

## Original Article

# Large and round tumor nuclei in osteosarcoma: good clinical outcome

Carlos E. de Andrea<sup>1</sup>, Antonio Sergio Petrilli<sup>2</sup>, Reynaldo Jesus-Garcia<sup>3</sup>, Luiz F. Bleggi-Torres<sup>4,5</sup>, Maria Teresa S. Alves<sup>1</sup>

<sup>1</sup>Department of Pathology, Universidade Federal de São Paulo, São Paulo, Brazil; <sup>2</sup>Instituto de Oncologia Pediátrica, Grupo de Apoio ao Adolescente e à Criança com, Câncer/Universidade Federal de São Paulo, São Paulo, Brazil; <sup>3</sup> Department of Orthopedics and Traumatology, Universidade Federal de São Paulo, São Paulo, Brazil; <sup>4</sup>Department of Pathology, Universidade Federal do Paraná, Curitiba, Brazil; <sup>5</sup>Instituto de Pesquisa Pelé Pequeno Príncipe, Curitiba, Brazil

Received January 20, 2011; accepted January 29, 2010; Epub January 30, 2011; published February 15, 2011

**Abstract:** Osteosarcoma is the most frequent primary malignant bone tumor. Distinct histological features are distinguishable based on the morphology of the tumor. Differences in nuclei size and shape are often observed in osteosarcoma reflecting its broad histopathological heterogeneity. This study explores the relevance of two nuclear parameters in osteosarcoma: large area and round shape. Computerized nuclear morphometry was performed in 56 conventional osteosarcoma preoperative biopsies. The mean patient follow-up time was 35.1 months. Based on the nuclear area, no significant difference ( $P = 0.09$ ) in overall survival between patients with large ( $\geq 42.5 \mu\text{m}^2$ ) and small ( $< 42.5 \mu\text{m}^2$ ) tumor nuclei was found. However, when cases with large and round nuclei were analyzed jointly ( $\geq 42.5 \mu\text{m}^2$  and coefficient of nuclear roundness  $\geq 0.7$ ), these two parameters together were likely to be a predictive factor ( $P = 0.05$ ). Osteosarcoma patients with large and round tumor nuclei had a better outcome than patients with small and polymorphic (ovoid or spindle-shaped) nuclei. In this study, nuclear morphometry proved to be a useful tool to shed light on the biology of osteosarcoma showing that some morphometric parameters can be easily applied to help identifying patients with a good prognosis.

**Keywords:** Large nuclei, round nuclei, nuclear morphometry, osteosarcoma

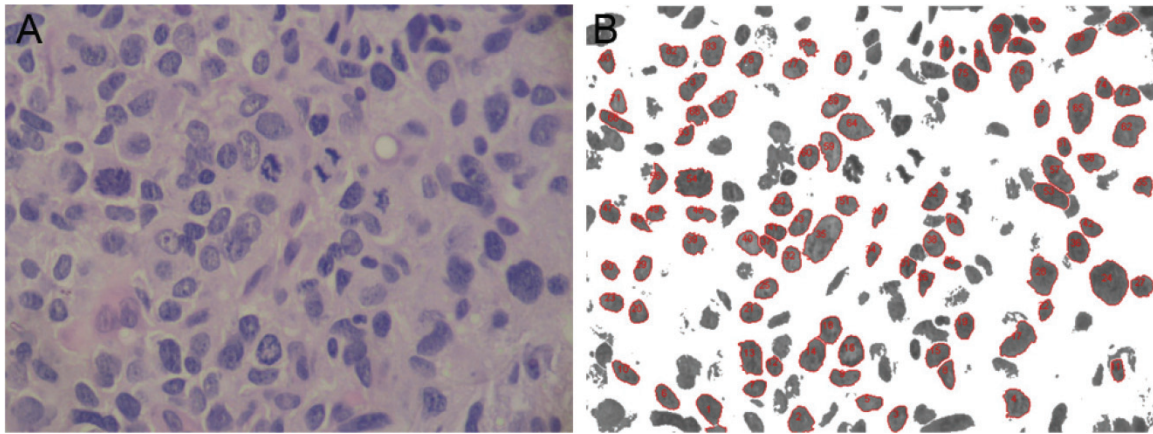
## Introduction

Osteosarcoma is the most frequent primary malignant bone tumor that primarily affects children and adolescents with an incidence of 4-5 per million [1]. Several histological subtypes are distinguishable based on the morphology of the tumor. The most common subtype is the conventional osteosarcoma, which accounts for 75% of the cases [2]. During daily routine, a broad histopathological feature is observed. Some of them are well known prognostic factor [3] (e.g., chemotherapy response, tumor size) and others are still unraveled. The presence of large tumor nuclei is described in osteosarcoma, especially in the anaplastic subtype [4]. However its biological relevance is still unclear. There has been report that cyto- or histomorphometric analysis may give reliable histopathological information regarding tumor behav-

our [5,6]. In 1982 Diamond et al. first used a measurement of the cell nucleus for estimation of prognosis in prostate cancer [7]. Later on, several other studies were carried out to establish the role of nuclear morphometry as a prognostic factor [6,8,9]. It has been shown that nuclear morphometry is valid and accurate in predicting relapse in early-stage renal cell carcinoma [10] and prognosis in rhabdomyosarcoma [6]. Taking advantage of the computerized nuclear morphometry previously described [6,11], the aim of this study was to clarify the relevance of two nuclear parameters: large area and round shape. In osteosarcoma, large and round tumor nuclei are likely to predict patient longer survival.

## Materials and methods

56 preoperative biopsies were obtained from



**Figure 1.** Computerized nuclear morphometry. **A.** Osteosarcoma with large tumor nuclei before image processing. H&E, high power. **B.** Example of the output after image processing. The color threshold module was used to remove the background which allowed a precise selection of the nuclei. Nuclear measurements were automatically performed regarding area and roundness.

56 patients with high grade osteosarcoma of long bones enrolled in the Brazilian osteosarcoma treatment group study 2000 [12], from January 2000 to May 2004, and treated in the Instituto de Oncologia Pediatrica, GRAACC/UNIFESP (Grupo de Apoio ao Adolescente e à Criança com Câncer/Universidade Federal de São Paulo). Patient data were obtained by reviewing pathological reports or clinical charts. All the cases were obtained from the Department of Pathology of the Universidade Federal de São Paulo and handled according to the ethical guidelines of that institution.

All the biopsies were fixed in formalin, decalcified and embedded in paraffin according to the standard procedures. 4  $\mu$ m-thick sections stained with Hematoxylin and Eosin (H&E) were used on the nuclear morphometric analysis.

#### *Assessment of patients- and tumor-related variables*

The following variables were evaluated: presence or absence of metastases at diagnosis, tumor size (< 12 cm or  $\geq$  12 cm), histological subtype, local recurrence, histological response to preoperative chemotherapy (good or poor responders) and overall survival. The subtype was classified following the criteria of the World Health Organization (WHO) classification for conventional osteosarcoma [1]. Good responder was defined as 90% tumor necrosis or more in the surgical specimen after preoperative che-

motherapy, and poor responder as less than 90% necrosis [13]. Overall survival was defined as the time interval between the date of enrollment onto the study and death from any cause, including secondary malignancies, or the most recent follow-up contact.

#### *Nuclear morphometry*

Nuclear morphometry was performed semiautomatically on H&E-stained sections using a computer connected to an Olympus BX 40 microscope with a x40 magnification lens as previously described [6,11]. The nuclei were manually selected and automatically measured by ImageJ software (NIH Image, Bethesda, MD). The color threshold module was used to remove parts of the image that not fall within the nuclear color range (Figure 1). For the nuclear morphometric evaluation the following parameters were measured: 1). Area of cell nucleus ( $\mu$ m<sup>2</sup>); 2). Coefficient of nuclear roundness (CNR) - it defines the degree of similarity of nuclear shape to the circle: if the shape of the nucleus is more round, the coefficient is closer to 1.

To reduce bias, the selection of the tumor nuclei was carried out without knowledge of the clinical or pathological report. For every sample at least 100 nuclei were evaluated and the mean of all measured nuclei was used (Table 1). As the histological response to chemotherapy is a good prognostic factor in osteosarcoma patients

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**Table 1.** Distribution of clinicopathological parameters and cross-tabulations with nuclear area and coefficient of nuclear roundness (CNR)

Variable	No. of cases	Nuclear Area		P-value	Nuclear Area and CNR		P-value	
		Small nuclei	Large nuclei		Small and ovoid/spindle-shaped nuclei	Large and round nuclei		
Roundness coefficient								
round nuclei	23	13	10	.00	-	-	-	
non- round nuclei	33	29	4		-	-		
Necrosis grade								
Poor responders	40	34	6	.00	36	4	.01	
Good responders	16	8	8		10	6		
Tumor size								
< 12 cm	25	17	8	.27	19	6	.28	
≥ 12 cm	31	25	6		27	4		
Local recurrence								
Yes	33	22	11	.08	26	7	.43	
No	23	20	3		20	3		
Metastasis at diagnosis								
Yes	26	22	4	.12	24	2	.06	
No	30	20	10		22	8		
Death								
Yes	26	17	9	.12	27	3	.09	
No	30	25	5		19	7		

[1], the mean of the nuclear area of the good responder cases was used as a cut-off to define the following types of cell nuclei: small nuclei and large nuclei. Nucleus was considered round when the roundness coefficient was above 0.70.

### Statistical analysis

For all statistical tests, SPSS 16.0 software was used. Survival curves were plotted using the Kaplan–Meier procedure and multivariable analysis was performed by using the Multivariate Cox Regression method. Correlations were studied by contingency tables and Pearson's chi-square ( $\chi^2$ ) test or one-way ANOVA. *P*-values <0.05 were considered statistically significant.

## Results

### Clinical features

The ages of the patients ranged from 8 to 26 years (mean age, 20 years) and 50% of them (28 of 56) were in the second decade of life. 38 patients were male and 18 were female. In 52 cases the lesion was located in long bones of

the lower extremity (34 femurs, 17 tibias and 1 fibula). Additionally, twenty-four patients (42%) had lung metastasis at the time of diagnosis. The mean follow-up time was 35.1 months (range, 9 to 74 months).

### Pathologic features

Pathological data for each of the 56 patients are summarized in Table 2. 62.5% of the cases were subclassified as osteoblastic, 23.2% as chondroblastic, 5.4% as fibroblastic and 8.9% as other rare variants. The proportion of good responders to preoperative chemotherapy was 46% and of poor responders was 54%.

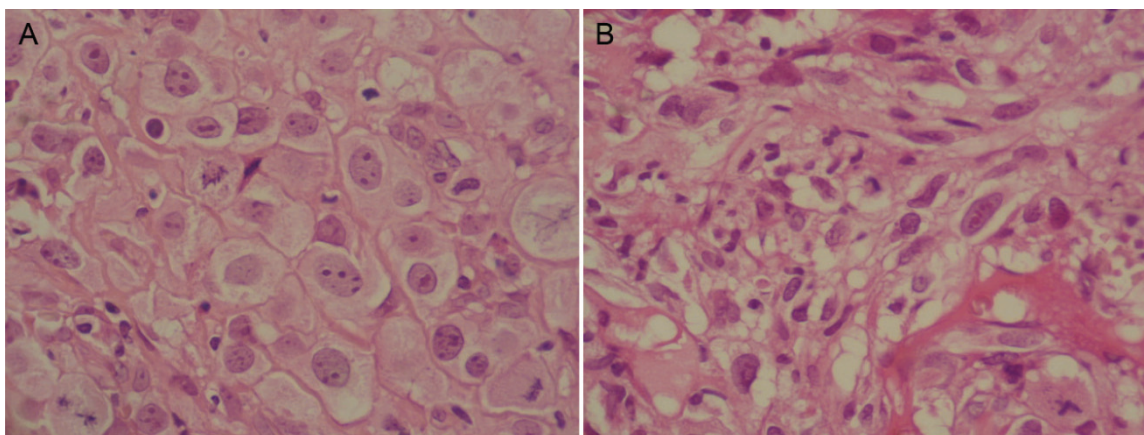
### Nuclear morphometry

The mean values of the nuclear morphometry are summarized in Table 1. No significant difference in the mean value of nuclear area between the osteosarcoma subtypes was observed (*P* = 0.10) (Table 2). Based on the mean of the nuclear area of the good responder cases, a cut-off was used to define cases with large ( $\geq 42.5 \mu\text{m}^2$ ) and small ( $< 42.5 \mu\text{m}^2$ ) nuclei. 42 osteosarcomas (75%) were classified as cases

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**Table 2.** Mean values of nuclear morphometry in the histological subtypes of osteosarcoma

	Area ( $\mu\text{m}^2$ )	Coefficient of nuclear roundness
Osteoblastic ( $n = 35$ )	35.17 $\pm$ 10.59	0.67 $\pm$ 0.03
Chondroblastic ( $n = 13$ )	31.47 $\pm$ 7.86	0.67 $\pm$ 0.03
Fibroblastic ( $n = 3$ )	34.91 $\pm$ 12.44	0.65 $\pm$ 0.02
Others ( $n = 5$ )	44.77 $\pm$ 6.85	0.72 $\pm$ 0.05



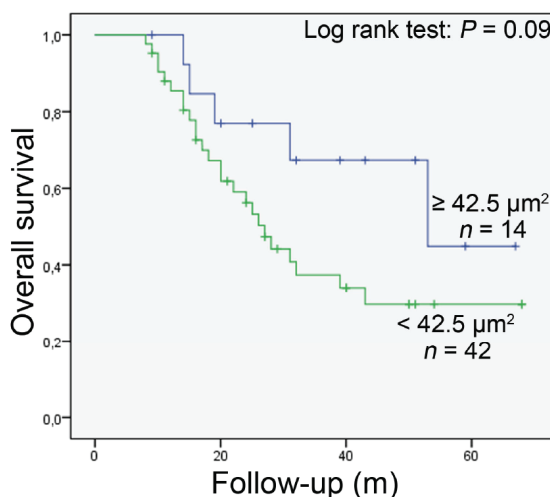
**Figure 2.** Osteosarcoma can display a variety of histopathological features. **A.** Osteosarcoma with large and round tumor nuclei. H&E, high power. **B.** Osteosarcoma with small tumor cell nuclei. H&E, high power.

with small nuclei and 14 (25%) as cases with large nuclei (**Figure 2**). The results are shown in **Table 2**.

As nuclear polymorphism is described in osteosarcoma, in all the studied samples, round, ovoid and spindle-shaped nuclei were observed. The coefficient of nuclear roundness was used to define tumors with round ( $\geq 0.70$ ) or non-round shape ( $< 0.70$ ). 10 tumors (18%) were classified as cases with large and round nuclei (mean area value  $\geq 42.5 \mu\text{m}^2$  and CRN  $\geq 0.7$ ) and 46 (82%) as cases with small and polymorphic (ovoid or spindle-shaped) nuclei (median area value  $< 42.5 \mu\text{m}^2$  and CRN  $< 0.7$ ). The results are shown in **Table 2**.

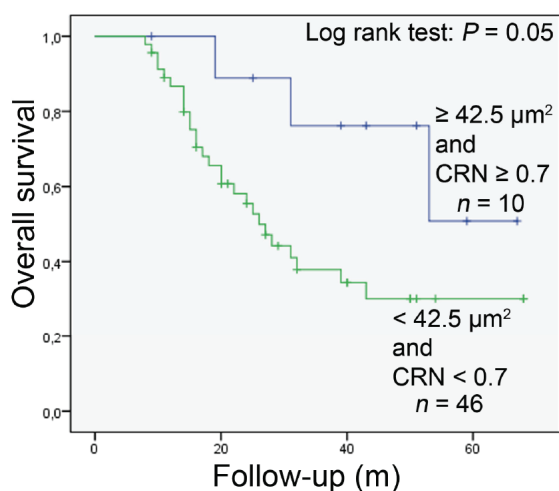
Among the 56 patients analyzed, 30 were alive at the time of the analysis (53.6%) and 26 had died (46.4%). No significant difference in overall survival between patients with large and small tumor nuclei was seen ( $P = 0.09$ ) (**Figure 3**). However, when cases with large and round nuclei were analyzed jointly, these two parameters together were likely to be a predictive factor ( $P$

$= 0.05$ ) (**Figure 4**). Osteosarcoma patients with large and round tumor nuclei had a better out-



**Figure 3.** Kaplan-Meier overall survival curves in all analysed osteosarcoma patients according to nuclear area of tumour cells (large nuclei:  $\geq 42.5 \mu\text{m}^2$ ; small nuclei:  $< 42.5 \mu\text{m}^2$ ). No differences in outcome between the patients was observed.

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**Figure 4.** Kaplan-Meier overall survival curves in all analysed osteosarcoma patients according to nuclear area and coefficient of nuclear roundness (CRN) of tumour cells (large and round nuclei:  $\geq 42.5 \mu\text{m}^2$  + CRN  $\geq 0.7$ ; small and ovoid/spindle-shaped nuclei:  $< 42.5 \mu\text{m}^2$  + CRN  $< 0.7$ ). Patients with large and round nuclei had better prognosis.

come than patients with small and polymorphic (ovoid or spindle-shaped) nuclei (**Figure 4**). Multivariate Cox regression analysis showed that large and round tumor nuclei was not an independent predictive factor for the overall survival of these patients.

### Discussion

Computerized nuclear morphometry has been shown as a good and cheap tool to predict patient outcome in several tumors [6,8]. During daily practice, differences in nuclei size and shape are often observed in osteosarcoma reflecting its broad histopathological heterogeneity. Large cells, up to  $100 \mu\text{m}$  in diameter, are described in the anaplastic subtype [14]. However the relevance of larger nuclei in osteosarcoma is still unclear. On the other hand, small cell osteosarcoma has a slightly worse prognosis [15]. It has shown that cell size and shape can predict the effect of chemotherapy in osteosarcoma [16].

In this study, large nuclei were not associated to the chondroblastic subtype indicating that this feature was present in others variants. Large nuclei, defined only by the nuclear area, can have a round-, ovoid- or spindle-shape. The co-

efficient of nuclear roundness was used to identify cases with large and round nuclei. The presence of large and round tumor nuclei in osteosarcoma is likely to be associated with longer survival. Interestingly, only large nuclei could not predict good outcome. In this series, patients with large and round nuclei responded better to the preoperative chemotherapy and had a longer survival which might indicate that these tumors were more drug-sensitive. The association between two nuclear parameters, large area and round shape, might reflect a subset of poorly differentiated osteosarcoma with more anaplastic features: abundant eosinophilic cytoplasm, minimal osteoid and cartilage production, multiple nucleolated nuclei with prominent nucleoli. In preoperative biopsy, a list of differential diagnosis might be considered in the cases with large and round cell/nuclei with minimal osteoid formation. This list has to include malignant lymphoma, malignant melanoma (metastatic), metastatic carcinoma and malignant fibrous histiocytoma. The immunoreactivity for osteocalcin might be useful in diagnosing osteosarcomas [14].

In rhabdomyosarcoma, a tumor also derived from mesenchymal tissue, nuclear morphometry has shown that the clinical outcome is better if the nuclei seen in biopsies are more spindle-shaped and, in the embryonal subtype, patients with small nuclei had a better outcome than patients with large nuclei [6]. It suggests that the relationship between nuclear size and prognosis seems to be tumor specific.

Taken all the findings together, nuclear morphometry proved to be a useful tool to shed light on the biology of osteosarcoma showing that some morphometric parameters can be easily applied to help identifying patients with a good prognosis.

### Acknowledgments

The authors thank the pathology department of the Federal University of São Paulo for the support and useful discussions. This study was partially supported by CAPES (Brazilian Higher Education Staff Training Agency).

**Please address correspondence to:** Maria Teresa S. Alves, MD, PhD, Department of Pathology, Universidade Federal de São Paulo Rua Botucatu 740, Postal Code: 04023-900, Sao Paulo, Brazil, Tel: +55 11

55727501, Fax: +55 11 55719295, E-mail: [mtsalmes@unifesp.br](mailto:mtsalmes@unifesp.br)

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