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Distribution of Mast cell in uterine smooth muscle tumour - An observational Study in tertiary care centre

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KEYWORDS

Uterine smooth muscle tumours; Mast cells; Leiomyoma; Leiomyosarcoma; Toluidine blue stain; WHO 2020 classification

ABSTRACT:

Background: Uterine smooth muscle tumours (USMTs) constitute a heterogeneous group of neoplasms ranging from benign leiomyomas to malignant leiomyosarcomas. Mast cells are important components of the tumour microenvironment and may influence tumour behaviour through their role in inflammation, angiogenesis, and tissue remodelling.

Objectives: To evaluate mast cell distribution in various histopathological types of uterine smooth muscle tumours and to correlate mast cell density with tumour type and functional classification as per the WHO 2020 criteria.

Materials and Methods: This retrospective and prospective cross-sectional study was conducted in the Department of Pathology, Integral Institute of Medical Sciences and Research, Lucknow, over a period of eighteen months. A total of 100 hysterectomy and myomectomy specimens diagnosed as uterine smooth muscle tumours were included. Routine histopathological evaluation was performed using hematoxylin and eosin–stained sections. Mast cells were demonstrated using 1% toluidine blue staining and counted in ten non-overlapping high-power fields. Tumours were classified according to the WHO 2020 criteria. Statistical analysis was carried out using ANOVA, Chi-square test, and Kruskal–Wallis test, with $p < 0.05$ considered significant.

Results: Leiomyoma was the most common diagnosis (85%). Mean mast cell count was significantly higher in benign tumours compared to malignant leiomyosarcomas ($p < 0.001$). Angioleiomyoma showed the highest mast cell density, while leiomyosarcoma demonstrated the lowest. A statistically significant inverse association was observed between mast cell density and tumour aggressiveness based on the WHO functional order.

Conclusion: Mast cell density shows significant variation across uterine smooth muscle tumours and is higher in benign lesions than in malignant tumours. Mast cell assessment may provide valuable insight into tumour biology and serve as a useful adjunct in the evaluation of uterine smooth muscle tumours.

INTRODUCTION

Uterine smooth muscle tumours (USMTs) constitute the most common mesenchymal neoplasms of the female genital tract and encompass a wide histopathological spectrum ranging from benign leiomyomas to highly aggressive leiomyosarcomas [1]. Leiomyomas affect a significant proportion of women of reproductive and perimenopausal age and are a major cause of morbidity due to abnormal uterine bleeding, pelvic pain, infertility, and pressure symptoms [2]. In contrast, leiomyosarcomas are rare but clinically significant because of their aggressive behaviour, high recurrence rate, and poor prognosis [3].

Accurate classification of USMTs is crucial, as management and prognosis differ markedly between benign, borderline, and malignant categories. The World

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Health Organization (WHO) classification of tumours of female genital organs emphasizes key histopathological parameters such as mitotic index, cytological atypia, and tumour cell necrosis for diagnostic stratification [4]. However, overlapping morphological features among certain variants—such as cellular leiomyoma, atypical (bizarre) leiomyoma, and mitotically active leiomyoma—pose diagnostic challenges, highlighting the need for adjunctive biological markers that may aid in understanding tumour behaviour [5].

Mast cells are bone marrow–derived immune cells that play an important role in inflammatory responses, angiogenesis, tissue remodelling, and tumour biology [6]. They exert their effects through the release of a wide array of mediators, including histamine, proteases, cytokines, and growth factors, which can influence tumour growth, vascularity, and stromal interactions [7]. Increasing evidence suggests that mast cells are actively involved in the tumour microenvironment of various benign and malignant neoplasms, where they may either promote or inhibit tumour progression depending on the tumour type and local milieu [8].

In gynaecological pathology, mast cell distribution has been studied in conditions such as endometrial carcinoma, cervical cancer, and ovarian tumours, with variable associations reported between mast cell density and tumour aggressiveness [9,10]. However, limited data are available regarding mast cell distribution in uterine smooth muscle tumours, particularly across different histopathological subtypes and functional categories defined by WHO criteria. Some studies have suggested a higher mast cell density in benign smooth muscle tumours compared to malignant counterparts, implying a possible inverse relationship between mast cell count and tumour aggressiveness [11].

Understanding the pattern of mast cell distribution in USMTs may provide insights into tumour biology, stromal–epithelial interactions, and mechanisms underlying benign versus malignant behaviour. Therefore, the present study was undertaken to evaluate mast cell density in various histopathological types of uterine smooth muscle tumours and to correlate mast cell counts with their cytological and functional classification as per the WHO 2020 guidelines.

MATERIALS AND METHODS

Place of Study

The present study was conducted in the **Department of Pathology, Integral Institute of Medical Sciences and Research (IIMSR), Lucknow, Uttar Pradesh, India**.

Study Duration

The study was carried out over a period of eighteen months, following approval from the Institutional Ethics Committee.

Study Design

This was a **retrospective and prospective cross-sectional study**.

Study Population

The study population comprised histopathological specimens of uterine smooth muscle tumours (USMTs) submitted to the Department of Pathology.

Inclusion Criteria

- Hysterectomy or myomectomy specimens with a histopathological diagnosis of smooth muscle tumour of the uterine corpus.

Exclusion Criteria

- Smooth muscle tumours of the uterine corpus diagnosed on curettage specimens, as limited tissue would not permit adequate evaluation of mast cell count.

Sample Size

Sample size was calculated using the formula:

$$n = \frac{z^2 \times (1 - p)}{d^2}$$

Where:

- n = sample size
- p = expected prevalence (40%)
- z = 1.96 at 95% confidence level
- d = margin of error (10%)
- Non-response rate = 10%

Based on the above calculation, the **final sample size was 100 cases**.

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Study Methodology

Histopathological Evaluation

Histopathological specimens were obtained from the archives and prospective submissions to the Department of Pathology, IIMSR, Lucknow. All specimens were fixed in 10% neutral buffered formalin. Routine tissue processing was performed using graded alcohols for dehydration, xylene for clearing, and paraffin wax for embedding. Paraffin blocks were prepared, and 3–5 µm thick sections were cut using a rotary microtome. Sections were routinely stained with hematoxylin and eosin (H&E). Ancillary immunohistochemistry was performed wherever required for diagnostic confirmation.

Microscopic evaluation was initially performed under low-power magnification (×4–×10) to assess overall architecture, tumour circumscription, growth pattern, cellularity, vascular invasion, tumour–host interface, and the presence of coagulative tumour cell necrosis. Detailed cytological assessment was carried out under high-power magnification (×20–×40) to evaluate nuclear atypia, cytoplasmic features, mitotic activity (mitotic count per 10 high-power fields), atypical mitoses, pleomorphism, and tumour cell necrosis. Based on these findings, the histopathological type of uterine smooth muscle tumour was determined.

Mast Cell Assessment

Representative tissue blocks from each confirmed case of uterine smooth muscle tumour were selected. Additional sections were subjected to mast cell staining using 1% toluidine blue, following standard histochemical protocols. Mast cells demonstrated characteristic metachromasia, with cytoplasmic granules staining purplish-red against a blue background, allowing clear identification.

Toluidine Blue Staining Procedure

- Formalin-fixed, paraffin-embedded tissue sections of 4–5 µm thickness were cut and mounted on clean glass slides.
- Deparaffinization was carried out using xylene in two changes, each for 5 minutes.
- Rehydration was performed through graded alcohols (95%, 80%, and 70%) for 2 minutes each.
- Sections were rinsed in distilled water.
- Slides were stained with 1% toluidine blue solution for 2–3 minutes.
- Gentle rinsing in distilled water was done to preserve metachromasia.
- Rapid dehydration was carried out through 70%, 95%, and absolute alcohol.
- Clearing was done using xylene in two changes for 2 minutes each.
- Slides were mounted with Dibutylphthalate Polystyrene Xylene (DPX) and coverslipped.

Microscopic Evaluation of Mast Cells

Microscopic assessment was performed using a light microscope. Initial scanning was done under low-power magnification (×10) to identify well-preserved and representative areas while avoiding necrosis, haemorrhage, or artefacts. Mast cell counting was subsequently performed under high-power magnification (×40). Mast cells were identified by their metachromatic granules and counted in ten non-overlapping high-power fields (HPFs). The total mast cell count per 10 HPFs was recorded for each case and used for further analysis.

Assignment of Functional Order of Tumours

All cases were assigned a functional order based on the WHO Classification of Tumours of Female Genital Tumours (2020) by two senior pathologists independently. The assignment was based on four parameters: mitotic count, cytological atypia, tumour cell necrosis, and WHO interpretation (benign or malignant). In cases of disagreement, a third pathologist was consulted to reach a consensus.

The histopathological types of uterine smooth muscle tumours were categorised as follows:

- **Leiomyoma**: <5 mitoses/10 HPF, absent or mild atypia, no tumor cell necrosis
- **Lipoleiomyoma**: <5 mitoses/10 HPF, absent atypia, no tumor cell necrosis

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- **Angioleiomyoma**: <5 mitoses/10 HPF, absent atypia, no tumor cell necrosis
- **Leiomyoma with myxoid change**: <5 mitoses/10 HPF, absent to mild atypia, no tumour cell necrosis
- **Cellular leiomyoma**: <5 mitoses/10 HPF, absent to mild atypia, no tumour cell necrosis
- **Mitotically active leiomyoma**: 5–15 mitoses/10 HPF, absent to mild atypia, no tumour cell necrosis
- **Atypical leiomyoma (bizarre nuclei)**: <10 mitoses/10 HPF, moderate to severe atypia, no tumour cell necrosis
- **Leiomyosarcoma**: ≥10 mitoses/10 HPF, moderate to severe atypia, presence of tumour cell necrosis

Ethical Considerations

The study protocol, data collection tools, and patient information documents were reviewed and approved by the Institutional Ethics Committee of Integral Institute of Medical Sciences & Research, Lucknow. All procedures were conducted in accordance with ethical standards, and modifications suggested by the committee were implemented.

Informed Consent

As the study involved analysis of archived and prospective histopathological specimens only, **direct patient consent was waived**, and approval for waiver of informed consent was obtained from the Institutional Ethics Committee.

Participant Confidentiality

Strict confidentiality was maintained throughout the study. Patients were assigned unique Patient Identification Numbers (PIDs), which were used for all records and data entry. All physical and electronic data were securely stored and accessible only to the researcher.

Data Collection Procedure

Data were collected using a semi-structured proforma, with one proforma per patient. The collected data were subsequently digitised using Microsoft Excel 2023.

Statistical Analysis

Statistical analysis was performed using IBM SPSS (latest version). Qualitative variables were expressed as frequency and percentage, while continuous variables were expressed as mean ± standard deviation.

- **ANOVA** was used to compare mast cell density across different histopathological types.
- **Chi-square test** was used to compare categorical variables.
- **Kruskal–Wallis test** was applied to assess the association between mast cell count and functional order of USMTs.

A **p-value <0.05** was considered statistically significant.

RESULTS AND OBSERVATIONS

The present study was undertaken to evaluate the distribution of mast cells and their potential role in uterine smooth muscle tumours (USMTs). A total of 100 histopathological specimens of USMTs were analysed, obtained from the histopathology laboratory.

The age distribution of the study population is summarised in Table 1.

Table 1: Age Profile of the Study Population (n = 100)

S. No.	Age Group (Years)	Number of Cases	Percentage (%)
1	≤30	4	4.0
2	31–40	35	35.0
3	41–50	38	38.0
4	51–60	18	18.0

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5	>60	5	5.0
—	**Total**	**100**	**100.0**

Mean age ± SD (Range): 44.72 ± 9.24 years (27–67)

Median age [Interquartile Range]: 44 [38–50] years

Table 2: Distribution of cases according to histopathological diagnosis

SN	Histopathological diagnosis	No. of cases (%)
1.	Leiomyoma	85
2.	Leiomyoma with bizarre nuclei	2
3.	Leiomyoma with mitotically active cells	1
4.	Leiomyoma with myxoid changes	1
5.	Leiomyosarcoma	4
6.	Lipoleiomyoma	3
7.	Angioleiomyoma	2
8.	Cellular leiomyoma	2

Table 3: Comparison of Mean Mast Cell count among different HPE Diagnosis

SN	Histopathological diagnosis	No. of cases (%)	Mean Mast cells/10 hpf±SD	Range (Min-Max)
1.	Leiomyoma	85	27.01±4.81	16-38
2.	Leiomyoma with bizarre nuclei	2	32.00±2.83	30-34
3.	Leiomyoma with mitotically active cells	1	43.00	43
4.	Leiomyoma with myxoid changes	1	12	12
5.	Leiomyosarcoma	4	9±1.41	7-10
6.	Lipoleiomyoma	3	44.33±5.77	44-45
7.	Angioleiomyoma	2	68.00±2.83	66-70
8.	Cellular leiomyoma	2	38.50±2.12	37-40

F=41.765; $p<0.001$ (ANOVA)

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Table 4: Distribution of different histopathological types of uterine smooth muscle tumours in different mast cell density categories

SN	Histopathological diagnosis	No. of cases (%)	Mast cell density/10 hpf				
			<10	11-20	21-30	31-40	40 or above
1.	Leiomyoma	85	0	4 (4.7%)	63 (74.1%)	18 (21.2%)	0
2.	Leiomyoma with bizarre nuclei	2	0	0	1 (50%)	1 (50%)	0
3.	Leiomyoma with mitotically active cells	1	0	0	0	0	1 (100%)
4.	Leiomyoma with myxoid changes	1	0	1 (100%)	0	0	0
5.	Leiomyosarcoma	4	4 (100%)	0	0	0	0
6.	Lipoleiomyoma	3	0	0	0	0	3 (100%)
7.	Angioleiomyoma	2	0	0	0	0	2 (100%)
8.	Cellular leiomyoma	2	0	0	0	2 (100%)	0

c2=227.56; p<0.001

Table 5: Mean mast cell count in accordance with order of cytological function status as per the WHO 2020 Classification of Uterine Smooth Muscle Tumours

Order	Tumor Type	WHO 2020 Cytological / Functional Status (Mitotic cell/10 hpf)	Mean Mast cell count±SD (/10 hpf)
1	Leiomyoma	Benign smooth muscle tumour with bland cytology and minimal mitotic activity	27.01±4.81
2	Lipoleiomyoma	Benign leiomyoma variant with adipocytic differentiation	44.33±5.77

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3	Angioleiomyoma	Benign smooth muscle tumor with prominent vascular component)	68.00±2.83
4	Leiomyoma with myxoid change	Benign tumor with myxoid stroma; requires exclusion of myxoid leiomyosarcoma	12
5	Cellular leiomyoma	Benign tumor with increased cellularity but low mitotic index	38.50±2.12
6	Leiomyoma with mitotically active cells	Benign tumor with increased mitoses without atypia or necrosis	43.00
7	Leiomyoma with bizarre nuclei (Atypical leiomyoma)	Marked nuclear atypia with low mitotic activity; benign behavior	32.00±2.83
8	Leiomyosarcoma	Malignant smooth muscle tumor with high mitotic rate, atypia, and tumor cell necrosis	9±1.41

H=35.71; p<0.001

Fig. 1: Mean mast cell count in accordance with order of cytological function status as per the WHO 2020 Classification of Uterine Smooth Muscle Tumours

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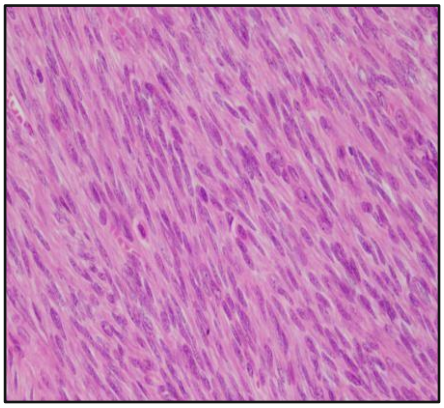
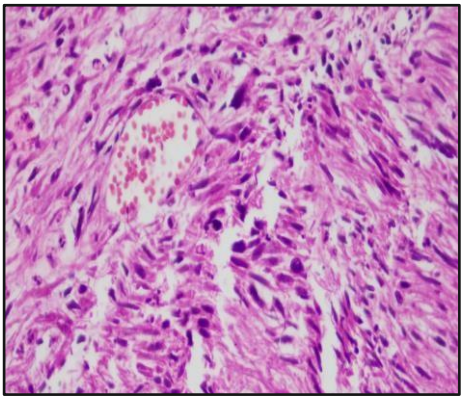


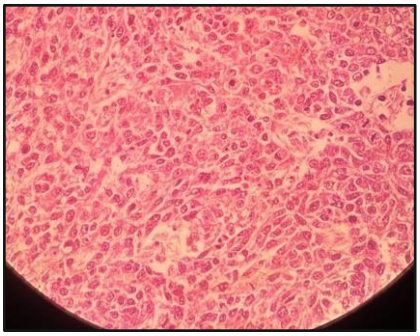
(A)



(B)

Figure A: H&E -stained section of Leiomyoma (40X)

Figure B: H&E -stained section of Atypical Leiomyoma (40X)



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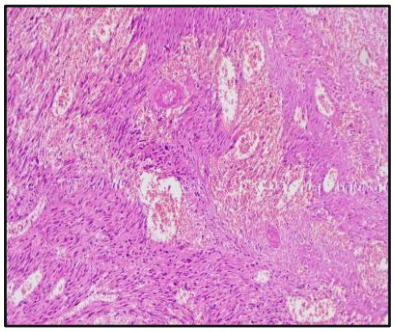

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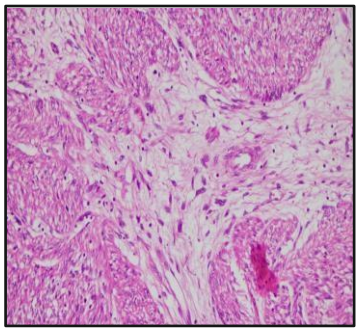
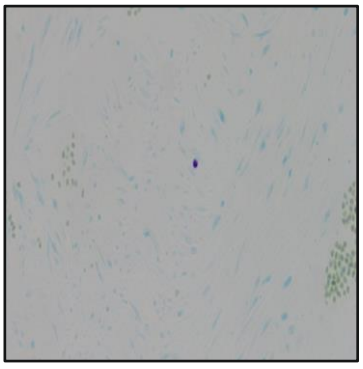


E

Figure C: H&E -stained section of Mitotically Active Leiomyoma (40X)

Figure D: H&E -stained section of Angioleiomyoma (10X)

Figure E: H&E -stained section of Myxoid Leiomyoma (40X)



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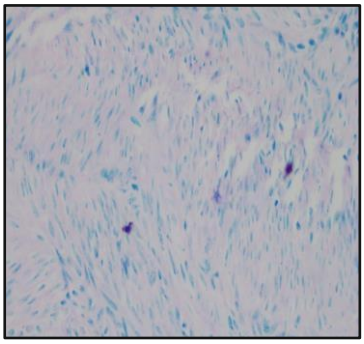

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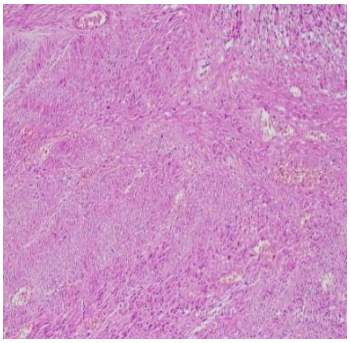

H

Figure F: H&E -stained section of Leiomyosarcoma with Necrosis (10X)

Figure G: Mast Cell Toludine Blue **Figure H: Toludine Blue Mast Cell Leiomyoma 40x**

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DISCUSSION

Uterine smooth muscle tumours (USMTs) represent a heterogeneous group of neoplasms with variable biological behaviour, ranging from benign leiomyomas to highly aggressive leiomyosarcomas. The present study evaluated mast cell distribution across different histopathological subtypes of USMTs and correlated mast cell density with tumour type and functional order as per WHO 2020 classification. The findings provide insight into the role of mast cells in the tumour microenvironment of uterine smooth muscle neoplasms.

In the current study, the majority of cases were leiomyomas (85%), which is consistent with the reported high prevalence of benign smooth muscle tumours in the uterus [12]. The peak incidence was observed in the fourth and fifth decades of life, in agreement with previous studies that report leiomyomas as most common during the reproductive and perimenopausal age groups [13]. Leiomyosarcomas constituted a small proportion of cases, reflecting their rarity among uterine neoplasms [14].

A key observation of this study was the significantly higher mast cell count in benign variants of USMTs compared to malignant leiomyosarcomas. Leiomyomas demonstrated a mean mast cell count of 27.01 ± 4.81 per 10 HPFs, whereas leiomyosarcomas showed markedly lower mast cell density (9 ± 1.41 per 10 HPFs). This difference was statistically significant, suggesting an inverse association between mast cell density and tumour aggressiveness. Similar observations have been reported in earlier studies, which noted reduced mast cell infiltration in malignant smooth muscle tumours when compared to benign counterparts [11,15].

Among the benign variants, angioleiomyoma exhibited the highest mast cell density, followed by lipoleiomyoma and mitotically active leiomyoma. The increased mast cell count in angioleiomyoma may be attributed to the prominent vascular component of this tumour, as mast cells are known to localise around blood vessels and promote angiogenesis through the release of pro-angiogenic mediators such as vascular endothelial growth factor (VEGF) and tryptase [16,17]. This finding supports the concept that mast cells play a role in vascular remodelling and stromal interaction in benign uterine smooth muscle tumours.

Leiomyomas with myxoid change showed a relatively low mast cell count in the present study. Myxoid stroma is characterised by abundant extracellular matrix and reduced cellularity, which may limit mast cell infiltration or retention. This observation is in line with previous reports suggesting variable mast cell distribution depending on stromal composition and tumour architecture [18]. Cellular leiomyomas and leiomyomas with bizarre nuclei demonstrated intermediate mast cell counts, reinforcing the notion that mast cell density does not merely correlate with cellular atypia but rather with overall tumour biology.

The functional order analysis based on WHO 2020 criteria further highlighted a progressive decline in mast cell density from benign to malignant tumours. The Kruskal–Wallis test demonstrated a statistically significant association between mast cell count and cytological/functional status of USMTs. These findings are concordant with studies in other organ systems, where mast cell density has been shown to decrease with increasing tumour grade and malignancy [19]. It has been postulated that mast cells may exert a protective or regulatory role in early or benign lesions, whereas malignant tumours may either inhibit mast cell recruitment or create a microenvironment unfavourable for mast cell survival [20].

The exact role of mast cells in tumour biology remains complex and context-dependent. While mast cells can promote tumour growth by enhancing angiogenesis and matrix degradation, they may also contribute to immune surveillance and tumour containment in certain settings [21]. In uterine smooth muscle tumours, the higher mast cell density observed in benign lesions and the marked reduction in leiomyosarcomas suggest that mast cells may be associated with benign behaviour and limited invasive potential.

The present study is limited by the relatively small number of malignant cases and certain rare variants, which may affect the generalizability of the results. Nevertheless, the statistically significant trends observed underscore the potential utility of mast cell assessment as an adjunct parameter in understanding the biological behaviour of uterine smooth muscle tumours.

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CONCLUSION;

The study demonstrates a clear variation in mast cell distribution across different histopathological types of USMTs, with significantly higher mast cell density in benign tumours compared to malignant leiomyosarcomas. These findings support the hypothesis that mast cells may play a role in modulating tumour behaviour and could serve as a useful indicator of tumour biology in uterine smooth muscle neoplasms.

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