The relationship of patients, giving or not giving a pathological full response, with YAP (Yes Associated Protein) in breast cancer cases to which neo-adjuvant chemotherapy is applied

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Sadi Yenel Isaogullari*, Ugur Topal**, Figen Ozturk***, Mustafa Gok*, Bahadir Oz*, Alper Celal Akcan*

*Department of General Surgery, Erciyes University Medical Faculty, Melikgazi, Kayseri, Turkey
**Department of Surgical Oncology, Erciyes University Medical Faculty, Melikgazi, Kayseri, Turkey
***Department of Pathology, Erciyes University Medical Faculty, Melikgazi, Kayseri, Turkey

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AIM: *We aimed to evaluate (immunohistochemically) the YAP expression in breast cancer patients undergoing neoadjuvant chemotherapy and to clarify the relationship between the molecular characteristics, treatment response and survival data and the YAP expression, and hence, to clarify the prognostic significance.*
MATERIAL AND METHODS: *One hundred and four patients who were diagnosed with Breast Cancer between 2015-2020 and underwent Neo Adjuvant Chemotherapy were included in the study. Estrogen Receptor(ER), Progesterone Receptor(PR), Human Epidermal Growth Receptor-2(HER2) and Ki-67. Expression are routinely stained immunohistochemically. In this study, existing immunohistochemical markers were reviewed and also, the relationship of YAP with these biological markers was evaluated by using immunohistochemistry and its effect on prognosis has been investigated.*
RESULTS: *The average age of the patients was 52.37. While YAP was positive in 78 patients (75%), it was negative in 26 patients (25%). In the evaluation after neoadjuvant therapy, pathological complete response (MillerPayne Grade5 response) in 28 patients (26.9%), relapse in 6 patients (5.8%), and exitus in 6 patients (5.8%) were detected. In the pathological evaluation, invasive Ductal Carcinoma was the most common one observed in 88 patients (84.6%). As a result of the statistical evaluation, no significant result was obtained between the parameters and YAP negative/positive.*
CONCLUSION: *As a result of staining with additional YAP in patients who were diagnosed with breast cancer and routinely stained with ER, PR, Cerb B2 and Ki-67 in pathology samples, we could not reach a result that would contribute positively to survival. Longer studies to be conducted prospectively will be meaningful.*

KEY WORDS: Breast Cancer, Chemotherapy, Neoadjuvant, Yes Associated Protein

Introduction

According to the Global Cancer Observatory (Globocan) 2018 data, breast cancer is the most common type of cancer diagnosed in women worldwide [1]. While it ranks second after lung cancer among cancer-related causes of death worldwide, it is the most common cause of cancer death in women. In Globocan 2018 data, while the total incidence of breast cancer is in the second place after lung cancer with 10.6%, the mortality rate is in the 5th place with 4.6%. When we examine only women in

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Corresponence to: Ugur Topal, MD, Department of Surgical Oncology, Erciyes University Faculty of Medicine, 38030 Melilgazi/Kayseri, Turkey (e-mail: sutopal2005@hotmail.com)

these statistics, breast cancer rises to the first place in both diagnosis and death rate [2].

Yes Associated Protein (YAP), a transcriptional co-activator protein, activates the transcription of genes involved in cell proliferation and apoptosis suppression. YAP was first defined as a 65-kDa proline-rich phosphoprotein capable of binding the Src homology-3 domain within the YES proto-oncogene [3,4]. Generally YAP, s widely expressed in a wide range of tissues except for mRNA peripheral blood leukocytes [5]. YAP is a powerful growth enhancer. The human chromosome has been shown to be in an 11q22 amplicon and has been identified as an oncogene [6].

Amplification or overexpression of the YAP gene has been demonstrated in various organ cancers in humans, and overexpression of YAP in mammalian cells has been shown to occur in a large number of oncogenic parameters [7]. Nuclear location of YAP in tumor biopsies has been associated with poor prognosis in cancer patients [8].

When the YAP is activated and passed to the nucleus, it regulates genes related to cell proliferation and viability. It sheds light on the mechanisms by which abnormal cell mechanics activate the onset of multiple diseases such as atherosclerosis, fibrosis, pulmonary hypertension, infection, muscular dystrophy and cancer [9-11].

It has been reported that transcriptional co-activator YES-related protein (YAP) acts as both an oncogene and tumor caregiver in breast cancers [10]. In addition, stronger YAP expression was observed in metaplastic carcinoma compared to triple negative breast cancer. YAP also correlates inversely with HER2 and Ki-67 levels and lymph node metastasis. Among patients with invasive ductal carcinoma, with regards to Luminal A breast cancer and HSK-related patients it was revealed that YAP expression was associated with HSK and GSK in luminal B (HER2-) and luminal B (HER2) breast cancers [12].

In our study, we aimed to evaluate the expression of YAP (immunohistochemically) in breast cancer patients treated with Neoadjuvant Chemotherapy and to clarify the prognostic significance of the relationship between the molecular characteristics, response to treatment and survival data and YAP expression.

Material and Method

Study Plan

This study has been performed by including 104 female patients who were planned to receive NACT as a result of clinical evaluation, among the breast cancer cases who applied to the Department of Breast and Endocrine Diseases Outpatient Clinic of Erciyes University Faculty of Medicine General Surgery Department after January 1, 2015.

By researching patient files retrospectively, demographic characteristics of the patients, localization of the tumor, axillary involvement, histological and pathological features of the tumor, the radiological stage of the tumor, the surgical method applied, recurrence and the current health status of the patients were determined.

Patients who are scheduled for NACT and not operated for various reasons, patients under the age of 18, pregnant and breastfeeding patients, patients with no pathological diagnosis, patients with insufficient tissue for immunohistochemical study and male breast cancer patients were excluded from the study.

Clinical and Histopathological Evaluation

Regarding the patients included in the study, for tumor size and localization definitions at the time of diagnosis, breast USG, MMG, MRI, PET/CT scans of the patients were used when needed.

Although the treatment regimens were anthracycline and taxane-containing regimens, they were selected according to the clinical and individual characteristics of the patients and all patients with HER-2neu expression were given taxane + trastuzumab ± pertuzumab as neoadjuvant therapy after anthracycline. After the operation, evaluation was made according to the pathological and clinical features, and adjuvant RT, hormonotherapy and KT planning were made in accordance with current guidelines. One year of adjuvant trastuzumab treatment was planned in all HER-2neu positive patients and adjuvant hormone therapy was planned according to the current guidelines during the treatment period in patients with any rate of hormone receptor positivity.

In the biopsies taken before the treatment, the ratio of ER, PR, HER2-neu, Ki-67 was taken as parameters. In the evaluation of ER and PR expression, 1% and above staining in cell nuclei in immunohistochemical staining was evaluated as a positive value. HER-2neu expression was considered negative in patients with Score 0 and Score 1 according to the rate of membrane positivity in immunohistochemical staining, and positive in patients with Score 3. HER-2neu gene expression analysis was requested from all patients who were reported to have score 2, by in situ hybridization method and they were considered positive or negative according to the result.

Pathological complete response evaluation was performed on the basis of no residual tumor detected in the surgical material and nodal involvement in the axilla.

During histopathological examination of hemotoxylin-eosin stained sections, for immunohistochemical staining, one block was selected from T-coded paraffin samples of each case.

From the selected paraffin blocks, 5 micron thick sections were prepared for YAP (Yes Associated Protein) immunohistochemical examination. Tissue samples taken on positively charged Poly-L-lysine slides were kept in

the oven at 56-60 C overnight for the first deparaffinization process.
Subsequently, immunohistochemical staining of the sections was performed in a fully automated immunohistochemistry device (VENTANA Benchmark/Ultra, Ventana Medical Systems, USA) that performs all staining steps, including antigen retrieval, under constant temperature and conditions.
Target proteins were made visible by dropping YAP monoclonal antibody (retrieval Citrat 60 min, dilution: 1/400 30 min. Incubation, Code: ab57222, Abcam) to the sections as primary antibody.
After the sections were washed, they were rehydrated through increasing amounts of alcohol solutions. The sections dried in air were kept in xylene for 15 minutes and closed with entellan.
Evaluation of immunohistochemically stained sections was performed under a light microscope (Olympus BX51) at x10 magnification. Staining of 25% of the image area was accepted as positive staining, and <25% staining was accepted as negative staining.
For statistical evaluation, patients were divided into two groups as YAP (+) staining and YAP (-) staining. Tumor characteristics, follow-up data and survival were compared in the groups. Final evaluations of the patients for survival analysis were made in June 2020.

Statistical Analysis

SPSS 23.0 package program was used for statistical analysis of the data. Categorical measurements were summarized as numbers and percentages, and continuous measurements as mean, deviation, and minimum-maximum. The conformity of the variables to normal distribution was examined using visual (histogram and probability graphs) and analytical methods (Kolmogorov-Smirnov / Shapiro-Wilk Tests). Chi-square test and Fischer's Precision Test were used for comparisons of categorical variables. Independent student's t-test was used for groups conforming to normal distribution, while Mann-Whitney U tests were used for groups that did not comply with normal distribution. Kaplan-Meier analysis and Log Rank tests were used for survival analysis. Statistical significance level was taken as 0.05 in all tests.

Results

The study was conducted by examining the data of 104 female patients who were diagnosed with breast cancer and referred to NACT after January 2015. Patients were divided into 2 groups as 78 patients in the YAP negative group and 26 patients in the YAP positive group.
The mean age of the patients in the study was (52.94 years vs 50.65 years p: 0.426).
In the patient group with negative YAP; ER positivity (73.1% vs 61.5% p = 0.265), PR positivity (47.4% vs 42.3% p = 0.650), Cerb B2 (Her2 neu) positivity (19.2% vs 15.4% p = 0.776) were detected higher and Ki-67 being over 20% (56.4% vs 65.4% p = 0.495) invasive lobular carcinoma (7.7% vs 0% p = 0.305) was found to be higher in those with YAP positive, but the differences were not statistically significant (p> 0.05) as shown in Table I.
When the groups were compared, the rates of tumor localization (p = 0.421), multicentrite (p = 0.906), multifocality (p = 0.595), axillary involvement (p =0.651) and applied surgical technique (p: 0.352) were similar in the groups. The frequency of occurrence in BIRADS Category 5 in patients with positive YAP (79.5% vs 96.2% p = 0.047) was found to be statistically significantly higher (p <0.05) is shown in Table II.
While it was determined that 98 (94.3%) of the patients in the study were alive, it was in 6 (5.7%) patients. Although complete response rates were higher in patients with YAP positivity in terms of pathological response compared to patients with YAP negativity (34.6% vs 24.4%), the differences between them were not statistically significant (p> 0.05) (Table III). Signs of

Table I - *Receptor and histological types of tumors*

		YAP Negative (n:78) n(%)	YAP Positive (n: 26) n(%)	p
Estrogen	Negative	21 (% 26,9)	10 (% 38,5)	0,265
	Positive	57 (% 73,1)	16 (% 61,5)	
Progesterone	Negative	41 (% 52,6)	15 (% 57,7)	0,650
	Positive	37 (% 47,4)	11 (% 42,3)	
CerbB2(Her2neu)	Negative	63 (% 80,8)	22 (% 84,6)	0,776
	Positive	15 (% 19,2)	4 (% 15,4)	
Ki-67	20% and below	34 (% 43,6)	9 (% 34,6)	0,495
	Above 20%	44 (% 56,4)	17 (% 65,4)	
Pathological Type	Invasive Ductal	64 (82,1)	24 (92,3)	0,305
	Invasive Lobular	6 (7,7)	0 (0,0)	
	Other	8 (10,3)	2 (7,7)	

* p<0,05, chi-square & Fisher exact test

Table II - *Tumor features and surgical technique*

		YAP Negative(n:78) n(%)	YAP Positive (n: 26) n(%)	p
BIRADS Score	4	16 (20,5)	1 (3,8)	0,047
	5	62 (79,5)	25 (96,2)	
Localization	Left	44 (56,4)	17 (65,4)	0,421
	Right	34 (43,6)	9 (34,6)	
Multi-centricity	None	50 (64,1)	17 (65,4)	0,906
	There is	28 (35,9)	9 (34,6)	
Multi-focality	None	48 (61,5)	16 (61,5)	0,595
	There is	30 (38,5)	10 (38,5)	
Axillary Involvement	None	38 (48,7)	14 (53,8)	0,651
	There is	40 (51,3)	12 (46,2)	
Surgical technique	Breast conserving surgery	27 (34,6)	12 (46,2)	0,352
	Mastectomy	51 (65,4)	14 (53,8)	

* p<0,05, chi-square and Fisher exact test

Table III - *Survival and follow-up results*

		YAP Negative(n:78) n(%)	YAP Positive (n: 26) n(%)	p
Survival	Ex	3 (3,8)	3 (11,5)	0,145
	Alive	75 (96,2)	23 (88,5)	
Pathological response	Residue	59 (75,6)	17 (65,4)	0,307
	Full response	19 (24,4)	9 (34,6)	
Presence of recurrence	None	73 (93,6)	25 (96,2)	0,627
	There is	5 (6,4)	1 (3,8)	
Average follow-up period (u)		25,45±10,85	22,55±10,46	
Average ±ss Median (Min-Max)		23,83 (9,65-53,55)	22,44 (10,28-40,84)	0,237

* p<0,05, t: Independent student t-test, u: Mann Whitney u test, Ki-kare & Fisher exact testi

Table IV - *Relationship of survival findings of the patients with the YAP groups*

	Average		% 95 confidence interval				
YAP Protein	Predicted average	Std. deviation	Lower limit	Upper limit	1 year survival %	3 year survival %	p
Negative	51,53	1,136	49,304	53,756	96,3	92,1	0,074
Positive	36,50	2,232	32,126	40,876	93,8	74,6	

* Kaplan-Meier analysis, ** Log rank test

recurrence were detected in 6 patients (5.7%) included in the study. The difference between the groups in terms of recurrence was not statistically significant (p> 0.05), the average follow-up period was similar in the groups (25.45 months vs 22.55 months p: 0.237).

When survival was compared in groups, it was seen to be higher in patients in the YAP negative group (51.53 ± 1.13 vs 36.50 ± 2.23 p: 0.074) but it was not statistically significant. Survival data are shown in Fig. 1 and Table IV.

YAP positivity was not an independent risk factor for survival (p> 0.05). Factors associated with survival are shown in Table V.

Discussion

Among some factors used in determining the prognosis of breast cancer; tumor size, lymph node involvement and number of lymph nodes involved, distant metastasis, presence of inflammatory carcinoma, histological grade, histological subtype, ER, PR, CerB-B2, IHC staining status, response after neoadjuvant therapy, gene expression status, lymph node ratio and DNA profile can be listed [13-15].

Shaul et al have demonstrated that YAP is involved post-translationally in stabilizing p73 by interfering with E3 ligase-dependent ubiquitylation [16,17], and that it is tumor

TABLE V - *Independent risk factors affecting survival*

Measurements		Univariate p	Multivariate HR (95% - CI)	Multivariate p
Age		0,063	0,940 (0,878-1,006)	0,074
Estrogen	Negative	0,846	1,000	0,846
	Positive		1,190 (0,206-6,859)	
Progesterone	Negative	0,299	1,000	0,313
	Positive		0,407 (0,071-2,329)	
Her 2 neu	Negative	0,916	1,000	0,917
	Positive		1,125 (0,124-10,225)	
YAP	Negative	0,174	1,000	0,165
	Positive		0,307 (0,058-1,624)	
Ki 67	None	0,181	1,000	0,236
	There is		0,267 (0,030-2,368)	
Localization	None	0,678	1,000	0,683
	There is		1,439 (0,251-8,231)	
Multicentricity	None	0,456	1,000	0,453
	There is		0,531 (0,102-2,776)	
Multifocality	None	0,788	1,000	0,791
	There is		1,267 (0,221-7,255)	
BIRADS	4	0,137	1,000	0,998
	5		0,487 (0,197-0,772)	
Surgical Technique	Breast conserving surgery	0,827	1,000	0,828
	Mastectomy		0,824 (0,144-4,724)	
Pathological Type	Inv. Ductal	0,058	1,000	0,065
	Inv.Lobular		0,095 (0,013-0,684)	0,019
	Other		0,176 (0,010-0,534)	0,239
Axillary Involvement	None	1,000	1,000	1,000
	There is		1,000 (0,192-5,200)	
Presence of Recurrence	None	0,323	1,000	0,269
	There is		0,269 (0,026-2,757)	
Pathological Response	Residue	0,541	1,000	0,566
	Full response		1,901 (0,212-17,028)	

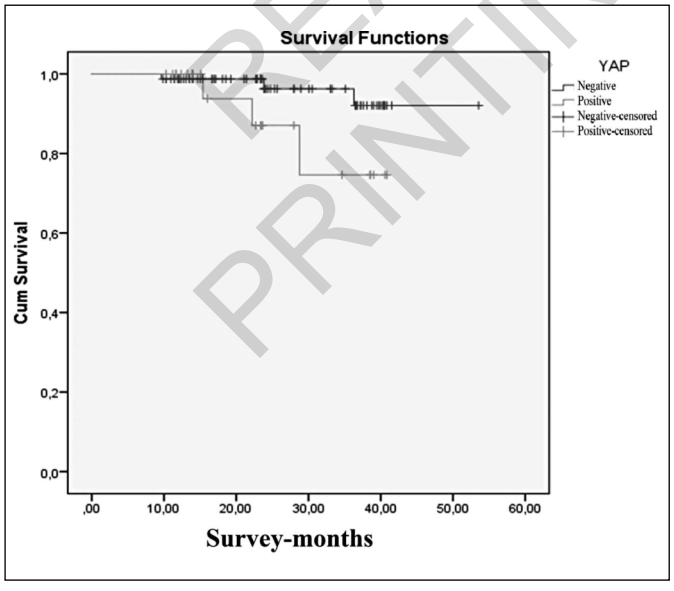

Fig. 1: Examination of the average survival curves of the patients in terms of YAP groups.

suppressor, and that it is the cause of protein loss in breast cancer. This implied that it was similar to Tip60, which is also involved in DNA damage and has recently been shown to be a tumor suppressor in which loss of monoallelic in the breast results in functional protein loss [18].

In the study that conducted, Kim et al have determined that there was involvement in more than 10% of tumor area and that there was positive reactivity in the form of moderate (2nd degree) or strong positive (3rd degree) cancer cell involvement [19].

Based on the same criteria, Sheen-Chen et al showed that 62% of breast cancers were moderately positive and only 6% were strongly positive [20]. In another study, Wang et al found that YAP was strongly positive (overexpression) in 29% of breast tumors and there was loss of expression in 24.6% compared to normal breast tissue (ductal and lobular). Yuan et al showed that YAP protein is lost in more than 60% of breast cancer tissue [12].

In our study, while 26 (25%) patients had YAP

positivity, 78 (75%) patients had YAP negativity. Although the mean survival time of patients with YAP negativity was 51.53 ± 1.13 years, being 36.50 ± 2.23 higher than the mean survival of patients with YAP positivity, the differences between them were not statistically significant (p> 0.05). We think that there was no statistical difference due to the low number of patients who developed mortality in both groups and the short follow-up period.

It is known that breast cancer is more common in the left breast than in the right. While it has been shown in many studies that there is no difference between the localization of the primary tumor and local recurrence, it has been shown that systemic metastases and deaths due to breast cancer increase 2-folds in medial-located tumors [21-23]. In our study, although the tumor was localized in the left breast in 61 patients (58.6%) and in the right breast in 43 patients (41.4%), there was no correlation between localization (p = 0.421) and negative/positive findings of YAP.

Multifocal and multicentric breast cancer is still a controversial and challenging issue for all physicians involved in the treatment of these pathologies. There are several reports stating that multifocal and multicentric breast cancers are associated with lower prognostic factors such as more frequent lymph node metastasis. Wolters et al. showed significantly lower survival parameters in multifocal and multicentric breast cancers compared to unifocal carcinomas [24]. In our study, it was found that 37 (35.5%) patients had multicentrite and 40 (38.4%) patients had multifocality.

When these values are examined, the differences between the patients' localization (p = 0.421), multicentrite (p = 0.906), multifocality (p = 0.595) rates and YAP negative/positivity findings were not statistically significant (p> 0.05). We can think that the behavior and formation pattern of the tumor is not affected by the YAP staining result.

In a study they conducted to investigate the positive predictivity of mammographic lymphography, Zhang et al investigated the findings of insufficient development, filling defect and dilatation in the axillary lymphatic duct in patients with BIRADS category 4 or 5 lesions in MMG and examined their relationship with breast cancer.

In this study, lymphatic duct filling defect was stated as loss of continuity in the duct after contrast injection, and no details were given about whether partial defect or complete obstruction was observed. As a result of the study, it was shown that there was a significant relationship with 89% positive predictivity between lymphatic duct defects and the presence of malignancy in patients with BIRADS category 4 (p = 0.02).

However, in patients with BIRADS category 5, despite the 100% Positive Predictivity Value, no statistically significant relationship was found (p = 1.00) [25]. In our study, in the YAP positive group, 1 (3.8%) patient in Category 4 and 25 (96.2%) patients in Category 5 were detected, and the frequency of BIRADS (p = 0.047) findings in Category 5 was found to be statistically significantly higher (p <0.05). Preoeperative radiological findings of patients with YAP positive staining suggests that tumor suspicion is higher.

High morbidity due to Halsted's radical mastectomy directed surgeons to protect the pectoral muscles by performing MRM first. The "breast cancer is a systemic disease" hypothesis developed by Fisher as a result of the studies conducted in the 1970s led the surgeon to protect the breast in breast cancer treatment [26]. In NSABP-B06 and Milan studies, BCS has been shown to provide survival rates similar to mastectomy, acceptable local recurrence rate, cosmetic result and functional results [27]. In our study, while mastectomy was applied to 65 patients (62.5%), it was found that BCS was applied to 39 patients (37.5%). It was found that the differences between surgical technique (p = 0.352) and YAP negative / positive findings were not statistically significant (p> 0.05). These results suggest that when planning surgical management for patients, location characteristics, diameter and patient preference of the tumor are at the forefront and that YAP protein is not effective.

The relationship between the histological and molecular features of the patients and the pathological complete response has been examined in many publications and it has been determined that the NACT response of invasive lobular carcinoma is worse than other histological types. However, despite poor treatment response, patients have a longer survival time than ductal carcinoma. Therefore, a separate parenthesis should be opened to this patient group and pathological complete response should not be considered as a prognostic factor [28].

Among the patients included in our work, it was found that 88 patients (84.6%) had IDC, 6 patients (5.8%) had ILC, and 10 patients (9.6%) had other pathological varieties. It was found that the differences between the patients' pathological type (p = 0.305) and YAP negative / positive findings were not statistically significant (p> 0.05). These results indicate that YAP protein staining is not directly related to the histological type of tumor.

In the literature, local recurrence rates after BCS are reported between 6-16% [29]. In a study they conducted, Komoike et al found the 10-year Ipsilateral Breast Cancer Recurrence (IBTR) rate to be 8.5% in those who received RT and 17.2% in those who did not receive RT [30]. In our study, as being similar to the literature, recurrence rate was 5.7% in 6 of 104 patients. In terms of recurrence (p = 0.627), although the presence of recurrence was high in the YAP negative group (5 patients), the difference between them was not statistically significant (p> 0.05). Although the local recurrence rate seems to be high in patients with YAP negative, it will not be correct to reach this result with a limited number of patients.

When the survival rates of patients diagnosed with breast

cancer are evaluated based on the data of SEER program, it is seen that the 5-year survival rate was 94% in Stage I patients, 85% in Stage IIa patients and 70% in Stage IIb patients, while the 5-year survival rate was 52% in Stage IIIa patients, 48% in Stage IIIb patients and 18% in Stage IV patients [31]. It was determined that the patients included in this study have been followed up for an average of 24.73 months since the time of diagnosis. The estimated average overall survival rate of the patients was determined to be 50.30 ± 1.26 months, whereas 1-year survival was 97.7% and 3-year survival rate was 88.2%.

Conclusion

As a result of staining with additional YAP in patients who were diagnosed with breast cancer and routinely stained with ER, PR, Cerb B2 and Ki-67 in pathology samples, we could not reach a result that would contribute positively to survival. However, we believe that prospective and longer-term studies on this subject are necessary in terms of enlightening the subject and that they will provide significant contributions to the literature.

Riassunto

Scopo: Valutazione immunoistochimica dell'espressione di YAP in pazienti con carcinoma mammario sottoposte a chemioterapia neoadiuvante e a chiarire la relazione tra le caratteristiche molecolari, la risposta al trattamento e i dati di sopravvivenza e l'espressione di YAP, e quindi, per chiarire il significato prognostico.
Nello studio sono state incluse centoquattro pazienti cui è stato diagnosticato un cancro al seno nell'intervallo 2015-2020 e sottoposte a chemioterapia neoadiuvante. Il recettore degli estrogeni (ER), il recettore del progesterone (PR), il recettore della crescita epidermica umana-2 (HER2) e l'espressione Ki-67 sono sottoposti a colorazione immunoistochimica di routine. In questo studio, sono stati esaminati i marcatori immunoistochimici esistenti e inoltre, è stata valutata la relazione di YAP con questi marcatori biologici utilizzando l'immunoistochimica ed è stato studiato il suo effetto sulla prognosi.
Risultati: L'età media dei pazienti era di 52,37 anni. Mentre YAP era positivo in 78 pazienti (75%), era negativo in 26 pazienti (25%). Nella valutazione dopo la terapia neoadiuvante, sono state rilevate una risposta patologica completa (risposta MillerPayne di grado 5) in 28 pazienti (26,9%), recidiva in 6 pazienti (5,8%) e exitus in 6 pazienti (5,8%). Nella valutazione patologica, il carcinoma duttale invasivo è stato il più comune osservato in 88 pazienti (84,6%). Come risultato della valutazione statistica, non è stato ottenuto alcun risultato significativo tra i parametri e YAP negativo / positivo.
Conclusione: a seguito della colorazione con YAP aggiuntivo in pazienti a cui è stato diagnosticato un cancro al seno e colorate di routine con ER, PR, Cerb B2 e Ki-67 sul pezzo anatomico, non siamo riusciti a raggiungere un risultato che avrebbe potuto contribuire positivamente alla sopravvivenza. Studi prospettici di più lunga durata saranno significativi.

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