

Morphometrical analysis of the spectrum of small round cell tumors in a tertiary care centre

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Abstract

Introduction: The term “small round-cell tumors” (SRCT) describes a group of highly aggressive malignant neoplasms composed of small, monotonous cells with high nucleocytoplasmic ratios. Microscopic tumor burden, pre and post therapy tumor necrosis quantification and mitotic index may help as an alternative method of assessing the prognosis in these patients.

Aims: Morphometrical analysis of the spectrum of small round cell tumors over a period of five years and its role in grading the tumors with respect to microscopic tumor burden, tumor necrosis and mitoses.

Materials and Methods: This study included 39 cases of small round cell tumors (19 NHL, 6 Ewings/PNET, 3 atypical carcinoid, 3 olfactory neuroblastoma, 2 cases each of rhabdomyosarcoma, wilms tumor, neuroblasoma and monophasic synovial sarcoma across all age groups. The study was confined to bone and soft tissues. The microscopic tumor burden and tumor necrosis was assessed using computer assisted morphometric analysis by digital processing. Concurrently the number of mitoses were studied and correlated.

Results: Thirty nine cases of small round cell tumors were morphometrically analyzed using image pro-plus analysis soft ware 5.0. The mean of microscopic tumor burden / necrosis / mitoses were compared among the four commonly encountered small round cell tumors namely atypical carcinoid, non-Hodgkins lymphoma (NHL), olfactory neuroblastoma, Ewings/PNET using Kruskal-Wallis Test. The difference was significant with respect to necrosis ($p=0.003$) and mitoses ($p=0.003$). Similarly inter group comparison of mitoses and necrosis were done for atypical carcinoid, NHL, olfactory neuroblastoma and Ewings/PNET using Mann-Whitney U Test. Inter group comparison of necrosis showed that the mean tumor necrosis of Ewings/PNET was more when compared with NHL, atypical carcinoid and olfactory neuroblastoma, showing a significant p value of 0.002, 0.020 and 0.018 respectively. The mean mitotic figures of Ewings/PNET when compared with atypical carcinoid and olfactory neuroblastoma were statistically significant ($p0.020$ and 0.020 respectively). Similarly, mitotic figures of NHL were more when compared with atypical carcinoid and olfactory neuroblastoma ($p0.006$ and 0.006).

Conclusion: The above results reveal that Ewings/PNET have relatively more tumor necrosis, mitosis compared to other SRCT. In the forthcoming years, it will be extremely useful to find out the reversal of these patterns as the tumors are subjected to chemotherapy and radiotherapy.

Keywords: Small round cell tumors (SRCT), Morphometry, Tumor necrosis, Microscopic tumor burden, Mitoses.

Introduction

The term “small round-cell tumor” (SRCT) comprises a group of highly aggressive malignant neoplasms composed predominantly of small and monotonous undifferentiated cells with high nucleocytoplasmic ratios on histology. This group includes Ewing’s sarcoma (ES), primitive neuroectodermal tumor (PNET) or extraskeletal Ewings sarcoma, neuroblastoma, rhabdomyosarcoma, desmoplastic small round cell tumor, non-Hodgkin’s lymphoma, small-cell osteosarcoma, small-cell carcinoma (either undifferentiated or neuroendocrine), olfactory neuroblastoma and mesenchymal chondrosarcoma. Their clinical presentation often overlap, thus making the diagnosis problematic in some cases. A clear understanding of their clinicopathologic features usually allows for a confident diagnosis, and immunohistochemistry is invaluable in narrowing the

differential diagnosis and the prognostication of these small round cell tumors.

Various studies have mentioned that small round cell tumor show necrosis of varying degree but no studies have been done on comparative morphometrical analysis of necrosis in these tumors. Morphometric analysis of microscopic tumor burden is also another area where no studies have been done. We also make an attempt to correlate and compare the mitotic rate with other parameters. The analysis of these parameters might throw greater insight into the behavior of these tumors and the overall effects on patient survival and quality of life. In the forthcoming years, it will be extremely useful to find out the reversal of these patterns as the tumors are subjected to chemotherapy and radiotherapy. Post treatment biopsies in case of residual or recurrent disease could be then evaluated and studied.

Materials and Methods

Institutional Ethics Committee approval was obtained before commencement of the study. Our study is a retrospective study. A total of 39 cases of small round cell tumours were included in our study. Clinical parameters of the study cases were recorded from the case files. Archived formalin-fixed, paraffin-embedded sections of all cases diagnosed as SRCT on small biopsies and resected specimen were retrieved from the department of Pathology of Sri Ramachandra medical college and research Institute.

Inclusion Criteria: The study was confined to small round cell tumors of bone and soft tissues. Age of the patient was taken as independent factor and included all age groups.

Exclusion Criteria: Small round cell tumors in the hematolymphoid and lymphoreticular system (i.e. bone marrow, spleen and lymph node) have been excluded from the study.

Decalcification was performed on bony tissue before the routine processing was done. H and E stained sections of all the cases were retrieved to confirm the tissue diagnosis and on the basis of these H and E stained sections and the histopathologic report of the respective cases including the immunohistochemical status, the various parameters were morphometrically studied. Morphometric analysis was done by digital processing. The microscopic tumor burden was assessed by calculating the number of tumor cells which showed positivity for the particular immunophenotype. An average was taken of five such areas and this was done for all the cases. Calculations were made at X200 magnification for all the cases.

Similarly, assessment of microscopic tumor necrosis was also done using morphometric image analysis software by digital processing. The area of necrosis was calculated on hematoxylin and eosin stained tissue sections. An average was taken of three different areas showing tumor necrosis. Also an average was taken of the total area. The percentage of tumor

necrosis was calculated by dividing the area of necrosis by total area and then multiplying by 100. These criteria were established on a microscope with a X200 magnification. All assessment by morphometry was done by two pathologists separately.

The number of mitosis was counted on H&E stained sections and an average of 10 different fields was taken. Mitoses was counted in the areas of highest mitotic activity and the fields selected for counts should be filled with as many as possible viable tumor cells. These criteria were established on a microscope with a X400 magnification.

The immunophenotypic markers which were used in the study to differentiate and categorize the small round blue cell tumors were CD45/LCA, CD20, CD3, CD99, desmin, EMA, CK, synaptophysin, chromogranin, GFAP. There being sufficient numbers of cases for NHL, Ewings/PNET, atypical carcinoid and olfactory neuroblastoma, statistical analysis was carried out only for the following cases. Immunophenotypic markers were not done for wilms tumor, medulloblastoma and hepatoblastoma because their specific clinical presentation and morphological growth pattern that made their diagnosis obvious.

Results

A total of 39 cases of small round cell tumors were identified and studied. The age of the patients with small round cell tumor ranged from 5 days to 73 years and 44% of the cases occurred between 15-45 years of age and 43(74%) were males. The mean of microscopic tumor burden / necrosis/mitoses were compared among these four common encountered small round cell tumors namely atypical carcinoid, NHL, olfactory neuroblastoma, Ewings/PNET using Kruskal-Wallis Test. The difference was significant with respect to necrosis ($p=0.003$) and mitoses ($p=0.003$). However the difference in microscopic tumor burden did not show any statistical significance (Table 1,2).

Table 1: Mean and Standard deviation of necrosis, mitoses and microscopic tumor burden for the four commonly encountered small round cell tumour

S. No	Diagnosis	N	Necrosis		Mitoses		Microscopic tumour Burden	
			Mean	SD	Mean	SD	Mean	SD
1	Non-Hodgkin's lymphoma	19	23.54	21.53	20.32	6.272	35.65	7.47
2	Ewings/PNET	6	59.59	9.587	19.67	7.967	36.98	11.46
3	Atypical Carcinoid	3	13.63	23.608	2.33	0.577	33.10	5.23
4	Olfactory Neuroblastoma	3	0.00	0.000	0.67	1.155	33.54	2.58

Table 2: Comparison of necrosis, tumor burden & mitosis between the 4 groups using the Kruskal-Wallis test. A p value of < 0.05 was considered to be statistically significant. Statistical significant difference was observed among the groups for "necrosis ($p=0.03$)" and "mitosis" ($p=0.03$)

	Necrosis	Tumour Burden	Mitosis
Chi-Square	14.100	2.612	14.266

Df	3	3	3
Asymp. Sig.	.003	.455	.003

Inter group comparison of mitoses and necrosis were done for four common small round blue cell tumor namely atypical carcinoid, NHL, olfactory neuroblastoma and Ewings/PNET using Mann-Whitney U Test (Table 3). The mean tumor necrosis of Ewings/PNET was more when compared with NHL, atypical carcinoid and olfactory neuroblastoma, showing a significant p value of 0.002, 0.020 and 0.018 respectively. The mean mitotic figures of

Ewings/PNET when compared with atypical carcinoid and olfactory neuroblastoma were more, showing a significant p value of 0.020 and 0.020 respectively. The mean mitotic figures of NHL were more when compared with atypical carcinoid and olfactory neuroblastoma, showing a significant p value of 0.006 and 0.006 respectively. However, although the mean mitotic count of non-Hodgkin's lymphoma was more than Ewing's/PNET showed no significance (Table 1).

Table 3: Comparison of necrosis and mitosis between the groups

Comparison between the groups	Necrosis (p value)	Mitoses (p value)
Ewings/PNET Vs NHL	0.002	0.823
Ewings/PNET Vs Atypical Carcinoid	0.020	0.020
NHL Vs Atypical Carcinoid	0.457	0.006
Ewings/PNET Vs Olfactory neuroblastoma	0.018	0.020
NHL Vs Olfactory neuroblastoma	0.071	0.006
Atypical Carcinoid Vs Olfactory neuroblastoma	0.317	0.099

Discussion

The small round cell tumors family of neoplasms has grown in an increasingly complex fashion in recent years. This group of neoplasms characterized by high cellularity, small cell size, generally diffuse growth pattern and widely distributed across age groups. The tumor cells have dark nuclei and scanty cytoplasm; this results in an overall blue appearance on routine hematoxylin and eosin stained sections. Ancillary diagnostic techniques like immunohistochemistry, flow cytometry and chromosomal markers have proven extremely useful when these undifferentiated tumors are not distinguishable morphologically.¹

In the present study we have compared the microscopic tumor burden, necrosis morphometrically and simultaneously correlated the mitotic counts. The study data of the 43 cases showed a wide age distribution ranging from 5 to 73 years, however 44% of cases were between 15-45 years of age. There was a male preponderance (74%) compared to females (26%). Of the 12 different cases of SRCT NHL was the predominant, accounting for 44.2% of the total cases. The others include Ewings/PNET (14%), rhabdomyosarcoma (4.7%), atypical carcinoid (7%), olfactory neuroblastoma (7%), neuroblastoma (4.7%), monomorphic synovial sarcoma (4.7%), wilms tumor (4.7%), medulloblastoma (2.3%), small (oat) cell carcinoma (2.3%), hepatoblastoma (2.3%) and small cell osteosarcoma (2.3%).

The mean of microscopic tumor burden/necrosis/mitoses were compared among the four commonly recurring small round cell tumor namely atypical carcinoid, NHL, olfactory neuroblastoma,

Ewings/PNET. The difference was statistically significant with respect to necrosis ($p=0.003$) and mitoses ($p=0.003$). However the difference in tumor burden did not show any statistical significance.

Statistical assessment of inter group for necrosis revealed that the mean tumor necrosis of Ewings/PNET was more when compared with NHL, atypical carcinoid and olfactory neuroblastoma, showing a significant p value of 0.002 (Ewings/PNET vs NHL), 0.020 (Ewings/PNET vs atypical carcinoid) and 0.018 (Ewings/PNET vs olfactory neuroblastoma) respectively. Studies have shown that Ewing's family of tumors (EFT) frequently undergoes necrosis and the residual viable cells show a "peritheliomatous" or a perivascular distribution.^{2,3}

Even NHL is known to have extensive necrosis and this finding may have a prognostic significance for patients with non-Hodgkin lymphomas. The patients with necrosis had significantly higher stages (Stage II or higher), greater International prognostic index (IPI of ≥ 2), and higher serum LDH levels than those without necrosis ($p = 0.001$, $p = 0.005$, and $p = 0.005$, respectively).⁴ We had found that the mean mitotic figures of Ewings/PNET was more when compared with atypical carcinoid and olfactory neuroblastoma, showing a significant p value of 0.020 (Ewings/PNET vs atypical carcinoid) and 0.020 (Ewings/PNET vs olfactory neuroblastoma) respectively.

We also noted that the mean mitotic figures of NHL was also more when compared with atypical carcinoid and olfactory neuroblastoma, showing a significant p value of 0.006 and 0.006 respectively.

Although monitoring the tumor progression or the

response to therapy can be approached using physical examination, radiological imaging studies, and serum tumor markers but these are limited in sensitivity and are inadequate measures. Studies have shown that histological response of Ewings sarcoma to preoperative chemotherapy in terms of the percentage of tumor necrosis and the oncological outcome is useful. Grading of necrosis by Huvos classification were the most important predictors of event-free survival. Some authors consider any degree of necrosis greater than 90% to be favorable. These indicators should be used to identify patients who are at high risk for metastasis as such patients may be candidates for more intensive or novel therapies.⁴⁵

Recent studies in olfactory neuroblastoma indicate that, in terms of overall survival, necrosis and mitosis were significant and mitosis was related with disease-free survival.¹⁶⁻⁸ In a cohort study by Tsuta K et al among 80 patients (68 with typical carcinoids and 12 with atypical carcinoids). Older age (P=0.002), pathologic stage III or IV disease (P=0.04), active fibroblastic proliferation (P=0.041), and comedo-like necrosis (P=0.001) were significantly associated with tumor recurrence or patient's death. Among the three mitotic counting methods, the overall mean number of mitoses was significantly correlated with recurrence-free survival (P<0.0001).⁹

We could not evaluate the prognosis for all these cases because follow up of cancer patients was not done in our institution. Further studies need to be done in more number of cases for an extensive evaluation of tumor burden, necrosis and mitosis.

Conclusion

The present study reveal that Ewings/PNET have relatively more tumor necrosis, mitosis compared to other SRCT. It will be extremely useful to find out the reversal of these patterns as the tumors are subjected to chemotherapy and radiotherapy. Post treatment biopsies in case of residual or recurrent disease could be then evaluated studied which might throw greater insight into the behavior of these tumors and the overall effects on patient survival and quality of life.

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