PEDIATRIC PATHOLOGY

The Contribution of Immunohistochemistry in the Diagnosis of Pediatric Neoplastic Lesions in the University of Puerto Rico, School of Medicine.

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Immunohistochemistry has revolutionized the field of diagnostic pathology in the past fifteen years. Since then, it has been increasingly used as an adjunct to morphological diagnosis. The purpose of this retrospective analysis is to examine the value of this technique in the diagnosis of pediatric neoplasias in our laboratory. Forty eight pediatric cases, collected from January 1998 until May 1999, were reviewed, and classified in one of four categories: confirmed the morphological diagnosis, provided the definite diagnosis from a list of probable diagnoses, contributed by excluding other entities, and non-contributory. Immunohistochemistry confirmed the morphological diagnosis in 29 cases (60.4%), provided the definite diagnosis from a list of probable diagnoses in 13 cases (27.1%), was contributory by exclusion of other entities in 2 cases (4.2%), and was non-contributory in 4 cases (8.3%). In this preliminary study, we conclude that immunohistochemistry is being used in our laboratory mostly as a confirmatory tool for the definitive diagnosis of the lesions and once more exalts the utility of this technology in the field of diagnostic pathology.

Key words: Immunohistochemistry, Pediatric neoplasias

Malignancy is the main cause of death from disease among children younger than 20 years of age, with approximately 1 in 475 children being diagnosed with cancer before the age of 15. In the United States, the incidence in 1992 of childhood cancer in children 15 years of age and under was 7800 new cases, according to the Surveillance Epidemiology and End Results (SEER) of the National Cancer Institute (1). Pediatric neoplasias differ from those in adults, who most commonly have tumors of epithelial origin. Most of the childhood cancers are of primitive embryonal tissue, resulting in sarcomas, leukemias, lymphomas and central nervous system (CNS) tumors. A large group of these tumors appear in light microscopy as small, round, blue cell tumors. Since distinct pathological entities have this appearance, such as neuroblastoma, lymphoma, rhabdomyosarcoma and primitive neuroectodermal tumors, ancillary studies are needed to arrive to the correct diagnosis (2,3). The optimum therapy can only be established when the neoplasm is diagnosed correctly.

In the past 20 years, immunohistochemistry has proved to be a useful adjunct to morphological diagnosis, especially in neoplastic lesions (4). Although most diagnoses can be reached using routine H&E stains, there remain a number of lesions whose nature cannot be established with certainty. This is especially true of lymphomas and poorly differentiated neoplasms (5-8). When a diagnostic dilemma arises, appropriate therapy cannot be instituted. Immunostains then provide a way of classifying these lesions by using a panel of antibodies directed against specific tissue antigens (9,10) present in the cells.

The purpose of this study was to examine the value of this technique in the diagnosis of pediatric neoplastic lesions in our laboratory. The value of the immunostains was evaluated by assigning each case one of four different categories.

Materials and Methods

Forty eight consecutive pediatric cases were selected from the accession book of the Immunohistochemistry Laboratory of the University of Puerto Rico School of

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Medicine from January 1998 until May 1999, referred to us because of diagnostic difficulties when examined histologically with H&E stains, to confirm a morphological diagnosis and to classify lymphomas as to cell lineages. The biopsies belonged to patients evaluated in the Universitary Pediatric Hospital, San Juan City Hospital, and *Hospital Interamericano de Medicina Avanzada.* The immunohistochemistry reports included clinical history, gross and microscopic findings, and the results and interpretation of the immunoperoxidase stains (Appendix 1).

The formalin fixed paraffin embedded tissue blocks were cut and the slides were placed in the oven for two hours at 75°C. They were deparaffinized in Xylol and rehydrated in graded ethanol and water. Each slide was properly labeled and placed in the Ventana Automated Slide Stainer (Ventana Medical System, Inc. Tuczon, AZ). The antibodies used are listed in Appendix 2.

The cases were classified as general pediatric pathology, neuropathology and hematopathology. All the slides were reviewed by at least two of the authors. The contribution of the immunohistochemical results was assessed by assigning them to one of the following four categories:

I. Confirmation of the morphological diagnosis
II. Definitive diagnosis from a list of probable diagnoses
III. Contributory by exclusion of other entities
IV. Non contributory

Results

The results are summarized in Table 1. In 29 of the 48 cases analyzed (60.4%), the immunohistochemistry was useful in confirming the morphological diagnosis. Of these cases, there were 10 of general pediatric pathology, 13 of hematopathology and 6 of neuropathology. The immunostains were done to confirm the cell lineages in these biopsies.

In 13 of the 48 cases (27.1%), immunohistochemistry provided a definitive diagnosis from a list of probable diagnoses. Hematopathology and general pediatric pathology cases made up most of these cases, with 5 and 5 cases respectively. They were especially useful to differentiate between lymphomas and small round cell tumors. The immunostains were done to identify neural, myogenic and/or histiocytic elements in the lesions in the general pediatric cases. In the three neuropathology cases, this technique provided the diagnosis of choroid plexus papilloma, anaplastic ependymoma, and pituitary adenoma.

Immunoperoxidases were contributory by exclusion of other entities in 2 cases (4.2%), both of general pediatric pathology. The negative immunostaining for S-100, myoglobin and muscle specific actin helped to exclude neurogenic or myogenic lesions in these cases.

Immunohistochemical staining was non-contributory in 4 cases (8.3%), 2 of general pediatric pathology and 2 of neuropathology. The two neuropathology cases fell in this category due to scant biopsy material for a definitive diagnosis. One of the general pediatric cases was a 38 days old baby girl with a probable diagnosis of Langerhan's cell histiocytosis. The biopsy was negative for S-100, however tissue histology did not provide any additional diagnostic possibilities. The other general pediatric case was a 3 month old girl with a granulosa cell tumor of the ovary, in which the immunostains were noncontributory to the diagnosis due to technical problems in the processing of the sample.

Table 1. Number of cases in each category according to the contribution of immunohistochemical analysis

Category	General	Neuropathology	Hematopathology	Total
I Confirmed diagnosis	10	6	13	29 (60.4%)
II Definitive diagnosis	5	3	5	13 (27.1%)
III Diagnosis by exclusion	2	0	0	2 (4.2%)
IV Non-contributory	2	2	0	4 (8.3%)
Total	19	11	18	48 (100%)

Discussion

The immunostains were found to be contributory in most cases, as found in other studies (11-13). Our results showed that immunohistochemical analysis was mostly used to confirm the morphological diagnosis. In the general pediatric cases, there were already one or two possible diagnoses based on the morphology. The immunostains confirmed that the cell types present were consistent with the working diagnosis. This was significant in the cases in which the diagnosis of sarcoma was confirmed. In the germ cell tumors, the staining results indicated the germ cell tumor components present in cases of mixed germ cell tumors. They were also useful to differentiate between two small round cell tumors. In the hematopathology cases, the specific panel of antibodies that reacted with the specimen enabled the classification of the lymphoma by identifying the cell lineages present. Definite diagnosis of 6 of 11 neuropathology cases was possible with H&E stains, but immunostains were done to confirm the origin of the neoplastic cells.

The immunostains provided the definitive diagnosis among a list of probable diagnoses in 27.1% of the cases.

Most of these biopsies were lesions in which it was difficult to differentiate between a small round cell tumor and a lymphoma or a tumor of another origin.

The immunostains were useful to exclude possible diagnoses in 2 general pediatric lesions, referred from the Dermatology Department. Antibodies against myoglobin and muscle specific actin were used to exclude myogenic lesions. Anti S-100 was similarly used to rule out lesions of neural origin.

In the cases where immunostains were non-contributory, they were due to technical difficulties. Two were neuropathology cases, a glioma by frozen section and a suprasellar mass. Neither could be properly analyzed because of scanty biopsy material. The other case was a suspected Langerhan's cell histiocytosis where an immunostain for S-100 was negative. However, tissue histology did not provide any additional diagnostic possibilities. The fourth case was a 3 month old infant with a granulosa cell tumor in which immunostains for placental alkaline phosphatase (PLAP), and alpha feto protein (AFP), were noncontributory due to technical problems in the processing of the sample.

Conclusions

The results of the immunostains were contributory in 92% of the cases. They were mostly used to confirm the morphological diagnosis. In the general pediatric cases, the stains confirmed that the cell types present were consistent with the preliminary diagnosis. They also indicated the germ cell tumor components present in mixed germ cell tumors. In addition, the stains were used to differentiate between two small round cell tumors. In the hematopathology cases, the specific antibody panel enabled the classification of the lymphoma by identifying the cell lineages present. In the neuropathology, the immunostains confirmed the origin of the neoplastic cells.

The immunostains were used to give a definitive diagnosis from a list of probable diagnoses in cases in which it was hard to differentiate between a small round cell tumor, a lymphoma or a tumor of another origin. They were also useful to exclude lesions of neural or myogenic origin. In the cases where the immunostains were non-contributory, they were due to technical difficulties.

Resumen

La immunohistoquímica ha revolucionado el campo de la patología diagnóstica en los últimos 15 años. Desde entonces, se ha utilizado como herramienta complementaria en el diagnóstico morfológico. El propósito de este análisis retrospectivo es evaluar el valor de esta técnica en el diagnóstico de neoplasias pediátricas en nuestro laboratorio. Cuarentiocho casos pediátricos, obtenidos entre enero de 1998 a mayo de 1999 fueron revisados y clasificados en una de cuatro categorías: confirmatorio del diagnóstico morfológico, proveedor del diagnóstico definitivo, contributorio por exclusión, y no contributorio. La immunohistoquímica confirmó el diagnóstico en 29 casos (60.4%), proveyó diagnóstico definitivo en 13 casos (27.1%), fue contributorio por exclusión en 2 casos (4.2%), y no contributorio en 4 casos (8.3%). En este estudio preliminar, concluimos que en nuestro laboratorio, la immunohistoquímica se utiliza mayormente para confirmar el diagnóstico definitivo de las lesiones y una vez más exalta la utilidad de esta tecnología en el campo de la patología diagnóstica.

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APPENDIX I: General Pediatric Pathology Cases				
Age	**Immunostains**	**Working diagnosis**	**Final Diagnosis**	**Category**
17 years	Myoglobin +;desmin +; actin -	Embryonal rhabdomyosarcoma	Recurrent embryonal rhabdomyosarcoma	I
9 months	Vimentin +; desmin+; S-100+;O13+;CD15-; lysozyme; chloroacetate esterase-; synaptophysin-; myoglobin-;smooth muscle actin-; NSE-;LCA-	Ewing sarcoma /alveolar rhabdomyosarcoma (SRCT)	Alveolar rhabdomyosarcoma	II
11 months	S-100+;lysozyme+	Langerhan's cell histiocytosis	Langerhan's cell histiocytosis	I
6 years	Alpha-1-antitrypsin+; alpha actin+;desmin-;S-100-;keratin-;smooth muscle actin-	Plexiform fibrohistiocytic tumor	Plexiform fibrohistiocytic tumor	I
14 years	PLAP+;AFP+;HCG+	Dysgerminoma/yolk sac tumor/mixed germ cell	Malignant mixed germ cell tumor	I
3 months	PLAP+;AFP+;keratin-	Germ cell tumor	Granulosa cell tumor	IV
7 years	Alpha-1-antitrypsin+;Muramidase+;S-100-	Fibrohistiocytic tumor	Benign fibrous histiocytoma	I
8 years	Alpha-1-antitrypsin+;Actin-;desmin-;NSE-; myoglobin-	Fibrohistiocytic tumor/myogenic lesion/neural lesion	Benign fibrous histiocytoma	II
16 years	Keratin+; alpha-1-antitrypsin+; PLAP+; AFP+;HCG-	Germ cell tumor	Malignant mixed germ cell tumor with mature and immature teratoma, embryonal carcinoma and yolk sac elements	I
15 years	Alpha-1-antitrypsin+; NSE-;myoglobin-; keratin; actin-;alpha smooth muscle actin-; PLAP-	Malignant fibrous histiocytoma/Rhabdomyosarcoma-/Germ cell tumor	High grade pleomorphic spindle cell sarcoma	II
4 years	CD3+;CD30+;lambda+;Kappa+;CD20-	Atypical lymphoid infiltrate	Pseudolymphoma	I
15 years	S-100+;desmin+;NSE-; LCA non reactive;myoglobin-; O13-	Myogenic sarcoma (SRCT)	Alveolar rhabdomyosarcoma	II
9 years	Vimentin+;myoglobin-; muscle specific actin-	r/o Rhabdomyosarcoma	Atypical fibrous histiocytoma	III
1 year	S-100-	Infantile digital fibroma	Infantile digital fibroma	I
8 years	Vimentin+;S-100-;alpha actin-;smooth muscle actin-; chromogranin-	r/o Neurogenic and myogenic lesion	Fibrous histiocytoma	III
38 days	S-100-	Langerhan's cell histiocytosis	Langerhan's cell histiocytosis	IV
1 year	S-100+;chromogranin+; NSE+	Neuroblastoma	Neuroblastoma	I
16 days	S-100+;actin+;myoglobin-desmin-	Spindle cell neoplasm	Infantile fibrosarcoma	II
13 years	Desmin+;actin+;Vimentin+;myoglobin-;S-100-	Alveolar rhabdomyosarcoma	Alveolar rhabdomyosarcoma	I

B. Neuropathology Cases				
Age	**Immunostains**	**Working diagnosis**	**Final diagnosis**	**Category**
17 years	S-100+;vimentin+	Neurofibroma	Orbital neurofibroma	I
9 years	GFAP+;synaptophysin-	Astrocytoma	Astrocytoma	I
15 years	GFAP-;synaptophysin+	Oligodendroglioma	Anaplastic oligodendroglioma	I
4 months	GFAP+;synaptophysin-	Astrocytoma	Anaplastic astrocytoma	IV
1 year	Synaptophysin+;NSE+;GFAP-	PNET	PNET	I
11 years	pankeratin+;EMA-;GFAP-; reticulum+;prealbumin-	Choroid plexus papilloma/ependymoma	Choroid plexus papilloma	II
3 years	S-100+;EMA+;Vimentin+	Chordoma	Atypical chordoma	I
6 years	GFAP+;synaptophysin-; prealbumin-	Neurocytoma/ Ependymoma	Anaplastic ependymoma, WHO grade III	II
7 months	GFAP+	Gliotic tissue	Non diagnostic	IV
10 years	GFAP-;GH+	Astrocytoma/pituitary adenoma	Pituitary adenoma	II
16 years	HCG+;prolactin+;ACTH+	Pituitary adenoma	Pituitary adenoma	I

C. Hematopathology Cases				
Age	**Immunostains**	**Working Diagnosis**	**Final Diagnosis**	**Category**
10 years	CD79+;CD45RO-; CD20+;CD3-	Malignant lymphoma, Burkitt's type	Malignant lymphoma, small non-cleaved cell, Burkitt's type	I
16 years	CD15+;CD30+;LCA+;CD20 non-specific	Hodgkin's disease, nodular sclerosis	Hodgkin's disease, nodular sclerosis, syncitial variant, left neck node	I
5 years	CD15-;CD3-;CD20-; CD79+;CD45RO+;CD30+; HDLM3+;CD4 3 non-specific	Hodgkin's disease	Hodgkin's disease, nodular sclerosis, partial involvement, neck node	I
6 years	Muramidase+;chloroacetate esterase-; CD15-; CD43+; CD3+; CD45RO-;CD45RA+; CD20-	Leukemia/lymphoma/granulocytic sarcoma	Malignant lymphoma, lymphoblastic T-cell diffuse	II
17 years	CD20+;CD79+;CD3-; CD45RO-	Malignant lymphoma, mainly large cell	Malignant lymphoma, mainly large cell, B cell phenotype, diffuse, neck node	I
9 years	CD45RO+;CD3+;CD20-; CD45RA-CD79-	T cell lymphoma/ Burkitt's lymphoma	Malignant lymphoma, lymphoblastic T cell, mediastinal lymph node	I
4 years	CD45RO+;CD20-;CD3+; LCA+;muramidase unspecific;chloroacetate esterase-	Malignant lymphoma/monocytoid lymphoid infiltrate	Malignant lymphoma, Tcell lineage, large cell, diffuse, thymus	I
5 years	CD45RO-;desmin-;S-100-; actin-;chromogranin-,LCA+; CD20+	Lymphoma/alveolarrhabdomyosarcoma	High grade B cell lymphoma, Burkitt's lymphoma	II
11 years	CD20+;CD79+;CD45RO-; CD3-	Malignant lymphoma	Malignant lymphoma, diffuse, B cell lineage, Burkitt's-like, parotid	I
16 years	CD45RO-;CD20+;LCA+	Malignant lymphoma	Malignant lymphoma, B cell lineage, mainly large cell, diffuse, tracheal lymph nodes	I
12 years	CD20-;CD30+;CD43-; CD45RO-;CD15-; lysozyme-;LCA-;S-100-; CD15-;HMB45-	Undifferentiated malignant tumor	Malignant lymphoma, pleomorphic, CD30 (Ki-1) positive, left neck	II
4 years	CD20-; CD45RO+; CD3+; CD79-	Leukemic infiltration	T cell leukemia, infiltrate testicles, bilateral	I
14 years	CD3-;CD45RO-;CD20+; CD45RA+;CD15+;CD30+	Malignant lymphoma	Hodgkin's disease, nodular sclerosis, syncitial variant, left neck nodule	I
9 years	CD45RO-;CD20-;LCA+; CD15-;S-100-;NSE-; chromogranin-;actin-	PNET/ rhabdomyosarcoma/ malignant lymphoma/ Ewing's sarcoma	Malignant lymphoma, lymphoblastic T cell	II
17 years	LCA+;S-100-;CD43+ synaptophysin-; NSE+; O13+;desmin-;myoglobin-;CD5-;CD79-;CD45RO-;CD3+;CD45RA+;CD20-	Small round cell tumor/Ewing's sarcoma/PNET	Malignant lymphoma, convoluted lymphoblastic T cell, spine tumor	II
3 years	CD79+;muramidase+; CD20+;CD45RO-	Reactive process/lymphoma	Malignant lymphoma, high grade B cell category	I
7 years	CD43+;CD45RO+;CD20-;CD45RA+	Malignant lymphoma	Lymphoblastic lymphoma, Tcell	I
15 years	CD43+;CD30+;LCA+;CD15-; CD45RO-;EMA-;CD3-; CD45RA-CD20-;CD79-	Malignant lymphoma	Large Tcell lymphoma,diffuse, CD30+	I

Appendix 2: Antibodies used in the immunostaining of the tissues.		
Antibody	**Clone**	**Company**
Epithelial membrane antigen	Mc 5	Ventana Medical Systems
Muscle actin	HUC 1-1	Ventana Medical Systems
Smooth muscle alpha actin	1A4	Dako
Pan-keratin	AE1/AE3/PCK26	Ventana Medical Systems
S-100	Rabbit polyclonal	Ventana Medical Systems
Vimentin	VIM 3B4	Ventana Medical Systems
Ki-67	MM	Ventana Medical Systems
Desmin	NCL-DE-R-11	Ventana Medical Systems
LCA	RP2/18	Ventana Medical Systems
NSE	BBS/NC/VI-H-14	Ventana Medical Systems
GFAP	Rabbit polyclonal	Ventana Medical Systems
Myoglobin	Rabbit polyclonal	Ventana Medical Systems
Synaptophysin	Rabbit polyclonal	Ventana Medical Systems
Lysozyme (muramidase)	Rabbit polyclonal	Ventana Medical Systems
Kappa light chain	Rabbit polyclonal	Ventana Medical Systems
Lambda light chain	Rabbit polyclonal	Ventana Medical Systems
Naphthol AS-D Chloroacetate esterase		Sigma Diagnostic
Placental alkaline phosphatase	Rabbit polyclonal	Ventana Medical Systems
HCG	Rabbit polyclonal	Ventana Medical Systems
Prealbumin	Rabbit polyclonal	Dako
CD3	PS1	Ventana Medical Systems
CD3	Rabbit polyclonal	Dako
CD4	1F6	Ventana Medical Systems
CD5	4C7	Ventana Medical Systems
CD8	1A5	Ventana Medical Systems
CD10	56C6	Ventana Medical Systems
CD15	MMA	Ventana Medical Systems
CD15	C3D-1	Dako
CD20	L26	Ventana Medical Systems
CD20	L26	Dako
CD23	1B12	Ventana Medical Systems
CD25/IL2-2Ra	IL2R-1	Neomarkers, Inc.
CD30	1G12	Ventana Medical Systems
CD30	Ber-H2	Dako
CD34	QBEnd/10	Biogenex
CD43	L60	Ventana Medical Systems
CD45RO	A6	Ventana Medical Systems
CD45RO	UCHL1	Dako
CD57	NK-1	Ventana Medical Systems
CD68	514H12	Ventana Medical Systems
CD79a	11E3	Ventana Medical Systems
Chromogranin	LK2H10	Ventana Medical Systems
Chromogranin	Rabbit polyclonal	Dako

Alpha-fetoprotein	C3	Ventana Medical Systems
Ewing's Sarcoma O13	O13	Signet Laboratories, Inc.
Alpha-1-antitrypsin	Rabbit polyclonal	Dako
Melanosome	HUB45	Ventana Medical Systems
Growth Hormone	Rabbit polyclonal	Dako
Human Neurofilament	2F11	Dako
Prolactin	Rabbit polyclonal	Dako
ACTH	Rabbit polyclonal	Dako