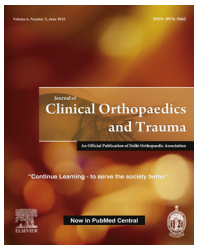
Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/jcot



Original Article

Histopathological, immunohistochemical, and image analytic parameters characterizing the stromal component in primary and recurrent giant cell tumor of bone

Charu Chandra Saxena [a], *Rajni Safaya* [a], *Neha Kawatra Madan* [b,*], *Shah Alam Khan* [c], *Venkateswaran K. Iyer* [a]

[a] *Department of Pathology, All India Institute of Medical Sciences, New Delhi, India*
[b] *Senior Research Associate, Department of Pathology, All India Institute of Medical Sciences, New Delhi 110029, India*
[c] *Department of Orthopaedics, All India Institute of Medical Sciences, New Delhi, India*

ARTICLE INFO

Article history:
Received 3 August 2015
Accepted 29 September 2015
Available online 26 October 2015

Keywords:
Giant cell tumor
Image analysis
Immunohistochemistry
Primary
Recurrent

ABSTRACT

Giant cell tumor (GCT) of bone is a benign locally aggressive tumor whose biological behavior is unpredictable. Currently, there are no definitive clinical, histological, biochemical, or immunological parameters that can predict its behavior. This study was undertaken to examine whether delineation of reactive and neoplastic stromal component of GCT can help in this regard. 55 cases of GCT (30 primary, 25 recurrent) were subjected to histopathological grading, immunohistochemistry, and image analysis. Spindling of stroma was more frequent in recurrent GCT with 64% cases having more than 50% spindled stroma ($p < 0.001$). Number of mitosis/10 HPF and higher grade were more in recurrent GCT. Mean percentage positivity for CD68 (38.36%) and α1-ACT (70.86%) was higher in primary than recurrent GCT. PCNA and MiB-1 labeling indices were higher in recurrent (42.62% and 9.18%, respectively) than in primary group (24.75% and 7.7%, respectively). A single numerical parameter encompassing stromal cell population and its proliferation was derived as ratio of PCNA/CD68 and PCNA/α1-ACT. Both ratios were higher in recurrent (0.81 ± 0.38; 1.58 ± 1.50) than in primary GCT (0.58 ± 0.62; 0.34 ± 0.29) ($p = 0.002$; 0.01). On image analysis, parameters significantly different between the two groups were nuclear area and nuclear integrated optical density. It was thus concluded that recurrent GCT shows higher grade, increased mitosis, more spindling, fewer reactive components, and higher proliferation than primary GCT. Delineation of reactive component (α1-ACT positive) and proliferating component (PCNA positive cells) using immunohistochemistry with calculation of the PCNA/ACT ratio delivers more information than image analysis.

* *Corresponding author*. Tel.: +91 9953236682.
E-mail address: nehakawatramadan@gmail.com (N. Kawatra Madan).

http://dx.doi.org/10.1016/j.jcot.2015.09.006

1. Introduction

Giant cell tumor (GCT) of bone is seen commonly in the 20 to 50-year age group, and is more common in females.[1] It occurs in the epimetaphyseal ends of long bones, with lower end of femur, upper end of tibia, lower ends of radio-ulna, and upper end of humerus being common sites.[2] The histopathology of GCT is characterized by numerous multinucleated giant cells, mononuclear stromal cells, and frank hemorrhage.[3] A range of morphological appearances are seen from specimen to specimen and from section to section.[4] The histogenesis and the relationship between multinucleated giant cells and mononuclear stromal cells of GCT have been recently better understood with both neoplastic and non-neoplastic reactive cells considered to be contributing to the stromal component. The neoplastic stromal cells are believed to arise from primitive osteoblastic cells while the reactive component is believed to arise from bone marrow-derived monocytic cells. Release of specific cytokines such as RANKL, M-CSF, IL-1, IL-6, and PGE2 by the neoplastic cells recruits reactive cells to the tumor stroma.[5] Fusion of these reactive mononuclear cells forms the characteristic multinucleated giant cells. The reactive cells express LCA, CD68, α1ACT, muramidase, MMP-1, MMP-9, and UCHM1, strongly suggesting that they are of hematopoietic origin.[6]

One of the most important characteristics of GCT is its unpredictable biological behavior. GCTs that are deemed to be histologically benign can recur and even metastasize. Many histopathological and radiological characteristics have been evaluated as indicators of prognosis.[7,8] Previous studies have not found any significant difference in the proliferation of neoplastic cells between primary and recurrent GCT.[4,9,10]

The present study was undertaken to evaluate whether delineation of reactive and neoplastic component of GCT using immunohistochemistry can help in deriving histopathological performance indicators which differ in primary and recurrent GCT. Nuclear morphometry of immunostained slides by image analysis was used for more accurate measurement and to assess the utility of this approach.

2. Material and methods

The study was conducted in the form of a retrospective and prospective evaluation including 55 cases of GCT (30 primary; 25 recurrent GCT) in the Departments of Orthopedic Surgery and Pathology, All India Institute of Medical Sciences, from 2004 to 2008. The cases were segregated into primary and recurrent GCT. The first group included primary cases presenting for the first time and without evidence of aggressive behavior in whom either a curettage or en-block resection was performed. The second group constituted recurrent cases of GCT in which a primary resection had been performed earlier at some other center and the tumor had recurred. Histopathological details of primary tumor resection were not available. A minimum follow-up of 6 months was available after the surgery was performed.

2.1. Histopathological analysis

All the H&E stained slides were reviewed by two observers for morphological features and presence of extra skeletal invasion. Nuclear grading was done (Grade 1 with stromal cells lacking pleomorphism and hyperchromasia, Grade 2 having stromal cells showing nuclear enlargement and pleomorphism without significant hyperchromasia or atypical mitosis, Grade 3 showing marked pleomorphism accompanied by hyperchromasia and presence of occasional atypical mitosis).[4] The number of mitotic figures was counted per 10 high power fields in the areas with highest mitotic activity. Percentage of spindle cells in the stroma was calculated by counting 500 cells under high power with examination of at least 10 fields.

2.2. Immunohistochemistry

Sections were cut from representative blocks of formalin fixed paraffin embedded tissue on poly-L-lysine coated slides. Sequential slides cut from the same block were subjected to immunohistochemistry for CD68, α1-Antichymotrypsin (α1-ACT), MIB-1, PCNA, VEGF, and p53 with LSAB2 streptavidin–biotin horseradish peroxidase detection with Diaminobenzidine (DAKO corporation, USA). Positivity was assessed by counting a minimum of 500 cells for each marker.

2.3. Image analysis

Morphometry was performed using Image Pro-Plus Analysis Software version 7.0 (Media Cybernetics Corporation, USA) and a 12-bit digital camera (Media Cybernetics Corporation, USA with inbuilt image grabber card). Multiple representative images were captured from α1-ACT and CD68-stained slides of the primary and recurrent cases of GCT. Enough images were captured in each case to permit analysis of at least 50 nuclei each of positive and negative immunostained cells per case. The software delivered the values of various parameters, which are enumerated in Table 1.

2.4. Statistical analysis

Univariate analyses for all parameters were done by parametric 'Students *t*-test' and 'Mann–Whitney' non-parametric test. *p*-Value for proportion was calculated by applying 'Fishers exact test'. Logistic regression was done by 'Chi-square test' to calculate the 'odds ratio' with 95% confidence limit and its *p* value. Multivariate analyses by multiple logistic regressions were employed to identify independent predictors of recurrence in GCT.

3. Results

3.1. Clinical characteristics

Both the groups had comparable age distribution with mean age in primary GCT of 29 years (range 15–59 years) and that in recurrent GCT of 27.8 years (range 15–45 years). Male:female ratio in primary GCT was 2:1, whereas in recurrent GCT it was 1:1.08. Overall male:female ratio was 1.39:1. The sites of