Immunophenotyping of Tumours

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Abstract

Background: Immunophenotyping has added a new dimension to improve the diagnostic accuracy of malignant diseases. The emphasis is on its usefulness in planning and institution of specific therapy besides helping in prognostication.

Methods: The study included 83/1385 biopsies of cancer patients over an 18 month period on which immunohistochemical staining (IHC) with monoclonal antibodies were performed. The technique was used to establish the histogenetic origins/ expression of the tumours. The study excluded haematolymphoid malignancies.

Result: Eighty three cases on whom IHC was performed included poorly differentiated tumours (15), metastatic tumours (16), soft tissue tumours (35), central nervous system tumours (9) and miscellaneous (6). Two cases could not be typed. The clinicopathological correlation in terms of the management and the problems related to its misinterpretation are discussed.

Conclusion: Immunophenotyping of tumours in an oncology set up is significant in the 'Final Diagnosis'.

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Key Words: Tumour markers; Diagnosis; Immunophenotyping

Introduction

Tumour diagnosis has undergone a sea change over the past two decades. It includes immunophenotyping and genetic analysis that improves objectivity and reduces inter-observer variation [1-4].

This paper presents the experience of utilizing immunophenotyping of tumours, to refine morphologic diagnosis and make it therapeutically relevant. It also highlights the problems of interpretation associated with this technique and how it can be used to advantage with awareness of cross-reactions.

Material and Methods

The study included all tumour biopsy reports from December 2002 to June 2004 at the Malignant Disease Treatment Centre, Pune. The tumours were grouped under the following selection categories for analysis by immunophenotyping:

Group A : Poorly differentiated tumours: eg: carcinoma/ sarcoma/ lymphoma

Group B: Source of primary in metastatic tumours

Group C: Subclassification of soft tissue tumours

Group D: Subtype of central nervous system (CNS) tumours

Group E : Miscellaneous

This study included all tumours except primary haematolymphoid neoplasms. In all cases a morphologic differential diagnosis dictated the choice of antibody panel keeping the clinico-radiologic context in mind.

The antibodies used were epithelial markers (keratin

cocktail (CK), epithelial membrane antigen (EMA)), mesenchymal markers (vimentin, desmin, smooth muscle antigen (SMA), muscle specific actin (MSA), myo D-I, leucocyte common antigen (LCA), neural markers (S-100, neuron specific enolase (NSE), neurofilament (Nf), synaptophysin, glial fibrillary acidic protein (GFAP), neuroendocrine marker chromogranin, melanoma marker Melan A, specific endocrine markers (thyroglobulin, calcitonin) and prostate specific antigen (PSA).

Immunohistochemical staining was carried out by the labelled streptavidin biotin method on paraffin sections using monoclonal antibodies and kits manufactured by DAKO Corporation. 3-3'-diaminobenzidine tetrahydrochloride (DAB) was used as chromogen. Antigen retrieval was done by microwave heat [3,4].

Results

During the study period, a total of 1385 biopsies of malignancy cases were reported. Immunophenotyping was performed in 125 cases and of these 83 cases fell in the categories enumerated as A to E. The distribution of cases in each category is illustrated in Fig. 1.

Group A consisted of 15 poorly differentiated tumours which included malignancies where the accurate labelling of a specific tumour type was therapeutically relevant, in contrast to the waste-bin diagnosis of 'poorly differentiated tumour'. In such malignancies, the primary looks were different from the usual histologic type seen at that site. Examples included sarcomatoid (spindle cell) tumours in oesophagus and urinary bladder which were adjudged as high grade carcinomas (and not sarcomas) by virtue of expression of epithelial markers.

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Fig. 1 : Diagnostic tumour categories for immunophenotyping

Fig. 2 : Thymic carcinoma. A. Poorly differentiated tumour composed of large cells in sheets with necrosis (H&E x 4). B & C. Tumour cells positive for epithelial markers (B. cytokeratin (IHC-DAB x10), C. Epithelial membrane antigen (IHC-DAB x20). D. Background lymphocytes express leucocyte common antigen (LCA) (IHC-DAB x10)

Other organs where immunophenotyping helped at the primary site of tumour were thymus (Fig. 2), lymph node and nasopharynx (lymphoma vs carcinoma), testis (seminoma vs lymphoma), and stomach (poorly differentiated carcinoma vs lymphoma).

Group B included 16 cases with source of primary in metastatic tumours. This assumes greater importance when despite metastasis the nature of the disease is biologically indolent and does not connote a terminal illness. Examples included: identification of prostatic carcinoma (by use of PSA staining) presenting as a bladder tumour (Fig. 3) and suspicion that a liver metastases was a carcinoid and not adenocarcinoma as diagnosed earlier: the use of chromogranin served to solve the issue. Similarly, a solitary metastasis presenting as fracture clavicle in a weight lifter, morphologically suspected to be a pheochromocytoma, was objectively proven and primary was found in the adrenal. A metastatic adenocarcinoma in a woman was suggested to be from the breast by virtue of its co expression of cytokeratin (positive in all carcinomas) and S-100 (otherwise a neural marker but cross-reacting with melanocytes, apocrine cells, chondrocytes, dendritic cells etc).

Fig. 3 : Prostatic adenocarcinoma presenting as a bladder tumour. A. Poorly differentiated tumour in sheets invading bladder muscle (H&E x20). B. Focally tumour cells show lobular and glandular patterns (H&E x 10). C. Tumour cells positive for prostate specific antigen (PSA) (IHC-DAB x 20)

Fig. 4 : Sub-typing of soft tissue tumours

Other examples included medullary carcinoma (amyloid-poor) metastases in node (calcitonin) and metastases to eyelid of choriocarcinoma (βHCG staining). Two patients, known cases of breast and prostate cancer respectively, came with lung lesions clinically suspected to be metastases. In both cases the clinical suspicion of metastatic disease from the original primary was ruled out and second malignancies in the form of malignant carcinoid and paraganglioma respectively were confirmed by immunophenotyping.

Despite all attempts two cases remained untyped. This may be due to poor antigenic expression or antigen destruction in processing.

Group C consisted of soft tissue tumours. This was a major area where morphology was invariably supplemented by IHC for establishing the histogenetic origin/expression, since a variety of soft tissue sarcomas share common microscopic patterns. The distribution of the 35 cases studied is depicted in Fig. 4. Three interesting cases represented myofibroblastic proliferations that were reactive in one (in a known malignant peripheral nerve sheath tumour suspected to be recurrent) and low grade in two bearing an excellent prognosis as compared to the other soft tissue sarcomas that were considered in the differential diagnosis. A childhood retroperitoneal sarcoma, referred as rhabdomyosarcoma, was phenotyped to reveal smooth muscle differentiation with three clinical implications viz. childhood leiomyosarcomas have a

Fig. 5 : Rhabdomyosarcoma Right arm: anaplastic variant A. Healed operation scar of tumour resected elsewhere and reported as high grade sarcoma NOS. B. Pleomorphic tumour with spindling and haemangiopericytoma-like vasculature (H&E x 4). C. Diffuse vimentin positivity of tumour cells (IHC-DAB x10). D. Strong desmin positivity of the tumour cells (SMA negative: not in picture) (IHC-DAB x20)

Fig. 6 : Posterior mediastinal mass: Malignant ganglioneuroma. A. Mitotically active acellular, spindle cell tumour suggestive of a malignant nerve sheath tumour (H&E x20). B. Focal ganglion like cells (H&E x20). C. Spindle and ganglion cells with S-100 positivity (IHC-DAB x20). D. The ganglion cells alone stained for neurofilament (Nf) (IHC-DAB x20)

worse prognosis, are not responsive to chemotherapy and some have a recognised association with HIV infection. On the other hand, a tumour in the arm of a child dubbed as a 'pleomorphic sarcoma' proved to be an anaplastic rhabdomyosarcoma with a better prognosis than the former because of well defined adjuvant chemotherapy protocols (Fig.5). An interesting case of soft tissue tumour with a mixed round cell and spindle cell pattern was referred as a primitive neuroectodermal tumour (PNET) because of CD 99 (MIC 2) positivity. However, it was observed that MIC 2 had a diffuse cytoplasmic staining (seen in round cell variant of synovial sarcoma) and was not membranous as in PNET. The clinical implications of this distinction are self evident. In three cases the diagnosis remained high grade sarcoma NOS (not otherwise specified).

In non-soft tissue tumours presenting at soft tissue sites, we had an orbital tumour that was diagnosed as embryonal

Fig. 7 : MALTOMA thyroid (hemithyroidectomy multinodular goiter) A. Diffuse infiltration by a monotonous small lymphocytic population (H&E x4). B. Formation of lymphoepithelial lesions in barely perceptible thyroid follicles (H&E x10). C. Cytokeratin highlights the thyroid follicles (IHC-DAB x10). D. Leucocyte common antigen positive neoplastic lymphocytes (IHC-DAB x20)

Fig. 8 : Multiple myeloma with generalized lymphadenopathy A. The lymph node was overrun by diffuse sheets of plasma cells (red arrows) leaving residual reactive follicles at the periphery (yellow arrows) (H&E x 10). B. Plasma cells with vesicular nuclei and nucleoli (H&E x 20). C. Plasma cells show EMA cross-reactivity (IHC-DAB x20). D. Residual lymphocytes express LCA while plasma cells are negative (IHC-DAB x20)

rhabdomyosarcoma but later confirmed as an acute leukaemia, which was treated successfully. Similarly, three cases of malignant melanoma (proven by IHC) presented on the dorsum of foot (previously diagnosed and treated as a squamous carcinoma), as an axillary mass (primary later found in the uvea) and paraspinal mass (history of previous excision of a mole a few years back was later elicited). These had been referred as poorly differentiated sarcoma/carcinoma by the referring hospitals.

Group D had nine cases with subtype of nervous system tumours. The diagnostic problems included high grade pleomorphic tumours where the distinction between astrocytic and non-astrocytic tumours especially metastases was to be rendered (a combination of glial (GFAP) and epithelial markers (CK/EMA) solved the situation). In addition,

clear cell tumours like oligodendroglioma resembling neurocytoma and clear cell ependymoma could be separated (by using a combination of glial, epithelial and neuronal markers). Distinction of lymphoma from other 'round cell' look-alikes in the CNS (like PNET and round cell variant of GBM (which may present as multifocal lesions on imaging like lymphoma) was important for deciding adjuvant chemotherapy/radiotherapy. A young adult treated for medulloblastoma five years back, who reported with cervical lymphadenopathy posed a diagnostic challenge. The clinical diagnosis favoured Non Hodgkins Lymphoma (NHL). Fine needle aspiration cytology showed necrosis with poorly preserved round cells. A biopsy showed part replacement of the node by a mitotically active, round cell tumour with areas of necrosis. Immunophenotyping showed negativity for leukocyte common antigen and positivity for neurofilament and synaptophysin (confirming extracranial spread of medulloblastoma to cervical lymph nodes). Similarly a spindle cell, mitotically active mediastinal mass showing ganglion cells was proven to be a malignant ganglioneuroma by combination of S-100 and neurofilament (Fig. 6).

Group E consisted of six miscellaneous cases where immunophenotyping was put to use in different ways including establishment of subtle bone marrow involvement by NHL by use of LCA, recognition of thyroid and thymus respectively as extranodal organs of origin of NHL by using LCA and CK that highlighted the destroyed parent organs (Fig.7), demonstration of suspected C-cell hyperplastic nodules in the thyroid by use of calcitonin and demonstration of actin positivity in the myoepithelial cells of a proliferative breast lesion thereby ruling out the referral diagnosis of malignancy. Another interesting use of antibodies was the exploitation of their property for cross reactions with different tissues. While this may lead to erroneous interpretations by an uninitiated it sometimes proves to be an economical and efficient way of utilizing a limited panel of antibodies: an example was demonstration that a lymph node overrun by plasma cells was the first manifestation of a myeloma and not an NHL by EMA positivity (a cross-reacting antigen) and LCA negativity (Fig. 8).

Discussion

The importance of the 'Final Diagnosis' is nowhere as relevant in pathology as it is in tumour diagnosis. Three major elements govern the decision: tumour type, degree of differentiation (grade) and local or distant spread of the tumour (stage). The use of immunological reagents (antibodies) to recognize tissue antigens (tumour antigens) referred to as immunophenotyping of tumours marked a milestone in tumour diagnosis [1,2]. It has enabled morphologic diagnosis to be objectively refined by actual demonstration of the cell lineage antigen.

In poorly differentiated tumours immunohistochemical applications have made it possible to assign at least a broad category of carcinoma (epithelial markers), sarcoma (mesenchymal markers) and lymphoma (lymphoid markers).

Till recently, finding metastases in a node from an unknown primary meant abandoning the patient for terminal care [5, 6]. Nowadays, if accurate diagnosis can pinpoint the organ, the prognosis is not so dismal [7]. Neuroendocrine metastatic tumours (that respond to chemotherapy) need to be separated from adenocarcinomas with neuroendocrine differentiation (that have a worse prognosis) [8, 9].

In soft tissue tumours the histologic diagnosis predicts expectancy of local recurrence/distant metastases [10,11]. While synovial sarcomas and malignant peripheral nerve sheath tumours uniformly carry a poor prognosis, epithelioid sarcomas are locally recurrent. Synovial sarcomas are often monophasic and hence necessitate IHC characterization [12]. A number of specific markers are available for determining smooth and skeletal muscle origin or nerve sheath histogenesis [13-15]. Round blue cell tumours of childhood can now be separated, with each having a completely different treatment protocol [16, 17]. Non-RMS soft tissue sarcomas in the paediatric age group, which defied typing by specific markers, still seemed to have a better prognosis than PNET [18]. Rhabdoid tumours (renal and extrarenal) can be differentiated from others by loss of INI1 expression [19]. Haematolymphoid proliferations and malignant melanoma (especially when pigment is scarce) can present at soft tissue locations and are invariably misdiagnosed as a poorly differentiated tumours despite excellent markers [20-22].

In CNS, poorly differentiated tumours need differentiation between primary CNS tumour and metastases [23,24]. Secondly, common cytologic appearances as in clear cell tumours may cause confusion between different primary tumours [25, 26]. Thirdly, it is difficult to pick up patterns on small stereotactic biopsies. Lastly, lymphomas can occur in the CNS and need to be identified from other small round cell tumours for therapy [27, 28]. IHC plays an important role in such diverse areas.

Miscellaneous uses of IHC include determining the invasiveness of a lesion in breast or prostate by highlighting absence of myoepithelial cells and basal cells respectively [29]. This study also illustrates that recognition of antigen cross reactions can be used to advantage when non-availability of a specific antibody (e.g. CD 138 for plasma cells or HMB45/Melan A for melanoma) can be substituted for by another with known cross-reactivity as in plasma cell demonstration by EMA and melanoma recognition by S-100. On the other hand, cross-reactivity can lead to mistakes in interpretation eg S-100 stains for a variety of tissues as schwann cells, melanocytes, skeletal muscle, salivary glands, dendritic cells etc. Similarly vimentin (a mesenchymal marker) is

coexpressed with keratins in some carcinomas (renal, endometrium). The localization of antigen in the cell is important eg. S-100, NSE, ER, PR, p53, MIB-1 are localized in the nucleus, while intermediate filaments are stained in cytoplasm and MIC-2 gives a membranous staining in PNET but stains cytoplasm in round cell synovial sarcoma.

It is vital that the accuracy of clinical evaluation and morphologic differential diagnosis will determine the success of IHC as a supplementary tool. The benefits of immunohistochemical applications more than justify the cost accrued [30].

Conflicts of Interest

None identified

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