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Role of Immunohistochemistry in Differential Diagnosis of Round Cell Tumor

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ABSTRACT

Immunohistochemistry has a significant role in the identification of tumors lacking evidence of lineage differentiation on the basis of routine light microscopic morphology alone. The aim of this study is to identify the true identity of round cell tumors by using minimal markers. This study was done in Department of pathology, Govt medical college, Surat from June 2010 to November 2012. 80 cases of small round cell tumors were studied. Primary staining with LCA, CK, vimentin and S100 was done. According to their results secondary markers (CD 117, myogenin, CD99, HMB45, etc) were used and we found 32.50% Non- Hodgkin Lymphoma, 18.75% Germ cell tumor, 12.50% Sarcoma, 8.75% each of melanoma and blastoma, 6.25% neuroendocrine tumor, 5% carcinoma, 2.50% plasma cell neoplasm, 1.25% mesothelioma and 3.75% undifferentiated round cell tumor. Immunohistochemistry is a valuable adjunct to routine hematoxylin and eosin staining for adequate and accurate categorization of round cell tumors.

Keywords : Immunohistochemistry; Round Cell Tumor; Differential diagnosis, Non Hodgkin's Lymphoma

Introduction:
Round cell tumors consist of a heterogenous group of neoplasms characterized by similar histopathological and cytological features. They are composed of uniform small round cells with round nuclei containing fine chromatin, scanty clear or eosinophilic cytoplasm. In some cases tumor cells are larger, ovoid or spindle with prominent nucleoli and irregular contours. [1]

Small round blue cell tumor is the name given to a group of highly malignant neoplasms that occur mostly in the pediatric age group, term could also apply to some adult neoplasms, notably small cell carcinoma of the lung. The name is derived from the primitive, highly cellular nature of these lesions, which typically present a vast sea of dark-blue nuclei in the sense that they have large hyperchromatic nuclei and a thin rim of cytoplasm on haematoxylin-based stains. Cytoplasmic abundance roughly correlates with cellular differentiation, which is often modest. 2, 3 As their name implies, these cancers are difficult to distinguish by light microscopy. 4 Ancillary techniques, such as histochemical stains, immunohistochemistry, electron microscopy, and cytogenetics and molecular techniques, reverse transcriptase polymerase chain reaction (RT-PCR) and Fluorescence in situ hybridization (FISH) have become important in the diagnosis of these tumors. [2]

For treatment purposes and prognostic evaluation it is crucial to determine whether the malignant small round cell tumor (MSRCT) is epithelial, mesenchymal, neuroendocrine, melanocytic or hematopoietic in nature but anatomic locations and microscopic details of these tumors and other aspects of their clinical presentations also helps in treatment and prognosis, immunohistochemical analysis may be tailored according to such considerations.[1]

diagnosis of round cell tumors include Ewing's sarcoma/ Primitive peripheral neuroectodermal tumor, Neuroblastoma, Pigmented neuroectodermal tumor, Esthesioneuroblastoma, Wilm's tumor (Nephroblastoma), Embryonal sarcoma of liver, Pleuropulmonary blastoma, Malignant melanoma, Desmoplastic small round cell tumor, Malignant peripheral nerve sheath tumor, Germ cell tumor, Histiocytosis, Rhabdomyosarcoma, Small cell osteosarcoma, Mesenchymal chondrosarcoma, Leukemia, Myeloma, Small cell carcinoma, Non Hodgkin's lymphoma, Small cell neuroendocrine carcinoma, Merkel cell carcinoma, Poorly differentiated Synovial sarcoma, Cellular myxoid/round cell liposarcoma, Medulloblastoma, Retinoblastoma, Hepatoblastoma, Melanotic neuroectodermal tumor, Rhabdoid tumor, Germ cell tumors, Hepatoblastoma, Sialoblastoma, Pancreatoblastoma, Neuroectodermal tumor, Angiosarcoma, Alveolar soft part sarcoma, Chondroblastoma, Clear cell sarcoma, Plasmacytoma, Solitary fibrous tumor and Neuroendocrine tumors.[2,5,6,7,8]

The histopathological assessment of SRCT is the initial step of the diagnostic procedure because of appreciable similarity of SRCT morphological picture. In differential diagnosis the immunohistochemical studies are essential. In 80-90% of cases diagnosis can be made on H & E staining slides coupled with IHC. However in 15- 20% there is a need for molecular or electron microscopic diagnosis. [1]

Materials and method:
The present study was undertaken to study the significance of immunohistochemistry for accurate characterization of round cell tumor and further sub typing of the tumor in the department of Pathology, Govt. Medical College, Surat.

Total 80 cases were studied, prospectively from June 2010 to November 2012, which were reported as round cell tumor using routine haematoxylin- eosin stains.

After histopathological examination, relevant panel of immunohistochemical antibodies was applied using peroxidase anti-peroxidase method. The final diagnosis was achieved after both histopathological and immunohistochemical findings.

The technique used was based on **PAP (peroxidase antiperoxidase) Method**. Monoclonal antibodies were used.

All specimens were fixed in 10% buffered formalin and embedded in paraffin (FFPE) according to standard procedures. Serial sections (4 µm in thickness) were used for haematoxylin and eosin staining (HE) and immunohistochemistry (IHC). Paraffin-embedded sections of the tumour were deparaffinized, rehydrated and heat-treated for antigen retrieval in a micro wave oven by giving 2-3 cycles at 95°C for 5 minutes and 100°C for 7 minutes in citric buffer solution, pH 9.0, 6.0 or 2.5. Subsequently, all sections were blocked in H2O2 blocking solution for 10 minutes and incubated with the primary antibody for 1 hour and then secondary antibody for 30 minutes at room temperature in a humidity chamber. For detection working DAB solution (1ml DAB buffer + 2drop of DAB chromogen) was used.

- Primary panel of antibodies consisting of CK, LCA, vimentin and S-100 was performed. According to their results, staining for various markers as shown in table was performed.
  In present study, weak/moderate/strong staining considered as positive staining and equivocal staining considered as negative staining.

Results:
Out of 80 cases of round cell tumor, the most common tumor was Non Hodgkin lymphoma (26 cases, 32.5%) followed by Germ cell tumor (15 cases, 18.75%). Varieties of RCT are given in Table 1. Each tumor was adequately categorized using the appropriate antibody panel. The recommended immunohistochemical panel for small round cell tumors is summarized in Table-2. Immunohistochemical staining was interpreted in the context of morphology in the appropriate cells concerned by comparing with the corresponding HE section.

Table 1: Distribution of cases on the basis of morphology

No.	Type	No of cases.	Percentage
1	Germ cell tumor	15	18.75%
2	Sarcoma (10 cases)		
	Rhabdomyosarcoma	3	3.75%
	Ewing's sarcoma/PNET	3	3.75%
	Undifferentiated round cell sarcoma	3	3.75%
	Poorly differentiated synovial sarcoma	1	1.25%
3	Melanoma	7	8.75%
4	Blastoma (7 cases)		
	Hepatoblastoma	1	1.25%
	Nephroblastoma	3	3.75%
	Pleuropulmonary blastoma	1	1.25%
	Retinoblastoma	1	1.25%
	Neuroblastoma	1	1.25%
5	Neuroendocrine tumors	5	6.25%
6	Carcinoma	4	5%
7	Non Hodgkin lymphoma	26	32.50%
8	Plasma cell neoplasms	2	2.50%
9	Mesothelioma	1	1.25%
10	Poorly differentiated round cell tumor	3	3.75%
	Total	80	100%

Table 2: Differential diagnosis of round cell tumor based on marker positivity

Primary marker	Differential diagnosis	Secondary markers	Final diagnosis
CK	GCT, Nephroblastoma, Carcinoma, Mesothelioma	PLAP/ CD 117/ AFP/ CD 30	Germ cell tumor
		EMA/CK 7	Carcinoma
		Calretinin	Mesothelioma
		vimentin/WT 1	Nephroblastoma
LCA	NHL		
Vimentin	Blastoma, Sarcoma	AFP	Hepatoblastoma
		CD 99	Ewing's sarcoma/ PNET
		Myogenin/ desmin	Rhabdomyosarcoma
		Chromo/ Synapto/ NSE	Neuroblastoma
		S 100	Pleuropulmonary blastoma
		no other marker	Round cell sarcoma
S 100	Melanoma, Neuroblastoma Neuroendocrine tumors	HMB 45	Melanoma
		NSE/Chromo/ synapto	Neuroendocrine tumors

1 case of retinoblastoma was positive for synaptophysin. 2 cases of plasma cell neoplasm were diagnosed based on serum electrophoresis and radiological investigations. IHC was not done due to lack of availability of plasma cell marker. 1 case was positive for vimentin, CK and EMA, CD 99 was equivocal, which was diagnosed as poorly differentiated synovial sarcoma.

Discussion:
Differential diagnosis of small round cell tumors (SRCT) constitutes frequently a difficult diagnostic problem. It appears that biopsy specimens of various neoplasms may present morphology of small round cell tumor (SRCT). [7] Accurate diagnosis of these cancers is essential because the treatment options, responses to therapy, and prognoses vary widely depending on the diagnosis. [4]

Immunohistochemistry is playing an increasing role in modern surgical pathology. The objective of performing immunohistochemistry (IHC) is to recognize cell constituents (antigens) and, consequently, to identify and classify specific cells within a cell population whose morphology is heterogeneous or apparently homogenous. The use of extensive panels of antibodies in all malignant undifferentiated neoplasms allows accurate histological diagnosis in more than 89% cases. [1]

In the present study we first applied primary panel of four antibodies: CK (epithelial marker), LCA (marker for lymphoma), vimentin (mesenchymal marker) and S 100 (marker for melanoma and neuroendocrine tissue). According to their results further IHC markers were applied.

Bashyal R et al [1], Ahmed Z et al [9] and present study found lymphoma as the most common tumor type (52.5%, 65.30% and 35% respectively) in round cell tumors. Thus our results correlate with other studies. Bianchini WA et al [10] found lymphoma and carcinoma as the most common tumor types (36.36% each). This is because they had done the study of head and neck tumors and carcinomas are the most frequent tumors in head and neck. [10]

IHC with a lymphoma panel of antibodies is a necessary test in most of SRCT cases. [7] All 26 cases showing LCA positivity were categorized as lymphoma. CD45 (LCA) is a surface antigen expressed by virtually all hematolymphoid proliferations, and monoclonal antibodies for this marker are reliably specific. [1] Lymphoma cases were diagnosed as T cell (4 cases) lymphoma and B cell (21 cases) lymphoma based on CD 3 and CD 20 positivity respectively. In 1 case markers for B and T cell were equivocal so it could not be differentiated accordingly. 1 case of B cell NHL was positive for CD 5 and negative for CD 23, which was diagnosed as Blastoid mantle cell lymphoma. 1 case was positive for both CD 5 and CD 23, and was diagnosed as SLL/CLL. In many studies, the distinction between MCL and SLL/CLL depends on the presence or absence of CD23. [11] Further categorization of other lymphoma cases was not possible due to unavailability of whole lymphoma panel.

Vimentin was found positive in cases of sarcomas and blastomas, which were further categorized by various markers. Cases positive for vimentin were diagnosed as EWS/PNET based on CD 99 positivity. Konrad P et al [7] showed 94.74%

cases and Bashyal et al [1] showed 100% cases of Ewing's sarcoma/ PNET to be to be positive for CD 99, correlating with our study. Immunohistochemically, strong membrane staining for CD99 is consistently seen in almost all cases of EWS/ PNET, although it is not very specific because it is shown in several other soft tissue sarcomas and lymphoblastic lymphomas. [1] Small cell Synovial sarcoma can be differentiated from EWS/PNET by positive staining for EMA(95-100%) of cases, CK(50%); it may also express CD99.[1] 1 case was positive for vimentin, CK and EMA, CD 99 was equivocal, which was diagnosed as poorly differentiated synovial sarcoma. 3 cases were diagnosed as Rhabdomyosarcoma based on myogenin and desmin positivity. Myogenin nuclear staining is considered very sensitive and specific for all variants of RMS. [7] Konrad P et al [7] showed 100% cases of Rhabdomyosarcoma to be positive for myogenin which correlates with our study. 3 cases were positive for vimentin. Panel of antibodies were performed but none was found reactive and further differentiation was not possible. So these cases were diagnosed as undifferentiated round cell sarcoma.

Vimentin positive cases of blastoma were further divided according to site and immunohistochemical profile. Hepatoblastoma is the most common primary hepatic tumor in children. Most tumor cells are also positive for EMA, pCEA, Hep Par-1 and α-fetoprotein (AFP). [12] In our study case of hepatoblastoma was found to be positive for vimentin and AFP. Nephroblastoma is the most common pediatric renal neoplasm. The blastemal component is typically reactive for vimentin. Nuclear staining for WT1 is positive in blastemal areas and in foci of early epithelial differentiation. More mature areas of epithelial differentiation such as tubules are typically reactive for cytokeratin. [12] In present study 3 cases of nephroblastoma were found to be positive for CK, vimentin and WT 1. Takagi et al [13] found 100% cases of Wilm's tumor to be positive for CK and vimentin, correlating with our study. Neuroblastoma shows immunoreactivity with NSE, synaptophysin, chromogranin-A, CD56, and NB84. [1] A fine network of cells that are positive for S-100 protein and vimentin surround the cells in a diagnostically distinctive fashion. [14] Herose et al [15] found synaptophysin and chromogranin to be reactive in 77% cases of Neuroblastoma. In present study case of neuroblastoma was positive for vimentin, chromogranin, synaptophysin and NSE; and negative for CD 99. CD99 is a useful marker for the distinction of neuroblastomas from other small, round, blue cell tumors. [12]

S 100 positive cases were further categorized as melanoma based on HMB 45 positivity and neuroendocrine tumors bases on positivity for NSE, synaptophysin and chromogranin. 7 cases were of melanoma showing positivity for S 100 and HMB 45. The S100 is a sensitive, albeit not a specific, melanoma marker, decorating more than 95% of MM's of primary and metastatic sites. The diagnosis of MM requires confirmation with a melanocyte-specific marker, including HMB-45, Melan-A. [1] Synaptophysin, NSE and chromogranin are markers for neuroendocrine differentiation. Okubo et al [16] showed 90% cases of neuroendocrine tumors to be positive for synaptophysin and 67.4% cases to be positive for chromogranin. Chromogranin is more specific, but less sensitive than synaptophysin. [12]

On performing further IHC on CK positive cases, those showing positivity for PLAP/ CD 117/ AFP/ CD 30 were diagnosed as germ cell tumor. The stains most often used for the diagnosis of malignant germ cell tumors include CD117, placental alkaline phosphatase (PLAP), Oct-4, α-fetoprotein, CD30, HCG and broad-spectrum antikeratins such as AE1/AE3. Cytokeratin AE1/AE3 stains many malignant germ cell tumors, and the pattern of staining can help with classification. [12] SK Lau et al [17] showed 94.74% cases of pure seminoma and 100% cases of mixed germ cell tumors to be CD 117 positive; and 100% cases of mixed germ cell tumors to be CD 30 positive. Cheville et al [18] showed 100% cases of seminoma to be PLAP positive. Immunoreactivity for PLAP has also been reported in 86% to 97% of embryonal carcinomas. [12] Kurman et al [19] showed immunoreactivity for AFP in 100% cases of yolk sac tumor. Our study correlates with above mentioned studies by showing positivity for PLAP/CD 117/ AFP/ CD 30 in cases of germ cell tumor according to the various components present. EMA is negative in most malignant germ cell tumors. [12] Cases showing CK, CK 7/EMA positivity were diagnosed as carcinomas. Few cases of carcinoma were also showing positivity for neuroendocrine markers. Small cell carcinomas may exhibit neuroendocrine, squamous or glandular differentiation. All 3 forms of small cell express cytokeratin reactivity. [20] 1 case was diagnosed as epitheloid mesothelioma which was positive for CK and calretinin. P.M. Cury et al. [21] also found 92% cases of mesothelioma to be positive for calretinin. Calretinin is specific marker for mesothelioma not present in adenocarcinoma. [22]

Retinoblastoma is commonest intraocular primary malignant tumor of childhood. [23] Synaptophysin a neural associated integral glycoprotein of presynaptic vesicle is concentrated in the synaptic regions of retina, and in the rosette forming cells of retinoblastoma. [24] Kenshi Yug et al [24] showed 100% of Retinoblastoma to be positive for synaptophysin. In our study also 1 case of retinoblastoma was found to be positive for synaptophysin.

In present study 3.75% cases were only defined as round cell tumor some due to exhaustion of the block during procedure or due to negative/unequivocal results of IHC markers. In a study by Konrad P et al [7] 25.95% cases and by Sajid H et al [25] 12.5% cases were inconclusive; only diagnosed as round cell tumor. In about ten percent (10%) of small round cell tumors particularly in the bones, the nature of the lesion remains undetermined even after immunohistochemical analysis. In such cases, the electron microscopy, cytogenetic studies and molecular techniques may be helpful. [25]

In this way our study correlated with various other studies in respect to most common tumor type and positivity of IHC markers in various cases of round cell tumor diagnosed. Our study also concluded that some cases of round cell tumor cannot be differentiated in any of the above mentioned categories by doing IHC alone, further correlating with other studies.

Conclusion:

The Round cell tumors are a heterogeneous group of malignant neoplasms. IHC represents a tool that can provide a clear distinction among the various tumor types. A panel approach is always recommended so that an antigenic profile of positive as well as negative markers will provide the most accurate characterization of tumor. Awareness of the diagnostic utility of several tissue or organ specific immunomarkers should help meet one of the most significant challenges in diagnostic pathology. In recent years, a better understanding of molecular genetic studies of these tumors allows molecular testing as a further valuable tool for definitive diagnosis in questionable cases.

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