# Analysis of ERBB2 and TOP2A gene status using fluorescence *in situ* hybridization versus immunohistochemistry in localized breast cancer*

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The aim of our study was to assess the ERBB2 and TOP2A gene status in breast carcinoma tissue using fluorescence *in situ* hybridization (FISH) and to compare their amplification with immunohistochemistry assay (IHC) of the ERBB2, resp. topoisomerase IIα proteins. TOP2A status is important in tailored treatment as topoisomerase IIα is the molecular target for topoisomerase IIα inhibitors. This study was conducted to determine whether the methods are equivalent in their assessment of TOP2A status and to correlate the genetic findings with basic tumor and disease characteristics.

Locus specific ERBB2, TOP2A genes and chromosome 17 centromeres (CEP17) probes were hybridized to 72 formalin-fixed paraffin-embedded (FFPE) tissue samples from patients with non-metastatic breast carcinoma (M0). The ERBB2, TOP2A and CEP17 signals were counted and gene numbers per nucleus or per CEP17 were calculated, respectively. Sections were also stained with commercial polyclonal antibody (HercepTest™), anti-topoisomerase IIα monoclonal antibody (clone SWT3D1) and scored for the presence of membrane/nuclear staining.

ERBB2 amplification was found in 20.3%, ERBB2 and TOP2A co-amplification was detected in 14.5% of cases. Deletion of the ERBB2/TOP2A gene was found in 1.4/2.8% of sections, respectively. Concordance of FISH and IHC techniques in the evaluation of ERBB2 and TOP2A status was found in 88.4% and 66.7%, respectively. The low concordance of FISH versus IHC in the evaluation of TOP2A status was mainly due to the presence of TOP2A amplified tumors in IHC negative or weakly positive specimens. Topoisomerase IIα expression was increased in bigger tumors, although direct correlation with tumor grading was not found. ERBB2 amplification was found in more aggressive breast cancers with grades 2 and 3, respectively. Interestingly, chromosome 17 polysomy was more frequently observed among older women (>55 years), suffering usually from less aggressive disease.

Our results confirm the high concordance of the ERBB2 and TOP2A gene co-amplification in breast carcinoma. Differences between FISH and IHC in the case of ERBB2 gene status were found only in IHC 2+ sections as reported in the literature. However, our study points to the importance of FISH examination of TOP2A gene status in all tumors with ERBB2 amplification.

*Key words: ERBB2, topoisomerase IIα, breast carcinoma, FISH, immunohistochemistry, anthracycline*

The ERBB2 gene also known as HER-2/neu is the most frequently amplified oncogene in breast carcinoma. A number of studies have shown that it is amplified in 20 to 35% of both breast and ovarian cancers. Further, the amplification is associated with shortened disease-free survival (DFS) and overall survival (OS) [1, 2]. For this reason, gene amplification and/or protein overexpression is routinely tested in breast cancers owing to the possibility of treatment using the humanized monoclonal antibody against the ERBB2 protein, trastuzumab (Herceptin, Genentech), which is active only against ERBB2 positive disease [3]. ERBB2 amplification has also been linked to benefit from anthracycline containing chemotherapy, although some publications report no association [1].

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Although the ERBB2 oncogene is considered to be biologically the most important breast cancer gene within the 17q12-q21 amplicon, it contains also other closely located genes, such as thyroid hormone receptor gene (THRA), retinoic acid receptor gene (RARA), metastatic lymph node genes (MLNs) 50, 51, 62, 64, and topoisomerase IIα gene (TOP2A). The latter is particularly important in breast carcinoma because topoisomerase IIα is the molecular target for topoII inhibitors. Anthracyclines and podophylotoxins stabilize DNA double strand breaks developed during topoisomerase mediated DNA relaxation, followed by proliferation inhibition and cell death. Mutation, deletion or decreased topoisomerase expression diminish the effect of cytostatics interacting with the topoII system and this leads to drug resistance [4]. *In vitro* studies have established that sensitivity to topoII inhibitors is dependent on expression levels of topoIIα in target cancer cells. Cells with a low concentration of topoIIα protein are less sensitive to topoII inhibitors than cells containing a high concentration of topoIIα and *vice versa* [1].

Accurate and reproducible evaluation of ERBB2 status is therefore crucial, in elucidating the increasing importance of the ERBB2 oncogene in clinical oncology. Known methods for identification of ERBB2 status include Southern, Northern and Western blottings, enzyme linked immunoglobulin assay, immunohistochemistry (IHC), chromogenic *in situ* hybridization (CISH), polymerase chain reaction and fluorescence *in situ* hybridization (FISH). Currently there are two types of FISH and two types of IHC ERBB2 assays approved by the Food and Drug Administration (FDA) in the USA. One test for CISH, a variant of FISH, also has the Confirmity European (CE) mark [5–12]. A comparison of FISH and IHC methods for evaluating ERBB2 status was made by WANG et al and subsequent statistical analysis showed the differences between them were primarily found in IHC medium positive specimens (IHC2+) with negative or positive FISH results. This study recommended for the first time a combination of both, in order to clarify ERBB2 status in IHC2+ and confirm the amplification in IHC3+ tumors using the FISH technique [12]. Current recommendations for ERBB2 evaluation in the United Kingdom and other countries is to confirm borderline IHC1+/2+ and 2+ tumors using another analytical system, usually FISH [13]. The importance of accurate determination of ERBB2 status to predict the clinical efficacy of trastuzumab was further underlined by SLAMON et al who showed in patients with IHC3+ tumors, greater efficacy of trastuzumab treatment than in those with IHC2+ breast cancers [14]. Recent information suggests that there is a high concordance between the finding of gene amplification and a score of 3+ in IHC staining [15, 16]. Taking these facts together one may conclude that the degree of ERBB2 amplification/overexpression determines the efficacy of trastuzumab [15, 17].

Although laboratory techniques for analysis of the TOP2A gene status are comparable to those for the ERBB2 gene, direct comparison of FISH versus IHC techniques has only been reported once before [18]. The aim of our study was to validate both methods, correlate the findings with basic clinical disease characteristics and to identify clinical situations where either one or both techniques are (not) equivalent.

## Patients and methods

*Patient and tumor characteristics.* We examined 72 samples obtained from women with breast carcinoma. At diagnosis patients were from 29 to 82 years old with a median age of 52.5 years. Sixty nine of 72 (95.8%) were primary non-metastatic, 3 of 72 (4.2%) were diagnosed with distant metastases after surgery. Twenty nine of 72 (40.3%) were node-positive: 26 patients were N1, 3 patients were N2. Forty of 72 (55.6%) women showed node-negative disease. In 3 of 72 cases (4.2%) N was not established. Grade G1 was established in 20 of 72 patients (27.8%), G2 in 34 of 72 (47.2%), and G3 in 18 of 72 (25%) patients. Positive estrogene receptors (ER) were detected in 45 of 72 (62.5%) patients, while 27 (37.5%) women were ER negative. Fifty one of 72 (70.8%) tumors were progesteron receptor (PgR) positive and and 21 tumors (29.2%) had negative PgR status. Fifty two of 72 (72.2%) women had invasive ductal carcinoma, while invasive lobular carcinoma was diagnosed in 13 (18.1%) patients. Other types of invasive carcinomas were detected in 5 patients (6.9%) and two of 72 patients had noninvasive tumors (2.8%). The patients were selected randomly from breast cancer sufferers within the period from April 1998 to April 2000. Twelve of 72 (16.7%) patients have already relapsed and 6 of these already died (8.3%).

*Tumor sections.* The samples were perioperatively obtained, formalin-fixed and paraffin-embedded (FFPE). Four to six μ sections were immobilized on "Plus Slides" (Superfrost Plus, BDH, Germany), baked overnight (56 °C) and deparaffinized before hybridization using hydrochloric acid and sodium thiocyanate pre-treatment [19, 20]. Protease treatment was then performed using pepsin solution (2 mg/ml in saline pH 2; catalytic activity 2500–3000 U/mg, Sigma, St.Louis, Missouri, USA). The slides were fixed in 10% buffered formaldehyde solution, washed twice in sodium saline citrate solution (SSC) and dried at 45–50 °C.

*Fluorescence in situ hybridization.* The genetic status of the ERBB2, TOP2A and chromosome 17 was analyzed using the three-color FISH on FFPE tissue sections after deparaffinization, applying directly labelled locus specific probes (LSI) and centromeric (CEP) DNA probes. Spectrum Orange labelled LSI ERBB2, SpectrumGreen labelled TOP2A and SpectrumAqua labelled CEP17 probes (Vysis, Downers Grove, Illinois, USA) were used for assessment of genetic status (Fig. 1). Tissue sections were hybridized with the probes overnight in a hybridizer (HYBrite™, Vysis, Downers Grove, Illinois, USA) at 80 °C. Slides were washed in 2xSSC/0.3% NP-40 and counterstained with DAPI III (Vysis, Downers Grove, Illinois, USA) [21–24]. FISH sig-

nals were evaluated using fluorescence microscopy Olympus BX60, and computer imaging system ISIS (MetaSystems, Altlussheim, Germany). Hybridization signals were counted in >100 non-overlapping nuclei per section. The results were expressed as absolute gene copy numbers per nucleus and/or relative numbers calculated as gene/CEP17 indices. Amplification/deletion of ERBB2 and TOP2A were diagnosed in cases where the gene/CEP17 index was ≥1.5 or ≤0.7, respectively.

*Immunohistochemistry.* A standardized IHC assay Hercep Test™ (DakoCytomation, Glostrup, Denmark) was used for detection of ERBB2 protein [13, 25]. Topoisomerase IIα was identified using monoclonal antibody SWT3D1 (Dako Carpinteria, CA, USA) [12, 26]. Evaluation of ERBB2 expression was performed using a 0 to 3+ scale according to intensity of staining (Fig. 1) as recommended by the producer. Topoisomerase IIα was semi-quantified using combined evaluation of expression intensity (from 0 to 3+) versus percentage of positive cells. The index of TOP2A expression was calculated as the number of positive cells x intensity of staining (grades 1–3, subgrades 0.5, 1.5, 2.5) (Fig. 1).

*Statistical methods.* A Spearman's correlation analysis and Mann Whitney test were performed using software Prisma, version 4.0, in order to show the concordance of the FISH and IHC techniques, any correlations among the genetic alterations of tumor cells and selected clinical/laboratory characteristics: patient age, TNM status and tumor histological grading.

## Results

Sixty-nine of 72 tumor sections (95.8%) were successfully deparaffinized and hybridized applying commercial ERBB2, TOP2A and centromere 17 probes. Three samples were not evaluable owing to weak signals.

Fourteen of the 69 (20.3%) successfully examined sections showed ERBB2 amplification, while co-amplification of the ERBB2 and TOP2A genes was found in 10 of the 69 sections (14.5%), i.e. in 71.4% of the ERBB2 amplified cases (Fig. 2). Deletion of the ERBB2 gene was found in 1 previously untreated patient (1.4%) with primary breast cancer. Deletion of the TOP2A gene was detected in one of ERBB2 amplified tumors (1.4%) and also in one ERBB2 deleted case (1.4%). No amplification of the TOP2A gene in ERBB2 non-amplified tumors was detected. Polysomy of chromosome 17 (≥3 CEP17/nucleus) was detected in 5 of the 69 sections (7.2%), of which 2 were IHC3+, 1 was IHC2+ and 2 were IHC0/1+ for ERBB2 protein.

IHC positive (IHC 2+, 3+) ERBB2 expression was found in 31.9% (22/69) of sections, and IHC positive TOP2A (IHC 1.5+ to 3+) was detected in 42.0% (29/69) of cases.

For comparison of FISH and IHC evaluations of the ERBB2 status (Tab. 1) we stratified patients into 4 groups according to IHC positivity (0, 1+, 2+, 3+). In the IHC3+ group (n=10) all women showed amplification of the ERBB2 gene; the concordance was 100%. In the IHC2+ group (n=12), 4 tu-

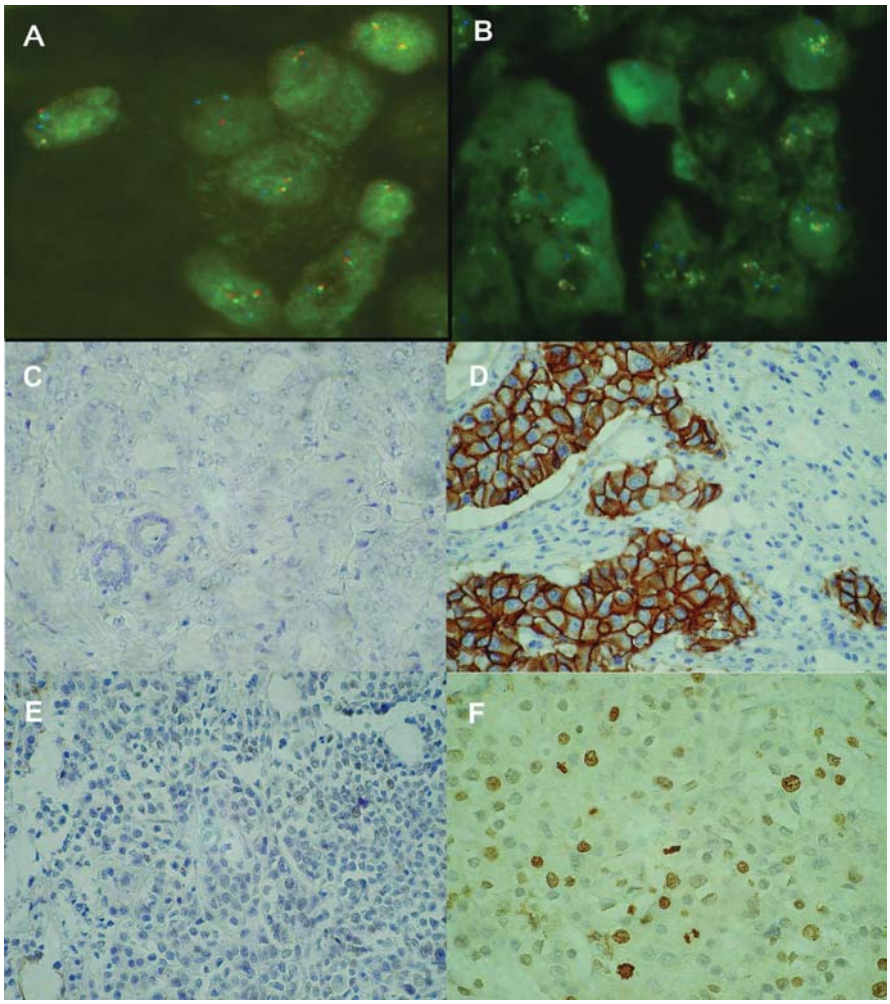


**Figure 1. Typical example of fluorescence *in situ* hybridization versus immunohistochemistry staining in breast carcinoma tissues. (A) Normal ERBB2 and TOP2A genes status (FISH); Red Signals: LSI ERBB2 labeled with SpectrumRed; Green Signals: LSI TOP2A labeled with SpectrumGreen; Blue Signals: CEP17 labeled with Spectrum Aqua. (B) Coamplification of ERBB2 and TOP2A genes (FISH). (C) Negative immunohistochemical staining (IHC0) for ERBB2 or topoIIα (E). (D) Positive immunohistochemical staining (IHC3+) for ERBB2 and (IHC2+) topoIIα (F).**

mors were amplified and 8 samples showed normal ERBB2 status. The concordance of expression versus amplification was 33.3%. In the IHC1+/0 groups (n=19/28, respectively) no tumors were amplified, and the concordance between FISH and IHC was 100% (Tab. 1). One patient with ERBB2 deletion was IHC negative. The overall concordance of the FISH versus the IHC in ERBB2 status evaluation was excellent and reached 88.4% (61/69). The statistical correlation between the ERBB2/CEP17 index and ERBB2 immunohistochemistry results was also highly significant (r=0.5930, p<0.0001) (Fig. 3).

As a next step we compared the applicability of FISH and IHC techniques for TOP2A status evaluation (Tab. 2). The patients were again divided into 4 groups according to the IHC results: 0, 0.5+ to 1+, 1.5+ to 2+, and 2.5+ to 3+. In the most IHC positive 2.5–3+ group, only 4/6 cases were amplified (66.7%), while 2/6 cases were not; concordance was 66%. In the medium IHC 1.5–2+ (n=23) tumors there were 4 amplified and 19 non-amplified cases. This group also included one patient with physical deletion of the TOP2A gene. We found concordance between the amplification and expression in the medium positive IHC1.5-2+ group in only 17.4% of cases. The lowest positive IHC0.5-1+ group (n=37) included two TOP2A amplified and 35 non-amplified specimens. The concordance of protein expression and non-amplified FISH status was 94.6%. There was only one patient included in the IHC 0 group and she had two copies of the TOP2A gene. Physical deletion of the TOP2A gene was found in two patients with IHC 2+. However in these two patients polysomy of chromosome 17 was also found. The overall concordance of FISH versus IHC in TOP2A status evaluation was much lower than in the case of ERBB2, 46/69 versus 61/69 of cases (66.7% versus 88.4%). The correlation between the ratio of TOP2A/CEP17 and immunohistochemistry was indeed statistically insignificant (r=0.1940, p=0.1156). However, we found a significant correlation between the ratio of TOP2A/CEP17 and the topoIIα IHC index (r=0.2628, p=0.0317, Fig. 3), which describes the protein expression more quantitatively.

The difference between FISH and IHC ERBB2 status results in our

**Table 1. The relationship between ERBB2 gene copy number (FISH) and protein expression (IHC)**

| ERBB2 | IHC0 | IHC1+ | IHC2+ | IHC3+ |
|---|---|---|---|---|
| Number of cases (69 total) | 28 | 19 | 12 | 10 |
| FISH non-amplified | 28* | 19 | 8 | 0 |
| FISH amplified | 0 | 0 | 4 | 10 |

*includes one deletion

**Table 2. The relationship between TOP2A gene copy number (FISH) and protein expression (IHC)**

| TOP2A | IHC0 | IHC0.5-1+ | IHC1.5-2+ | IHC2.5-3+ |
|---|---|---|---|---|
| Number of cases (67 total) | 1 | 37 | 23 | 6 |
| FISH non-amplified | 1 | 35 | 19* | 2 |
| FISH amplified | 0 | 2 | 4 | 4 |

*includes two deletions

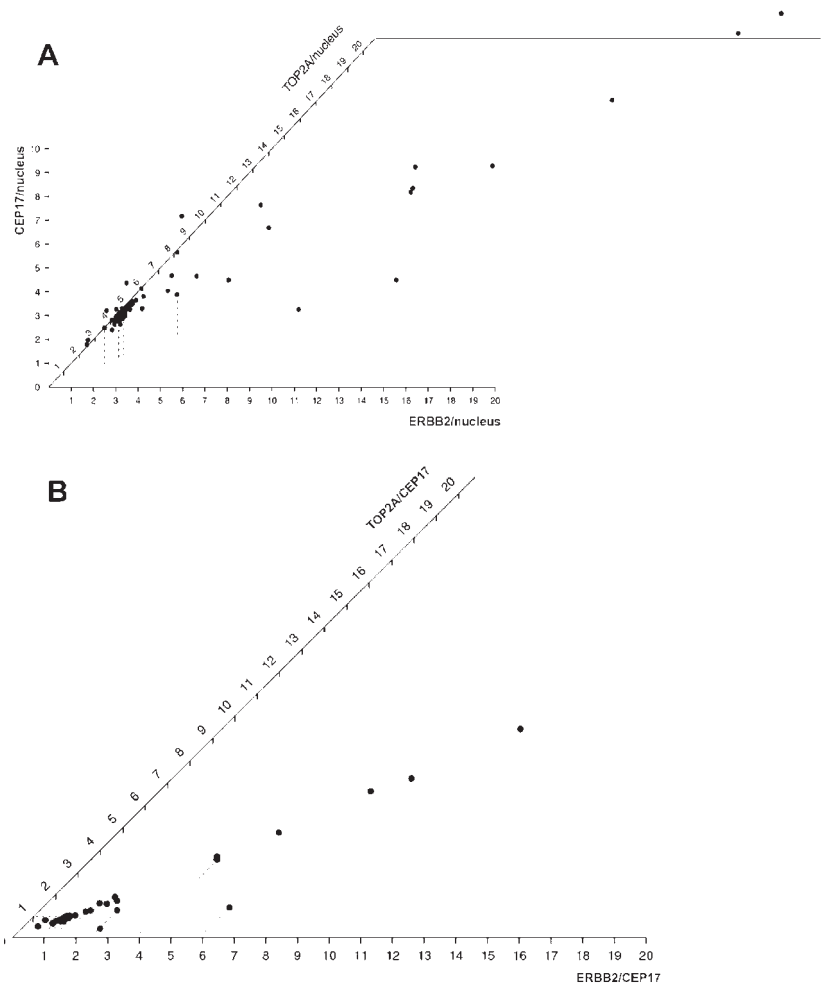


**Figure 2. Distribution of patients population according to tumor genetic status. (A) Distribution according to copies of ERBB2/nucleus, TOP2A/nucleus and CEP17/nucleus. (B) Distribution according to gene copy/CEP17 indexes of ERBB2/CEP17 and TOP2A/CEP17.**

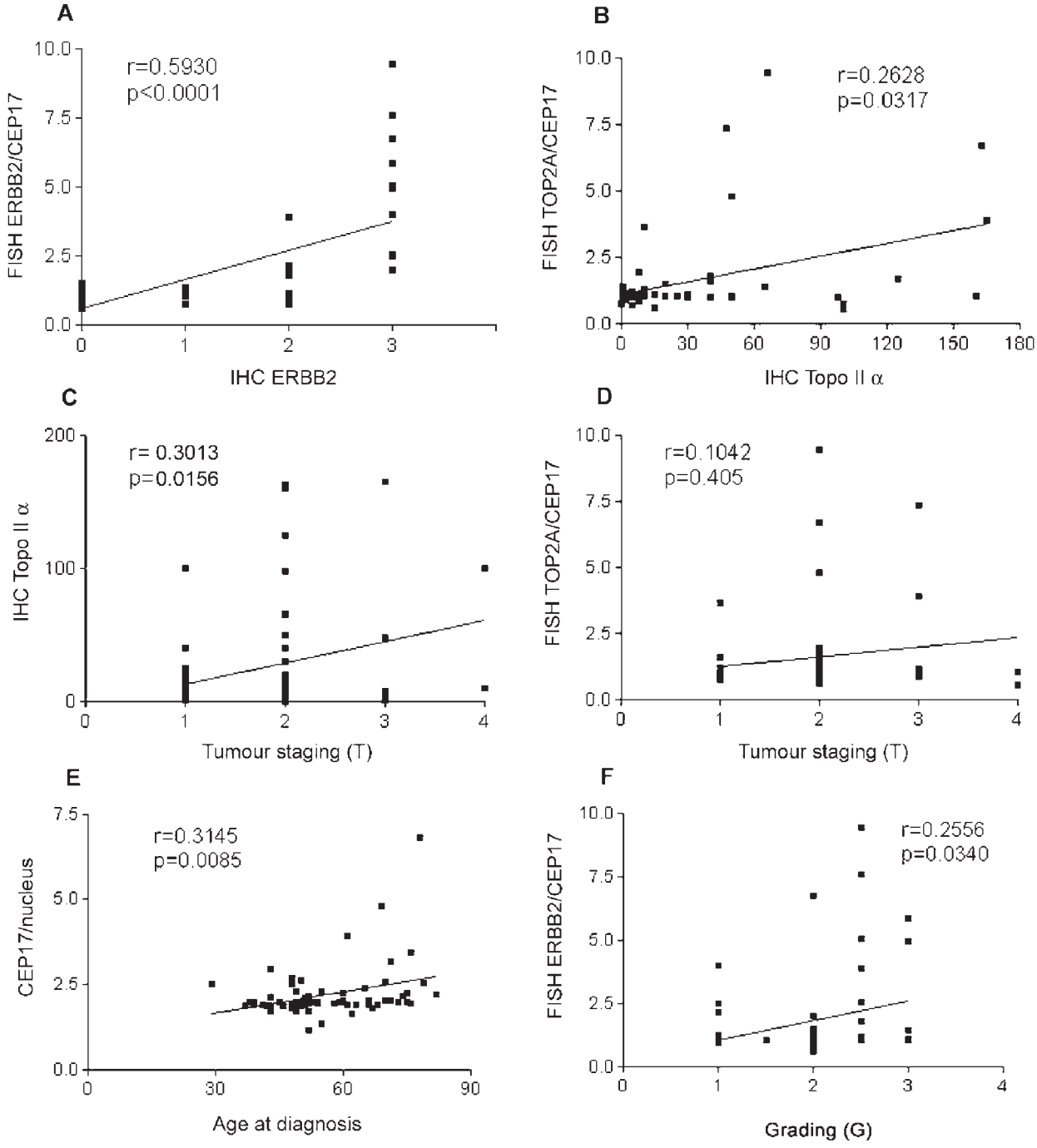


**Figure 3. Correlation analysis of FISH and IHC results for ERBB2, TOP2A and chromosome 17 values. Only significant correlations listed in Table 3 or otherwise important relationships were plotted. (A) IHC of ERBB2 versus ERBB2/CEP17. (B) IHC of topoIIα versus TOP2A/CEP17. (C) Tumor staging (T) versus IHC of topoIIα. (D) Tumor staging (T) versus FISH of TOP2A/CEP17. (E) Age at diagnosis versus CEP17/nucleus. (F) Tumor grading (G) versus FISH of ERBB2/CEP17.**

study was found only in patients recruited from the IHC2+ group (Tab. 1) and thus we confirm previously published data showing that FISH is crucial in the identification of ERBB2 gene amplified tumors in IHC 2+ cases [12, 13, 16]. These patients can benefit to a greater degree from treatment with trastuzumab [14, 15]. However, comparison of FISH and IHC techniques for evaluation of TOP2A gene status versus expression showed significant disconcordance in every

group with the exception of IHC0 (Table 2). According to these results TOP2A status should be evaluated by FISH regardless of the findings of the immunohistochemistry.

Statistical analyses of the relationship between gene status, and basic clinical or histological findings (TNM status, grading, etc.) were also performed (Fig. 3 and 4). A significant correlation of histological grading versus ratio of ERBB2 copy number and centromere 17 was found in this study (r=0.2556, p=0.034, Fig. 3). Indeed, tumors with the highest grade were more frequently ERBB2 amplified compared to cancers with lower grades (p=0.0171/0.0126, Fig. 4). The statistics also revealed an association between IHC for topoIIα and tumor staging (r=0.2804, p=0.0216, Fig. 3), although the only significant difference in topoIIα IHC was found between T1 and T2 tumors (p=0.0217, Fig. 4). Interestingly, no concordance of TOP2A/CEP17 ratio and tumor staging was noted (r=0.1042, p=0.405, Fig. 3). There was also a highly significant tendency to chromosome 17 polysomy in tumors from older patients (r=0.3145, p=0.0085, Fig. 3), and in fact, breast cancer patients older 55 years demonstrated significantly higher CEP17 copy number per nucleus (p=0.0237, Fig. 4).

## Discussion

FISH is an accurate, quantitative and extremely reliable molecular cytogenetics method for the assessment of gene status. It is widely employed in hemato-oncology diagnostics where it is used for both leukemia/lymphoma classification and/or treatment indication. Although solid tumors may account for 92% of all malignant diseases, published karyotypes of solid tumors represented only 27% of all cases until the year 1995 [27]. Hence, there is an urgent need for ongoing cytogenetic studies of solid tumors to describe cytogenetic changes important in the diagnostics and treatment of such tumors. FISH in solid tumors can be performed on interphase nuclei using smear preparations from fine needle aspirates, cytospins from effusions, cultured cells and paraffin-embedded tissue sections [28]. The last option enables us to examine tissue architecture as well and this is useful for examining archival samples when fresh biopsy material is unavailable.

Differences between FISH and IHC of the ERBB2 gene in our study was only found in patients from the IHC2+ group. 33.3% of these tumors were amplified in our cohort. In other

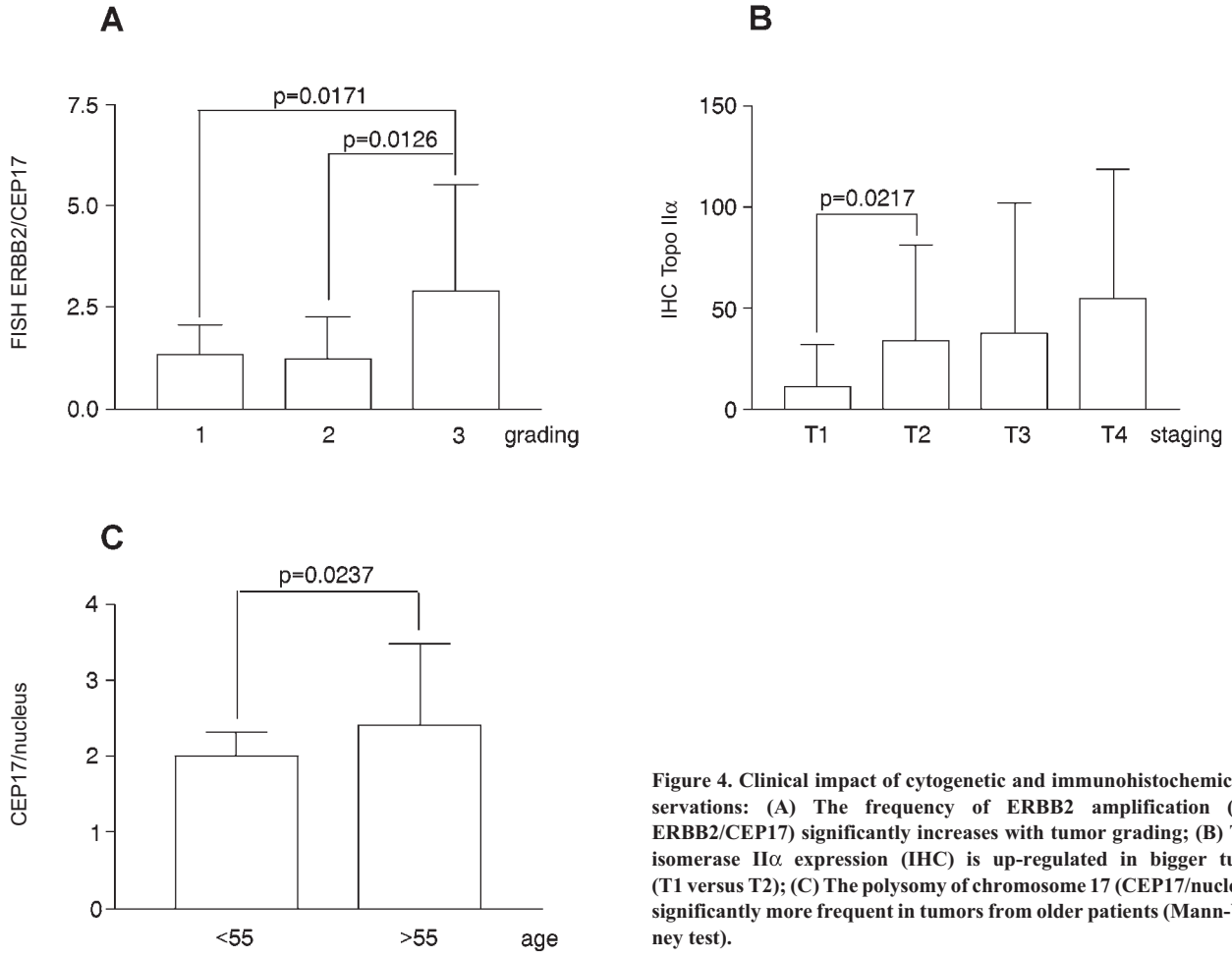


**Figure 4. Clinical impact of cytogenetic and immunohistochemical observations: (A) The frequency of ERBB2 amplification (FISH ERBB2/CEP17) significantly increases with tumor grading; (B) Topoisomerase IIα expression (IHC) is up-regulated in bigger tumors (T1 versus T2); (C) The polysomy of chromosome 17 (CEP17/nucleus) is significantly more frequent in tumors from older patients (Mann-Whitney test).**

**Table 3. Spearman correlation analysis between clinical and cytogenetic parameters**

| | Staging (T) | Staging (N) | Staging (M) | Grading | PCNA | ER | PR | Bcl-2 | CEP17 /nucleus | FISH ERBB2 /CEP17 | FISH TOP2A /CEP17 | IHC Topo2α | IHC ERBB2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Age | 0.26* | 0.03 | –0.06 | 0.09 | 0.22 | –0.08 | –0.06 | 0.01 | 0.31** | –0.01 | –0.08 | –0.08 | –0.01 |
| Staging (T) | | 0.28* | 0.13 | 0.31* | 0.26* | –0.12 | –0.14 | –0.22 | 0.24 | 0.17 | 0.10 | 0.30* | 0.17 |
| Staging (N) | | | 0.29* | 0.02 | 0.06 | 0.08 | 0.09 | 0.03 | –0.04 | –0.19 | –0.19 | 0.06 | 0.08 |
| Staging (M) | | | | 0.20 | 0.32** | 0.11 | 0.15 | 0.14 | –0.29* | –0.11 | –0.19 | 0.07 | –0.16 |
| Grading | | | | | 0.24* | –0.34** | –0.32** | –0.32** | –0.01 | 0.27* | 0.09 | 0.30* | 0.10 |
| PCNA | | | | | | 0.01 | 0.00 | 0.00 | 0.03 | 0.04 | –0.08 | 0.17 | –0.02 |
| ER | | | | | | | 0.39*** | 0.54*** | –0.03 | –0.18 | –0.14 | –0.04 | –0.03 |
| PR | | | | | | | | 0.41*** | –0.04 | 0.05 | 0.15 | –0.18 | –0.12 |
| Bcl-2 | | | | | | | | | –0.18 | –0.23 | –0.06 | –0.17 | –0.24* |
| CEP17/ nucleus | | | | | | | | | | 0.30* | 0.17 | 0.17 | 0.29* |
| FISH ERBB2 /CEP17 | | | | | | | | | | | 0.78*** | 0.43*** | 0.59*** |
| FISH TOP2A /CEP17 | | | | | | | | | | | | 0.26* | 0.44*** |
| IHC Topo2α | | | | | | | | | | | | | 0.53*** |

*, **, *** – p value <0.05, <0.01, <0.001, respectively

studies a concordance of FISH and IHC in IHC2+ samples between 0% and 30.8% has been reported [12, 16, 29, 30]. Discordant HercepTest/FISH results are most commonly IHC false-positive samples with a score 2+. TUBBS et al assessed IHC2+ false-positive sections via evaluation of specific mRNA expression and demonstrated that all 2+ false positive cases were mRNA-negative [25]. The 2+ score as defined in the guidelines for the FDA-approved HercepTest should not be used as a criterion for trastuzumab therapy unless confirmed by FISH. Determination of the ERBB2 gene copy number by FISH is a more accurate and reliable method for selection of the best candidates for trastuzumab therapy [25]. Moreover, clinical studies suggest that ERBB2 gene amplification determined by FISH may be a better predictor of clinical benefit from trastuzumab therapy than overexpression evaluated by IHC [12, 16, 17, 30]. Deletion of the ERBB2 gene in one sample was found in the present study (1.4%) and this tumor showed IHC0 similar to the findings of PEREZ et al [16]. As anticipated, ERBB2 amplification was increased in more aggressive breast cancers with grades 2 and 3 (Fig. 3 and 4). Interestingly, chromosome 17 polysomy was more frequently found in older women, which usually suffer from less aggressive disease (Fig. 3 and 4). However, further studies are needed to validate prognostic value of chromosome 17 polysomy in ERBB2 positive breast cancer patients.

Comparing FISH and IHC TOP2A status we found some disconcordance in every group except for that of IHC0. Variation of the topoisomerase IIα expression level during the cell cycle is the most probable reason for the differences found. Topoisomerase IIα expression increases during the cell cycle [31] and it is down-regulated by the end of mitosis [32–35]. This hypothesis is indirectly supported with our finding of higher topoIIα expression in more proliferating and thus bigger tumors, although direct correlation with tumor grading was not found (Fig. 3 and 4, Tab. 3). Another important factor is intratumoral heterogeneity described for TOP2A status using FISH. JÄRVINEN et al described 8/70 primary tumors with high level ERBB2 amplification that showed a new type of heterogeneity with two different cell clones characterized by either high level amplification or deletion of TOP2A found adjacent to each other in the same tumor [36]. Our results confirm a study showing no correlation between FISH and TOP2A protein expression [18].

On the other hand we found ERBB2 and TOP2A co-amplification in 71.4% (10 of 14) and deletion in 7.1% (1 of 14) ERBB2 amplified and previously untreated tumors. JÄRVINEN et al described coamplification detected by FISH in 44% and deletion in 42% ERBB2 amplified tumors [1]. In another study using the Southern blot technique, TOP2A co-amplified with ERBB2 in 12% of ERBB2 amplified cases [35]. TOP2A changes were detected nearly always in ERBB2 amplified samples. In different studies TOP2A amplification was detected in 12% to 50% of ERBB2 amplified tumors [1, 35–39] and TOP2A deletion was found in up to 43% of ERBB2 amplified cancers [1, 36, 38]. A low frequency (3–8%) of TOP2A changes in ERBB2 non-amplified samples is described in some recent studies [40–42]. The relative discrepancy in percentage of co-amplification in these studies is probably due to differences in patient population characteristics, namely staging, grading and pre-treatment status. Although deletion of the TOP2A gene has been previously assumed to predict resistance to topoisomerase IIα based chemotherapy, the majority of these observations are based on *in vitro* investigations. A typical example is the study of JÄRVINEN et al who showed physical deletion of two alleles of TOP2A (2 copies of TOP2A per 4 chromosomes 17) in one of five ERBB2 amplified cell lines. This observation was unexpected and described in breast cancer cell lines for the first time. The type of TOP2A deletion in these MDA-361 cells

was identical as it was in most clinical tumors that showed TOP2A deletion in this report [1].

In conclusion, we have shown here that both FISH and IHC examinations of the ERBB2 and TOP2A genes/proteins can be successfully performed on paraffin-embedded archival tissue from breast carcinomas. However, although the concordance of IHC and FISH is very good for the ERBB2 gene with the exception of IHC2+ samples, IHC failed to determine precisely the TOP2A gene status. For this reason we recommend the FISH technique in further clinical work to determine the significance of this gene for tailored treatment of breast cancer patients.

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