SIGNIFICANCE OF IMMUNOHISTOCHEMISTRY IN ACCURATE CHARACTERIZATION OF MALIGNANT TUMORS

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Background: To determine in a large series of surgical biopsies the role and significance of immunohistochemistry in the adequate and accurate characterization of malignant tumors. **Methods:** A retrospective study of 20,000 consecutive surgical biopsies reported in the Section of Histopathology, AKU in 2003. Data was obtained by retrieving the filed surgical biopsy reports in the section. **Results and Conclusions:** Out of the 20,000 biopsies, 6534 (32.67%) were neoplastic. 4726 neoplasms (72.33%) were malignant, and 1808 (27.67%) were benign. Immunohistochemistry was performed on 29.49% of malignant tumors, and 4.97% of benign tumors. Immunos were performed on only 2.82% of routine squamous cell carcinomas and adenocarcinomas of various organs, and in only 1.9% of infiltrating breast carcinomas, the commonest malignant tumors in females. In contrast, immunos were performed on 97.12% of non-Hodgkin's lymphomas, 97.94% of Hodgkin's lymphomas, 98.09% of malignant spindle cell neoplasms, 87.96% of small round blue cell tumors of childhood, 87.30% of neuroendocrine neoplasms, and 84.37% cases of malignant melanomas. In addition, immunos were performed on all cases of malignant undifferentiated neoplasms and were able to resolve the issue in over 89% of such cases. Immunos were also performed on 54.74% of metastatic tumors. Lymph nodes were the commonest organs on which immunos were performed i.e. 96.50% of lymph node tumors, followed by CNS and renal neoplasms with 33.01% and 25.92% respectively.

Key Words: Malignant tumours, Karachi, Immunochemistry

INTRODUCTION

The histologic diagnosis of cancer and the categorization of the proper tumor type is essential for the adequate treatment of malignant tumors. Histologic subtyping with the help of immunohistochemical characterization of tumors has resulted in a level of distinction between diagnoses which was not previously possible. In addition to substantiating the diagnosis of malignancy, this subtyping provides information which is essential in guiding therapy. The distinction between histologically similar tumors is often critical as therapeutic options often differ.[1]

Immunohistochemistry has emerged as the most valuable adjunct to Hematoxylin and Eosin (H&E) staining in diagnostic histopathology.[2] No other method during the past fifty years has had such a major impact on histopathology.[3] This technique has equipped the histopathologist with the tools needed to tackle the most common diagnostic problems in tumor pathology especially the characterization of the undifferentiated or poorly differentiated malignant tumors, whether primary or metastatic.[4-6] The impact of immunohistochemistry has been enhanced by the large number of good quality antibodies that are available commercially and improvements in antigen retrieval techniques.[7]

No modern histopathology laboratory can hope to function without adequate immunohistochemistry facilities. The section of Histopathology at the Aga Khan University Hospital (AKUH) has state of the art immunohistochemistry facilities and a large, diverse and ever-expanding panel of antibodies is available. This has proved invaluable in the development of our section as the major referral center especially for tumor pathology in Pakistan.

The aim of our study was to determine in a large series of surgical biopsies, the role and significance of immunohistochemistry in the adequate and accurate characterization of malignant tumors.

MATERIAL AND METHODS

A retrospective study of 20,000 consecutive surgical biopsies reported in the section of Histopathology, AKUH in 2003. Data was obtained by retrieving the filed surgical biopsy reports in the section.

The immunohistochemistry technique used is a combination of immunocytochemistry, which attaches the tracer to the specific antigen within the tissue sections, and standard enzyme histochemistry which visualizes the tracer for bright field or electron microscopy.

Several procedures are available. The two most commonly used are the peroxidase antiperoxidase immune complex method and the biotin avidin immunoenzymatic technique. In the latter, the high affinity of avidin for biotin is used to couple the peroxidase label to the primary antibody.

We use the envision system. This is a two step immunohistochemistry staining technique. This is based on an HRP labeled polymer against which primary antibodies produced in mice react well. These primary mouse antibodies are supplied by the user. The combination then reacts with the antigens in paraffin embedded tissues cryostat tissues etc. Tissues processed in a variety of fixatives may be used. The procedure used is described in table 1.

It is of utmost importance to use fresh solutions; make sure antibody is not expired, run appropriate controls for every batch of each antibody. It is recommended that immunos are done on all cases of Non-Hodgkin's and Hodgkin's lymphoma, all cases of undifferentiated malignant neoplasms, all sarcomas for further characterization, in metastatic tumors, sometimes even in benign lesions for adequate characterization and on cytology cell blocks.

RESULTS

Out of the 20,000 consecutive biopsies, 6534 (32.67%) were neoplastic and 13,466 (67.33%) were non-neoplastic. Of the neoplastic lesions 4726 (72.33%) were malignant, while 1808 (27.67%) were benign. Immunohistochemistry was performed on 29.49% of malignant tumors.(Table-1)

Very few (2.82%) of the routine squamous cell carcinomas, adenocarcinomas of various organs required immunohistochemistry. These carcinomas comprised a large chunk of malignant tumors. There were 1665 cases of such ordinary types of carcinoma and another 736 infiltrating carcinomas of the breast. Together, these 2401 tumors comprised 50.80% of all malignant tumors. But immunohistochemistry was required in only a tiny percentage of these cases. Infiltrating carcinomas of the breast, which comprise the commonest malignant tumors in females required immunohistochemistry in only 1.9% cases. On the other hand, immunohistochemistry was performed on 97.12% of Non Hodgkin's lymphomas, 97.94% of Hodgkin's lymphomas, 98.09% of malignant spindle cell neoplasms, 87.96% of small round blue cell tumors of childhood, 87.30% of neuroendocrine neoplasms, and 84.37% cases of malignant melanomas. (Table-2)

Malignant undifferentiated neoplasms required immunohistochemistry in all cases (n=213). Immunohistochemistry allowed accurate diagnosis in 190 of these cases (89.20%). In less than 11% such cases, immunohistochemistry was unhelpful. Metastatic tumors (n=316) comprised 6.69% of all malignant tumor. Immunohistochemistry was performed in 173 out of 316 metastatic tumors or 54.74%.

Table-1: Distribution of cases on the basis of biological behavior and morphology

Category of Lesion	Total Number	Number of cases on which immunos were performed	Percentage of cases on which immunos were performed
Malignant	4726	1394	29.49%
Benign	1808	73	4.97%
All Tumors	6534	1467	22.45%
Non-neoplastic	13,466	59	0.44%
All Lesions	20,000	1526	7.63%

Table 2: Distribution of cases on the basis of morphology

Morphology			
Non-Hodgkin's lymphoma	382	371	97.12%
Hodgkin's lymphoma	97	95	97.94%
Malignant Spindle cell neoplasms	157	154	98.09%
Small round blue cell tumors	108	95	87.96%
Neuroendocrine tumors.	63	55	87.30%
Malignant melanomas	32	27	84.37%

Table 3: Distribution of cases on the basis of site of origin

Organ / Tissue	Total Number	Number of cases on which immunos were performed	Percentage of cases on which immunos were performed
Lymph nodes	372	359	(96.50 %)
CNS	212	70	(33.01 %)
Kidney	54	14	(25.92 %)
Testis	44	11	(25 %)
Bone	137	27	(19.71 %)
Liver	60	7	(11.66 %)
Ovary	242	26	(10.74 %)
Thyroid	69	6	(8.69 %)
Salivary Gland	71	6	(8.45 %)
Skin *	143	11	(7.69 %)
U. Bladder	129	8	(6.20 %)
Vascular	142	8	(5.63 %)
Prostate	126	5	(3.97 %)

*squamous cell carcinomas not included

In 73 of these 173 cases (23.74%), CK 7 and CK 20 antibodies were utilized to give accurate information about the site of the primary tumor. Certain neoplasms like gastrointestinal stromal tumor (GIST), anaplastic large cell (Ki 1) lymphoma (ALCL), mesothelioma etc. required immunohistochemistry in all cases. Among specific organs, immunohistochemistry was performed on 96.50% of lymph node neoplasms, followed by CNS

neoplasms (33.01%) and renal neoplasms (25.92%). (Table-3).

DISCUSSION

The optimal treatment of patients with cancer depends on establishing accurate diagnoses by using a complex combination of clinical and histopathological data. In some instances, this task is difficult or impossible because of atypical clinical presentation or histopathology.

With advances in the treatment of cancers, surgeons and oncologists now demand accurate characterization of malignant tumors. And with histopathology also developing spectacularly as a science, it has now become possible in the large majority of cases to accurately subtype malignant tumors histologically. Immunohistochemistry is the most important tool that has made this possible. Its advantages include its remarkable sensitivity and specificity, its applicability to routinely processed, formalin fixed material, and compatibility to most common fixatives.[8]

Recognizing the importance of immunohistochemistry in tumor pathology, the section of Histopathology, AKU has developed excellent immunohistochemistry facilities, which are provided in cases which require immunohistochemistry, without any additional charge from the patient, despite the use of one to more than ten antibodies used per case, with an average of four to five.

However, there is no over-dependence on immunohistochemistry. Immunos were performed on only 29.49% of all malignant tumors where accurate diagnosis and characterization of tumors was not possible. In Pakistan, where histopathology is still evolving as a science and most centers lack immunohistochemistry facilities, it is imperative that a major referral center like AKU should utilize this technique in order to provide accurate diagnosis. A study conducted by Ahmed et al[9] showed that lack of immunohistochemistry facilities can result in significant differences in diagnosis in difficult cases.

As shown in our results, immunohistochemistry is performed in all malignant undifferentiated neoplasms and the use of extensive panels of antibodies in such cases allows accurate histologic diagnosis in more than 89% cases. Immunohistochemistry is performed in all cases of suspected Non-Hodgkin's and Hodgkin's lymphoma. The few cases (Table 2) in which it was not performed were those cases in which only slides were sent for second opinion and no blocks were available. All Non-Hodgkin's lymphomas are phenotyped into B or T cell types according to the WHO / REAL classification of lymphoid neoplasms.[10,11] Similarly Hodgkin's lymphomas are confirmed by immunohistochemistry.

In some cases it may be difficult to differentiate Hodgkin's lymphomas from anaplastic large cell (ki 1) lymphoma (ALCL) or diffuse large B cell lymphoma. ALCL can only be diagnosed by immunohistochemistry. In all suspected cases of Non-Hodgkin's and Hodgkin's lymphomas, a panel of antibodies including LCA (CD45), CD 20 and CD 79 (Pan B markers), CD 3 and UCHL 1 (Pan T markers), CD15 (GAA) and CD 30 (ki 1) are used. In suspected cases of ALCL, Epithelial membrane antigen (EMA) and ALK protein an also used.[12,13] We are now diagnosing a large number of cases of ALCL which in the past may have been misdiagnosed as Hodgkin's lymphoma or Diffuse Large B cell lymphoma.

Antibodies such as bel-2 and CD 10 can distinguish between reactive follicular hyperplasia and follicular lymphoma in difficult cases.[14,15] There are prognostic and therapeutic differences between B and T Non-Hodgkin's Lymphomas as well as Hodgkin's lymphoma which make immunohistological characterization very important. Immunohistochemistry is also performed on most cases of malignant spindle cell neoplasms (soft tissue sarcomas) for their accurate characterization (Table 2). The few cases in which immunos were not performed were those in which only slides were received or external blocks which showed marked processing artifact. On the basis of immunohistochemistry, soft tissue sarcomas can be accurately classified as fibrosarcomas, leiomyosarcomas, malignant peripheral nerve sheath tumors etc. The specific types of soft tissue sarcomas have prognostic and therapeutic difference which makes their accurate characterization very important. Also, the diversity of these tumors, their differentiation along several lines, and the difficulties often faced in their differential diagnosis with benign pseudosarcomatous lesions and non-mesenchymal malignant tumors makes their accurate typing with immunohistochemistry very important.[2]

Gastrointestinal stromal tumors (GISTs), distinctive type of stromal tumors occurring in the GIT, must be distinguished from other mescenchymal tumors arising in the GIT. Immunohistochemistry is essential in the diagnosis of these neoplasms.

The CD117 (c-kit) is a proto-oncogene which encodes a transmembrane tyrosine kinase receptor is normally expressed by the interstitial cells of Cajal in GIT. In GISTs, a c-kit mutation occurs and is thought to be the direct cause of the tumor.[16] CD 117 is positive in 85-100% cases of GISTs[17] and this positivity requires to be demonstrated for the patient to receive the new effective treatment i.e. STI

571.[18] With the use of this antibody in all suspected cases of GIST, we are now diagnosing these tumors with increasing frequency. These tumors in the past would have been characterized as smooth muscle or nerve sheath tumors.

Similarly immunohistochemistry is performed in the overwhelming majority of small round blue cell tumors of childhood (Table 2). The few cases in which immunos were not performed included lesions such as retinoblastoma, nephroblastoma (Wilm's tumor) etc. However, immunos are essential in differentiating between rhabdomyosarcoma, ewing's sarcoma/primitive neuroectodermal tumor (PNET), lymphoma etc. In few other cases, immunos were not performed for the same reasons outlined above for other tumors.

Neuroendocrine neoplasms comprise another diverse category which often present difficulties in diagnosis and also need to be accurately classified for prognostic and therapeutic reasons. Again, immunos were performed in a large majority of these cases in the form of a panel of antibodies including S-100 protein, Neuron specific enolase (NSE), chromogranin, synaptophysin and Neurofilament resulting in their accurate diagnosis (Table 2).

Malignant melanomas, in most cases, are also confirmed by immunohistochemistry by using a panel of antibodies i.e. cytokeratins (negative in melanoma), S100 profein, vimentin and HMB 45 (positive in melanoma). HMB 45 is a highly specific marker for melanoma.[19]

Immunohistochemistry is essential for the diagnosis of malignant mesothelioma of the pleura and to differentiate if from metastatic lung adenocarcinoma. We perform immunos on all pleural biopsies with malignant neoplasms using a panel of antibodies, Calretinin-Thrombomodulin-Cytokeratin 5/6 (positive in mesothelioma, negative in adenocarcinoma) and Ber EP4 (negative in mesothelioma, positive in adenocarcinoma).[20-22]
Similarly, immunohistochemistry is almost always performed in other biphasic neoplasms such as synovial sarcoma for confirmation by employing antibodies such as EMA, cytokeratins, bcl-2 and CD99 (MIC2).[2, 23, 24]

As shown in the results, very tiny percentages of ordinary carcinomas of various organs (e.g. Skin, esophagus, lungs, stomach, colorectum, endometrium etc) needed immunohistochemistry for confirmation (only less well differentiated cases) and similar was the case with infiltrating ductal carcinomas of breast which required immunos only when there was very little material or crushed material in trucut biopsy specimens and when there was need to exclude malignant lymphoma in such small specimens. So although these non-specific carcinomas of various organs (including breast) comprised a large chunk of malignant tumors, immunos were required in very few cases.

According to the results, immunohistochemistry was performed on 54.74% of metastatic tumors. esp. tumors in lymph nodes, brain, liver etc to rule out primary neoplasms of these organs. In lymph nodes, cytokeratins together with lymphoid markers were used. In the brain, cytokeratins were used together with glial fibrillary acidic protein (GFAP) to rule out a high grade astrocytoma.[25] In the liver, cytokeratins CAM 5.2 and AE1/AE3 were used in poorly differentiated cases. These two antibodies are of great value in distinguishing between a primary hepatocellular carcinoma and metastatic carcinoma in difficult cases as hepatocellular carcinoma is positive for CAM 5.2 and negative for AE1/AE3, while metastatic carcinomas to liver are positive to both markers.[26] In 23.74% of metastatic tumors, CK7 and CK20 were used especially in cases of metastases occurring in omentum, mesentery etc. These antibodies often allowed us to predict the site of origin of the primary tumor since specific tumors are either positive or negative to one or both these markers.[4] CK7 and CK 20, together with Prostate Specific Antigen (PSA) are also very useful in cases of poorly differentiated carcinoma of bladder and prostate where it cannot be determined histologically whether the tumor is of bladder or prostatic origin. Urothelial carcinomas of bladder are CK7 and CK20 positive, and PSA negative, while the opposite is true for prostatic adenocarcinoma. In spinal metastases, we use PSA to conform that a metastatic adenocarcinoma represents a primary from the prostates.[27]

Table 3 shows the numbers and percentages of malignant tumors in specific organs on which immunohistochemistry is performed. Not surprisingly, lymph nodes are at the top of the list owing to the fact that immunos are performed in almost all cases of Non-Hodgkin's and Hodgkin's lymphomas. Tumors of the CNS are next due to the diversity of CNS neoplasms and the common occurrence of metastases in the CNS. More surprising is the fact that renal neoplasms are number three on the list, but it must be kept in mind that these include not only renal cell carcinomas, but other tumors as well. Other organs on which immunos are commonly performed include testis, bone, liver etc.

As shown in the results, immunohistochemistry was also performed on 4.97% of benign tumors. These were those tumors which proved difficult to characterize accurately by H&E alone. Immunohistochemistry was also performed on 0.44% of non-neoplastic cases. These were those

cases on which H&E could not prove conclusively whether they were neoplastic or not.

The accurate characterization of neoplastic lesions, both malignant and benign, is also important from an academic view point. Being a major referral and research center for Histopathology in Pakistan, we need to have a complete and accurate data base of all neoplasms for research and academic purposes, and for our data to be acceptable internationally. Histopathology has now advanced to a stage where accurate characterization of most neoplasms is possible and should be attempted. Immunohistochemistry is the most valuable tool available to us for this purpose. If we do not update ourselves according to new techniques, we will be left far behind. Yet another transformation in histopathology is now occurring which is the application of molecular techniques to histopathology specimens. We must welcome these and adopt them but with a firm realization that all these are invaluable adjuncts to meticulous gross and microscopic examination, but cannot replace them.

CONCLUSION

Immunohistochemistry has emerged as the most valuable adjunct to routine H&E staining for accurate characterization of malignant neoplasms, esp in difficult and challenging cases. The accurate typing of malignant as well as benign tumors is important from diagnostic, prognostic, therapeutic, academic and research viewpoints, and immunohistochemistry is the main tool which has enabled the histopathologist to achieve this goal. The process is not cost-effective for all laboratories especially those in smaller cities, therefore it is suggested that better referral laboratory services should be available to give population coverage.

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