ORIGINAL PAPER

Immunohistochemical study of p53 and Ki67 in a group of patients with mammary carcinoma

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Abstract

The study was done over a period of 10 years, 1996–2005 and it included 562 mammary cancer patients. Of the 562 cases, 100 cases of invasive mammary carcinoma included in this study were investigated from an immunohistochemical point of view. The p53 overexpression was more frequently seen in patients under 50 (23 cases, that is 54.76%), compared to those over 50 years old (19 cases, that is 45.24%). The positive p53 tumors were more often over 2 cm big. The invasive ductal carcinomas were p53-positive in 40 cases (44.44%) of all invasive ductal carcinoma cases, and the invasive lobular carcinomas were only positive in two cases (20%) of all mammary invasive lobular carcinoma cases. Most cases that had the overexpression of the p53-protein (30 cases that is 71.43%) had a high histological degree (G3), and only 12 cases (28.57%) had a low histological degree (G1 and G2). The overexpression of the p53-protein was present in all cases that had a heterogeneous phenotype (with one of the hormonal receptors being negative), in over half of the cases that had both hormonal receptors negative (59.37% of ER-/PR- phenotype cases) and in only 21.05% of cases that had ER+/PR+ phenotype. The association of the p53 overexpression (p53 over 10%), with the HER2 (2+ or 3+ score) overexpression was seen in seven patients of the 100 invasive mammary carcinoma cases included in this study. Consequently, 16.66% of p53 positive cases had associated positivity for HER2. Most cases that were p53 positive had an increased proliferation activity, as determined with Ki67. The Ki67 immunostaining analysis has made it clear that this marker has positivity presence in all cases. The vast majority of cases had a nuclear marking to Ki67, but two cases (2% of cases) had a cytoplasmatic / membrane staining.

***Keywords*:** breast cancer, Ki67, p53, prognosis factors.

Introduction

The suppressor gene p53 encodes a nuclear protein that is involved in adjusting cell proliferation. This protein is normally undetectable by immunohistochemical evaluation, due to its very short lifetime. Still, a third of breast cancers have p53 gene mutations that in turn determine the production of a stable p53 protein that accumulates in the nucleus. Thus, the p53 overexpression through routine immunohistochemical staining identifies the tumors with mutations of the p53-gene. The analysis of the status of p53 through immunohistochemical methods has later on proven to be a powerful and independent prognosis factor in breast cancer. The immunopositivity of p53 is associated to aggravating prognosis factors, like high histology grading, increased cell proliferation rate and aggressive clinical behavior.

P53 can also be a predictive marker through identifying the most likely patients to respond to chemotherapy. The loss and (or) alteration of p53 protein, because of gene rearrangements, can cause an unbalance in cell growth through replicating errors and genetic accumulations. If the DNA is altered with, p53 blocks replication, favoring the activation of repairing genome systems. When cell repair fails, p53 induces self-destruction of the cell through apoptosis. Immunohistochemical detection of the p53 protein can now be done using antibodies, the most used one being CM1, PAb1801, DO1 and DO7. Immunohistochemical methods are based on the accumulation of p53 protein inside cells.

Ki67 is a nuclear protein found in the G1-phase of cell cycle and it is considered a useful marker of cell proliferation. Many studies have found a link between the percentage of positive Ki67 cells and the clinical evolution. These studies suggest that the measuring of Ki67 expression can be useful in stratifying patients into two categories, good prognosis and bad prognosis [1, 2]. The Ki67 antigen is a useful non-histonic protein, used to identify prolific cells that, not having phase specificity, is expressed in all active phases of the cell cycle (Ki67 is not expressed in the G_0 phase) [3]. Although there are presently more antibodies that can be used in paraffin sections (MM1, NCL-ki-67p, Rah Ki-67), studies show that the MIB-1 antibody has the highest

sensitivity, offering a better visual staining, most nuclei being intensely and diffusely stained, the reproducing of staining index being just as good for any of these antibodies [4]. An increase in Ki67-expression indicates an increase in mitotic cell activity and proliferation [5].

Material and Methods

Research material

The material researched in this study was human material, mammary tissue that came from patients at the Oncology Clinic in Drobeta Turnu Severin, between 1996–2005, that have had a simple mastectomy, an axial ganglion evidation mastectomy, sectorectomy or punction biopsy. These surgical ablation materials were fixated in formalin and initially processed through the usual technique of paraffin enclosure in the Pathological Anatomy Laboratory at the Emergency County Hospital of Drobeta Turnu Severin, being brought to the stage of paraffin block. Afterwards, an immunohistochemical processing was done in the Laboratory of Histological, Histopatological and Immunohistochemical Techniques, of University of Medicine and Pharmacy of Craiova.

All 100 cases of invasive mammary carcinoma included in this study were investigated from an immunohistochemical point of view. These correspond to the usual staining of the following histological types:

- invasive ductal carcinoma: 90 cases (90%);
- invasive lobular carcinoma: 10 cases (10%).

Working methods

In the morphological study, we used the classic histology technique of paraffin enclosure. Next, the prepared materials were stained with Hematoxylin–Eosin, the staining technique being done by going through the following stages.

Results of staining

- nuclei, stained in dark blue;
- pink-red cytoplasm;
- pale pink collagen fibers;
- elastic and reticulin fibers do not come out.

The method used in the immunohistochemical study was one of the methods based on soluble immunoenzymes complexes, called LSAB/HRP (*Labelled Streptavidin Biotin*). The kit used was DAKO LSAB 2 System HRP (Universal DAKO Labeled Streptavidin Biotin 2 System Horseradish Peroxidase) (Figure 1).

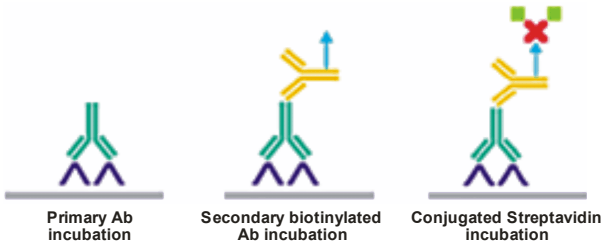

Figure 1 – ***Comprehensive presentation of processing using LSAB method [6].***

The LSAB/HRP method (with Streptavidin Biotin) is one of the methods called ABC (*Avidin–Biotin Complex*), where Avidin is replaced by Streptavidin and is based on the direct conjugation of Streptavidin with enzyme molecules [6]. Streptavidin is a tetrametric analogue of Avidin, with a molecular weight of 60 kD, extracted from *Streptomyces avidinii* bacteria, that is capable to bind, with great affinity, the Biotin molecules. This affinity is, in theory, ten times greater than that of antibodies for their antigens, leading to a specific and intense detection and an amplifying of the antigen–antibody links. Now Streptavidin is preferred instead of Avidin. From the paraffin blocks, we took 3-4 μm thick sections that we applied on polylysine treated slides and then we dried them at lab temperature, for 12 hours.

The LSAB (HRP) work procedure implies the following sequences [6]: deparaffinising, alcohol rehydrating, endogenous peroxidase inhibition, incubation with peroxide in 3% distilled water, incubation with the primary antibody (the negative control), in the optimal solution, incubation with the secondary biotinylated species specific antibody (serum) for the primary antibody, incubation with the peroxidated Streptavidin, chromogen developing (DAB – 3,3'-diaminobenzidine) in the dark, counterstaining with Mayer's Hematoxylin, for 15–30 seconds, alcohol dehydrating, with increasing concentrations, xylol clarification, then mounting with Canada Balm. The result is visualizing the investigated antigens, with the DAB chromogene, that determines a brown solution at their levels (negative nuclei are stained in light blue with Hematoxylin).

Markers used and their main characteristics

In this study, we used concentrated antibodies from DAKO Cytomation Company, in Denmark, whose solutions and pre-treatments are presented in Table 1.

Table 1 – ***Antibodies, clone, dilution and preprocessing***

Antibody	Clone	Dilution	Pre-processing	Incubation time
Ki67	MIB-1	1:10	Five cycles MW in citrate buffer	30 minutes TA
P53	DO7	1:50	Five cycles MW in citrate buffer	30 minutes TA

To obtain the optimal solution, the antibodies were diluted in PBS–azide solution when used.

Control of immunohistochemical reactions

To validate the results of immunostaining, we must use reagents and control-tissue, without which the interpretation is without value. The staining results are valid if all interference is excluded, that may cause an unspecific staining (negative control does not become stained) and if the technique sensitivity is guaranteed (positive control-tissue is positive, it containing the studied antigen in low concentration).

For each used antibody, we used both external positive control and external negative control, using the same work technique. We also followed, on diagnosis specimens, the presence of internal positive control. If this internal positive control is present, there is no need for an external positive control.

Methods of interpreting results

For Ki67 immunostaining we only considered posi-

tive the cells that had an undoubtedly positive nuclear staining, while the cells where the staining was not clear were considered negative (Figures 2 and 3). We then calculated an index of Ki67 staining. The Ki67 index was calculated by comparing the number of positive cells (nuclei), with the total number of cells (positive and negative), multiplying the result by 100. There were at least 1000 nuclei for each case (40×-objective), being interpreted as positive the ones that were brown to black. A Ki-67 index of 0–15% was considered low, between 16–30% was medium and a 31–100% index was a high one.

The reaction for p53 was considered positive (the overexpression of p53-protein) when over 10% of tumor cells had a clear nuclear staining, no matter the intensity (Figures 4 and 5). The percentage of positive cells, with p53 protein accumulation, was calculated by determining the number of positive nuclei in ten different microscopical fields. Fields that had necrosis, hemorrhage infiltrate or section folding were not interpreted.

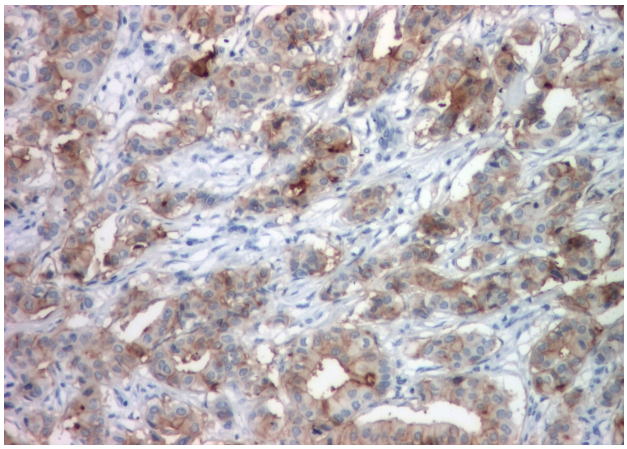

Figure 2 – ***Ki67 cytoplasmic staining with accentuated membrane staining, ×40.***

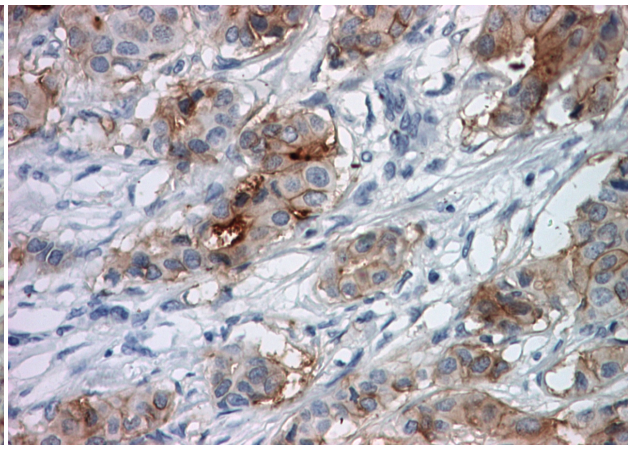

Figure 3 – ***Ki67 cytoplasmic staining with accentuated membrane staining, ×200.***

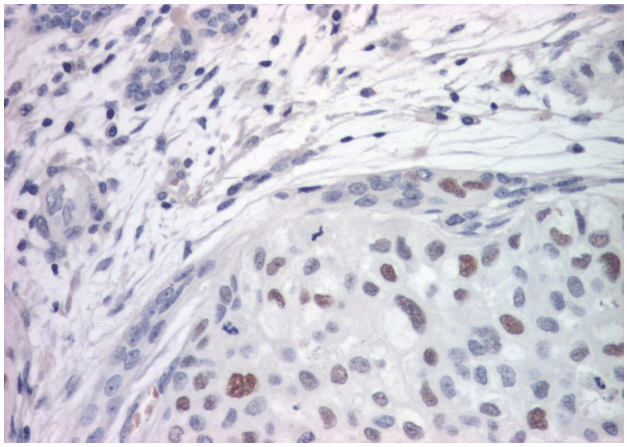

Figure 4 – ***P53 overexpression: CDI, ×200.***

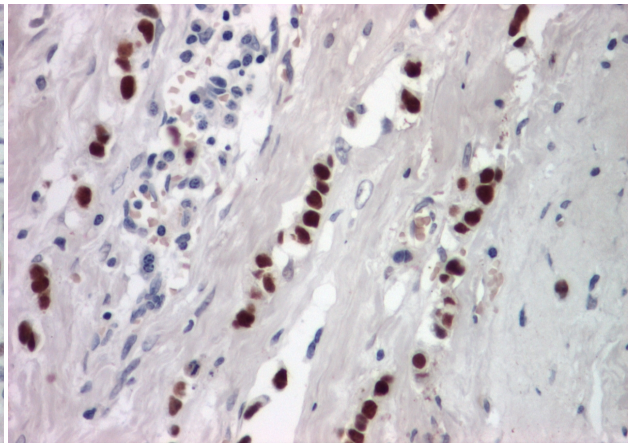

Figure 5 – ***P53 overexpression: lobular carcinoma, ×100.***

Results

All 100 cases of invasive mammary carcinoma studied between 1996–2005 were processed. The morphological study on the Hematoxylin–Eosin staining allowed the selection and framing of analyzed cases in the categories in Table 2, according to *WHO* principles.

The analyzed cases were mammary tumors belonging to patients with the ages between 28 and 78-year-old (37 patients under 50 and 60 patients over 50-year-old). The tumors were smaller or equal to 2 cm, in 35 cases and larger than 2 cm, in 65 cases (Table 2).

The *morphological study* on the Hematoxylin–Eosin coloration allowed a categorization of the cases according to the *WHO* into:

- NOS invasive ductal carcinomas: 90 cases;
- invasive lobular carcinomas: 10 cases (nine cases the classic type and one case of histiocytoid invasive lobular carcinoma).

Table 2 – ***Characteristics of tumors and patients***

Characteristics	No. of cases	%
Age [years]		
<50	37	37
≥50	63	63
Size of tumors [cm]		
<2	35	35
>2	65	65
Histological types		
Invasive ductal carcinoma	90	90
Invasive lobular carcinoma	10	10
Histological grading		
G1	14	14
G2	46	46
G3	40	40

The tumor differentiation grading was evaluated according to the *Nottingham Grading System*, the tumors being classified into (Table 2):

- G1 invasive mammary carcinomas: 14 cases;
- G2 invasive mammary carcinomas: 46 cases;

▪ G3 invasive mammary carcinomas: 40 cases.

The analysis of p53 immunostaining indicated the presence of the overexpression of p53-protein (over 10% of tumor cells marked with the antibody) in 42 cases (42% of studied cases) (Figures 2 and 3) (Table 3).

P53-overexpression was more frequently encountered in patients under 50, 23 cases (54.76%), compared to the ones over 50, 19 cases (45.24%).

P53 positive tumors were generally larger than 2 cm. Thus, 28 of them measuring over 2 cm (66.64%) were p53 positive, while only 14 tumors smaller than 2 cm (33.33%) overexpressed the p53-protein (Table 3).

Table 3 – *P53 expression in relation with clinico-pathological parameters*

Characteristics	P53 overexpression	
	No. of cases=42	%
Age [years]		
<50	23	54.76
≥50	19	45.24
Size of tumors [cm]		
≤2	14	33.33
>2	28	66.64
Histological type		
Invasive ductal carcinoma	40	95.24
Invasive lobular carcinoma	2	4.76
Histological grading		
G1/G2	12	28.57
G3	30	71.43
Estrogen receptors		
ER positive	16	38.10
ER negative	26	61.90
Progesterone receptors		
PR positive	19	45.24
PR negative	23	54.76
HER2 expression		
HER2 positive (score 2+ or 3+)	7	16.66
HER2 negative (score 0 and 1+)	35	83.34
Proliferative index (Ki67)		
>15%	32	76.19
>30%	23	54.74

Invasive ductal carcinomas were positive for p53 in 40 cases, 44.44%, of all cases, and invasive lobular ones in only two cases, 20% of all invasive lobular carcinoma cases. Thus, the overexpression of p53 was less present in invasive lobular carcinomas (4.76%), than invasive ductal ones (95.24%) (Table 3).

Concerning the histological grading, most cases that had the overexpression of p53-protein (30 cases, 71.43% respectively) were high grading (G3), and only 12 cases (28.57%) had a low grading (G1 and G2).

Of all 42 cases with invasive mammary carcinoma that overexpressed protein p53, 26 cases (61.9%) had no estrogen receptor expression, only 16 cases (38.1%) having the immunoexpression of estrogen receptors. Also, 23 cases (54.76%) with p53 overexpression were negative for PR, while only 19 cases (45.24%) were PR positive. All ER+/PR- phenotype cases overexpressed p53 protein (Table 3).

The overexpression of p53 protein was present in all cases with a heterogeneous phenotype (with one of the hormone receptors negative), in over half of the cases that had both receptors negative (59.37% of ER-/PR- phenotype cases), and in only 21.05% of the cases with ER+/PR+ phenotype (Table 4).

Table 4 – *P53 expression in relation with hormonal receptors phenotype*

Phenotype		Cases with p53 overexpression
ER+/PR+	57 cases	12 (21.05%)
ER-/PR-	32 cases	19 (59.37%)
ER-/PR+	7 cases	7 (100%)
ER+/PR-	4 cases	4 (100%)

The association of p53-overexpression (more than 10% p53) to the overexpression of HER2 (score 2+ or 3+) was encountered in seven patients. Thus, the coexpression of p53 and HER2 was encountered in seven of 100 cases of this study. As a result, 16.66% of cases that had positive p53 had associated positivity for HER2 and most of them, five cases (71.42%) had a cell proliferation index of over 30%.

The immunohistochemical overexpression of p53 was seen in 46.66% of the 15 cases that were HER2 positive (score 2+ and 3+), and in only 35 cases (41.17%) of the 85 cases that were HER2 negative (score 0 and 1+). The overexpression of p53 (corresponding to a high value of p53 protein, IHC determined) was more frequent in HER2-positive carcinomas compared to the one in HER2-negative. (46.66% *vs.* 41.17%).

Most cases that were p53-positive had an increased proliferative activity, determined with Ki67. Thus, 76.19% (32 cases) that were p53-positive had a proliferation index of over 15%, and more than half the cases (23 cases, 54.76% respectively) had a proliferation index of over 30%.

The analysis of immunostaining in Ki67 (MIB1)

This analysis emphasized the presence of positivity to this marker in all cases that were studied. Most cases had a nuclear staining to the Ki67, but two cases, (2%) had a cytoplasmic/membrane staining (Figures 4 and 5). One of the cases had a cytoplasmic pattern of reactivity with membrane accentuation, and the other one a predominantly apical pattern.

The specific staining to Ki67 is the nuclear one and was seen in 98 cases. For these cases, we have made correlations between the Ki67-index and the morpho-clinical parameters. Thus, we have seen that in patients under 50 a high Ki67-index was more common (over 15% in tumor cells being Ki67-positive), in comparison to patients over 50 years old (60% of cases *vs.* 55.55%). Also, patients with tumors larger than 2 cm had more frequently a high Ki67-index compared to those with tumors under 2 cm (71.42% *vs.* 51.43%) (Table 5).

Concerning the histological type of the analyzed carcinomas, it was seen that the lobular type had a low Ki67 index in all cases, while the invasive ductal type had a lower Ki67 index in 43.87% of cases and a high index in 56.12% of cases (Table 5).

In relation to the histological grading, the tumors with a high grading (G3) always had a high Ki67 index, in comparison with the tumors with a low grading (G1/G2) that have had a proliferation index increased in only 8.62% of cases. A high proliferation activity (a high Ki67-index) was seen more frequently in tumors with no estrogen receptors, in comparison with those that were ER-positive (48.72% *vs.* 27.11% of all cases

had a high proliferation index). Also, the tumors that were progesterone-negative had a higher proliferation activity, determined with Ki67, compared to the progesterone positive ones (38.88% *vs.* 30.64% of cases had a high Ki67-index) (Table 5).

A high proliferation index was seen in most HER 2+ and 3+ score tumors. Thus, 93.34% of HER2-positive tumors had a proliferative activity that was over 15%, compared with 6.66% of tumors with under 15% proliferative activity (Table 5).

Table 5 – ***Correlation between Ki67 index and morphoclinical parameters***

Characteristics	Ki67 index <15%	Ki67 index >15%
	No. of cases (%)	
Age [years]		
<50	14 (40%)	21 (60%)
≥50	28 (44.45%)	35 (55.55%)
Size of tumors [cm]		
≤2	17 (48.57%)	18 (51.43%)
>2	18 (28.57%)	45 (71.42%)
Histological type		
Invasive ductal carcinoma	43 (43.87%)	45 (56.12%)
Invasive lobular carcinoma	10 (100%)	0 (0%)
Histological grading		
G1/G2	53 (91.38%)	5 (8.62%)
G3	0 (0%)	40 (100%)
Estrogen receptors		
ER positive	43 (72.88%)	16 (27.11%)
ER negative	20 (51.28%)	19 (48.72%)
Progesterone receptors		
PR positive	43 (69.35%)	19 (30.64%)
PR negative	22 (61.12%)	14 (38.88%)
HER2 expression		
HER2 positive (score 2+ or 3+)	1 (6.66%)	11 (93.34%)
HER2 negative (score 0 and 1+)	17 (20.48%)	66 (79.52%)

Discussion

The p53 oncoprotein is a phosphoprotein of 53 kD, encoded by the p53 gene, located on the short arm of chromosome 17. Under normal conditions, the p53 gene has the role of "genome guardian", that is to monitor the DNA integrity during cell division. The protein product of normal allele (wild-type) of the p53-gene negatively regulates the growth and cell proliferation, blocking cells in G1 cell cycle phase. The loss or altering of p53-protein, because of gene rearranging, can cause the unbalancing of cell growth through replicating errors and genetical accumulations. If the DNA is altered, p53 blocks replication, favoring the activation of genome repairing systems. When cell repair fails, p53 induces destruction through apoptosis. P53 is often mutated, and corresponding protein products have altered regulation properties. An excess of mutant proteins could neutralize a normal protein. In addition, some mutant forms could manifest new properties responsible with the growth of their oncogenity. Generally, wild-type form and mutant forms of p53 are different concerning their immunoreactivity to anti p53 monoclonal antibodies [7].

A rise of the intercellular concentration of p53, that is frequent, but not systematical, associated with the mutation of p53 protein, is apparently accompanied by a poor prognosis in some tumors and a weak response to treatment and radiotherapy resistance [8, 9].

Immunohistochemical detection of p53-protein can now be done with many antibodies, the most used being CM1, PAb1801, DO1 and DO7.

In mammary carcinoma, the mutations of p53 are associated with a more aggressive behavior and with a lower survival rate. Still, the frequency of the p53 mutations is lower in mammary carcinoma in comparison with other solid tumors [10].

In this study, the overexpression of p53 protein was encountered in 42% of cases of studied mammary carcinoma, the result well correlated with the data from other studies that varies between 16% and 48% percent of positive p53 cases in invasive mammary carcinoma [11, 12]. Still, the overexpression of p53, immunohistochemically determined, does not reflect accurately the appearance of p53 mutations, given that the existing antibodies determine both wild and mutant types of p53 gene, this wild type possibly accumulating in some tumors as a response to DNA-alteration.

Analyzing the p53 overexpression in this study cases, it was determined that it was more frequently encountered in patients under 50 compared to the ones over 50 (54.76% *vs.* 45.24%), with tumors generally larger than 2 cm (66.64% *vs.* 33.33%). Studies published before have found correlations, more or less significant, of p53 immunopositivity with young ages. Bartley AN and Ross DW have shown, in a study in 2001 [11], that p53-positivity was detected in five out of seven patients under 43, Al-Moundhri M *et al.* have shown, in 2003 [12], that p53 overexpression tends to appear in patients younger than 40 and pre-menopause patients, and Pietiläinen T *et al.*, in 1995 [13], that p53 nuclear positivity is significantly correlated to age factors, the highest percentage being seen in the group of patients under 50-year-old. These have suggested that the poor prognosis in mammary cancers in young patients can be correlated to p53 abnormalities and some of these tumors could be directly linked to the mutations of p53, the data in literature being few and suggesting no correlation between the parameters [12].

In regard to the histological grading of invasive carcinoma analyzed, in most cases the p53-overexpression was associated with the ductal type, compared to the lobular one (95.24% *vs.* 4.76%) and with poorly differentiated tumors (G3) compared to the ones that were moderately / well differentiated ones (28.57% *vs.* 71.43%). These results agree with recent studies that have proven that mammary tumors that have a larger quantity of p53 (IHC measured), are more frequently of a high histological and nuclear grading [12, 14] and much rarer of lobular type [15].

The same results have been obtained when specialists considered the genically determined mutations of p53, instead of the p53-protein accumulation [15].

The p53-immunopositivity was correlated with the lack of estrogen and progesterone receptors: 61.9% had no receptor expression for estrogens *vs.* 38.1% having the immunoexpression of estrogen receptors. Also, 54.76% cases with p53-overexpression were negative for PR, while only 45.24% cases were PR-positive.

All studies done before have noticed that mammary tumors with increased p53 immunoexpression or genetically determined mutations of p53 were much more

often progesterone and estrogen receptor negative, most studies finding significant statistically differences [12, 14–16].

The presence of the p53 protein overexpression in all cases with a heterogeneous phenotype (with one of the hormone receptors negative), in over half of the cases that had both receptors negative, and in only 21.05% of the cases with ER+/PR+ phenotype can explain a bad prognosis in mammary carcinoma with one or both types of receptors missing in comparison to the double positive phenotype (ER+/PR+).

Cases that have p53 mutations of are moderately or poorly differentiated tumors, ER and PR negative and have a significant number of MIB1-positive cells [17].

The coexpression of p53 and HER2 was seen in 7% of all invasive mammary carcinoma cases that were included in this study. This percent reflects the rarity of this double genetic defect. The rate of p53 and HER presence varied in past studies between 7% and 19.5% of all examined cases, but the number of cases was small (between four and 18 cases), with the exception of two studies when the number of cases was 47 (of 717, 6.5%) and 42 (of 543, 7.7%) [15, 18–21].

Sidoni A *et al.* [22] have identified a rate of simultaneous immunohistochemical expression of the two proteins in 3% of cases. This study has a significant value because it is a comparative study of cases with coexpression with a separate group that manifested none of these alterations. The study proved that the coexpression of p53 and HER2 appears in young patients that have aggressive types of cancers (poorly differentiated, lymph-node positive, high proliferation activity and negative for estrogen and progesterone receptors). In this study, most cases with p53–HER2 double expression (five cases being 71.42%) had a proliferation index of over 30%, this supporting the theory that says the altered expression of p53 and HER2, even though they have different onset mechanisms, give cancers an accentuated malignity by affecting the inhibition mechanisms.

Studies regarding the prognosis importance and the predictive value of the p53 and HER2 coexpression are however controversial, because of: the small number of cases analyzed, variations of the methods used to identify the expression of the markers, the short follow-up, the non-homogenous character of the series chosen in regards to the treatment, histological type, stage, etc. However, Bull SB *et al.* study, in 2004 [15], that analyzed the risk of recurrences and mortality in patients that had p53 mutations and HER2 amplification determined by genetic methods, has proven that there is a significant risk of recurrences in patients that have mutations of the p53-gene associated with the amplifying of HER2, compared to the patients that have only one of these alterations. Apparently, the determining of cases with p53 mutations select a sub-group of patients with HER amplification that run a greater risk of recurrences and death.

The overexpression of p53 is more frequent in HER2-positive carcinoma, compared to the ones where HER2 is negative (46.66% *vs.* 41.17%). Still, considering the facts mentioned above, we consider that the testing for any existing p53 mutations in patients with HER2 increases would be useful (even through indirect methods like the immunohistochemical ones), to identify that certain sub-group of patients that have more aggressive tumors and that could later benefit from a more aggressive treatment.

Ki-67

Ki67 is considered a useful marker of cell proliferation. An increase of the Ki-67 indicates an increase of cell mitotic activity and cell proliferation [23]. The proliferation study was completed with this antibody precisely because it is a reliable marker of the mitotic activity and it is not expressed inside cells in case DNA repairs take place (as if another cell proliferation marker, PCNA, does).

The immunohistochemical expression of Ki67 has a good correlation with the growth fraction and does not seem to express itself during the DNA repair process [24]. In mammary cancers, Ki67 is used to stratify patients into categories with a favorable and unfavorable prognosis and it was reported that it correlates with the clinical response to chemotherapy [25, 26]. Still, the optimal value of the cutoff that makes the distinction between high proliferation and low proliferation activity in a clinically relevant manner when it is IHC determined in mammary cancers was not universally established. Also, the relation between the Ki67 expression, IHC determined, and the profile of the genic expression of this protein has not fully been studied [27].

The Ki67 immunomarking is usually nuclear localized. In this study, Ki67 was mostly nuclear localized, 98%, but in 2% of cases, there was a cytoplasmic / membrane staining. Very recently, an unusual pattern was described in the hyalinizing trabecular adenoma of the thyroid, the pulmonary sclerosing hemangioma and in the parotid pleomorphic adenoma, pattern that helps the diagnosis [28]. Also, the membrane reactivity of the MIB1 anticlonal antibody was recently described in mammary carcinomas, in 8% of cases [28]. This type of reactivity was either cytoplasmic with membrane accentuation (85% of cases), or predominantly apical (15% of cases) [28]. Faratian D *et al.* have proven that this type of reactivity to Ki67 is significantly associated with a high grading of mammary tumors, with HER2 amplification and ER absence [28].

The mechanism of the cytoplasmic/membrane reactivity is unknown. It can be explained by: crossed reactivity with other proteins, technical artifacts or Ki67 relocation during the cell cycle [29]. The first two variants seem to be excluded by Leonardo E *et al.* [29] that also proved that membrane reactivity appears only when MIB1 clone is used and absent in other clones. The membrane localization of Ki67 was emphasized *in vitro* by Schmidt MH *et al.*, in 2002 [30], but further studies will be necessary to investigate the functional forms of the Ki67 antigen, and its subcellular localization *in vivo*.

Conclusions

In mammary carcinoma, the mutations of p53 are associated with a more aggressive behavior and with a

lower survival rate. Ki67 is considered a useful marker of cell proliferation. An increase in the Ki67 expression indicates an increase in the mitotic cell activity and proliferation. In mammary carcinoma, Ki67 can be useful in stratifying patients into two categories, good prognosis and bad prognosis, being reported to correlate with the clinical response to chemotherapy.

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