Evaluation of the predictive value of topoisomerase II α in patients with breast carcinoma

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Although the increasing body of knowledge in the field of immunohistochemistry and molecular biology has allowed for better understanding of the biology of breast cancer; nevertheless, a search is still in progress for new factors that would make it possible to preselect patients and employ target therapies. Predictive factors that arouse interest include topoisomerases.
The authors evaluated the response to treatment and survival rates depending on expression of topoisomerase II α (TOP2A) and other predictive factors, as well as assessed treatment effectiveness employing therapeutic schemes based on anthracycline depending on TOP2A expression.
The investigations demonstrated that the response to treatment and survival of patients with TOP2A expression were similar to the response and survival of those with tumours demonstrating the presence of other recognized predictors.
The employed adjuvant therapy according to anthracycline therapeutic protocols was markedly more effective in TOP2A-positive patients as compared to the remaining individuals. Thus, TOP2A constituted a predictive factor. The study demonstrated that in adjuvant therapy employed in infiltrating ductal carcinoma of the breast, not only HER2 but also TOP2A determination in cancer cells was of importance; for this reason, routine determinations by immunohistochemistry should be performed, especially when anthracycline treatment is planned.

Key words: topoisomerase II α, breast carcinoma.

Introduction

In the last decade, in order to increase therapeutic effectiveness in infiltrating breast carcinoma, methods of preselecting patients for appropriate therapeutic modalities were widely introduced. Such methods include evaluation of the status of steroid receptors (ER and PR) in hormonal treatment and HER2 status in patients on trastuzumab therapy, as well as optimization of hormonal treatment. The effectiveness of such an approach to cancer treatment, which is termed "target treatment" and in which determining the presence or disturbances in the amount of a specific protein constitutes the foundation for selecting a therapeutic method has led to a search for new parameters that would affect the therapeutic outcome. Such a search is associated with both new chemotherapeutic agents and with pharmaceuticals that have been employed for decades; in both instances, a detailed understanding of molecular foundations of their activity has allowed for commencing studies aiming at determining predictive factors [1-5].

Such predictive factors newly discovered by molecular studies include class I and II topoisomerases [6-8].

If topoisomerases are inhibited, DNA trans-cription processes in the cell are distorted, which hinders or renders impossible cell division processes [5, 6, 8, 12].

This phenomenon can be used in the treatment of cancer. Commonly used pharmaceuticals, antibiotics belonging to a group of anthracyclines, such as doxorubicin and daunomycin, inhibit class II topoisomerases, while a plant toxin, camptothecin, blocks the relaxing action of class I topoisomerases [2, 6, 8, 10].

When the double-stranded DNA is separated and then reattached again, topoisomerase II α (TOP2A) and DNA form covalent bonds, and the thus formed cleavable complexes are the site of activity for numerous therapeutic agents belonging to aminoacridines, anthracyclines and epi-podofilotoxins, e.g. etoposide and teniposide (TOP2A inhibitors). These agents stabilize the cleavable complexes, thus preventing TOP2A from reuniting the two DNA strands. Such DNA damage leads to apoptosis and necrosis of cancer cells through complex mechanisms that are not yet fully understood – the process most likely occurs with the participation of TP53 protein, cyclins and cyclin-dependent kinases [2, 5, 6, 8].

Irrespectively of the type of cancer, TOP2A is determined in carcinoma cells by immunohistochemistry and fluorescence *in situ* hybridization FISH or CISH [4, 10-15].

The TOP2A protein-encoding gene is situated on chromosome 17, similarly as the gene that encodes HER2 [4, 6, 9, 13, 16-20] and in a considerable percentage of cases, co-amplification of these genes may occur. Investigations assess the number of copies of genes that encode these proteins [4, 9, 16].

In recent years, numerous researchers have been involved in evaluating the role of TOP2A as a predictor in carcinoma of the breast, chiefly in conjunction with assessment of the HER2 status, most commonly employing the technique of *in situ* hybridization and immunohistochemistry [4, 9, 10, 13, 15, 20-24].

In HER2 and TOP2A-positive cases, the predominantly employed treatment consists in the use of anthracyclines, such as doxorubicin and daunomycin or their derivatives, as well as TOP2A inhibitors [22, 23, 26-30]. Therapeutic effects are generally positive and indicate that TOP2A may be considered a predictive factor, which is also confirmed by studies on the mechanisms of the effect exerted by these therapeutic agents on TOP2A, i.e. inhibition of the ability to undergo cell division and triggering DNA damage [1-6, 25, 32].

The objective of the investigations was to evaluate the response to treatment and survival rates depending on the expression of TOP2A and other prognostic factors, as well as to assess treatment effectiveness employing therapeutic programs with anthracyclines depending on TOP2A expression.

Material and methods

The investigations were carried out on archival material originating from 151 patients with infiltrating ductal carcinoma of the breast, who were treated surgically in the Podkarpacki Oncology Center in Brzozów in 1999–2001 by mastectomy with lymphadenectomy and subsequently received chemotherapy at the Outpatient Cancer Chemotherapy Clinic of the above Center.

The age of the patients ranged from 28 to 70 years, with the mean age of 56.5 years and a standard deviation of ±7 years. The group with age ranging from 49.5 to 63.5 years included 82 females (54.3%), 30 patients (19.8%) were below 49.5 years of life and 39 women (25.8%) were aged above 63.5 years.

Of the original group of 151 females, 33 patients (21.8%) died within less than 5 years after treatment, while 5-year survival was noted in 118 women (78.2%), including 95 females (62.7%) with clinically event-free survival.

Local postoperative recurrent disease was observed in 18 patients (11.9%), while relapses, i.e. recurrent and/or metastatic disease, were detected in 38 women (23.8%).

Postoperatively, in comprehensive adjuvant therapy, anthracyclines were employed in 50 patients (33.1%). The morphological characteristics of the material was presented in the previous paper [33].

Patients with infiltrating ductal carcinoma of the breast were evaluated in keeping with the pTNM staging system according to the WHO classification [45]. Histopathological malignancy was graded according to the 1-3 Bloom-Richardson scale as modified by Elston and Ellis [34].

Oestrogen (ER) and progesterone (PR) receptors as well as HER2 were determined immunohistochemically, using commercially available kits manufactured by DAKO and adhering to procedures described by the manufacturer. The threshold for ER and PR positivity was established as 10%.

In keeping with the previously published criteria of DAKO, the HER2 status was described as negative (0 and +) or positive (++ and +++). The criteria were altered in 2006 and at present, the HER2 status is defined as negative (0 and +), positive (+++) and borderline (++) that requires verification by FISH.

Topoisomerase II α was determined in paraffin sections selected from the same paraffin blocks that served for immunohistochemical determinations of ER, PR and HER2. Immunohistochemical staining for TOP2A was performed at the Department of Pathology, Center of Oncology, Warsaw.

Immunohistochemical reactions were carried out in a DAKO Autostainer Plus according to

the enclosed instructions using DAKO REAL™ Envision™ Detection System, Peroxidase (DAB+) Rabbit (Mouse, Catalog No. K5007).

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

Grading of expression intensity was based on the criteria developed by the authors [33]:

- 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 a reactive tonsil were used as a positive tissue control; reagent negative controls were also employed.

To describe the analyzed material, the authors 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

While evaluating immunohistochemically stained preparations originating from 151 patients with infiltrating ductal carcinoma of the breast, TOP2A presence of varying intensity was noted in 99 females (65.6%) (Fig. 1, Fig. 2).

Based on the adopted criteria of TOP2A expression evaluation, 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%).

Tables I, II and III present the assessment of the response to treatment and the analysis of patient survival rates depending on the expression of TOP2A and other factors.

As Table I demonstrates, the percentage of local recurrent disease showed no association with TOP2A expression intensity. On the other hand, the percentage rate of relapses and deaths within less than 5 years increased with an increasing TOP2A expression intensity, while the percentage of patients with 5-year survival clearly decreased.

Response to treatment as dependent on the status of TOP2A and other recognized predictive morphological factors is presented in Table II.

Local recurrent disease in carcinoma of the breast was found to be independent of the TOP2A status; the differences were statistically non-significant. On the other hand, the percentage rate of relapses and deaths within less than 5 years was dependent on

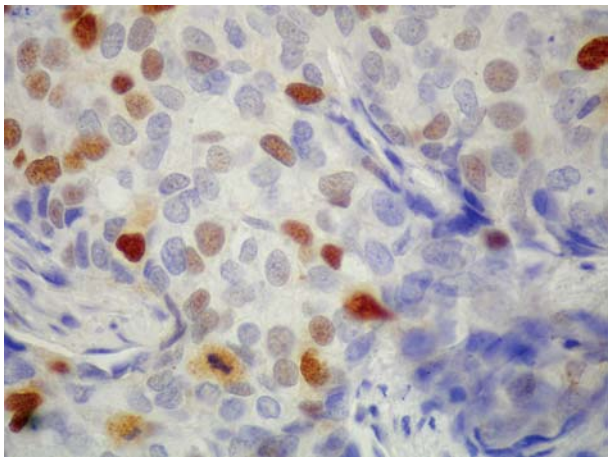

Fig. 1. Topoisomerase II α expression – 1+

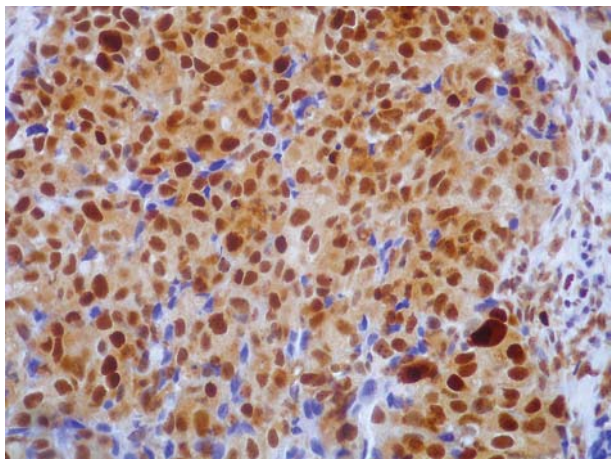

Fig. 2. Topoisomerase II α expression – 3+

Table I. Assessment of the response to treatment and analysis of survival rates depending on intensity of TOP2A expression

TOP2A expression (no. of patients)	Local recurrent disease	Relapses	Deaths < 5 years	Survival > 5 years	
				Total	Event-free
0 (n = 52)	5 (9.6%)	5 (9.6%)	4 (7.7%)	48 (92.3%)	42 (80.7%)
1+ (n = 24)	3 (12.5%)	3 (12.5%)	2 (8.3%)	22 (91.7%)	18 (75.0%)
2+ (n = 26)	4 (15.4%)	10 (38.4%)	8 (30.7%)	18 (69.2%)	12 (46.0%)
3+ (n = 49)	6 (12.2%)	20 (40.8%)	19 (38.8%)	30 (61.2%)	23 (46.9%)
TOTAL 151	18 (11.9%)	38 (25.2%)	33 (21.8%)	118 (78.2%)	95 (62.9%)

Table II. Response to treatment and survival depending on the TOP2A status and other basic morphological factors

Number of patients	Local recurrent disease	Relapses	Deaths < 5 years	Survival > 5 years	
				Total	Event-free
Total 151	18 (11.9%)	38 (25.2%)	33 (21.8%)	118 (78.2%)	95 (62.9%)
Negative TOP2A status (n = 52)	5 (9.6%)	5 (9.6%)	4 (7.7%)	48 (92.3%)	42 (80.7%)
Positive TOP2A status (n = 99)	13 (13.1%)	33 (33.3%)	29 (29.3%)	70 (71.7%)	53 (55.5%)
pT1 (n = 61)	4 (6.7%)	4 (6.7%)	4 (6.7%)	56 (91.8%)	49 (80.3%)
pT2 (n = 58)	5 (11.9%)	13 (30.9%)	10 (23.8%)	46 (79.3%)	39 (67.2%)
pT3 (n = 18)	5 (13.9%)	14 (38.9%)	13 (36.2%)	10 (55.5%)	6 (33.3%)
pT4 (n = 14)	4 (30.8%)	7 (53.8%)	6 (46.1%)	6 (42.2%)	1 (7.1%)
G1 (n = 12)	1 (8.3%)	2 (16.7%)	1 (8.3%)	11 (91.7%)	9 (75.0%)
G2 (n = 89)	10 (11.2%)	20 (22.5%)	18 (20.2%)	71 (79.8%)	61 (68.5%)
G3 (n = 50)	10 (20.0%)	16 (32.0%)	14(28.0%)	30 (72.0%)	18 (38.0%)
N0 (n = 60)	4 (6.7%)	4 (6.7%)	4 (6.7%)	56 (93.3%)	52 (86.6%)
pN1 (n=42)	5 (11.9%)	13 (30.9%)	10 (23.8%)	32 (76.2%)	24 (57.1%)
pN2 (n = 36)	5 (13.9%)	14 (38.9%)	13 (36.2%)	25 (63.8%)	19 (52.0%)
pN3 (n = 13)	4 (30.8%)	7 (53.8%)	6 (46.1%)	7 (53.9%)	2 (15.4%)

the TOP2A status and the differences were statistically significant ($p < 0.005$).

The death rate among TOP2A-positive patients was higher as compared to females with the TOP2A-negative status. These observations indicate that the clinical course and deaths within less than 5 years are associated with the TOP2A status. Among TOP2A-negative patients, the event-free 5-year survival rate was higher and statistically significant.

Among patients with pT1 tumours, local recurrent disease (8.2%), relapses (11.5%) or deaths within less than 5 years (6.6%) were rare, while 80.3% of the patients survived 5 event-free years. With an increasing tumour size, the percentage rate of recurrent disease, relapses and deaths within less than 5 years increased. On the other hand, the percentage of cases characterized by 5-year event-free survival decreased, reaching 7.1% in women with pT4 tumours.

With a higher grade of histological malignancy (G), the percentage of recurrent disease increased, especially in cases classified as grade G3, similarly as the percentage of deaths within less than 5 years, whereas the rate of 5-year event-free survival decreased. Statistical calculations demonstrated that the differences in the percentage rates of local recurrent disease as depending on grade of malignancy were statistically non-significant. On the other hand, the percentage of recurrent disease at grade G1 and G3 was statistically significant, similarly as the 5-year event-free survival rate. In cases classified as G1, local recurrent disease, relapses and deaths within less than 5 years were very rare. The differences in the percentage rates of deaths occurring within less than 5 years in patients with various malignancy grades G1, G2, G3 were statistically significant.

Among cases with no metastases to the lymph nodes (pN0), local recurrent disease, relapses and deaths within less than 5 years were very infrequent. On the other hand, 93.3% of the patients survived longer than 5 years, including 86.6 %with event-free survival. In patients with extensive pN 2 and pN 3 metastases, local recurrent disease, relapses and deaths within less than 5 years were more frequent, while a low percentage of the women showed event-free survival of more than 5 years. The differences for these parameters were statistically significant ($p < 0.005$).

As it follows from the data presented in Table III, patients with negative ER/PR receptors more frequently demonstrated local recurrent disease (23.1%), relapses (69.2%) and deaths within less than 5 years (69.2%), while 5-year event-free survival was observed only in 7.7% of the patients. The results describing the percentage of relapses, deaths within less than 5 years and event-free survival of more than 5 years in the ER/PR-positive and negative groups were statistically significant ($p < 0.005$).

Local recurrent disease, relapses and deaths within less than 5 years were much more frequent in

patients with the HER2-positive as opposed to HER2-negative status, which was statistically significant ($p < 0.005$). On the other hand, the percentage of HER2-negative cases among patients with 5-year event-free survival was more than two times higher as compared to females with the HER2-positive status, showing statistical significance ($p < 0.005$).

Evaluation of effectiveness of chemotherapy with anthracyclines

In the group of 151 patients treated surgically by mastectomy with axillary lymphadenectomy, adjuvant chemotherapy was employed. Fifty cases (33.1%) included females who underwent chemotherapy with anthracyclines, while in the remaining 101 cases (66.9%), another type of chemotherapy was employed. Table IV presents treatment evaluation.

As the table shows, the percentage of patients on chemotherapy with anthracyclines who died within less than 5 years was considerably lower as compared to females receiving another type of chemotherapy and the difference was statistically significant ($p < 0.005$). The percentage of patients with 5-year event-free survival (74.0%) was also higher among females treated according to therapeutic protocols with anthracyclines as compared to patients on another chemotherapy and the difference was statistically significant. On the other hand, the differences in the remaining evaluated parameters were statistically non-significant.

The effect of the immunohistochemical TOP2A status on the outcome of anthracycline therapy is presented in Table V.

The analysis of the clinical course in women treated in keeping with anthracyclines-including protocols demonstrated that the percentage of recurrent disease, relapses and deaths within less than 5 years was lower in females whose tumour cells were TOP2A-positive. However, only the differences in the percentage of recurrent disease and deaths within less than 5 years were statistically significant. On the other hand, the event-free above 5-year survival rate was considerably lower among patients showing both the negative TOP2A status and treated with anthracyclines, the difference being statistically significant ($p < 0.005$). The results achieved in patients with the negative TOP2A status were poorer as compared to the average clinical course evaluated in the entire material.

Table III. Evaluation of response to treatment and analysis of survival depending on the ER/PR and HER2 receptor status

Number of patients	Relapses	Local recurrent disease	Deaths < 5 years	Survival > 5 years	
				Total	Event-free
Total 151	18 (11.9%)	38 (25.2%)	33 (21.8%)	118 (78.2%)	95 (62.9%)
Positive ER/PR status (n = 125)	12 (9.6%)	20 (16.0%)	15 (12.0%)	110 (88.0%)	93 (74.4%)
Negative ER/PR status (n = 26)	6 (23.1%)	18 (69.2%)	18 (69.2%)	8 (30.7%)	2 (7.7%)
Negative HER2 status (0 and +) (n = 95)	7 (7.4%)	14 (14.7%)	10 (10.5%)	85 (89.5%)	74 (77.8%)
Positive HER2 status (++ and +++) (n = 56)	11 (19.6%)	24 (42.8%)	23 (41.1%)	33 (58.9%)	21 (37.5%)

Table IV. Evaluation of response to treatment and analysis of survival depending on the use of chemotherapy with anthracyclines

Adjuvant therapy Number of patients	Local recurrent disease	Relapses	Deaths < 5 years	Survival > 5 years	
				Total	Event-free
Total 151	18 (11.9%)	38 (25.2%)	33 (21.8%)	118 (78.2%)	95 (62.9%)
Protocols with anthracyclines (n = 50)	5 (10.0%)	9 (18.0%)	6 (12.0%)	44 (88.0%)	36 (72.0%)
Another type of chemotherapy (n = 101)	13 (12.9%)	29 (28.7%)	27 (26.7%)	74 (73.2%)	59 (58.4%)

Table V. Analysis of associations between the clinical course, deaths of patients on anthracycline therapy and the immunohistochemical TOP2A status

Patients on anthracycline therapy	Local recurrent disease	Relapses	Deaths < 5 years	Survival > 5 years	
				Total	Event-free
Total 151	5 (10.0%)	9 (18.0%)	6 (12.0%)	44 (88.0%)	36 (72.0%)
Positive TOP2A status (n = 35)	3 (8.5%)	4 (11.4%)	2 (5.7%)	33 (94.2%)	28 (80.0%)
Negative TOP2A status (n = 15)	2 (13.3%)	5 (33.3%)	4 (26.7%)	11 (73.3%)	8 (53.3%)

Discussion

The analyzed group of 151 cases morphologically consisted of patients with infiltrating ductal carcinoma of the breast involving at least 90% of the tumour architecture, which resulted in the fact that one prognostic factor, i.e. the histological tumour type, was constant, while other factors were assessed in relation to TOP2A presence and intensity of expression. From a clinical point of view, the group represented patients who had not been preoperatively subjected to chemotherapy or irradiation therapy, which eliminated the possible effect of these therapeutic modalities on microscopic pictures and immunohistochemical reactions.

When evaluating the response to treatment, the authors took into consideration local recurrent disease as well as relapses, i.e. recurrent and/or metastatic disease; the analysis also included survival rates, breaking up the patient group into females that died within less than 5 years and those that survived event-free more than 5 years. The data were obtained from medical records collected by the Podkarpacki Oncology Center in Brzozów.

Of 151 patients with infiltrating ductal carcinoma, local recurrent disease was seen in 18 females (11.9%), relapses in 38 (25.2%), 33 patients died within less than 5 years (21.8%) and 118 survived 5 years (78.2%), including 95 women (62.9%) with event-free survival.

Evaluating the response to treatment and survival rates depending on the presence and expression intensity of TOP2A, the authors noted that local recurrent disease was not associated with TOP2A presence and expression intensity, while the percentage rate of relapses and deaths within less than 5 years increased parallel to increasing TOP2A expression intensity, and the event-free survival rate decreased. The percentage of relapses and deaths within less than 5 years was associated with TOP2A status and the differences in the increased percentage rates were statistically significant ($p < 0.005$), whereas the 5-year event-free survival rate was considerably higher in TOP2A-negative patients, the difference being statistically significant.

As it follows from observations and statistical data, the clinical course and deaths within less than 5 years are associated with the TOP2A status, which is an unfavourable predictive factor.

The above presented results of studies on the response to treatment combined with the analysis of survival rates indicate that simultaneous expression of ER/PR receptors in tumour cells suggests a favourable prognosis. In 100 patients from the investigated group, local recurrent disease was rare (9%), similarly as relapses (11%) and deaths within less than 5 years (8%), while 92% of the patients survived 5 years, including 80.0% females with event-free survival.

Unfavourable factors were represented by absence of expression of both ER and PR receptors in tumour cells, since such cases were characterized by frequent recurrent disease (23.1%), relapses (69.2%) and deaths within less than 5 years (69.2%). On the other hand, 5-year survival was noted only in 30.7% of the patients from this group, with only two of them showing event-free survival.

The statistical analysis demonstrated that the differences in the rates of relapses, recurrent disease and deaths within less than 5 years were statistically significant ($p < 0.005$) in the group showing expression of at least one steroid receptor and in the group characterized by absence of expression as compared to patients with expression of both ER/PR receptors.

The present observations have been confirmed in numerous reports published in recent years [34, 35]. It has been demonstrated that ER/PR receptor-positive patients respond well to treatment in 80-90% of cases. On the other hand, ER/PR-negative tumours show only a slight response to treatment in less than 10% of cases [34-36].

In the presently analyzed material, the results describing the response to treatment were similar to these found in the literature on the subject. The probability of 5-year event-free survival depended on

the acknowledged factors indicating a good prognosis, such as a small size of the tumour, low histological grade of malignancy, absence of metastatic disease or presence of metastases characterized by low extensiveness (N1), simultaneous ER/PR expression, negative HER2 status and additionally, as it was demonstrated by the present authors, also negative TOP2A status.

A decrease in the risk of developing local recurrent disease, relapse or death within less than 5 years occurred proportionally to a decrease in the tumour size, decreased grade of histological malignancy, decreased extensiveness or absence of metastases to regional lymph nodes (pN), to a slightly lesser degree in proportion to ER/PR and HER2 expression and, as it was demonstrated in the present report, to TOP2A status. Prognostic factors indicating an unfavourable prognosis included tumour size, extensiveness of metastases (N2 and N3), a high grade of histological malignancy, negative ER/PR receptors, HER2 positivity and positive TOP2A status.

The authors assessed the effectiveness of anthracycline therapy taking into consideration its effect on the clinical course and deaths within less than 5 years, as well as event-free survival above 5 years as dependent on TOP2A expression.

Of 151 patients described in the present report, chemotherapy was employed in all females, while 50 women (33.1%) additionally received anthracyclines. As it follows from the analysis of responses to therapy and 5-year survival rates when adjuvant therapeutic protocols with anthracyclines and other chemotherapy protocols were taken into consideration, patients treated with anthracyclines less frequently died within less than 5 years (12%) as compared to females treated according to other chemotherapy protocols (26.7%) and the difference was statistically significant.

The percentage of patients treated with anthracyclines who survived 5 event-free years (72.0%) was higher as compared to the remaining females (58.4%) and the difference was also statistically significant. On the other hand, no statistically significant differences were observed among cases with local recurrent disease and relapses.

A comparison of therapeutic effects of anthracycline treatment in patients with the positive immunohistochemical TOP2A status (35 women) and in TOP2A-negative females (15 patients) indicates, as it follows from Table II, that TOP2A-positive individuals demonstrated a considerably better reaction to anthracycline treatment, since both the percentage of recurrent disease and the number of deaths within less than 5 years were lower as compared to patients with the negative status, the difference being statistically significant.

Among patients on anthracycline treatment, the 5-year event-free survival rates were lower in women with the negative TOP2A status (53.3%) as compared to TOP2A-positive patients (80.0%) and the difference was statistically significant.

The results of the present investigations indicate that employment of the therapeutic protocol with anthracyclines was more effective among patients with the positive immunohistochemical TOP2A status and confirm its being a predictive factor in breast cancer treatment.

Anthracyclines have been employed in treating neoplastic diseases, including carcinoma of the breast, for several score years. Numerous analogues of the first anthracycline antibiotics: doxorubicin and daunorubicin have been evaluated. Numerous molecular mechanisms explaining the cytotoxic and cytostatic activity evoked by anthracyclines have been proposed. The contribution of anthracyclines to free radical generation, lipid peroxidation and evoking changes in the structure of cellular membranes is well known [2, 5, 37]. However, in recent years, researchers have understood their effect on TOP2A activity and interactions with DNA through intercalation complexes and covalent bonds, as well as modification of nitrogenous bases, which is supposed to lead to abnormalities in replication or transcription, initiation of DNA repair mechanisms and apoptosis [1-3, 5].

The activity of anthracyclines has been demonstrated to be associated with initiation of numerous, hitherto not fully elucidated mechanisms: DNA stabilization may occur through formation of an uncleavable helix [1, 2].

Topoisomerase II α is a transcription enzyme encoded by the *TOP2A* gene, which occurs in the nuclei of cells undergoing the active cell division cycle [6, 38]. In recent years, this has been believed to be the target of activity of anthracyclines and other chemotherapeutic agents employed in the treatment of carcinoma of the breast, such as topoisomerase I inhibitors – irinotecan and topotecan, and TOP2A inhibitors – etoposide and teniposide [27, 31, 38].

Inhibition of TOP2A activity is among the major mechanisms of the toxic effect of anthracyclines on tumour cells. Anthracyclines bind the enzyme-cleaved complex and thus, when the broken DNA fragment is covalently bound to TOP2A, reunion of the DNA strand is impossible.

The most recent communications indicate that in HER2-positive and TOP2A-positive patients, even at higher tumour stages, relatively good target therapy results are achieved [13, 19, 22, 26, 29, 40, 42].

In the planned adjuvant therapy, the determination in breast cancer cells of not only HER2, but also TOP2A is of great importance and for this reason, routine immunohistochemical determinations should be commonly employed. In immunohistochemically HER2 and TOP2A-positive cases, amplification of the genes should also be determined. Establishment of an algorithm of HER2 and TOP2A assessment in carcinoma of the breast is postulated [4, 9, 43, 44].

Authors of numerous molecular biology papers, experimental and clinical reports [2, 20, 22, 25, 27, 28, 30, 39, 45, 46, 48] are generally in agreement that TOP2A is a predictive factor and treatment of cancers where it is present should be geared towards inhibiting TOP2A activity through the use of anthracyclines or topoisomerase-blockers in comprehensive treatment, either systemic or monotherapeutic. Isolated critical reports are, however, also published [13, 20, 47].

Problems associated with evaluation and understanding of new prognostic and predictive factors constitute an important part of reports focusing on treatment of breast cancer. The results of these studies lead to improvement of therapeutic methods and – in combination with assessment of numerous predictive factors – are reflected in target therapies, which represent a tremendous progress achieved in the past few years.

Most recent literature worldwide has been a forum for a discussion on the prognostic and predictive importance of the newly recognized factors. There is much information indicating that these investigations may improve selection of therapeutic agents and therapeutic effectiveness in carcinoma of the breast.

Conclusions

1. The response to treatment and survival of patients with TOP2A expression was similar to the reaction observed in patients demonstrating other recognized predictive factors.

2. Adjuvant therapy in keeping with therapeutic protocols with anthracyclines was considerably more effective in patients with the positive TOP2A status, which confirms that TOP2A is a predictive factor in treatment of infiltrating ductal carcinoma of the breast.

3. Routine immunohistochemical determinations of not only HER2, but also TOP2A should be employed in infiltrating ductal carcinoma of the breast, especially when anthracycline treatment is planned.

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