Research Article

Role of immunohistochemistry in the differential diagnosis of malignant small round cell tumor: a study of 38 cases

Amita Patel, Mubin Patel*, Vasudha Bhagat, Kinnari Naik

Department of Pathology, Government Medical College, Majura gate, Surat, Gujarat, India

Received: 30 October 2015
Accepted: 20 November 2015

*Correspondence:
Dr. Mubin I Patel,
E-mail: mubin.smimer@gmail.com

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ABSTRACT

Background: Immunohistochemistry play a very important role in modern surgical pathology especially for identification of tumors lacking the evidence of lineage differentiation on the basis of routine Hematoxylin and Eosin (H&E) stain alone. More than 90% of the tumor with diagnostic difficulties by routine H & E stain could be very well classified by using IHC. The aim of present study is to classify and identify MSRCT.

Methods: The study was carried out in Department of Pathology, Government Medical College in a period from August 2008 to November 2011. Total 38 cases of MSRCT are selected for Immunohistochemical staining (IHC) and they are classified and categorized accordingly after IHC.

Results: Out of 38 cases of MSRCT, there are 18 cases (47.36%) of non-Hodgkin lymphoma, 3 (7.89%) cases of Neuroblastoma, 3 (7.89%) cases of Synovial Sarcoma, 2 (5.26%) cases of Ewing Sarcoma, 2 (5.26%) cases of Undifferentiated neuroendocrine carcinoma, 2 (5.26%) case of Desmoplastic Small Round Cell Tumor, 1 (2.63%) case of Primitive Neuroectodermal Tumor, 1 (2.63%) case of Amelanotic Melanoma (Small cell variant), 1 (2.63%) case of Anaplastic Dysgerminoma, 1 (2.63%) case of Osteosarcoma, 1 (2.63%) case of Wilms tumor, 1 (2.63%) case of Dendritic cell Tumor Testis, 1 (2.63%) case of Undifferentiated Nasopharyngeal Carcinoma, 1 (2.63%) case of Embryonal rhabdomyosarcoma.

Conclusions: IHC is very valuable tool for adequate and accurate categorization of MSRCT.

Keywords: IHC, Malignant Small Round Cell Tumor, Non-Hodgkin Lymphoma

INTRODUCTION

Malignant Small Round Cell Tumor (MSRCT) is defined as a highly undifferentiated neoplasm composed of monotonous population of small round cells with high nuclear to cytoplasmic ratio. Tumors that show good differentiation are generally easy to diagnose, but when a tumor is poorly differentiated, identification of the morphological features is difficult. Because of undifferentiated appearance of Malignant Small Round Cell Tumor, accurate diagnosis is difficult with routine H & E stain alone and so IHC is a valuable tool for all these cases. For treatment purposes and prognostic evaluation it is very important to differentiate whether the MSRCT is epithelial, mesenchymal, neuroendocrine, melanocytic, and hematopoietic. In more than 90% cases accurate diagnosis is made with the help of IHC along with routine H & E stain, but remaining cases require help of other ancillary technique like molecular or genetic study. Differential diagnosis of Malignant Small Round Cell Tumor are Non-Hodgkin lymphoma, Lymphoblastic Lymphoma, Retinoblastoma, Hepatoblastoma,
Neuroblastoma, Synovial Sarcoma, Ewing Sarcoma/Primitive Neuroectodermal Tumor, Undifferentiated Neuroendocrine carcinoma, Desmoplastic Small Round Cell Tumor, Dysgerminoma, Osteosarcoma, Wilm's tumor, Mesenchymal Chondrosarcoma, Malignant Melanoma (Small Cell variant), Dendritic cell Tumor, Nasopharyngeal Carcinoma and Rhabdomyosarcoma.¹⁻⁴

For treatment purposes, it is crucial to determine whether an undifferentiated neoplasm is epithelial, mesenchymal, or hematopoietic. Treatment may include surgery, radiotherapy, chemotherapy, hormone therapy or a combination. For example, squamous cell carcinoma is treated with radiotherapy while adenocarcinomas require primary surgery since it is not as radiosensitive. For many types of sarcomas, combining surgery with radiation treatment has proven more effective than treatment with surgery alone. Chemotherapy is used to treat three types of sarcomas, all of which tend to occur in children under the age of 18: Osteosarcomas (bone sarcomas), Ewing’s sarcomas (bone and soft-tissue sarcomas) and Rhabdomyosarcoma (muscle sarcomas) and radiation may be combined. Chemotherapy is now the main stay of therapy for most cases of lymphoma with local radiotherapy. Targeted therapy like anti-CD20 monoclonal antibody has been shown to be an effective therapy for CD20 positive B-cell lymphoma. Malignant melanoma is treated by surgery/ palliation/ interferon. Extra gonadal germ cell tumors and neuroendocrine carcinomas are treated by chemotherapy. In general, the diagnosis of lymphoma for an undifferentiated tumor predicts a better clinical outcome compared with that of carcinoma.⁵

METHODS

The study was carried out in Department of Pathology, Government Medical College, in a period from August 2008 to November 2011. Total 38 cases reported as Malignant Small Round Cell Tumor were selected and IHC study has been done for conclusive diagnosis. Age group of the patient is between 2 years to 70 years. Out of 38 cases, 23 were males and 15 were females. The immune-profile selected for this study were Pan cytokeratin (CK), Vimentin, S-100, CD 45 (LCA), HMB 45, CD 99, EMA, Synaptophysin, Chromogranin, NSE, NF, CEA, Desmin, Myogenin, Calretinin, BCL-2, CD117, CD68, CD34, SMA, PLAP. IHC markers for secondary panel for Lymphoma used in present study were CD3, CD5, CD10, CD15, CD20, CD23, and CD30.

The technique used was based on PAP (peroxidase anti-peroxidase) Method. The protocol for IHC staining used in our laboratory is as following.⁶⁻¹⁴

1. Cut tissue section 4 mm thick and spread sprinkle free on to the poly-L-lysine coated slides (temperature of wax water bath should be at 60° C).
2. Put the slides in oven at 95° C for 10 minutes.
3. For deparaffinisation give 3-changes of xylene 10 minutes each.
4. Rehydrate the tissue with graded isopropyl alcohol (100%, 80%, 70%, 50%) 5 minutes each.
5. Then Transfer the slides in running tap water for 10 minutes.

Antigen retrieval:

1. Prepare fresh citric buffer solution of pH 6 for all markers except (a) Myogenin, Ki67, WT1 on 2.5 pH and (b) CD5, CD10, CD3, CD30, CD99 on 9 pH.
2. Put the slides in citric buffer solution and keep this bowl in microwave oven for antigen retrieval.
3. For Antigen retrieval :Give THREE cycles at 95° C temp for 5 min for all markers except (a) CD68: Give two cycle at 95° C temp for 5 min and (b) CD10 : Give three cycle at 95° C temp for 5 min and give second cycle at 100 °C temp for 7 min.
5. Then put the slides in wash buffer.
6. Wipe the area aside the section and put the slides in humidity chamber.
7. Then add peroxide blocking solution on the slide for 10 min. Then put the slides in wash buffer for 10 min. Wash with Tris buffer and give 2-changes each 10 min.
8. Cover the tissue with power block for 10 min. Do not give wash after power block.
9. Mark the tissue with marker pen.
10. Add the primary antibody for 1hr/Incubation depend on literature.
11. Wash three times with tris buffer (each wash 10 min).
12. Add secondary antibody for 30 min. Wash three times with Tris buffer (each wash 10 min).
13. Prepare the working DAB solution (1ml DAB buffer + 2 drops of DAB chromogen).
14. Add working DAB solution for 5 min.
15. Give wash with tap water for 2-3 min.
16. Counter stain with Hematoxylin for 1min.
17. Wash under running tap water and air dry.
18. Put the slide rack in alcohol for 2 min.
19. Air dry and deep the rack in xylene.
20. Air dry and mount the slide in DPX (DisrteneDibutyl-Pthalate Xylene) and then observe the slide.

Interpretation of staining:

1. The positive control was examined for the presence of colored end product at the site of target antigen (DAB chromogen – brown end product). The presence of these colors was interpreted as a positive staining result, indicating proper performance of kit reagents)
2. Test specimen stained with the primary antibody was then examined. The absence of a colored end product was interpreted as a negative result.
3. Only intact cells were examined for presence or absence of staining since necrotic and degenerated cells often stain non-specifically.

RESULTS

Out of 38 cases of malignant small round cell tumor, there were 18 cases of (47.36%) of Non-Hodgkin lymphoma, 3 (7.89%) cases of Neuroblastoma, 3 (7.89%) cases of Synovial Sarcoma, 2 (5.26%) cases of Ewing Sarcoma, 2 (5.26%) cases of Undifferentiated neuroendocrine carcinoma, 2 (5.26%) case of Desmoplastic Small Round Cell Tumor, 1 (2.63%) case of Primitive Neuroectodermal Tumor, 1 (2.63%) case of Amelanotic Melanoma (Small cell variant),1 (2.63%) case of Anaplastic Dysgerminoma, 1 (2.63%) case Osteosarcoma, 1 (2.63%) case of Wilm’s tumor, 1 (2.63%) case of Undifferentiated Nasopharyngeal Carcinoma, 1 (2.63 %) case of Embryonal rhabdomyosarcoma.

<table>
<thead>
<tr>
<th>Differential Diagnosis of Malignant Small Round small tumor</th>
<th>Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non Hodgkin Lymphoma (18 cases)</td>
<td>Nasopharynx (2), Cervical (5), Axillary (1), Stomach (2), Ileal (1), Mesenteric (1), Rectal (1), Inguinal (2), Ovarian (1), Testis (2)</td>
</tr>
<tr>
<td>Synovial Sarcoma (3 cases)</td>
<td>Mesenteric mass (1), Supraclavicular mass (1), Retropertoneal mass (1)</td>
</tr>
<tr>
<td>Ewing's Sarcoma (2 cases)</td>
<td>Left thigh (1), Left Shoulder (1)</td>
</tr>
<tr>
<td>Undifferentiated neuroendocrine carcinoma (2 cases)</td>
<td>Testicular mass (1), Abdominal mass (1)</td>
</tr>
<tr>
<td>Desmoplastic Small Round Cell Tumor (2 cases)</td>
<td>Retropertoneal mass (1), Abdominal mass (1)</td>
</tr>
<tr>
<td>Primitive Neuro-Ectodermal Tumor (1 case)</td>
<td>Omental mass (1)</td>
</tr>
<tr>
<td>Amelanotic melanoma (Small Cell Variant) (1 case)</td>
<td>Skin- abdomen (1)</td>
</tr>
<tr>
<td>Anaplastic Dysgerminoma (1 case)</td>
<td>Left Ovarian Mass (1)</td>
</tr>
<tr>
<td>Osteosarcoma (1 case)</td>
<td>Nasal mass (1)</td>
</tr>
<tr>
<td>Wilm's tumor (1 case)</td>
<td>Right Renal mass (1)</td>
</tr>
<tr>
<td>Dendritic cell Tumor (1 case)</td>
<td>Testicular mass (1)</td>
</tr>
<tr>
<td>Undifferentiated Nasopharyngeal Carcinoma (1 case)</td>
<td>Nasal mass (1)</td>
</tr>
<tr>
<td>Embryonal rhabdomyosarcoma (1 case)</td>
<td>Axillary mass (1)</td>
</tr>
</tbody>
</table>

Main role of IHC is to recognize cell antigen and to identify and classify specific cell within cell population. After addition of Fluorochrome conjugate or enzyme to the antibody, visualization of Antigen-antibody complex is made possible, which is seen under the microscope. There is use of extensive primary and secondary panel of Antibody for categorization of undifferentiated tumor.

The most common MSRCT in present study was Non-Hodgkin Lymphoma (18 cases). The tumor cells show Cytoplasmic and Membranous reactivity for CD45 (LCA). CD45 is a surface antigen expressed by virtually all haematolymphoid proliferations, and antibodies for this marker are specific.15

In present study, 18 cases of NHL were found. Out of 18 cases, 9 cases were nodal and 9 cases were extra-nodal. There were 14 cases of B cell Lymphoma and 4 cases of T cell Lymphoma. In all suspected cases of Non-Hodgkin’s lymphomas, a panel of antibodies including LCA (CD45), CD20 (Pan B markers), CD3 (Pan T markers), CD5, CD10 and CD30 are used. ALCL can only be diagnosed by immunohistochemistry because it is difficult to differentiate Anaplastic large cell lymphoma (ALCL) from diffuse large B cell lymphoma (DLBCL). In suspected cases of ALCL, Epithelial membrane antigen (EMA) and CD30 are also used. Now a day’s large number of cases of ALCL are diagnosed which in the past may have been misdiagnosed as Hodgkin’s lymphoma or Diffuse Large B cell lymphoma. In present study 1 case of ALCL was diagnosed which showed reactivity for LCA, CD 3, Vimentin and non-reactive for CD5, EMA, NSE and Chromogranin. There are prognostic and therapeutic differences between B cell and T cell Non-Hodgkin’s Lymphomas which make immune-histological characterization very important and can be used for targeted therapy.15

DISCUSSION

In present study 3 cases of Neuroblastoma was found. Out of three, one case was presented as a nasal mass, was reported as MSRCT (In routine H & E stain) with two differential diagnosis: (1) Olfactory Neuroblastoma and
(2) Undifferentiated Sino nasal carcinoma, IHC study shows NSE (+), NF (+), CK (+), S-100 (+), LCA (-), Vimentin (-), EMA (-), so the final diagnosis was Olfactory Neuroblastoma.

Table 2: IHC findings in different types of Non-Hodgkin Lymphoma (NHL) found in present study.

<table>
<thead>
<tr>
<th>Various Non-Hodgkin Lymphoma</th>
<th>No. of cases</th>
<th>IHC panel- Positive (Results of IHC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLBCL (Diffuse large B cell Lymphoma)</td>
<td>10</td>
<td>LCA 10, Vimentin 4, CD20 10, CD3 0, CD5 0, CD30 1</td>
</tr>
<tr>
<td>B cell NHL</td>
<td>3</td>
<td>LCA 1, Vimentin 3, CD20 1, CD3 0, CD5 0, CD30 1</td>
</tr>
<tr>
<td>Blastoid NHL</td>
<td>1</td>
<td>LCA 1, Vimentin 1, CD20 0, CD3 1, CD5 0, CD30 1</td>
</tr>
<tr>
<td>Natural Killer cell NHL (NK NHL)</td>
<td>1</td>
<td>LCA 1, Vimentin 1, CD20 0, CD3 1, CD5 0, CD30 1</td>
</tr>
<tr>
<td>T cell ALL</td>
<td>1</td>
<td>LCA 1, Vimentin 1, CD20 0, CD3 1, CD5 0, CD30 1</td>
</tr>
<tr>
<td>Peripherial T cell Lymphoma (PTCL)</td>
<td>1</td>
<td>LCA 1, Vimentin 1, CD20 0, CD3 1, CD5 0, CD30 1</td>
</tr>
<tr>
<td>Anaplastic Large Cell Lymphoma (ALCL)</td>
<td>1</td>
<td>LCA 1, Vimentin 1, CD20 0, CD3 1, CD5 0, CD30 1</td>
</tr>
</tbody>
</table>

Other case was also presented as nasal mass and reported as MRSCRT (In routine H & E stain) with two differential diagnosis: (1) Neuroblastoma and (2) Undifferentiated Carcinoma, IHC study shows NSE (+), S 100 (+) while CK (-), LCA (-), Vimentin (-), EMA (-), so the final diagnosis was Neuroblastoma.

Table 3: IHC findings in different cases of MRSCRT (Other than NHL) found in present study.

<table>
<thead>
<tr>
<th>DD of MRSCRT in present study (Other than NHL)</th>
<th>Positive IHC marker</th>
<th>Negative IHC marker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuroblastoma (3 cases)</td>
<td>CK (1/3), NSE, NF, S-100</td>
<td>CK (2/3), LCA, Vimentin, CD99, EMA, Desmin</td>
</tr>
<tr>
<td>Synovial sarcoma</td>
<td>Vimentin, CK, BCL2, Calretinin</td>
<td>S-100, LCA, CD34, CD99</td>
</tr>
<tr>
<td>Ewing sarcoma</td>
<td>Vimentin, CD99, S-100</td>
<td>CK, LCA</td>
</tr>
<tr>
<td>Poorly differentiated Neuroendocrine carcinoma</td>
<td>CK, Vimentin, NSE, CD56, Synaptophysin(1/2), Chromogranin(1/2)</td>
<td>Desmin, Synaptophysin (1/2), Chromogranin (1/2), CD 99, Myogenin, SMA, CD 20, Calretinin, WT-1, Inhibin</td>
</tr>
<tr>
<td>Desmoplastic Small Round Cell Tumor (DSRCT)</td>
<td>Vimentin, Desmin, EMA, NSE</td>
<td>CK, LCA, S-100</td>
</tr>
<tr>
<td>Primitive Neuroectodermal Tumor (PNET)</td>
<td>Vimentin, NSE</td>
<td>CK, S-100, LCA, NF, Myosin</td>
</tr>
<tr>
<td>Amelanotic melanoma (Small cell variant)</td>
<td>Vimentin, S-100, HMB45, Synaptophysin, NSE</td>
<td>Desmin, Chromogranin, EMA, BCL2, CD34, CD99, Myogenin</td>
</tr>
<tr>
<td>Anaplastic Dysgerminoma</td>
<td>Vimentin, PLAP, CD117</td>
<td>CK, LCA, S-100, CD30</td>
</tr>
<tr>
<td>Osteosarcoma</td>
<td>Vimentin, Desmin, CD99</td>
<td>CK, LCA, S-100, EMA, SMA</td>
</tr>
<tr>
<td>Wilm’s tumor</td>
<td>Vimentin, EMA, NSE</td>
<td>CK, S-100, WT1</td>
</tr>
<tr>
<td>Dendritic cell tumor</td>
<td>CD68, LCA, CD3, CD23, Synaptophysin, S-100</td>
<td>CK, CD10, CD79a, CD56</td>
</tr>
<tr>
<td>Undifferentiated Nasopharyngeal carcinoma</td>
<td>CK, NSE</td>
<td>Vimentin, LCA, S-100</td>
</tr>
<tr>
<td>Embryonal Rhabdomyosarcoma</td>
<td>Vimentin, S-100, Desmin, Myogenin</td>
<td>NF, Synaptophysin, EMA, SMA, CD99, CEA</td>
</tr>
</tbody>
</table>

In present study, 3 cases of Synovial sarcoma found, all cases were immunopositive for CK, BCL 2, Vimentin, and Calretinin, while they were immunonegative for S-100, LCA, CD34, CD99, Desmin and HMB 45.

In present study, 2 cases of Ewing sarcoma was found, out of that one case was reported as MRSCRT (In routine H & E stain) with two differential diagnosis: (1) Ewing sarcoma and (2) Alveolar Rhabdomyosarcoma, IHC study shows CD 99 (+), NSE (+), while Desmin (-), Myosin (-), EMA (-). Because of the negativity of the muscle marker Desmin and Myosin, the possibility of Alveolar RMS was ruled out, and diagnosis given was Ewing sarcoma.

In present study, 2 cases of Undifferentiated Neuroendocrine carcinoma was found, one case of Testicular mass was reported as MRSCRT (In routine H & E stain) with three possibilities: (1) Alveolar Rhabdomyosarcoma, (2) Desmoplastic Small Round Cell Tumor (DSRCT) and (3) Ewing sarcoma/PNET. IHC study shows NSE (+), CD 56 (+), while Desmin (-), Myosin (-), CD99 (-), Calretinin (-), SMA (-), Synaptophysin (-), Chromogranin (-). In this case with
the help of IHC, the final diagnosis was neuroendocrine carcinoma which was entirely different from previous morphological diagnosis, in this case NSE and CD56 were positive, while other neuroendocrine markers like Synaptophysin and Chromogranin were negative, which suggests that at least three markers for neuroendocrine lineage should be performed before rule out the possibility of neuroendocrine carcinoma.

The other case of Neuroendocrine carcinoma was reported as MSRCT (In routine H & E stain) with three possibilities: (1) Alveolar Rhabdomyosarcoma, (2) Neuroendocrine carcinoma and (3) Malignant melanoma. IHC study shows NSE (+), Synaptophysin (+), Chromogranin (+), while Desmin (-), Myosin (-), SMA (-), HMB45 (-). And the final diagnosis was neuroendocrine carcinoma in this case.

In present study, 2 cases of Desmoplastic Small Round Cell Tumor (DSRCT) was found, they were reported as MSRCT (In routine H & E stain) with two possibilities: (1) Lymphoma and (2) DSRCT. IHC study shows Vimentin (+), EMA (+), Desmin (+), NSE (+) while LCA (-), CK (-). LCA was negative in this case so Lymphoma was ruled out; furthermore there were characteristic co-expression of mesenchymal and epithelial marker which confirms the diagnosis.

In present study, 1 case of Primitive Neuroectodermal Tumor (PNET) was found, which was reported as MSRCT with two differential diagnosis (1) DSRCT and (2) Primitive neuro-ectodermal Tumor and IHC study shows Vimentin (+), CD99 (+), NSE (+) while CK (-), S-100 (-), LCA (-), NF (-) so final diagnosis was PNET.

In present study, 1 case of Amelanotic melanoma was reported, the case was reported as MSRCT (In routine H & E stain), IHC study shows HMB45 (+), S-100 (+), Vimentin (+), Desmin (-), LCA (-), CK (-). Therefore the case was reported as Amelanotic melanoma- small cell variant (Because of positivity of HMB45) though there was absence of melanin pigment in this case, it was reported as Melanoma, and this was possible with the use of IHC study.
In present study, 1 case of Anaplastic Dysgerminoma was found, IHC study shows Vimentin (+), PLAP (+), CD117 (+), while CK (-), LCA (-), S-100 (-), CD30 (-).

In present study, 1 case of Osteosarcoma was found, IHC study shows Vimentin (+), Desmin (+), CD99 (+) while CK (-), LCA (-), S-100 (-), EMA (-), SMA (-).

In present study, 1 case of Wilm’s tumor was found, IHC study shows Vimentin (+), EMA (+), NSE (+), while CK (-), S-100 (-), WT1 (-). Wilm’s tumor although show WT1 positivity, but in our study it was WT1 negative.

In present study, 1 case of Dendritic cell tumor was found, IHC study shows CD68 (+), LCA (+), CD3 (+), CD23 (+), Vimentin (+), S-100 (+) while CK (-), CD10 (-), CD79a (-), CD56 (-).

In present study, 1 case of Undifferentiated Nasopharyngeal carcinoma was reported. This case is a Malignant Small round cell tumor (In routine H & E stain) with three possibilities: (1) Ewing sarcoma /PNET, (2) Undifferentiated Carcinoma and (3) Lymphoma. IHC study shows CK (+), Vimentin (+), NSE (+) while LCA (-), CD99 (-), S-100, Chromogranin (-). LCA was negative in this case so Lymphoma was ruled out furthermore CD99 was negative so Ewing sarcoma /PNET were ruled out, and final diagnosis was nasopharyngeal carcinoma.

In present study, 1 case of Embryonal Rhabdomyosarcoma was found, IHC study shows Vimentin (+), Desmin (+), Myogenin (+), S-100 (+), while NF (-), Synaptophysin (-), EMA (-), SMA (-), CD99 (-), CEA (-), CD99 (-). Although differential diagnosis of Malignant Small Round Cell Tumor also includes Lymphoblastic Lymphoma, Retinoblastoma, Hepatoblastoma, Mesenchymal Chondrosarcoma but we did not get these cases in our study.

CONCLUSION

Malignant Small Round Cell Tumor are a group of neoplasm that are very well classified and diagnosed with the help of IHC, to ensure the appropriate and specific treatment. Because IHC can provide this with high precision, it must be performed with high standard. But in questionable cases in addition to IHC, molecular & genetic studies can use as a valuable tool.
Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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