Cytopathology in Focus: Synergy in cytopathology and molecular microbiology

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August 2018—In today's less-is-more world, health care consumers and providers often seek explicit and detailed information from minimally invasive procedures and tiny samples. Over are the days of "malignant cells present" and on to the next case. Cytopathologists and cytotechnologists are embracing and integrating novel techniques and applying new methods to the diagnosis and classification of essentially every imaginable form of neoplasia. The 2018 WHO publications confirm that 29 percent of deaths worldwide (more than 10 million people annually) are attributable to communicable diseases.[1,2] This means the purpose of procuring many specimens is not to just rule out malignancy but also to diagnose infectious etiologies. Awareness of current and potential future synergies between traditional cytopathology practices and molecular microbiologic approaches may help pathologists and their patients sleep better at night.

When many physicians "think cytology" their minds turn immediately to concepts of cervical cancer prevention by Pap testing. No cancer screening test has contributed more to the well-being of humans than good old-fashioned exfoliative cervicovaginal cytology. Cotesting and reflex testing of liquid-based cervicovaginal cytology samples for human papillomavirus have become standard of care, and a burgeoning literature exists that evaluates and compares various commercially available and laboratory-developed techniques for detecting nucleic acids and proteins that are specific to certain strains of HPV.[3,4] With the recent Food and Drug Administration approval of the first HPV molecular test for primary screening for cervical cancer, even the lay press (*Time*) has published articles covering the synergistic applications of molecular microbiologic techniques and liquid-based cytology samples.[5,6]

Non-morphologic and non-culture–based testing platforms have also emerged as the gold standard for diagnoses of other cervicovaginal infections, including *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. These pathogens can be readily identified from PreservCyt liquid-based Pap samples.[7,8] Some laboratories are also using commercially available, non-amplified nucleic acid probe-based tests on cervicovaginal cytology samples to identify the etiologic agents of vaginitis, including *Candida* species, *Gardnerella vaginalis*, and *Trichomonas vaginalis*.[9,10] Molecular testing for HPV can also prove valuable in nongynecologic cytologic samples derived from anal carcinomas and squamous cell carcinomas of the head and neck.[11,12]

Molecular testing of cytology samples for oncogenic viruses is not limited to HPV. Other viruses such as Epstein-Barr are known to be carcinogenic, and confirmation of EBV in conditions such as certain B-cell lymphomas and undifferentiated nasopharyngeal carcinomas can definitely be achieved from cytology samples.[13,14]

Many infectious organisms can be identified by cytomorphology using routine or special staining. Historically, further workup of bacterial, mycobacterial, and fungal infections in body fluids and needle aspiration samples was based on conventional cultures with colony morphology and relevant biochemical testing. Today, bacterial and fungal infections that are morphologically detected in cytology samples can be identified using matrix-assisted laser desorption ionization time of flight or polymerase chain reaction techniques.[15,16] While the direct identification of fungi from specimens without the need for culture first is still largely limited to selected *Candida* species or to single-target PCR assays, multiplex PCR panels, broad-range PCR followed by DNA sequencing, and even metagenomic applications are now being used to detect and characterize fungal pathogens.[17] Applying molecular techniques to the identification of mycobacterial infections has the potential to improve the rapidity and accuracy of tuberculosis diagnosis and management in the developed and developing worlds. Identifying *Mycobacterium tuberculosis* DNA is feasible in percutaneous fine needle aspiration, endobronchial ultrasound-guided FNA, and sputum slides, with molecular testing capable of not only confirming a diagnosis but also in some settings

identifying genes linked to specific types of drug resistance.[18-22]

From the perspective of sample volume, cerebrospinal fluid is a cytology sample type in which laboratorians are often asked to do more with less. PCR testing of CSF is possible for single pathogens or as meningitis/encephalitis panels. *Cryptococcus neoformans* meningitis can be readily confirmed by PCR.[23] In addition, rapid and accurate diagnoses of acute pyogenic meningitis due to *Streptococcus pneumoniae, Haemophilus influenzae*, and *Neisseria* meningitidis are feasible from small-volume samples.[24] The same techniques used for *M. tuberculosis* diagnosis in other cytology samples can be applied to aliquots of CSF.[25]

Cytopathologists who perform and interpret FNAs sometimes encounter clinical situations or morphologic features that suggest infectious etiologies. Certain infectious agents are difficult to culture and are sometimes difficult to identify by traditional special biochemical stains. *Bartonella henselae*, the pathogen in cat-scratch disease, can be confirmed by PCR assay.[26,27] In a similar sense, *Francisella tularensis*, the pathogen in tularemia, is also identifiable by molecular means in cytology specimens.[28]

In an excellent recent review in *Diagnostic Cytopathology*, Canberk, et al., cogently and concisely cover three main categories of nucleic acid testing related to the identification of specific infections in cytology samples, including amplified nucleic acid techniques, non-amplified techniques, and microarrays. In their closing remarks, the authors write, “The integration of nucleic acid testing methods with cytopathology provides improved diagnostic protocols and in some cases a correct diagnosis more rapidly for life saving treatment.”[29] One day in the not-so-distant future, high-throughput sequencing capable of producing massive sets of parallel data may allow for a universal or unbiased molecular microbiologic approach to the diagnosis of infectious diseases.30 Even with this highly advanced technology, close communication between cytopathologists, microbiologists, and the clinical team is of paramount importance. Today it is necessary to assess which tests to use, where and when to use them, and how to best combine molecular microbiologic methods with cytopathologic findings to maximize diagnostic potential and ensure optimal benefit as we aim to provide the highest quality patient care.[29] In some instances, tiny samples may be enough to do it all.

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