



Breast cancer molecular subtypes based on MRI diagnosis and its impact on surgical plan: A Pilot Study

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Abstract. Background: Breast cancer (BC) is the most common cancer affecting women worldwide. In 2022, about 2.3 million new cases and 670,000 deaths were reported globally according to World Health Organization, making it a major public health problem. BC is divided into four molecular subtypes according to the way certain genes express themselves, including hormone receptors (estrogen and progesterone receptors), HER2 expression and proliferation index. Dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) has shown crucial role in identifying the characteristic morphological pattern of the different molecular subtypes. Aim of the study was to assess the impact of MRI on the choice of optimal surgical plan for different molecular subtypes of breast cancer. **Methods:** This prospective study included forty patients diagnosed with breast cancer who underwent immunohistochemistry followed by contrast enhanced MRI. They were all candidates for upfront surgery. **Results:** The study was done at time interval between June 2022-October 2023. The mean age of selected patients was 51.6 ± 9.8 years. The most dominant molecular subtype was luminal (26/40) followed by HER2 (10/40) and triple negative (4/10). Luminal subtype was strongly associated with spiculated margins, while HER2/neu tumors and TNBC showed circumscribed and irregular margins respectively ($p=0.0077$). Heterogeneous enhancement was dominant in luminal and HER2/neu cancers, while TNBC showed rim enhancement ($p=0.0059$). Regarding management, luminal subtypes were mostly managed by breast conservative surgery, while HER2/neu and triple negative were managed by mastectomy. A significant association was found between the molecular subtype and the surgical plan ($p < 0.013$). **Conclusion:** MRI morphological pattern of different molecular subtypes can be an acceptable predictor for the optimal surgical plan for each type.

Keywords: Breast Cancer, Luminal, MRI, Multifocal, Multicentric.



Introduction

Breast cancer (BC) is the most common cancer affecting women worldwide. In 2022, about 2.3 million new cases and 670,000 deaths were reported globally according to World Health Organization, making it a major public health problem (1).

BC includes a heterogeneous group of diseases with distinct phenotypes. It is divided into a number of biological subgroups, each of which exhibits unique behaviors and therapeutic responses. They are categorized according to the way certain genes express themselves through hormone receptors (estrogen and progesterone receptors), human epidermal growth factor 2 (HER2) expression and proliferation index (Ki67). These subtypes include luminal cancers, HER2 enriched and triple negative breast cancer (TNBC) (2, 3).

Dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) has shown vital role in identifying the characteristic morphological pattern of the different molecular subtypes in various studies and thus serves as an optimal preoperative imaging modality to guide the appropriate surgical plan (4).

Historically, mastectomy was the primary surgical procedure performed on patients with multifocal and multicentric breast cancer. While unifocal cancer traditionally was managed by conservative surgery. However, decades of research showed that surgical management of breast cancer patients depend, not only on tumor focality, but on many other factors. Furthermore, nowadays conservative surgeries are prioritized in appropriate settings for the sake of cosmesis and post operative well-being (5, 6).

Aim of the study was to assess the diagnostic impact of MRI on the choice of optimal surgical plan of breast cancer patients based upon the tumor molecular subtype.

Methods

Patient population

This prospective pilot study was conducted upon forty patients presented to MRI unit of our University Hospital at time interval between June 2022- October 2023. All patients underwent conventional imaging and core biopsy followed by histopathological analysis and immunohistochemistry. All patients were managed by upfront surgery.

Written informed consent was taken from the patients to perform MRI. The study was approved by ethics committee of our university hospital.

Inclusion criteria encompassed: a- patients diagnosed with breast cancer including unifocal and multifocal/multicentric cancer with full histopathological data including immunostaining. b- All patients eligible for upfront surgery

Exclusion criteria included: a- patients with contraindications to MRI. b- patients eligible for



chemotherapy regimen. c- patients with missing histopathological data or those who didn't perform immunostaining.

Magnetic resonant mammography (MRM)

- a- MRI breasts:** done on 3 Tesla MRI machine (Ingenia Philips, Netherland).
- b- MRI protocols:** Routine sequences of MRI breasts and their parameters were done including:
 - i- T2-weighted fat-saturated sequence (STIR) (TR 5000, TE 60, FOV 270-340, Matrix 320x314, slice thickness 2mm, gap 0.8mm).
 - ii- T1-weighted non-fat-saturated sequence (TR shortest, TE 4.7, FOV 270-340, Matrix 448x323, slice thickness 1mm, gap 0.8mm).
 - iii- Echo-planar DWI before contrast injection with b values 0, 400, and 800 and computer-generated ADC map (TR shortest, TE 95, FOV 270-340, Matrix 192x192, slice thickness 4mm, gap 2mm).
 - iv- 3D T1-weighted fat suppression gradient echo sequence performed before and after intravenous injection of gadolinium chelate (0.2 mmol /kg at a rate of 3–4mL/s), using dynamic T1 high-resolution isotropic volumetric examination (THRIVE) sequence (TR shortest, TE shortest, FOV 270-340, Matrix 336x448, slice thickness 1mm, gap 0.2mm).

The whole study duration: 30-35 minutes.

Image post-processing

Imaging post processing was performed on an MR workstation by two qualified consultant radiologists, with 17 and 12 years of experience each. They examined and interpreted the MR images independently. They were both blinded from final histopathological findings. After evaluation, discussion was done and minor differences in final results were resolved by consensus.

Image post-processing done initially by image subtraction where pre-contrast images were subtracted from each post-contrast image. Maximum intensity projection (MIP) views were acquired generating sagittal, coronal, and axial projections. Morphological analysis was done for detected lesions using MRI BIRADS lexicon descriptors 5th edition, 2013 (7). Kinetic analysis was done by generating time intensity dynamic curves.

Statistical analysis

Data were collected and entered into the Statistical Package for Social Science (Rstudio) version 2.3.2. Qualitative data were displayed as numbers and percentages. Quantitative data with parametric distribution were assessed using mean, standard deviations, and ranges and with non-parametric distribution using median with inter-quartile range (IQR). Shapiro test was



used to examine normality of quantitative data. Fisher exact test and Anova test were used to test for statistical significance. The confidence interval was set to 95% and the margin of error accepted was set to 5%. P-value was considered significant if $p < 0.05$ and highly significant if $p < 0.01$.

Results

The age range of the patients was 34-58 years with a mean of 51.6 ± 9.8 years. The most dominant molecular subtype was luminal 26/40 (65%) followed by HER2/neu 10/40 (25%) and triple negative 4/10 (4%).

As regards demographic and clinical variables, no significant statistical significance was found between molecular subtypes and patients' age ($p = 0.16$), tumor laterality ($p = 0.44$) or family history of breast cancer ($p = 0.89$) table (1).

As regards histopathological criteria, histologic grading did not differ significantly among subtypes ($p = 0.58$). Most of luminal subtypes showed grade II, in 16/26 (61.5%). HER2/neu showed 60% grade II and 40% grade III with no reported low-grade cases. TNBC showed an even distribution among grade II and grade III.

In histopathological types, invasive ductal carcinoma (IDC) predominated across all subtypes but was more exclusively represented in TNBC (100%) and HER2/neu (90%) groups. Invasive lobular carcinoma (ILC) was only seen in luminal subtype. Likewise, IDC with ductal carcinoma in situ component (DCIS) were exclusively seen in luminal subtypes 5/26 (19.2%). A statistically significant association was observed between molecular subtypes and histopathological types ($p = 0.024$) table (1).

DCE-MRI analysis revealed mass findings in 35/40 patients ($n=35$), while five patients presented with only non-mass enhancement (NME), these included: one case with focal NME (luminal) and four patients presented with segmental NME (1 luminal and 3 HER2/neu) with no other mass findings. Spiculated margins were exclusively observed in luminal subtype, while circumscribed margins appeared more commonly in HER2-positive tumors. TNBC only showed irregular margins. These findings were statistically significant with ($p=0.0077$). Heterogeneous enhancement predominated in luminal, figure (1) and HER2/neu, figure (2). While TNBC exclusively showed rim enhancement, figure (3). Internal enhancement pattern showed statistically significant association with molecular subtypes ($p=0.0059$).



Table 1- Demographic, clinical and histopathological criteria of different molecular subtypes

	Luminal (n=26)	HER2/neu (n=10)	TNBC (n=4)	p-value
Age				
- Min-max	34-73	43-70	52-58	0.16 ^A
- Mean \pm SD	49 \pm 10	56 \pm 8	55 \pm 3	
- Median	47 (42, 56)	54 (54,54)	55 (52, 58)	
Laterality				
- Right	11	6	3	0.44 ^f
- Left	15	4	1	
Family history				
- Positive	11	4	1	0.89 ^f
- Negative	15	6	3	
Histologic grading				
- Grade I	4 (15.4%)	0	0	0.58 ^f
- Grade II	16 (61.5%)	6 (60%)	2 (50%)	
- Grade III	6 (23.1%)	4 (40%)	2 (50%)	
Histological type				
- IDC	12 (46.2%)	9 (90%)	4 (100%)	0.024^{*f}
- ILC	9 (34.6%)	0	0	
- IDC+DCIS	5 (19.2%)	0	0	
- DCIS	0	1 (10%)	0	

*: statistically significant $p < 0.05$; F: fisher exact test; A: Anova test

As regards tumor multifocality, luminal cancers and HER2/neu showed predominantly multifocal disease figures (1,2), while TNBC presented more cases with multicentric disease, seen in 50%. No significant association was found between tumor focality and different molecular subtypes ($p=0.51$).

In terms of dynamic curves, type I curve was only observed in luminal subtypes, while type III was the dominant curve pattern, seen nearly even among all molecular subtypes ($p=0.42$). Furthermore, no significant associations were observed in diffusion weighted imaging activity (DWI) ($p=0.26$).

As regards non mass enhancement patterns, segmental NME was predominant in both luminal and HER2/neu cancers. TNBC wasn't expressed by any cases of NME. There was no significant association between molecular subtypes and NME ($p=0.32$).

Ductal extension and nipple invasion were predominant among HER2/neu group. Pathological nodes were observed the most in HER2/neu and TNBC subtypes with no significant association, table (2).

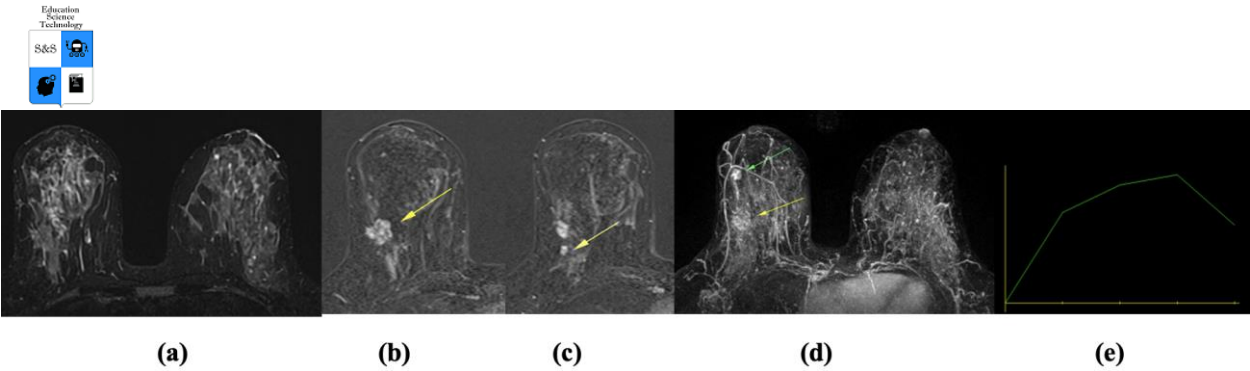


Table 2 - MRI features of molecular subtypes of breast cancer

	Luminal (n=26)	HER /neu (n=10)	TNBC (n=4)	P value
Unifocality	6 (23%)	1 (10%)	0	0.51 ^f
Multifocality	16 (61.5%)	6 (60%)	2 (50%)	
Multicentric	4 (15.4%)	3 (30%)	2 (50%)	
Masses (n=35)				0.0077*^f 0.0059*^f
⇒ Margins				
- Circumscribed	0	2 (20%)	0	
- Spiculated	11 (42%)	0	0	
- Irregular	13 (50%)	5 (50%)	4 (100%)	
⇒ Enhancement				
- Homogeneous	3 (11.5%)	0	0	
- Heterogeneous	18 (69%)	4 (40%)	0	
- Rim enhancement	3 (11.5%)	3 (30%)	4	
Dynamic curve				
- Type I	3 (11.5%)	0	0	0.42 ^f
- Type II	1 (3.8%)	2 (20%)	0	
- Type III	22 (84.6%)	8 (80%)	4 (100%)	
DWI				0.26 ^f
- Positive	15 (57.7%)	9 (90%)	3 (75%)	
- Negative	9 (34.6%)	1 (10%)	1 (25%)	
NME				0.32 ^f
- Present (n=12)				
○ Focal	1 (3.8%)	0	0	
○ Segmental	4 (15.3%)	3 (30%)	0	
○ linear	3 (11.5%)	1 (10%)	0	
- Absent (n=28)	18 (69.2%)	6 (60%)	4 (100%)	
Nipple invasion	3 (11.5%)	2 (20%)	0	0.77 ^f
Ductal extension	4 (15.4%)	4 (40%)	0	0.21 ^f
Pathological nodes	10 (38.5%)	5 (50%)	2 (50%)	0.79 ^f

*: statistically significant $p < 0.05$, F: fisher exact test

Figure (1): 42 years old lady presented with right lump. (a) Axial STIR showed upper outer intermediate signal mass with related distortion. (b,c) Axial post contrast subtraction showed index mass at 9 o'clock with a nearby posterior satellite (d) Axial MIP image showed third lesion anteriorly along 8 o'clock axis (green arrow). All lesions showed heterogeneous enhancement. (e) TIC showed type III curve. Patient went for BCS, pathology revealed: multifocal IDC grade II with foci of DCIS, luminal subtype.



Regarding the type of surgical procedure, breast conservative surgery (BCS) was most frequently performed in luminal subtype. On the other hand, HER2/neu and TNBC were almost exclusively managed by mastectomy. A significant association was identified between molecular subtype and surgical management ($p = 0.013$) Table 3.

Table 3 - Correlation between molecular subtypes and type of surgery

	Luminal (n=26)	HER2/neu (n=10)	TNBC (n=4)	p-value
BCS	14	1	0	0.013* ^f
Mastectomy	12	9	4	

*: statistically significant $p < 0.05$; F: fisher exact test

Figure (2): 70-year-old lady presented with right lump and nipple retraction. (a) Axial STIR showed retroareolar mass with related edema and nipple retraction. (b) DWI showed mild restriction with ADC of (1.0x10⁻³mm²/sec). (c) Axial subtracted DCE MIP images showed three irregular heterogeneous masses at 12 o'clock and retroareolar region. (d) TIC showed type III curve in all lesions. Patient did total mastectomy and revealed: multifocal HER2/neu IDC grade II.

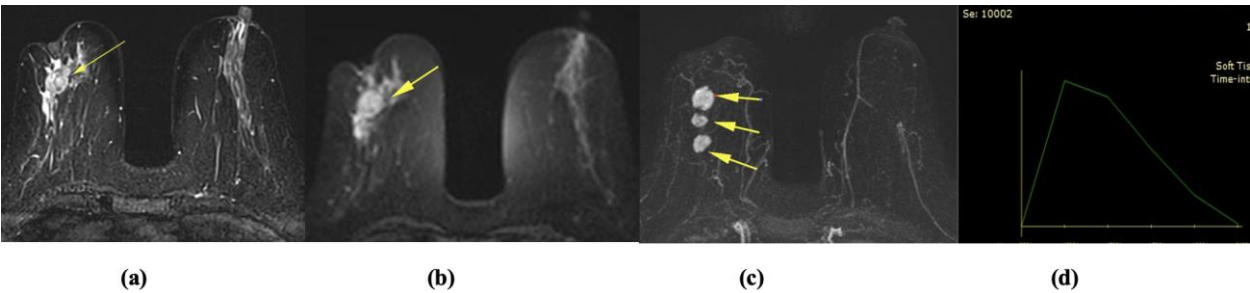
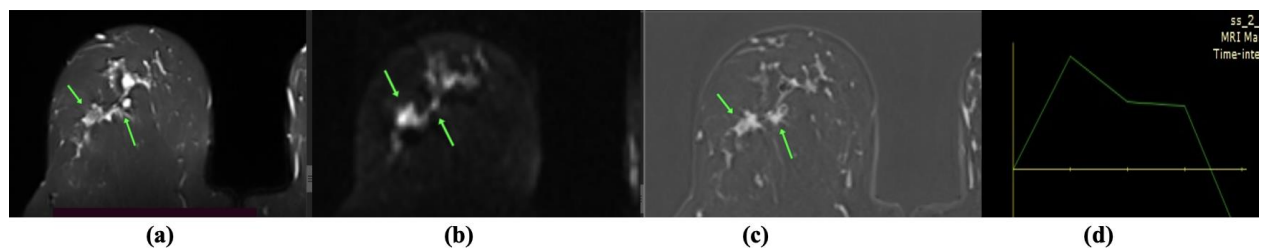




Figure (3): 58 years old lady presented with right lump. (a) Axial STIR showed right two upper outer and central masses. (b) DWI showed restriction with ADC value of $(0.8 \times 10^{-3} \text{mm}^2/\text{sec})$. (c) Axial DCE images show two irregular masses with enhancing rim at masses at 11 o'clock and 12 o'clock axis (green arrows). (d) TIC showed type III curve in both lesions. Patient did mastectomy and revealed: multifocal TNBC, IDC NOS grade II.



Discussion

Breast cancer is a polymorphic disease with various histological and phenotypical types. The use of pre-operative MRI has shown to aid in assessing different morphological patterns of breast cancer molecular subtypes. The National Comprehensive Cancer Network (NCCN) even recommended the use of DCE-MRI in preoperative assessment of breast cancer to define disease extent and identify candidates for BCS (8).

The aim of this study was to examine the association between breast cancer molecular subtypes with various clinical, histopathological, and imaging features, as well as with the type of surgical intervention.

Demographic and clinical data did not show statistically significant differences between subtypes. Specifically, there was no significant association between patients' age and molecular subtype ($p = 0.16$). These findings suggest that these demographic variables are evenly distributed across molecular classifications. Our results coincide with Mohamed et al. who didn't find significant age difference among different molecular subtypes ($p=0.472$) (9).

Regarding histological grading, high grade tumors were more prevalent in HER2/neu (60%) and TNBC (50%). While in luminal, intermediate grade were the most dominant. These findings coincide with Issar et al. (10) and Boisserie-Lacroix et al. (11) that also showed similar results to our study.

The current study showed statistically significant association between molecular subtypes and histopathological types ($p = 0.024$). While IDC predominated across all subtypes, ILC was exclusively seen in luminal cancers. This implied that certain histological patterns are more frequently associated with specific molecular types, reinforcing the biological heterogeneity of breast cancer. In line with our results, El-Rawy et al. (12) showed ILC in only luminal cancers. However, conversely, their results



showed no significant association ($p=0.625$) between histopathological data and different molecular subtypes.

Our results show prevalent multifocal and multicentric breast cancer in HER2/neu and TNBC, while luminal cancers predominantly showed multifocal disease. No significant association was found between tumor focality and different molecular subtypes ($p=0.51$). These findings are consistent with Bitencourt et al. (3) who also reported multiplicity in non-luminal cancers with no significant statistical association.

Morphologically, spiculated margins were observed exclusively in luminal cancers (42%). While irregular margins were predominant in HER2/neu (50%) and TNBC (100%). Circumscribed margins were reported only in HER2/neu (20%). Our results showed significant association between tumor margins with different molecular subtypes ($p=0.0077$). Our results align with Shokeir et al. (2) who showed spiculated margins in luminal cancers, however circumscribed margins were seen in TNBC ($p < 0.001$).

Regarding internal enhancement pattern, TNBC showed exclusive rim enhancement coinciding with few studies (13, 14). Luminal and HER2/neu cancers showed predominantly heterogeneous enhancement in 69% and 40% respectively. On the other hand, homogeneous enhancement was exclusively seen in luminal cancers. Significant association was found between molecular subtypes and internal enhancement pattern ($p=0.0059$). Shokeir et al. (2) and Vilar et al (15) showed also similar results with significant statistical association between molecular subtypes and tumor internal enhancement.

Luminal cancers showed type III curves in 84.6% followed by type I in only three cases (11.5%). Unlike Shokeir et al. (2) who showed type II and type III curves to be dominant in luminal cancers. Moreover, their results showed statistical significance ($p=0.0004$), unlike our results which yielded no significant association between dynamic curves and molecular subtypes.

However, in line with our results, Szep et al. (13) also showed predominant washout curves in high grade tumors as HER2/neu and TNBC.

Non mass enhancement was observed in 12 cases in our study with no significant statistical association ($p=0.32$). Segmental NME was mainly observed in HER2/neu in three patients (30%), as well as luminal cancers in four patients (15.3%). TNBC didn't show any cases with NME. El-Rawy et al. (12) also reported that 50% of HER2/neu patients showed NME, mainly of segmental distribution. Moreover, Vilar et al. [15] observed NME in HER2/neu more than TNBC which usually presented as mass lesions rather than non-mass ones. Dogma et al. (16)



found no significant association between molecular subtypes and NME, coinciding with our results.

Pathological axillary nodes were more frequent in HER2/neu (50%) and TNBC (50%) than in luminal subtypes (38.5%). Unlike our results, Ning Liu (17) reported that TNBC were more frequently node negative. This may be explained by small sample size of our TNBC cases and later stage of disease discovery. However, El-Rawy et al (12) showed node positive results in cases of HER2/neu and TNBC rather than luminal subtypes.

In the past two decades, the demand of mastectomy has risen due to the increased diagnosis of multiple ipsilateral breast cancer (MIBC), previously known as multifocal/multicentric breast cancer. However, in appropriate circumstances, BCS has been the preferred surgical choice for the sake of cosmesis, less hospital stay and for the well-being of the patient (6).

In the current study, many factors contributed to the choice of the final surgical plan, these included: patient's and surgeon's decision, the tumor grading, size and extension as well as molecular subtypes of the breast cancer.

The present study showed a significant statistical association between different molecular subtypes and the choice of surgical plan ($p=0.013$). Luminal cancers were mostly managed by BCS in 14/26 (53.8%), while mastectomy was the preferred surgical choice in HER2/neu (90%) and TNBC (100%). The seven unifocal cases were all managed by BCS.

It is worth mentioning that although luminal cancers showed multifocality and multicentricity in 76.9%, they were mostly managed by BCS. This was explained by their lesser aggressive morphological, histological and functional behaviors, when compared to HER2/neu and TNBC. Luminal cancer showed NME in 7/26 (26.9%) of cases, compared to HER2/neu which in turn showed NME in 40% of cases. Furthermore, nipple invasion (20%) and ductal extension (40%) were the most prevalent in HER2/neu cancers. On the other hand, all four cases of TNBC showed multiplicity which mandated mastectomy as the final surgical decision.

Our results concur with Verma et al. (18) who also supported that BCS was more feasible in luminal cancers, while mastectomy was the preferred choice in HER2/neu and TNBC.

Limitations in this study are exclusion of some comparable tumor features between different molecular subtypes, such as tumor size and T2 signal of the tumor.

We recommend further studies in the future with larger population and longer duration of follow up with more MRI tumor features to validate the results.

In conclusion, DCE-MRI is a valuable tool to assess the morphological pattern and tumor behavior of the various molecular subtypes of breast cancer. Thus, it can serve as an acceptable predictor of the optimal surgical plan tailored for each type.



List of abbreviations

BC	:	Breast cancer
BCS	:	Breast conservative surgery
DCE-MRI	:	Dynamic contrast enhancement magnetic resonance imaging
DWI	:	Diffusion-weighted imaging
HER 2	:	Human epidermal growth factor
MIBC	:	Multiple ipsilateral breast cancer
MIP	:	Minimal intensity projection
NME	:	Non-mass enhancement
TIC	:	Time intensity curve
TNBC	:	Triple-negative breast cancer

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Ethics approval and consent to participate:

This study was approved by the local Ethical Committee at faculty of medicine, Alexandria University Hospital in Egypt in 2021. Written consents were obtained from all patients. Reference number: 0201587, IRB number 00012098.

Conflict of interest: None

Author contribution:

Data collection and statistical analysis were done by BA. The study was designed by BA and HH. MRI images were interpreted by HH and EB. Manuscript was edited and revised by AAM, MH and MZ. All authors read and approved the final manuscript.

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