Review

Newer vistas in Pathology: A leap into future.

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Abstract: Now that we practice in the current era of evidence based Medicine, there has been exponential rise in armamentarium of such tools in the discipline of Pathology , which has revolutionized the diagnostic skills with highly fair degree of confidence. This review highlights some of such tools and their practical implications in diagnosis and management. To cite a few such modalities Immunohistochemistry (IHC), Flow cytometry, Flourescence in Situ Hybridisation(FISH) and Molecular assay are index representations in this review.

Key Words: Hybridoma technique, Cluster Differentiation, Fluorescent signals, Reverse Transcriptase polymerase chain reaction.

JK-Practitioner 2019;24(3-4): 8-10

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Indexed:
Scopus, INMED, EBSCO and Google Scholar among others
Cite This article as::
Atta A, Rashid A, Sahaf B. Newer vistas in Pathology: A leap into future. JK-Practitioner2019;24(3-4): 8-10
Full length article available at **JK-Practitioner.com** one month after publication

Introduction: Ever since times immemorial analysis of body fluids would provide a lead towards disease cause and effect relationship. With advent of microscope ability of curious human eye to see beyond got realized and a term got coined by the name of "FINAL DIAGNOSIS" which became sine quo non of discipline of Pathology. Modern century is witness to a quantum leap in diagnostic ability of discipline of Pathology, noteworthy among them being in the form monoclonal antibodies and their subsequent use either as solo in immunohistochemistry (IHC)or in Flow cytometry. FISH(Fluorescence in Situ Hybridrisation) and molecular assays by Polymerase chain reactions equally deserve a mention.

Monoclonal antibodies with their ever increasing availability, have found a space in diagnostic Pathology. Basic concept of producing and harvesting monoclonal antibodies is asfollows. Murine myeloma cell has inherent property of immortality, when splenic lymphocytes from an immunized mouse are fused with murine myeloma cell, in hypoxanthine aminotryptine (HAT) medium, desired monoclonal antibodies are harvested. This technique is called Hybridoma Technique. Immunohistochemistry in diagnostic pathology gainfully utilizes these monoclonal antibodies [1].

Flow cytometry: This modality is now routinely being used in most laboratories. Basic design of a flow cytometer involves four major elements: Optics, fluidics, electronics, and computer with soft ware. The optical system utilizes one or more light sources that produces monochromatic light and serves as an excitation beam. At the opposite side of optical bench, light generated from cells that have intersected the excitation beam is collected; filters and dichroic mirrors set in fixed locations are linked to photo detectors to allow quantization of emitted light at specific wave lengths.To ensure that all cells are analysed, cell suspension is injected. The interplay of cells with excitation beam produces cell specific and characteristic light scatter non fluorescent signals. Additional fluorescent signals are generated by flouro chromes that typically are linked to specific reagents. The two reagent independent, nonflourescent parameters are forward angle light scatter which is marker of cell size whileas side angle light scatter is an index of cellular granularity . Beauty of combination of these two parameters allows for discrimination among three major types of leukocytes as well as evaluation of red blood cells and platelets in whole

blood samples. The fluorescent data collected by a flowcytometer are the result of either cell surface or intracellular binding of specific monoclonal antibodies conjugated directly to flourochromes. There are currently many different flourochromes used like flouresceinisothiocynate (FITC), PHYCOERYTHRIN(PE), Peridinchlorophyl protein (perc CP) and allophycocyanin. Florochromes complementing each other are used in daily practice of Flowcytometry. Research flowcytometrs with the availability of host of new flourochromes have greatly extended multicolor limits.

DATA PRESENTATION AND INTERPRETATION:

Current flowcytometrs yield graphic results of cell frequency versus light intensity for one or more parameters by means of specific computer software. Usually histogram depicting quantitative distribution of cells on y-axis versus light intensity of single parameter on X-axis. On an average 10,000 to 20,000 events are collected to provide sufficient number of cells for meaningful data.

The clinical applications of flow cytometry saw its earlier use as a supplement to the morphological classification of Leukemias and Lymphomas ,affording not only lineage information but also state of differentiation and or maturation. In addition flowcytometry provided the best prognosticator in human immunodeficiency virus(HIV) infection based on absolute CD4 T-Cell numbers. Now on there is a huge list of indications and applications,some of which are listed as under[2,3]

Based on cluster differentiation(CD) designation:

CD 1a: cortical thymocytes, dendritic cells, Langerhan cells.
CD 2: T cells, thymocytes, NK –cell subset.
CD 3: T cells, thymocytes.
CD 4: T cell subset,thymocyte subset, monocytes/ macrophage.
CD 5: T cell, B Cell subset.
CD7: Thymocytes, T Cell, NK cells.
CD8: T cell subset,thymocyte subset, NK Cell subset.
CD 10: Early B Cell (CALLA Positive)
CD 11b: Monocytes, Granulocytes, NK Cells.
CD 11c: Myeloid cells, Monocytes.
CD 13: Myelomonocytic cells.
CD 14: Monocytes, myelomonocytic cells.
CD 15: Granulocytes,Monocytes, Endothelial cells.
CD 16: NKCells,Granulocytes,Macrophages.
CD 19: B Cells.
CD 20: B cells.
CD 21: Mature B Cells,follicular dendritic cells.
CD 22: Mature B cells.
CD 23: Activated B cells.
CD 25: Activated T Cells, Activated B cells, Regulatory T Cells.
CD 27: Memory B Cells.
CD 30: Activated T, B ,NK Cells,Reed Sternberg cells.
CD 33: Myeloid cells, Myeloid progenitors, Monocytes.
CD 34: Hematopoietic precursor cells.
CD 36: Platelets, monocytes/macrophages.
CD 41: Megakaryocytes, platelets.
CD 42b: Megakaryocytes, platelets.
CD 45: Leukocytes.
CD 45 RA: T Cells (Naïve) subset.
CD 45 RO: T cells(memory) suset.
CD 56 : NK Cells.
CD 61: Megakaryocye , Macrophage.
CD 79 a: B cells.
CD 103: Intestinal epithelial Lymphocytes.
CD 117: Myeloid Blast cells.
Glycophorin: erythrocytes, & precursors.

Fluorescence In Situ Hybridisation (FISH):

FISH is applied methodology useful in characterization of structural chromosomal abnormalities and identification of chromosomes of uncertain origin. The technique can be applied to Blood, marrow, bodyfluids, tissue touch preprationsas well as to paraffin embedded tissues. In FISH Flourescent labeled single stranded probes are hybridized to the nuclei of metaphase or interphase cells attached to glass slides. The use of probes labeled with different dyes allows for multicolor FISH on a single slide. For balanced translocations, probes spanning individual breakpoints are used. Dual colour/dual fusion probes or single fusion / dual color FISH probes target sequences located at opposite ends of two breakpoints[4]. In addition , two color break apart probes recognizing DNA sequences from 3' and 5' ends of a single gene can be applied. These probes yield combined yellow signal in normal germline configuration, while two colors are seen when target sequences are separated because of translocation. FISH is more reliable for detection of duplication in chromosomes than deletions[5,6].

Translocations And Deletions detected by FISH

Disease	**chromosomal abnormality**
CLL/SLL	del t3q 14,del 11q 22
LPL	t(9:14)
MZL	t(11:14), t(1:14), t(14:18)
FL	t(14:18)
MCL	t(11:14)
DLBCL	del 3q27, t(14:18)
BL	t(8:14), t(2:8),t(8:22)

ALL	t(12:21), t(11q23),t(9:22)
AML	t(11q23),t(8:21),inv(16).
CML	t(9:22).
APML	t(15:17)

ALL, Acute lymphocytic leukemia; AML, Acute myeloid leukemia, BL ;Burkitt Lymphoma; CLL; Chronic lymphocytic leukemia; CML, Chronic myeloid leukemia, DLBCL, Diffuse large B cell Lymphoma, FL, Follicular lymphoma; LPL ;Lymphomaplasmacytoid lymphoma; MCL; Mantle cell Lymphoma; MZL ;Marginal zone lymphoma; SLL, Small lymphocytic lymphoma.

POLYMERASE CHAIN REACTION:

PCR based assays have found a wide applicationin diagnosis of malignant disorders associated with specific translocations in genetic material. Its main advantage being high specificity and sensitivity ,of course it is to be ensured to have an immaculate protocol to avoid contamination. DNA primers are designed to flank the specific translocated region, producing a PCR product with a characteristic size, while in the absence of specific translocation the amplification product is not generated.RQ PCR/ RTPCR implies quantitative and qualitative assays respectively.

In clinical practice, PCR technology has proven predictive of therapy response and relapse. For certain Hematological malignancies with translocation coding for specific targets,cytogenetic and molecular remissions have been defined as specific end points of therapy. Most common being RQPCR for BCR/Abl in chronic myeloid leukemia, PML RARA in acute promyelocytic leukemia (APML), Cyc D1/IgH and IgH/bcl2 for mantle cell and follicular lymphoma respectively [1,5].

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