



Pediatric Radiology

## Rhabdomyosarcoma disease spread evaluation on CT scans: Association with primary tumor size and Ki-67 proliferation marker

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## ABSTRACT

**Purpose:** Ki-67 is a marker of cellular proliferation that is commonly used for the assessment of rhabdomyosarcoma. The aim of this study was to investigate the associations between Ki-67 expression and primary tumor diameter with CT evidence of lymph node and solid organ metastatic spread in rhabdomyosarcoma.

**Materials and methods:** An institutional review board approval was granted for this study.

A retrospective search for rhabdomyosarcoma patients was conducted. Pathology reports were examined for Ki-67 expression. Chest-abdomen CT was assessed for radiological evidence of lymph node and metastatic spread. The maximal primary tumor diameter (termed *tumor size*) was also measured in different modalities CT, MRI, PET-CT and US. Ki-67 levels and primary tumor maximal diameters were compared to CT evidence of lymph node and organ metastatic spread.

**Results:** Twenty-four patients with rhabdomyosarcoma were included. CT evidence of lymph node spread was associated with Ki-67 levels (AUC = 0.896,  $p = 0.006$ ) and to a lesser extent with tumor size (AUC = 0.790,  $p = 0.030$ ). However, organ metastatic spread was associated only with tumor size (AUC = 0.854,  $p = 0.006$ ) and not with Ki-67 levels (AUC = 0.604,  $p = 0.469$ ). A combination of tumor size  $\geq 50$  mm and Ki-67 levels  $\geq 60\%$  was significantly associated with CT evidence of lymph node spread ( $p = 0.004$ ).

**Conclusion:** In conclusion, this study demonstrates radiological-pathological correlation in RMS. Lymph node spread detected by radiological images is associated with Ki-67 values. Lymph node and metastatic spread are associated with primary tumor size.

## 1. Introduction

Rhabdomyosarcoma (RMS) is the most common childhood soft tissue sarcoma and is responsible for 7% of all pediatric cancer and 40% of all soft tissue sarcomas [1]. Patient management depends on multi-variant factors including TNM staging, histologic subtype, and clinical group. The initial staging evaluation is mainly based on radiologic evaluation [2]. Several prognostic factors have been identified for unfavorable disease: primary tumor site, age younger than 1 year and older than 9 years, bone or bone marrow metastases and multiple metastases as well as multiple sites of metastases [3]. Although prognosis has improved in last two decades the 5-year overall survival is limited

to 70% [4,5].

Immunostaining of Ki-67 is used as a marker for cellular proliferation. It has been shown that Ki-67 expression strongly correlates with prognosis and clinical behavior of soft tissue sarcomas [6,7]. Nevertheless, its strength as a prognostic factor in RMS is still controversial [8–12], and previous studies have not evaluated the correlation of Ki-67 expression with imaging findings in RMS.

The purpose of this study was to investigate the associations between Ki-67 expression and primary tumor diameter with computed tomography (CT) evidence of either lymph node spread or solid organ metastatic spread in rhabdomyosarcoma.

**Abbreviations:** RMS, rhabdomyosarcoma; CT, computed tomography; ROC, receiver operating characteristic; AUC, area under the curves; PPV, positive predictive values; NPV, negative predictive values

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## 2. Materials and methods

Institutional review board (IRB) approval was granted to this retrospective study and informed consent was waived.

### 2.1. Study cohort

A retrospective computerized search in our institution's Radiology Information System (RIS) (Carestream Vue RIS) was conducted for RMS patients (12/2011–11/2016).

We included patients with pathology proven RMS who were treated in our hospital. We excluded patients without pre-treatment radiological evaluation, patients initially presented with a recurrent disease and patients with pathology reports that were conducted outside of our institution.

Clinical data was retrieved from patients' electronic medical records and included: patients' demographics, clinical presentation, primary disease site, histological subtype (embryonal, alveolar, and undifferentiated), Ki-67 values, treatment regimens and mortality.

### 2.2. Imaging evaluation

All patients underwent chest-abdomen staging CT, and in some cases MRI, F-18 FDG PET-CT and ultrasound.

#### 2.2.1. CT technique

Patients were scanned in our institution using: Philips Brilliance 256, Philips Brilliance 64 and GE Discovery 64. Patients received intravenous contrast agent (Iohexol 350 mg/ml up to 2 ml/kg) and oral contrast agent (Iohexol 350 mg/ml 52 ml diluted in 2 l water).

#### 2.2.2. MRI technique

MR scans were performed using 3 T MR system (HDX GE Medical Systems Milwaukee, WI, USA) and 1.5 T MR system (HDX GE Medical Systems) using a standard tumor protocol.

#### 2.2.3. PET-CT technique

Whole-body FDG-PET/CT scans were performed using a combined PET/CT scanner with a 16 detector row helical CT scanner (Philips Gemini GXL). Patients received an intravenous injection of 370 MBq F-18 FDG after 4–6 h of fasting.

#### 2.2.4. Ultrasound technique

Patients were examined on a Philips iU22 (Phillips Medical Systems, Bothell, WA, USA) using a linear probe, L 12-5 (5–12 MHz), and convex probe, C5-1 (1–5 MHz), as well as on a Siemens s2000 (Siemens, Erlangen, Germany) using linear probe, L 9-4 (4–9 MHz), and a convex probe, C6-1 (1–6 MHz).

Examinations were analyzed using our institution's [picture archiving and communication system](#) (PACS) (Carestream Vue PACS). A board certified radiologist (EK, with seven years of experience) assessed the examinations for the following imaging features: location of involved disease, CT evidence of lymph node spread and organ metastatic spread, tumor heterogeneity (homogenous vs. heterogeneous appearance) and measurement of the maximal diameter of the primary lesion (termed tumor size). Primary tumor diameters were measured by identification of the longest diameter of the target lesions in the axial plane in cross sectional examinations (in the staging chest-abdomen CT, the CT examination of the staging PET-CT or MRI for patients with head and neck tumors) or by the assessment of the long axis in ultrasound of patients with paratesticular lesions, according to the RECIST criteria [13]. In accordance to the literature, an upper limit of 10 mm in the short axis was used to define normal lymph nodes [14,15]. In cases of head and neck tumors, MRI examinations were also assessed for lymph node spread. Primary tumor heterogeneity was assessed in patients who underwent contrast-enhanced CT scan.

### 2.3. Statistical analysis

Analysis was performed using IBM SPSS statistics (Version 20.0) (Armonk, NY, USA). Descriptive statistics were presented as means  $\pm$  standard deviations for continuous variables and percentages for categorical variables. A two-tailed p-value  $< 0.05$  was considered statistically significant.

The correlation between Ki-67 expression and tumor size was evaluated (Pearson's correlation coefficient). The associations between Ki-67, tumor size, CT evidence of disease spread and tumor heterogeneity were evaluated (Mann Whitney *U* test, Fisher's exact test). The abilities of Ki-67 and tumor size to identify CT evidence of disease spread were assessed using receiver operating characteristic (ROC) curve analysis and area under the curves (AUC) were calculated. ROC curve analysis was used to find cut-off levels of tumor size and Ki-67 that are suggestive of CT evidence of lymph node spread. The association between the combination of tumor size and Ki-67 cut-off values with lymph node spread detected by CT was evaluated (Fisher's exact test), and sensitivity, specificity, and positive and negative predictive values (PPV and NPV) for CT evidence of lymph node spread were calculated.

## 3. Results

Records of 36 consecutive RMS patients who were treated in our hospital during the study period were retrieved. Twelve patients were excluded due to: absence of pre-treatment radiological imaging (six patients), initial presentation with a recurrent disease (four patients) and pathology reports that were conducted outside of our institution (two patients). Ki-67 values were available for 18 patients. Measurements of the maximal diameter of the primary lesion were feasible in 23 patients, as one patient underwent surgical intervention for his primary lesion prior to imaging. Evaluation of tumor heterogeneity was available in 17 patients.

The demographic and clinical data of the study cohort are presented in [Table 1](#). Lymph node spread was evident in seven patients and involved the retroperitoneal ( $n = 2$ ), inguinal ( $n = 1$ ), pelvic ( $n = 1$ ), supradiaphragmatic ( $n = 1$ ), cervical ( $n = 1$ ) and supraclavicular ( $n = 1$ ) lymph nodes. Solid organ metastasis was evident in eight patients and involved the lungs ( $n = 4$ ), vertebra ( $n = 2$ ), omentum ( $n = 2$ ), liver ( $n = 1$ ) and bone marrow ( $n = 1$ ); in two patients more than one distant organ was involved.

[Fig. 1](#) illustrates a CT scan of a 19 year old woman with a large 9.5 cm right ischiorectal mass and right inguinal lymphadenopathy.

**Table 1**  
Demographic and clinical data of the study cohort.

Characteristic	Findings
Median age, years (range)	9 (0–36)
Gender	
Male	16
Female	8
Disease site	
Favorable	14
Unfavorable	10
Histopathologic type	
Embryonal	18
Alveolar	6
Lymph node metastasis	7
Organ metastasis	8
Mortality	4
Median size, mm (23 patients)	53
$\leq 50$ mm	11
$\geq 50$ mm	12
Median Ki-67 values (%) (18 patients)	67.5
$\leq 60\%$	8
$\geq 60\%$	10

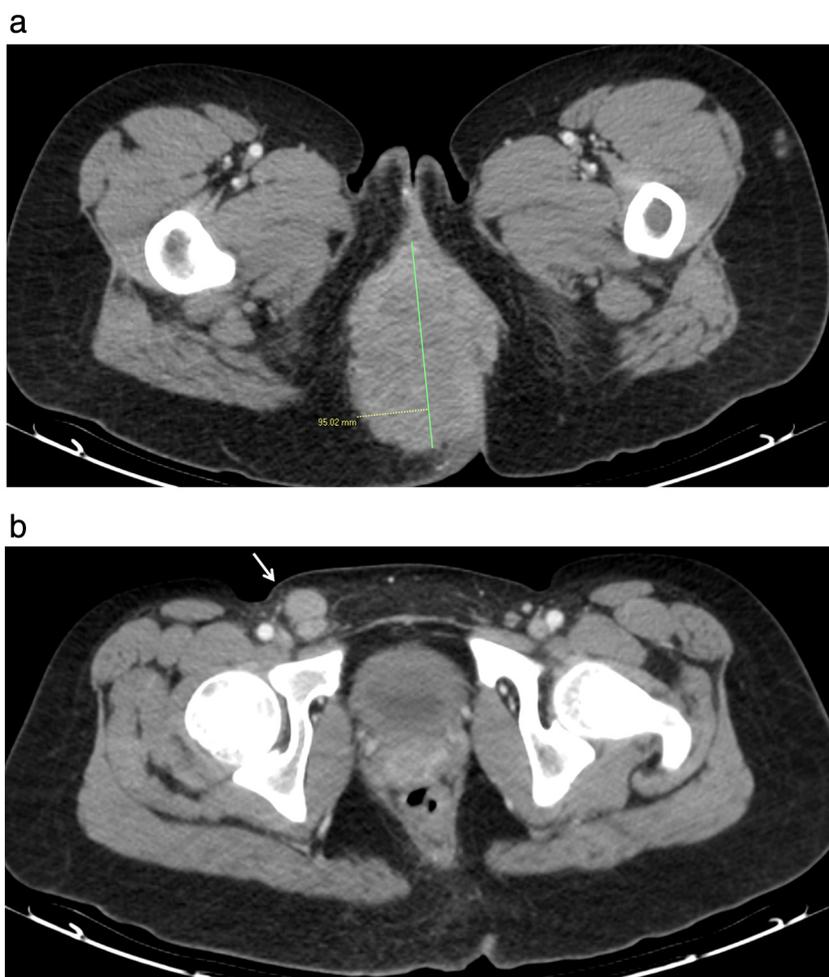


Fig. 1. A – a 19 year old woman with a large 9.5 cm right ischioanal mass. Ki-67 value was 90%. B – Same patient as in A. Right inguinal lymphadenopathy (arrow).

Table 2

Associations of Ki-67 expression and of tumor size with investigated imaging features. Significant associations ( $p < 0.05$ ) are bolded. (Abbreviation: vs. = versus).

	Ki-67 (%)	Tumor size (mm)
Tumor size (mm)	$r = 0.353$ $p = 0.091$	–
Lymph node spread	<b><math>50.0 \pm 20.5</math> vs.</b> <b><math>78.6 \pm 8.5</math></b> <b><math>p = 0.004</math></b>	<b><math>54.7 \pm 32.5</math> vs.</b> <b><math>96.1 \pm 38.7</math></b> <b><math>p = 0.027</math></b>
Organ metastatic spread	$58.2 \pm 21.0$ vs. $65.7 \pm 24.0$ $p = 0.479$	<b><math>51.2 \pm 30.6</math> vs.</b> <b><math>97.5 \pm 35.6</math></b> <b><math>p = 0.004</math></b>

The associations between tumor size, Ki-67 and CT evidence of disease spread are presented in Table 2. Tumor size was not significantly correlated with Ki-67 ( $p = 0.091$ ). Tumor heterogeneity was associated only with tumor size ( $p = 0.005$ ). While tumor size was associated with both lymph node spread and organ metastatic spread ( $p = 0.027$  and  $p = 0.004$ , respectively), Ki-67 levels were only associated with lymph node spread ( $p = 0.004$ ).

ROC curve analysis showed that Ki-67 values had a higher diagnostic accuracy for prediction of CT evidence of lymph node spread ( $AUC = 0.896$ ,  $p = 0.006$ ) in comparison to tumor size ( $AUC = 0.790$ ,  $p = 0.030$ ); however, in regards to the prediction of organ metastatic spread, tumor size ( $AUC = 0.854$ ,  $p = 0.006$ ) was superior to Ki-67 values ( $AUC = 0.604$ ,  $p = 0.469$ ). Figs. 2 and 3 present the ROC curves of Ki-67 values and tumor size diagnostic accuracy for lymph node and

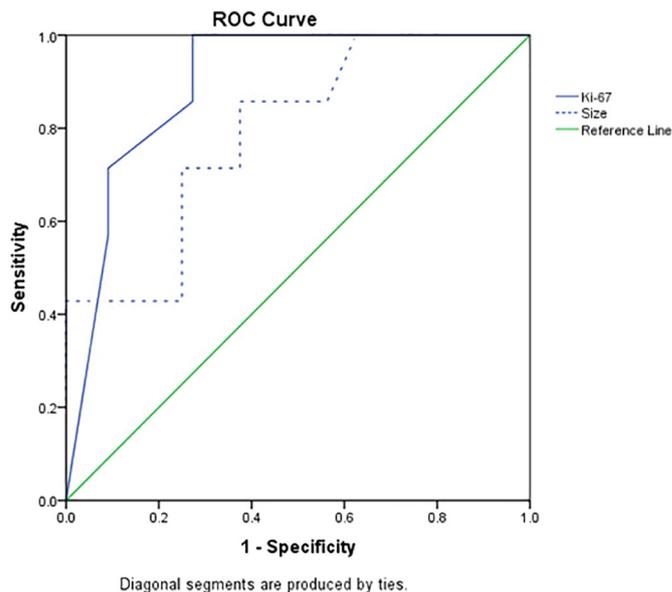


Fig. 2. ROC curve for the diagnostic accuracy of Ki-67 values and tumor size to lymph node spread identification.

organ metastatic spreads identification, respectively. An analysis of ROC curves showed that a Ki-67 value of 60% had a sensitivity of 100% and specificity of 72.7% to predict lymph node spread. Tumor size of

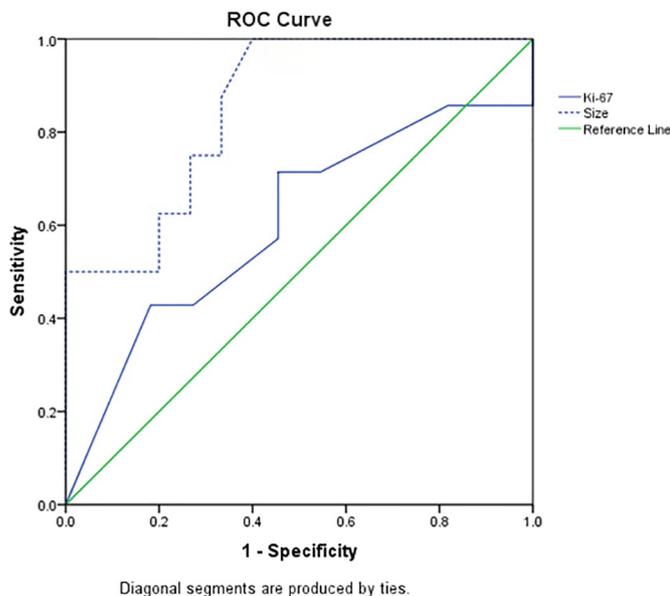


Fig. 3. ROC curve for the diagnostic accuracy of Ki-67 values and tumor size to organ metastatic spread identification.

52.5 mm had a sensitivity of 85.7% and specificity of 62.5% for lymph node spread. Tumor size of 52.5 mm had a sensitivity of 87.5% and specificity of 67.7% for organ metastatic spread.

The combination of Ki-67  $\geq$  60% and tumor size  $\geq$  50 mm was associated with lymph node spread ( $p = 0.004$ ), Fig. 4. Six of seven patients (85.7%) with lymph node spread exhibited this combination, while only 1/10 (10.0%) of the patients without this combination exhibited lymph node spread. Thus, this combination showed sensitivity, specificity, PPV and NPV of 85.7%, 90.0%, 85.7%, 90.0%, respectively, for lymph node spread.

Fig. 5 presents one patient with Ki-67 of 50% and tumor diameter

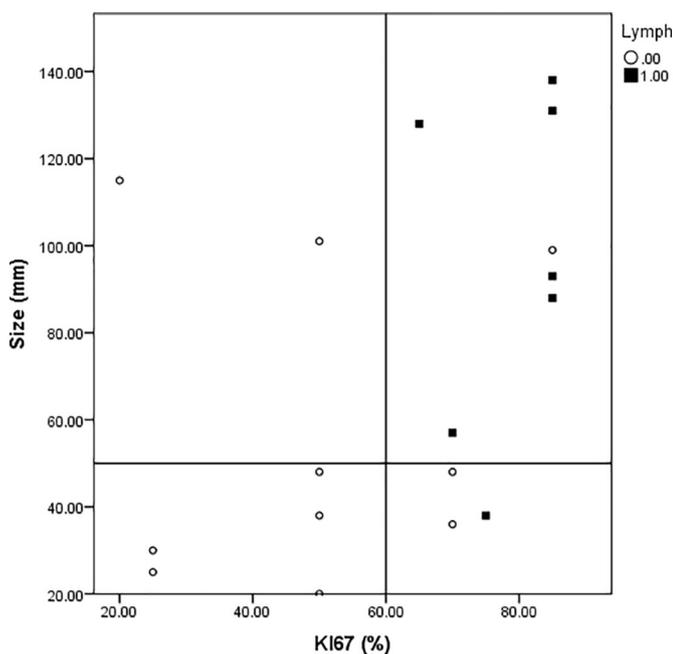


Fig. 4. Distribution of Ki-67 values and tumor size (mm) in the entire study cohort. Patients with lymph node spread are depicted with rectangles; patients without lymph node spread are marked with circles. Notice that the majority (6/7) of patients with the combination of Ki-67  $\geq$  60% and tumor size  $\geq$  50 mm exhibit lymph node spread.

size of 2 cm and another patient with Ki-67 of 90% and tumor diameter size of 12.4 cm.

#### 4. Discussion

In this study we aimed to elucidate the relationship between radiologic features and pathology findings in RMS. Our results demonstrate a strong correlation between radiologically evident lymph node spread with Ki-67 as well as radiologically evident lymph node and metastatic spread with tumor size.

Ki-67 protein is a marker for cellular proliferation and an integral part of the pathology panel staining in RMS diagnosis. Although physicians attribute importance to this marker, its role as a predictor of clinical behavior and prognosis is not clear. Moreover, no previous study has examined the radiological-pathological correlation of Ki-67 in RMS. Few studies evaluated Ki-67 prognostic value in RMS, but the results were contradictory. In a study by Miguel-Fraile et al., high Ki-67 values were an indicator of a worse disease [12]. In contrast, in a report by Staibano et al., high Ki-67 values were associated with relapse-free survival, suggesting Ki-67 index is a predictor of favorable clinical outcomes [8]. In a research by Noguchi et al., no association was found between the prognostic variable and Ki67 values [11].

Unlike prior reports, the focus of our study was on the correlation between radiological findings and Ki-67 values. Ki-67 values were highly associated with CT evidence of lymph node spread but not with tumor size or metastatic spread. We also observed an association between tumor size and both lymph node and metastatic spread. ROC curve analysis demonstrated that Ki-67 value of 60% as well as tumor size of 52.5 mm showed high accuracy for predicting lymph node spread. A study by Lawrence et al. from 1987 found tumor size to be the only predictive factor for lymph node spread in RMS, showing no correlation between lymph node spread and age, sex, or histopathologic type [16]. Our results are consistent with their findings and incorporate Ki-67 expression as another prognostic factor. Interestingly, in RMS the size 50 mm is designated in the literature as the cut-off point to differ between large and small lesions, and it is an integral part of the TNM staging [2,17].

One of the systems used in order to stratify RMS patients' prognosis is TNM. The presence of lymph node metastasis classifies the patient in a more advanced stage. RMS has a high probability for lymph node involvement compared to other types of sarcomas, demonstrating lymph node spread in up to 36% of cases [18]. In our research 29% of the cases presented lymph node involvement. In a study by Weiss et al., it was demonstrated that patients with no lymph node involvement and tumors  $<$  50 mm had no lung metastasis [19]. It was shown that patients with lymph node spread had a high rate of metastases and are at 90% higher risk of death than those without [16,19]. Hence, presence of lymph node involvement is crucial for prognosis evaluation and treatment selection for RMS patients. In our study, the combination of Ki-67  $\geq$  60% and tumor size  $\geq$  50 mm were strongly associated with lymph node spread. Our study contributes to the trend towards a better characterization of RMS prognostic factors, which is particularly significant as RMS patients are varied by clinicopathologic subgroups [3,20].

Our study has several limitations. Like many prior studies in RMS, the number of patients in our study is relatively small. This is due to the rare nature of this disease. Also, this is a retrospective study, and therefore has innate flaws. In order to maximize the uniformity, patients with workup outside of our institution were excluded.

In conclusion, this study demonstrates radiological-pathological correlation in RMS. Lymph node spread detected by radiological images is associated with Ki-67 values. Lymph node and metastatic spread are associated with primary tumor size. A combination of Ki-67 and tumor size was found to be predictive of lymph node spread.



**Fig. 5.** A - A two year old child with a 2 cm prostate mass and a Ki-67 value of 50%. No lymph node spread or organ metastatic spread were evident. B - A 23 year old woman with a large 12.4 cm uterine mass with bilateral pelvic lymph nodes (arrows) and a Ki-67 value of 90%. The patient also had solid organ metastatic spread to the vertebra and omentum.

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### Declarations of interest

None.

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