Review Article

Newer Diagnostic Methods in Oncology

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Introduction

Over the last few years there have been an increasing number of newer methods available to the physician for the diagnosis and staging of neoplasm. Many of these technologies have entered and even established their role in routine clinical practice [1]. The present review will focus on some of the important newer diagnostic methods already available as well as emerging technologies [2-7].

The diagnostic methods will be categorized under the following categories: (1) Surgical pathology (2) Flow cytometry (3) Cytogenetics (4) Molecular diagnostics (5) Imaging studies.

Surgical pathology

A. Immunohistochemistry: Histopathology (HP) is the standard for the diagnosis of neoplasia. In last few years its role has been defined further by widespread introduction of immunohistochemistry (IHC). This method increases the diagnostic power of the HP by detecting specific antigens in the tumor tissue by using nonflourescent chromogens that can be seen by conventional microscopy [2,3]. This technique basically uses various highly specific monoclonal antibodies against the antigen to be evaluated in the tumor tissue. IHC can differentiate a malignant from benign tumors by use of κ and λ light chain restriction by establishing clonality.

The antigens chosen may be used initially for the broad classification of tumor for example into carcinomas (cytokeratin), sarcomas (desmin, vimentin) and lymphomas (Leukocyte common antigen-LCA or CD45 positive). The next approach can be to use further more specific antibodies (Table 1). This technique can now be even performed on archival tissue blocks. The area where IHC has a special role is in classification of hematolymphoid neoplasms on basis of lineage specificity. IHC is of immense importance for proper diagnosis of Small round blue cell tumors (Table 2) and lymphomas (Table 3). The pattern of IHC staining on light microscopy can be diagnostic in certain tumors. A relatively new application of IHC is to detect micro metastases in lymph nodes in breast cancer and in malignant melanoma. IHC also is helpful for determining various prognostic markers like proliferation rate (Ki 67), estrogen/progesterone receptor status and HER-2/*neu*.

Table 1

Immunohistochemical Tumor Markers of Diagnostic Importance

Antigen	Tumor Type
Alpha-fetoprotein	Germ cell tumor, trophoblastic tumors, hepatocellular carcinoma.
Carcinoembryonic antigen	GIT, pancreas, cervix, uterus lung, breast.
Chromogranin	Neuroendocrine tumors
Cytokeratin	Carcinomas and sarcomas
Desmin	Sarcomas (smooth or skeletal muscle)
Epithelial membrane antigen(EMA)	Sarcomas and carcinomas
Glial filiary acid protein (GFAP)	Astrocytoma,ependymoma and giomas
HMB-45	Melanoma,nerve sheath tumors
Human chorionic gonadotropin (hCG)	Trophoblastic, germ cell tumors
Leukocyte common antigen (LCA,CD 45)	Lymphomas, leukemias, histiocytic tumors
Prostate specific antigen (PSA)	Prostate
Vimentin	Sarcomas; renal cel carcinoma; melanoma
Thyrolobulin	Thyroid(except medullary)
Smooth muscle actin (SMA)	GIST, leiomyosarcoma, mesothelioma
Plancental alkaline phosphatase (PLAP)	Trophoblastic tumors, testis, dysgerminoma

B. Frozen section and sentinel lymph node biopsy: Frozen section is a great aid to surgeon if the

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Table 2

Use of IHC in diagnosis of malignant small round cell tumors

Tumor Type	CK	EMA	CD99	S-100	NSE	VIM	LCA	DES
Lymphoma	0	±	0	0	0	0	+	0
Neuroblastoma	0	0	0	±	+	0	0	0
PNET/ Ewings	0	0	+	±	±	+	0	0
Rhabdomyosarcoma	0	0	0	0	0	+	0	+
DSRCT	+	+	±	0	±	0	0	±
Synovial cell sarco.	+	+	±	0	0	+	0	0

CK- cytokeratin, EMA- epithelial membrane antigen, NSE – neuron-specific enolase, DES- desmin, VIM – vimentin, DSRCT- desmoplastic round cell tumor.

diagnosis of a neoplasia is suspect. The newer application of the frozen section technique is sampling of the sentinel lymph node and if the node is positive a definitive lymph node dissection can be carried out.

Flow Cytometry

This technique involves the study of cellular antigens in a cell suspension. With the availability of newer multiple laser high speed cell sorters the technique of flow cytometry has become the standard for the diagnosis and classification of acute leukemias, chronic leukemias and certain Non hodgkins lymphomas [6-9]. It is also used for the detection of minimal residual disease. It also gives prognostic information (e.g CD 38 in chronic lymphocytic leukemia). There is emerging data for the use of flow cytometry in plasma cell dyscrasias (CD 38 and CD 138 positive). In solid tumors the flow can be used to assess the DNA content and thus help in prognostification.

In acute leukemias the flow cytometry is helpful in following parameters (1) Distinction of myeloid(CD13, CD 33) vs. lymphoid (2) T cell(CD2,CD3,CD5,CD7) vs. B cell(CD 22) lineage in acute lymphoblastic leukemia, (3) Identification of subtypes of Acute myeloid leukemia (e.g. CD 14 in AML M4/M5, CD41/61 in AML M7, glycophorin A in AML M6) and (4) Diagnosis of biphenotypic leukemias by scoring system. In chronic leukemias the flow cytometry is especially important in diagnosis of CLL and hairy cell leukemias (Table 3).

Cytogenetics

The use of cytogenetics for the diagnosis of neoplasms in recent times has increased due to development of newer, faster and more sensitive methods of detecting abnormalities in the chromosomes [10,11]. The newer methods are fluorescence in situ hybridisation (FISH), multicolor (M)-FISH, spectral karyotyping (SKY), or comparative genomic hybridization (CGH). SKY and CGH are quite expensive and their use is restricted to research laboratories.

Fluorescence in situ hybridisation: This is the technique used for assessment of a specific genetic abnormality in the genome. In this test specific probes (fluorochrome labeled DNA sequences) are used which are hybridised to the DNA to be analysed. This binding to the complementary DNA provides with the information of the region under investigation and any abnormality can be detected. Chromosomal deletions, duplication, rearrangements, aneuploidy, amplifications etc. can be detected with FISH.

The various abnormalities that can be detected in the various neoplasms are given in Table 4.

Molecular diagnostic methods

This is the technology in which the diagnostic material is processed for its nucleic acid composition so as to detect unique signatures/sequences which are specific for a known neoplasm [12,13]. The newer methods being used for diagnostic and prognostic purposes in oncology practice are: (1) Polymerase chain reaction (PCR) and (2) Gene expression profiling/microarrays.

A. Polymerase chain reaction: PCR is an application in which amplification of DNA can be done so as to use that amplified DNA for diagnostic purposes. If the process starts from RNA the technique is called RT-PCR. In this technique the RNA is first incubated with enzyme reverse transcriptase (RT) so as to

Table 3

Use of IHC in the diagnosis of lymphomas

Neoplasms	CD5	CD10	CD23	CD43	CD103	Cyclin D1	sIg, CIg
B-CLL/SLL	+	—	+	+	—	—	+; –/+
Lymphoplasmacytic lymphoma	—	—	—	+/-	—	—	
Splenic marginal zone lymphoma	—	—	—	—	+	—	+; –/+
Hairy cell leukemia	—	—	—	+	+ +	+/-	+;-
Follicular lymphoma	—	+/-	-/+	—	—	—	+;-
Mantle cell lymphoma	+	—	—	+	—	+	+;-
MALT lymphoma	—	—	-/+	-/+	—	—	+; +/-
Diffuse large B-cell lymphoma	—	-/+	NA	-/+	NA	—	+/-; -/+
Burkitts lymphoma	—	+	—	—	NA	—	+; -

+, positive; +/–, >50% positive; –/+, <50% positive; –, < negative ; CIg, intracytoplasmic immunoglobulin; sIg, surface immunoglobulin.

Table 4
Important Cytogenetic abnormalities in selected cancers

Disease	Chromosome Abnormality	Genes Involved
ALL B-precursor	t(9;22)(q34;q11.2)	BCR/ABL
	t(12;21)(p13;q22)	TEL/AML1L
	t(1;19)(q23;)p13.3)	E2A/PBX1
CLL	+12	
	del(13q14) or -13	RB1
	del(17p13)	TP53
Follicular lymphoma	t(14;18)(q32;q21)	IgH/BCL2
MALT lymphoma	t(11;18)(q21;q21)	API2/MLT
Mantle cell lymphoma	t(11;14)(q13;q32)	CCND1/IgH
Burkitts lymphoma	t(8;14)(q24;q32)	c-MYC/IgH
	t(8;22)(q24;q11.2)	c-MYC/IgL
Anaplastic large cell lymphoma	t(2;5)(p23;q35) and variants	ALK/NPM
RCC, clear cell	-3 or del(3p)	VHL, other
Breast	d-min, hsr	HER2/neu
Alveolar RMS	t(2;13)(q37;q14)	PAX3/FKHR
	t(1;13)(p36;q14)	PAX7/FKHR
Neuroblastoma	del(1p), +17q, dmin, hsr	n-MYC
Ewings/PNET	t(11;22)(q24;q12) & variants	FLI1/EWS
DSRCT	t(11;22)(p13;q12)	WT1/EWS
Synovial cell sarcoma	t(X;18)(p11;q11)	SSX/SYT
AML	t(8;21)(q22;q22)	ETO/AML1
	inv(16)/t(16;16)	CBFB/MYH11
	t(15;17)(q22;q21)	PML/RARA
	t(11q23;v)	MLL
CML	t(9;22)(q34;q11.2)	BCR/ABL
MDS	5q-	
	-7 (deletion)	
	-5/5q-; -7/7q-together	
	+8	

synthesize complementary DNA (cDNA) which can be amplified later on as in regular PCR assay.

The PCR can be applied clinically for diagnosis of chronic myeloid leukemia (Bcr-abl); acute promyelocytic leukemia (PML-RAR±); acute lymphoblasic leukemia (TEL-AML-1); PCR for Ig/TcR for minimal residual disease (MRD) studies in ALL and chronic lymphocytic leukemia; in NHL for example bcl-2 and Alk-1; solid tumors for example EWS-FL1 for PNET/Ewing's sarcoma [4,5,12].

The MRD studies can be done to one tumor cell in 10,000 to 10,00,000 cells using the real time quantitative PCR. This is extremely useful in following up patients with specific molecular aberrations specific for the tumor eg. Patients with CML on imatinib of following hematopoietic stem cell transplantation.

B. DNA microarrays: This technology involves the study of expression at DNA, RNA and protein levels. By using DNA microarrays the level of transcription of genes (expression profiling) can de done.

The general principle of microarrays involves first the extraction of RNA. The mRNA is then utilized to generate cDNA using RT and flourochome labeled nucleotides, thus generating a labeled cDNA.

This labeled cDNA is applied to a surface of microarray which may contain from a few genes to thousands of cDNA or oligonucleotides probes which are of interest. After incubation, labeled cDNA molecules hybridize to microarray spots. After hybridisation, array is washed and scanned using a fluorescence microscope and degree of fluorescence is noted. The data analysis is usually done by computers. The differential expression can also be made out if the reference DNA labeled with another flourochrome is also hybridized with the test material. For each specific disease a specialized 'chip' can be made after scanning thousands of genes and narrowing the choice of genes to an important few. This chip than can be commercially made and new patients samples can be analysed. These chips usually provide excellent prognostic information and can divide a disease which looks similar on conventional tests into various subdivisions with dissimilar outcomes. This approach has been successfully used in ALL (25 gene chip) [14], diffuse large B-cell lymphoma (70 gene chip) [15] and breast carcinoma (113 gene

chip) [16]. This technique normally requires tissue that is immediately snap frozen with liquid nitrogen. Now this technique can also be applied on paraffin blocks: tissue microarrays.

Imaging Studies

A. Magnetic resonance spectroscopy (MRS): MRS is also known as nuclear MRS and has been used in functional imaging of brain tumors[17]. From last few years its use has been tried in many other tumors including esophagus[18], colon [19], prostate[20], pancreas[21], breast[22] and cervix [23]. In MRS instead of detecting the magnetic resonance of water (as done in conventional MRI); various other chemical compounds are detected. The compounds used are Carbon 13(13C), deuterium (2H), fluorine (19F), hydrogen 1(1H), phosphorous 31 (31P), sodium 23 (23Na), tritium (3 H), etc.

In brain tumors hydrogen (1H) MRS can be used to detect the alteration in the levels of choline, creatine, lactate, myoinositol and n- acetlyaspartate leading to improved diagnostic accuracy. It is especially helpful for follow up of the patient after surgery and radiotherapy. For prostate imaging, MRS is helpful in distinguishing between benign prostatic hyperplasia and adenocarcinoma. It may even differentiate between a slowly growing tumor from an aggressive one. In breast carcinoma MRS can detect increase in concentration of phosphocholine intracellularly and early malignant lesions can be accurately identified. Pancreatic cancer can be very difficult to distinguish clinically and radiologically from chronic focal pancreatitis. MRS is an emerging tool to differentiate these two entities based on analysis of lipid content (higher lipid content in chronic focal pancreatitis). MRS can differentiate normal esophageal epithelium from Barrett's esophagus as well as adenocarcinoma. This is differentiated on basis of choline to creatinine ratio. In cervical cancers based on lipid levels early invasive cancers can be distinguished from preinvasive cervical lesions.

In summary MRS is a very promising functional biological imaging that is coming into clinical use in centers where the expertise is available. The main issues that prevent its wider applications are lack of standardization, need for expensive equipment and need for separate protocols for each neoplasm. The advantages are that it is non-invasive and radiation free.

B. Newer Computed tomographic (CT) imaging applications: With the wider availability of 16 slice helical CT scanners some newer imaging modalities have come in application, which are noninvasive in nature.

(i) Virtual Colonography: This can be done nonivasively in a patient who cannot be taken up for conventional colonoscopy. This test is commonly used for screening. The accuracy of CT colonography is comparable to conventional colonoscopy for detection of polyps >6mm in size with a few false-positives. The average reported sensitivity of CT colonography for large polyps (i.e. ≥ 1 cm in size) is 92% with a specificity of 97%. For large and medium sized polyps combined, the average sensitivity is 86.4% with a specificity of 86.1% [24]. It is an excellent imaging modality for detection of colorectal carcinoma as well as synchronous/metachronous lesions. The main issue of controversy is radiation exposure and standardisation of the procedure.

(ii) Virtual bronchoscopy (VB): This investigation is being used in specific clinical situations as an adjunct to conventional bronchoscopy. It is especially useful in evaluation of patient with significant airway stenosis. Finkelstein *et al.* [25] examined the potential role of VB and found that the sensitivity of VB was 100% for detection of obstructive lesions and 83% for endoluminal nonobstructive lesions, but the sensitivity for mucosal abnormalities was 0%. The specificity of VB was 100%.

Thus VB is good for detecting lesions which are infiltrative in nature and larger but inadequate for smaller lesions confined to the mucosa. Its advantage is that it is noninvasive and the area distal to the obstruction can be evaluated.

(iii) Low dose spiral CT: This imaging modality is being increasingly advocated for screening of lung cancer in high risk groups. The problems with its general applicability in India will be due to presence of many more benign granulomatous lesions than encountered in the developed countries.

C. Newer applications of Magnetic resonance imaging in breast cancer: Contrast enhanced MRI is being used in adjunct to mammography for diagnosis of breast cancer. It is especially useful for screening and diagnosis of younger women who generally have dense breasts, (which decreases the sensitivity of mammography). It is also useful in detection of post chemotherapy changes and postoperative residual disease. Other indications for breast MRI include evaluating palpable masses in the silicone augmented breast, evaluation for recurrent cancer in the post treatment breast, identifying clinically or mammographically occult primary tumor in the patient presenting with axillary breast cancer [26].

D. PET scan and integrated PET-CT: PET scan is the novel, functional imaging technique in current oncologic practice. PET scan is a metabolic imaging technique detecting high glycolytic activity in malignant cells, using positron emitter (fluorine 18) radiolabelled glucose analogue 2 fluro-2-deoxy-D-glucose (FDG).

FDG is avidly taken up by the tumour cells, where inside the cells FDG is converted to phosphorylated form. This FDG-P is abnormally trapped, as it can not be metabolised further in the glycolytic pathway. The scanning of positron emitted from fluorine conjugated to FDG helps for accurate detection of malignancy.

The most important drawback of PET scan in its effective use for evaluation of various tumours is the lack of spatial resolution. This has been overcome by integration of hardware for CT scan and PET scan-available in clinical practice as PET-CT, wherein PET scan is superimposed on the anatomic background obtained by whole body CT scan. Thus, areas of active FDG uptake, indicating tumour sites, can be identified with greater sensitivity as well as specificity.

FDG uptake, apart from malignant cells, can be high physiologically in certain areas like brown fat, and pathologically in areas of infection and inflammation. Hence cautious interpretation is needed while use of PET or PET-CT scan in oncologic evaluation.

PET or PET-CT scan has been evaluated in variety of tumours and has been approved for staging, response assessment, detection of recurrent disease, restaging in lymphoma, NSCLC, colorectal, oesophageal, head & neck cancer, breast, and melanoma.

(i) Lymphoma : PET Scan has emerged as a valuable non-invasive imaging technique for accurate staging and restaging in lymphoma- both Non-Hodgkin's and Hodgkin's lymphoma. PET scan has been shown to have the sensitivity and specificity of 89-90% in pre-treatment staging of lymphoma. Sensitivity and specificity of PET Scan is slightly higher in Hodgkin's lymphoma as compared to Non-Hodgkin's lymphoma. Changes in clinical staging are noted in 10-40% of patients with PET Scan as compared to staging done by conventional imaging technique. 8-17% of patients are upstaged while down staging is observed in 2-23%. Upstaging with PET Scan has been correlated with more aggressive clinico-biologic behaviour and in turn poorer outcome in terms of PFS and OS [27]. PET Scan is less reliable in diagnosis and staging of low grade or indolent NHL like follicular, SLL, marginal zone lymphomas, with lower detection rates in tune of 50-90 % [28]. Differentiation between indolent vs. aggressive NHL has been suggested with help of quantification of SUV. SUV > 10 has been correlated with aggressive nature of NHL.

Achieving CR after first line chemotherapy in lymphoma would be the most important prognostic factor for long term progression free survival. However, defining CR with conventional imaging can be difficult at many instances, as residual masses can be identified in 30-60% of patients of lymphoma after 1st line chemotherapy and only up to 18% of these patients may relapse suggesting residual or recurrent disease. Differentiating active viable tumour from necrotic, fibrotic masses poses an obstacle in defining CR and predicting long term progression free survival. PET Scan, in these situations has proven to be valuable, achieving high positive predictive value and no false positive results for residual or recurrent disease [29]. Thus, PET scan is a valuable tool for post treatment response assessment and restaging of lymphoma.

Review of published retrospective as well as prospective studies have demonstrated higher sensitivity as compared to conventional imaging in detecting disease sites and accurate pre-treatment staging in lymphoma [29].

(ii) Lung Cancer: PET Scan has emerged as a valuable, complementary imaging technique in NSCLC, especially for diagnosis of SPN (Solitary Pulmonary Nodule), staging and restaging of primary tumour, mediastinal disease as well as evaluation of metastatic disease. In the diagnosis of SPC, FDC PET has the sensitivity of 96.8% and specificity of 87%. However false positive results due to infection, granuloma, sarcoidosis and false negative results with well differentiated adenocarcinoma, bronchoalveolar cell carcinoma, carcinoid tumour and lesion <1 cm are the limiting factors for effective use of PET scan for SPC.

Role of FDC PET for evaluation of primary tumour (T) stage is limited due to lack of spatial resolution. However specificity for metastatic pleural effusion is more than 90%. In the evaluation of mediastinal nodal status (N) stage PET scan has sensitivity of 84% and specificity of 89%. PET scan has been shown to decrease rate of futile thoracotomies by 20% in patients staged as stage I-III with conventional imaging [30].

Integrated PET + CT have achieved higher sensitivity and specificity for T and N staging in NSCLC.

PET scan achieves specificity/ sensitivity rates similar to other conventional techniques like Bone scan/ CT for evaluation of skeletal and hepatic metastases. Decline in SUV by 20% post chemo has correlated with overall response rate estimated by RECIST criteria and thus can be used for evaluation of response to treatment to avoid false positive results due to post apoptotic inflammatory activity in tumor. PET scan should be done 1 month after last CT.

(iii) Colorectal Cancer: PET scan / especially integrated PET and CT scan have established its role in various management issues of colorectal cancer. High sensitivity and specificity (> 90%) has been achieved in early detection, accurate staging, diagnosis and staging of recurrent disease and monitoring response to treatment [31]. Along with tumour marker correlation

PET scan can be used for early detection of colorectal CA in asymptomatic individuals. Differentiation from benign adenomas can also be suggested with PET scan [32]. Detection of occults or suspicious hepatic metastatic disease with PET scan has impact on staging and management of local colorectal cancer. PET or PET-CT has a high sensitivity and specificity for detection of early recurrences at local as well as distant sites [33].

(iv) Oesophageal Carcinoma: Conventional imaging techniques like CT scan and endoscopic ultrasound are the current standard modalities for evaluation and staging of CA oesophagus. Integrated PET-CT is shown to have superior specificity as well as sensitivity for detection of nodal and metastatic disease and evaluation of localised disease. Staging done by baseline PET scan has been shown to correlate with post treatment outcome in terms of OS and DFS[34]. Post treatment response evaluation by PET scan correlates well with the pathologic response, suggesting its valuable role in response evaluation [35].

(v) Head and Neck Cancer: PET scan has sensitivity and specificity, superior to CT scan and similar to MRI for accurate staging of squamous cell carcinoma in Head and Neck region. PET scan is valuable in planning radiotherapy by virtue of its ability for accurate delineation of viable tumour extent. Post chemotherapy response evaluation, early detection of recurrent disease at post surgical or post RT local site, as well as distant sites is the other approved indications for PET scan in Head and neck cancer. PET scan is also useful in evaluation of metastasis to cervical lymph node from unknown primary [36]. Overall success in this situation is around 27% after all other modalities have failed [37].

(vi) Breast Cancer: PET scan can be a valuable technique for staging of CA breast, especially in detection of distant metastatic sites while evaluating clinically local disease. However most established indication of PET scan in CA breast is to evaluate response to therapy in locally advanced CA breast, post CT PET scan evaluation has been shown to correlate well with pathologic response and in metastatic setting PET scan identify responders vs non responders early in the course of treatment, as early as at the end of 1 # of chemotherapy [38]. PET scan has higher sensitivity and specificity for detection of recurrence and restaging in CA breast.

(vii) Melanoma: PET scan is highly sensitive for detection of metastasis in melanoma as compared to CT scan, especially metastatic disease in skin, lymph node and abdomen, hence valuable in surgical treatment plans. PET scan has been approved for staging and restaging of melanoma[39].

Large number of studies with PET scan are available in the literature, suggesting emerging role in other tumors like ca cervix, ca ovary, ca prostate, mesothelioma etc.

Conclusions

The clinician is currently faced with an array of diagnostic techniques and options for each patient suspected to have a malignancy. Most of them are not cheap and some of them are available only at specialized centers. The challenge is to ensure that these possibilities are utilized in the most cost effective manner. For this one has to pay particular attention to the diagnostic and prognostic importance of each test as well as their specificity and sensitivity when compared to each other. The frequency or repeating them at the time of follow up is also being hotly debated. The importance of some of these tests in pharmacogenomics is being recognized only recently. Such insights can have widespread ramifications. For instance Caucasian patients with NSCLC have a 10 % possibility of response to gefitinib – particularly among women, non smokers and those with adenocarcinoma histology [41] . However the same drug is effective in as many as 46 % of Indian patients and similar number of Asian cases – including men, smokers and those with histology other than adenocarcinoma [42]. The exact opposite is the challenge while using the PET scans. Since it shows a positive result in malignancies as well as in active inflammation/infection, the specificity in developing countries is likely to be less than reported. India specific data will therefore be of immense benefit for the optimal application in our patients.

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