Topoisomerase II α – a fundamental prognostic factor in breast carcinoma

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Because of the introduction of modern diagnostic methods, numerous prognostic and predictive factors have been recognized and are today considered classic, yet they seem to be insufficient in assessment of prognosis, hence the need for further investigations. Among factors newly discovered by molecular techniques, there are class I and II topoisomerases, the role of which as prognosticators has not been fully determined. The objective of the present investigation was the assessment of topoisomerase II α (TOP2A) expression in patients with infiltrating breast carcinoma, as a prognostic factor in correlation with other recognized prognosticators and patient survival. The study was carried out in 151 patients treated by mastectomy and lymph node excision followed by adjuvant chemotherapy. The material was evaluated histopathologically according to the pTNM system, taking into consideration such parameters as grade of malignancy (G); the ER, PR as well as HER2 and TOP2A receptors status – all of them were assessed immunohistochemically. TOP2A was expressed with varying intensity in the majority of infiltrating ductal carcinomas studied, more frequently in large T3 and T4, grade G2 and G3 tumours, in patients with extensive metastases to regional N2 and N3 lymph nodes, a positive HER2 and negative ER and PR status. Five-year mortality rates were higher and 5-year symptom-free survival rates were lower in patients with TOP2A-postive tumours as compared to individuals with a negative TOP2A status. The study indicates that TOP2A expression is a negative predictive factor and may be recognized as a prognostic factor.

Key words: breast carcinoma, topoisomerase II α.

Introduction

Prognostic factors allow one to evaluate the risk of relapse or progression of the disease leading to a fatal outcome assuming no systemic adjuvant therapy has been employed [1-8].

Prognostic factors are associated with the tumour biology, the patient and environmental exposure. The best understood and defined prognosticators are those associated with selected carcinoma features; among them there are those evaluated by pathomorphologists, referred to as pathomorphological prognostic factors [2, 3, 5, 7-9].

The introduction of new techniques to pathomorphology, and especially the advent of immunohistochemistry, molecular biology and cytometry, has made a contribution to our discovering and describing several new factors that characterize tumours and to recognizing these factors as prognostic or predictive [3, 4, 6, 9-11].

In 2000, the College of American Pathologists reviewed prognostic and predictive factors based on the body of information on their clinical value and on results of research [11-13], dividing the factors into three groups:

I – tumour size, lymph node status, pTNM, histological type, grade of histological malignancy, assessment of ER and PR receptors,

II – proliferation markers (MIB1, S phase of the cell cycle, HER2/c-erb-B2, PT53, invasion of lymphatic and blood vessels),

III – angiogenesis, epidermal growth factor (EGF), transforming growth factor α (TGF-α), BCL-2, PS2, cathepsin D.

In 2005, group I was extended and now it includes HER2 [4].

According to the authors of the classification, group I represents fundamental factors, whose prognostic value has been fully proven and which should be taken into consideration while planning further treatment and determining the prognosis.

Other prognostic factors in breast carcinoma that play a significant role both in carcinogenesis and tumour development are presently commonly used in view of the lack of uniform standards of evaluation and evidence of their clinical value; these factors are, nevertheless, subject to extensive research.

Higher importance in selecting a therapeutic modality and prognosis is ascribed to prognostic rather than predictive factors; the mechanism underlying the activity of the latter has not been fully understood and it requires further studies [3, 5, 12, 13].

Although numerous prognostic and predictive factors believed to be classic prognosticators have been identified and understood, they seem to be insufficient to evaluate prognosis in individual patients in view of the varied clinical course in tumours with similar parameters. Further investigations on breast tumour carcinogenesis are required, especially since therapeutic methods employed to date do not lead to anticipated results [2-5, 7, 14-16].

Among such factors that have been newly discovered by means of molecular studies are class I and II topoisomerases [16-24]. A complex DNA structure in the nuclei requires extremely precise mechanisms responsible for spatial organization of DNA molecules in the nucleus. Numerous active enzymes present in the nuclei participate in these processes, decreasing the degree of DNA spiralization, giving access to particular regions – in spite of intense packing – in order for transcription, translation and recombinant repair to occur. In addition to other substances, these functions are exercised by topoisomerases I and II [16, 17, 19, 21, 24-26].

Class I topoisomerases are responsible for relaxing tensions resulting from coiling and uncoiling DNA molecules. Class II topoisomerases specialize in untangling DNA in the nucleus, allowing one DNA helix to pass through the other one, cutting both strands of one DNA helix, passing the other DNA strand through the gap and subsequently reattaching both formerly cut ends of the DNA strand [17, 19, 21, 24-27].

Topoisomerase II has two isoforms encoded by various genes: α and β, which differ by their location in the nucleus, type of DNA binding and amount of enzyme, which depends on the physiological state of the cell. The level of topoisomerase II β is almost constant throughout the entire cell cycle, but the level of topoisomerase II α clearly increases in an unplanned manner in dividing cells [17, 19, 21, 24, 25, 28, 29].

Topoisomerase determinations in neoplastic tumours may be performed in archival paraffin blocks both by immunohistochemistry and FISH, which allows for retrospective assessment of the patients [15, 17, 18, 22, 29-34].

The literature evaluating the prognostic value of TOP2A status determinations is extremely scant, and the results of such studies are frequently divergent [30, 35-40].

Reported data indicate that evaluation of TOP2A status using immunohistochemistry and hybridization *in situ* yields varying, at times discordant results [15, 29, 32, 38, 41-43].

Various methods of evaluating TOP2A status in breast carcinoma, as well as absence of uniform criteria of assessing the level of overexpression or even of an unequivocal definition of borderline values of the number of gene copies or results of immunohistochemistry, justify the necessity of further research.

A review of the literature indicates that, despite the theoretical background, the prognostic importance of TOP2A in breast carcinoma has not been sufficiently documented to date, which favours further studies.

The objective of the study was assessment of topoisomerase II α (TOP2A) expression in patients with breast carcinoma as a prognostic factor in correlation with other recognized prognosticators, such as tumour size (pT), metastases to regional lymph nodes (pN), histological grade of malignancy (G) and expression of oestrogen (ER) and progesterone (PR) receptors, as well as HER2.

Material and methods

The study was carried out in archival material originating from 151 patients treated surgically in Podkarpacki Oncology Centre in the years 1999-2001 by means of mastectomy combined with lymph node excision followed by chemotherapy. Patients without preoperative chemotherapy or radiotherapy and with histopathologically confirmed infiltrating ductal breast carcinoma were selected for the investigation.

The study did not include patients with other histopathological types of breast tumours or with mixed types, in which another component accounted for more than 10% of the tumour structure, or patients above 70 years of age in view of problems involved in establishing the cause and time of death. In this way, a relatively clinically and morphologically homogeneous group was formed.

The age of the investigated patients was within the range of 28-70 years, mean age 56.5 years, standard deviation ±7 years. In the age range from

49.5 to 63.5 years were 82 females (54.3%), while 30 patients were below 49.5 years (19.8%), and 39 were above 63.5 years (25.8%).

Postoperative materials originating from patients with infiltrating ductal breast carcinoma were assessed histopathologically in keeping with the pTNM staging system, according to the WHO classification [8], while the grade of histological malignancy was evaluated following the three-score grading system developed by Bloom and Richardson and modified by Elston and Ellis [8], which is presented in Table I.

Oestrogen and progesterone receptors were determined by immunohistochemistry using commercially available kits produced by Dako and following procedures recommended by the manufacturer. Immunohistochemical assessment of preparations was performed in keeping with the manufacturer's recommendations, determining the percentage of stained nuclei per 10 fields of vision (40 ×) and employing a four-score scale:

- 0 – 0-10% of stained nuclei, 1^+ – 11-30% of stained nuclei,
- 2^+ – 31-60% of stained nuclei, 3^+ – more than 60% of stained nuclei.

HER2 was assessed by immunohistochemistry on slides obtained from paraffin blocks, employing a Herceptest kit by Dako (K5207) and following the manufacturer's recommendations.

The assessment of HER2 status was performed in keeping with the Dako scale based on staining intensity of nuclear membranes of tumour cells:

- 0 – no staining of nuclear membranes,
- 1^+ – poor reaction in nuclear membranes in at least 10% of cells,
- 2^+ – moderate reaction in nuclear membranes in at least 10% of cells,
- 3^+ – strong reaction in at least 10% or more tumour cells.

In agreement with the previously published Dako criteria, HER2 status was regarded as negative when the score was 0 and 1^+, and positive with the score of 2^+ and 3^+. The criteria were modified in 2006 and at present, HER2 status is regarded as negative at 0 and 1^+, positive at 3^+ and dubious at 2^+ – in this case verification by FISH is recommended.

Topoisomerase II α (TOP2A) was determined in paraffin sections obtained from the same paraffin blocks as used for immunohistochemical determinations of ER, PR and HER2. Immunohistochemical staining for TOP2A was performed at the Department of Pathomorphology, Centre of Oncology, Warsaw, Poland.

Immunohistochemical reactions were performed using an automatic Dako Autostainer Plus – according to the enclosed instructions – with the DAKO REAL™ Envision™ Detection System, Peroxidase (DAB+) Rabbit (Mouse, catalogue no. K5007).

The specific antibody Mouse Monoclonal Anti-Human Topoisomerase II α Clone Ki-Si (catalogue no. M7186) diluted 1 : 50 was used.

Grading of expression intensity was based on the following criteria (similar to those used in assessment of ER/PR receptors):

- Grade 0 – 0-5% of stained nuclei,
- Grade 1^+ – 6-30% of stained nuclei,
- Grade 2^+ – 31-60% of stained nuclei,
- Grade 3^+ – above 60% of stained nuclei.

In each staining series, sections from reactive tonsil were used as a positive tissue control; reagent negative controls were also employed.

To describe the analyzed material, the author used standard statistical tools, such as: frequency tables for categorical variables, mean standard deviation and statistical significance of differences between particular determinations calculated by the χ^2 test. Randomness of differences amounting to $p < 0.005$ was considered statistically significant.

Results

Assessment of topoisomerase II α expression in breast carcinoma

In 151 women with infiltrating ductal breast carcinoma, the author analyzed slides stained immunohistochemically for TOP2A. The assessment focused on the intensity of tumour cell nuclei staining.

In six cases nuclear staining was completely absent; in the remaining cases, the observed reactions were characterized by a varying number of stained nuclei, ranging from 1.5% to 98.5% per 10 high power

Table I. Morphological characteristics of the analyzed tumours

pT	G1	G2	G3	N0	N1	N2	N3
TOTAL 151	12 (7.9%)	89 (58.9%)	50 (33.1%)	60 (39.7%)	42 (28.8%)	36 (23.8%)	13 (8.6%)
T1 61	6 (9.8%)	42 (68.8%)	13 (21.3%)	40 (65.6%)	12 (19.7%)	8 (13.3%)	1 (1.6%)
T2 58	5 (8.6%)	37 (63.8%)	16 (27.6%)	18 (31.0%)	19 (32.7%)	15 (25.8%)	6 (10.3%)
T3 18	1 (5.5%)	5 (27.7%)	12 (66.7%)	2 (11.1%)	10 (55.5%)	4 (22.2%)	2 (11.1%)
T4 14	0 (0.0%)	5 (35.7%)	9 (64.3%)	0 (0.0%)	1 (1.7%)	9 (64.3%)	4 (28.6%)

fields (40 ×), as well as by diversified staining intensity. The number of brown-stained nuclei within the infiltrative component was calculated, irrespectively of staining intensity: from light brown, through various darker hues to an almost black colour of the "inkblot" nucleus (microscopic pictures are presented in Figures 1-3). Discarding negative cases with less than 5% of stained nuclei, a positive reaction was seen in 99 patients (65.6%).

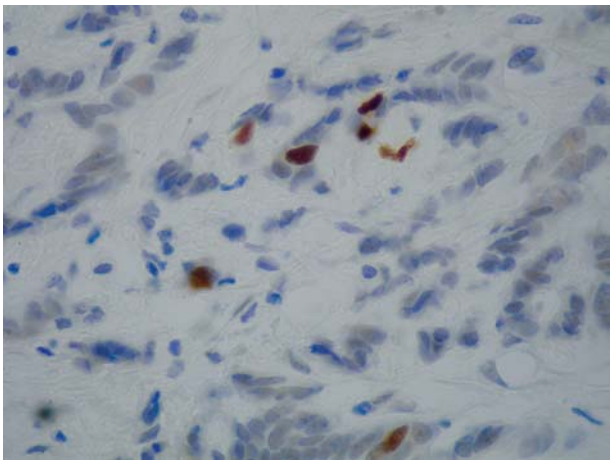

Fig. 1. Topoisomerase II α expression – 1^+

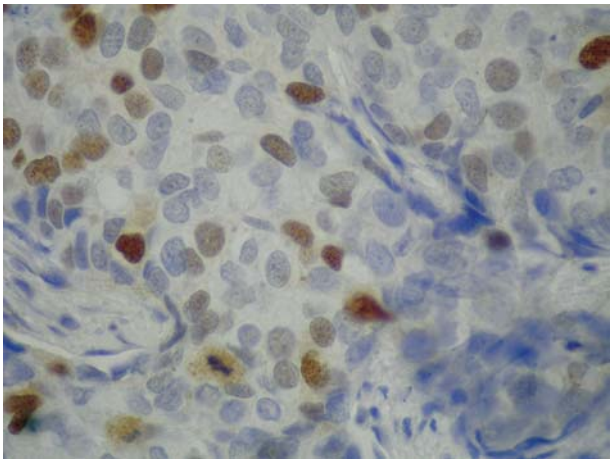

Fig. 2. Topoisomerase II α expression – 2^+

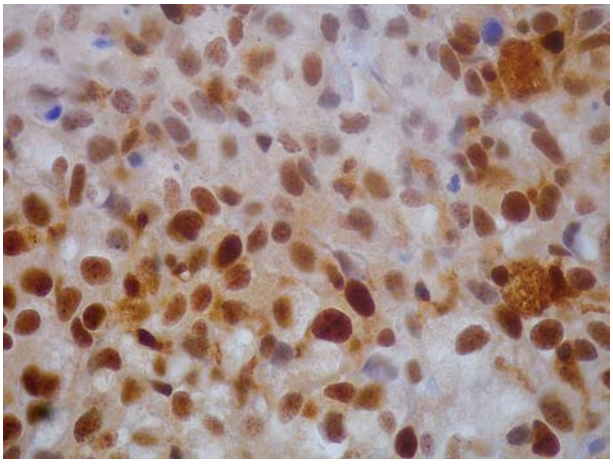

Fig. 3. Topoisomerase II α expression – 3^+

On the basis of the adopted criteria of TOP2A expression assessment, the following results were obtained:
- Grade 0 – 52 patients (34.4%),
- Grade 1+ – 24 patients (15.9%),
- Grade 2+ – 26 patients (17.2%),
- Grade 3+ – 49 patients (32.5%).

Correlation of topoisomerase II α expression with other prognostic factors

The correlation between TOP2A expression and intensity and other recognized morphological prognostic factors, such as tumour size (pT), histological grade of malignancy (G) and lymph node status (pN), as well as oestrogen (ER) and progesterone (PR) receptors and HER2 determined by immunohistochemistry, is presented in Tables II, III and IV.

As follows from Table II, an increased tumour size was accompanied by increased TOP2A expression and an increased, statistically significant ($p < 0.005$) percentage of cases with a positive TOP2A status.

Grade 3^+ expression of TOP2A and the percentage of cases positive for TOP2A were considerably and statistically significantly higher among patients with histopathological grades of malignancy G2 and G3 as compared to cases classified as G1. Among cases with grade G1 malignancy, a high percentage of cases were represented by patients with a negative TOP2A status.

With an increasing extent of involvement of regional lymph nodes, there was observed an increase of both the number of cases with TOP2A overexpression and the percentage of patients with a positive TOP2A status; the increase was statistically significant ($p < 0.005$).

Tables III and IV demonstrate that in cases of breast carcinoma in which no ER expression was detected (38 cases), TOP2A expression (34 cases) was 8.5 times higher as compared to patients with TOP2A-negative tumours (4 cases). No such correlation was noted at various expression rates of ER and TOP2A. TOP2A expression was observed much more frequently in the case of ER receptor-negative breast carcinomas (89.5%) in comparison to ER receptor-positive breast cancers (57.5%), the difference being statistically significant ($p < 0.005$).

Similar results were obtained when evaluating the correlation between TOP2A expression and PR receptor expression. Among PR receptor-negative cases (39 patients) TOP2A expression (35 cases) was approximately 8.7 times higher as compared to cases negative for TOP2A expression (4 cases). No such clear-cut dependence was observed at various intensities of PR and TOP2A expression. Similarly, TOP2A expression was noted more frequently in patients

with PR receptor-negative disease (89.8%) in comparison to women with PR receptor-positive breast tumours (57.2%), the difference being statistically significant ($p < 0.005$).

When comparing TOP2A expression with HER2 expression using the 4-score scale, no unambiguous dependencies were observed, and the too small number of cases in particular groups did not allow for statistical calculations.

Further analysis of the tables indicates that at a negative HER2 status there were no correlations with TOP2A status, but in cases with a HER2 positive status a positive TOP2A status occurred frequently (80.4%), while a negative status was observed approximately four times less commonly (19.6%); the differences were statistically significant ($p < 0.005$). At a positive HER2 status a positive TOP2A status was seen in 80.4% of the patients, while in HER2-negative tumours, TOP2A positivity was observed in 56.8% of the cases, with the difference being statistically significant ($p < 0.005$). At a negative HER2 status, a positive TOP2A status was more common (56.8%) as compared to TOP2A negativity (43.1%). The differences were non-significant.

Table II. Intensity of expression and status of TOP2A vs. other recognized morphological factors

No. of patients	No. (percentage) of patients with TOP2A expression				No. (percentage) of patients with TOP2A presence	
	0	1+	2+	3+	negative	positive
TOTAL 151	52 (34.4%)	24 (15.9%)	26 (17.3%)	49 (32.4%)	52 (34.4%)	99 (65.6%)
T1 (n = 61)	31 (58.8%)	14 (24.6%)	9 (14.7%)	7 (11.5%)	31 (50.8%)	30 (49.2%)
T2 (n = 58)	18 (31.0%)	6 (10.3%)	10 (17.2%)	24 (41.3%)	18 (31.0%)	40 (70.0%)
T3 (n = 18)	2 (11.1%)	2 (11.1%)	6 (33.3%)	8 (44.4%)	2 (11.1%)	16 (88.9%)
T4 (n = 14)	1 (7.1%)	2 (14.3%)	1 (7.1%)	10 (71.4%)	1 (7.1%)	13 (92.9%)
pG1 (n = 12)	8 (66.7%)	1 (8.3%)	2 (16.6%)	1 (8.3%)	8 (66.7%)	4 (33.3%)
pG2 (n = 89)	32 (35.9%)	14 (15.7%)	15 (16.8%)	28 (31.5%)	32 (35.2%)	57 (64.0%)
pG3 (n = 50)	12 (24.0%)	9 (18.0%)	9 (18.0%)	20 (40.0%)	12 (24.0%)	38 (76.0%)
N0 (n = 60)	27 (45.0%)	12 (20.0%)	10 (16.7%)	11 (16.3%)	27 (45.0%)	33 (55.0%)
N1 (n = 42)	13 (30.9%)	8 (19.0%)	10 (23.8%)	11 (26.2%)	13 (30.9%)	29 (69.0%)
N2 (n = 36)	10 (27.8%)	3 (8.3%)	4 (11.1%)	19 (52.8%)	10 (27.8%)	26 (72.2%)
N3 (n = 13)	2 (15.4%)	1 (7.7%)	2 (15.4%)	8 (61.5%)	2 (15.4%)	11 (84.6%)

Table III. Comparison of TOP2A expression and ER, PR receptor and HER expression

No. of patients	No. (percentage) of patients with TOP2A expression				No. (percentage) of patients with TOP2A presence	
	0	1+	2+	3+	negative	positive
TOTAL 151	52 (34.4%)	24 (15.9%)	26 (17.3%)	49 (32.4%)	52 (34.4%)	99 (65.6%)
TOTAL 151	52 (34.4%)	24 (15.9%)	26 (17.3%)	49 (32.4%)	52 (34.4%)	99 (65.6%)
ER-0 (n = 38)	4 (10.5%)	7 (18.4%)	7 (18.4%)	20 (52.6%)	4 (10.5%)	34 (89.4%)
ER-1+ (n = 38)	18 (47.4%)	4 (10.5%)	5 (13.7%)	11 (28.9%)	18 (47.4%)	20 (52.6%)
ER-2+ (n = 47)	18 (38.2%)	7 (14.9%)	10 (21.3%)	12 (25.5%)	18 (38.2%)	29 (61.7%)
ER-3+ (n = 28)	12 (42.8%)	6 (21.4%)	4 (14.3%)	6 (21.4%)	12 (42.8%)	16 (57.1%)
PR-0 (n = 39)	4 (10.2%)	7 (17.9%)	8 (20.5%)	20 (51.3%)	4 (10.2%)	35 (89.8%)
PR-1+ (n = 37)	18 (48.6%)	5 (13.5%)	4 (10.8%)	10 (27.0%)	18 (48.6%)	19 (51.4%)
PR-2+ (n = 49)	18 (36.7%)	7 (14.3%)	10 (20.4%)	14 (28.6%)	18 (36.7%)	31 (63.2%)
PRp3+ (n = 26)	12 (46.1%)	5 (19.2%)	4 (15.4%)	5 (19.2%)	12 (46.1%)	14 (53.9%)
HER2-0 (n = 80)	40 (50.0%)	14 (17.5%)	14 (17.5%)	12 (15.0%)	40 (50.0%)	40 (50.0%)
HER2-1+ (n = 15)	1 (6.6%)	2 (13.3%)	2 (13.3%)	10 (66.2%)	1 (6.6%)	14 (93.4)
HER2-2+ (n = 15)	1 (6.6%)	1 (6.6%)	3 (20.0%)	10 (66.6%)	1 (6.6%)	14 (93.4)
HER2-3+ (n = 41)	10 (24.4%)	7 (17.0%)	7 (17.0%)	17 (41.4%)	10 (24.4%)	31 (74.6)

Table IV. Comparison of presence and absence of ER, PR and HER2 expression in correlation with TOP2A expression

NO. OF PATIENTS	NO. (PERCENTAGE) OF PATIENTS WITH TOP2A PRESENCE	
	NEGATIVE	POSITIVE
TOTAL 151	52 (34.4%)	99 (65.6%)
ER negative (n = 38)	4 (10.5%)	34 (89.5%)
ER positive (n = 113)	48 (42.4%)	65 (57.5%)
PR negative (n = 39)	4 (10.2%)	35 (89.8%)
PR positive (n = 112)	48 (42.8%)	64 (57.2%)
HER2 negative ($0,1^+$) (n = 95)	41 (43.1%)	54 (56.8%)
HER2 positive ($2^+,3^+$) (n = 56)	11 (19.6%)	45 (80.4%)

Among 52 patients with TOP2A-negative tumours, 4 (7.7%) died within less than 5 years, while 5-year symptom-free survival was observed in 42 cases (80.7%). Of 99 patients with a positive TOP2A status, 29 females (29.3%) died within 5 years, while 53 patients (55.5%) survived for 5 symptom-free years. Both the difference in mortality rates in less than 5 years and the 5-year symptom-free survival rates were statistically significant.

Discussion

The investigations were carried out on archival material, with the author attempting to distinguish a group that would be uniform with respect to both morphological and clinical presentation. The study group of 151 cases was composed of women with infiltrating ductal breast carcinoma constituting at least 90% of the tumour texture; in consequence, one prognostic factor, i.e. tumour type, was constant, while other prognosticators were analyzed in relation to TOP2A expression and intensity. Clinically, the patients had not been subjected to preoperative chemotherapy or radiotherapy, which eliminated the possible effect of these treatment modalities on macroscopic findings and immunohistochemical reactions. Although the distinguished group of patients was not representative and the collected data lacked epidemiological value, nevertheless, in view of the fact that the distribution of cases in keeping with the pTNM classification system and ER/PR or HER2 expression was similar to that described in numerous publications [3, 8, 40], the author was capable of carrying out comparative studies and performing a statistical analysis.

No unambiguous criteria for assessing the degree of TOP2A expression intensity, developed by a significant research team, were found in the available literature. That is why for the purpose of the present report the author discarded negative cases characterized by up to 5% of stained nuclei in the average number of 10 fields of vision (40 ×), similarly to the practice of some other investigators [29, 38].

In the analyzed material of 151 cases of breast carcinoma, TOP2A expression assessed by immunohistochemistry was observed at varying reaction intensities and varying percentage of stained nuclei in the majority of patients, i.e. in 99 cases (65.6%), the percentage being statistically significant ($p < 0.005$). The reported immunohistochemical TOP2A determinations were performed in diversified clinical and morphological material, using various methods, employing Dako or other antibodies and using a method identical to that applied in the present material, yet at varying solutions of specific antibodies (1 : 50, 1 : 100 or 1 : 200), which may explain the differences between results obtained in various centres. In the majority of publications, TOP2A was determined in cases with a positive HER2 status, employing FISH or CISH complemented by immunohistochemistry. Numerous reports have demonstrated that evaluation of TOP2A and HER2 status by these methods yielded different results [15, 29, 32, 35, 41, 42, 44-47].

The author analyzed the correlations between TOP2A expression and other prognostic factors, such as tumour size (pT), presence and extent of metastases to regional lymph nodes (pN), grade of histological malignancy (G), presence and intensity of ER/PR expression and HER2 status.

The results of these studies, as well as the analyses and statistical calculations, indicated that the presence and expression of TOP2A correlated with numerous recognized prognostic factors, as witnessed by statistical significance ($p < 0.005$). Topoisomerase II α expression was much more frequently observed in advanced T3 and T4 tumours than in less advanced T1 and T2 carcinomas, the difference being statistically significant ($p < 0.005$). With increasing tumour size, the percentage of cases with 3^+ TOP2A expression also increased.

When analyzing the association between TOP2A and grade of histological malignancy G, the author noted that G1 cases were characterized by negative TOP2A reactions (66.7% of cases). On the other hand, in the case of G2 and G3 tumours, the percentage of the presence and expression intensity of TOP2A was considerably higher as compared to the G1 group, with the difference being statistically significant ($p < 0.005$).

Evaluating TOP2A presence and expression intensity among cases characterized by different degrees of lymph node involvement (N), the author noted that with an increased extent of regional lymph node involvement (Table IV), the percentage of cases with 3^+ TOP2A overexpression was also increased.

The increased percentage of cases with a positive TOP2A status at N2 and N3 was statistically significant ($p < 0.005$). Among N0 cases, TOP2A expression was noted in 55% of the cases, while the mean percentage for the entire group was 65.6%, being thus higher, yet the difference was non-significant.

No investigations have been found in the literature that would be directed at evaluating interrelations between the presence and expression of TOP2A and other prognostic factors; nevertheless, in some reports [14, 29, 49-54] attention was drawn to the more frequent TOP2A presence in large tumours, with a higher grade of histological malignancy, and among patients with an increased extent of N2 and N3 nodal involvement.

Comparing in the investigated group the expression and presence of steroid receptors ER and PR with TOP2A presence and expression, the author observed that among patients with steroid receptor-negative tumour, TOP2A was present in approximately 90% of cases, while in steroid receptor-positive cases it was present in approximately 57% of cases only. The differences were statistically significant ($p < 0.005$), and the percentage values for ER and PR were similar. In ER or PR receptor-negative cases, TOP2A expression was approximately 8 times more frequently positive than negative.

Correlating the immunohistochemical status of TOP2A with HER2 status, the author noted that TOP2A-positive cases were much more common among HER2-positive cases (80.4%) as compared to patients with HER2-negative carcinomas (56.8%), the difference being statistically significant ($p < 0.005$). Among HER2-positive cases, TOP2A positivity was approximately four times as common (80.4%) as TOP2A negativity (19.6%), and the difference was statistically significant. Observations of other authors confirm the present results [15, 29, 38, 42].

Recently, numerous papers have been published that evaluate both *HER2* and *TOP2A* status by immunohistochemistry, fluorescence *in situ* hybridization (FISH) and CISH [15, 18, 29, 32, 35, 41, 50, 62-65]. This trend is associated with the HER2 and TOP2A genes being situated on the same chromosome, so they may frequently coamplify [15, 45, 53, 66-68]. The results of research presented in the literature indicate that breast carcinomas are characterized by a high correlation between positive HER2 and TOP2A status, ranging from approximately 30% to more than 90% of cases [15, 29, 38, 42, 45-49]. A proposal has been suggested to develop an algorithm of HER2 and TOP2A evaluation in breast carcinomas [15, 32, 36, 50, 69]. The literature still lacks a definite, uniform agreement concerning the prognostic value of TOP2A determinations by immunohistochemical methods or by determination of gene amplification. Assessment of the status of both TOP2A and HER2 by immunohistochemistry and FISH at times yields discordant results, which points to the necessity of conducting further studies. A search of the literature has failed to find investigations focusing solely on the effect of the status and expression of TOP2A on the prognosis in breast carcinoma; nevertheless, numerous reports emphasize the prognostic importance of TOP2A expression, which indicates a poorer prognosis [22, 29, 43, 45, 46, 52, 55-57, 64].

The results of the present study and data from the literature suggest that TOP2A may be added to the other known prognostic factors. The following data support this statement:

- correlation with other recognized prognostic factors, such as tumour size (pT), grade of histological malignancy (G), metastases to lymph nodes (pN), ER, PR and HER2 receptor expression,
- statistically significant, higher mortality within 5 years among patients with a positive TYP2A status,
- according to the literature, TOP2A actively participates in cell proliferation in late S phase with a further increase in G2/M phase, but it is not detected in G0 phase, when the cells do not proliferate [17, 19, 21, 24, 39, 58-60, 62, 63, 70].

Thus, TOP2A expression in cellular nuclei is a factor indicating intensified proliferation and may be regarded as a prognostic factor.

Assessment and understanding of new prognostic factors is an important element of studies focusing on therapeutic management of breast carcinoma. The results of such studies allow us to improve the therapeutic methods and in combination with assessment of numerous predictors can be implemented in target therapy, which is an enormous achievement of the past several years.

Conclusions

1. Expression of topoisomerase II α was correlated with numerous known prognostic factors, such as tumour size (pT), grade of histological malignancy (G), presence and extent of metastases to regional lymph nodes (pN) and expression of ER, PR and HER2 receptors.

2. Topoisomerase II α was considerably more frequently present in large tumours (T3 and T4), in cases with higher histological malignancy grade (G2 and G3), as well as in patients with extensive lymph node metastases (N2 and N3), positive HER2 status and negative ER/PR status, which points to TOP2A being a negative prognostic factor.

3. Topoisomerase II α expression in breast carcinoma may be regarded as a fundamental prognostic factor.

4. Immunohistochemical determinations of TOP2A in tumour cells should be routinely employed in patients with infiltrating ductal breast carcinoma.

References

1. Bagwell CB, Clark GM, Spyratos F, et al. Optimizing flow cytometric DNA ploidy and S-phase fraction as independent prognostic markers for node-negative breast cancer specimens. Cytometry 2001; 46: 121-135.
2. Clark GM. Prognostic and predictive factors. In: Diseases of the Breast. Harris JR, Lippman ME, Morrow M, et al. (eds). Lippincott-Rayen Publishers, Philadelphia 1996; 461-485.
3. Hayes DF. Breast Cancer. In: Prognostic Factors in Cancer. Gospodarowicz MK (ed.). 2006; 27: 207-212.
4. Hayes DF. Clinical importance of prognosis factors. Moving from scienting to clinical useful. In: Principles of Molecular Oncology. Bronchud MH et al. (eds). 2004; 51-72.
5. Olszewski WT. Patomorfologiczne czynniki prognostyczne w raku piersi. Nowa Med 2002; 6: 119.
6. Olszewski WT. Patomorfologiczne czynniki prognostyczne. W: Onkologia kliniczna. Krzakowski M (red.). Warszawa 2001; 258-273.
7. Pierzchała RP, Paszcz-Walczak G, Jeziorski A. Nowe czynniki rokownicze w raku piersi – przegląd piśmiennictwa. Współcz Onkol 2004; 8, 9: 429-434.
8. Tayassoli FA, Deville P. Tumors of the Breast and Female Genital Organs. WHO Classification of Tumors, 2003.
9. Allred DC, Harvey JM, Berardo M, Clark BM. Prognostic and predictive factors in breast cancer by immunohistochemichal analysis. Mod Pathol 1998; 11: 155-168
10. Domagała W. Klasyczne i nowe czynniki prognostyczne w raku sutka u kobiet. Nowotwory 1996; 47: 23.
11. Hammond MEH. College of American Pathologists Conference prognostic factors which, how and so what ? Arch Path Lab Med 2000; 124: 958.
12. Fitzgibbons PL, Page DL, Weaver D, et al. Prognostic factors in breast cancer. College of American Pathologists Consensus Statement 1999. Arch Pathol Lab Med 2000; 124: 966-978.
13. Fitzgibbons PL. Breast Cancer In Prognostic Factors in Cancer. Gospodarowicz MK (ed.). New York 2001; 153: 465-466.
14. Buzdar A. Adjuvant Chemotherapy for High-Risk Operable Breast Cancer 2007; JCO 25: 1642-1644.
15. Olszewski WP, Mrozkowiak A, Suszyło K, et al. Oznaczanie statusu genów TOPA 2 i HER 2 w raku piersi metodą fluorescencyjnej hybrydyzacji in situ (FISH). Zakład Patologii Centrum Onkologii w Warszawie. Klinika Nowotworów Piersi i chirurgii Rekonstrukcyjnej Centrum Onkologii w Warszawie. Program Badań – zadanie wieloletnie 2006.
16. Ravdin PM. Prognostic factors in breast cancer. In: Textbook of breast cancer. A clinical guide to therapy. Bonadonna G, Hortobagyi GN, Giani M, Dunitz M (eds). London 1997; 35-63
17. Bakshi RP, Galande S, Muniyappa K. Functional and regulatory characteristics of eukaryotic type II DNA topoisomerase (review). Crit Rev Biochem Mol Biol 2001; 36: 1-37.
18. Bar JK, Grelewski P, Gabryś M. Expression of topoisomerase I and II alpha in ovarian neoplasms. Pol J Pathol 2007; 2: 110.
19. Boege F, Andersen A, Jensen S, Zeidler R, Kreipe H. Proliferation-associated nuclear antigen Ki-S1 is identical with topoisomerase IIα. Am J Pathol 1995; 146: 1302-1308.
20. Costa MJ, Hansen CL, Holden JA, Guinee DJ. Topoisomerase II alpha: prognostic predictor and cell cycle marker in surface epithelial neoplasms of the ovary and peritoneum. Int J Gynecol Pathol 2000; 19: 248-257.
21. Goodsell DS. Topoizomerazy. Biotechnolog. Pl, Serwis biotechnologiczny Copyright 2006.
22. Hellemans P. Immunohistochemical study of topoisomerase II alpha expression in primary ductal carcinoma of the breast. JCO 1995; 48: 147-150.
23. Murphy DS, McHardy P, Coutts J, et al. Interphase cytogenetic analysis of ERBB2 and TOP2A co-amplification in invasive breast cancer and polysomy of chromosome 17 in ductal carcinoma in situ. Int J Cancer 1996; 64: 18-26.
24. Steward C, Ratain M. Pharmacology of cancer chemotherapy. Topoisomerase interactive agents. In: Principles and Practice in Oncology. De Vita, et al. (eds). Philadelphia 2001; 415-418.
25. Binaschi M, Capranico G, Dal Bo L, et al. Relationship between lethal effects and topoisomerase II mediated double – stranded DNA breaks produced by anthracyclines with different sequence specifity. Mol Pharmacol 1997; 51: 1053-1059.
26. Binaschi M, Farinosi R, Borgetto ME, et al. In vivo site specificity and human isoenzyme selectivity of two topoisomerase II poisoning anthracyclines. Cancer Res 2000; 60: 3770.
27. Siedlecki JA. Biologia molekularna nowotworów. W: Onkologia kliniczna. Krzakowski M (red.). Borgis, Warszawa 2006; 61.
28. Adjei AA, Hidalgo M. Intracellular signal transduction pathway proteins as targets for cancer therapy. J Clin Oncol 2005; 23: 5386.
29. Bhargava R, Lal P, Chen B, et al. HER 2/neu and Topoisomerase II alpha Gene Amplification and Protein Expression in Invasive Breast Carcinomas, Am J Clin Pathol 2005; 123: 889-895.
30. Durbecq V, Leo A, Cardoso F, et al. Comparison of topoisomerase II alpha gene status between primary breast cancer and corresponding distant metastatic sites. Breast Cancer Res Treat 2003; 77: 199-204.
31. Kreipe H, Heidebrecht HJ, Hansen S, et al. A new proliferation – associated nuclear antigen detectable in paraffin-embedded tissues by the monoclonal antibody Ki-S1. Am J Pathol 1993; 142: 3-9.
32. Olszewski WP, Mrozkowiak A, Kosińska-Bauer, et al. Coamplification of HER2 and topa 2 alpha genes in selected cases of breast carcinoma. Pol J Pathol 2007; 2: 130.
33. Olszewski WT. Diagnostyka nowotworów – patomorfologiczne nowe techniki specjalne. Nowa Medycyna – Onkologia V 2000; 10.
34. Płuciennik E, Byczewska M, Seta K, et al. Determination of gene expression in breast cancer. Pol J Pathol 2007; 2: 133.
35. Isola J, Tanner M, Forsyth A, et alInterlaboratory comparison of HER-2 oncogene amplification as detected by chromogenic and fluorescence in situ hybridization. Clin Cancer Res 2004; 10: 4793-4798.
36. Järvinen TA, Kononen J, Pelto-Huikko M, Isola J. Expression of topoisomerase IIalpha is associated with rapid cell proliferation, aneuploidy, and c-erbB2 overexpression in breast cancer. Am J Pathol 1996; 148: 2073-2082.
37. Lynch BJ, Guinee DG, Holden JA. Human DNA topoisomerase II alpha: a new marker of cell proliferation in invasive breast cancer. Hum Pathol 1997; 28: 1180-1188.
38. Mueller R. Amplification of the TOP2A gene does not predict high levels of topoisomerase II alpha protein in human breast tumor samples, genes, chromosomes, Cancer 2004, 39: 288-297.
39. Nakopoulou L, Lazaris AC, Kavantzas N, et al. DNA topoisomerase II alpha immunoreactivity as a marker of tumor aggressiveness in invasive breast cancer. Pathobiology 2000; 68: 137-143.
40. Veronesi U. Breast cancer. Lancet 2005; 365: 1727-1741.
41. Arnould L, Denoux Y, MacGrogan G, et al. Agreement between chromogenic in situ hybridization (CISH) and FISH in the determination of HER 2 status in breast cancer. Br J Cancer 2003; 88: 1587-1591.
42. Arpino G, Ciocca DR, Weiss H, et al. Predictive value of apoptosis, proliferation HER 2 and topoisomerase II alpha for anthracycline chemotherapy in locally advanced breast cancer. Breast Cancer Res Treat 2005; 92: 69-75.
43. Tanner M, Järvinen P, Isola J. Amplification of HER-2/neu and topoisomerase IIalpha in primary and metastatic breast cancer. Cancer Res 2001; 61: 5345-5348.
44. Harris LN, Yang L, Liotcheva V, et al. Induction of topoisomerase II activity after ErbB-2 activation is

associated with a differential response to breast cancer chemotherapy. Clin Cancer Res 2001; 7: 1497-1504.
45. Durbeq V, Leo A, Cardoso F, et al. Correlation between topoisomerase-IIalpha gene amplification and protein expression in HER-2 amplified breast cancer. Int J Oncol 2004; 25: 1473-1479.
46. Olszewski WT, Krzakowski M, et al. Rekomendacje Polskiej Grupy Badawczej ds. HER 2. Nowotwory 2004, 54: 500-505.
47. Petit T, Wilt M, Velten M, et al. Comparative value of tumour grade, hormonal receptors, Ki 67, HER 2 and topoisomerase II alpha status as predictive markers in breast cancer patients treated with neoadjuvant anthracycline-based chemotherapy. Eur J Cancer 2004; 40: 205-11.
48. Järvinen TA, Tanner M, Bärlund M, et al. Amplification and deletion of topoisomerase II alpha associate with Erb-2 amplification and affect sensitivity to topoisomerase II alpha inhibitor doxorubicin in breast cancer. Am J Pathol 2000; 3: 839-846.
49. Cardoso F, Durbecq V, Larsimont D, et al. Correlation between complete response to anthracycline-based chemotherapy and topoisomerase II-alpha gene amplification and protein overexpression in locally advanced/metastatic breast cancer. Int J Oncol 2004; 24: 201-209.
50. Di Leo A, Gancberg D, Larsimont D, et al. HER-2 amplification and topoisomerase IIalpha gene aberrations as predictive markers in node-positive breast cancer patients randomly treated either with an anthracycline-based therapy or with cyclophosphamide, methotrexate, and 5-fluorouracil. Clin Cancer Res 2002; 8: 1107-1116.
51. Di Leo A, Larsimont D, Gancberg D, et al. HER 2 and topoisomerase II alpha as predictive markers in a population of node-positive breast cancer patients randomly treated with adjuvant CMF or epirubicin plus cyclophosphamide Ann Oncol 2001; 12: 1081-1089.
52. Järvinen TA, Liu ET. HER-2/neu and topoisomerase IIalpha–simultaneous drug targets in cancer. Breast Cancer Res Treat 2003; 78: 299-311.
53. Järvinen TA, Tanner M, Bärlund M, et al. Characterization of topoisomerase II alpha gene amplification and deletion in breast cancer. Genes Chromosomes Cancer 1999; 26: 142-150.
54. Krzakowski M. Podstawy kliniczne hormonoterapii nowotworów. W: Onkologia kliniczna. Krzakowski M (red.). Borgis, Warszawa 2006; 193.
55. Jacobson KK, Morrison LE, Henderson BT, et al. Gene copy mapping of the ERBB2/TOP2A region in breast cancer. Genes Chromosomes Cancer 2004; 40: 19-31.
56. Rudolph P, Olsson H, Bonatz G, et al. Correlation between p53, c-erbB-2, and topoisomerase II alpha expression, DNA ploidy, hormonal receptor status and proliferation in 356 node-negative breast carcinomas prognostic implications. J Pathol 1999; 187: 207-216.
57. Simpson JF, Gray R, Dressler LG, et al. Prognostic value of histologic grade and proliferative activity in axillary node-positive breast cancer: results from the Eastern Cooperative Oncology Group Companion Study, EST 4189. J Clin Oncol 2000; 10: 2059-2069.
58. Leonard D. Molecular Pathology in Clinical Practice. Springer Verlag 2007.
59. Loeb L, Loeb K, Anderson JP. Multiple mutations in cancer. Proc Natl Sci U S A 2003; 100: 776.
60. Villman K, Stahl E, Liljegren G, et al. Topoisomerase II alpha expression in different cell cycle phases in fresh human breast carcinomas. Mod Pathol 2002; 15: 486-491.
61. Isaacs RJ, Davies SJ, Sandri MI, et al. Physiological regulation of eukaryotic topoisomerase II. Biochem Biophys Acta 1998; 1400: 121-137.
62. Szuławska A, Czyż M. Molekularne mechanizmy działania antracyklin. Postępy Higieny Dośw 2006; 60: 78-100.
63. Baak JP, Path FR, Hermsen MA, et al. Genomics and proteomics in cancer. Eur J Cancer 2003; 39: 1199-1215.
64. Houlbrook S, Addison CM, Davies SL, et al. Relationship between expression of topoisomerase II isoforms and intrinsic sensitivity to topoisomerase II inhibitors in breast cancer cell lines. Br J Cancer 1995; 72: 1454-1461.
65. Olsen KE, Knudsen H, Rasmussen BB, et al.; Danish Breast Cancer Cooperative Group. Amplification of HER 2 and TOP 2A and deletion of TOP2A genes in breast cancer investigated by new FISH probes. Acta Oncol 2004; 43: 35-42.
66. Olszewski WP. HER2 status in breast carcinoma – practical information for physicians. Nowa Medycyna – Badania nad rakiem 2005; 1.
67. Stacey DW, Hitomi M, Chen G. Influence of cell cycle and oncogene activity upon topoisomerase II alpha expression and drug toxicity. Mol Cell Biol 2000; 20: 9127-9137.
68. Goswami PC, Sheren J, Albee LD, et al. Cell cycle-coupled variation in topoisomerase IIalpha mRNA is regulated by the 3'-untranslated region. Possible role of redox-sensitive protein binding in mRNA accumulation. J. Biol Chem 2000; 275: 384-392.
69. Mrozkowiak A, Olszewski WP, Suszyło K, et al. The Usefulness of fluorescent in situ hybridization (FISH) in the algorithm of diagnosis management in Breast cancer. Pol J Pathol 2007; 2: 128.
70. Wang JC. Cellular roles of DNA topoisomerases a molecular perspective Nat Rev Mol Cell Biol 2002; 3: 430-440.

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