Original Article

Immunophenotyping of Tumours

Col R Lakhtakia*, Brig SK Nema +

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-l, 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.

*Senior Advisor (Pathology), CH (CC) Lucknow, +DDMS, HQ 10 Corps, C/o 56 APO.
Received : 07.06.2005; Accepted : 24.03.2006 email : ritulakhtakia@hotmail.com

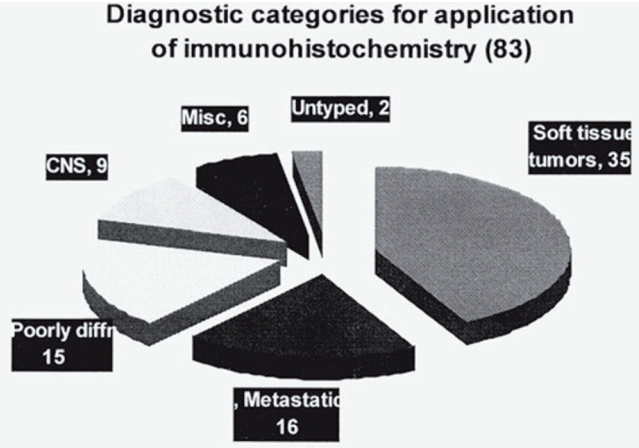

Fig. 1 : Diagnostic tumour categories for immunophenotyping

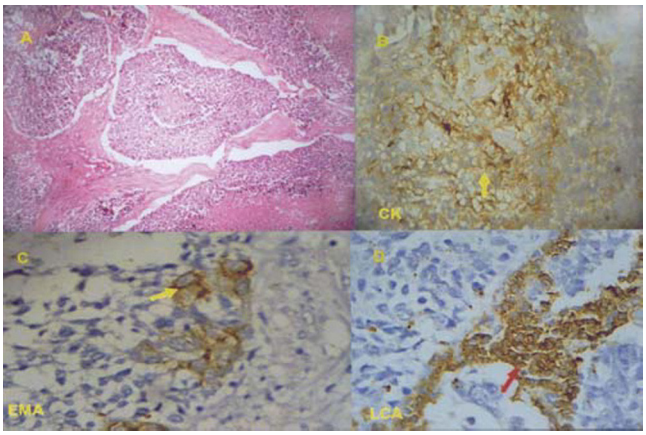

Fig. 2 : Thymic carcinoma. A. Poorly differentiated tumour composed of large cells in sheets with necrosis (H&E x 4). B & C. Tumour cells positive for epithelial markers (B. cytokeratin (IHC-DAB x10), C. Epithelial membrane antigen (IHC-DAB x20). D. Background lymphocytes express leucocyte common antigen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

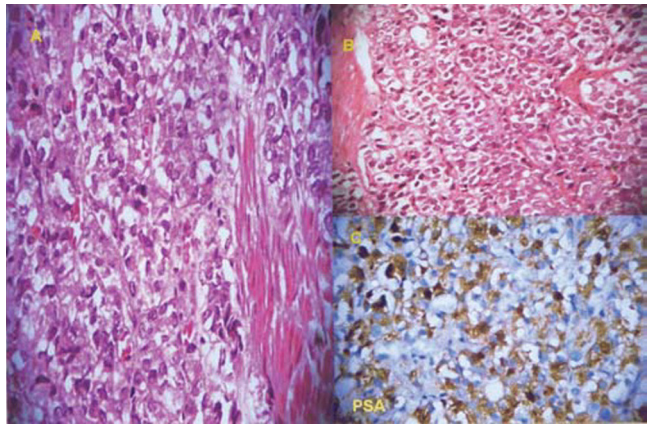

Fig. 3 : Prostatic adenocarcinoma presenting as a bladder tumour. A. Poorly differentiated tumour in sheets invading bladder muscle (H&E x20). B. Focally tumour cells show lobular and glandular patterns (H&E x 10). C. Tumour cells positive for prostate specific antigen (PSA) (IHC-DAB x 20)

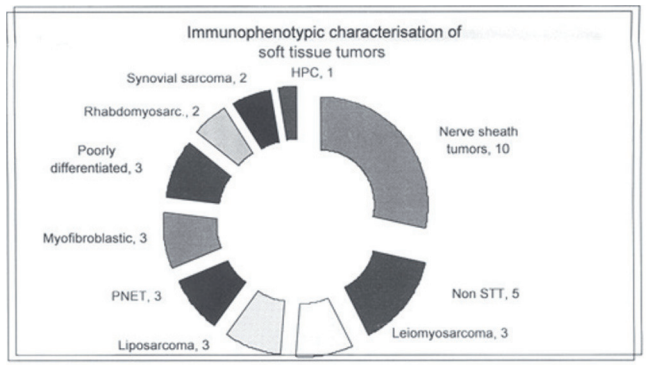

Fig. 4 : Sub-typing of soft tissue tumours

Other examples included medullary carcinoma (amyloid-poor) metastases in node (calcitonin) and metastases to eyelid of choriocarcinoma (ßHCG 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