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Editorial

Reimagining cytopathology in the molecular era: Integration or fragmentation?

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Dear Editor,

Cytopathology has been embraced for years in diagnostic pathology because of its simple, quick, cost-effective, and minimally invasive technique for diagnostic precision.[1] Almost solely based on morphology, it provides expeditious and minimally invasive diagnostic insights to the pathologists and clinicians. However, the current understanding of disease has accelerated with the evolution of next-generation sequencing, whole exome sequencing, DNA methylation, etc., and placed cytopathology at a crossroads.[2] So, the core question arises: Should cytopathology become an integrated pillar of personalized medicine, or should fragmentation be risked by remaining tied to obsolete paradigms?

THE PROMISE AND TENSION OF THE MOLECULAR ERA

Cytopathology's promising strength has been its dogged persistence in providing rapid and minimally invasive diagnosis with crucial morphometric assessment. However, in the era of precision medicine, where targeted therapy plays a remarkable role in cancer therapy management, morphology alone cannot reveal the accurate genomic picture of cancer.[3] The power of high-throughput molecular technologies often overshadows the cytopathologic morphologic foundation.[4] Even with occasional cells scraped from cytopathology slides, molecular profiling can reveal actionable mutations and nuanced genetic alterations. The demand is rising for DNA and RNA analysis from cytology slides with increasing demand.[5]

These advancements raise a crucial question: should cytopathology remain a "support" discipline – or can it become a leading player in integrated diagnostics? There is a genuine risk of fragmentation if molecular testing separates from morphologic interpretation, with cytopathologists marginalized as mere suppliers of "material" for molecular laboratories.

INTEGRATION: A NECESSARY IMPERATIVE

A vital step in clinical utility and sustainability is the integration of cytopathology with molecular pathology.[6] Liquid-based cytology preparations, cell blocks, and residual needle rinses are being increasingly used for molecular testing, such as mutation and gene fusion. Fine-needle aspiration cytology (FNAC) cell blocks for epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK) testing in lung adenocarcinoma, cervical cytology samples for HPV genotyping, and thyroid cytology samples for BRAF V600E mutation detection are some examples where cytology samples are precious.[7] This integration aids in patient care

for determining targeted therapy without further invasive techniques, thus reducing turnaround time and providing personalized treatment plans.

There are evidences of improved diagnostic accuracy with the integration of cytology with molecular studies. Studies have shown that fine-needle aspiration cytology in cases of tubercular lymphadenitis, if combined with GeneXpert Mycobacterium tuberculosis/rifampicin and reverse transcription polymerase chain reaction (PCR), greatly improves diagnostic performance compared to FNAC alone. It reduces the need for lymph node excision or biopsy. When combined, sensitivity reached 100% and specificity also highly improved.[8] Rapid-on-site-evaluation (ROSE), when combined with EBUS TBNA, can reduce the number of repeat procedures or biopsy rate for diagnosis of molecular alterations such as EGFR, ALK, ROS proto-oncogene 1, and receptor tyrosine kinase (ROS1).[9,10] In the era of targeted therapy, it is a significant improvement in cytopathology sampling. Cytopathological analysis of indeterminate thyroid nodules, when combined with ThyGenX/ThyraMIR and ThyroSeq, reduces the surgery rate by 68.6%.[11]

The seamless integration model is shown in Figure 1.

BARRIERS AND BRIDGES

While integration is not easy to perform continuously with diagnostic precision, several barriers exist.[12] These include:

(a) Pre-analytical variability: Due to diverse fixation methods such as air-drying, formalin, and alcohol, they can affect molecular techniques.
(b) Sample adequacy and preservation: For standardized molecular testing to be successful, a sufficient quantity and quality of nucleic acid is required, which is often difficult to collect from cytology samples.
(c) Standardization deficit: The lack of standardized protocols for cytology sample processing for molecular techniques hampers universal protocol structure making
(d) Training gaps: Limited exposure to molecular testing and validation is another hurdle faced by cytopathologists.

Core recommendations and solutions:

(a) Standardized pre-analytical protocols for cytological samples: Specific protocols concerning sample collection timing, handling, and transport with minimal DNA damage should be followed strictly.[13] Fixation of tissue and preservation of material should be standardized for molecular assays. Alcohol-based fixatives like ethanol have proved to be the best fixative for molecular testing[13,14]
(b) Sample adequacy and quality for molecular testing: For morphological assessment and molecular interpretation, specimen triaging by ROSE has dramatically improved adequacy checking.[9] Definite cellularity threshold criteria should be standardized and followed (e.g., minimum number of preserved cells for better DNA quality).[13] Many ancillary techniques, such as cell blocks, liquid-based cytology, and needle-rinsed collections, should be optimized for nucleic acid extraction[10,13]
(c) Protocols for integrated reporting and workflow: A standard integrated reporting system where combined cytology and molecular reports, with interpretation guidance and actionable mutations, should be implemented.[15] Seamless data transfer between the cytology and molecular labs should be established using LIS.[16]

THE RISK OF FRAGMENTATION

Suppose cytopathology and molecular pathology are not interlinked and do not go together. In that case, we risk creating a bifurcated system, where cytopathologists are only constrained to morphology, and molecular pathologists or oncopathologists will be restricted by the genetic data without the necessary background morphological knowledge or correlation. This disintegration can attenuate the interpretative benefit of cytopathology – its potential to complement cellular morphology with molecular alterations in a single, cohesive diagnostic framework.

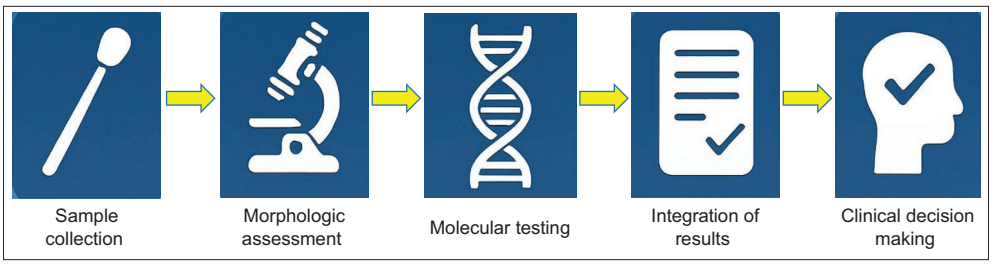

Figure 1: Workflow demonstrating the integration of cytopathology with molecular diagnostics in the molecular era, from sample collection to clinical decision-making.

TOWARD A HYBRID DIAGNOSTIC MODEL

A hybrid model should be adopted to avoid fragmentation in cytology, where cytopathologists are the key contributors to molecular pathology.[4,6]

- Upstream involvement: Cytopathologists can help in specimen triage during on-site evaluation to ensure sufficient material for morphology as well as molecular testing
- Standardized protocols: Multidisciplinary meeting to develop consensus guidelines for the handling of cytology samples in the molecular lab[17]
- Cross-training: Integration of molecular pathology modules into the cytopathology fellowship program
- Digital and AI integration: With the increasing use of digital pathology and artificial intelligence with good sensitivity and specificity, the diagnostic precision of both gynecological and non-gynecological cytopathology will benefit.[18]

THE PROMISE OF MULTIMODAL CYTOPATHOLOGY

The ultimate goal of cytopathology is to make a blended and holistic approach where early screening morphology meets the ultimate genetic signature for management and therapy.[6] Now let's think of a situation where we make a diagnosis of adenocarcinoma on FNAC material, ensure sample adequacy, and send it for PCR/next-generation sequencing test for EGFR/ALK/ROS1 mutation in a molecular cytology lab, and integrate AI for risk stratification, helping the clinician with personalized therapeutic management.[19] This whole integration can prevent further invasive biopsies for molecular testing.

This flawless model aligns perfectly with tailored cancer treatment, where timely, comprehensive diagnostic information drives therapy selection.

CALL TO ACTION

The molecular upgrade does not depreciate cytopathology's value but enriches it.[20] However, to remain central, cytopathology must endorse its application in diagnostic molecular pathology, team up with the molecular laboratory, and invest in training and research on cytology-molecular collaboration.

SUMMARY

Cytopathology is stepping into a world where its traditional capability can be amplified rather than outdated by modern molecular techniques. Cytopathologists have to be forward-looking and embrace the future of molecular innovation, thus strengthening a better integrative future. The coming era of cytopathology provides an opportunity to redesign cytopathology from merely a morphology-based service to a dynamic platform that incorporates cell morphology details with genetic and epigenetic signatures and artificial intelligence to one flawless patient report. Choosing collaboration over compartmentalization will ensure that cytopathology remains relevant and indispensable in guiding timely, tailored, and effective treatment decisions in modern cancer care.

AVAILABILITY OF DATA AND MATERIALS

No new data were generated.

ABBREVIATIONS

ALK: Anaplastic lymphoma kinase
BRAF V600E: v-raf murine sarcoma viral oncogene homolog B1 V600E
EGFR: Epidermal growth factor receptor
FNAC: Fine-needle aspiration cytology
HPV: Human papilloma virus
NGS: Next-generation sequencing
PCR: Polymerase chain reaction
ROS1: ROS proto-oncogene 1, receptor tyrosine kinase
EBUS-TBNA: Endobronchial Ultrasound-guided Transbronchial Needle Aspiration

AUTHOR CONTRIBUTIONS

SD: Concept or design of the study; SD, NKR, BD, PK: Acquisition and interpretation of data, drafting the article or revising reviewing it critically for important intellectual content, final approval of the version to be published, and aptitude to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All the authors meet ICMJE authorship requirements.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

EDITORIAL/PEER REVIEW

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