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Color Doppler Combined Ultrasound Findings Compared to Histopathological Diagnosis of Breast Carcinoma

Research Article

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Abstract

Aim: To identify criteria suggestive of histological diagnosis of breast cancer in the study of the internal vascularization of the breast lesion using ultrasonography.

Material and Methods: A descriptive observational cross-sectional study was performed in 115 women aged 23 to 83 years old, with a diagnosis of a breast lump who underwent a biopsy between September 2018 and August 2019. Two study groups were formed, one consisting of positive cases for malignancy and the other negative cases for malignancy in the histopathological study. The nodule was evaluated first in B-mode and then by Doppler to study the internal vascularization. To investigate the association of US and Doppler variables with positive histopathological diagnosis, Pearson's chi-square test, Fisher's exact test, Student's t-test and Mann-Whitney test for 95% confidence interval were used.

Results: Among the positive results, 1/27 (3.7%) did not exhibit internal vascularization; and 26/27 (96.3%) had internal vascularization (p : 0.001). The presence of central distribution presented an odds ratio (OR) of 13.58 (3.96 - 46.51) ($p < 0.001$). For the presence of peripheral distribution vascularization, the OR was 5.96 (1.53 - 23.24) ($p = 0.01$). The vascular morphology of the penetrating type showed a significant association with positive histopathological diagnosis (OR : 23.19 [7.07 - 75.99], $p < 0.001$). Pulsatility Index (PI), Resistance Index (RI) and Systolic Peak (SP) analysis showed significantly higher rates in malignant lesions ($p < 0.0001$).

Conclusion: In breast nodules with suspicious characteristics in the B-mode, the color Doppler examination of vessels of peripheral and central distribution, as well as vessels with penetrating morphology and high spectral indexes, is strongly associated with a positive histopathological diagnosis.

Clinical Significance: The results demonstrate it is possible to identify ultrasound characteristics combined with color Doppler in histopathologically proven cases of breast cancer

Keywords: Ultrasonography; Doppler; Color; Breast neoplasms; Blood vessels

Introduction

Despite significant advances in research, breast cancer is still the most prominent health problem and a priority in biomedical research [1]. It is the most common malignancy in women worldwide, and their incidence and mortality rates are expected to increase significantly over the next five to ten years [1,2]. In Brazil, 59,700 new cases of breast cancer for each year of 2018-2019 are estimated, with an estimated risk of 56.33 cases per 100,000 womenm [3].

Breast cancer screening is carried out in the following ways: self-examination, clinical examination, and imaging methods. Imaging methods include: mammography, ultrasound, and magnetic resonance imaging, based on breast density and risk factors [4]. Mammography is currently the primary method for breast cancer screening. It has the ability to detect cancer at an early stage, with a better prognosis of treatment [5]. The diagnostic accuracy of mammographic screening strongly depends on the radiographic density of the evaluated breast

tissue. In radiographically dense breasts, non-calcified cancers are more likely to be not detected. Therefore, mammographic screening will not be effective in these breast types [4,5].

Ultrasonography (US) is used as an adjunct method to mammography in cases of radiographically dense breasts due to the reduced sensitivity of the mammography [5,6]. In the US it is also performed in patients under 40 years of age when mammographic screening is not yet indicated, pregnant patients and patients with palpable breast abnormalities [7,8]. Some critics fear that the regular performance of complementary ultrasound in women at medium risk will also increase the rate of false positives leading to a higher number of unnecessary biopsies [5]. To improve diagnostic accuracy, color Doppler [7] could be used.

The most crucial advantage of Doppler in breast ultrasound, among many other applications, is to assist in the classification of breast lesions as probable benign or probable malignant. This is from the detection and characterization of the neovascularization of the breast tumor, especially regarding its distribution and morphology [9]. However, this function is not well defined. The objective of the study was to identify ultrasound and Doppler characteristics in proven cases of breast carcinoma and whether there are criteria in the study of the internal vascularization of the ultrasound lesion which are associated with the histological diagnosis of breast cancer.

Materials and Methods

Study Design

A descriptive observational cross-sectional study was performed in 115 women who had a diagnosis of a breast lump and who underwent a histopathological study. They visited the mastology service at the Assis Chateaubriand Maternity School between September 2018 and August 2019.

Study Population

In the study women between 23 and 83 years old visiting the Mastology Service of the Assis Chateaubriand Maternity School (MEAC) were included. They underwent a breast ultrasound visualization, classified according to BI-RADS US, grade IV or V, and agreed to participate in the research. Pregnant women, women undergoing breast cancer treatment, and those who refused to participate in the study were excluded.

Ultrasound Examination Techniques

All ultrasound examinations were performed before the invasive procedure to avoid altering the findings and all were performed by the same experienced professional. The patient was positioned supine, with her arms raised above her head. The pressure exerted with the transducer on the breast examined was as low as possible so as not to occlude the small vessels investigated. The patient was often asked to stop breathing so as not to miss the small vessels. The device used for the ultrasound examination was a GE-Voluson brand, using a 13mhz linear probe, considered high frequency, suitable for the study of superficial structures, enabling a better resolution [10]. HD-flow software was used, with the following parameters: PRF = 0.6khz; wall filter (WMF) = Low 1; frequency = Low; flow resolution = high.

The nodule was first evaluated in B-mode and then by Doppler. In B-mode, all the nodule characteristics, according to the American College of Radiology Breast Imaging Reporting and Data System, or BI-RADS, were analyzed, such as size, shape, orientation, margin, echogenicity pattern, posterior acoustic characteristic. If one or more features were identified, such as irregular shape, larger axis perpendicular to the skin, non-circumscribed margins (angled, microlobulated, undefined), thin calcifications inside, posterior acoustic shadow (except calcified fibroadenoma identified on mammography), markedly hypoechoic nodules or complex, they were classified as BIRADS 4. Subdivision into 4A, 4B, and 4C was not used in the study since, according to the BIRADS lexicon, the recommendation for the three subdivisions consists of tissue diagnosis. The irregularly shaped nodules and spiculated margins were classified as BIRADS V. Then the color Doppler was inspected to evaluate the internal vascularization of the nodule.

The color box (ROI) was adjusted to include the lump and a small margin of healthy tissue adjacent to it. The lesion was then classified for vascularization as absent or present. When present, vascularization was classified according to its distribution in peripheral and central and could be exclusively peripheral, exclusively central and peripheral/central. For morphology the classification used was linear, branching, punctual, and penetrating. The spectral analysis was performed, evaluating Resistance Index (RI), Pulsatility Index (PI), and Systolic Peak (SP) in cm/s. The nodules classified as grade IV and V, according to BI-RADS®, were then referred to the core biopsy, and the fragments of the lesion were sent for pathological study. The piece was packed in 10% formaldehyde and sent to the pathology laboratory for processing and confection of lamina for study in hematoxylin-eosin staining by an experienced pathologist.

Statistical Analysis

To investigate the association of ultrasound and color Doppler variables of breast nodules with positive histopathological results, Pearson's chi-square test and Fisher's exact test were used for categorical variables; and Student's t-test and Mann-Whitney test for continuous variables, conditioned to adherence to a normal distribution, for 95% confidence interval. Logistic regression models were adjusted with the independent variables: distribution and morphology of internal vascularization and histopathological dependent. A significance level of 5% was adopted. The analyses were performed using software R, version 3.1.1.

Ethical Aspects

All patients signed the Informed Consent Form before the examination and were informed of the purpose, risks, and benefits associated with the study and ensured of the full confidentiality of the records. It was approved by the Assis Chateaubriand Maternity School Research Ethics Committee (CEP/MEAC) number: 2,855,509; Date: 08/21/2018.

Results

Of the 115 biopsied nodules, 27 (23.47%) were diagnosed as malignant and 88 (76.52%) as benign. Among the malignant results, 04 were ductal carcinoma in situ, 19 invasive ductal carcinomas, 02 tubular carcinomas, and 02 invasive lobular carcinomas. Among the benign lesions, 31 were fibroadenomas, 39 were non-proliferative lesions such as adenosis and fibrosis, 13 proliferative lesions without atypia (5 papillomas), two proliferative lesions with atypia, 02 inflammatory disorders and 01 phylloid tumor.

Regarding socio-demographic data, it can be observed that the study group had a mean age of 52.2 (+1.8) and the control group of 50.9 (+11.2) (p = 0.212). Hormone therapy was reported by 2/27 (7.41%) patients in the study group and 2/88 (2.27%) (p = 0.234). The personal and family history of breast cancer, respectively, were 2/27 (7.41%) and 13/27 (48.15% in the study group and 12/88 (13.64%) and 37/88 (42.05%) in the control group (p not significant) The ultrasound examination was categorized as diagnostic in 24/27 (88.89%) and as screening in 3/22 (11.11%) in the group with histopathological cancer outcome (positive), and as a diagnosis in 55/88 (62.50%) and as a screening in 33/88 (37.5%) in the control group (p : 0.010). /27 (62.96%) in the study group and 61/88 (69.32%) in the control group (p : 0.536) (see Table 1).

Table 1: Bivariate analysis of socio-demographic and historical data of breast cancer patients and the control group attended at Assis Chateaubriand Maternity School Mastology Service (Fortaleza-Brazil) from September 2018 to August 2019.

Histopathology			
Variables	**Positive (n (%))**	**Negative (n (%))**	**p**
Age (mean ± standard deviation)	54.2 ± 13.8	50.9 ± 11.2	0.212a
Hormonal Therapy			0.234d
Yes	2 (7.41%)	2 (2.27%)	
No	25 (92.59%)	86(97.73%)	
Breast Cancer Familiar History			0.576c
Yes	13 (48.15%)	37 (42.05%)	
No	14 (51.85%)	51 (57.95%)	
Breast Cancer Personal History			0.514d
Yes	2 (7.41%)	12 (13.64%)	
No	25 (92.59%)	76 (86.36%)	
US Indication			0.010c
Diagnosis	24 (88.89%)	55 (62.50%)	
Screening	3 (11.11%)	33 (37.50%)	
Mammography			0.536c
Yes	17 (62.96%)	61 (69.32%)	
No	10 (37.04%)	27 (30.68%)	

The sonographic characteristics of the nodules, according to the histopathological diagnosis, are shown in (Table 2). Among the positive cases, the median size of the nodules was 1.5cm (1.10 - 2.30); and among the control group this was 0.90cm (0.70 - 1.30) (p : 0.001). Regarding the shape of the nodules, in the study group 2/27 (7.41%) were rounded; 18/27 (66.67%) were irregular, and 7/27 (25.93%) were oval. In the control group, on the other hand, the rounded, irregular and oval shapes were respectively 4/88 (4.55%), 6/88 (6.82%) and 78/88 (88.64%) ($p < 0.001$). The largest diameter nodule in relation to the skin, i.e., parallel or non-parallel to the skin for the study group was 1/27 (3.7%) and 26/27 (96.3%) respectively; and for the control group it was 3/88 (3.4%) and 85/88 (96.6%), without statistical significance. Regarding the nodule margins studied, the circumscribed margins in the positive group were 1/27 (3.7%), and in the control group was 19/88 (21.6%); the microlobulated margins in the positive result were 12/27 (44.4%); and negative result was 43/88 (48.9%), the angled margins in positive cases were 2/27 (7.4%) and negative result 24/88 (27.3%). In the indistinct margins the results were respectively for the study group and control group 1/27 (3.7%) and 2/88 (2.3%). Regarding spiculated margins, the study group was 11/27 (40.7%); and the control group 0/88 (0%) ($p < 0.001$). In the standard internal echo criterion, the complex type for the study group was 2/27 (7.4%). The heterogeneous pattern showed results respectively for the positive and negative groups of 11/27 (40.7%) and 12/88 (13.6%). For the hypoechoic internal standard characteristic, the study group was 14/27 (51.9%) (p: 0.008). In the posterior echo pattern variable, the absent variable was for the positive group 23/27 (85.2%) and for the control group 83/88 (94.3%). The posterior acoustic reinforcement variable was 0/27 (0%) for the positive group and for the 2/88 (2.3%) control group. The posterior acoustic shadow criterion showed the following results: positive group 4/27 (14.8%) and 3/88 (3.4%) (p: 0.103). Regarding the axillary lymph nodes, in the study group 4/27 (14.81%) were altered and 23/27 (85.19%) normal. The BIRADS 4 classification showed 17/27 (62.96%) for the study group and BIRADS 5 10/27 (37.04%). For the control group the result was respectively 87/88 (98.86%) and 1/88 (1.14%) ($p < 0.001$).

Doppler flow study of nodules comparing cases with positive histopathological diagnosis with control groups is shown in (Table 3). Regarding the present or absent internal vascularization variable, it was observed that in the positive group, 1/27 (3.7%) presented absent vascularization, while 26/27 (96.3%) showed internal vascularization. In the control group the results were respectively 49/88 (55.7%) and 39/88 (44.3%) (p : 0.001). In the peripheral distribution variable among the positive group, 24/27 (88.89%) exhibited peripheral vascularization, and 3/27 (11.11%) did not exhibit. Among the control group 39/88 (44.32%) exhibited peripheral vascularization, and 49/88 (55.68%) did not ($p < 0.001$). Central distribution showed the following results for the study group: 15/27 (55.56%) showed central vascularization, and 12/27 (44.44%) did not exhibit this. Among the control group 5/27 (5.68%) showed central flow, and 83/88 (94.32%) did not show central flow. Regarding the morphology of the internal vessels, the linear morphology variable among the positive cases 6/27 (22.22%) presented and 21/27 (77.78%) did not exhibit this morphology. for negative cases the results were respectively 22/88 (25.00%) and 66/88 (75.00%) ($p = 0.769$). In the punctiform morphology, among the positive groups 3/27, (11.11%) exhibited punctiform morphology. Among the control group the results were respectively 12/88 (13.64%) and 66/88 (86.36%) ($p > 0.999$). Branching morphology is observed among the positive group 5/27 (18.52%), and 22/27 (81.48%) did not present this. Among the control cases, 5/88 (5.68%) presented and 83 / 88 (94.32%) did not present (p: 0.053). The penetrating morphology showed that among the positive cases 17/27 (62.96%) exhibited this morphology, and 10/27 (37.04%) did not. Among the control group the numbers were respectively 6/88 (6.82%) and 82/88 (93.18%) ($p < 0.001$).

(Table 4) presents the values of sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) of the variables present/absent vascularization, peripheral distribution, central distribution, and penetrating morphology. For vascularization the values were respectively: 96%, 56%, 40% and 98%. For the peripheral distribution variable, we have the following results for sensitivity, specificity, PPV, and NPV: 88%, 55%, 39%, and 94%, respectively. Regarding the central distribution variable this is 55%, 94%, 75%, and 87%, respectively. In addition, for the penetrating morphology variable this was 62%, 93%, 73%, and 89% respectively for sensitivity, specificity, PPV, and NPV.

Table 2: Bivariate analysis of data related to ultrasound characteristics of B-mode nodules of breast cancer patients and control group attended at Assis Chateaubriand Maternity School, Mastology Service (Fortaleza, Brazil), between September 2018 and August 2019.

Histopathology			
Variable	**Positive (n (%))**	**Negative (n (%))**	**p**
Size	1.50 (1.10-2.30)	0.90 (0.70-1.30)	0.001b
Form			< 0.001d
Roudend	2 (7.41%)	4 (4.55%)	
Irregular	18 (66.67%)	6 (6.82%)	
Oval	7 (25.93%)	78 (88.64%)	
Orientacao			> 0.999d
Not parallel	1 (3.7%)	3 (3.4%)	
Parallel	26 (96.3%)	85 (96.6%)	
Margins			< 0.001d
Circumscribed	1 (3.7%)	19 (21.6%)	
Micro lobulated	12 (44.4%)	43 (48.9%)	
Angled	2 (7.4%)	24 (27.3%)	
Indistinct	1 (3.7%)	2 (2.3%)	
Spiculated	11 (40.7%)	0 (0%)	
Internal Eco Standard			0.008c
Complex (solid / cystic)	2 (7.4%)	13 (14.8%)	
Heterogeneous	11 (40.7%)	12 (13.6%)	
Hypoechoic	14 (51.9%)	63 (61.6%)	
Posterior echo pattern			0.103d
Absent	23 (85.2%)	83 (94.3%)	
Posterior acoustic reinforcement	0 (0%)	2 (2.3%)	
Back acoustic shadow	4 (14.8%)	3 (3.4%)	
Lymph Nodes			0.211d
Abnormal	4 (14.81%)	5 (5.68%)	
Normal	23 (85.19%)	83 (94.32%)	
Birads			< 0.001d
4	17 (62.96%)	87 (98.86%)	
5	10 (37.04%)	1 (1.14%)	

a: Student's t-test

b: Mann-Whitney test

c: Pearson chi-square test

d: Fisher's exact test

Table 3: Bivariate analysis of data related to flow Doppler characteristics of nodules in breast cancer patients and control group attended at Assis Chateaubriand Maternity School, Mastology Service (Fortaleza, Brazil) between September 2018 and August 2019.

Histopathology			
Variable	**Positive (n (%))**	**Negative (n (%))**	**p**
IV Vascularization			0.001c
Absent	1 (3.7%)	49 (55.7%)	
Present	26 (96.3%)	39 (44.3%)	
IV Peripheral Distribution			< 0.001d
Yes	24 (88.89%)	39 (44.32%)	
No	3 (11.11%)	49 (55.68%)	
IV Central Distribution			< 0.001d
Yes	15 (55.56%)	5 (5.68%)	
No	12 (44.44%)	83 (94.32%)	

IV Linear Morphology			0.769c
Yes	6 (22.22%)	22 (25.00%)	
No	21 (77.78%)	66 (75.00%)	
IV Punctiform Morphology			> 0.999d
Yes	3 (11.11%)	12 (13.64%)	
No	24 (88.89%)	76 (86.36%)	
IV Branching Morphology			0.053d
Yes	5 (18.52%)	5 (5.68%)	
No	22 (81.48%)	83 (94.32%)	
IV Penetrating Morphology			< 0.001c
Yes	17 (62.96%)	6 (6.82%)	
No	10 (37.04%)	82 (93.18%)	

a: Student's t-test

b: Mann-Whitney test

c: Pearson chi-square test

d: Fisher's exact test

Table 4: Study of sensitivity, specificity, PPV, and NPV in ultrasound and Doppler flow assessment of breast nodules for the histological diagnosis of breast cancer.

Variable	Sensibility	Specificity	PPV	NPV	p
Distribution (present / absent)	96%	56%	40%	98%	0.001
Peripheral distribution	88%	55%	38%	94%	< 0.001
Central distribution	55%	94%	75%	87%	< 0.001
Penetrating morphology	62%	93%	73%	89%	< 0.001

In the multivariate analysis, with the variables of the type of internal vascularization distribution, the presence of central distribution had an odds ratio (OR) of 13.58 (3.96 - 46.51) ($p < 0.001$). For the presence of peripheral distribution vascularization, the OR was 5.96 (1.53 - 23.24) (p : 0.01). In the multivariate analysis of the nodule internal vessel morphology variables, the penetrating type showed a significant association with positive histopathological diagnosis (OR : 23.19 [7.07 - 75.99], $p < 0.001$) (Table 5).

Table 5: Analyzes the logistic regression of the nodule vascular distribution in relation to the positive histopathological diagnosis and evaluation of Internal Vascularization morphology of breast nodules among patients diagnosed with breast cancer and control group.

Independent Variables: IV-Distribution	OR (IC95%)	P
Peripheral	5.96 (1.53 - 23.24))	0.01
Central	13.58 (3.96 - 46.51)	< 0.001
Independent Variables: IV-Morphology	**OR (IC95%)**	**P**
Branching	1.85 (0.31 - 10.99))	0.495
Penetrating	23.19 (7.07 - 75.99)	< 0.001
Point-shaped	1.66 (0.34 - 8.12)	0.529

Discussion

Breast cancer is the cancer that most affects women worldwide, in both developing and developed countries, and despite significant advances in research, it remains a significant health problem [1-3]. Early detection remains the cornerstone of breast cancer control [11]. Ultrasound is of great importance in the diagnosis of breast diseases and is considered a useful complementary tool to mammography in breast cancer screening [12-14]. In this study the age, the use of hormone therapy and family and personal history of breast cancer in both groups showed no difference [15-17].

The statistical analysis of the median size of the nodules showed a significant association with the positive histopathological diagnosis [18,19]. The shape of the nodule was significantly associated with the positive histopathological result ($p < 0.001$). This finding corroborates that of another study [20,21]. Characteristics of lesion margins were significantly associated with positive histopathological findings ($p < 0.001$). Spiculated and microlobulated margins were more associated with malignancy (40.7% and 44.4%, respectively), in agreement with other studies [20,21].

Color Doppler is widely used for the evaluation of vascularity in

breast lesions 7, but to date, there is no standardization of criteria in Doppler evaluation for differentiating malignancy and benignity 22. Regarding the presence or absence of internal vascularization in the nodules, there was a significant association with a diagnosis of malignancy ($p = 0.012$). Of the 27 malignant nodules, 61% showed internal vascularization. The study showed high sensitivity (96%) and low specificity (56%). The positive predictive value of 40% and the negative predictive value of 98% could be explained by the low prevalence of false-positive cases shown in the study. In another study (7), 132 breast lesions analyzed with Doppler were retrospectively studied. Of the 58 malignant lesions, only three did not show internal vascularization compared to the same technique used in the present study. In a study by Yongfeng 13, a semi-quantitative evaluation of the flow inside the nodule was performed, showing that the flow signal was observed in 38 of 41 malignant lesions (92.6%) 23. Observed the presence of vascularization in 95% of cancers and 46% of benign lesions, in agreement with the present study. Vessel distribution within the nodules was significantly associated with the histopathological diagnosis of malignancy ($p < 0.001$). In the present study, of the 27 malignant nodules, 88.89% had peripheral distribution; and 55.56% presented central distribution.

In this study, we showed that the presence of peripheral distribution increases the chance of positive histopathological diagnosis 5.96 times. The central distribution increases the likelihood of positive histopathological diagnosis 13.58 times. This finding is in agreement with Yongfeng's 13 study, which showed more microvessels in the center of the malignant lesions, with sensitivity, specificity, PPV, and NPV of 85.4%, 92.6%, 83.3%, and 93.5% respectively 22. Showed central and peripheral vascular distribution in malignant lesions.

Regarding the morphology variable, we observed in this study that in positive cases for malignancy, 62.96% presented the penetrating morphology, also showing high specificity and negative predictive value (93% and 89% respectively). In the multivariate analysis of the morphology variables, the internal flow of the nodules, the penetrating type was significant for positive histopathological diagnosis ($p < 0.001$), where the penetrating morphology of the vessel corresponds to a 23. 19-fold chance of a positive result than nodules without vessels of penetrating morphology. The morphology of the vessels of solid breast lesions seen using power Doppler was first described by Raza and Baum [20]. They showed that the sensitivity, specificity, positive predictive value, and negative predictive value of penetrating vessels in predicting malignancy were respectively 68%, 95%, 85%, and 88%, in agreement with our study.

Our study was also in agreement with an investigation by [20], which showed that there was a significant correlation between the presence of penetrating vessels and malignancy ($p < 0.05$). The sensitivity, specificity, positive predictive value, and negative predictive value of the existence of penetrating vessels in predicting malignancy were respectively 76.5%, 80%, 76.5%, and 80.0% in the study described above. In our research, we have better specificity and a positive predictive value. The study by [21] showed that penetrating vessels were observed in 37 of 41 malignant lesions, with a sensitivity for the diagnosis of malignancy of 90.2% and a specificity of 62.8%. In another study 23, in 21 cases of breast cancer, 16 tumors had penetrating vessels (76.2%) [21]. Found that the morphology of malignant vessels, including penetrating and branching vessels, showed higher specificity when compared to distribution (86.9% and 44.8%).

Regarding the analysis of the spectral curve, we observed that lesions with positive histopathological results for malignancy presented higher Resistance Index (RI), Pulsatility Index (PI), and Systolic Peak (SP) ($p < 0.0001$). In this variable, our results agree with [12,14]. There are some limitations to our study. Ultrasonography is a total operator-dependent method. We assessed microvessels that often increase slightly with the pressure made with the probe on the lesion which could lead to occlusion leading to false avascularity. Furthermore, discrete respiratory movements performed by the patient could make it difficult to evaluate the flow indices.

The results demonstrate it is possible to identify ultrasound characteristics combined with color Doppler in histopathologically proven cases of breast cancer, such as the presence of peripheral flow, central flow, the presence of penetrating vessels and high resistance indices, and pulsatility indices. This may represent additional information to be taken into consideration in the selection of lesions indicated for histopathological study.

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