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PROGNOSTIC SIGNIFICANCE OF CA 15-3 TUMOR MARKER IN BREAST CANCER PATIENTS

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Summary

The research objectives was to establish pre-operative values of the tumor marker CA 15-3 in serum and to establish connection between serum level of the tumor marker CA 15-3 in patients with breast cancer with other prognostic factors for breast cancer (age, tumor size, lymph node status, histological grading, hormone receptor status). In this work, a retrospective study in 100 breast cancer patients who started their medical treatment in year 2007. on Department of Surgical Oncology, University Hospital for Tumors, Sestre milosrdnice Clinical Hospital Center, has been made.

The concentration of tumor marker CA 15-3 was measured in serum by immunochemical method. When breast cancer was diagnosed, 11 patients had elevated tumor marker CA 15-3, while 89 patients had CA 15-3 within the normal range (values ≤28 kUI/L were considered within the reference range and values >28 kUI/L were considered elevated). Patients who developed metastases had significantly higher values of pre-operative tumor marker CA 15-3 and that patients died from the same disease (mean, CA 15-3=30.67 kUI/L) . High levels of CA 15-3 had patients with positive lymph nodes (status N3, mean CA 15-3=52,78). Results from this research shown that correlation between pre-operativne value tumor marker CA 15-3 with age, ER expression, HER2 status and histologic grade were not found.

Elevated value of tumor marker CA 15-3 is at the time of diagnosis unfavorable prognostic factor, connected with other unfavorable prognostic factors. Justification of this study was confirmed by the value of preoperative tumor marker CA 15-3 in breast cancer patients and after determining its connection with other prognostic factors CA 15-3 indicates that is safer to extract subgroup of breast cancer patients in which metastases can be expected. As such, CA 15-3 can early focus on access to treatment by aggressive protocol.

KEY WORDS: *breast cancer, CA 15-3, prognostic significance*

PROGNOSTIČKO ZNAČENJE TUMORSKOG BILJEGA CA 15-3 U BOLESNICA S RAKOM DOJKE

Sažetak

Cilj istraživanja bio je utvrditi predoperativne razine tumorskog biljega CA 15-3 u serumu te ustanoviti povezanost razine tumorskog biljega CA 15-3 kod bolesnica s rakom dojke s ishodom liječenja, te s drugim prognostičkim čimbenicima za rak dojke (dob, veličina tumora, status limfnih čvorova, histološki i hormonski status tumora). U ovom se radu izradila retrospektivna studija na uzorku od 100 bolesnica koje su započele liječenje zloćudnog tumora dojke na Zavodu za onko-

lošku kirurgiju, Klinike za tumore, KBC Sestre milosrdnice tijekom 2007. godine. Koncentracija tumorskog biljega CA 15-3 mjerena je u serumu imunokemijskom metodom.

U trenutku dijagnoze karcinoma dojke, 11 bolesnica imalo je povišen tumorski marker CA 15-3, dok je 89 bolesnica imalo vrijednosti CA 15-3 unutar granica normale (gdje su vrijednosti ≤28 kUI/L smatrane u granicama referentnih vrijednosti a vrijednosti >28 kUI/L povišene). Bolesnice koje su razvile metastaze imale su značajno veće predoperativne vrijednosti tumorskog biljega CA 15-3 te su iste imale i smrtni ishod liječenja (srednja vrijednost, CA 15-3=30.67 kUI/L). Veće vrijednosti CA 15-3 imale su i bolesnice s zahvaćenim limfnim čvorovima (status N3, srednja vrijednost CA 15-3=52,78). Povezanost predoperativne razine tumorskog biljega CA 15-3 s dobi, statusom estrogenskih receptora, HER2 receptorom i histološkim gradusom nije nađena. Povišena vrijednost tumorskog biljega CA 15-3 je u trenutku dijagnoze nepovoljan prognostički pokazatelj, povezan s drugim nepovoljnim prognostičkim pokazateljima.

Ovo istraživanje je potvrdilo opravdanost određivanja predoperativne razine tumorskog biljega CA 15-3 kod bolesnica oboljelih od raka dojke jer je nakon određivanja njegove povezanosti s ostalim prognostičkim pokazateljima ukazalo da taj tumorski biljeg sigurnije izdvaja podgrupu bolesnica kod kojih se može očekivati pojava udaljenih metastaza. Kao takav, CA 15-3 može rano usmjeriti na pristup liječenja prema agresivnijem protokolu.

KLJUČNE RIJEČI: *karcinom dojke, CA 15-3, prognostičko značenje*

INTRODUCTION

Breast cancer is most common cancer site in women worldwide. In Europe, 464,000 new breast cancer cases a year are detected in women (13.4% new cancer cases in women) (1). In Croatia, 25,574 new female cancer cases (26% of total new female cancer cases) were recorded in 2013, and 994 (16.8%) of total female deaths are caused by breast cancer. Male breast cancer is rare. The male breast cancer incidence is 1-2 in 100 cancer cases (2).

Risk factors related to the breast cancer development are numerous, including genetic, hormonal, nutritional and environmental.. Regardless of all available risk factor data, the said risk factors have not been confirmed in 75% women with breast cancer (3).

The most recent classification of invasive breast carcinoma based on biological characteristics includes: Luminal A, Luminal B, HER2 positive and triple-negative breast carcinoma (4,5).

Determination of the so called prognostic factors is important for its multiple role: it renders the disease biology data, points to possible disease outcome in terms of survival probability or disease recurrence, could be used for therapy planning or point to specific sensitivity to a specific therapy agent.

The most important prognostic factors for breast cancer are tumor size and differentiation and histological grade (the most common is Bloom-Richarson grading system with 3 prognostic grades), histological type of tumor, axial node status (involvement), cell proliferation factors such as percentage of cells in S phase, proteins related to degree of tumor invasive properties of tumors (cathepsin D, plasminogen activators and nm23) and hormone receptors – estrogen (ER) and progesterone (PR) receptors. Tumors with high level of these receptors are hormone-dependent tumors responsive to hormonal therapy. Thus, for example ER+/PR+ tumors respond to hormonal therapy in about 75% of cases. Molecular markers, such as mucinous epithelium, oncogenes and tumor suppressor genes also need to be mentioned (6).

Determination of gene expression and introduction of new molecular biology methods sheds a new light on hormone receptor – ER positive tumors. Several subgroups could be determined within ER positive and negative tumors with different prognosis and response to hormonal therapy. For example, ER positive tumors (luminal type) could be subdivided into three subtypes according to their expression which is related to the frequency of TP53 mutation (A,B and C) while ER negative tumors (basal) could be subdivided into tumors with HER2 expression and HER2 negative tumors.

Breast cancer treatment depends on tumor grade, tumor extension, tumor and host properties, and it could be local (surgery or radiation) or systemic.

Markers

In broader sense of the word, markers encompass all the ingredients and products of tumor cells that enable tumor to be detected (diagnosed),

classified and its behaviour during treatment monitored. In narrower sense of the word, markers are products of tumor cells that can be measured in extracellular environment, primarily in bodily liquids. Determination of tumor marker concentration in serum is considered particularly important in modern tumor diagnostics and therapy effect monitoring. Concentration of tumor antigen in serum is determined by sensitive immunochemical methods, enzyme-linked immunosorbent assay (ELISA) or turbidimetry. Data on serum level of markers is used as complementary method in making diagnosis, assessment of the disease extent, and for treatment effect monitoring (7).

CA 15-3 is one of the first prognostic breast cancer factors relying on circulating tumor cells. CA 15-3 is a glycoprotein, which occurus particulary in the cells of mammary glands. Its concetration in blood may be measured in laboratory from semple of venous blood.

Preoperative value of this marker could be combined with other prognostic factors to predict outcome of the disease in patients with newly diagnosed breast carcinoma. However, it is particularly clinically significant for monitoring metastatic disease response to chemotherapy (8).

In breast carcinoma diagnostics, elevated value of CA 15-3 tumor marker is an indication of locally advanced breast cancer and therefore faster recurrence and poorer treatment outcome (9,10).

CA 15-3 determined individually could have a prognostic significance in newly diagnosed female breast cancer patients, and be a strong prognostic indicator in patients with advanced breast cancer (10, 11).

Low sensitivity and specificity of this marker rule it out as a marker for early breast cancer diagnosis. In patients diagnosed with cancer in early stage of the disease, CA 15-3 levels correspond to a large degree with CA 15-3 levels in healthy women or women with benign breast cancer. Elevated CA 15-3 levels point to a metastatic disease, thus additional tests are necessary to exclude such a possibility. About 5-10% of women are diagnosed with advanced breast cancer. Although elevated CA 15-3 tumor marker levels are determined in the majority of patients with advanced breast cancer, its high concentrations might also be detected in case of some other types of advanced adenocarcinoma. Aside from breast cancer, advanced carcinoma that could result in elevated CA 15-3 levels include ovarian, pancreatic, lung and intestinal cancer. Rarely, some non-malignant diseases such as chronic hepatitis, cirrhosis of the liver, sarcoidosis, megaloblastic anemia could cause elevated CA 15-3 levels (12,13). Depending on the determination method, and reagent and analyser manufacturer, normal levels of serum marker CA 15-3 for men and women is less than 25-30 kUI/L.

The aim of this study are to determine the preoperative serum CA 15-3 tumor marker levels and to establish correlation between the CA 15-3 level in breast cancer patients and treatment outcomes, and other breast cancer prognostic factors

SUBJECTS AND METHODS

Subjects

A retrospective study was made of 100 female patients who started their breast cancer treatment at the Oncological Surgery Department, University Hospital for Tumors, Sestre milosrdnice University Hospital Center in 2007. All the patients included in the study had operable breast carcinoma, namely subjects were patients with disease stage I-III.

The study was approved by the Ethics Committee of the Sestre milosrdnice University Hospital Center (No.: EP-6820/13-10).

The preoperative basic serum values of tumor marker CA 15-3 and patients data, were retrieved from the University Hospital for Tumors archives keeping all the histories of the female patients. The data on the state of health of a part of patients regularly checked at the University Hospital for Tumors was retrieved from the University Hospital for Tumors database, while the data on patient with fatal outcome was obtained from the database of the Croatian Institute of Public Health Cancer Registry.

Information on the state of health of other patients was obtained by telephone survey.

Methods

The CA 15-3 marker concentration was measured in serum obtained by centrifuging whole blood at 3,500 rpm for 10 minutes. The measuring was based on chemoluminiscence principle use, where reagent components are specifically de-

signed antibodies lining paramagnetised micro particles. During the first incubation, antigen from the sample (CA 15-3) bonds specifically to bound antibodies proportional to its presence in sample. During the secondary reaction, also referred to as indicator reaction, a conjugate (secondary antibody to which a marker of chemiluminiscent properties is bound) bonds to the formed At-Ag complex. After excessive unbound conjugate is washed and magnetic separation of bound complex from micro particles carried out, in the end, a dramatic change of pH (addition of high molar NaOH) activates chemiluminiscence whose intensity is proportional to antigen (CA 15-3) concentration in primary reaction. CA 15-3 concentration was measured by automated immunoassay analyser Architect i2000SR manufactured by Abbott (Abbott Laboratories, Abbott Park, Illinois, USA). Reagents (Ca15-3 RGT;2K44-20), calibrator (CA 15-3 CAL; 2K44-01)) and controls (Tumor Marker –MCC Lyophilised; 6E21-10) by the same manufacturer were used.

Statistical analysis

The data stored in spreadsheets was processed using descriptive and inferential statistics methods.

Kaplan-Meier estimator of survival function and Nelson-Aalen estimator of risk function were used for descriptive survival analysis. Mean and median survival times with related limits were also calculated.

Cox regression for survival analysis was used in evaluation of predictive (prognostic) validity of CA 15-3 marker.

RESULTS

Characteristics of 100 patients involved in the study are given in Table 1.

In majority of patients (59) the smallest tumor T1 (to 2 cm) was determined. In 38 patients tumor size was T2 (2-5cm), and 3 patients had tumor larger than 5 centimetres. Axillary nodes (N0) were not affected in majority of patients (n=47), while 28 patients had lymph node status N1 (1-3 positive lymph nodes), 16 patients had N2 (4-9 positive lymph nodes), while in 9 patients more than 9 lymph nodes were positive. The majority of patients (57) had moderately differentiated tumors (grade II), 15 patients had grade I and 28 patients had grade III tumors. As regards histological diagnosis, carcinoma ductale invasivum was dominant (84 patients), while incidence of other carcinomas was lower (CLI 11, CMI 5 patients). Estrogen receptors were positive in the majority of patients (n=82), and 68 patients had progesterone receptor positive tumor. Incidence of breast cancer is present in elderly women (>50 years of age). Total 76 patients that developed breast cancer were older than 50 years, while 24 patients were younger than 50 years. HER2 positive tumor was diagnosed in 39 patients, and HER2 negative breast cancer was diagnosed in 61 patient.

At the time breast cancer was diagnosed, 11 patients had elevated CA 15-3 marker, and 89 patients had CA 15-3 marker values within normal range (where values ≤28 kUI/L are considered as within the reference value range and values >28 kUI/L as elevated). Mean preoperative value of CA 15-3 marker was 19.47 kUI/L.

Table 1. shows that CA 15-3 value was significantly statistically different if the patients were classified by criterion N (p=0.01), where testing of difference in CA 15-3 level with regard to the lymph node status indicates that the patients with affected lymph nodes (status N3) had statistically significantly higher CA 15-3 level (Kruskal-Wallis test); and PR (p=0.04) where the results also indicated that the patients with negative progesterone receptors had statistically significantly higher CA 15-3 marker values (Mann-Whitney test).

Results of testing the difference in CA 15-3 level with regard to the tumor size (T_1-T_4) indicated that the patients with tumor T3 (p=0.01) had significantly higher preoperative value of CA 15-3 marker (87.50 kUI/L) than the patients with T_1 and T_2 tumor (Kruskal-Wallis test).

Testing the difference in CA 15-3 level between T_1 and T_2 tumors (Mann-Whitney test) resulted in marginal statistical significance (p=0.055).

Correlation between CA 15-3 and tumor size expressed in centimetres is statistically significant (p=0.002). Mean size of tumor in patients participating in the study was 2.2 cm. Patients with tumor larger than 2.2 cm had higher values of CA 15-3 marker compared to the patients with tumor smaller than average.

Results obtained by descriptive statistic method indicated that preoperative value of CA 15-3

Table 1.
CHARACTERISTICS OF PATIENTS INVOLVED IN THE STUDY

	Number of patients (n)	CA 15-3 (mean)	p
T			
T1	59	16.32	
T2	38	21.11	0.055
N			
N0	47	14.85	
N1	28	16.14	
N2	16	20.13	0.01
N3	9	52.78	
HG			
I	15	23.33	
II	57	16.93	p>0.05
III	28	22.57	
ER			
pos	82	18.48	0.1
neg	18	24.00	
PR			
pos	68	15.82	0.04
neg	32	27.22	
Histological diagnosis			
CDI	84	19.36	
CLI	11	22.82	p>0.05
CMI	5	14.00	
AGE			
<50	24	16.25	0.8
>50	76	20.49	
HER2			
pos	39	23.77	0.1
neg	61	16.72	
Marker (preoperative level)			
CA 15-3 < 28 kUI/L	89		
CA 15-3 > 28 kUI/L	11		

T, tumor size; N, lymph node status; HG, histological grade; ER, estrogen receptor; PR, progesterone receptor; HER 2, human epidermal growth factor receptor 2; CA 15-3, tumor antigen 15-3; p, statistical significance determined by Kruskal-Wallis and Mann-Whitney test

marker and age is statistically insignificant (p=0.2). There is also no statistical significance in CA 15-3 level in patients under/over 50 years of age (Mann-Whitney test).

No statistically significant difference was determined in measured CA 15-3 values in patients classified using HG, ER, histological diagnosis and HER2 status.

Table 2.
ADJUVANT THERAPY ADMINISTERED

Treatment method	Number of patients (n)
chemotherapy	
yes	73
no	23
hormonat therapy	
yes	81
no	19
radiation therapy	
yes	78
no	22
chemotherapy + hormonal	56
chemotherapy + hormonal + radiation	47

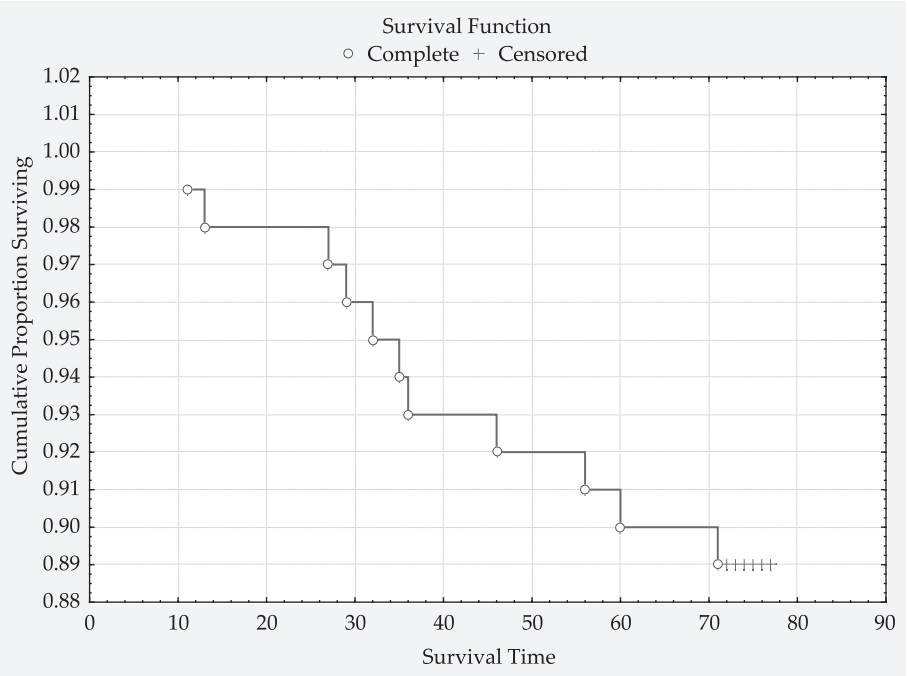
Table 3.
NUMBER OF PATIENTS WITH LOCAL RECURRENCE, METASTASES AND FATAL OUTCOME

	Number of patients (n)	CA 15-3 (mean)	p
local recurrence			
yes	4	26.50	p>0.05
no	96	19.18	
metastases			
yes	9	30.67	0.01
no	91	18.36	
exitus			
yes	11	22.91	p>0.05
no	89	19.04	

Table 2. shows which therapy was administered. Seventy-three patients were administered chemotherapy, eighty-one hormonal therapy and seventy-eight patients received radiation therapy. Combination of chemotherapy and hormonal therapy was administered to fifty-six patients while the combination of chemotherapy, hormonal and radiation therapy was administered to forty-seven patients.

Table 3. shows local recurrence in 4 patients, metastases in 9 patients, and fatal outcome in 11 patients.

CA 15-3 value in the group of patients with metastases was statistically significant (p=0.01) as compared with the group of patients without metastases, namely the patients with metastases had higher preoperative level of CA 15-3 (30.67 kUI/L)



Graph 1. Kaplan-Meier survival curve

Table 4.

COX REGRESSION FOR SURVIVAL ANALYSIS

N = 100	Dependent variable: time (months) Censored variable: exitus Chi2 = 8.82264 df = 7 p = 0.26568					
	Beta	Standard. Error	t-value	Exponent beta	Wald Statist.	p
Age	0.02414	0.028069	0.85994	1.024431	0.739491	0.389831
Tumor size	0.01041	0.146405	0.007108	1.010461	0.005052	0.943334
ER	0.062634	0.855409	0.73221	1.870744	0.536127	0.464048
PR	-1.14444	0.712940	-1.60524	0.318403	2.576787	0.108452
HER2	0.73305	0.624799	1.17326	2.081422	1.376539	0.240700
Metastases	1.58006	0.770597	2.05044	4.855246	4.204293	**0.040330**
Preoperative CA 15-3	-0.00840	0.014560	-0.57692	0.991635	0.332831	0.564001

than the patients without metastases (Mann-Whitney Test).

CA 15-3 did not differentiate the patients with regard to the presence of local recurrence or exitus (p>0.5).

Cox regression for survival analysis did not show correlation between the patients who died and their age, tumor size, status of estrogen and progesterone receptors, HER2 status and CA 15-3 level. Only development of metastases in patients was shown as significant variable in fatal outcome of the treatment (p=0.04).

Graph 1. shows the survival curve for patients with metastases (Kaplan-Meier). Survival time is measured by moths.

DISCUSSION

A retrospective study was made of 100 female patients who started their breast cancer

treatment at the Oncological Surgery Department, University Hospital for Tumors, Sestre milosrdnice University Hospital Center in 2007 and were followed up for 5 years. Preoperative values of CA 15-3 marker were determined, and correlation between its level and treatment outcomes and other breast cancer prognostic factors was analysed.

CA 15-3 is the tumor marker routinely used in breast cancer diagnosis and monitoring of treatment results. Determination of its value in initial treatment stage when diagnosis is made is an almost standard approach. CA 15-3 is one of the first prognostic breast cancer factors relying on circulating tumor cells and when determined individually it has prognostic value. (9,11) Its prognostic value in combination with other tumor markers is frequently studied. In breast cancer detection, elevated CA 15-3 marker levels are an indication of locally advanced carcinoma and consequently faster recurrence and fatal outcome of the disease (14).

The patients included in this study who developed metastases had elevated values of CA 15-3 ($p=0.01$) and consequentially fatal treatment outcome. The results obtained correspond with results of other studies (15,16).

The results presented in this study also demonstrate correlation between the preoperative CA 15-3 marker levels and tumor size (less than 2.2 cm). An average tumor size of the studied group was more than 2.2 cm. The patients with tumor larger than average had statistically higher (preoperative) level of CA 15-3.

The patients with tumor between 5 and 10 cm (T_3 tumors) had statistically significantly higher CA 15-3 marker level. However, because of a small number of patients in the group (2 patients with T3 tumor and one patient with T4 tumor), the result is difficult to interpret. Also, testing of difference between T_1 and T_2 tumors with regard to CA 15-3 level has not shown statistical significance.

Patients with N_3 tumor had significantly higher values of CA 15-3 marker.

Results obtained by Park *et al.* and Moazzezy *et al*. are similar. In their study they established a correlation between CA 15-3 (and CEA) level and tumor size (>5cm) and lymph node status (more than 4 nodes involved).(10,16) Duffy *et al.* got the results showing a significantly higher CA 15-3 marker levels in patients with larger tumors and patients with positive lymph nodes, unlike the patients with N_0 status (14).

Additionally, the conducted study detected no correlation between the preoperative level of CA 15-3 marker and age, estrogen receptor status, HER2 receptor or histological grade. The same results were obtained by Park *et al.* in their study. (10) Ebeling *et al.* study shows that tumor size, lymph node status, histological grade and hormonal status are prognostically significant (9). Duffy *et al.* have not established a correlation between the CA 15-3 level with estrogen positive or negative receptors (which corresponds with the results of this study). On the other hand, their obtained results, of CA 15-3 level, was significantly higher in patients aged 50 years and older, while in our study the difference in CA 15-3 levels in patients older/younger than 50 years of age was not statistically significant (14).

The study result indicate correlation between the negative progesterone receptor levels and CA 15-3 level (considerably higher). The result is difficult to interpret since negative progesterone receptors do not have as significant prognostic value as negative estrogen receptors. However, when estrogen receptors are positive, negative progesterone receptors often have poorer prognostic significance (17,18).

Multivariate analysis did not statistically indicate correlation of CA 15-3 and traditional prognostic indicators with treatment outcome. As expected, only development of metastases was the statistically significant indicator of fatal outcome of patient treatment. Since the study dealt with early breast carcinoma, the result complies with the results of other studies where the most difficult task is to single out a subgroup of patients with poorer prognosis who will benefit from aggressive adjuvant therapy (18,19,20).

As regards this study, it should be underscored that the patients who developed metastases had significantly higher preoperative values of CA 15-3 marker and fatal outcome of treatment. Higher CA 15-3 values were recorded in patients with larger tumors (>2.2cm), affected lymph nodes (status N3) and in patients with negative progesterone receptors.

In diagnostic process, elevated values of CA 15-3 tumor present an unfavourable prognostic indicator and they are correlated with other unfa-

vourable prognostic indicators (such as tumor size, lymph node status, hormonal status).

The present study confirmed that determination of preoperative level of CA 15-3 marker in breast cancer patients is justified since, once its correlation with other prognostic indicators is established, it serves for safer singling out of patient subgroup expected to develop distant metastases. As such, CA 15-3 could result in early decision of treatment under more aggressive protocol.

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