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Recent Advances in Cancer Diagnosis: On an Overview of Diagnostic Cytopathologic Modalities and Ancillary Techniques

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= Abstract =

From the concepts of cellular pathology and of exfoliative cytology, as elucidated by Virchow and Papanicolaou respectively in the late 19th and early 20th century, have evolved the primary methods for the diagnosis of cancer today. From Papanicolaou's concept of exfoliative cytology developed fine needle aspiration biopsy in the early 1960's ; this has become a major diagnostic procedure and has contributed to a significant reduction in open biopsies and, therefore, to medical cost-effectiveness. Immunobiochemical techniques provided us with a supplement to cancer diagnosis in the 1980's. The immunoperoxidase method, using monoclonal antibodies, is applied primarily as an ancillary measure to elucidate the nature of cancers. The availability of specific monoclonal antibodies has greatly facilitated the identification of cell products or surface markers. For example, antibodies directed against intermediate filaments have proved to be of value in determining the histogenesis of poorly differentiated neoplasms. Tumor markers may serve as biochemical indicators of the presence of a neoplasm. They can be detected in plasma and other body fluids. Their concentration can be applied as a diagnostic test, for monitoring the clinical course of known cancer, and as a screening measure to detect certain cancers in a population at risk.

Flow cytometry is a useful tool for distinguishing several cell characteristics, such as the immunophenotype of leukemia-lymphoma cells, the DNA content of neoplastic cells, and cell proliferation rate.

Molecular biologic techniques provided a giant step for the management of cancer patients encompassing diagnosis, prognostic evaluation, and therapy. Nucleic acid hybridization techniques are utilized as Southern, Northern, and dot blots and in situ hybridization. Molecular biology and its techniques may bring a bright new horizon for understanding cancer biology and in designing therapy on the basis of gene manipulation.

Key words: *Cancer diagnosis, Cytopathologic modalities.*

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Introduction

Cellular pathology pioneered by Virchow in the late 19th century has evolved into modern day histopathology, which constitutes the framework for pathologic diagnosis and will remain so for the foreseeable future[1,2].

In the early 1950's, electron microscopy was introduced into diagnostic pathology, resulting in ultrastructural features being recognized as making a contribution to pathological diagnosis.

Since Papanicolaou's presentation of the diagnostic significance of exfoliative cytology in 1928, diagnostic cytology had remained under the shadow of histopathology the next 30 years. Its ultimate acceptance made a remarkable contribution to the reduction in the mortality of cervical cancer. The concepts of exfoliative cytology finally evolved to include fine needle aspiration cytology in the 1970's.

Since conventional histopathology by routine microscopy has its limitations, efforts have been made to reinforce the routine simple histopathologic approach to diagnosis by more sophisticated methods. Consequently, over the last two decades remarkable diagnostic modalities and techniques have been introduced to modern laboratory medicine, including physical, biochemical, and recombinant nucleic acid techniques. The major objectives of this presentation are to present an overview of the concepts behind these new modalities.

Cytology

Exfoliative Cytology

Papanicolaou's observation of cancer cells desquamated from cancer of the uterine cervix in the 1920's[3] has evolved into the discipline of modern cytopathology, including fine needle aspiration biopsy. Subsequently, exfoliative cytology expanded its application to respiratory secretions, serous fluids, cerebrospinal fluid and urine. Exfoliative cytology has become an indispensable diagnostic procedure in the evaluation of many patients suspected of having cancer. With adequate sampling, the validity of interpretation of cervical cytology specimens ranges from 93 to 97%[4,5].

Sputum cytology can establish a diagnosis of lung cancer in 60% of cases examined on the first specimen increasing to 89% after examination of three consecutive specimens[6]. Furthermore, sputum cytology can determine the tumor type with a high degree of accuracy[7]. However, based on a project sponsored by the National Cancer Institute in 1971, screening for cancer of the lung is not recommended except as a part of general health evaluation[8].

Esophageal cancer is nor common in the United States and the Western world; it is, however, relatively common in other areas of the world, particularly in Northern Iran and Northern China. Cytologic screening for esophageal cancer in Northern China proved to be valuable in early detection of cancer, with a detection rate of 94% with the balloon/cytologic brushing technique[9].

Since cancer of the stomach is now a rare disease in the USA, screening programs have not been developed here. Gastric brushing combined with biopsy under fiber optic endoscopy can, however, establish a diagnosis in 98% of cases[10].

Carcinoma of the urinary bladder is one of the most common neoplasms in North America

and Europe. Since the detection rate by urinary cytology of low grade tumors is low, screening of the general population is not a practical measure. However, cytologic screening of voided urine of high risk patients for bladder cancer is highy recommended, providing an overall diagnostic sensitivity of 82.5%, with detection rates of 94% for high grade tumors, 72 to 75 % for grade II tumors, but only 17% for low grade tumors[11]. Cytologic examination of voided urine is also of great value in monitoring for recurrence of bladder cancer.

Cytology of serous fluid is extremely important in that identification is a finding of utmost gavity, signifying that a patient has advanced and usually incurable cancer. The fluid is collected into a clean, dry container and sent to the laboratory as soon as possible. If not processed immediately; it should be kept in a refrigerator at 4℃ and not allowed to freeze. Several cytopreparatory techniques may be applied to serous fluids to enhance the diagnostic yield, including electron microscopy and immunocytochemistry[12].

Cerebrospinal fluid normally contains a small number of mature lymphocytes and monocytes, up to 5 to 10 cells per mm^3[3]. The choice of cytopreparatory techniques for cerebrospinal fluid is generally determined by the preference of the individual laboratory. A commonly used technique is that of membrance filltration using Millipore, Nuclepore or Gelman filters, which provide excellent cell retrieval. The technique requires considerable skill and experience. In contrast, cytocentrifugation is simpler and less time consuming than membrane filtration, although some degree of cell loss is a disadvantage. Cerebrospinal fluid cytology may be valuable in the diagnosis of some infectious diseases and for the diagnosis of leukemia-lymphoma of the central nervous system, but it is not likely to be successful with primary or metastatic solid neoplasms unless the neoplasm has extended into the ventricles or subarachnoid space[13,14]. A most valuable diagnostic approach to solid neoplasms of the central nervous system is fine needle aspiration[15].

Fine Needle Aspiration Cytology

Fine needle aspiration(FNA) cytology became popular in North America in the 1970's because of the availability of sophisticated imaging techniques. Its application brought about great advances in cancer diagnosis, treatment and overall cost-effective management. FNA can avoid open biopsy in patients with unresectable cancer or with non-neoplastic disease mimicking cancer.

Aspiration techniques depend on the anatomic site of the lesion. Aspiration may be performed with or without local anesthesia on superficial lesions, which are those of breast, thyroid gland, lymph node, salivary glands and subcutaneous tissue. The aspiration procedure can be performed on superficial anatomic sites in an outpatient clinic or in the physician's office[16]. A small caliber aspiration needle should be used(22 to 25 gauge) to minimize trauma and bleeding.

FNA for deeply situated organs are performed under radiologic imaging guidance, usually by computed tomography(CT); the technique can be applied to virtually any organ site. The commonly targeted visceral organs are lung, liver, pancreas, retroperitoneum and brain. In the interest of safety, use of 21 or 22

gauge needles is also recommended for deeply situated organs.

Lung: Almost one-third of all FNA procedures on deeply situated viscera are performed on the lung. The transthoracic FNA procedure, combined with sophisticated radiologic imaging, has revolutionized the diagnosis of pulmonary diseases and has saved many patients the trauma and expense of open biopsy by thoracotomy. The primary diagnostic method for suspected lung cancer is a matter of personal or institutional preference. The time-tested conventional method cytology of sputum or bronchial secretion provide a high diagnostic yield and in most cases should be used before resorting to FNA. On the basis of its diagnostic sensitivity and specificity, and its low rate of complications, FNA frequently takes priority over other methods, particularly for peripheral and metastatic pulmonry neoplasms[17,18]. Contraindications for FNA of the lung are uncontrollable cough, hemorrhagic diathesis, or possible echinococcal cyst.

Liver: Fine needle aspiration is the method of choice for any mass in the liver suspected of being malignant. The procedure is performed under either ultrasonography or computed tomography. In North America and other Western countries, most neoplasms in the liver are metastatic in origin. Whereas in many Asian and African countries, most hepatic neoplasms are hepatocellular carcinomas. The diagnostic sensitivity for hepatic neoplasms is over 90%[19,20].

Pancreas: Because of its location, investigation of a pancreatic mass is difficult. Open biopsy, either wedge or core needle biopsy, misses the diagnosis in a considerable number pf cases and risks a significant rate of complications, including hemorrhage, fistula formation, pancreatitis or pseudocyst formation.

FNA of the pancreas is performed under ultrasonographic or computed tomographic guidance, and has a diagnostic rate of 80 to 90 %. Complications of the procedure are minimal[21-23].

Kidney and Other Retroperitoneal Organs: The indications for FNA of the kidney, adrenal gland, and retroperitoneal space are for identification of a mass, staging of neoplasm, or therapeutic aspiration of a cystic lesion. Metastasis to the kedney and adrenal gland occurs two to three times more frequently than primary neoplasms of these organs. The most common retroperitoneal neoplasm is metastatic carcinoma, followed by lymphoma and sarcomas. Transperitoneal FNA of retroperitoneal lymph nodes or masses are performed under CT or ultrasonography[24].

Breast: Breast lesions are among the most common clinical abnormalities of women in North American and European countries. The application of FNA biopsy to palpable breast lesions or to lesions detected by mammography has increased the accuracy of diagnosis of breast cancer, achieving a diagnostic accuracy of 90% in cases of breast carcinoma[25]. FNA of breast lesions can also be used for ancillary tests, such as estrogen binding assay and DNA ploidy analysis. In many patients, FNA is an acceptable alternative to open biopsy for the diagnosis of palpable breast lesions, and has achieved considerable cost-effectiveness[26].

Lymph Nodes: Lymph node enlargement is a common clinical finding; it may represent an infectious process, a primary neoplasm of the lymph nodes, or metastatic neoplasm. Diag-

nosis by FNA of enlarged lymph nodes is highly efficient, reliable and cost-effective. The aspirate is also suitable for microbiologic investigative workup. The aspirate can identify metastatic neoplasms in patients known to have or have had a primary neoplasm and it is also suitable for the diagnosis of metastatic neoplams without any known primary neoplasm. The diagnostic accuracy of FNA of metastatic neoplasm in lymph nodes ranges from 90 to 96%[27,28].

The cytodiagnosis of lymphomas by FNA is based on the presence of a monomorphic population of lymphoid cells. Such cells may be small cleaved, small non-cleaved, large cleaved or large non-cleaved, or they may be mixed small cleaved cells and large cells. The limitations of FNA for a lymphoma workup are difficulty in architectural evaluation, and low diagnostic accuracy as compared to that of metastatic neoplasms in lymph nodes. If cytologic features of lymphoma are not distinct, special ancillary techniques such as flow cytometry and Southern blot hybridization[29,30] can be performed on the aspirate.

Thyroid: FNA of the thyroid gland is primarily applied to thyroid nodules, and has markedly reduced thyroid surgery, with accompanying economic benefit. Recent compiled data on the sensitivity, specificity, and positive predictive value of FNA of the thyroid by Bedrossian et al. indicate a mean sensitivity of 94%, a specificity of 96%, and a positive predictive value of 93%[31].

The aspiration is usually performed with 22- to 25-gauge needles. Two to 3 aspirations for lesions larger than 3 cm and even more for multinodular lesions should be performed. The cytologic features of nodular goiter include small to mederate numbers of uniform follicular cells in sheets and isolated in a colloid milieu. The smears of aspirates of neoplastic lesions tend to have a large number of cells in clusters of fronds. Colloid is usually absent or scanty. Most malignant neoplasms of the thyroid are cytologically straightforward. However, the cytologic discrimination between follicular adenoma and well differentiated follicular carcinoma may be difficult. Consequently, in such cases the use of the generic designation "follicular neoplasm" followed by surgical excision of the neoplasm is recommended[32]. Immunocytochemical application to the aspirate may be valuable in distinguishing between primary or metastatic carcinomas and lymphomas.

Immunocytochemisry

The techniques of immunocytochemistry (ICC) originated with immunofluorescent labeling of antibodies and fluorescence microscopy in the 1940s. Today, enzyme ICC methods are in common use, based on peroxidase, alkaline phosphatase, biotin-avidin and gold silver techniques. These methods can be combined with a hematoxylin counterstain to delineate nuclei and require only light microscopy. These enzyme ICC methods are applied to specimens of tissue, FNA specimens, serous fluids, and cerebrospinal fluid. Tissue samples are fixed in 10 % buffered formalin or frozen for specific antigen preservation. Cytologic samples are fixed in 95% ethanol.

Immunobiochemical techniques provided us with a supplement to cancer diagnosis in the 1980's. These techniques are primarily applied

as ancillary measures to elucidate the nature of a cancer by the immunoperoxidase method using monoclonal antibodies. For example, antibodies directed against intermediate filaments have proved to be of value in determining the epithelial histogenesis of many poorly differentiated neoplasms. Immunocytochemical techniques are widely used in situ in human cell and tissue samples to detect cell specific antigenic molecules in order to identify the nature and origin of a cell. Immunocytochemical data have to be considered together with the clinical data and the morphologic features of cells and tissue; they must not be employed as the sole method of diagnosis.

Tumor markers may serve as biochemical indicators of the presence of neoplasm. They may be detected in plasma or other body fluids. They can be used as a diagnostic test, for monitoring the clinical course of cancer, and as a screening device for some cancers in high risk populations, such as prostate specific antigen for prostate cancer and alpha-fetoprotein for hepatocellular carcinoma in endemic areas.

Flow Cytometry

Flow cytometry is useful for demonstrating several cell characteristics, such as the immunophenotype of leukemia-lymphoma cells, and for measuring the DNA content of neoplastic cells and the cell proliferative fraction. It may become a practical necessity in the management of certain cancers. The flow cytometer is an instrument for measuring rapidly, reproducibly and quantitatively certain morphologic cellular parameters such as size, shape and molecular composition of the intact cell.

Flow cytometry is complementary and supplementary to the conventional light microscopic, histologic and cytologic examination. The principle of flow cytometry is that cells stained with a fluorescent dye and suspended singly are passed through a sensing region where optical or electrical signals are generated and measured. Photomultiplier tubes convert light into an analog electrical signal, which is changed into a digital signal. The signals are processed by the computer and frequency histograms are generated.

1. Immunology

Whole blood samples or single cell suspensions of lymphoid tissue are labeled with cell-surface phenotypic markers by direct or indirect immunofluorescence. T- or B-cell subpopulations may be rapidly quantitated to determine the monoclonality of lymphoma or leukemia and the quantitation of CD4:CD8 ratios in AIDS patients or other immunocompromised patients[33,34].

2. DNA Content and Cell Cycle Analysis

The measurement of nuclear DNA content and cell-cycle analysis are useful in assessing the prognosis of a variety of neoplasms. The principle of their measurement is based on a linear relationship between the amount of DNA in the nucleus and the fluorescence emission intensity. The frequency histogram of fluorescence(DNA content) versus cell number has a characteristic pattern, which allows the proportion of cell nuclei in G_0/G_1, S, and G_2/M phases to be calculated by computer software. The presence of DNA aneuploidy or high in-

dex of cell proliferation in malignant neoplasms tend to be associated with a poor prognosis[33,34].

Cell Image Analysis and Morphometry

Cell image analysis is applied for nu meric measurement of certain cell features: cell size and shape, nucleocytoplasmic ratio, nuclear size and shape, quality and quantity of nuclear chromatin, and the number and size of nucleoli.

Computer analysis of digitized imagery will be a remarkable supplement to light microscopic cytodiagnosis. The operating system consists of microphotometry augmented by micromorphometric procedures, implemented on videophotometers and supported by personal computers. Cytologic samples are fixed in 95% ethanol. Solid tissue samples should be prepared for thin section or as monolayer cell smears on slides. The Feulgen procedure is the usually recommended stain.

Clinical Applications

Diagnosis: DNA aneuploidy is the most reliable marker of malignancy, and provides pivotal information in cases where histologic or cellular features by light microscopy are equivocal.

Prognosis: DNA ploidy assessment provides prognostic and treatment information.

Quantitation of Immunologic Staining: With a computerized image analysis system, an immunostain of specimen can be evaluated more objectively and conclusively. Estrogen receptor and progesterone receptor in breast cancer tissue can be detected by monoclonal antibodies[35~37].

Cell Proliferation Rates: The monoclonal antibody Ki-67 is a highly sensitive marker of proliferative status of cells in tissues or in cellular samples. The labeled cells by Ki-67 immunostaining are distinct and closely related to tumor grade and aggressiveness[38].

Contextual Karyometry: The contextual analysis of nuclei encompassing shape, area, polarity, ploidy and mitotic density, provides a confirmation of samples as being benign or malignant as well as prognostic information.

Some of the tests can be performed by flow cytomety, immunocytochemistry or cell image analysis. Selection of a particular test is based on personal preference, availability of the method at the facility, and cost-effectiveness.

Molecular Biologic Techniques

Human genome project has been isolating and sequencing disease-associated genes, and at the present time about 5,000 of these genes have been encoded on nuclear DNA. These genes will eventually provide us with information related to developmental biology, etiology and pathogenesis DNA is a duplex molecule composed of two complimentary polynucleotide strands which can be reversibly separated by denaturation under high temperature or alkaline treatment(DNA denaturation). The denatured single stranded DNA is reversible under appropriate conditions, in which the two separated complimentary strands reform into a double helix(DNA renaturation, reassociation or hybridization). By introducing an exogenous but complimentary labeled polynucleotide stran-

d(probe) to the milieu of the denatured single stranded DNA, it will, under appropriate renaturation condition, combine with the target polynucleotide strands to form a labeled hybrid DNA duplex. Gross deletions and rearrangements in genomic DNA can be detected by hybridization with cloned probes on filters (Southern, Northern and dot blots) and in situ in histologic sections[39~41].

Lymphoma cells rearrange their DNA identically, and the unique abnormal gene rearrangement can be detected on a Southern blot. The detection of a monoclonal cancerous cell gene rearrangement by Southern blot facilitates the diagnosis of leukemias and lymphomas. The gene rearrangements occur always in lymphocytic malignant diseases, and occur sometimes in nonlymphocytic malignant diseases.

In situ hybridization can detect complementary DNA or RNA targets in tissue sections or cytologic preparation. In situ hybridization techniques allow identification of a specific nucleic acid sequence in a particular cell type with routinely fixed and paraffin-embedded tissue in small biopsy specimens or cellular samples. The technique enables viral identification, such as hepatitis B virus, Epstein-Barr virus, human papilloma virus, herpes virus, cytomegalovirus and JC virus, as well as the oncogenes of some cancers.

The polymerase chain reaction(PCR) is a new technique for in vitro DNA amplification, and it is used in conjunction with Southern blot analysis. PCR offers advantages with speed and flexibility in small, impure, or degraded samples.

The clinical applications in molecular biologic techniques are viral identification in association with malignancy and infectious disease, gene rearrangement of lymphoma-leukemia, and amplification and identification of oncogenes. Molecular biology and its techniques may herald a new age in the understanding of cancer biology and in designing therapy[42].

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= 국문 초록 =

세포병리학적 기초에 의한 암진단의 발전: 진단방법과 보조기법

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19세기말과 20세기초에 각각 비르효와 파파니콜로에 의해 명료하게 된 세포병리학과 탈락세포학의 개념에서 오늘날의 암진단의 일차적인 방법이 발전해 왔다. 파파니콜로의 탈락세포학의 개념에서 1960년대 초반에 세침흡인 세포검사가 개발되었다. 이 세침흡인 세포검사는 주된 진단방법이 되어져, 절개생검을 감소하게 하고 의료비용의 효과적인 이용에 공헌하였다.

1980년대에는 면역생화학적 기술들이 암 진단에 보충역활을 하게 되었다. 단클론 항체를 이용하는 면역과산화효소법이 먼저 암의 본성을 밝히는 보조적인 방법으로 쓰여졌다. 특정 단클론 항체들이 이용가능하게 되어 세포산물이나 표면표지자들을 인지하는 것을 훨씬 용이하게 하였다. 예를 들면 중간세사에 대한 항체들이 분화가 나쁜 종양의 조직기원을 결정하는데 가치가 있는 것이 증명되었다. 종양표지자들은 종양존재의 생화학적 표시자로 이용될 수도 있는데 이러한 종양표지자들은 혈장이나 다른 체액들에서 검출할 수 있다. 이 종양표지자들을 농출한 것을 진단적 검사에 이용하여 이미 진단된 암의 임상 경과를 추적하고 암 발생의 위험이 있는 집단에서 특정 종양을 발견해 내기 위한 선별검사로써 이용할 수 있다.

유세포 검사는 백혈병이나 림프종 세포들의 면역표현형을 알아내고, 종양세포들의 DNA함유량을 알아내며, 세포증식율을 알아내는 등의 몇가지의 세포의 특성을 분류해내는데 유용한 도구이다.

분자생물학적 방법들은, 암 환자를 진료하는데 있어 진단, 예후평가 및 치료 등의 분야에서 일보 전진하게 하였다. 핵산교잡법이 Southern blots, Nothern blot, Dot blot 및 *in situ hybridization*으로 이용된다. 분자생물학 및 그 기술이 암종 생물학을 이해하고 유전자 조작을 기초로한 치료법을 계획하는데 밝은 새로운 지평선을 열어줄 수 있을 것이다.