Histopathological and Immunohistochemical Approach for Characterization of Malignant Round Cell Tumors

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ABSTRACT

Background and Objectives: To analyze the role of immunohistochemistry in characterization of malignant round cell tumors

Patients and Methods: Immunohistochemical staining (IHC) with (monoclonal or polyclonal) antibodies was performed on 127 cancer cases reported as malignant round cell tumors over a 12 month period from July 2008 through August 2009.

Setting: Department of histopathology, Duhok Central Labs

Results: Malignant round cell tumors were more frequently located in the respiratory tract 30 (23.6%) followed by gastrointestinal tract 25 (19.7%), lymph node 19 (14.9%), and bone/soft tissue 19 (14.9%). Among these, 75 (82.7%) cases were primary and 22 (17.3%) metastatic. Application of immunohistochemistry resulted in characterization of 112 (88.2%) cases. Non-Hodgkin lymphoma 21 (16.5%) was at the top of the diagnosed list followed by adenocarcinoma 20 (15.7%), sarcoma 17 (13.4%), and small undifferentiated carcinoma 15 (11.8%).

Conclusions: Immunohistochemistry is a very helpful tool for characterization of malignant round cell tumors.

Key words: Malignant round cell tumor, immunohistochemistry .

INTRODUCTION:

Malignant round cell tumors include a diverse group of cancers that appear morphologically as round cells. These may be large undifferentiated malignant neoplasms which are generally, but not always, presenting as a lymph node based process and usually suggesting the differential diagnosis of poorly differentiated carcinoma, high grade melanoma, or high grade lymphoma [1, 2]. More commonly malignant round cell tumors include cancers consisting of small to intermediate cells having a dark, hyperchromatic nuclei and scant or indistinct cytoplasm; these malignant blue cell tumors include primitive neuroectodermal tumor (PNET)/Ewing's sarcoma family, lymphoma/leukemia, malignant melanoma, neuroendocrine carcinoma, small undifferentiated carcinoma (SUC), Merkel cell carcinoma, neuroblastoma, medulloblastoma, desmoplastic small round cell tumor (DSRCT), synovial sarcoma, and rhabdomyosarcoma, etc… [3-8]. The optimal treatment of patients with cancer depends on establishing accurate diagnosis by using a complex combination of clinical and histopathological data. In malignant round cell tumors, this task is difficult or impossible because of similar morphological appearances in histopathology [9, 10]. Through the identification of specific cellular components, using specific monoclonal or polyclonal antibodies, immunohistochemistry (IHC) has emerged as the most practical adjunct tool to the histopathology making accurate diagnosis possible[9, 10,11]. However, the complex distribution patterns of many antigens and loss of some differentiation antigens in malignant tumors often necessitate the use

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the role of IHC in determining conclusive diagnoses of malignant round cell tumors. The occurrence of these tumors and the way in which they were distributed according to the localization were also evaluated.

METERIALS AND METHODS:

During a 12 month period, from August 2008 through July 2009, a total of 127 cases were diagnosed as malignant round cell tumors in the department of histopathology, Duhok Central Labs, Duhok, Iraq. Data including age and site of the tumor was obtained from histopathology request forms. The IHC technique used was carried out by the labelled streptavidin biotin method on manufactured by DAKO Corporation (Dako Denmark A/S), 3-3'-diaminobenzidine tetrahydrochloride (DAB) was used as chromogen, and the antigen retrieval was done by microwave heat (12-16). Three µm sections were mounted on silanized slides and allowed to dry overnight at 56°C, deparaffinized with xylene and blocked for endogenous peroxidase with 3% H_2O_2. The buffer used was Tris-buffered saline (TBS, 0.05 M) and the counterstain was Harris hematoxyllin (12-17). Appropriate positive and negative controls were run in all cases. The primary antibodies used were of rabbit or mouse origin depending on the target antigen. A basic panel of antibodies was applied first with respect to the patient's age, tumor location, and cell pattern (Table 1).

Table 1. Immunohistochemical staining of malignant round cell tumors (1-12)

Type of tumor	Vimentin	S-100	Desmin	CK (pan)	CD99	NSE	NFP	WT1	TTF1	LCA	Actin	HMB45	Chromogranin	EMA
Ewing's/PENT	+	+/-	-	-	+	+/-	-/+	-	-	-	-	-	-	+/-
DSRCT	-	-	+	+	+/-	+/-	-	+	-	-	-	-	-	+
Neural tumors	-	+/-	-	-	-	+	+/-	-	-	-	-	-	-	-
SUC	-	-	-	+	-/+	+/-	+/-	-	+	-	-	-	+	+
Neuroendocrine Ca.	-	-	-	+	+/-	+	-	-	+/-	-	-	-	+	+/-
Rhabdomyoarcoma	+	-	+	-	-	-	-	-	-	-	-	-	-	-
Melanoma	-	+	-	-	-	-	-	-	-	-	-	+	-	-
Synovial sarcoma	+	-	-	+	+	-	-	-	-	-	-	-	-	+
Lymphoma	-	-	-	-	+/-	-	-	-	-	+	-	-	-	+/-
Mesothelioma	-	-	-	+	-	-	-	+	-	-	-	-	-	+
Merkel cell carcinoma	-	-	-	+	-	+	+	-	-	-	-	-	-	+
Adenocarcinoma	-	-	-	+	-	-	-	+	-	-	-	-	-	+

CK: Cytokeratin, NSE: Neuron specific enolase, NFP: Neurofilament protein, WT-1: Wilm's tumor-1, TTF-1: Thyroid transcription factor-1, LCA: Leukocyte common antigen, EMA: Epithelial membrane antigen, Ca: Carcinoma.

Then additional antibodies were used for final diagnosis; in lymphoma the antibodies used were Kappa and Lambda immunoglobulin light chain, CD20 and CD79a (Pan B markers), CD2, CD3, CD4, CD8, and CD43 (Pan T markers), CD15 (GAA), and CD 30 (ki-1). In suspected cases of anaplastic large cell lymphoma (ALCL), CD30, EMA and ALK-1 protein were applied. CD10 with Ki67 were used for giving the diagnosis of Burkitt's lymphoma (CD10+, Ki67+ in 100%), CD56 for natural killer (NK)/T-cell lymphoma, CD5, CD23, and cyclinD1 were applied to distinguish Mantle cell lymphoma (CD5+, CD23-, cyclin D1+) from small cell lymphoma (CD5+, CD23+, cyclin D1-). In suspected leukemic infiltration, certain markers were applied (CD117, CD13, CD34, and myeloperoxidase) [12, 17, and 18]. In metastatic tumors, antibodies were applied according to the patient's age, pattern of cells, and site of the organ involved to exclude primary tumors; cytokeratins were used together with lymphoid markers to rule out lymphomas in the lymphoid organs and with GFAP to rule out high grade astrocytoma in the brain [8, 12, 18, 20, 22]. In the liver, the cytokeratins CAM 5.2 and AE1/ AE3 were applied to distinguish primary hepatocellular carcinoma (CAM 5.2+, AE1/ AE3-) from metastatic carcinoma (CAM 5.2+, AE1/AE3+) [12,23]. CK7 and CK20 were used to predict the site of origin of the primary tumor whether colonic (CK20+, CK7-) or gastric (CK7+, CK20+/-) [8, 12]. CK7 was also used with lactalbumin for diagnosis of breast carcinoma and with CA-125 for diagnosis of ovarian malignancy [7, 8,12]. PSA was applied to confirm a metastatic prostatic adenocarcinoma [24]. The immunos profile used for categorization of malignant blue cell tumor cases included antibodies specific for small undifferentiated carcinoma, neuroblastoma, medulloblastoma, Merkel cell carcinoma, rhabdomyosarcoma, DSRCT, synovial sarcoma, and panel of antibodies, S-100 protein, HMB45 and MelanA were used to confirm the diagnosis of malignant melanomas [8, 12, and 26]. Immunohistochemistry was also applied to differentiate mesothelioma from metastatic adenocarcinoma by using a panel of antibodies, calretinin, thrombomodulin, and CK 5/6, which are positive in mesothelioma and negative in adenocarcinoma [8, 12, 27].

RESULTS:

The diagnosis of malignant round cell tumors was given for 127 cases. Among specific organs involved, respiratory tract 30 (23.6%) was at the top of the list followed by gastrointestinal tract 25 (19.7%), lymph node and bone/soft tissue 19 (14.9%) each (Table 2). Metastatic tumors formed 17.3% (n=22) of all cases. Table 3 shows the final histopathological diagnoses. Non-Hodgkin lymphoma was the most frequently identified pathology 21 (16.5%). Of these, 6 (28.6%) were nodal and 15 (71.4%) extranodal. Only 2 (9.5%) cases were of T-cell type, one nodal ALK -1+ anaplastic large T cell lymphoma (ALCL) and one nasal natural killer (CD56+) T cell lymphoma. The remainders (90.5%) were B-cell lymphomas: 12 diffuse large, 3 Burkitt's, 1 Mantle cell, 1 small cell (SLL/CLL), 1 T cell-rich B cell lymphoma and the last case was leukemic infiltration of the skin (Table 4). Poorly differentiated adenocarcinoma was the second most frequently identified pathology 26 (20.5%). Of these, 12 were primary and 8 metastatic (Table 5). As shown in table 6, sarcomas 17 (14.4%) were classified as Ewing's/ PNET, embryonal rhabdomyosarcoma, round cell variant of liposarcoma, monophasic synovial sarcoma, osteogenic sarcoma, and malignant peripheral nerve sheath tumor. The diagnosis of small undifferentiated carcinoma was given in 15 (11.8%) cases, 10 primary and 5 metastatic (Table 7).

Table 2. Distribution of the study cases on the basis of location.

Organ/tissue	Number	Percentage
Respiratory T	30	23.6
Gastrointestinal T	25	19.7
Lymphoid tissue *	19	14.9 (*Including 2 cases of bone marrow)
Bone/Soft tissue	19	14.9
Female genital T	7	5.5
CNS	6	4.7
Male genital T	4	3.1
Eye	4	3.1
skin	4	3.1
Kidney	3	2.4
Adrenal	2	1.6
Breast	2	1.6
Bladder	1	0.8
Ear	1	0.8

Table 3 . Distribution of the study cases on the basis of morphology

Morphology	Number	Percentage
Non-Hodgkin lymphoma	21	16.5
Adenocarcinoma	20	15.7
Sarcoma	17	13.4
Small undifferentiated carcinoma	15	11.8
Nasopharyngeal carcinoma	7*	5.5
Neuroendocrine carcinoma	7	5,5
Squamous cell carcinoma	5	3.9
Neuroblastoma	4	3.1
Malignant melanoma	3	2.4
Mesothelioma	3	2.4
Merkel cell carcinoma	3	2,4
Retinoblastoma	2	1.6
Medulloblastoma	2	1.6
Miscellaneous	3**	9.4
Uncertain	15	11.8

*** Five nasopharyngeal carcinoma were primary, 2 metastatic in cervical lymph nodes**

**** Wilm's, GM, & DSRCT**

Table 4. Distribution of lymphoma cases on the basis of site (n=21)

Organ/tissue	*Number*	*Percentage*
Lymph node	6	28.6
Skin	2*	9.5
Stomach	2	9.5
Salivary gland	2	9.5
Others	9**	38.1

*One case is leukemic infiltration

** Colon, sinonasal, small intestine, liver, testis, ovary, bone, bone marrow, and breast

Neuroblastoma was identified in 4 (3.1%) cases (adrenal, renal, sinonasal, and cervical LN), retinoblastoma in 2 cases in the eye, one almost completely calcified leaving a peripheral rim of malignant blue cells and the other formed a big mass involving the eye and extending to the peiorbital region making the diagnosis by H&E impossible. Two cases of medulloblastoma were diagnosed in the cerebellum; one seeding via the CSF down to the lower vertebrae and the other was so undifferentiated making the differentiation from Glioblastoma multiforme (GM), PNET, and metastatic carcinoma very difficult. The diagnosis of malignant melanoma was given for 3 cases in the rectum, nose, and inguinal LN.Mesothelioma was diagnosed in 3 cases, 2 pleural and 1 mesenteric and Merkel cell carcinoma was seen in 3 cases in the skin, LN, and kidney. Poorly differentiated squamous cell carcinoma was diagnosed in 5 (3.9%) cases and there was 1 extrarenal monophasic wilm's tumor, 1 cerebellar GM, and 1 abdominal DSRCT. In 15 (11.8%) cases, the diagnosis was given as malignancy of uncertain histogenesis after application of extensive panels of antibodies.

Table 5. Distribution of adenocarcinoma cases on the basis of site (n=20)

Type	Organ/tissue	Number (%)
Primary (n=12)	Stomach	5 (35,7)
	Colon	3 (21.4)
	Prostate	2 (14.3)
	Others	2 (28.6) (Lung and skin)
Metastatic (n=8)	Lymph node & BM	5 (41.7)
	Pleura	2 (16.7)
	Bone	1 (41.7)

Table 6. Distribution of sarcoma cases (n=17)

Type	Number (%)
Ewing's/PNET	10 (58.8)
-Extraskeletal	8*
- Skeletal	2**
Rhabdomyosarcoma	3 (17.6)
Others***	4 (23.6)

*8 extra-skeletal (2 thigh, 1 retroperitoneum, 1 shoulder, 1 CNS, 1 eye, and 2 metastatic in LN and bone)

** Vertebra and pelvis

*** Others: liposarcoma (round cell variant), monophasic synovial sarcoma, osteogenic sarcoma, and MPNST*

Table 7. Distribution of small undifferentiated carcinoma cases (n=15)

Type	Site	Number (%)
Primary (10)	Sinunasal	5 (33.3)
	Larynx	3 (20)
	Lung	2 (13.3)
Metastatic (5)	LN	3 (20)
	Others*	2 (13.3) (*Pleura and liver)

DISCUSSION:

This study indicated that Non-Hodgkin lymphoma (16.5%) was at the top of the diagnosed malignant round cell tumors; we demonstrated that the positive rate of IHC in lymphoma is much higher than carcinoma in undifferentiated round cell tumors. This finding is correlated with that reported by some authors [20, 21] but higher than 5.2% reported by others [8]. We also found high frequent B cell types of non-Hodgkin lymphoma (90.5%). Therefore it is suggested that IHC clearly characterizes malignant round cell tumors especially in difficult and challenging cases of non-Hodgkin lymphoma. Adenocarcinomas of various organs (including the breast) comprise a large chunk of malignant tumors. In the present study, only 20 cases challenged the routine histopathological diagnosis. These cases were represented as a very little or crushed material of true-cut or endoscopic biopsy specimens. An important application of IHC is to detect and characterize micrometastases [8,19,20]. In the current study, definite diagnosis was given in 22 (17.3%) metastatic malignant round cell tumors. This finding may be of benefit for the search of new cases different from the classical once based on their unlimited histogenesis. On the basis of immunohistochemical analysis, using panels of antibodies, malignant blue cell tumors were accurately diagnosed and subcategorized into EWS/PNETs, neuroblastoma, emryonal rhabdomyosarcoma, DSRCT, synovial sarcoma, and Merkel cell carcinoma. Again IHC was extremely helpful for the diagnosis of 15 cases of small undifferentiated carcinoma and 7 cases of neuroendocrine carcinomas which comprise another diverse category those often present difficulties in diagnosis. Immunos performed in the form of a panel of antibodies including S-100 protein, HMB45 and MelanA resulted in accurate diagnosis of 3 cases of malignant melanoma. In the current study, IHC was also essential to mesothelioma and to exclude metastatic adenocarcinoma in the pleura and mesentery. Application of extensive panels of antibodies in the current study resulted in definite diagnosis of 88.8% of malignant round cell tumors. Site and distribution patterns of immunos staining whether nuclear, cytoplasmic, membranous, or any combination were of a great importance for final diagnosis. For example, almost all lymphoma markers, EMA, and CD99 produce cell membrane staining; WT-1, TTF-1 are nuclear; S-100 protein is nuclear +/- cytoplasmic (only cytoplasmic is nonspecific); CK, GFAP, NFP, and HMB45 are cytoplasmic [1-12]. The most interesting finding in this study is the diagnosis of malignant round cell tumors in uncommon locations like malignant melanoma in rectum and nose, Merkel cell carcinoma in the kidney and lymph node, leukemic infiltration in the skin, and monophasic synovial sarcoma near the tarsal bones of the foot. Nevertheless, conclusive diagnosis was impossible in 11.2% of cases, probably due to limitations of the technique, antigen changes during tissue fixation or true absence of cellular differentiation. However, we should keep in mind that this study is only definitive and a small sample sized research.

CONCLUSIONS AND RECOMMENDATIONS:

Immunohistochemistry is very useful for accurate characterization of malignant round cell tumors, especially in difficult and challenging cases, whereas in some cases even after application of extensive panels of antibodies, diagnosis may still be problematic because of the presence of overlapping morphologic and immunophenotypic features. In such cases, molecular techniques may able the pathologist to achieve the goal.

REFERENCES:

1. Gatter KC, Alcock C, Heryet A, Pulford KA, Heyderman E, Taylor – Papadimitriou J, Stein H, and Mason DY. The differential diagnosis of routinely processed anaplastic tumors using

monoclonal antibodies. Am J Clin Path. 1984;82:33 -43.
2. Delellis RA and Dayal Y. The role of immunohistochemistry in the diagnosis of poorly differentiated malignant neoplasms. Semin Oncol 1987;14:173-192.
3. Sebire NJ, Gibson S, Rampling D, Williams S, Malone M, and Ramsay AD: Immunohistochemical findings in embryonal small round cell tumors with molecular diagnostic confirmation. Appl immunohistochem Mol Morphol. 2005;13:1-6.
4. Halliday BE, Slagel DD, Elsheikh TE, and Silverman JF. Diagnostic utility of MIC-2 immunocytochemical staining in the differential diagnosis of small blue cell tumors. Diagn Cytopathol. 1998;19:410-6.
5. Katz RL, Quezado M, and Senderowicz AM. An intraabdominal round cell neoplasm with features of primitive neuroectodermal and desmoplastic round cell tumor and a EWS/FLI-1 fusion transcript. Hum Pathol. 1997;28:502-9.
6. Ambros IM, Ambros PF, Strehl S, Kovar H, Gadner H, and Salzer-Kuntschik M. MIC2 is a specific marker for Ewing's Sarcoma and peripheral primitive neuroectodermal tumors. Cancer. 1991;67:1886-1893.
7. Lakhtakia CR and Nema BSK. Immunophenotyping of tumours. MJAFI 2008;64:16-20.
8. Ahmad Z, Azad NS, and Bhurgari Y. Significance of immunohistochemistry in accurate characterization of malignant tumors. www.toodoc.com/Immunohistochemistry-ebook.html.
9. Chan JK. Advances in immunohistochemistry: impact on surgical pathology practice. Semin. Diagn. Pathol. 2000;17:170-177.
10. Slapak CA and Kufe DW. Principles of cancer therapy. In Isselbacher KJ, Braunvald E, Wilson JD, Martin JB, Fauci AS, Kasper DL eds: Harrison's Principles of Internal Medicine, 13th Ed. Vol 2, 1994; McGraw-Hill, Inc. 1826-1840.
11. Raab SS. Cost effectiveness analysis in pathology. Clin. Lab. Med. 1999;19:757-71.
12. Special techniques in Surgical Pathology. In Rosai J, 9th Ed: Vol 2, Rosai and Ackerman's Surgical Pathology. New York, Mosby. 2004. 45-63.
13. Taylor CR. The total test approach to standardization of immunohistochemistry. Arch. Pathol. Lab. Med. 2000;124:945-51.
14. Larsson L. Tissue preparation methods for light microscopic immunohistochemistry. Appl. Immunohistochem. 1993;1:2-16.
15. Hsu SM, Raine L, and Fanger H. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. J. Histochem. Cytochem. 1981;29:577 -80.
16. Shi SR, Cote RJ, and Taylor CR. Antigen retrieval
17. Chan JKC, Banks PM, Cleary ML, Delsol G, De Wolf-Peeters C, Falini B, and Gatter KC. A Revised European–American Classification of lymphoid neoplasms proposed by the International Lymphoma Study Group. A summary version. Am J Clin Pathol 1995;103:543-560.
18. Jaffe ES, Harris NL, Stein H, and Vardiman JW. Tumors of hematopoitic and lymphoid tissues, pathology and genetics. World Health Organization classification of tumors, Lyon IARC Press, 2001.
19. Bianchini WA, Altemani AM, and Paschoal JR. Undifferentiated head and neck tumors: the contribution of immunohistochemical technique to differential diagnosis. Sao Paulo Med J 2003; 121(6):244-247.
20. Coindre JM, Tanguy F, Merlfo JP, De Mascarel I, De Mascarel A, and Trojani M. The value of immunohistological techniques in undifferentiated cancers. Tumori. 1986;72:539-44.
21. Gatter KC, Alcock C, Heryet A, and Mason DY. Clinical importance of analyzing malignant tumours of uncertain origin with immunohistological techniques. Lancet. 1985;1:1302-5.
22. Kleihues P, Davis RL, Ohgaki H, Burger PC, Westphal MM, and Cavanee WK. Diffuse astrocytoma. In Kleihues P, Cavanee W (eds): World Health Organization classification of tumors. Pathology and genetics – tumors of the nervous system. 2000; lyon, IARC Press, 22-26.
23. Johnson DE, Herndier BG, Medeiros LJ, Warnke RA, and Rouse RV. The diagnostic utility of the keratin profiles of hepatocellular carcinoma and cholangiocarcinoma. Am J Surg Pathol. 1988;12:187-197.
24. Papsidero LD, Croghan CA, Asirwattham J, Gaeta J, Abenoza P, Englander L, and Valenzuela L. Immunohistochemical demonstration of Prostate Specific Antigen in metastases with the use of monoclonal antibody. Am J Pathol. 1985;121:451- 454.
25. Frierson HF, Mills S, Fechner R, etal. Sinonasal undifferentiated carcinoma: an aggressive neoplasm derived from schneiderian epithelium and distinct from olfactory neuroblastoma. AmJSurgPathol. 1986;10:771-779.
26. Yaziji H and Gown AM. Immunohistochemical markers of melanocytic tumors. Int J Surg Pathol. 2003;11:11-15.
27. Ordonez NG. Value of cytokeratin 5/6 immunostaining in distinguishing epithelial mesothelioma of the pleura from lung adenocarcinoma. Am J Surg Pathol. 1998;22:1215-1221.