rate between IHC and CISH results varied according to the antibodies used for IHC (Table 2). Correlation between CISH and IHC for almost all antibodies was above 93 and 91% for 0/1+ and 3+ immunostaining, respectively. Breast tumors IHC 0/1+ evaluated by the SP3 antibody were non-amplified in 86% of the samples, while Herceptest 3+ was associated with amplification in 89% of the cases. Higher agreement values were observed for SP3 and Herceptest antibodies in the IHC 3+ and 0/1+ groups, respectively. Three studies (49,53,55) used the SP3 antibody in a total of 282 cases. Two of these used tissue microarray and observed a correlation between CISH and IHC results of 84 and 93%
Table 1. Comparative analysis of chromogenic in situ hybridization (CISH), immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) results for breast cancer samples.

| Study                        | CISH agreement with IHC [% (n/n)] | CISH agreement with FISH [% (n/n)] |
|------------------------------|-----------------------------------|-----------------------------------|
| Arena et al. (69)            | 100 (40/40)                       |                                   |
| Arnould et al. (23)          | 81 (61/75)                        | 96 (72/75)                        |
| Bartlett et al. (70)         | -                                 | 89-100 (40-45/55)                 |
| Bartlett et al. (71)         | -                                 | 83-100 (25-30/30)                 |
| Bassarova et al. (24)        | 100 (16/16)                       | -                                 |
| Bhargava et al. (72)         | -                                 | 100 (102/102)                     |
| Bilous et al. (25)           | 55 (27/49)                        | 94 (46/49)                        |
| Bozhanov et al. (26)         | 86 (6/7)                          | -                                 |
| Cayre et al. (27)            | 69 (38/55); 69 (38/55)            | 94 (50/53); 93 (51/55)            |
| Chang et al. (28)            | 75 (97/130)                       | -                                 |
| Dandachi et al. (29)         | 96 (164/171)                      | 100 (38/38)                       |
| Di Palma et al. (30)         | 88 (141/161)                      | 100 (24/24)                       |
| Francis et al. (17)          | 96 (220/228)                      | -                                 |
| Garcia-Caballero et al. (32) | 97 (121/125)                      | 100 (167/167)                     |
| Gong et al. (73)             | -                                 | 91 (73/80)                        |
| Gong et al. (33)             | Na                                | 99 (192/194); 99 (213/215)        |
| Gupta et al. (74)            | -                                 | 84 (26/31)                        |
| Hanna and Kwok (34)          | 59 (151/254)                      | 95 (242/254)                      |
| Hauser-Kronberger and Dandachi (14) | 96 (164/171)                      | 100 (38/38)                       |
| Hyun et al. (35)             | 66 (204/309)                      | 97 (299/309)                      |
| Isola et al. (75)            | -                                 | 94 (180/192)                      |
| Kapila et al. (36)           | 67 (8/12)                         | Na                                |
| Kato et al. (76)             | -                                 | 97 (39/40)                        |
| Kim and Oh (16)              | 85 (23/27)                        | -                                 |
| Kostopoulou et al. (37)      | 72 (114/159)                      | 99 (158/159)                      |
| Kumamoto et al. (38)         | 94 (17/18)                        | -                                 |
| Laakso et al. (77)           | -                                 | 91 (40/44)                        |
| Leong et al. (39)            | 76 (44/58)                        | Na                                |
| Leong et al. (40)            | 69 (33/48)                        | 88 (28/32)                        |
| Lin et al. (78)              | -                                 | 92 (23/25)                        |
| Li Ning et al. (41)          | 55 (30/55)                        | 97 (31/32)                        |
| Loring et al. (42)           | 82 (90/110)                       | 99 (109/110)                      |
| Madrid and Lo (43)           | 86 (138/160)                      | -                                 |
| Mayr et al. (44)             | 52 (105/202)                      | 95 (122/129)                      |
| Moelans et al. (45)          | 91 (291/321)                      | 91 (61/67)                        |
| Moelans et al. (46)          | 93 (204/219); 93 (203/219)        | -                                 |
| Ni et al. (47)               | 85 (69/81); 98 (52/53)            | 90 (73/81); 92 (49/53)            |
| Ntoulia et al. (48)          | 95 (81/85)                        | -                                 |
| Nunes et al. (49)            | 83 (70/84); 76 (64/84); 93 (78/84); 94 (79/84); 95 (70/74); 96 (81/84) | - |
| Park et al. (79)             | -                                 | 94 (177/188)                      |
| Peiró et al. (50)            | 88 (52/59); 85 (50/59)            | 82 (9/11)                         |
| Peiró et al. (51)            | 92 (147/159)                      | 95 (38/40)                        |
| Pothos et al. (52)           | 79 (72/91)                        | 100 (88/88)                       |
| Ricardo et al. (53)          | 86 (138/161); 83 (134/161)        | -                                 |
| Rietdorf et al. (54)         | 69 (280/403)                      | 95 (379/399)                      |
| Rosa et al. (55)             | 84 (31/37)                        | 100 (8/8)                         |
| Sáez et al. (56)             | Na                                | 95 (165/174)                      |
| Sapino (57)                  | 64 (68/106); 83 (88/106)          | -                                 |
| Sartelet et al. (58)         | 93 (87/94)                        | Na                                |

Continued on next page
and 93% and 100% (49) for 0/1+ and 3+ immunostaining, respectively. Rosa et al. (55) showed agreement in 85 and 100% of cases presenting IHC 0/1+ and 3+, respectively. Putative explanations for SP3 false-negative cases are the loss of the extracellular domain due to HER2 cleavage and the shedding of the SP3 binding site, despite gene amplification by CISH (53) or gene overexpression by qRT-PCR (55).

Herceptest 3+ was detected in 552 cases from 16 studies (14,17,27,29,30,34,38,42,45,46,49,59,60,62-64), in which 60 samples were not amplified by CISH. Nine studies evaluated the same cases by FISH (14,27,29,30,34,42,45,59,64). Among these, 7 presented adequate data that permitted comparison. Of the 23 discordant cases between CISH and IHC, 100% were also non-amplified by FISH, confirming the CISH results. The discrepancy between CISH and IHC 3+ analyzed by Herceptest may be due to false-positive IHC signals, probably due to overstaining (54). Confirmatory evaluation in IHC 3+ cases by CISH or FISH will ensure that patients receive the most appropriate therapeutic approach, avoiding expensive and cardiotoxic treatment for cases presenting IHC 3+ and non-amplified tumors.

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\begin{array}{|c|c|c|}
\hline
\text{Study} & \text{CISH agreement with IHC [% (n/n)]} & \text{CISH agreement with FISH [% (n/n)]} \\
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\text{Słičczak-Kuta et al. (59)} & 64 (35/55)^a & \text{Na} \\
\text{Sumiyoshi et al. (60)} & 81 (47/58) & - \\
\text{Tanner et al. (18)} & 95 (149/157) & 94 (147/157) \\
\text{Tanner et al. (61)} & 99 (93/94) & - \\
\text{Todorovic-Rakovic et al. (62)} & 77 (43/56) & - \\
\text{Todorovic-Rakovic et al. (63)} & 79 (84/107) & - \\
\text{van de Vlij et al. (64)} & 68 (144/211)^a & 91 (193/211) \\
\text{van der Vegt et al. (65)} & 91 (221/242); 95 (231/243); 96 (224/234); 94 (212/225)^a & 96 (230/240) \\
\text{Vera-Román and Rubio-Martínez (66)} & \text{Na} & 94 (47/50) \\
\text{Vocaturo et al. (67)} & 77 (86/111) & 95 (105/111) \\
\text{Wixom et al. (68)} & 93 (57/61) & 97 (32/33) \\
\text{Zhao et al. (15)} & 97 (60/62); 94 (58/62); 84 (52/62)^a & 100 (62/62) \\
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\end{array}
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\(\text{Na} = \text{data not available to calculate the percentage}; (-) = \text{method not performed}; ^a \text{Different antibodies were used}; ^b \text{Data from different laboratories}; ^c \text{No IHC 2+ cases}; ^d \text{Two different groups of samples or different sites from the same tumor}; ^e \text{No IHC 0-1+ cases}; ^f \text{CISH was performed on cytology samples.}\)

\(\text{CISH compared to FISH. Forty-two studies presenting 4460 breast cancer cases compared CISH to FISH analysis (Table 1). The overall agreement was 96%. Three reports (7%) revealed <90% concordance between the two methodologies (40,50,74). Peiro et al. (50) performed CISH and FISH on 11 paraffin-embedded samples and observed two IHC 2+ cases amplified by FISH, but not by CISH. The authors suggested that CISH could have a lower sensitivity compared to FISH and recommended that IHC 2+ cases without amplification by CISH analysis should be later submitted to the FISH methodology. Gupta et al. (74) observed that most (3/4) low-level amplification cases showed chromosome 17 polysomy by FISH, explaining the presence of more than 5 copies per nucleus. CISH was repeated in one case that presented a weak signal and was non-amplified. The false-positive case of amplification was caused by overinterpretation of the signal in the presence of background, which in this case was due to an endogenous peroxidase. An agreement ranging from 90 to 95% was observed for 48% of studies, while 45% presented >96% concordance. These findings were expected, since CISH has been confirmed by many investigations as a robust and}

**Figure 1.** Forty-seven studies (14-18,23-30,32,34,55,57-65,67,68) comparing CISH to IHC methodology for breast cancer samples. A, Agreement between the two methodologies ranging from 50 to 100%. B, Percent agreement based on IHC subgroups (0-1+, 2+, and 3+) compared to amplified and non-amplified HER2 status by CISH. CISH = chromogenic in situ hybridization; IHC = immunohistochemistry.
Table 2. Concordance between immunohistochemistry (IHC) and chromogenic in situ hybridization (CISH) according to the antibodies tested for HER2 staining.

| HER2 antibodies | IHC(0-1+)/CISH (NA) (%) | IHC (2+)/CISH (A) (%) | IHC 3+/CISH (A) (%) |
|-----------------|------------------------|-----------------------|--------------------|
| Herceptest      | 97                     | 36                    | 89                 |
| A0485           | 96                     | 21                    | 90                 |
| CB11            | 96                     | 37                    | 93                 |
| 4B5             | 95                     | 26                    | 91                 |
| SP3             | 86                     | 55                    | 94                 |
| TAB250          | 93                     | 69                    | 91                 |

NA = non-amplification; A = amplification. For these comparisons, 40 studies with available data (14-18,24,26-30,34-39,41-51,53,55,57-65,68) were analyzed.

Viable test to assess HER2 status (14,25,41,54,55). Moreover, CISH has some relevant advantages over FISH: 1) the analysis is faster; 2) interpretation is performed using equipment that already exists in routine histopathology laboratories, such as a standard light microscope; 3) it permits simultaneous evaluation of copy number alterations, tumor cell and surrounding tissue morphology on the same slide; 4) morphology is easier to analyze, particularly for distinguishing invasive cancer cells and in situ components; 5) permanent staining is produced allowing the samples to be archived indefinitely; 6) CISH is also easier to interpret for pathologists who are not familiar with fluorescence (9,15,18,23,25).

In FISH analysis, tissue morphology and gene amplification are primarily independent because tumor cells for copy number evaluation are based on nuclear DAPI or propidium iodide staining, which does not always permit adequate histopathological evaluation of the cells (14). FISH slides must be stored at a temperature of 4°C or lower and are subject to quenching of the fluorescent signal, whereas CISH-stained slides can be stored in standard slide files and the reaction product is permanent (15).

When grouping the 42 studies, disagreement between CISH and FISH results was observed in 194 cases (4%). Data were available for 132 of these, with 67 being amplified by FISH and not by CISH and the inverse occurring in 65. Explanations for this disagreement are differences in sample preparation (34), scoring system (25,35), tumor heterogeneity (44,75), material thickness (23) or the absence of a specific probe for the chromosome 17 centromere, which would be able to distinguish amplification from chromosomal polysomy by CISH analysis (27,73). A limitation of determining chromosomal polysomy is the time lost to retest the chromosome 17 centromere probe by CISH on a serial section. With the advent of dual-color CISH (dc-CISH), information regarding polysomy and gene amplification has been obtained in a single assay using probes for HER2 and the chromosome 17 centromere and has shown very strong agreement with FISH results (32,44,71,76,77). However, even using dc-CISH, disagreement with FISH results has been observed for high-level amplification cases, because the signal appears as a typical peroxidase-positive ‘cluster’, in which the number of gene copies cannot be counted (32,76).

CISH compared to SISH. In a study of 230 breast cancer cases, Francis et al. (17) found a concordance rate of 96% for CISH and SISH using HER2 single-probe analysis and 95% for CISH and SISH with single- and dual-probe analysis, respectively. Similar results were observed by Park et al. (20) in a study in which 96% of 257 cases presented concordant data between CISH and SISH. According to the authors, the advantage of using SISH is the shorter time (6 h) needed to perform the procedure and automation of the method compared to CISH and FISH.

CISH compared to qRT-PCR. Rosa et al. (55) observed a correlation between transcript expression analysis and CISH in 90% of cases. Two discordant samples were amplified by CISH and non-overexpressed by qRT-PCR. This was probably caused by a difference in sample material; paraffin-embedded and fresh tumor tissue for CISH and qRT-PCR, respectively, or by the presence of normal cells. Kostopoulos et al. (37) reported mRNA overexpression in all cases amplified by CISH. In the IHC 2+ group, cases presenting or not polysomy showed similar mean mRNA values, while IHC 2+ cases without amplification or polysomy had mRNA expression values closer to normal samples. High concordance between gene expression and amplification status has also been observed in other studies using FISH (11,37,55).

Conclusion

Since the FDA (September 1998) approval of the use of the monoclonal antibody Tratuzumab for breast cancer treatment in HER2-positive cases, significant improvements in the overall prognosis for patients have been reported. Different methods have been developed to evaluate HER2 copy number alterations, including FISH, CISH, SISH, and qRT-PCR. CISH has been compared to non-routine and routine diagnostic methods, such as IHC, and has been used as an efficient alternative method to FISH for HER2 gene status elucidation.

Acknowledgments

Research supported by FAPESP and CNPq.
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