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RESEARCH

# Prognostic Value of *TOP2A* Gene Amplification and Chromosome 17 Polysomy in Early Breast Cancer

**Anna Żaczek · Aleksandra Markiewicz · Anna Supernat · Natalia Bednarz-Knoll · Burkhardt Brandt · Barbara Seroczyńska · Jarosław Skokowski · Jolanta Szade · Piotr Czapiewski · Wojciech Biernat · Marzena Wełnicka-Jaśkiewicz · Jacek Jassem**

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**Abstract** The aim of this study was to analyze the occurrence of *TOP2A* gene amplification and chromosome 17 polysomy in patients with early breast cancer and to correlate the status of these alterations with the prognostic significance expressed as patients' clinical features and survival. Such concurrent analyses of *TOP2A* gene status and chromosome 17 polysomy have not been performed before. Study group included 149 consecutive stage I-III patients administered standard multimodality treatment. *TOP2A* abnormalities were examined by standard fluorescence in situ hybridization (FISH) and developed by our group quantitative real-time PCR (qPCR). *TOP2A* amplification and deletion assessed by FISH were found in 23% and 7% of the tumours, respectively, and by qPCR in 31% and 11% of the tumours, respectively. Chromosome 17 polysomy was detected in 40% of the cases. *TOP2A* amplification (by qPCR) correlated with shorter disease-free survival ($p$=0.03) and overall survival ($p$=0.047), and the prognostic value of *TOP2A* was confirmed in the multivariate analysis (HR=3.22, 95% CI 1.09–9.56, $p$=0.03). *TOP2A* gene amplification, but not chromosome 17 polysomy, carries negative prognostic information in early breast cancer. Given the aforementioned results, qPCR might serve as a prognostic tool in determining the patient's prognosis.

**Keywords** Breast cancer · Chromosome 17 polysomy · *TOP2A* amplification · qPCR · FISH · Prognostic factor

A. Żaczek (✉) · A. Markiewicz · A. Supernat
Laboratory of Cell Biology, Department of Medical Biotechnology, Intercollegiate Faculty of Biotechnology, University of Gdańsk and Medical University of Gdańsk, Dębinki 1, 80-211 Gdańsk, Poland
e-mail: azaczek@gumed.edu.pl

A. Markiewicz
Postgraduate School of Molecular Medicine, Medical University of Warsaw, Warsaw, Poland

N. Bednarz-Knoll · B. Brandt
Institute of Tumour Biology, University Medical Centre, Hamburg-Eppendorf, Martinistrasse 52, 20-246 Hamburg, Germany

B. Seroczyńska · J. Skokowski
Bank of Frozen Tissues and Genetic Specimen, Medical University of Gdańsk, Dębinki 1, 80-211 Gdańsk, Poland

J. Szade · P. Czapiewski · W. Biernat
Department of Pathomorphology, Medical University of Gdańsk, Dębinki 7, 80-211 Gdańsk, Poland

M. Wełnicka-Jaśkiewicz · J. Jassem
Department of Oncology and Radiotherapy, Medical University of Gdańsk, Dębinki 7, 80-211 Gdańsk, Poland

## Introduction

Topoisomerase IIα is an enzyme encoded by *TOP2A* gene localized on chromosome 17q21 in close proximity to *HER2* gene. In *HER2*-amplified/overexpressing tumours, the rate of *TOP2A* aberrations (amplifications and deletions) is in the range of 27–90% [1–4]. TOP2A overexpression [5] and amplification [3, 6, 7] were shown to correlate with better anthracycline response. Furthermore, *TOP2A* aberrations, when combined with HER2 positive status, were found to correlate with shorter recurrence-free survival [8]. Cases with deletion were characterised by the worst prognosis in comparison with amplification and normal status, the latter being the most favourable condition [8, 9]. The study of Park et al., however, did not find any correlation between *TOP2A* status and clinical outcome [10].

Cytogenetic techniques, such as fluorescence in situ hybridization (FISH) or comparative genomic hybridization (CGH), have been used to show common chromosome 17 abnormalities including gene and chromosome copy number changes together with losses of alleles or structural rearrangements [11, 12]. Genes involved in breast cancer development including *HER2*, *TOP2A*, *P53* and *BRCA1* are localised on chromosome 17. Therefore, the clinical significance of chromosome 17 polysomy has been a subject of extensive investigations and its reported incidence ranges between 8 and 68%, depending on criteria used for definition of polysomy [13–15]. Polysomy of chromosome 17 is widely discussed in the context of HER2 status [16–19]. There is growing evidence that chromosome 17 polysomy with or without *HER2* gene amplification is associated with poor prognosis breast cancer [15–17]. However, the biological and clinical relevance of chromosome 17 polysomy remains to be elucidated. There is also a paucity of data on the correlation of chromosome 17 aneusomy with TOP2A status and with clinicopathological characteristics of breast cancer patients. The main aim of this study was to determine the correlation between *TOP2A* gene amplification and chromosome 17 polysomy in patients with early breast cancer and correlate the status of these biomarkers with patients' clinical features and survival. We have also investigated whether *TOP2A* gene status measured by qPCR might serve as a prognostic tool.

## Patients and Methods

### Patients

Study group included 149 consecutive breast cancer patients treated between 2001 and 2008 at two Polish centres. Inclusion criteria were stage I-III breast cancer and signed informed consent. The majority of patients (62%) underwent primary surgery followed by systemic treatment, radiotherapy or both. Twenty-six percent of patients were administered induction chemotherapy. Tumour samples were collected by surgical excision or excisional biopsy prior to any systemic treatment, immediately frozen in liquid nitrogen and stored at −80°C for further analysis.

The mean age of the patients was 57.3 (range 27–86 years). Thirty percent of the patients were premenopausal and seventy percent post-menopausal (Table 1). HER2 protein expression was determined by immunohistochemistry using HercepTest (DAKO, Denmark) in accordance with the manufacturer's guidelines. Positive result was considered as 3+ score, negative as 0 and 1+. 2+ score was not verified by fluorescence in situ hybridization (FISH), it was treated as equivocal and excluded from the statistical analysis. Survival analysis was performed for all patients. After a median follow-up of 2.7 years

**Table 1** Patient characteristics ($N$=149)

| Variable | Number of cases (%) |
|---|---|
| Menopausal status | |
| premenopausal | 44 (30) |
| postmenopausal | 105 (70) |
| T stage | |
| T1 | 50 (34) |
| T2 | 81 (54) |
| T3 | 8 (5) |
| T4 | 9 (6) |
| Missing data | 1 (1) |
| N stage | |
| N0 | 68 (46) |
| N1 | 50 (34) |
| N2 | 22 (15) |
| N3 | 6 (4) |
| Missing data | 3 (2) |
| Grade | |
| G1 | 41 (27) |
| G2 | 68 (46) |
| G3 | 40 (27) |
| Histological type | |
| Ductal | 120 (81) |
| Lobular | 17 (11) |
| Other | 12 (8) |
| ER | |
| Negative | 70 (47) |
| Positive | 77 (52) |
| Missing data | 2 (1) |
| PgR | |
| Negative | 61 (41) |
| Positive | 86 (58) |
| Missing data | 2 (1) |
| HER2 protein status | |
| Negative (0, 1+) | 81 (54) |
| Weakly positive (2+) | 25 (17) |
| Strongly positive (3+) | 22 (15) |
| Missing data | 21 (14) |

(range 0.13–8.6 years), 14 patients (9%) experienced recurrence of the disease and 8 died (5%).

Patients were examined every six months after the inclusion into the study, with the last follow-up in May 2010. The study was accepted by the Ethics Committee of the Medical University of Gdańsk.

## Controls

Pooled leukocyte DNA isolated from fresh or frozen blood samples of five healthy donors was used for qPCR assay optimization and as a calibrator. The donors signed informed consents. DNA isolated from SKBR3 breast cancer cell line served as a positive control as it exhibits *TOP2A* amplification [20].

## DNA Isolation

DNA from peripheral blood (0.2 ml per sample) and from breast cancer samples (20 mg per sample) was isolated with QIAamp DNA Mini Kit (Qiagen, Germany) using blood or tissue protocol, in accordance with the manufacturer's instructions. After the isolation, DNA concentration and purity were determined by Spectrophotometer ND-1000 (NanoDrop, USA). Good quality DNA was defined as $A_{260nm/280nm}$ ratio between 1.70 and 1.90.

## Real Time PCR (qPCR)

*TOP2A* gene copy number was determined by qPCR with hydrolysis probes [21]. Briefly, *TOP2A* copy number was calculated using Pfaffl quantification method, which relates the gene dosages of *TOP2A* and a reference gene (amyloid precursor protein gene—*APP*) in the tumour tissue and a calibrator (leukocyte DNA). In these experiments, thermocycler CFX96$^{TM}$ (Bio-Rad, USA) equipped with appropriate filters was used. Reactions were performed in duplicates on 96 well plates; on each plate two negative controls for each gene and two calibrators were included.

We used experimentally determined cut-off values calculated using critical difference parameter, as described previously [21]. Amplification was classified as *TOP2A* gene dosage higher than 1.15 and deletion as values lower than 0.85.

## Fluorescence In Situ Hybridization (FISH) on Tissue Microarrays (TMA)

TMAs were constructed from formalin-fixed, paraffin-embedded surgical resection tumour specimens. Briefly, two 1.5 mm-diameter cores from each tumour were obtained from the most representative areas using tissue-arraying instrument (MTA-I, Beecher Instruments, USA), and then reembedded in microarray blocks. Punches of normal breast tissue samples were added to the "tumour array", to introduce built-in internal controls to the system. TMAs of 41 cases per block were made. Consecutive 4 μm-thick TMA sections were cut and placed on charged polylysine-coated slides (Superfrost Plus, BDH, Germany) for subsequent FISH analysis.

To determine *TOP2A* gene dosages, Food and Drug Administration approved TOP2A FISH pharmDxt Kit (DAKO, Denmark) was applied on the TMA specimens in accordance with the manufacturer's protocol with some minor modifications (10 min. pepsin digestion).

FISH slides were evaluated using a Zeiss Axioscope epifluorescence microscope (Carl Zeiss, Inc, Thornwood, NY). Signals were counted using a DAPI, Texas Red and FITC filter set to visualize nuclei, SpectrumRed TOP2A, and SpectrumGreen CEN-17, respectively. In each TMA tissue, core signals for each probe were counted in 30–60 individual intact cell nuclei with malignant morphology.

Experimentally determined cut-off values were used to assess *TOP2A* gene status. Amplification of *TOP2A* was defined as *TOP2A*/*CEN17* ratio higher than 1.25 or when a large gene copy cluster was seen in over 30% of the nuclei in the cancer cells. Deletion was defined as *TOP2A*/*CEN17* ratio lower than 0.7. Chromosome 17 polysomy was defined as at least three *CEN17* copies per nucleus in more than 30% of the evaluated tumour cells [15].

## Statistical Analysis

All statistical analyses were performed using the STATISTICA software, version 9. In order to compare FISH and qPCR methods Spearman's Rank Correlation Coefficient was used. *TOP2A* gene aberrations and chromosome 17 polysomy, according to clinicopathological parameters, were assessed by Crosstabs statistics with Pearson's chi-square test. The endpoints for the study were disease-free survival (DFS) and overall survival (OS). DFS was defined as the time from tumour sample collection to an event or censoring. An event was defined as relapse (local or distant), second malignancy or death, whichever came first. A censoring was defined as loss of follow-up or alive without relapse at the end of follow-up. OS was defined as the time from sample collection to death or censoring. DFS and OS Kaplan-Meier curves for subgroups of patients were compared using the log-rank test. Cox proportional hazards regression analysis was used to identify the independent predictors of DFS. Univariate predictors significant with a value of $p \leq 0.10$ were entered into a step-wise multivariate model to identify those with independent prognostic information. Statistical significance was assumed when $p<0.05$. The study was performed in accordance with the REMARK criteria [22].

## Results

### TOP2A Gene Dosage Assessed by FISH

The average *TOP2A/CEN17* ratio in the tumour samples was 1.17 (range 0.41 to 3, median 1.03). Amplification and deletion of *TOP2A* was found in 23% and 7% of tumours, respectively (Table 2). The rate of *TOP2A* deletions and amplifications in HER2-positive subgroup (3+ by HercepTest) was 14% and 41%, respectively, and in HER2-negative subgroup 5% and 20%, respectively ($p$=0.0025).

### TOP2A Gene Dosage Assessed by qPCR

*TOP2A* gene dosage in clinical samples ranged from 0.2 to 3.25, with an average of 1.16 and a median of 1.05. *TOP2A* gene amplifications and deletions were detected in 31% and 11% of the patients, respectively (Table 2). The rate of deletions and amplifications in HER2-positive subgroup (3+ by HercepTest) was 5% and 36%, respectively, and in HER2-negative 15% and 25%, respectively ($p$=0.31).

### Correlation Between qPCR and FISH

The results obtained by qPCR and FISH correlated, although the concordance was relatively weak (53%, $r^2$= 0.27, $p$<0.01). Average gene dosage of *TOP2A* determined by qPCR was 0.97±0.19 (average ± SD) for *TOP2A* deletion determined by FISH; 1.07±0.35 for normal gene status and 1.34±0.6 for amplification.

### Chromosome 17 Polysomy

Chromosome 17 polysomy, measured by FISH, was detected in 59 patients (40%), 35 of whom (59%) had polysomy without *TOP2A* abnormalities (Table 2). The occurrence of chromosome 17 polysomy correlated with *TOP2A* status assessed by FISH ($p$=0.009) and qPCR ($p$= 0.04). *TOP2A* amplifications together with polysomy were found in 16 cases (47%). The majority of deletions (8/10, 80%) occurred in tumours with polysomy.

### Correlation of Examined Biomarkers with Clinical and Pathological Data

*TOP2A* status determined by FISH correlated with tumour grade, estrogen receptor status and HER2 expression (Table 3). Deletions were found in 17.5% (7/40) of the G3 tumours and in 2.7% (3/109) of the G1-2 tumours ($p$= 0.004). *TOP2A* amplifications were more frequent in ER-negative cases (28.6%, 20/70) compared to ER-positive cases (16.8%, 13/77; $p$=0.03). *TOP2A* amplifications occurred two times more frequently in HER2-positive than in HER2-negative tumours (41%, 9/22 vs. 20%, 16/81; $p$= 0.025). Similarly, chromosome 17 polysomy occurred more frequently in HER2-positive (55%, 12/22) than in HER2-negative tumours (30%, 24/81, $p$=0.03).

### Survival Analysis

In the univariate analysis, *TOP2A* amplification assessed by qPCR correlated with shorter DFS ($p$=0.03; Fig.1a) and OS ($p$=0.047; Fig. 1b). Recurrence occurred in 8 out of 46 (17.4%) patients with *TOP2A* amplification and in 4 out of 87 (4.6%) patients with normal *TOP2A* level. In the multivariate regression analysis including menopausal status, T stage, lymph node involvement, grade, histological type, ER and PgR status, *TOP2A* amplification (by qPCR and FISH), and chromosome 17 polysomy (Table 4), only *TOP2A* amplification (by qPCR) and T stage were independent prognostic factors for DFS (HR=3.22, 95% CI 1.09–9.56, $p$= 0.03 and HR=7.08, 95% CI 2.42–20.7, $p$=0.0003, respectively). Multivariate analysis could not be performed for OS due to insufficient number of events. No correlation with DFS and OS was observed for *TOP2A* status determined by FISH and for chromosome 17 polysomy.

**Table 2** *TOP2A* gene copy number, as measured by FISH and qPCR, according to chromosome 17 polysomy status (N—number of patients)

| Chromosome 17 Polysomy | *TOP2A* status | by qPCR | | $p$ | by FISH | | $p$ |
|---|---|---|---|---|---|---|---|
| | | N | % | | N | % | |
| Absent ($N$=90) | Deletion | 2 | 2 | | 12 | 13 | |
| | Normal | 70 | 78 | | 57 | 63 | |
| | Amplification | 18 | 20 | | 21 | 23 | |
| Present ($N$=59) | Deletion | 8 | 14 | | 4 | 7 | |
| | Normal | 35 | 59 | 0.04* | 30 | 51 | 0.009* |
| | Amplification | 16 | 27 | | 25 | 42 | |
| Total ($N$=149) | Deletion | 16 | 11 | | 10 | 7 | |
| | Normal | 87 | 58 | | 105 | 70 | |
| | Amplification | 46 | 31 | | 34 | 23 | |

*$p$ value expresses the correlation between the occurrence of chromosome 17 polysomy and *TOP2A* status, assessed by FISH and qPCR

**Table 3** *TOP2A* status and chromosome 17 polysomy, as measured by qPCR and FISH, according to clinicopathological parameters (N—number of patients)

| Variable | *TOP2A* by qPCR | | | | | *TOP2A* by FISH | | | | | Chromosome 17 polysomy | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Deletion $N=16$ | Normal $N=87$ | Amplified $N=46$ | Total $N=149$ | $p$* | Deletion $N=10$ | Normal $N=105$ | Amplified $N=34$ | Total $N=149$ | $p$* | Absent $N=90$ | Present $N=59$ | Total $N=149$ | $p$* |
| T stage | | | | | | | | | | | | | | |
| T1-2 | 13 | 76 | 42 | 131 | 0.55 | 8 | 93 | 30 | 131 | 0.67 | 80 | 51 | 131 | 0.52 |
| T3-4 | 3 | 10 | 4 | 17 | | 2 | 11 | 4 | 17 | | 9 | 8 | 17 | |
| N stage | | | | | | | | | | | | | | |
| N0 | 8 | 40 | 20 | 68 | 0.92 | 5 | 49 | 14 | 68 | 0.77 | 43 | 25 | 68 | 0.49 |
| N1-3 | 8 | 45 | 25 | 78 | | 4 | 55 | 19 | 78 | | 45 | 33 | 78 | |
| Grade | | | | | | | | | | | | | | |
| G1-2 | 12 | 59 | 38 | 109 | 0.18 | 3 | 78 | 28 | 109 | 0.004 | 66 | 43 | 109 | 0.95 |
| G3 | 4 | 28 | 8 | 40 | | 7 | 27 | 6 | 40 | | 24 | 16 | 40 | |
| Histological type | | | | | | | | | | | | | | |
| Ductal | 11 | 69 | 40 | 120 | 0.3 | 10 | 82 | 28 | 120 | 0.2 | 74 | 44 | 118 | 0.71 |
| Lobular | 3 | 11 | 3 | 17 | | 0 | 15 | 2 | 17 | | 10 | 7 | 17 | |
| Hormone receptor status | | | | | | | | | | | | | | |
| ER(+) | 8 | 44 | 25 | 77 | 0.81 | 5 | 59 | 13 | 77 | 0.03 | 44 | 33 | 77 | 0.65 |
| ER(−) | 8 | 41 | 21 | 70 | | 4 | 46 | 20 | 70 | | 45 | 25 | 70 | |
| PgR(+) | 7 | 47 | 32 | 86 | 0.23 | 4 | 62 | 20 | 86 | 0.07 | 51 | 35 | 86 | 0.89 |
| PgR(−) | 9 | 38 | 14 | 61 | | 5 | 43 | 13 | 61 | | 38 | 23 | 61 | |
| HER2 | | | | | | | | | | | | | | |
| 3+ | 1 | 13 | 8 | 22 | 0.31 | 3 | 10 | 9 | 22 | 0.025 | 10 | 12 | 22 | 0.03 |
| 0-1+ | 12 | 49 | 20 | 81 | | 4 | 61 | 16 | 81 | | 57 | 24 | 81 | |

*$p$ values express correlation between *TOP2A* status or chromosome 17 polysomy and a given variable

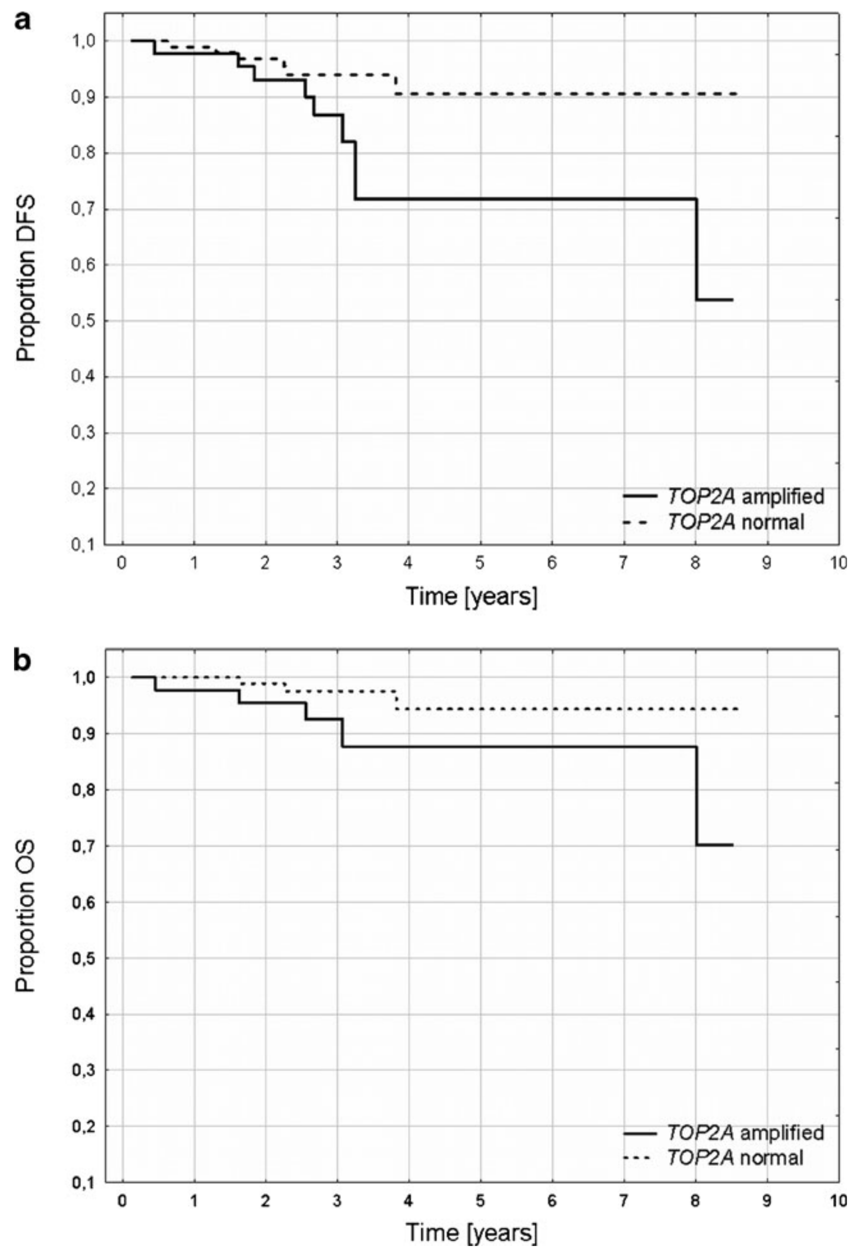


**Fig. 1** **a** The difference in disease-free survival with normal and amplified *TOP2A* gene as measured by qPCR ($p$= 0.03), number of patients: 133. **b** The difference in overall survival with normal and amplified *TOP2A* gene as measured by qPCR ($p$=0.047), number of patients: 133

## Discussion

In the current study, we analyzed the occurrence of *TOP2A* gene amplification and chromosome 17 polysomy in patients with early breast cancer and correlated the status of these alterations with patients' clinical features and survival. To our knowledge, this is the first joint analysis of chromosome 17 polysomy and *TOP2A* status determined by qPCR in breast cancer.

In our study, *TOP2A* aberrations were evaluated in consecutive breast cancer patients, including HER2-positive and HER2-negative tumours. It has been shown that measured occurrence of *TOP2A* alterations depends not only on the analysis technique and chosen cut-off level but also on the intrinsic molecular subtypes of the tumour, with the lowest alteration levels in basal-like and normal-like [23]. *TOP2A* amplification prevalence of 36% in HER2-positive tumours in this series is comparable with the previously published results ranging from 30% to 50% [3, 24, 25]. In this subset of patients, the proportion of samples showing deletion was also comparable to that reported by O'Malley et al. [6]. Interestingly, as opposed to the majority of other studies [10, 26, 27], in our series *TOP2A* amplifications were also found in HER2-negative cases. The highest previously reported rate of *TOP2A* amplifications in the HER2-negative population was 6.4% [6], compared to 26% in our study. This difference might have resulted from higher sensitivity of qPCR to detect local gene rearrangements in comparison to FISH used by others [21]. Our results might suggest that evaluating *TOP2A* status only in HER2-positive

**Table 4** Univariate analysis for disease free survival of patients with early breast cancer

| Variable | N | Hazard ratio (95% confidence interval) | *p* |
|---|---|---|---|
| Menopausal status (post vs. pre) | 149 | 2.6 (0.58–11.73) | 0.2 |
| Tumor size (T3-4 vs. T1-2) | 148 | 1.09 (1.04–1.14) | 0.0004 |
| Lymph nodes (positive vs. negative) | 147 | 3.15 (0.87–11.45) | 0.08 |
| ER (negative vs. positive) | 147 | 0.83 (0.28–2.48) | 0.74 |
| PgR (negative vs. positive) | 147 | 0.93 (0.31–2.77) | 0.89 |
| Histological type (ductal vs. lobular) | 143 | 0.64 (0.14–2.98) | 0.57 |
| Grade (G3 vs. G1-2) | 149 | 0.6 (0.16–2.12) | 0.42 |
| TOP2A by qPCR (amplified vs. normal) | 149 | 3.08 (1.05–9.02) | 0.04 |
| TOP2A by FISH (amplified vs. normal) | 149 | 1.23 (0.34–4.46) | 0.75 |
| Chromosome 17 polysomy (yes vs. no) | 149 | 1.94 (0.67–5.62) | 0.22 |

patients may miss *TOP2A* abnormalities in a proportion of HER2-negative tumours. Aberrant *TOP2A* status, independently of HER2, could indicate patients with higher probability of benefit from anthracycline-containing regimens.

Our study detected chromosome 17 polysomy in 40% of cases. This number is within the wide range of 5–68% reported by other authors [17, 28–36]. Such a wide range of chromosome 17 polysomy may result from various scoring criteria [15]. Analysis of 11,000 breast tumours revealed the best discriminatory ability for defining chromosome 17 polysomy when at least 30% of nuclei had 3 or more centromere 17 signals [15]. This level for polysomy classification was therefore used in our study. Another reason for diverse results of polysomy in particular studies [37] may be the complexity of chromosome 17. The centromere itself does not necessarily need to be representative for the entire chromosome, which results in *TOP2A/CEP17* ratio bias [13, 14]. It was even postulated that chromosome 17 polysomy is likely to be a rare event in breast cancer and that *CEP17* copy number greater than 3.0 in FISH analysis is frequently related to the gain or amplification of the centromeric region, but not to the higher chromosome 17 number [38].

In this study, the occurrence of polysomy correlated with *TOP2A* gene copy number determined by both qPCR and FISH. Chromosome 17 polysomy was accompanied by *TOP2A* amplification determined by qPCR and FISH in 54% and 27% of cases, respectively. In the series of Beser et al. [39] including 50 patients, in no case was *TOP2A* amplification accompanied by polysomy, but this might have been due to the low number of patients examined. Other reports suggested that chromosome 17 polysomy was usually more frequent in *HER2*-amplified (10–88%) than in *HER2*-nonamplified tumours (6–55%) [15]. Likewise, in our study, polysomy occurred significantly more frequently in HER2-positive than in HER2-negative tumours. Similarly to the findings of Beser et al. [39], in this series *TOP2A* deletions (determined by FISH) were frequently accompanied by polysomy. Hence, the coexistence of *TOP2A* deletions and chromosome 17 polysomy may confirm the hypothesis that *TOP2A* deletions occur immediately before genome polyploidisation [15, 39, 40].

Chromosome 17 abnormalities can result in disruption of key genes involved in cancer development, such as *P53*, *HER2*, *BRCA1* and *TOP2A*. In consequence, the impact of *TOP2A* amplification and chromosome 17 polysomy on the tumour behaviour and prognosis has been extensively investigated in breast cancer [26, 41–43]. In concordance with the results obtained by other groups, in this study *TOP2A* amplifications determined by FISH correlated with ER-negativity and HER2 overexpression [6, 7]. This relation indicates that tumours carrying amplification of *TOP2A* are intrinsically more aggressive [10]. Indeed, in our series *TOP2A* amplification assessed by qPCR correlated with shorter DFS and OS. These results confirm our previous preliminary findings showing for the first time prognostic value of *TOP2A* determined by qPCR [21]. Similarly to other groups [10, 42, 44] we did not find adverse effect of *TOP2A* amplification determined by FISH, although other reports demonstrated such correlation [8, 9]. Our results show that *TOP2A* amplification or deletion determined by qPCR and FISH do not completely overlap, and that the rearrangements of *TOP2A* measured by qPCR may better predict survival.

*TOP2A* deletions remain one of the most intriguing genetic events occurring in the 17q21 amplicon [2]. They were reported to determine adverse prognosis [9, 12, 42, 45], even stronger than *TOP2A* amplifications [9]. In our study, *TOP2A* deletions occurred more frequently in less differentiated tumours, which might suggest that, like *TOP2A* amplifications, they are associated with more aggressive tumour behaviour. We were unable to examine prognostic role of *TOP2A* deletions since their number was not sufficient for survival analysis. The impact of *TOP2A* deletions on prognosis reported in other studies may be related to bystander effect. Indeed, *TOP2A* gene is located on chromosome 17 close to many important genes including *HER2*, Gene Rich Region (17q21 GRR) and *BRCA1* locus. Of these, especially BRCA1 loss seems to be associated with stem-like phenotype of cancer cells and their dedifferentiation [46]. If deletion of *TOP2A* covers a larger segment of 17q21, the observed clinical correlations might be rather due to the loss of *BRCA1* and not *TOP2A*, which could change tumour aggressiveness and alter the response to different therapeutic agents.

Polysomy 17 may reflect increased chromosomal instability and was found to correlate with adverse prognostic tumour features, such as high grade, high Nottingham Prognostic Index (NPI), ER negativity [17] and lymph node status [47]. In our work, polysomy 17 occurred more frequently in HER2-positive tumours ($p=0.03$), but similarly to the results of Vanden Bempt [18] it did not carry adverse prognosis. Those authors concluded that clinicopathologic impact of polysomy 17 is not as strong as that of *HER2* gene amplification in breast cancer and that the behaviour of tumours showing polysomy 17 in the absence of *HER2* gene amplification is similar to that of HER2–negative rather than to HER2–positive tumours [18]. This can explain the lack of correlation of polysomy 17 with reduced survival in our study and in other series [17, 18] and indicates that this abnormality cannot be considered a prognostic marker in early breast cancer.

In summary, our study suggests that *TOP2A* gene amplification, but not chromosome 17 polysomy, carries negative prognostic information in early breast cancer. Thus, qPCR might serve as a prognostic tool in determining the patient's prognosis. A translational study with patients treated with anthracyclines might provide further evidence on the clinical applications of *TOP2A* status determined by qPCR.

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**Conflict of Interest** The authors declare that they have no conflict of interest.

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