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Review Article

Laboratory Diagnosis of cancer in 21st century: A brief review

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Abstract

Laboratory diagnosis of cancer is one of the most challenging part of cancer management. Diagnosis lies at the heart of cancer management and accurate diagnosis is the key to the successful management of cancer. The majority of patients have the diagnosis of cancer confirmed on tissue pathology. An important principle is to obtain diagnostic material via the least invasive approach. The diagnosis of cancer by the least invasive procedure (FNAC or core biopsy) facilitates appropriate staging investigations, planning of the definitive treatment and discussion of these treatment recommendations with the patient and their support person(s). Historically, histopathology and cytopathology have been the main tools utilized in the diagnosis of cancer. For the last couple of decades histopathology remains the gold standard for cancer diagnosis. But now the concept is changing. Current regular evaluation of tumors by immunocytochemistry (IHC) to confirm tumor histogenesis and subtype is promising. Advances in technology, especially our understanding of genomics, proteomics, DNA microarrays, and bioinformatics have led to great expectations about the future of cancer detection, diagnosis, tailored therapy, and surveillance after treatment.

Key words: Laboratory diagnosis, Cancer, Histopathology, Cytopathology, molecular diagnosis

Introduction

Laboratory diagnosis of cancer is one of the most changeable part of cancer management. Diagnosis lies at the heart of cancer management and accurate diagnosis is the key to the successful management of cancer. Every year the approach to laboratory diagnosis of cancer becoming more complex, more sophisticated, and more specialized. Every doctor, medical students as well as health related personnel should have at least basic knowledge regarding the laboratory diagnosis of cancer topic.

Materials and Methods

We performed a structured literature review identifying the studies focusing on the recent techniques of laboratory diagnosis of cancer. Searches were conducted both manually and electronically. A manual exploration was made by methodically examining the books available in the library of the institution; documents and data were retrieved from the online databases PubMed, Google Scholar and researchgate.

Cytologic Methods

The beginnings of the cytology of the female genital tract can be traced to the middle of the 19th century. Since then it is one of the most famous modality of cancer diagnostics. Diagnostic cytology is based on four basic sampling techniques[1]

1. Exfoliative cytology: Exfoliative cytology is based on spontaneous shedding of cells derived from the lining of an organ into a body cavity, whence they can be removed by nonabrasive means. The advantage of exfoliative cytology is the facility with which multiple samples can be obtained from bronchus, peritoneal fluid, plueral fluid, sputum etc.
2. Aspiration cytology: Also known as Fine needle aspiration cytology. It is the most widely used cytological technique.
3. Abrasive cytology: The method allows direct sampling of specific targets, such as the surface of the uterine cervix or a bronchus by simple abrasion.

Pap's smear is the most widely used abrasive cytology technique used for cervical cancer screening.

4. Intraoperative cytology: Applicable to all organs and tissues done intraoperatively. Squash cytology from central nervous system specimen and imprint cytology from ovarian specimen are now widely used as an adjunct with histological specimen.

Fine Needle Aspiration Cytology

This method is used most commonly for the assessment of readily palpable lesions in sites such as the breast, thyroid, and lymph nodes. CT and USG guided FNAC permit extension of the method to lesions in deep-seated structures, such as lung, liver as well as small or deep seated lesions of breast and thyroid.

Advantages of FNAC

1. Is an economical outpatient procedure
2. Wide patient acceptance because it is less traumatic then the conventional tissue biopsy
3. Result can be available in 20-30 minutes in urgent case
4. It is extremely reliable, rapid and useful
5. Highly accurate in experience hand
6. Even when diagnosis is not definite, certain diagnosis will often provide useful information to guide the clinician to choose future investigation
7. Can be repeated as often as necessary
8. It can be done in any place where modern facilities are not available
9. Aspirated material is suited for bacteriologic including Gene Xpert study to detect mycobacterium tuberculosis, Electron microscopic, immunocytochemical and other studies
10. Can be used intra-operatively in frozen sections, particularly in carcinoma of pancreas.

Disadvantages and limitation

- Not suitable for investigation of many non-neoplastic condition where detailed examination of tissue architecture is necessary for diagnosis.
- Small and deep seated lesions can be missed
- Can't categorize tumors. Ex- Spindle cell tumor, lymphoma
- Considerable training and experience are required before the result become reliable
- Procedure may produce complicatons, eg: Bleeding, infection, shedding of tumour cells
- Sometimes differentiation between benign and malignant tumor is difficult where tissue invasion is required for malignant diagnosis. Ex- follicular adenoma and follicular carcinoma

Histologic Methods

Histologic subtyping of tumors based on microscopic pattern recognition by a pathologist. For the last couple of decades histopathology remains the gold standard for cancer diagnosis. But now the concept is changing. The roles of the clinician often help the pathologist in facilitating the correct diagnosis. Clinical data are invaluable for accurate pathologic diagnosis, but often clinicians underestimate its value.[2] several sampling approaches are available for histopathology:

1. Incisional biopsy: A biopsy in which only a sample of the suspicious tissue is cut from a mass (incised) and removed for purposes of diagnosis.
2. Excisional biopsy: is aimed at the complete surgical removal of an entire lesion, usually a tumor for diagnostic and therapeutic purpose.
3. Punch biopsy: Performed in uterine cervix, Oral cavity, esophagus, stomach, intestine, bronchus
4. Core needle biopsy: Done by wide bore biopsy needle. Now widely used in breast, liver, kidney and Prostate
5. Curettage biopsy: Usually done for endometrial tissue.

Limitations of histopathology

- Cannot categorize lymphoma, soft tissue tumors and undifferentiated carcinoma
- Cannot detect primary site in case of carcinoma of unknown primary (CUP)
- Diagnosis cannot be made in small or non-representative samples

- Adequate clinical and radiological information is required particularly in case of bone and CNS tumor
- Inter observer variation

Immunohistochemistry

Immunohistochemistry (IHC) combines anatomic, immunologic, and biochemical techniques to identify specific tissue components using a specific antigen-antibody reaction labeled with a visible reporter molecule.[3] There is probably no other method that has so revolutionized the field during the past 50 years as the immunohistochemical technique.[4] The advantages are obvious: remarkable sensitivity and specificity, applicability to routinely processed material, and feasibility of an accurate correlation with the traditional morphologic parameters.[5] It is compatible with most of the fixatives currently in use.[6]

Role of immunohistochemistry in tumor diagnostics

- Categorization of undifferentiated malignant tumors, lymphoma, germ cell tumor and soft tissue tumor
- Determination of site of origin of metastatic tumors
- Detection of molecules that have prognostic or therapeutic significance.
- ER, PR, HER-2 and Ki 67 in case of carcinoma breast
- CD 20 in case of Non hodgkin lymphoma
- Bcl-6, Mum 1 and CD 10 in case of follicular lymphoma
- ZAP-70 in case of Chronic lymphocytic leukemia.
- The pattern of immunoglobulin, T-cell receptor and cluster designation (CD) antigen expression helpful in diagnosis and classification of lymphoma.
- Can distinguish between reactive hyperplasia with lymphoma
- Tumor aggressiveness can be demonstrated: By-cell Proliferation marker Eg: Ki-67 index in case of central nervous system tumor grading.
- Therapeutic response i.e detection of molecules that have therapeutic significance. eg: Trastuzumab in case of HER-2 positive breast carcinoma, Tamoxifen in ER (Eostrogen receptor) positive breast cancer, Imatinib in c-Kit positive gastrointestinal stromal tumor.
- To demonstrates amyloid and some infective agents (CMV, HSV, H. pylori)

Flowcytometry

Flow cytometry is widely used to detect clonal hematopoietic populations including leukemia and lymphoma. It can be performed on peripheral blood, bone marrow, and lymph nodal tissue[3] The technique of flow cytometry consists of the measurement of various parameters while a suspension of cells flows through a beam of light past stationary detectors.[7]

Uses of flow cytometry

1. Categorization of lymphoma and leukaemia by identification of cell surface antigen
2. Provide Prognostic information independent of stage and grade
3. Provide useful identification & classification of tumour arising from T & B lymphocyte & from mononuclear Phagocytic system.
4. Detects minimal residual disease
5. Monitor response to therapy
6. Detection of ploidy in tumour
7. Cell sorting

Frozen section techniques

It is a histological technique to provide intra operative diagnosis. In frozen section there is no use of fixative solution, dehydrating solution, Clearing agent and embedding media. Although some pathologies are not suited to frozen section, it remains vital to surgical practice with an accuracy of 98.7%.[8]

Uses of Frozen section

1. Is most commonly used for urgent histological or 'On-table' diagnosis of malignancy
2. Depending on result, surgeon can plan the next course of operation
3. Determine whether margins are free of malignancy
4. Determine whether a breast nodule is benign or malignant and decide to perform lumpectomy or mastectomy
5. Determine presence or absence of ganglion cells in case of Hirchsprung's disease in surgical pathology

Limitations of frozen section

1. Adequate clinical information must be needed.
2. Adequate and representative sample must be submitted

Molecular and cytogenetic diagnosis of tumor

Molecular tests are now widely relied upon as standard of care assays in the diagnosis of a variety of pathologic conditions including tumors. During the past quarter century, the revolutionary advances made in molecular biology are having a major impact on the practice of surgical pathology particularly tumor diagnosis, prognosis and therapy decision. It has the potential to change the practice in a way that no other techniques ever had before.

The immediate significance that molecular techniques have for the surgical pathologist derives from the fact that they can be performed in tissues handled as part of routine work, including formalin- fixed, paraffin-embedded material.[9]

In situ hybridization

In situ hybridization (ISH) consists of the detection of specific DNA or RNA sequences in tissue sections or cell preparations using a labeled complementary nucleic acid sequence or probe.[10] ISH can also be used for the detection of viral infections (such as HPV, EBV, and HIV), taking advantage of the ability of the technique to identify directly the viral genome within the infected cell. An advantage this offers over immunohistochemistry is that it can detect not only productive but also latent infections. [11] Sometimes fluorochrome incorporated into the probe commonly known as fluorescent in situ hybridization (FISH). It goes without saying that in neoplasia the identification of cytogenetic markers is of high clinical significance for diagnostics, follow-up studies and prognosis.[12] Examples include:

- Detection of numerical chromosome abnormalities, such as in renal cell carcinomas, which commonly show loss or gain of whole chromosomes.
- Detection of gene amplification, such as *HER2* in breast and gastric cancer and *MDM2* amplification aiding in diagnosis of atypical lipomatous tumor
- Demonstration of chromosomal translocation/ gene fusion, most notably for diagnosis of hematolymphoid neoplasms, soft tissue sarcomas, and some other tumors.[5]

Polymerase chain reaction

Polymerase chain reaction (PCR), a revolutionary technique first introduced in 1985, allows millions of copies of any specific DNA sequence to be generated within a few hours.[5]

The PCR technique can also be used to amplify RNA so that gene expression can be analyzed. Real-time (quantitative) PCR represents an alternative PCR technique whereby incorporation of fluorescent markers in the reaction mixture permits real-time monitoring of the amplification process. The advantages over conventional PCR include shorter procedural time, higher sensitivity, higher specificity as a result of use of a fluorescent internal probe, possibility of quantitation, and reduction of risk of laboratory contamination because post-PCR manipulation is not required.[1]In the field of tumor pathology, the uses of PCR are the following:

- Detection of immunoglobulin(Ig) or T cell receptor(TCR) gene rearrangements as a means to determine the clonality of B- or T-cell proliferations[14]
- Detection of the chimeric transcripts resulting from chromosomal translocations in hematologic and solid malignancies e.g., t(14;18) in follicular lymphomas. This can be applied for primary diagnosis or for the detection of 'residual minimal disease' after treatment[15]
- Detection of gene amplifications, such as *MYC* in neuroblastoma and *HER2* in breast carcinoma.[16]
- Detection of microsatellite instability, which is a key feature of tumors occurring in patients with hereditary nonpolyposis colorectal cancer syndrome[17]
- Detection of circulating tumor cells in peripheral blood, through the identification of mRNA for thyroglobulin in thyroid carcinoma, for tyrosinase in melanoma, for PSA in prostatic carcinoma, and others[18]

DNA sequencing

DNA sequencing is the determination of the precise sequence of nucleotides in a sample of DNA usually in a PCR-amplified product. This is essential for defining the type of mutations, deletions or insertions in the DNA, and sometimes for characterization of gene fusions/translocations. Previously laborious manual methods were used. Nowadays, the procedure is typically performed in an automated DNA sequencer.[19]

Conclusion

With advances in molecular technologies, instrumentation, computing power, analytic software and informatics, we have entered the new genomic era, whereby the entire DNA complement or expressed RNA of cells can be fully sequenced, revolutionizing the molecular approaches to diseases. The cost of such high throughput sequencing is going down quickly, such that the technology will soon become highly affordable. But histological and cytological techniques are still the mainstay of cancer diagnosis in the third world countries like Bangladesh.

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